Non-exercise micro-interventions to mitigate sedentarism induced poor health

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Non-exercise micro-interventions to mitigate sedentarism induced poor health

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Abstract

Introduction: Displacing sedentary behaviour (SB) with light intensity physical activity (LIPA) enhances health in older adults. However, neither the underpinning physiologic mechanisms nor any impact of LIPA pattern are clear. Therefore, the current thesis investigated the effects of chronically displacing SB, with two distinct patterns of LIPA (intermittent vs continuous) on musculoskeletal health outcomes in older women. It was hypothesised that both LIPA implementations would improve health, with SB fragmentation (SBF) resulting in greater enhancements.

Methods: Thirty-six community-dwelling older women were recruited (73±5 years). Following lifestyle (physical behaviour & diet) and health (body composition, muscle morphology, strength, activation capacity and physical function) assessments, participants were allocated to either: 1) SBF (2 minutes LIPA for every 30 minutes SB) (*n* = 14), 2) continuous LIPA (45–50-minute LIPA bout) (*n* = 14), or 3) control (*n* = 8). Assessments were repeated at week 8.

Results: SB displacement with LIPA was successfully implemented and overwhelmingly perceived as palatable and achievable. Irrespective of prescribed pattern, displacing SB with LIPA resulted in enhanced physical function, plantar flexor (PF) isometric maximum voluntary contraction (iMVC), thoracic spine bone mineral density (BMD), and increased intake of nutrients promoting anabolism. Interestingly, increased PF iMVC was mediated via divergent neuromuscular adaptation pathways, dependant on prescribed LIPA pattern (SBF: reduced dorsiflexor antagonist co-activation, LIPA: increased PF activation capacity), and occurred irrespective of apparent PF maladaptation (reduced gastrocnemius medialis muscle volume). Furthermore, SBF reduced habitual dietary glucose intake and increased intake of nutrients promoting bone health. Accordingly, significantly greater increases in leg BMD and trends toward greater peak handgrip strength were observed following SBF.

Conclusions: Ultimately, the results from the current thesis suggest some advantage of intermittent vs continuous LIPA implementation. Notably, despite its conventional designation as suboptimal, 8-weeks of LIPA implementation enhances overall musculoskeletal health in older adults.

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Abbreviations

aCSA: anatomical Cross-sectional area

AC%: Activation capacity

AgCoA: Antagonist co-activation

ANOVA: analysis of variance

AT: Achilles Tendon

BFP%: Body fat percentage

BMD: Bone mineral density

BMI: Body mass index

CSA: Cross-sectional Area

DF: Dorsi Flexor

EI: Echo Intensity

EE: Energy Expenditure

GS: Gait speed

GM: *Gastrocnemius Medialis*

GL: *Gastrocnemius Lateralis*

HGS: Handgrip Strength

ICC: Intra-class correlation coefficient

LIPA: Light intensity physical activity

Lf: Fascicle Length

MVC: Maximum Voluntary Contraction

MVPA: Moderate to Vigorous Physical activity

PF: Plantar flexor

RT: Resistance training

SB: Sedentary Behaviour

SBF: Sedentary Behaviour Fragmentation

VL: *Vastus Lateralis*

Chapter 1 – Literature Review

The Distinct effects of Sedentary Behaviour and Physical inactivity on health in Older Adults

Sedentary behaviour (SB) is defined as any waking physical behaviour, characterised by low energy expenditure (≤1.5 Metabolic equivalents), and a seated/ reclined posture (1, 2). SB does not merely reflect a lack of physical activity. Physical inactivity is independently associated with adverse health outcomes (e.g. cardiovascular disease, Type 2 diabetes mellitus) (3, 4). However recommended engagement in moderate to vigorous physical activity [≥ 3.0 metabolic equivalents (MVPA)], does not appear to fully mitigate the health risks associated with high levels of SB (5, 6). Furthermore, recommended MVPA engagement, merely constitutes ~2% of waking hours (7), potentially leaving 98% of waking hours spent in physical behaviours of a much lower energy expenditure (8). Therefore, SB is now recognised as an independent determinant of health (9-11). However, the lowest risk of adverse health outcomes are observed in those performing regular MVPA, and minimising time spent in SB (Active-ambulator), compared with those who perform no MVPA and sit routinely (Inactive-couch potato) (12, 13), suggesting both physical behaviours have a synergistic positive effect on health, which is more powerful than either behaviour alone. Nevertheless, SB poses an independent health risk, and has hitherto received little scientific attention compared with physical inactivity.

Interestingly, light intensity physical activity [light walking, household tasks, etc, 1.5-3.0 metabolic equivalents (LIPA)] is associated with positive health benefits (14-16), and is strongly inversely correlated with time spent in SB (r = -0.99 to -0.96) (7, 17-19). Therefore, the detrimental effects of SB could plausibly be due to the spontaneous displacement of LIPA with SB. Furthermore SB tends to be accumulated in prolonged uninterrupted bouts (20), with such a pattern suggested to be more detrimental, compared with greater fragmentation (shorter sitting bouts, frequent standing breaks etc) (11, 21, 22). However, the current accelerometery methodology widely employed across studies fails to account for posture (stood vs seated) (23), and thus may underestimate physical behaviour nuance, given the mere quantification of raw movement signal [quantifying counts per minute (cpm)] (24, 25). For example, using arbitrarily defined movement thresholds (≤99cpm = SB, ≥100cpm LIPA) (26, 27), can cause inaccurate classification of light upright activity (stationary standing) as SB (23, 28). However, it can be concluded based on current evidence that, an absence of

lower intensity movement irrespective of posture (stationary behaviour), which typically results in prolonged uninterrupted periods of SB, is detrimental to health.

Older adults (herein defined as ≥ 65y), spend ~65-80% of their waking hours performing SB (10, 29, 30). When compared to younger populations older adults are consistently more sedentary (31-33), and there is a concerning trend of decreasing physical activity (30, 34-36), and increasing SB (5, 37-39) with age. Interestingly, older adults consistently underestimate SB time using self-report (questionnaire), compared with accelerometery (40), suggesting an unawareness of daily SB time. Pooling of objective data from numerous studies, suggests older adults spend 9.4-10.5h.day (63-80% of waking hours) performing SB on average (41, 42), assuming 16 hours waking time. However, it must be acknowledged that this study assumed 16h waking time due to the lack of objective data for wake and sleep time (41, 42), considering this is typically determined through a subjectively reported wake/ sleep diary.

Prolonged SB engagement in older adults is strongly associated with chronic metabolic morbidity including obesity, metabolic syndrome, and type 2 diabetes mellitus (43-45). SB also exhibits a dose response relationship with all-cause and cardiovascular disease mortality in older adults (11, 46, 47). Consequently, prolonged uninterrupted bouts of SB in older adults are associated with increased risk of chronic metabolic morbidity, and all-cause mortality. A further consequence of SB engagement in older adults, is compromised quality of life (48, 49). SB is independently associated with increased risk of frailty (50), sarcopenia (the age related loss in muscle mass/ function) (51-53); and physical disability (54) in older adults. Accordingly, SB is proposed to elicit maladaptation to the muscle-tendon complex (MTC) (10, 55, 56), suggesting a potential mechanism for decreased physical function. Consequently, prolonged uninterrupted bouts of SB are proposed to exhibit a detrimental effect upon physical function (51, 53, 57), which may ultimately compromise quality of life, and lower the chances of ageing successfully (58, 59).

Therefore, the aim of this review is to discuss the effects of SB on physical aspects of older adult's health, with focus on body composition, physical quality of life, and the potential mechanisms responsible. Novel non-exercise micro-intervention strategies to counteract such negative effects will also be discussed, as well as directions for future research.

The effects of sedentary behaviour on all-cause mortality in older adults

Self-reported SB is consistently associated with mortality risk in older adults (60-63). Notwithstanding methodological heterogeneity between studies, self-reported SB is also limited by question misinterpretation, the difficulty in accurately recalling SB, as well as a strong social desirability bias influencing participant responses (40, 42, 64). However, the association between SB and mortality in older adults persists within studies utilising accelerometery (12, 65, 66). Despite such methodological progress establishing a clear dose response relationship between SB and all-cause mortality in older adults (46), such studies failed to account for a large amount of confounding lifestyle variables (smoking status, socioeconomic status, habitual diet). Recent iso-temporal substitution modelling studies have observed lower all-cause mortality risk, of ~11% when displacing 30 minutes SB with LIPA (67, 68).

Importantly, excessive MVPA engagement does not appear to offset the mortality risk associated with high TV viewing time (>5h.day) (6, 13, 69). MVPA does not appear to protect against TV viewing, due to the fact that TV viewing is specifically also linked with unhealthy dietary behaviours (70, 71). However, MVPA does in-fact protect against general SB, considering the mortality risk of high SB time (≥8h.day) is offset with excessive MVPA engagement (60-75min.day) (6, 13, 69). Nevertheless, mortality risk offers little insight as to the quality of life experienced up until the point of death, thus people may be living longer but not necessarily healthy, when performing high levels of MVPA in conjunction with high SB. Furthermore, the protective effect of excessive MVPA is based largely upon analysing self-reported studies that also failed to account for SB bout length. Therefore, it is still unclear whether excessive MVPA protects against chronic SB in a prolonged uninterrupted or more fragmented seating pattern. Accordingly, older adults engaging in a prolonged uninterrupted SB pattern, exhibit a 32% higher risk of all-cause mortality compared to those who accumulate SB time a more fragmented pattern (11). However, SB breaks were recently found not to be associated with all-cause mortality in older adults (68). A potential explanation for this discrepant finding may be the aforementioned reliance on inadequate accelerometer methodologies (25, 72).

However, the failure to account for variations in body composition is the major limitation of the current SB-mortality evidence base. Previous studies have merely

used body mass index (BMI) as a confounding variable (11, 68), despite the fact that BMI is not an infallible assessment of body composition (73-75). Increased body weight may be a mechanism through which SB exerts the increased mortality effect, and thus controlling for BMI may subsequently diminish the association between SB and mortality (76). However, BMI does not have a clear linear relationship with mortality in older adults, with many studies showing J and U shaped curvilinear relationships (77).

The effects of sedentary behaviour on body composition in older adults

BMI is consistently negatively associated with all-cause mortality risk in older adults (obesity paradox) (78). This counterintuitive effect is likely primarily driven via a detrimental effect of reduced lean body mass in the lower BMI range, as opposed to a protective effect of adiposity (77, 78). Accordingly, both high levels of adiposity and decreased lean body mass are independently and jointly associated with mortality in older adults (77), with the latter serving as a plausible explanation for the obesity paradox (79). Chronological ageing is also associated with a reduction in bone mineral density (BMD) increasing the risk of fracture (80), and related fragility (81) in older adults. Therefore, determining the effect SB has on body composition in older adults is highly important in elucidating the overall impact of SB on long-term health outcomes.

Reduced lean body mass is the primary concern regarding body composition and mortality risk in older adults, given the age related decline in skeletal muscle mass (strength and function) (82, 83) termed sarcopenia. Inactivity is associated with accelerated age related reductions in lean body mass (83, 84). Accordingly, ~0.5-0.6% of total muscle mass is lost per day during ~10-42 days of severe muscle disuse (bed rest) in older adults (85, 86). Furthermore, ~14 days of step reduction (↓~76%) in older adults, decreases leg lean body mass by ~1.5-4.0%, and blunts the muscle protein synthetic response to feeding (87-89). Interestingly, both self-reported (51) and objectively assessed (53) SB time/ pattern are negatively associated with lean body mass (pre-sarcopenia), in older adults independent of MVPA accumulation. Promisingly, frequent LIPA interruptions to prolonged SB significantly stimulates skeletal muscle, compared with prolonged sitting (90, 91). Of note, lower body muscle

activity [expressed as a % of maximum voluntary contraction (MVC)], increases by ~8% during 2 minutes of LIPA (91).

Self-reported TV viewing is positively associated with adiposity in older adults (92). However this appears to be primarily driven by residual confounders, such as unhealthy dietary behaviours (70, 71). Prolonged uninterrupted bouts of SB are also positively associated with BMI in older adults (92). Furthermore, each 60 minute increase in objectively assessed SB time (adjusted for MVPA time) in 124 older adults, was found to be positively associated with increased visceral [0.62 Litres (L)], total abdominal (1.74 L), and subcutaneous (1.14L) adiposity, as well as a 1.86% increase in liver fat as determined by magnetic resonance imaging (93). Breaking up SB (every 20 mins) with frequent LIPA bouts (~2 mins), significantly increases net energy expenditure (EE) by ~0.33 and 0.29 kcal/min, compared to continuous sitting and standing breaks respectively (91, 94, 95).

Reduced BMD increases the risk of fractures, falls, frailty syndrome, and exacerbated mortality risk in older adults (96, 97). Increased physical activity is associated with a maintenance of BMD over time (98-101), primarily due to the frequent mechanical loading stimulus (102, 103). Such mechanical loading specifically increases lower body BMD (104-106), and reduces fracture risk (107, 108) in older adults. Conversely, activity cessation results in decreased lower body (104, 105) and spine (109, 110) BMD in older adults. Accordingly, sedentary behaviour engagement (especially in a prolonged uninterrupted pattern) is strongly associated with decreased BMD in older adults independent of concurrent MVPA time (111, 112). Promisingly, BMD losses during bed rest are perturbed with frequent light lower limb activity (109, 113). Furthermore, LIPA is associated with enhanced BMD (112), and reduced fracture risk (107), in older adults. However, whether long-term displacement of SB with LIPA results in a sufficient stimulus to enhance lean body mass, adiposity, and BMD in older adults, is as yet undetermined.

The effects of sedentary behaviour on physical function in older adults

SB is associated with greater functional impairment and reduced self-reported physical health-related quality of life in older adults (58, 59). Ultimately, SB strongly exacerbates frailty risk in older adults (114-116), compromising vitality and independence across the lifespan (58). Interestingly, the negative association

between SB and physical function (117) appears independent of MVPA (118, 119), and is exacerbated when SB is accumulated in a prolonged vs fragmented pattern (21, 57, 120). Specifically, SB is associated with reduced unipedal stance ability and increased falls risk in older adults (121, 122). Furthermore, SB has been identified as a mediator for the association between obesity and falls risk (123). Interestingly, past experience of a fall and fear of falling in older adults, are associated with increases in SB time of ~22 and 45 minutes per day respectively (122, 124) . This suggests an adverse event sequence, whereby SB increases the risk of a fall, and a fall [or concern of one (re)occurring] increases SB. Alas, increased SB is associated with diminished sit-to-stand ability/ gait speed in older adults (122), both independent predictors of mortality risk (125, 126). SB is also linked to upper body functional markers like handgrip strength (HGS) (52, 127). Despite consistent associations between SB and physical function in older adults (≥65y), SB time does not appear to be as detrimental to younger adults (<65y) (128-130). Nevertheless, SB time is associated with diminished function in early old age (65y) (131). This suggests the 'Sit-Less' message may possess greater utility with both advancing chronological age and frailty status. In fact a recent consensus statement concluded clear associations exist between SB and geriatric health outcomes, despite the evidence base being mostly epidemiological at present (48). Promisingly LIPA time is positively associated with gait speed in adults aged ≥50y (132), suggesting LIPA implementation could enhance physical function in older adults.

The effects of sedentary behaviour on muscle-tendon structure and function in older adults

Negative alterations to the structural and functional aspects of the muscle tendon complex (MTC) are proposed to be a primary mechanism mediating the aforementioned detriments in physical function with prolonged sedentary behaviour in older adults, but this has yet to be systematically demonstrated. Structural and functional aspects of the plantar flexors explain the majority of the variance in postural stability in older adults (133). Furthermore, higher levels of muscle quality in the lower limb plantar flexors are consistently associated with functional capacity in older adults (134-136). Accordingly, *Triceps Surae* weakness is specifically associated with diminished stability during ambulation, and reduced ability to recover from balance perturbations (137). Therefore, continued investigation of the *Triceps Surae* and the

Achilles tendon is highly warranted. Alas, the association between SB and diminished functional performance in older adults may be due to deterioration in MTC function. Chronological ageing is associated with a progressive loss of muscular strength (138). Aside from sarcopenia and increased tendon compliance, age-related strength losses are partially due to increased antagonist co-activation (133, 139, 140), motor-neuron loss (141)/ denervation (138) and reduced agonist activation capacity (138, 142).

Lower levels of physical activity are associated with greater age-related declines in muscle function (142-144). Furthermore, physical activity is a modulator of neural activation (145), and reduced fibre-specific tension can be accounted for through reductions in physical activity (146). However, SB is proposed to affect skeletal muscle function independent of concurrent MVPA time (10), likely mediated via reduced contractile activity during SB (91, 147). Accordingly, SB is negatively associated with knee extensor strength in older adults (148). Furthermore, extreme disuse (bed rest) in older adults, results in a reduction in knee extensor voluntary activation capacity (~7%), and isometric knee strength (10-13%) (149-151). Notable also, is the fact that women tend to exhibit larger reductions in strength following disuse compared to men (152-154). Interestingly, prolonged SB time is counterintuitively associated with enhanced 'muscle quality' older adults (155). However, the authors definition of 'muscle quality' failed to account for several parameters essential to muscle function calculation (antagonist co-activation, agonist drive, muscle architecture) (55).Therefore, despite the lack of high quality evidence not permitting definitive conclusions, SB does appear to exhibit a detrimental impact on MTC size and function in older adults.

The efficacy and preliminary health effects of sedentary behaviour reduction interventions in older adults

Most interventions in older adults have reported mixed efficacy regarding the ability to reduce SB (71). For example, a group of obese older adults (n=25, 60-84 years old, BMI 34kg/m^2, 70% female, 91% white, USA) took part in an 8-week theory-based intervention, involving motivational phone calls, graphical feedback (physical behaviour), and self-monitoring (setting reminders to cue breaks from sitting) (156). Impressively, participants reduced daily SB time (~27 minutes), increased daily standing time (~25 minutes), and performed 2 additional sit to stands per day.

Participants also significantly improved physical function post intervention, considering a reduction in the time taken to complete a 3-m walking course (0.42s on average).

Furthermore, a group of overweight older adults (n=38, 62-74 years old, BMI 29kg/m^2, 71% female, 85% white, USA), took part in a 12-week two-armed randomised intervention study, involving phone calls, in person visits, and digital feedback (157). Participants were randomly allocated to either an MVPA intervention (n=19) or 'Sit Less' intervention (n=19) (157). Despite not achieving the desired reduction in SB time (1 hour), the 'Sit Less' group was the only group to improve sit to stand ability (0.4 average point improvement). The authors speculated this was due to a specificity of training effect, as trying to reduce SB time specifically improved the ability to stand up from a chair.

A further group of frail older adults (n=23, 69-90 years old, BMI 27.3kg/m2, 67% female, UK), took part in a 10-week two-arm randomised intervention pilot study (158). Participants were randomly allocated to either a behaviour change (motivational interviewing, home visits, and feedback) intervention (n=11) or behaviour change with the addition of real time feedback intervention (n=12) (157). This involved a novel accelerometer vibration, that prompted the participant after a pre-determined amount of SB time (e.g. 30 minutes). The chosen interval length was individually determined based on participant goals. Despite no change in SB, both groups experienced an improvement in gait speed (4s reduction in the timed up and go test), as well as sit to stand ability (2 additional sit-stands performed in 30 seconds).

A major limitation of the current evidence base is that none of the studies discussed utilised a control group for comparison (39, 156, 157). Furthermore, previous interventions merely prescribed a generic reduction in SB time, without specifying which behaviour should replace SB (no instruction or merely instructed to perform more standing and moving) (39, 156, 157). Nevertheless, general increases in light walking time over 6 (159), and 12 (160) weeks enhances gait speed in older adults, suggesting LIPA may play a role. Moreover, previous interventions have not controlled prescribed SB pattern (no instruction, self-determined alarm, reminder, and accelerometer prompt intervals) (39, 156, 157). This despite authors noting that the pattern of SB appears more changeable and speculating improvements in physical function are specifically a result of breaking up SB pattern (fragmentation) (157, 158).

Therefore, determining both the optimal SB displacement behaviour (standing or LIPA), as-well as the optimal fragmentation pattern, could improve the efficacy of future SB reduction interventions in older adults. As such, investigating SB fragmentation [(SBF) regular sit-to-stand transitions, and frequent bouts of LIPA] (39, 157) in older adults could help to elucidate these unknown factors.

Older adults find SB reduction interventions acceptable, easy to incorporate (161), and experience minimal adverse events (e.g. low dropout rate) (39, 156, 157) during such interventions. Moreover, considering SB reduction specifically enhances chair stand ability in older adults, whereas increased MVPA fails to improve physical function (157), this further suggests SBF may be a more feasible and sustainable means of improving physical function in older adults. Accordingly, one sit-to-stand transition (followed by 10 minutes of sitting) results in a relatively low energy cost (1.49kcal/min) (90), compared to structured lower body exercise [bodyweight squatting for 10 repetitions per minute (6kcal/min) (162), 5 minutes of parallel squatting, 40% of one repetition maximum (8-11kcal/min) (163)]. However, it should be noted that the aforementioned studies investigated energy cost in younger adults (95, 162, 163), and protocols to assess sit-to-stand transitions are poorly standardised in the literature (164).

Nevertheless, a relatively lower energy cost during SBF, may result in a greater level of long-term compliance in older adults compared to MVPA. This is ideal considering older adults have poor long-term tolerance for MVPA (165, 166), which makes recommendations to engage in structured exercise at best impractical, costly (time and or support-wise) or at worst unachievable (167). Despite a relatively low energy cost, sit-to-stand transitions require a similar level of force production as lower body exercise, and thus generate a similar level of muscle activity (95). Aside from a specificity of training effect, comparable muscle activity may potentially be a further reason for improvements in physical function following SB reduction (e.g. increased chair stand ability). Therefore, it can be anticipated that displacing SB with LIPA would be a safer, less effortful, and more sustainable means of improving physical function in older adults compared to structured MVPA.

Conclusion

In conclusion, SB is ubiquitously prevalent in older adults, and long-term engagement in such behaviour exhibits a very clear dose response relationship with all-cause mortality, and a negative impact upon lean body mass and BMD. Despite the relationship between SB and adiposity in older adults being less clear, interrupting prolonged SB with frequent bouts of LIPA stimulates marked increases in both energy expenditure and muscle activity. SB is markedly consistently associated with exacerbated loss physical function and quality of life in older adults. Despite not yet being systematically demonstrated maladaptation to structural and functional aspects of the MTC are likely implicated in this process. Current published SB reduction interventions in older adults have shown that where the pattern of SB is changeable, this has positive effects upon physical function. However, it is still unclear whether the positive effects of SBF are primarily due to decreasing SB, or due to SB displacement spontaneously increasing LIPA time. Furthermore, the physiological mechanisms underpinning such changes are still as yet undetermined, but likely include, learning to better perform functional assessments (e.g. practicing sit-to-stands), neural adaptations (independent of muscular adaptation), muscular adaptations, or in fact a combination of all three. Consequently, interventions aiming to manipulate the SB-LIPA interchange in older adults with the intention of improving a wide range of health outcomes are currently limited and warrant a long-term intervention trial.

Chapter 2 – Methodology, Experimental Design, and Experimental procedures

Experimental Design

A randomised controlled trail was chosen to build upon the previous SB reduction intervention studies in older adults [one 8-week pre-post intervention (156), & two randomised intervention studies (39, 157)]. Considering, a major limitation of the current evidence base is the lack of a control group (39, 156, 157), a control group was utilised to improve the reliability of evidence suggesting SB displacement is an effective intervention in older adults. Furthermore, previous studies have used non-specific instructions, regarding both the physical behaviour that replaces SB time (standing or LIPA), as-well as varied instructions regarding the pattern of breaking up SB (39, 156, 157). Therefore, two experimental groups were also utilised to investigate confounding factors which may have impacted previous results (standardised SBF pattern and specific SB displacement behaviour). The chosen study design was therefore a randomised controlled trial with 3 groups (2 experimental groups and 1 control), and 2 time points (pre and post).

Sample Size and Power Calculation

A power calculation was performed with G*power before the study commenced. Gait speed was the primary outcome measure used to assess sample size. Gait speed was chosen considering previous SB reduction interventions have consistently observed improvements in gait speed (39, 156, 157). Furthermore, gait speed is a key indicator of sarcopenia and frailty in older adults (84). As mentioned, an SB reduction intervention in obese older adults (n=25) achieved a reduction in the time taken to complete a 3-m walking course [-0.42±0.64s, Cohens *d* effect size = 0.52 (moderate)]. Another SB reduction intervention in frail older adults (n=23) also increased gait speed [0.04±0.61m/s, Cohens *d* effect size = 0.51 (moderate)]. Such information suggested previous studies had achieved a moderate improvement in gait speed following SB reduction. This information was entered into G*power to calculate required sample size to detect a significant change in gait speed (a= 0.05, b=0.80, Cohens f moderate effect size =0.26) for a two-way mixed design analysis of variance (3 groups and 2

time points). G*Power estimated the main effect of time would require 119 participants, whilst the main effect of group and interaction effects would both require 146 participants. Rounding up to multiples of 3 (due to a 3-group study design), this suggested that 120-150 participants (40-50 in each group) were required to observe a significant moderate improvement in gait speed. Considering a high retention rate in previous SB reduction studies (39, 156, 157) no adjustment was made envisaging a low dropout rate. Furthermore, previous low-intensity resistance training studies in older adults, deemed total sample sizes of 17 (168), and 18 (169), adequate to detect changes in tendon and muscle size respectively. The proposed sample size 120-150 participants fell well within this range.

Participant recruitment

The study was approved by the ethical committee of Manchester Metropolitan University in March 2018 [approval code: 230118-ESS-DG-(2)]. Previous SB reduction interventions studies in older adults used a higher proportion of female participants (67-71%) compared to men (29-33%) (39, 156, 157). Furthermore, women exhibit greater anabolic resistance (154, 170), greater reductions in strength following disuse (152), and enhanced benefits from lower intensity loading (171-173) compared to men. Therefore a stronger rationale exists to study the effects of SB displacement in older women specifically, compared to men. For these reasons, participant inclusion criteria for this study specified female gender. Accordingly, all 271 contacts were older women who resided in the local community of Cheshire, situated near the Manchester Metropolitan University Cheshire campus. This was important as a long journey time is considered a barrier to recruitment (174).

The entire recruitment process was managed by the principal investigator. The primary strategy used to contact and recruit older female participants was through recruitment packages that were sent out through the post. 271 recruitment packages (which included health questionnaires, participant information sheets, and a pre-paid return envelope, please see appendices), were sent to all contacts on a pre-existing research database. Based on the k calculation (n= 120-150), this meant 44-55% of the contacted participants (n=271) needed to be recruited to achieve the desired sample size. Altruism and self-education (especially about one's own health), are key reasons why participants sign up to research projects (174). Therefore, the covering letter for

the recruitment package (please see appendices) highlighted the benefits of participation both to the participant themselves (e.g. using state of the art equipment to assess participants health), but also to others (e.g. helping the researchers uncover how activities affect the health of the population). Furthermore, the exclusion criteria was not explicitly mentioned in the recruitment letter, as not limiting the recruitment pool during initial recruitment has been shown to be an effective strategy to increase recruitment (174). Instead, participants who had an interest in in participating were asked to fill in a health questionnaire, and eligibility screened following return of the recruitment package.

The contacts on the research database had been retained from previous research studies conducted with the sport and exercise science department at Manchester Metropolitan University, specifically focusing on older adult women. This strategy was chosen considering targeting the demographic of interest (in this case older adult women) (174), as-well as recruiting participants from a pre-existing database (within an existing research community) (175), have both been shown to be optimal strategies for recruitment. However, considering the general data protection regulation deadline on 25/05/2018, such contacts also needed to provide or withdraw consent on whether their contact information could continue to be used for recruitment purposes. Therefore, recruitment packages were sent out prior to the general data protection regulation deadline on 25/05/2018 and included “General Data Protection Regulation” opt in/out permission slips”. Whilst this was not considered a direct recruitment strategy, having an opt in/ opt out procedure has also been shown to be an effective strategy for improving recruitment (176).

The secondary strategy involved recruiting from the local community. Considering the large amount of recruitment packages that were sent out (n=271) this recruitment strategy was not actively pursued (through community speaking engagements, posters, or posted information leaflets). Nevertheless, certain participants (n=2) were recruited after expressing an interest during routine in person conversation with the main investigator in community settings (e.g. a local gymnasium). Aside from this, participants who were either currently taking part in the study or had taken part, referred the study to their friends (n=8), who then contacted the principal investigator expressing an interest to participate. In such scenarios, participants were sent the

same recruitment package mentioned above. Promisingly, 131 of the 271 participants who were sent recruitment packages replied (48%). Furthermore, 70 of the 131 who replied (53%) stated a desire to participate. Combined with the participants recruited directly from the local community (n=10), this meant 80 participants were screened for potential eligibility. Following screening of health questionaries returned from the recruitment packages, certain participants (n=16) were immediately excluded based on the exclusion criteria (please see table 2.1 and figure 2.1), leaving 64 eligible participants.

Table 2.1 Inclusion exclusion criteria table

Inclusion criteria	**Exclusion criteria**
Female gender	History of lower limb muscle disorder in the past six months
Above the age of 65	History of lower limb tendon disorder in the past six months
Under the age of 85	History of lower limb joint disorder in the past six months
Physically active status (<150 minutes of MVPA per week, or ≥ 150 minutes MVPA per week)	Currently suffering from active cancer
Not knowingly allergic to the physical behaviour monitoring equipment	Currently suffering from cardiovascular disease
Not partaking in structured resistance training at baseline	Currently suffering from uncontrolled diabetes
[redacted]	Currently using prescribed medication likely to affect ability to perform movement

MVPA; Moderate to vigorous physical activity.

Experimental Phases

Considering limited resources during data collection (one researcher manging the entire process), the number of eligible participants was unmanageable from a logistical perspective. Therefore eligible participants (n=64) were re-contacted by phone prior to 4 separate experimental phases, each lasting 3-months. Eligibility based on the exclusion criteria was re-confirmed during such phone consultations, specific to the timing of the experimental phase. During phone consultations a further 9 participants were excluded leaving 55 eligible participants.

The experimental phases were as follows; phase 1: June 2018-August 2018, phase 2: September 2018- December 2018, phase 3: January 2019-March 2019, and phase 4: April 2019-July 2019. A manageable number of participants were recruited for each experimental phase (n= 8-10). If a participant was unavailable during one experimental phase (e.g. away on holiday), they were re-contacted for a future experimental phase. During phone consultations, prior to each experimental phase, a further 19 participants were unable to participate for a variety of reasons including lack of time, moved out of the local area, or simply declined to participate. No further recruitment cycles or experimental phases were permitted beyond July 2019, considering the closure of the

Manchester Metropolitan University Cheshire campus, in July 2019. Experimental data collection phases could not continue at the main Manchester Metropolitan University Cheshire campus, considering both the principal investigator and eligible participants resided in the Cheshire community, as well as various logistical challenges (differences in equipment between campuses etc).

Control Group Recruitment

Considering, both the, imminent campus closure deadline as well as the additional logistical challenges (more time required) of experimental participants (please see physical behaviour interventions) priority was given to experimental participants. Therefore, all eligible participants contacted during the first 3 experimental phases (June 2018-April 2019) were randomised to the two experimental groups: 1) Sedentary behaviour fragmentation (SBF) (*n* = 14), and 2) Single bout Light intensity physical activity (LIPA) (*n* = 14). Furthermore, all eligible participants contacted during the 4th experimental phase were allocated to the third group 3) Control i.e. no lifestyle change (*n* = 8). This means the design of the study is not entirely reflective of the consolidated reporting of clinical trials (CONSORT) statement (177), considering participants did not have an equal chance of being allocated to any group. It should therefore be acknowledged, that this decision does slightly compromise the integrity of the randomisation process. However, it must also be acknowledged this decision was taken under difficult circumstances (campus closure deadline) where an experimental prioritisation was entirely justified.

Experimental Process, Familiarisation, and Missing Data

All eligible participants who agreed to take part following the telephone consultation (n=36) visited the laboratory for familiarisation. Participants were asked to re-read a participant information sheet. All participants had been sent out a copy of the participant information sheet as part of the initial recruitment package, meaning all participants had a sufficient amount of time (> 7 days) to consider whether they wished to consent to take part in the study. Nevertheless, after reading the participant information sheet again, participants were asked if they had any further questions or if they wished to clarify any issues, before they signed an informed consent form. Prior to any procedures taking place participants gave written informed consent by signing the informed consent form, in line with the declaration of Helsinki. Participants were

then asked to complete additional questionnaires and screening [falls risk assessment tool (FRAT)].

During the familiarisation session participants underwent physical function (see below for more details), muscle strength (see below for more details) and neuromuscular assessments (see below for more details). Participants completed each assessment a minimum of 3 times during familiarisation, as 3 attempts is the point at which functional performance begins to stabilise in older adults (178). Participants were also asked if they were comfortable with performing each assessment before continuing. One participant (n=1) refused to undergo activation capacity assessment due to disliking electrical stimulation (see below for more details). For all testing occasions (familiarisation, pre-test, & post-test) postural balance assessments took place before all other assessments to ensure accumulative fatigue did not affect balance ability and thus internal validity. Physical function data from familiarisation was recorded and retained for all participants (n=36), except for balance posturography where only a sub-sample was available (n=10) due to time constraints. Furthermore, familiarisation data was only recorded and retained for a sub-sample of participants for muscle strength/ neuromuscular function (n=6). Finally, a sub-sample of participants data was retained for ultrasonographic assessment of muscle-tendon complex morphology (architecture & tissue related quality), (see below for more details) during familiarisation (n=8).

Before leaving, participants were provided with monitoring equipment (habitual physical behaviour and habitual dietary assessment). After seven days habitual monitoring, participants returned to undergo pre-tests. In order of procedure during the testing day, participants underwent fasted body composition analysis (10-h to 12-h overnight), ultrasonographic assessment of muscle-tendon complex morphology (architecture & tissue related quality), balance posturography, muscle strength/ neuromuscular function assessment, and remaining physical function assessments (gait speed, sit to stand ability, and handgrip strength). Before leaving, participants were then allocated (see above for more information) to one of three groups: 1) Sedentary behaviour fragmentation (SBF) (*n*=14), 2) Single bout continuous light activity (LIPA) (*n*=14), or 3) Control i.e. no lifestyle change (*n*=8) (see control group recruitment). Participants were given specific instructions depending on their

intervention group. Participants returned to the laboratory after 8-weeks to undergo post-tests as above, as well as a questionnaire, assessing intervention palatability. Physical behaviour was objectively assessed throughout the entire 8-week intervention period, whilst habitual diet (4-day food diary) was assessed at week 0 and week 8.

Throughout data collection loss of data for various outcome measures was encountered (please see figure 2.1). Accordingly, the number of participants missing data for the following outcome measures was as follows; food diary data [n=1, (control n=1)], AC% [n=1, (control n=1)], antagonist co-activation (AgCoA) [n= 5, (SBF: n=1, LIPA: n=3, control: n=1)], net plantar flexor (PF) [maximum voluntary contraction (MVC) n= 5, (SBF: n=1, LIPA: n=3, control: n=1)], *Gastrocnemius Medialis* (*GM*) muscle architecture [n=2 (LIPA: n=1, control: n=1)] and balance posturography [n= 5, (SBF: n=2, LIPA: n=3)]. The reasons for such instances of data loss (n=19), were, participant refusal to undergo assessment [food diary assessment and AC% (n=2)], and routine equipment malfunction throughout data collection [AgCoA, *GM* muscle architecture, and net PF MVC (n=17)]. Considering the logistical challenges outlined previously (campus closure, intervention design) further opportunities to obtain data missed due to equipment malfunction were not available.

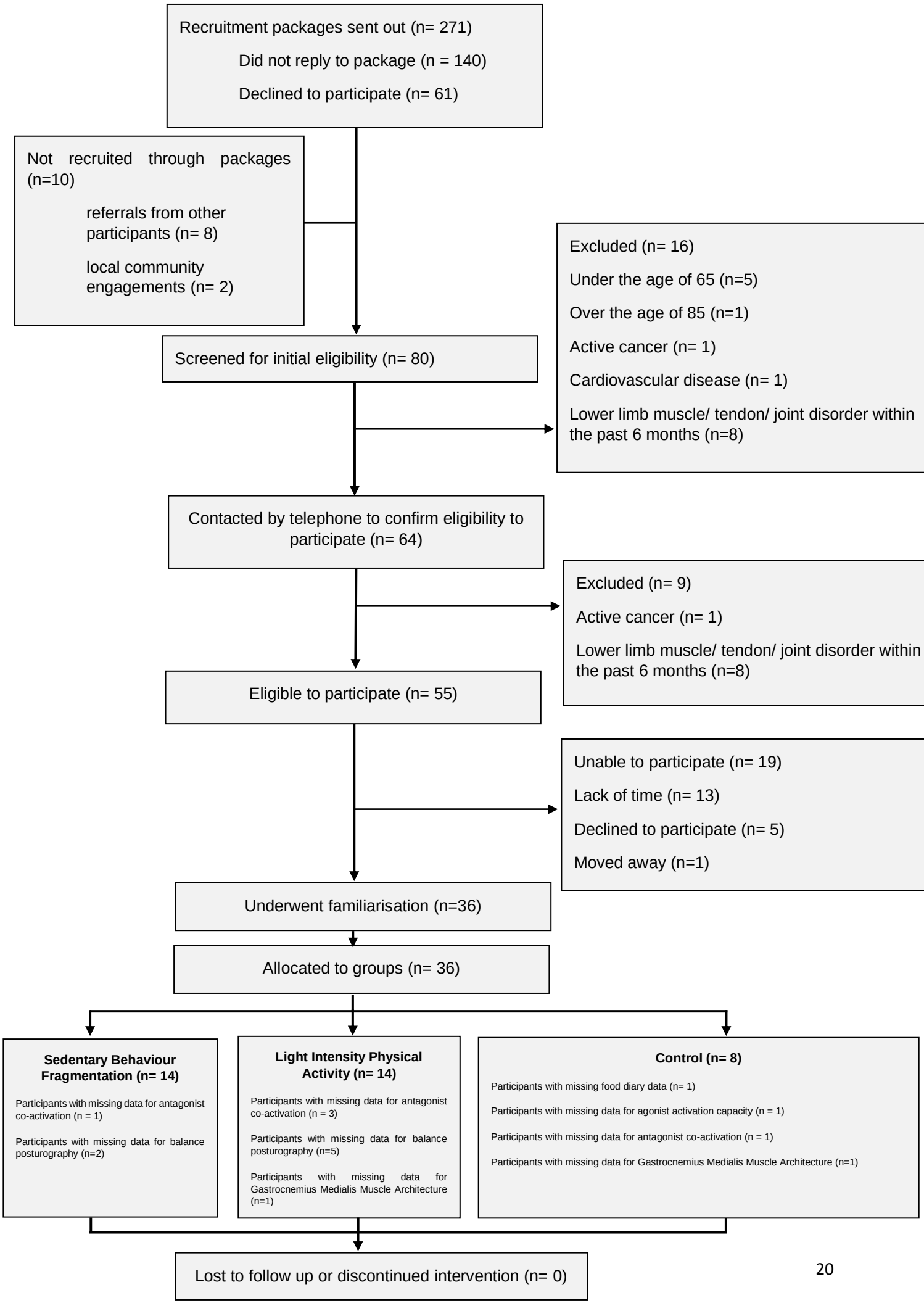

Figure 2.1: Consolidated reporting of clinical trials (CONSORT) flowchart detailing recruitment process, exclusion, allocation, and missing data

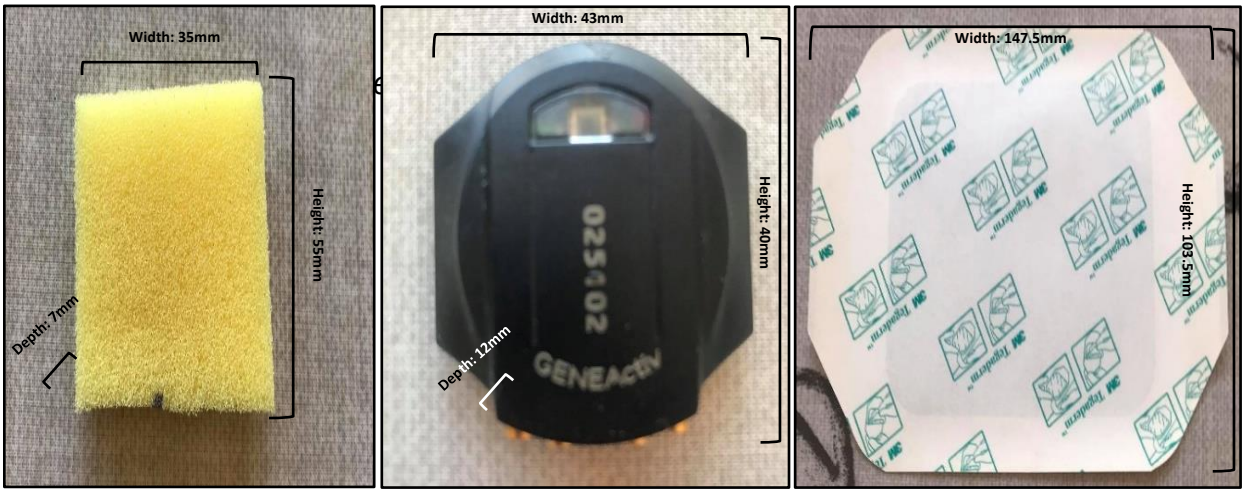

Figure 2.2- Photographic representation of physical behaviour monitoring equipment. From left to right, soft sponge, GENEA acceleromter, & TEGADERM adhesive patches.

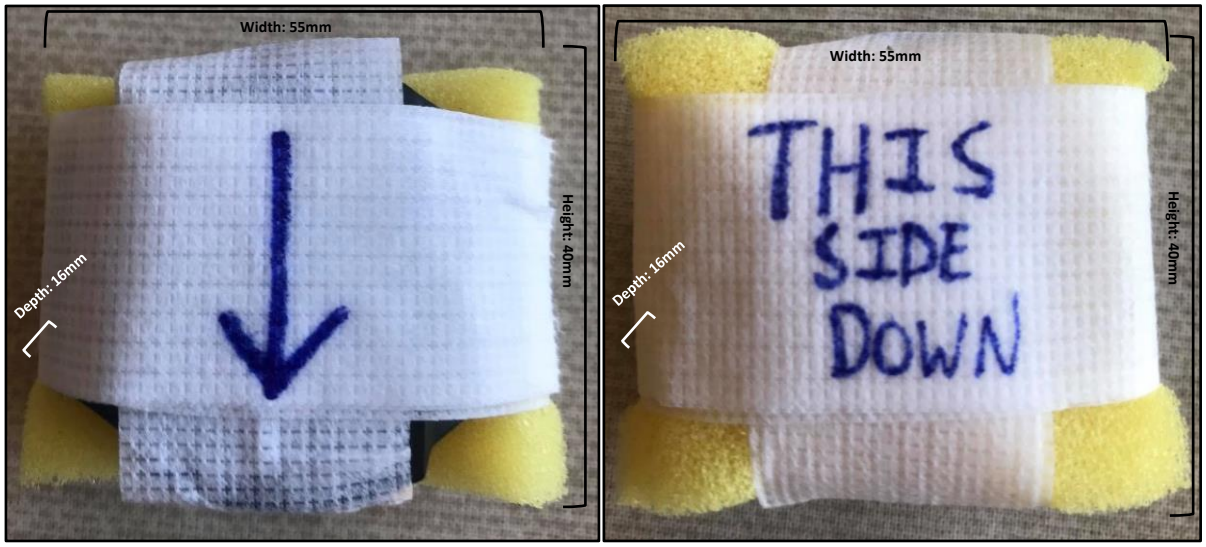

Figure 2.3- Photographic representation of assembled physical behaviour monitoring equipment. From left to right, Anterior view, of assembled GENEA device (Note: arrow to remind participant of correct orientation), Posterior View (Note: written instruction to remind participant of correct orientation).

Physical behaviour was objectively assessed with a blinded GENEActiv original (GENEA, Activinsights Ltd, Kimbolton, UK) triaxial accelerometer (43.0 mm x 40.0 mm x 16.0 g), that did not provide feedback to the participant (please see figure 2.2). The GENEActiv has previously been validated for objective measures of free-living physical behaviour (24, 179, 180). Forgoing the conventional wrist position of the GENEActiv (24), the thigh was selected as the mounting point. Such a site is considered the gold standard for SB assessment (181-183), due to the change in thigh orientation during sit-to-stand transitions and vice versa. Protocols of 2-3 days (adjusted for weekends), give reliable estimates of older adults' physical behaviour

using hip accelerometery (184). In contrast to previous intervention studies, the GENEA device was used to objectively determine physical behaviour and monitor compliance throughout the intervention period, giving nine monitoring periods per participant (baseline, week 1, week 2, week 3, week 4, week 5, week 6, week 7, & week 8). This not only allowed the conventional comparison between baseline and week 8, but also intra-week variability.

Briefly, the GENEA was pre-configured using GENEActiv software (Version 3.2, Activinsights Ltd, Kimbolton, UK). The device was connected via the USB enabled port (please see figure 2.5). Each device required ≥95% battery life prior to configuration, thus limiting the odds of data loss. Once charged each device was pre-configured (please see figure 2.6) to capture and record PB, at a frequency of 60 Hertz [frequency per second, (Hz)], and set to the maximum monitoring period of 12 days. For a given monitoring period, the device began recording at midnight (00:00:00) on the day following configuration, ensuring concise 24-hour monitoring periods were captured. Following configuration, a 35 × 55 × 7mm soft sponge (Vitrex, Burton-upon-Trent, Staffordshire) was attached to the back of the GENEA using two microporous strips (3M, Minnesota, USA). This ensured maximum participant comfort once attached (please see figure 2.3). The GENEA was mounted on the participant's leg (anterior, 50% of femur length) using two Tegaderm Films 1626W (100mm x 120mm) (3M, Minnesota, USA). Participants pulled downwards on their thigh stretching the skin, allowing better adhesion between the skin and the Tegaderm Films (please see figure 2.4). The gold connection prongs were always positioned downwards to ensure the correct orientation of the GENEA axes. Participants were provided with two spare films in case the original films began to peel away. Participants were instructed not to remove the film as this could disrupt the GENEA recording. Instead the spare films should be placed over the disrupted area.

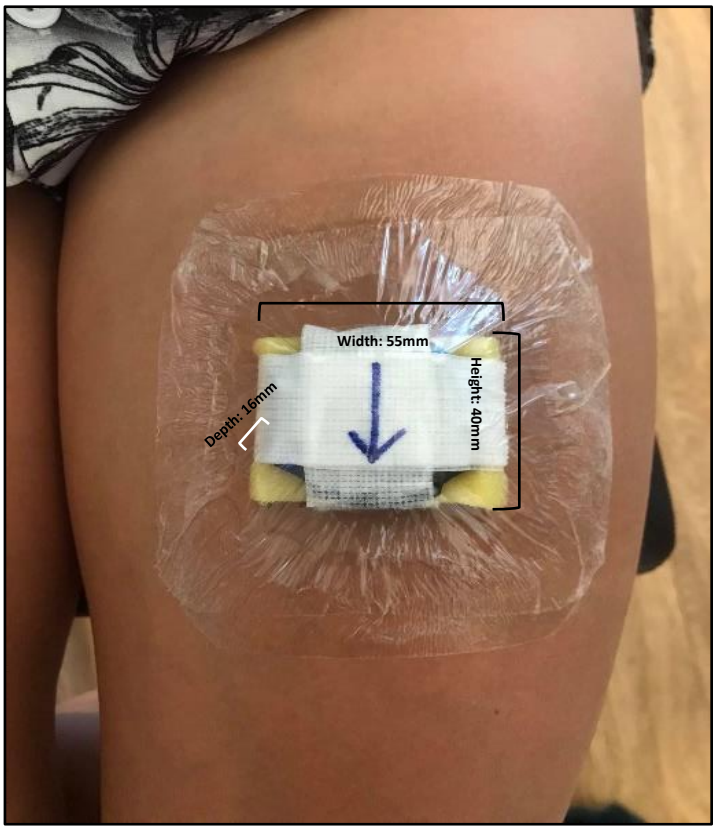

Figure 2.4- Photographic representation of fully assembled GENEA device mouted on the skin with TEGADERM patches.

Figure 2.5– GENEA devices connected to computer via USB docking station.

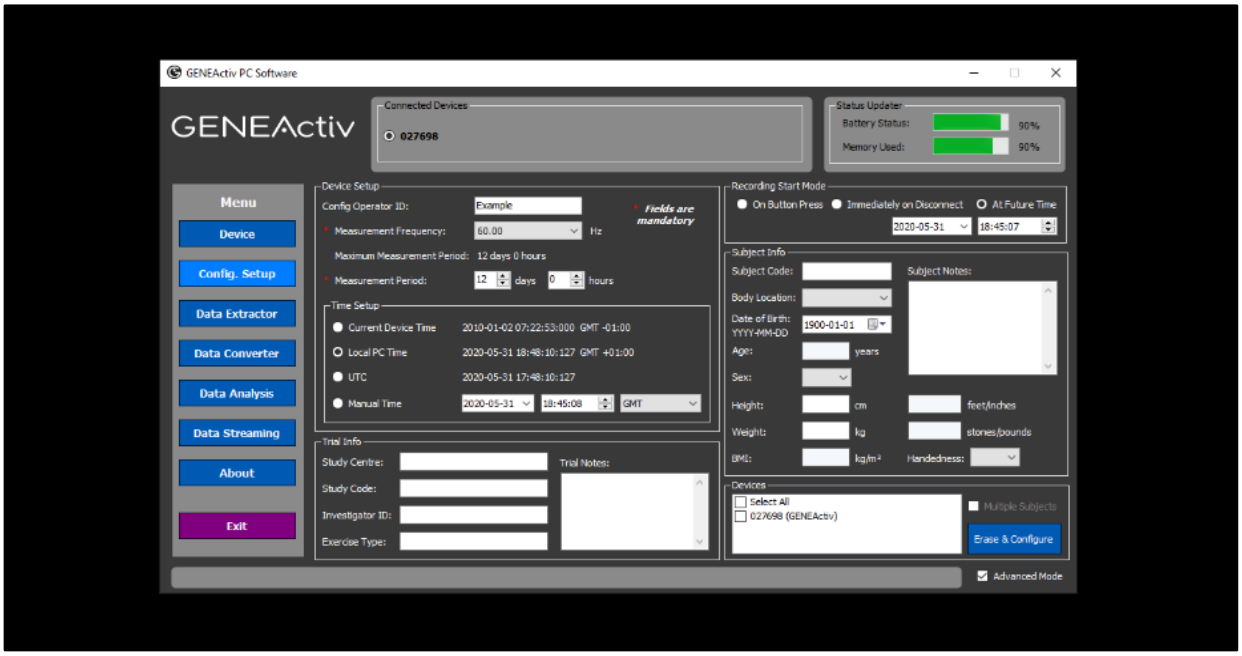

Figure 2.6– Screen grab of GENEA PC software, with configutation screen.

A diary was provided, so participants were able to record waking/ sleep (time they turned the lights off to go to sleep at night) times. Such times were used to triangulate sleeping hours. In cases of missing wake/sleep times, the epoched data was manually assessed to determine when daily movement ceased. If such a time was similar to wake/ sleep times of other days then it was assumed at the researcher's discretion, that this was the wake/ sleep time for that particular day. Participants were asked to record any device disruption (Date/Time device was removed/ re-attached). This allowed such a period to be removed from analysis if anomalies were observed, again at the researcher's discretion.

Every participant received a fortnightly home visit from the principal investigator. This was to re-secure a newly configured and fully charged (please see above) GENEA device, ensuring maximum physical behaviour recording time. GENEA mounting limb was alternated with every fortnightly investigator visit. This ensured each mounting sight did not become too irritated. Thus, alternating mounting limb ensured one mid-thigh site was given ~14 days to recover whilst the device was mounted on the opposite leg. Secondly, home visits secured physical behaviour data stored on the GENEA device. The device was removed and secured in a plastic container. The GENEA device was then removed from the sponge and thoroughly cleaned. The data was downloaded onto a computer using the GENEA software. The .bin file was smoothed in the 'Data Convertor' Stage, into 10-second (s) epochs. 10 s epochs were chosen over 60 s to increase the sensitivity to detect changes in physical behaviour and allow for comparisons with other studies (185-187).

A G value (1 G = 9.81 m/s^2) is defined as the force of the earths gravitational pull. The GENEA unit is able to detect within ±8 G's meaning it is highly sensitive to capture most human movements (including MVPA) (188). All GENEA devices underwent rigorous calibration within the laboratory after purchasing from the manufacturer. As performed previously (189), gravity's pull was recorded for one minute through each axis (X,Y, & Z) for each device, to determine if any unit fell outside of a previously suggested 5% variability limit (190). Any unit that fell outside of this limit was not used in the study. Short Epochs are recommended as a small accumulation of high intensity movement (counts per minute, G values, sum of vector magnitude), within an epoch

that consists mostly of SB could cause a larger average epoch output leading to an over/ under estimation of physical behaviour intensity (186, 191).

The Cheshire Algorithm for Sedentarism (192) was used for off-line analysis. Similar to the previously established sedentary sphere (24), each axis recording from the GENEA device (X,Y, & Z) was screened to determine which had the lowest G value. Accordingly, the participant was deemed to be in a seated/ supine, stood, or side lying orientation should the lowest value of the G- axes be X, Y, or Z, respectively. Once posture was established the residual G was automatically calculated to determine any movement that was occurring within that posture. Residual G is a similar measure of total movement (133), and is calculated according to the following equation:

$$Residual\ G = \sqrt{(SD\ x^2 + SD\ y^2 + SD\ z^2)}$$

Where:

- x is the medio-lateral axis
- y is the vertical axis
- z is the anterior-posterior axis
- SD is the standard deviation
- $\sqrt{\ }$ is the square root

The Cheshire Algorithm for Sedentarism

Briefly, The Cheshire Algorithm for Sedentarism has previously been validated by calculating the incremental metabolic cost (calculated from expired gas and heart rate) of ten everyday tasks in 40 healthy older adults (~74y) (e.g. lying down, brisk treadmill walking etc) (192). The Cheshire Algorithm for Sedentarism uses regression analysis to identify specific physical activity intensity ranges utilising metabolic equivalent thresholds (SB: <1.5 metabolic equivalents, LIPA: 1.5-3.0 metabolic equivalents, MVPA: >3.0 metabolic equivalents) mapped against the concurrently recorded GENEActiv gravitational pull and acceleration data (192). These methods, match up against metabolic equivalent cut off points for LIPA (Any standing posture that elicits

≥1.5 – ≤3.0 metabolic equivalents), and moderate to vigorous physical activity (MVPA) (Any standing posture that elicits ≥ 3.0 metabolic equivalents) (192, 193). Specifically, the balanced accuracy for estimating physical behaviour from the total movement method of the Cheshire Algorithm for Sedentarism (analysing G values from the GENEA accelerometer), meets a critical threshold of ≥80% for all movements essential to physical behaviour quantification (SB, standing, MVPA) with the exception of LIPA (192). However, LIPA classification from participant-specific balanced accuracies, demonstrated an acceptable balanced accuracy of 57.5%, from The Cheshire Algorithm for Sedentarism estimation, near perfect scores for standing (92.5%) and a perfect score for SB and MVPA (100%) (192). Accordingly, the Residual G cut off points for a 10-s epoch in a mixed sex older adult population were 0.057 Residual G for SB – LIPA (1.50 metabolic equivalents, physical activity below this threshold was classified as standing), and 0.216 Residual G for LIPA – MVPA (3.0 metabolic equivalents).

The Cheshire Algorithm for Sedentarism provided several physical behaviour parameters, such as SB (hrs.day^{-1}), Standing (hrs·day^{-1}), LIPA (hrs·day^{-1}), & MVPA (hrs·day^{-1}). Specifically for SB, the Cheshire algorithm for sedentarism provided SB Breaks (*n*·day^{-1}), <5min SB bout (*n*·day^{-1}), >5min SB bout (*n*·day^{-1}), True mean SB bout (mins·day^{-1}), Alpha (Power law exponent used to describe sedentary behaviour accumulation) (Alpha·day^{-1}), The bout duration above and below which half of all sedentary time is accrued (mins·day^{-1}), SB% (%·wakinghrs·day^{-1}). Specifically for PA, the Cheshire algorithm for sedentarism provided, physical activity Bouts (*n*·day^{-1}), Daily Sum of physical activity Bout time (mins·day^{-1}), True Mean physical activity Bout (mins·day^{-1}), $_{10}$MVPA Bouts (n·day^{-1}), Total Week $_{10}$MVPA (hrs·week^{-1}), Standing% (%·wakinghrs·day^{-1}), LIPA% (%·wakinghrs·day^{-1}), MVPA% (%·wakinghrs·day^{-1}) (194). Given the constrained monitoring periods (≤12 days), this meant crossover between monitoring periods. Thus, intervention weeks were calculated relative to each participants intervention starting date. This process often resulted in a 7-day/ 5-day split. As previously mentioned, protocols of 2-3 days hip accelerometery have been shown to give reliable estimates of older adults physical behaviour (184). Thus, if an intervention week did not contain ≥3 valid days, it was removed. Importantly, if ≥3 valid days was not present for the final intervention week (week 8), then week 7 or the next closest week containing ≥3 valid days was used as the baseline comparison. Following

physical behaviour analysis, participants were classified as either sedentary (≥8h/day) or an ambulator (<8h/day) depending on their average daily sedentary behaviour time. Participants were also further classified as physically active (≥150mins/week $MVPA_{\geq 10minute\ bouts}$), or non-physically active (<150mins/week $MVPA_{\geq 10minute\ bouts}$). Such limits were selected as classification thresholds given that sedentary time appears to be exponentially hazardous above 8h/day (195, 196), and the world health organisation recommends a weekly MVPA engagement time of ≥150mins/week in bouts of ≥10 minutes (197).

Physical behaviour data was split into three distinct categories, Absolute PB, intra-week co-efficient of variation, and intra-week individual variance. The standard deviation of a particular physical behaviour variable on a particular week was divided by the average for that week, giving the co-efficient of variation. Furthermore, intra-week individual variance was then calculated using the following equation:

$$(\Sigma(x_i - x_{Mean})^2)/n)$$

Where:

- Σ is the sum
- X_i is the daily value for that particular physical behaviour parameter
- X_{mean} is the weekly mean physical behaviour parameter value for that particular intervention week
- n is the number of valid days for that particular intervention week

Individualised variance was used alongside co-efficient of variation to give a secondary measure of variance. Individualised variance was also used to offset the weaknesses of the co-efficient of variation, mainly sensitivity to outliers, and smaller means being more sensitive to change.

Assessment of habitual dietary intake

Participants were instructed to record their habitual dietary intake on 3-week days and 1 weekend day, during the baseline week, including any supplements consumed habitually (please see food diary in appendices). Self-reported assessment of dietary intake has many limitations (198), with ~35 days needed to accurately estimate energy intake in women (199). Therefore, steps were taken to maximise self-reported accuracy. Digital weighing scales (Salter, Kent, United Kingdom) were provided allow weighing of food/drink to the nearest gram. Each diary was checked by the principal investigator with uncertainties clarified by the participant. If the participant was unavailable, quantity was estimated from previous diary entries. Diaries were analysed with Nutritics software (Version 5.0, Nutritics Ltd, Dublin, Ireland) to produce a comprehensive report of energy, macro, and micronutrient intakes. In the event a consumed item was missing from the Nutritics database, the data was retrieved from the manufacturer (e.g. Kellogg's) and entered into the Nutritics database. Accordingly, participants were asked to return any packaging from foods they regularly consumed (Breakfast cereals, canned foods, etc), to estimate intake as accurately as possible. Where available, barcodes were scanned, and nutritional information digitally logged using MyFitnessPal software (MyFitnessPal, San Francisco, USA). Such information was cross referenced against the Nutriritcs database. In the event of a discrepancy the MyFitnessPal data was utilised. During the current intervention, participants did not undertake nutritional counselling.

Recommended daily intake and health enhancing nutrients

Nutrient intake thresholds recommended for older women (65-74y) (200, 201) were used to evaluate intake of all nutrients with criteria available (Please see appendices i). Furthermore, previous research has identified specific nutrients as principal mediators of musculoskeletal health in older adults (202, 203). Accordingly, skeletal muscle health in older adults is modulated by habitual intake of protein (204), vitamin D (205, 206), vitamin E (207), as-well as omega-3 and omega-6 fatty acids (208, 209). Therefore, all five nutrients were grouped as key nutrients promoting anabolism. Similarly, bone health in older adults is specifically modulated by habitual intake of

calcium (210), zinc (203), magnesium (211), phosphorus (212), Vitamin C (203), Vitamin D (213), protein (214), and omega-3 fatty acids (215). Therefore, all eight nutrients were grouped as key dietary components promoting bone health.

Energy balance

The Harris-benedict formula (216) was used to calculate basal metabolic rate of all participants, given that this method has previously been shown to be valid in older adults (217). Basal metabolic rate was then multiplied by an activity factor to give total daily energy expenditure.. Activity factor was determined based upon each participants objectively determined physical behaviour profile as opposed to using physical activity classification, given that intense activity contributes minimally to total daily energy expenditure (8). Specifically, basal metabolic rate was multiplied by an activity factor of 1.2 and 1.375, when a participant was classified as sedentary or ambulator, respectively. Secondly, the Schofield equation (218) was also used to calculate basal metabolic rate. Basal metabolic rate calculated from the Schofield equation was then also multiplied by activity factors of 1.3 and 1.5, depending on whether a participant was classified as sedentary or ambulator, respectively. This gave a secondary estimate of TDEE. Both total daily energy expenditure methods were then separately subtracted from total daily energy intake to give two estimates of energy balance. Both Harris-benedict and Schofield have previously been used by the world health organisation as reference standards for energy intake (219).

Body Composition Analysis

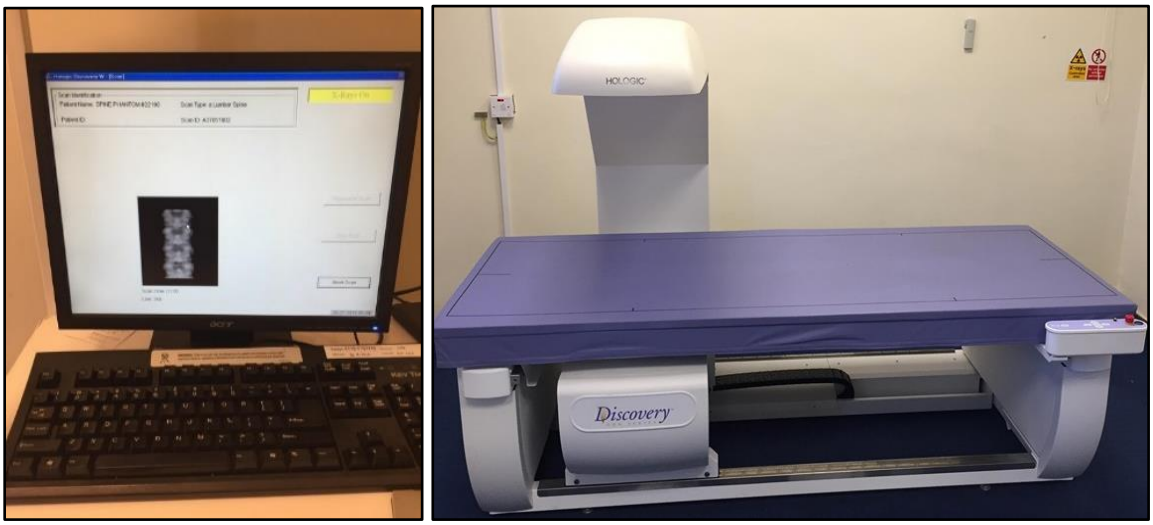

Figure 2.7– Hologic Discovery: Vertec Dual X-Ray Absorptiometry scanner and accompanying workstation.

Participants arrived at the laboratory in a fasted state (10-h to 12-h overnight) and changed into a hospital style gown (unshod) in a private scanning room, before undergoing a *Dual X-ray absorptiometry* scan. Height was measured to the nearest 0.1m using a stadiometer (Seca model 213 portable stadiometer, Seca, Germany). Briefly, participants were asked to stand with their heels against the stadiometer backboard, take a deep breath in, and out, before the scale was lowered onto their head. Participant mass was then measured with digital scales (Seca model 873, Seca, Germany), to the nearest 0.1kg. Waist (cm) and hip (cm) measurements were also manually assessed using a tape measure, as the bodily circumference at the point of the navel, and the maximum circumference of the buttocks, respectively. Waist to hip Ratio was calculated as waist/ hip.

Figure 2.8 - Hologic DXA quality control phantom.

Figure 2.9- Representative image of participant fully prepared to undergo Dual X-Ray Absorptiometry scanning (Note: Arms placed in prone position).

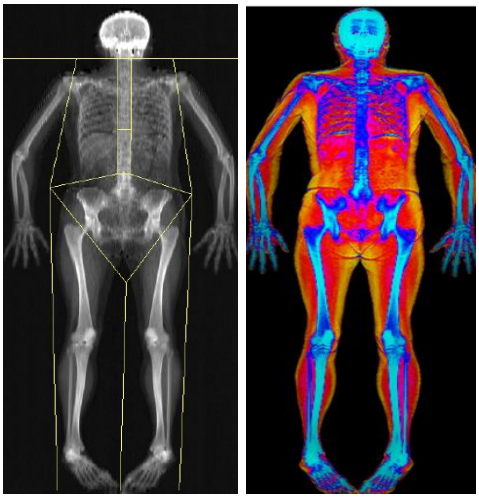

Figure 2.10- Representative Dual X-Ray Absorptiometry image. Different body composition tissues are represented in panel B as Blue (Bone), Red (Lean body mass), and yellow (adipose tissue).

A Dual X-Ray Absorptiometry scanner (Hologic Discovery: Vertec Scientific Ltd, UK) was used to ascertain whole-body composition (please see figures 2.7, 2.9 & 2.10). Before each scan, calibration was performed using a Hologic DXA quality control phantom (Hologic Discovery: Vertec Scientific Ltd, UK) (please see figure 2.8). Participants were further instructed to remove all metal objects (e.g. jewellery). However, hearing aids were permitted so that participants could hear any instruction from the practitioner. Briefly, participants assumed a supine position on the scanning bed, avoiding any contact between the trunk and the appendicular mass. Both hips were also internally rotated, so that the toes pointed inwards (220) (whole body procedure, EF 8.4 lSv) (please see figure 2.9). Variations in hand position (Prone vs. mid prone) have previously been shown to affect BMD, lean body mass, and fat mass during Dual X-Ray Absorptiometry scanning (221). However, considering the spatial constraints of scanning obese participants, consistently positioning the arms in a favourable prone orientation (please see. figure 2.9) was not always feasible. Accordingly, great take was taken to ensure internal consistency of hand position (prone vs mid prone). Participants were asked to remain as still as possible whilst the slow moving ‘arm’, scanned the body over the course of 7 minutes. Hologic software was then used to delineate body segments thus identifying regions of interest (Appendicular mass, Trunk, Android: Gynoid, etc) (222, 223), and hence Dual X-Ray

Absorptiometry derived BFP%, lean body mass and BMD (please see figure 2.10). For comprehensive body composition definitions please see chapter 7 appendices i.

Ultrasonography

Participants lay on a height adjustable physiotherapy bed in a prone position. The ankle joint of the dominant leg was positioned on top of a cushion and secured in neutral angle (90° referred to hereafter as 0°) against a footplate (please see figure 2.11). Ankle angle was measured with goniometry and re-standardised at 0° before each assessment, to ensure consistency between measures. In order to minimise fluids shift, all images were taken after 20 minutes rest in this prone position (224-226). During scanning, participants were asked to remain still and relaxed. Briefly, water-soluble transmission gel (Aquasonic 100 Ultrasound Transmission gel; Parker Laboratories Inc., Fairfield, NJ, USA), was applied to the skin and the ultrasound probe (38mm wide, frequency: 7.5mHz) placed mid-sagitally and transversely. Light pressure was applied to avoid compression of the dermal surface and thus muscle during all scans. Anatomical landmarks of the *GM* and *GL* muscles were identified on screen and marked on the skin with a pen marker, including the proximal/distal insertions, medial/lateral borders, and discrete muscle sites (25, 50, and 75% of muscle length). Proximal and Distal endpoints of the Achilles tendon were identified, and length markers drawn on in 1cm increments from the calcaneal insertion to the

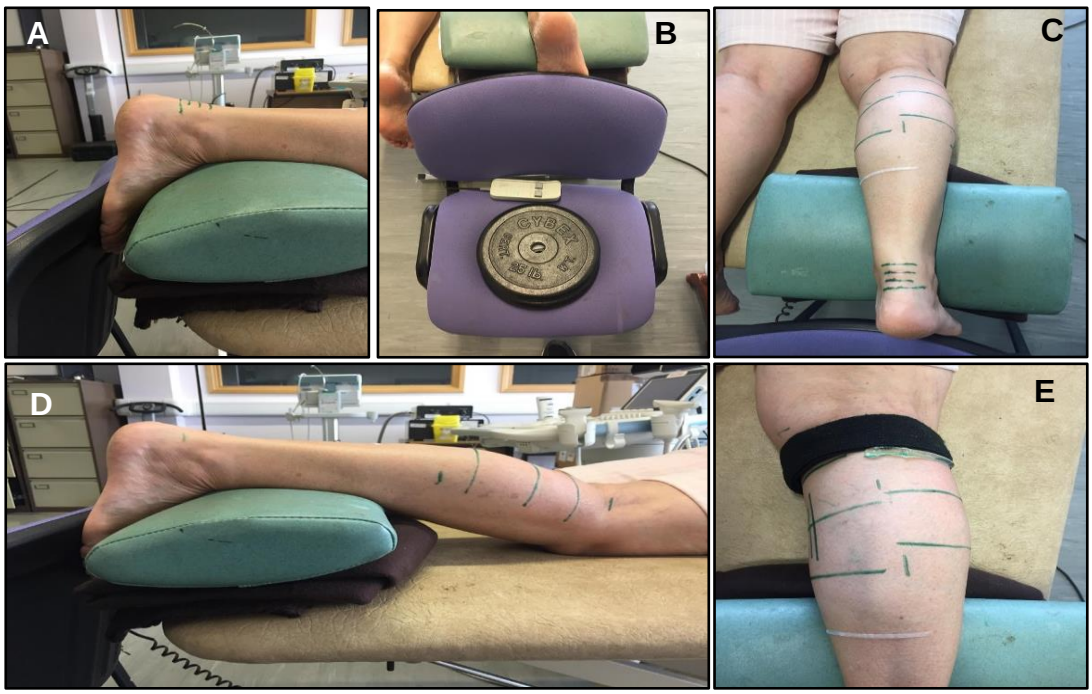

Figure 2.11- *Representative images of the laboratory set up for assessment of the* GM, GL, and Achilles tendon. Please note the ankle secured at 90, the weighted plate securing the footplate in place, and the Velcro strap/ ultrasound gel in panel E. *GL*; *Gastrocnemius lateralis*, *GM*; *Gastrocnemius Medialis*. Please note ankle angle was measured with goniometry and re-standardised at 0° before each assessment, to ensure consistency between measures.

GM muscle tendon junction. *GM* and Achilles tendon lengths were then combined to grant muscle-tendon unit length.

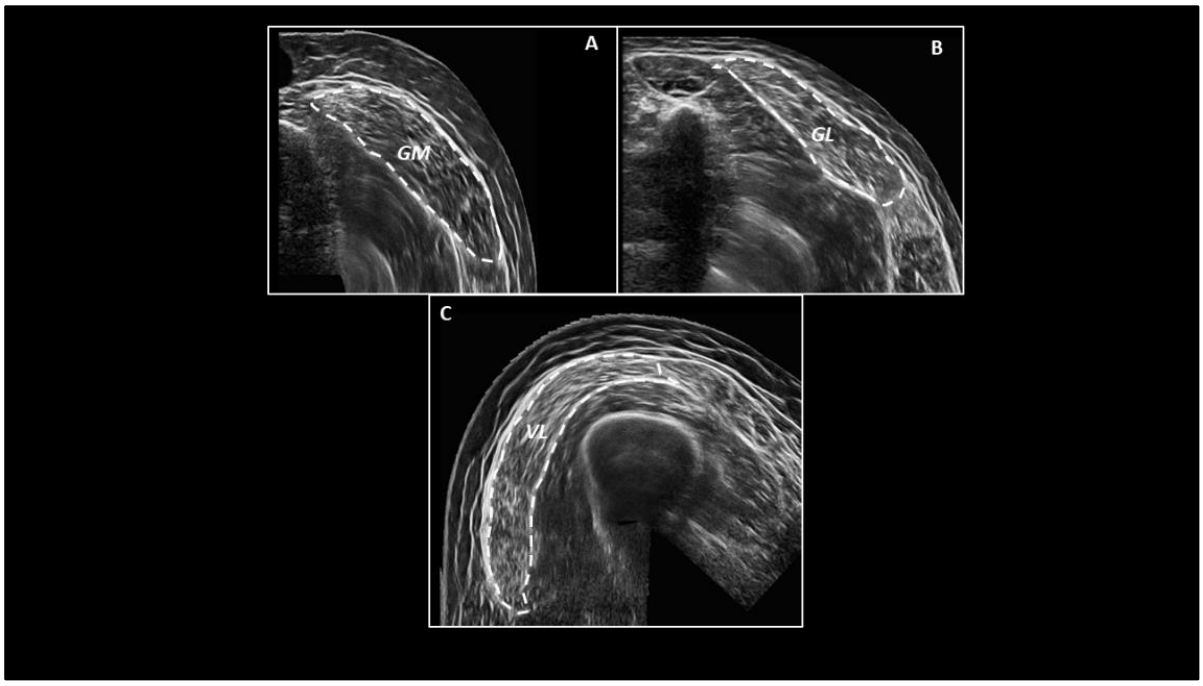

Figure 2.12- Representative ultrasound images following panoramic ultrasound imaging. Panel A represents a transverse image of GM ACSA (outlined for effect) at 50% of muscle length, Panel B, represents a transverse image of GL ACSA (outlined for effect) at 50% of muscle length, and Panel C, represents a transverse image of VL ACSA (outlined for effect) at 50% of muscle length. *GL*; *Gastrocnemius lateralis*, *GM*; *Gastrocnemius Medialis*, *VL*, *vastus lateralis*.

Muscle cross-sectional area

VPAN imaging procedure 2 was employed (please see chapter 6 appendices i) and originally granted *GM/GL* anatomical cross-sectional area (ACSA) (please see figure 2.12). Proximal images were obtained first (75% of *GM* and *GL* length), followed by distal sites (50 and 25% of *GM/GL*). Participants then switched to a supine position, with the knee fully extended and the hip angle raised to 45°, on top of a 30cm platform (please see figure 2.13). The proximal and distal insertions of the *VL* were identified, and 50% of *VL* length marked on the skin. Three more panoramic imaging images of the *VL* head and thus *VL* ACSA were then obtained, as described previously. Offline analysis was performed using IMAGEJ (1.45 s; National Institutes of Health, Bethesda, MD, USA) in a non-blind fashion (please see figure 2.12). echo intensity was determined by computer-aided grey-scale analysis, using the standard histogram function. Briefly, the same pre-selected polygon outlined and used to calculate CSA, was used to determine mean echo intensity value, typically ranging from 0 to 255 arbitrary units (AU) (black = 0, white = 255), as described previously (227). Considering alterations in probe tilt of as little as 2° can decrease echo intensity in the

lower limb musculature by ~5% (228), the aforementioned Velcro strap aided with maintaining echo intensity consistency. Furthermore despite echo intensity exhibiting variability between different ultrasound systems, standardised acquisition parameters offset such variation (229).

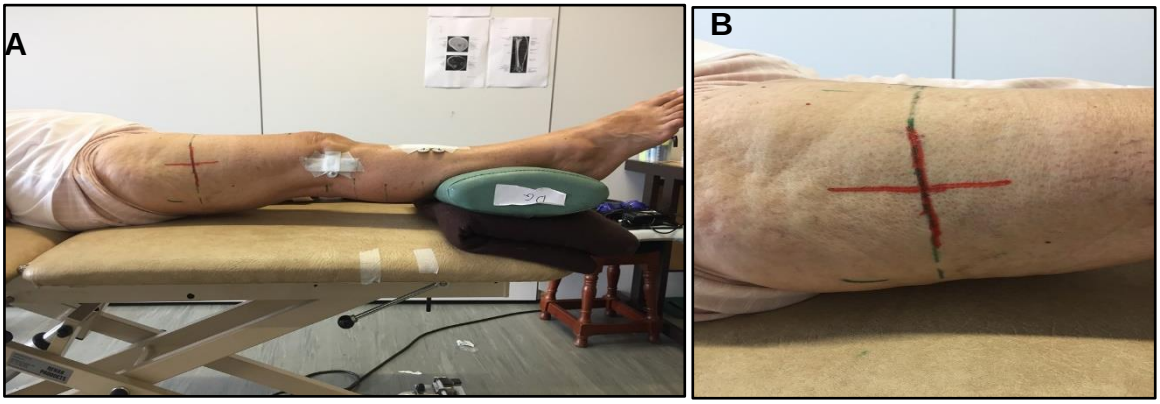

Figure 2.13- Representative images of the laboratory set up for assessment of the VL. Please note the raised platform in Panel A. Panel B represents the point from which VL muscle architecture was imaged. VL, vastus lateralis.

Achilles Tendon *cross sectional area*

Achilles Tendon cross-sectional area was obtained from representative transverse images at 0, 1, 2 and 3cm, of Achilles tendon length, whilst the participants ankle angle was secured at 0° (prone orientation) (please see figure 2.14). Determination of tendon ACSA using this method has previously demonstrated good validity and reliability (230, 231). Tendon echo response has been linked to tendon mechanical quality (232).

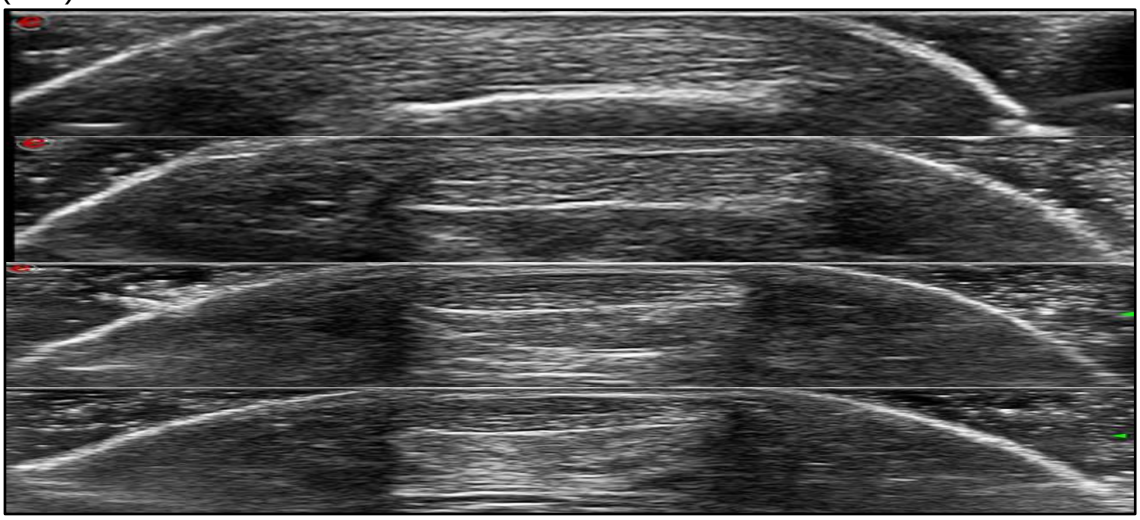

Figure 2.14– Typical transverse cross-sectional area images of the Achilles Tendon 0, 1, 2, & 3cm of length (top to bottom).

Muscle Architecture

The ultrasound probe was then positioned along the mid-sagittal line, at 50% of the GM muscle length, to record resting muscle architecture. Images of both resting fascicle pennation angle (FPA) and resting fascicle length (Lf), were then analysed using ImageJ (1.45s; National Institutes of Health). Three fascicles (defined from the deep to the superficial aponeurosis) of the GM were recorded and the mean value of both fascicle pennation angle and Lf determined (please see figure 2.15). Linear extrapolation of fascicles was carried out where fascicles extended beyond the reach of the probe, as described previously (220). This method has previously demonstrated good validity and reliability (233, 234). *VL* muscle architecture (FPA & Lf) was then determined, as previously described for the *GM*. Lf was also divided by muscle length to give normalised fascicle length.

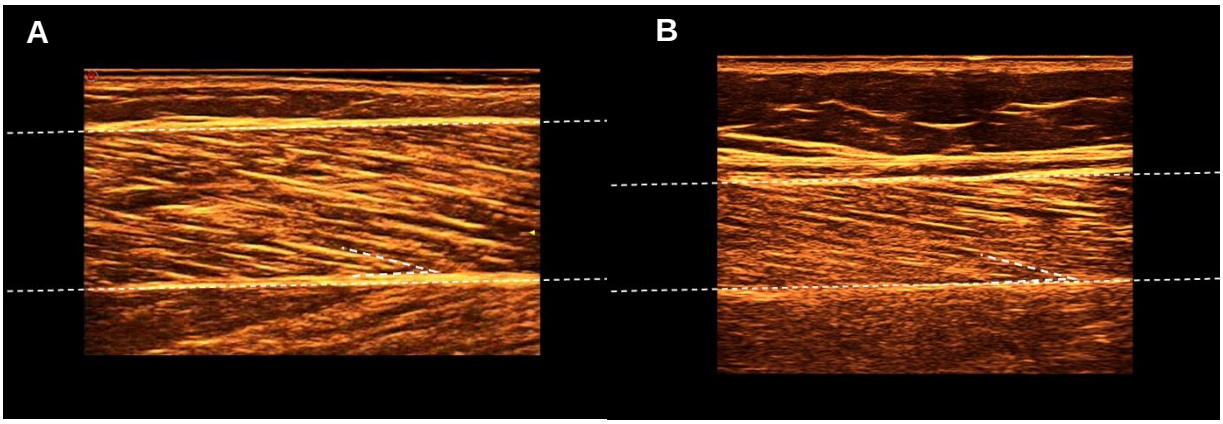

Figure 2.15- A representative mid-sagittal ultrasound image, highlighting the fascicular path, fascicle pennation angle, and the upper and lower aponeuroses. Panel A represents the *GM* muscle, whereas Panel B represents the *VL* muscle.GM; Gastrocnemius Medialis, VL, vastus lateralis.

Calculation of muscle volume and physiological cross -sectional area

GM and *GL* muscle volume was calculated by treating the muscles as a series of truncated cones (235, 236), through the construction of several ACSAs taken at discrete muscle sites (25, 50 and 75% of *GM* and *GL* length). Each of the four truncated cones was calculated using the following equation:

$$muscle\ volume = \frac{1}{3}\ .d.\left[a + \sqrt{(a.b)} + b\right]$$

Where:

- d is the distance between the two ACSA's (a and b)

The sum of the four cones provided *muscle volume* for *GM* and *GL*. *VL* muscle volume was calculated from a single ACSA re-construction at 50% of *VL* length and extrapolated to calculate overall muscle volume. This method of calculating muscle volume from a single ACSA has been validated previously (237). Physiological cross-sectional area was then calculated for both *GM* and *VL* using the following equation, as described previously (220):

physiological cross-sectional area = muscle volume / Lf

The same experienced sonographer performed all scans and demonstrated moderate to excellent good inter-day reliability (*n*=8). Specifically, the panoramic ACSA imaging of the *GM* [75% (intra-class correlation co-efficient: 0.89, systematic error: 14%, typical error: 30.54 cm^2), 50% (intra-class correlation co-efficient: 0.71, systematic error: 12%, typical error: 53.76 cm^2), 25% (intra-class correlation co-efficient: 0.76, systematic error: 15%, typical error: 49.95 cm^2) of length, and average of all three sites (intra-class correlation co-efficient: 0.72, systematic error: 10%, typical error: 25.79 cm^2)] exhibited moderate to excellent inter-day reliability. GM volume (intra-class correlation co-efficient:0.77, systematic error: 9%, typical error, 22.5 cm^3), and GM physiological cross-sectional area (intra-class correlation co-efficient: 0.61, systematic error: 16%, typical error, 0.7 cm^2), also exhibited moderate to excellent inter-day reliability.

Furthermore, the panoramic ACSA imaging of the *GL* [75% (intra-class correlation co-efficient: 0.89, systematic error: 11%, typical error: 14.6 cm^2), 50% (intra-class correlation co-efficient: 0.92, systematic error: 8%, typical error: 28.97 cm^2), 25% (intra-class correlation co-efficient: 0.21, systematic error: 24%, typical error: 62.3 cm^2) of length, and average of all three sites (intra-class correlation co-efficient: 0.52, systematic error: 11%, typical error: 21.02 cm^2)] exhibited poor to excellent inter-day reliability.

Panoramic ACSA imaging of the *VL* at 50% of length (intra-class correlation co-efficient: 0.92, systematic error: 7%, typical error: 30.87 cm^2), as-well as VL volume

(intra-class correlation co-efficient:0.99, systematic error: 5%, typical error, 29.4 cm^3), and VL physiological cross-sectional area (intra-class correlation co-efficient: 0.97, systematic error: 8%, typical error: 0.82 cm^2), all exhibited excellent inter-day reliability.

Assessments of muscle architecture including *GM* Lf (intra-class correlation co-efficient: 0.91, systematic error: 7%, typical error: 3.8cm), *GM* fascicle pennation angle (intra-class correlation co-efficient: 0.80, systematic error: 5%, typical error: 1.1°), *VL* Lf (intra-class correlation co-efficient: 0.96, systematic error: 5%, typical error: 3.3cm), and *VL* fascicle pennation angle (intra-class correlation co-efficient: 0.87, systematic error: 6%, typical error: 1.0°), also exhibited excellent inter-day reliability.

Imaging of the Achilles Tendon including ACSA at 0cm (intra-class correlation co-efficient: 0.87, systematic error: 9%, typical error: 6.97cm^2), 1cm (intra-class correlation co-efficient: 0.93, systematic error: 7%, typical error: 6.1 cm^2), 2cm (intra-class correlation co-efficient: 0.92, systematic error: 8%, typical error: 6.6cm^2), 3cm (intra-class correlation co-efficient: 0.76, systematic error: 16%, typical error: 12.7cm^2), and average of all sites (intra-class correlation co-efficient: 0.97, systematic error: 4%, typical error: 3.7cm^2).

Regarding tissue related quality, good inter-day reliability was observed for *GM echo intensity* [75% (intra-class correlation co-efficient: 0.92, systematic error: 7%, typical error: 6.7 AU)' 50% (intra-class correlation co-efficient: 0.91, systematic error: 4%, typical error: 3.97AU)'' 25% (intra-class correlation co-efficient: 0.88, systematic error: 7%, typical error: 7.9 AU) and average of all three sites (intra-class correlation co-efficient: 0.92, systematic error: 5%, typical error: 5.11 AU)], *GL echo intensity* [75% (intra-class correlation co-efficient: 0.76, systematic error: 7%, typical error: 7.23 AU), 50% (intra-class correlation co-efficient: 0.59, systematic error: 6%, typical error: 5.91 AU), 25% (intra-class correlation co-efficient: 0.78, systematic error: 7%, typical error: 7.85 AU), and average of all three sites (intra-class correlation co-efficient: 0.53, systematic error: 6%, typical error: 6.28 AU)], and *VL echo intensity* [at 50% of length (intra-class correlation co-efficient: 0.90, systematic error: 6%, typical error: 5.6 AU)].

This was also the case for Achilles Tendon echo intensity [0cm (intra-class correlation co-efficient: 0.57, systematic error: 6%, typical error: 6.13 AU), 1cm (intra-class correlation co-efficient: 0.73, systematic error: 6%, typical error: 5.71 AU), 2cm (Intra-

class correlation co-efficient: 0.67, systematic error: 5%, typical error: 4.7 AU), 3cm (Intra-class correlation co-efficient: 0.87, systematic error: 4%, typical error: 3.62 AU), and the average of all sites (intra-class correlation co-efficient: 0.64, systematic error: 3%, typical error: 2.49 AU)].

Postural Balance Assessment

Participants performed a single leg balance test with their eyes either open or with visual feedback removed through utilising blacked out goggles to isolate proprioceptive feedback (238). The single balance postural test is well established within the literature including in research using older persons, with documented reliability (133, 239). The test was performed on a wireless balance board (Wii balance board, Nintendo, Kyoto Japan) (please see figure 2.16), which has been previously been shown to be a valid indicator of postural sway and balance in older adults, when compared to conventionally used laboratory grade force plates (240-246). The Wii board was synchronised with previously validated software [BrainBLoX Software, Version 1.0, (247)] via a research laptop (using Bluetooth). Each trial began with participants placing their hands on a physiotherapy bed (Set to the participant's hip height) and standing on one leg (please see figure 2.17). During such time, 5s of baseline displacement was recorded. The physio bed allowed participants to reach their hands out in the event of a severe loss of balance, thus preventing an injurious fall. Nevertheless, the researcher was on hand to assist throughout. Participants were then instructed to slowly place their hands by their side. Test cessation occurred when participants placed their elevated foot on the ground, or their arms were no longer fixed by their side (Figure 3.2, Panel B). Trial duration (maximum 30.0s) was manually assessed with a stopwatch. In total twelve trials were performed [Three on each leg for both conditions (eyes open/ closed)], in a random order to minimise learning effects. To prevent fatigue, participants rested in a seated position for ~60s following every 4 trials. BrainBLoX Software (247) calculates centre of pressure displacement in both anterior-posterior, and medio-lateral directions in mm. Total displacement (mm) was subsequently calculated using the following equation (133):

$$\textit{Total Displacement} = \sqrt{(RMS_{AP})^2+(RMS_{ML})^2}$$

Where RMS = root mean square, AP = anterior-posterior, and ML = medio-lateral

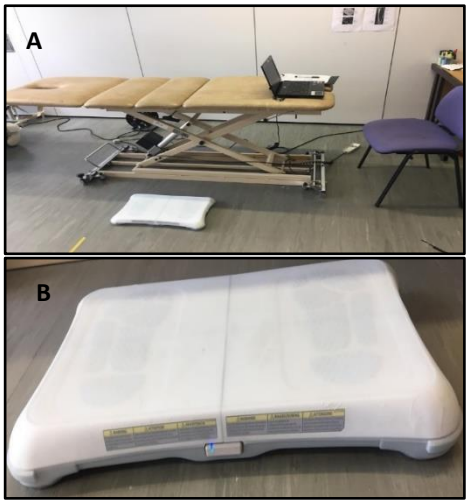

Figure 2.16- Setup for postural balance assessment. Panel A represents the testing area including the researcher's position. Panel B represents the Wii Board used to assess posturography.

Figure 2.17- Participant setup for postural balance assessment. Panel A represents the starting position (hands on bed). Panel B represents the testing position (hands by side).

The longest duration achieved on the best performing leg was used for each condition. For quality data processing purposes, the first 5s of each trial was removed as this involved the participants hands on the physio bed. Accordingly, the first and last 5% of data for the remaining trial duration was also discarded, and the remaining 90% used for analysis. Discarding the first and last 5% was selected over discarding 5s either side, due to the relatively short trial durations during eyes closed trials (1-5s). Three outcomes were then determined for each trial: duration (seconds), total displacement (mm), and sway frequency [total displacement expressed relative to trial duration ($mm.s^{-1}$)]. These detailed posturography data were only available for a sub-sample of participants (n=29).

Regarding single leg stance time (manually assessed with stopwatch), excellent inter-day reliability was exhibited for eyes open single leg stance time [left leg (intra-class coefficient: 0.95, systematic error: 13%, typical error: 2.94s), right leg (intra-class

coefficient: 0.93, systematic error: 15%, typical error: 3.37s)] and good inter-day reliability for eyes closed single leg stance time [left leg (intra-class coefficient: 0.58, systematic error: 75%, typical error: 2.53s) right leg (intra-class coefficient: 0.82, systematic error: 52%, typical error: 1.73s)] (n=34).

Regarding the sub-sample posturography analysis (n=29), 10 participants underwent reliability assessment. Good to excellent inter-day reliability was exhibited for all posturography variables for both eyes open [duration (intra-class coefficient: 0.95 systematic error: 11%, typical error: 2.4s), total displacement (intra-class coefficient: 0.75, systematic error: 28%, typical error: 6.11mm) and sway frequency (intra-class coefficient: 0.97, systematic error: 42%, typical error: 0.73mm/s)], and eyes closed [duration (intra-class coefficient: 0.65, systematic error: 36%, typical error: 1.6s) total displacement (intra-class coefficient:0.68, systematic error: 27%, typical error: 7.47mm) sway frequency (intra-class coefficient: 0.82, systematic error: 42%, typical error: 3.30mm/s)]. conditions.

Muscle Strength and Neuromuscular Function Assessments

Muscle Function Assessments- All muscle function assessment s were assessed with an isokinetic dynamometer (Cybex Norm, Cybex International, New York, NY, USA), interfaced with an analogue-to-digital converter (Biopac Systems Inc, Santa Barbara, USA), sampled at 2,000 Hz and displayed on the screen of an iMac computer (Apple, Cupertino, USA) using AcqKnowledge software (Version 3.9, Biopac Systems). Isometric MVC (iMVC) torque of the Plantar flexors (PF) and Dorsi Flexors was assessed in the dominant limb. Participants were seated with a hip angle of 85°, their leg fully extended, and the ankle positioned anatomically neutral at 0°. The lateral malleolus of the dominant foot was aligned with the centre of rotation of the dynamometer lever arm and secured to the footplate using inextensible straps. To minimise extraneous movement participants were firmly strapped at the hip, distal thigh, and chest with inextensible straps (please see figure 2.18).

PF iMVC- Participants completed a series of warm-up PF and dorsiflexor contractions. Following this, four isometric (2 x PF, 2 x dorsiflexor) MVC's were performed, with a rest period of ~30-45s given between efforts. If >10% difference between iMVC attempts was observed, assessments were repeated, ensuring true iMVC was reached (please see figure 2.20).

Offline analysis was performed on the highest PF and dorsiflexor iMVC, using Acknowledge software (Version 5, Biopac Systems, Norfolk United Kingdom) (please see figure 2.19).

Figure2.18- Experimental setup for muscle strength assessment.

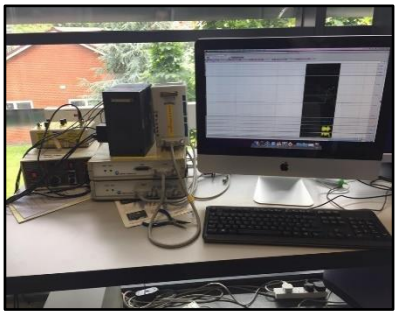

Figure 2.19- Computer setup for muscle function assessments. Note Acknowledge software open on screen, and Biopac hardware interface.

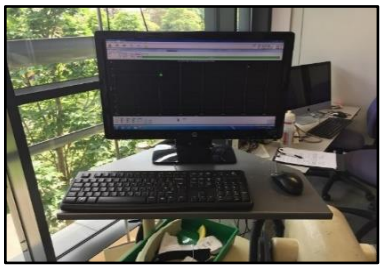

Figure 2.20- Computer setup for isometric strength assessment. Note the Biofeedback provided to the participant regarding their force output.

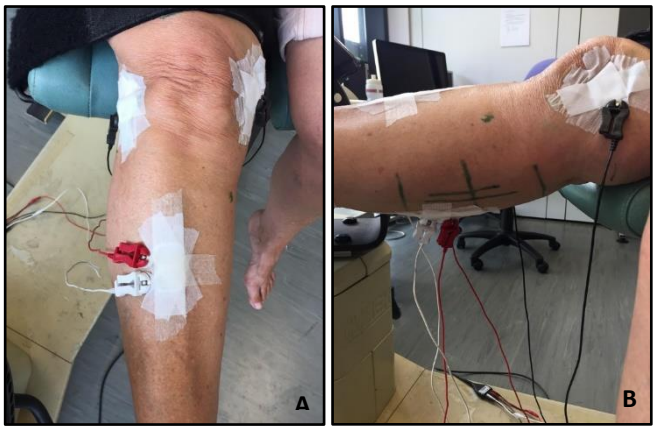

Figure 2.21- Experimental setup electromyography (EMG) assessment during all contractions. Panel A represents *Tibialis Anterior* (Antagonist) electromyography assessment, whilst panel B represents GM (Agonist) electromyography assessment. Note the two reference electrodes on the medial and lateral condyles. GM; Gastrocnemius medialis.

Antagonist muscle co-contraction- Surface electromyography (EMG) of the *Tibialis Anterior* was recorded during all iMVC's to calculate antagonist muscle co-contraction, during PF iMVC. *Tibialis Anterior* muscle length was defined as the distance from the Lateral Tibial condyle to the first metatarsal. These insertion points were visually identified, palpated for, marked on the skin, and then measured with a tape measure. Two electrodes [skin contact size 30mm x 22mm (unipolar pre gelled electrodes)] (Ambu®, Neuroline 720, Cambridgeshire, United Kingdom) were placed proximally at

one third of the tibialis anterior muscle length (mid muscle belly), with a 1-2mm gap separating each electrode. A reference electrode (Medicost, Denmark) was also placed at the head of the fibula (please see figure 2.21 Panel A). Raw electromyography was then recorded at 2,000 Hz, with the band pass filter set at 10-500 Hz and notch at 50 Hz, as demonstrated previously (75). AgCoA% (%) was calculated utilising the raw electromyography signal [computed as root mean square (RMS)] 500ms either side of the instantaneous peak torque of the *Tibialis Anterior* during PF iMVC divided by electromyography during dorsiflexion iMVC. Co-contraction torque (Nm) was then calculated as the product of percent co-contraction and maximal dorsiflexion torque. This method assumes that the dorsiflexion electromyography/ torque relationship is linear (248).

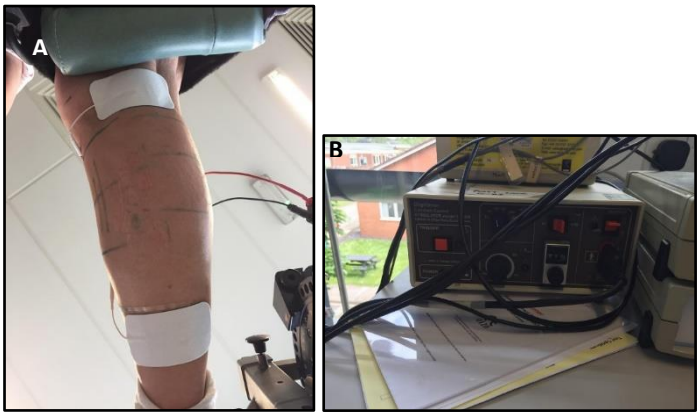

Figure 2.22- Experimental setup for assessing voluntary activation capacity, with the interpolated twitch technique. Panel A represents the participants leg with rubber stimulation pads attached to the skin at proximal and distal endpoints of the *GM*. Panel B represents the stimulation box. Note the amplitude, set to the starting amplitude for resting stimulations of 50mA. GM; Gastrocnemius medialis.

Agonist Muscle Activation- GM AC% was estimated using the interpolated twitch technique, with percutaneous electrical stimulation (142). Two stimulation pads (50 mm×100 mm; American Imex, Irvine, CA, USA), were placed transversely distal to the popliteal crease and myotendinous junction of the soleus (please see figure 2.22). Percutaneous stimuli doublets (DSV Digitimer Stimulator, Digitimer, Herts., UK), were then briefly applied to the *GM* via the stimulation pads. Stimuli amplitude [~50 volts (v)] was determined in the relaxed state, prior to interpolation. Twitches started at ~

50 mA, with subsequent increments of 50–100 mA, until no further increase in twitch torque was observed. The identified supramaximal doublets were then superimposed during a maximal PF iMVC, with an additional stimulation applied ~2-5 seconds post PF iMVC whilst the participant was resting. *GM* AC% was then determined by the following equation [as described previously (142)]:

= [1-(superimposed stimulation torque/ post MVC resting stimulation torque)] x 100

Calculation of NET PF MVC and muscle quality

AgCoA corrected MVC was calculated as the sum of the observed maximal PF torque and absent co-contraction torque. AgCoA corrected MVC was then multiplied by the missing agonist drive capacity providing the absent torque diminished through reduced AC%. Thus, net PF MVC was calculated as the sum of AgCoA corrected MVC and absent AC% torque.

Finally, *GM* muscle quality (Nm/cm^3) was calculated, firstly by calculating GM muscle volume. *GM* muscle volume was calculated by treating the muscles as a series of truncated cones (235, 236), through the construction of several ACSAs taken at discrete muscle sites (25, 50 and 75% of *GM* and *GL* length). Each of the four truncated cones was calculated using the following equation:

$$muscle\ volume = \frac{1}{3}\ .d.\left[a + \sqrt{(a.b)} + b\right]$$

Where:

- d is the distance between the two ACSA's (a and b)

The sum of the four cones provided *muscle volume* for *GM* (please see above for further details on *GM* muscle volume calculation). Net PF iMVC was then divided by GM muscle volume (cm^3). This value was then multiplied by 0.25, assuming the ***GM's*** contribution towards total PF iMVC is 25% (202, 249).

Assessment of both PF MVC (intraclass correlation coefficient: 0.97, systematic error: 9%, typical error: 6.7 nm) (n=6) and dorsiflexor MVC (intraclass correlation coefficient: 0.88, systematic error: 21%, typical error: 3.15 nm) (n=6)] exhibited moderate to

excellent inter-day reliability. Furthermore AgCoA (intraclass correlation coefficient: 0.88, systematic error: 21%, typical error: 2%) (n=6)], and agonist drive (intraclass correlation coefficient: 0.65, systematic error: 6%, typical error: 5%) (n=5), exhibited moderate inter-day reliability. Reliability was therefore deemed acceptable for all neuromuscular assessments.

Gait speed, and Sit-to-stand parameters

Considering chair height mediates the variation in sit-to-stand ability in older adults (164, 250), the height of an adjustable stool was standardised to the length of each participants lower leg (distance in cm from the tibio-femoral junction, to the bottom of the participants footwear worn during the test) (please see figure 2.23). Participants were further instructed to wear the same comfortable footwear (e.g. trainers) for pre and post testing. Finally, a modified pressure sensor (Tekescan, South Boston, USA), was attached to the stool as a method of automated timing, linked via hardwire to an external laptop (please see figure 2.23). During offline analysis, the average baseline pressure and standard deviation was determined from the first 5s of each recording. The participant was deemed to have stood up once the pressure had decreased by 2 standard deviations below the 5s baseline seated average. The same process was repeated for the first 5s when force reappeared (i.e. when the participant resumed the seated position) and was used to accurately judge the time standing and sitting (accurate to 0.01 of a second).

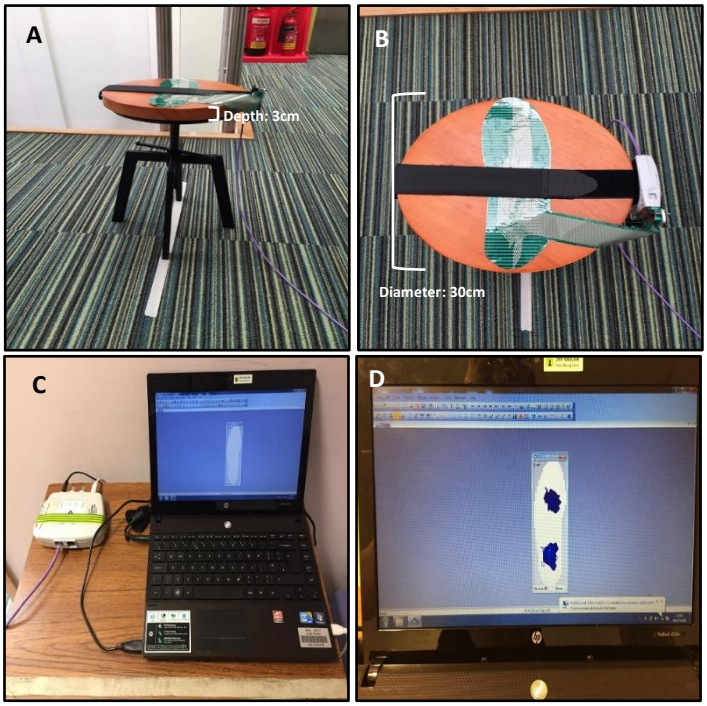

Figure 2.23- Setup for gait speed and sit to stand ability assessment. Panels A and B represent the height adjustable stool and modified pressure sensor, respectively. Panels C and D represent the Tekscan software used to accurately determine time between sitting bouts. Note the visual representation of pressure in panel D.

Gait speed was assessed through the previously validated timed “Up and Go” test (251-253). The test was completed in an open space to limit potential hazards. Participants began in a seated position, with their hands rested on their knees for 5s (please see figure 2.2). When instructed participants rose from the chair and walked at maximum self-selected pace up to a 1m x 1m box marked out on the floor with masking tape (where the nearest edge of the box was approximately 6m away from the chair), before returning and re-assuming the seated position. A box was used at the end of the course instead of a cone (similar to a previous study) (254) to avoid participant confusion (not knowing whether to walk in line, around or over the cone). Participants also received the following clear instructions “*After the countdown 3,2,1, Please get up on the word go, stand upright and walk as quickly and as safely as possible to the box marked on the floor, turn around inside the box, then walk back to the chair and sit down*”. The test was repeated 3 times with 60s rest in-between, during which time the participant was seated. Total time was divided by the total course distance (12m) to calculate average gait speed [metres per second (m/s), accurate to 0.01 of a s]. This is important as the minimally clinically important difference in gait speed has previously been identified as ~0.10 to 0.20 m/s in multiple populations (255). Average gait speed was also used to classify sarcopenia severity classifying participants as either non-sarcopenic (gait speed >0.8 m/s), or low functional performance (gait speed ≤0.8 m/s) (84).

Sit-to-stand ability was then determined, through the one sit-to-stand time test (functional speed), and the 30 second sit-to-stand test (functional endurance). Participants began in a seated position, with their hands across their chest for 5s allowing baseline pressure to be recorded (please see figure 2.25). Participants were then instructed to rise from the chair as quickly as possible until the knee joint was fully extended and then return to a seated position. For 1STS, the pressure sensor permitted accurate timing (0.01 of a second) between standing and resuming the seated position in a rapid fashion. Furthermore, automated timing permitted accurate assessment of the number of sit-to-stand transitions performed within an approximate 30.00s period. Sit-to-stand transitions were counted as every time force zeroed, following the first sit-to-stand transition.

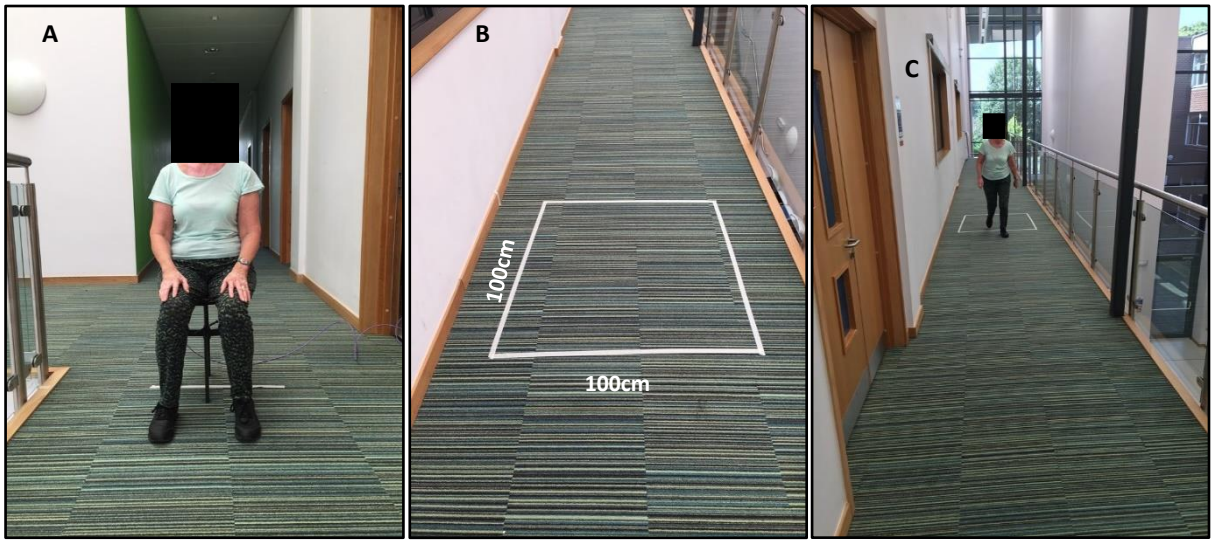

Figure 2.24- Participant setup for gait speed assessment. Panel A represents the starting position (note hands on knees). Panel B represents the box marked 6m away from the starting position. Panel C represents a participant performing a trial.

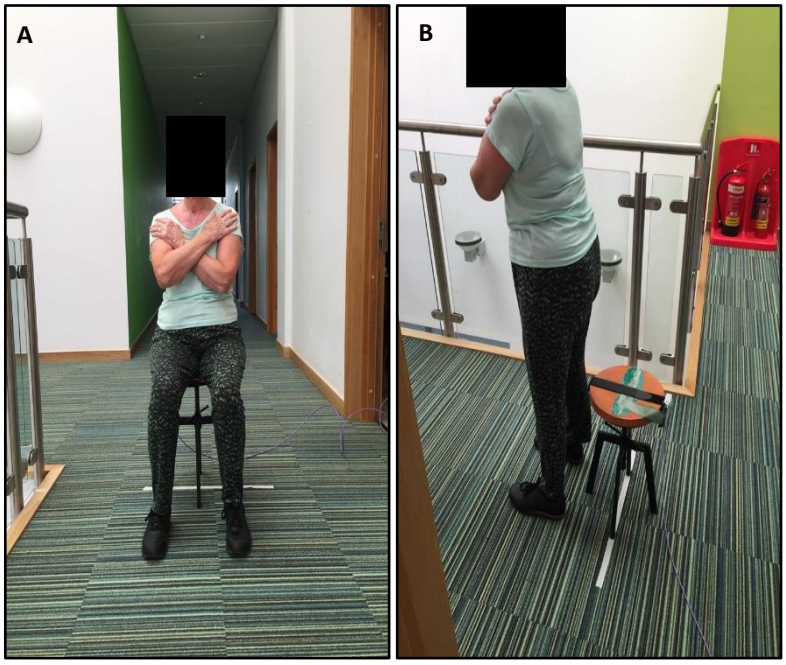

Figure 2.25- Participant setup for sit to stand assessment. Panel A represents the starting position (note hands across chest). Panel B represents a participant performing a trial.

Both average (intra-class coefficient: 0.91, systematic error: 6%, typical error: 0.1m/s), and peak (intra-class coefficient: 0.88, systematic error: 7%, typical error: 0.1m/s) gait speed exhibited excellent inter-day reliability within the whole cohort (n=36). This was also the case for 30 second sit-to-stand (intra-class coefficient: 0.83, systematic error: 10%, typical error: 2 sit to stands), and 1 sit-to-stand time (intra-class coefficient: 0.80, systematic error: 19%, typical error: 0.44s).

Handgrip Strength

A handgrip dynamometer (Takei Hand Grip Dynamometer, Takei Scientific Instruments, Niigata, Japan) was used to assess grip strength (please see figure 2.26). Participants gripped the dynamometer in one hand and squeezed the handle with maximum voluntary effort. Three trials were performed on each hand in a randomised order to minimise any learning effects. Peak HGS was defined as the maximum value achieved across both arms, and the average of three trials used to provide an average of both arms. Hand grip dynamometry is both a reliable and valid measure of strength in older adults (256, 257).

Figure 2.26- Hand-grip dynamometer (Takei) used to assess handgrip strength.

Both average HGS (intra-class coefficient: 0.97, systematic error: 5%, typical error: 1.1kg), and peak HGS (intra-class coefficient: 0.93, systematic error: 5%, typical error: 1.42kg) exhibited excellent inter-day reliability within the whole cohort (n=36).

Physical behaviour interventions

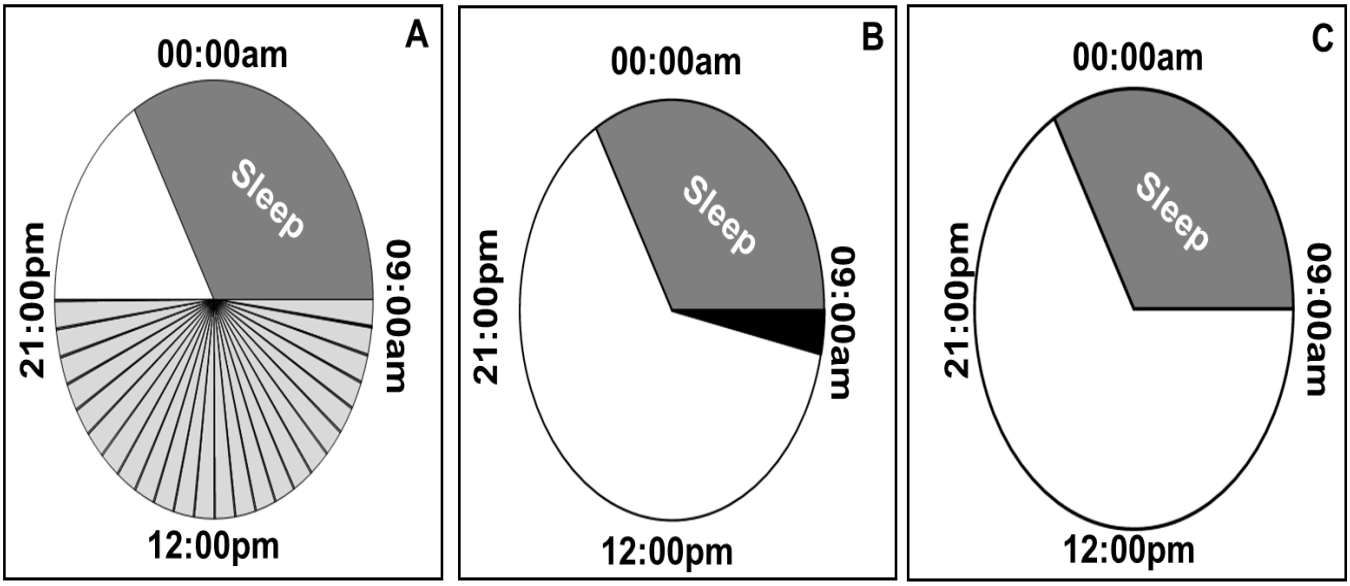

Figure 2.27- 24-hour visual representation of prescribed daily physical behaviour. Grey segments represent habitual sleep time, Black segments, prescribed intervention light intensity physical activity, and white segments, free leisure time. Panels A, B, & C represent Sedentary behaviour fragmentation, light intensity physical activity, and control, respectively.

The purpose of the two intervention groups was to displace SB with LIPA. The interventions were confined to a 12-hour period between 09:00 and 21:00. The prescribed amount of LIPA (45-50 minutes) was based upon two key points. First, the WHO's MVPA recommendation (197) gives a theoretical starting point for what physical activity amount may be beneficial. Consequently, 150min/week translates into ~21 min/day moderate activity, (~64 metabolic equivalents Mins/day), meaning the same number of metabolic equivalents ·mins/day, performed in LIPA (with a minimum intensity of 1.6 metabolic equivalents), would theoretically total ~40 min/day. Furthermore, the SBF group was instructed to fragment sitting time every 30 minutes over a 12h period (09:00-21:00), based on recent epidemiological evidence linking a more prolonged sedentary accumulation pattern (≥30min bouts) with greater all-cause mortality (258). Consequently, this totalled a maximum of 24 2-minute LIPA bouts throughout the day (48 minutes). Envisaging a varied compliance response, the LIPA group was prescribed a range for their single continuous bout. Accordingly, the prescribed amount of LIPA (an additional 45-50 minutes per day), was equally matched between the two groups, whereas the prescribed pattern (intermittent micro-bouts vs single continuous bout) was different.

Both interventions involved a booklet, which contained illustrated activity suggestions. The list contained simple activities (e.g., light walking, side-to-side shuffling, and hanging out washing) compiled from the compendium of physical activities (28). Participants could perform as few or as many of the activities as suited them. The most important aspect was additional LIPA implementation but not MVPA. Accordingly, instructions included avoiding MVPA stimulating movements (heat, perspiration, and breathlessness generating as-well as high speed). Experimental participants were requested to avoid MVPA when displacing SB, thus ensuring specific displacement with LIPA. Importantly, habitual MVPA (e.g., brisk walks, exercise classes, and sports clubs), was strongly encouraged to continue with participants explicitly told as much. The prescribed increase in LIPA was explicitly stated as additional to physical activity already present at baseline. Control participants were only instructed to maintain their habitual routines. All groups recorded wake/sleep times with the aforementioned diary and received fortnightly home visits from the principal investigator. Instructions varied depending on the group participants to which they were allocated.

SBF group - Participants allocated to the SBF group were given a brief background, by the principal investigator explaining the adverse health effects of SB. Participants were explicitly instructed that the purpose of their intervention was to reduce SB time (sitting, lying, or reclining) especially in prolonged uninterrupted bouts. Participants were also instructed to reduce SB bout length to ≤30 minutes, with 2 minutes of upright LIPA performed for every 30-minute SB bout (please see figure 2.27). The potential risks associated with SBF were discussed along with the countermeasures in place. SBF participants also had an additional accelerometer (activPAL3™ triaxial physical activity logger, PAL technologies Ltd, Glasgow, Scotland, United Kingdom) (34mm x 55mm x 6mm) (please see figure 2.29) mounted alongside the GENEA unit, as described above (please see Physical Behaviour assessment) (please see figure 2.30). The activPAL3™ was pre-configured using activPAL software version 7.0 (PAL technologies Ltd, Glasgow, Scotland, United Kingdom) (please see figure 2.28), to prompt movement following 30 minutes of SB, in the form of a vibration against the skin. The advantage of the activPAL3™ over other prompting devices is the ability to specifically prompt movement, following SB [classified through both postural inclination (thigh angle) and lack of movement signal]. Such a sophisticated classification mechanism avoids alarm fatigue, which could lead to participants

potentially ignoring movement prompts and negatively affecting intervention compliance. Participants were instructed to comply between the hours of 9:00 and 21:00. Participants also received a booklet to keep (please see appendices i), which contained the aforementioned LIPA suggestion booklet. The booklet also contained a diary with 30-minute time increments. Specifically participants, marked the blank space in the diary next to the appropriate time with an (X) if they complied to a given accelerometer prompt, a (O) if they did not comply, or leave the space blank if they were already up and moving. A new activPAL3™ device was also fitted to the participants thigh during the fortnightly visits, in accordance with the aforementioned procedure for trading the GENEA devices every fortnight.

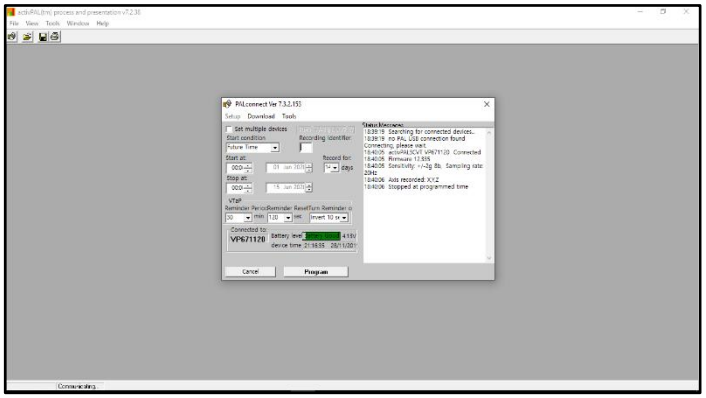

Figure 2.28– Screen grab of ActivPAL software version 7.0 (PAL technologies Ltd.

Figure 2.29– activPAL3™ triaxial physical activity logger.

Figure 2.30- Photographic representation of fully assembled SBF monitoring (Note: additional ActivPal device).

LIPA Group - Participants allocated to the LIPA group were given a brief background explaining the adverse consequences of not performing LIPA. The framing of this conversation was aimed toward increasing LIPA with no specific instruction provided on SB. LIPA participants were explicitly instructed the purpose of their intervention was to increase LIPA time. Participants were requested to perform a single continuous bout of 45-50 minutes LIPA, every morning for the duration of the 8-week intervention (please see figure 2.27). Temporal prescription was however not strict as the morning instruction was aimed at enhancing compliance. Participants could implement the additional LIPA at any point between 09:00 and 21:00. During the 45-50-minute period participants were instructed to avoid sitting down and continuously be moving, thus utilising LIPA. LIPA participants also received a booklet to keep (please see appendices i), which contained the aforementioned LIPA suggestion booklet. The booklet also contained a compliance diary which the participant marked with an (Y) if they complied to the single LIPA bout for that day, or (N) if they did not. Participants were also encouraged to record the amount of LIPA implemented during a complied bout

Control Group - Participants allocated to the control group were given a brief background on the importance of all PB. The framing of this conversation was aimed toward purely tracking PB, and not intervening. Control participants were explicitly instructed that the overall purpose of the study was to track physical behaviour and examine the corresponding relationship with health markers (please see figure 2.27). Control participants also received a booklet with basic information (accelerometer maintenance, wake/sleep time tracking, etc) (please see appendices i).

Physical Behaviour Intervention Palatability Assessment

Participants were asked to complete a custom designed palatability questionnaire post-intervention (designed by the principal investigator). The first step of scale development in health, social, and behavioural research is specifying the boundaries of the domain, and identifying appropriate questions that fit the boundaries of the identified domain (259). The general domain to be investigated was the barriers, facilitators, and future implications of SB displacement in older adults. Considering the current investigation represents (to the authors knowledge) the first intervention to displace SB time in two separate patterns in older adults, no existing instruments were available at the time of data collection that fulfilled this specific purpose. Therefore, previous qualitative studies investigating the potential barriers to reducing SB in older adults were reviewed to identify specific domains (161, 260, 261). Specific domains included locational compliance (e.g. at home), experience of fatigue, experience of muscle/ joint soreness, self-perception of health, social factors (e.g. potential embarrassment), and future implications (e.g. likelihood of continuing intervention). Further domains included feedback on the specific components of the intervention (helpfulness of compliance diary, and understanding of instructions), and self-perception of physical function (confidence to perform household tasks, and perception of balance improvement). Questions were asked in a retrospective manner (e.g. did you find the instructions easy to follow?), thus encouraging the participant to reflect on their intervention experience.

A Likert response scale was chosen, as this is appropriate to assess respondent experience or perception (259). Participants were asked to rate a specific aspect of their intervention, on a 5-point Likert scale by circling one of five responses: *"Definitely not", "Fairly not", "Undecided", "Fairly",* or *"Definitely".* A 5-point Likert scale was chosen as this has been shown to improve reliability of participant responses, compared to 2-3 point scales (262). The five responses were presented from left to right on the questionnaire page ("*Definitely Not"* to "*Definitely"*), with equal gaps and no overlap between responses (please see appendices), to ensure a meaningful scale that was interpreted the same by each participant (259). Participants were asked to circle the most appropriate response, to make the questionnaire as undemanding as possible. Where appropriate, participants were also given the opportunity to provide individualised feedback, for example: *"Were there any places/ environments you*

struggled to implement the intervention (please list 5)". All groups received the same questionnaire and thus answered the same questions, with one exception. The SBF group were given one additional question regarding the ActivPal device (see physical behaviour interventions), which was "*Did you find the accelerometer prompt (vibration) helpful?*".

The questionnaires were scored by the principal investigator, and the responses collated. Considering the lack of validation and multiple specific domains investigated, there was no overall score for the palatability questionnaire. The number of respondents who circled a certain response (e.g. definitely) was compared (using Chi squared analysis) between groups to highlight response trends. This allowed a particular barrier or facilitator to SB displacement in older adults to be highlighted based upon the prescribed pattern of SB, and in relation to a control group who received no intervention. Due to the lack of time prior to the imminent campus closure (see experimental phases) as well as considering the fact that one researcher oversaw the entire experimental process, there was insufficient time to fully validate the palatability questionnaire [focus groups, interviews, expert judge analysis, initial sampling, correct answer rate determination, rigorous statistical testing (dimensionality, reliability, and validity)]. Therefore, it should be noted that this questionnaire design is only partially validated, meaning the results can only be considered good pilot data on the barriers, facilitators, and future implications of SB displacement in older adults.

Reliability analysis

All statistical analyses were carried out using SPSS (Version 26, SPSS Inc., Chicago, IL, USA). Data collected from familiarisation and pre-test were used for reliability analysis. First of all, intra-class co-efficient were determined for the two data sets for a particular variable using scale reliability analysis. Next, typical error was calculated as the standard deviation of the differences between the two data sets (between familiarisation and pre-test) divided by the square root of two. Importantly, 1.5 to 2.0 times the typical error is the threshold outside of which a meaningful change is considered to have occurred (263). Finally, the typical error was then divided by the grand mean (average of familiarisation and pre-test) to give an indication of systematic error [expressed as a percentage (%)].

Statistical Analyses

Normal distribution and equality of variances between groups were checked using the Shapiro– Wilk and Levene's tests, respectively (please see appendices i). Baseline group differences were subsequently examined with a one-way analysis of variance (ANOVA) or a Kruskall-Wallis ANOVA as appropriate, with post-hoc pairwise comparisons conducted using the Fishers test of Least Significant Difference, or Mann-Whitney U test, respectively. Fishers test of least significant difference was used for post-hoc comparisons instead of the Bonferroni adjustment, considering Fishers least significant difference corrects based on multiple comparisons across groups (on account of a 3-group design), whereas for the Bonferroni only corrects for the worst-case scenario (independent comparisons).

The effects of the interventions were mostly determined using a 2×3 split plot ANOVA [2-time phases (pre & post intervention) and 3 intervention groups (SBF, LIPA, and control)], with post-hoc comparisons again conducted using the least significant difference. In cases of non-normally distributed data, within group comparisons were made using the Wilcoxon-Sign Rank test, whilst, between group pairwise differences were analysed through a Kruskall-Wallis ANOVA on the relative changes from baseline, with post-hoc pairwise comparisons examined by the Mann-Whitney U test. This included physical behaviour variables, where for simplicity, effects across time were analysed by examining physical behaviour at baseline and comparing it to physical behaviour at week 8. Nevertheless, Spearman bivariate correlations, were

utilized to investigate associations between physical behaviour at baseline, and each subsequent week of data collection (week 1, week 2, week 3, week 4, week 5, week 6, week 7, and week 8). Spearman was utilised in favour of Pearson correlations based on the assumption that ≥5 data collection weeks were non-normally distributed as was true in the majority of cases.

Due to the observed discrepancies between the experimental and control participants at baseline (please see results), a sub-sample analysis was conducted on the absolute physical behaviour variables (not co-efficient of variation or individual variance) for experimental participants (n=28) only. Due to there only being two groups in this sub-sample (SBF and LIPA), baseline group differences were examined with either an independent samples T-test or Mann-Whitney U test (SBF vs. LIPA) as appropriate. Accordingly, the effects of the interventions were determined using a 2×2 split plot ANOVA [2-time phases (pre & post intervention) and 2 intervention groups]. In cases of non-normally distributed data, within group comparisons were made using the Wilcoxon-Sign Rank test, whilst, between group differences were analysed through the Mann-Whitney U test on the relative changes from baseline. In addition, a sub-analysis was run on nutritional data for participants who positively shifted classification from sedentary to ambulator post intervention (n=8), using a paired samples T-test or Wilcoxon-Sign Rank test as appropriate.

In cases where groups were unmatched at baseline, the baseline values were added into the statistical analysis model as a co-variate. Previously identified co-variates (Total Fat tissue, BMI, & Android to Gynoid ratio) (112, 203), were added into the statistical models for all bone mineral density variables. The effects of the interventions on *Gastrocnemius Medialis* and *Gastrocnemius Lateralis* (GL) muscle echo intensity were determined using a split plot ANOVA or 2x3x3 [2 phases, 3 cross-sectional area regions (75%, 50%, & 25% of muscle length), & 3 groups]. Furthermore, both echo intensity and cross-sectional area assessment of the Achilles tendon used a split plot ANOVA OR 2x4x3 [2 phases, 4 cross-sectional area regions (0,1,2, & 3cm from the calcaneal insertion), & 3 groups].

Spearman correlation analysis was also used to investigate any association between the relative change in SB and LIPA (see chapter 3), and the relative change from baseline for physical function outcomes, neuromuscular assessment outcomes,

muscle strength outcomes, muscle tendon complex morphology (architecture and tissue related quality) outcomes, and body composition outcomes.

A Chi squared test was used to compare between group differences for nominal data (physical behaviour classifications, proportion classified as low function vs. normal, right leg dominance vs. left leg, prone vs mid-prone arm orientation). Chi squared analysis was also used to analyse the results from the palatability questionnaire. Briefly, the number of respondents who circled a certain response (e.g. Definitely) was compared (using Chi squared analysis) between groups to highlight response trends. A significant difference between groups highlighted a difference between groups regarding number who selected a given response.

Data are reported as Mean±SD (or Median, IQR for non-parametric data). Statistical significance was accepted when $P \leq 0.05$. Furthermore, a statistical trend was deemed to present when p was between 0.05 and 0.10 (264). 95% confidence intervals were calculated of significant results that were detected with parametric statistics. However, for those calculated with non-parametric statistics a 95% confidence interval calculation was not permitted, considering there is no consensus on what methodology should be used. Effect sizes were Pearson *r* correlation (*r)*, Cohens d effect size (*d*), or partial eta squared (np^2). Pearson *r* correlation was used to calculate effect size for the Wilcoxon signed-ranked test, and the Mann-Whitney U test. For the Wilcoxon signed ranked test, *r* was calculated by dividing the Z value for the test by the square root of the total number of observations. For the Mann-Whitney U test, *r* was calculated by dividing the Z value for the test by the square root of the total sample size. An *r* value of 0.1, 0.3, and 0.5, is considered a small, medium, and large effect size respectively (265). Cohens *d* effect size was used to calculate effect size for the paired samples t-test. For the paired sample t-test, *d* was calculated by dividing the mean difference by the standard deviation of the difference. A *d* value of 0.2, 0.5, and 0.8, is considered a small, medium, and large effect size respectively (265). Partial eta squared (np^2) was used to calculate effect size for the two-way mixed design analysis of variance, and two-way mixed design analysis of co-variance. For such tests effect size was calculated automatically by SPSS software with the values displayed in the output. An np^2 value of 0.01, 0.06, and 0.14, is considered a small, medium, and large effect size respectively (265).

Finally, Z-scores were calculated for each dietary nutrient, and unit weighted composite z-scores for groups of nutrients to enable a) the nutrients grouping comparisons at baseline versus post-intervention for a diet promoting anabolism and a diet promoting bone health data reduction analysis; b) comparison of the diet composition change in those participants classified as sedentary pre-intervention, who changed to ambulators post-intervention. Z-scores were calculated as the average population value for a specific dietary nutrient at a particular point in time (average pre-test carbohydrate value for SBF) minus the average population value for a specific dietary nutrient (e.g. carbohydrate), divided by the population standard deviation for a specific nutrient (e.g. carbohydrate).

Chapter 3 – The efficacy of displacing sedentary behaviour with light intensity physical activity in older women, and compensatory lifestyle behaviours

Data from the current chapter are published in/ presented at (please see. research outputs in appendices ii):

Grant, D., Tomlinson, D., Tsintzas, K., Kolic, P. and Onambele-Pearson, G., 2020. Displacing Sedentary Behaviour with Light Intensity Physical Activity Spontaneously Alters Habitual Macronutrient Intake and Enhances Dietary Quality in Older Females. *Nutrients*, *12*(8), p.2431. **https://doi.org/10.3390/nu12082431**

Chapter take home message: Promisingly, within the sub-sample of 28 experimental participants significant reductions in Sedentary behaviour (SB) and average SB bout length were observed, as-well as a significant light intensity physical activity (LIPA) increase. Glucose exhibited a group×time interaction, mediated by a reduction following SB fragmentation (SBF). SBF was also the sole experimental group to increase nutrients promoting bone health intake, whereas both experimental groups increased nutrients promoting anabolism consumption.

Abstract

The efficacy of displacing Sedentary Behaviour (SB) with light intensity physical activity (LIPA) is still unclear regarding palatability, and potential behavioural compensations (MVPA, Sleep, habitual diet etc). Therefore, the aim of this chapter was to examine the efficacy of SB displacement with LIPA in older women and identify any compensations in other lifestyle behaviours. It was hypothesised SB displacement would be perceived as palatable and result in minimal behavioural compensations. Thirty-six older women (73±5 years) were allocated to one of three groups: 1) sedentary behaviour fragmentation (SBF) (*n* = 14), 2) continuous LIPA (*n* = 14), or 3) control (*n* = 8). Habitual diet and physical behaviour were assessed at weeks 0 and 8. Despite no significant main effects in the main cohort (n=36), stable levels of MVPA/ sleep were observed, and likelihood of long-term compliance. However, individual intra-week variance for both % of physical activity time spent in SB (p=0.029), and LIPA (p=0.047), exhibited a significant group×time effect, with the control group becoming more homogenised. Promisingly, within the sub-sample of 28 experimental participants significant reductions in SB (p=0.006) and average SB bout length (p=0.045) were observed, as-well as a significant LIPA increase (p=0.04). Glucose intake exhibited a group×time interaction (p=0.03), mediated by a reduction only in SBF (-31%). SBF was also the sole experimental group to increase nutrients promoting bone health (SBF: 17%, LIPA: -34%. control: 21%), whereas both experimental groups consumed more nutrients promoting anabolism (SBF: 13%, LIPA: 4%, control: -34%) (Z-scores). New ambulators (n=8) also consumed more nutrients promoting bone health (16%)/ anabolism (2%) (Z-scores), and Zinc intake (p=0.05, 29%). In conclusion, displacing SB with LIPA (irrespective of prescribed pattern) into the daily routine of older women, is achievable, palatable, and spontaneously enhances habitual dietary quality. Furthermore, SB fragmentation appears more advantageous for various dietary outcomes.

Introduction

Sedentary behaviour (SB) is defined as any waking physical behaviour, characterised by low levels of energy expenditure (EE) (≤1.5 metabolic equivalents), and a seated or reclined posture (23, 193). Interestingly, population moderate to vigorous physical activity (MVPA) did not significantly decline from 2008-2016, whereas SB significantly rose (~0.7-1.0h/day) (266, 267). Accordingly, studies that further controlled for the confounding effect of physical activity intensity, have found that SB is an independent determinant of health (46, 196). Furthermore, meeting the world health organisation's MVPA recommendation (150min/week), does not fully offset high SB time (8h-day) (195, 268). Light intensity physical activity [1.5-3.0 metabolic equivalents (LIPA)] is associated with positive health outcomes (269, 270), and is inversely correlated with SB (- r = -0.99 to -0.96) (18, 19).Thus, it is unclear whether the displacement of LIPA, may be mediating the aforementioned effects. Furthermore, SB accumulation pattern, also appears to play a role (258, 271). Accordingly, a more prolonged sedentary accumulation pattern (≥30min bouts) is associated with greater all-cause mortality (258), compared to a more fragmented pattern (<30min bouts).

Older adults (herein defined as ≥ 65y), are reported to be the most sedentary population (272). Comparing self-reported physical behaviour to an objective method, older adults underestimate SB by ~41% (40), suggesting an overt unawareness of SB time. Pooling of objective data, suggests older adults spend ~65-80% of their waking hours performing SB (71). SB is associated with adverse health outcomes in older adults (273), including cardiovascular disease, all-cause mortality (46), and frailty (116). Novel statistical moderation analyses, predict a necessary MVPA increase of ~29% and ~180%, relative to current recommendations (~150min.week), in order to respectively offset the risk of frailty (274) and all-cause mortality (268) associated with high SB. However, the necessary MVPA increase required to promote longevity (268) is substantially greater (151%) compared to the increase required to offset frailty (274). Thus, it is unclear whether longevity promoting excessive MVPA may indirectly compromise vitality. Therefore, despite enhanced longevity, those performing excessive MVPA with high SB, may not necessarily be healthier, given that longevity offers little insight as to the quality of life experienced up until the point of death .Furthermore, older adults overestimate MVPA (~15.7-fold), comparing self-report to

accelerometery (40). Importantly, enhanced longevity following excessive MVPA/ high SB, was exhibited following collation of self-reported studies (268), and failed to account for SB accumulation. Therefore, it remains unclear whether excessive MVPA (potentially overestimated due to self-report) merely protects against a fragmented pattern, or perhaps more impressively a prolonged pattern. In-fact pooling of objective data suggests physical activity (independent of intensity) is negatively associated with all-cause mortality (196). Ultimately, older adults exhibit a poor long-term tolerance for MVPA (166), making efforts to attain current recommendations, at best impractical and costly [time and or social/family support-wise; (275)] or at worst unachievable (276).

Most SB reduction interventions in older adults have reported mixed efficacy. Early studies initially utilised self-report physical behaviour assessments (277-279). Such studies reported significantly reduced TV viewing (~6 mins) (277), and total SB time (~100min.day) (279), as-well as a significant increase in physical activity (278). Despite such reductions in self-reported sedentary time being small in magnitude (6-minute reduction in TV viewing time), minor increases in physical activity may still hold some benefits, especially in those who perform little to no MVPA. This is adequately reflected in the 2019 UK chief medical officer's recommendations for physical activity in older adults (280), where it is stipulated that any amount of activity is now seen as providing a benefit and worth implementing. This highlights the practical significance of minor increases in physical activity, for older adults.

In any case, when self-reported SB reductions were compared to accelerometery data, older adults drastically overestimated reductions by ~85% (281), consistent with the under-reporting of habitual SB (40). Accordingly, SB is firmly embedded into older adults' habitual routine, suggesting attaining the required awareness to alter SB is difficult (282). Thus, objectively assessed SB intervention studies, may hold greater value, through providing a more accurate depiction of SB changeability. A recent review concluded the current evidence base lacked gold standard methodologies (e.g. triaxial accelerometers), and a control group (283). Nevertheless, two intervention studies utilising tri-axial accelerometery in older adults, reduced daily SB by 23-60min.day over 12-24 weeks (284, 285). However, such interventions were performed in diabetic patients, and utilised a behavioural modification intervention, limiting the

generalisability of the findings. Furthermore, a 12-week primary care-based walking intervention in older adults, decreased SB time by 48min.day, whilst improving self-reported physical quality of life (286). Interestingly, another intervention study reduced %SB time by ~3%, and increased breaks in SB per day by 4 (287). However, individual changes in %SB ranged from -13.6% to +8.0%, highlighting the heterogenous response to SB reduction in older adults (287). Furthermore, previous intervention studies merely prescribed the generic goal of decreasing SB time, without providing adequate instruction on which physical behaviour should displace SB. SB displacement appears to be spontaneously accompanied by increased physical activity time of varied intensity (LIPA, MVPA, Step count) (284-287). Previous studies have also failed to acknowledge the intra-week variability of PB, or how consistent/ sporadic a given behaviour is at baseline, and how this changes in response to an intervention. In other words, if an individual performs SB for 8h.day on a given day of the week, what is the likelihood they perform this same amount of SB on the other 6 days of the week. Intra-week variability has previously only been utilised as a method of monitoring athletes' training load (288-290), however the intra-week variability has not yet been considered in the context of older adults PB. Beyond assessing absolute physical behaviour in older adults, intra-week variability permits further investigation of how stable a particular behaviour is. Such in depth data mining may have implications for health outcomes and long-term adherence to physical behaviour interventions in older adults.

Is tolerance for (MV)PA the main limitation to physical behaviour intervention adherence?

Given the benefits of MVPA (196), together with the issue of sustained MVPA tolerance (166) in older adults, limited insight can be gained from displacing SB time with MVPA. Therefore, the specific role standing, and LIPA plays in the health promoting efficacy of displacing SB time, is still undetermined, and is difficult to elucidate in the absence of studies utilising gold standard tri-axial accelerometery. Nevertheless, two such studies observed significant SB reductions of between -2 to -5% per day (156, 277), with one observing a concurrent increase in walking time (LIPA) (277), and another standing time (156). Therefore, it remains unclear which strategy fosters the greatest health promoting utility. Acute experimental studies have

demonstrated greater health promoting utility of LIPA based SB displacement compared to stationary upright standing (89, 291, 292), likely mediated via higher muscle activity (91) and energy expenditure (91, 94).The majority of intervention studies that do exist have displaced SB with LIPA, and have observed increased gait speed (156), improved postural balance (293), and enhanced sit-to-stand ability (158). Interestingly, such improvements are observed when SB pattern is interrupted with frequent bouts of LIPA [SB fragmentation (SBF)] (158, 293). However, it remains unclear whether such improvements could still be achieved if the same absolute amount of LIPA displaced SB time through a single daily bout (analogous to conventionally recommended exercise), or whether the specific stimulus of frequent LIPA micro-bouts (SBF) is necessary.

Additional SB displacement interventions are required to determine whether the efficacy of such approaches, is dependent on SBF, or whether the mere increase in LIPA (irrespective of prescribed pattern) can foster comparable long-term adherence. Given compromised MVPA tolerance (166), determining how interventions are received by older adults is another important consideration. Promisingly, older adults find SB displacement acceptable, easy to incorporate, and perceive the intervention to have a positive impact on their health (161). In-fact, compared to structured lower body exercise [bodyweight squatting for 10 repetitions per minute (6kcal/min) (162), 5 minutes of parallel squatting, 40% of one repetition maximum (8-11kcal/min) (163)], one sit-to-stand transition (followed by 10 minutes of sitting) results in a relatively low energy cost (1.49kcal/min) (90) despite a similar level of force production (95). However, it should be noted the aforementioned studies investigated energy cost in younger adults (95, 162, 163), and protocols to assess sit-to-stand transitions are poorly standardised in the literature (164). Nevertheless, a relatively low energy cost during SB displacement may improve tolerance in older adults, compared to MVPA interventions. Interestingly, interventions that have displayed particular promise have focused on decreasing SB, proposed restructuring of the physical environment, and included some form of self-monitoring (283). Following a 12-week primary care-based walking intervention, older adults reported the social support by the nurse and the pedometer-based feedback were very helpful (286). However, it is unknown whether the prescribed pattern of SB displacement with LIPA (SBF vs. continuous LIPA),

affects older adults' tolerance, long-term adherence and overall perception of a given intervention.

What is the role of subconscious compensation in physical behaviour interventions?

Current interventions have also failed to account for, the potential for compensatory alterations in other lifestyle behaviours. Accordingly, the most promising health outcomes are observed in those performing MVPA, and minimising SB (Active-ambulator), compared with those who perform little to no MVPA and sit routinely (inactive-couch potato) (12, 13). Such evidence suggests both physical behaviours (increased MVPA and decreased SB) have a synergistic positive effect on health, which is more powerful than either behaviour alone (active-couch potato or inactive-ambulator).

SB displacement could also potentially cause compensations in other physical behaviours (71), as a result of conscious/subconscious compensation. Subconscious compensation, is consistent with the 'Activity Stat Hypothesis', which states that following physical activity manipulation in one intensity domain, there is a compensatory change in another, designed to stabilise energy expenditure (294). Despite mixed results (295), older adults reduce LIPA by ~35 minutes per day following increased MVPA (296). Interestingly, older adults were unaware they were compensating perhaps indicating subconscious regulation (296), yet attributed compensations to fatigue and muscle soreness following MVPA. This adequately highlights the major limitation of current physical activity recommendations, given that achieving recommended MVPA time requires a mere ~2% of waking hours (197), leaving ~98% unaccounted for (7). Consequently, despite the 'Activity Stat Hypothesis' not yet being systematically demonstrated (295), displacing SB with LIPA may cause subconscious MVPA reductions. Conscious physical behaviour compensation on other hand, represents a compensatory health belief (CHB), where the positive effects of a potentially healthy behaviour (displacing SB), are perceived to compensate for or neutralise the negative effects of an unhealthy lifestyle behaviour (reducing MVPA time) (297). Such perceptions may restrict an individual's progress towards overall achievement of health, given a perpetual cycle of implementation and compensation (297). Therefore, successfully displacing SB time with LIPA, whilst reducing MVPA time, may trade off some health promoting utility, through simply trading MVPA for

LIPA. Consequently, there is a requirement to investigate the long-term efficacy of SB displacement with LIPA of different prescribed patterns (SBF vs LIPA) in older adults and determine with gold standard methodologies (Tri-axial accelerometery) whether any compensatory changes in other physical behaviours occur following such approaches.

Does SB displacement influence habitual diet?

It is also unknown whether displacing SB time worsens/ enhances healthy diet-related practices. This is important, as older adults typically present with various adverse dietary practices, such as reduced energy intake over time (298), driven by a lack of hunger (299). Conversely, positive energy balance (energy intake exceeding EE), could facilitate adiposity accumulation (83, 300). Older adults consistently under consume protein (299, 301) , and exhibit a higher saturated fatty acid to polyunsaturated fatty acid intake ratio, as-well as a specific deficiency in omega-3 fatty acids like alpha-linolenic acid (298, 301, 302), with both dietary patterns strongly associated with cardiovascular disease mortality (303, 304). Deficiencies in vitamins B, C, and D, as well as key minerals such as calcium, magnesium, and zinc (301, 302, 305, 306) are also exhibited. Reductions in dietary quality over time are highlighted by the fact that older adults exhibit serving size reductions in food of high dietary quality (i.e. consisting of a good balance of starchy root vegetables, proteins, dairy products, as well as variety of fruit/ vegetables) (298), whereas correcting such deficiencies can enhance vitality/longevity (307).

Promisingly, physical activity has been identified as a gateway to the adoption of further healthy behaviours (308), with those consistently adhering to adequate physical activity levels more likely to exhibit healthier dietary practices (309). Accordingly, metabolic balance is defined as ‘The extent to which one’s physical behaviour profile influences nutritional intake and vice versa’ (202)]. Various subtypes of sedentary behaviour are consistently linked with unhealthy eating behaviours, including a) high driving time (≥3h/day), associated with reduced fruit/ vegetable intake (310) and b) adults who engage in ≥2h/day TV viewing time consume significantly more calories (2033kcal/ day) than adults who engage in 1-2h/day (1962kcal/day) and <1h/day (1896kcal/day) (311). However, within this sample of US adults (n=9157), more individuals engaged in ≥2h/day TV viewing time (n=5544, 61%) compared to

adults who engage in 1-2h/day (n=2317, 25%) and <1h/day (n=1296, 14%) (311). Whilst this may have contributed to differences in energy intake between groups, it does highlight the prevalence of excessive TV viewing time.

Interestingly, high self-reported standing time has been associated with reduced risk of obesity in middle aged women (55-65y) (312). Acutely displacing sedentary time in younger adults with standing marginally increases energy expenditure (300, 313), suggesting reduced obesity risk with high standing time, may be due to reduced energy intake. In-fact, rodents that were implanted with weighted capsules for 15 days (15% and 3% of bodyweight) spontaneously consumed less food, which then resulted in a decrease in body weight (8-20%) (314). Crucially, this suppression of food intake was not observed in osteocyte-depleted mice (314), suggesting increased bodyweight activates a load sensitive osteocyte strain detection mechanism of the weight-bearing bones (termed the gravitostat) (315). However, such a mechanism would theoretically not be activated in obese humans during insufficient loading of the lower body bones, (e.g. in a seated position). In contrast, standing or LIPA could hypothetically activate the gravitostat, manage food intake, and reduce bodyweight.

Promisingly, after 3 weeks of wearing a weighted vest (11% of bodyweight), 35 obese adults (BMI: 32.3±1.6 $kg.m^2$) experienced a 3% reduction in bodyweight, and a 4% reduction in fat mass (316). Despite the human pilot study failing to assess energy intake, participants self-reported wearing the weighted vest whilst standing for 4.8-5.9h.day (316), suggesting increased loading of the gravitostat with standing time may reduce bodyweight in obese adults. In support, a further study observed a 39% reduction in relative energy intake following a LIPA breaks protocol, compared to continuous SB (317). Therefore, despite all supportive human data having been observed in younger adults, or with extreme loading protocols (weighted vests), it can be reasoned that displacing SB with upright activity in older adults, may activate the gravitostat and manage food intake. Combined with chronic improvements in markers of appetite control (serum Ghrelin, PPY) following physical activity in older adults (165), ‘the gravitostat’ provides a further mechanism for reduced energy intake following SB displacement. However, changes in dietary quality are generally implemented in the long-term. Therefore, whilst previous findings must be interpreted

carefully, they do identify a promising trend of improved dietary quality following improved physical behaviour profile (greater activity).

Therefore, the aim of this chapter was to examine the feasibility and efficacy of displacing SB in older women with two distinct LIPA based interventions, which differ in terms of prescribed physical activity pattern (SBF vs continuous LIPA). The current chapter also aimed to identify any potential compensatory lifestyle behaviours that accompany SB displacement. The primary hypothesis was that 1) SB displacement would be implemented successfully irrespective of prescribed pattern and be perceived as a feasible alternative to structured exercise. It was further hypothesised that: 2) SB displacement would not cause spontaneous MVPA reductions, 3) SB displacement would be accompanied by improved dietary quality, 4) SBF would exhibit greater efficacy, and be perceived as more palatable and 5) SBF would result in fewer compensatory behaviours compared to continuous LIPA.

Results

Whole cohort (n=36) Baseline differences

Groups were matched at baseline for Age (p=0.15), weight (p=0.66), BMI (p=0.52), and proportion who lived alone/ cohabitate (p=0.19) (please see table 2.1). However, MVPA (p=0.04), MVPA% (p=0.024), PAMVPA% (p=0.037), and SPMVPAMins (p=0.038), were significantly different between groups at baseline (please see table 2.1). Following post-hoc pairwise comparisons, all the aforementioned variables were significantly higher in the control group compared to experimental at baseline. Furthermore, SB exhibited a trend towards being different between groups at baseline (p=0.051), similarly mediated through the control group displaying lower SB. Regarding intra-week variability co-efficient of variation the only variable that exhibited a significant difference between groups at baseline was PAMVPA% (p=0.037). Following post-hoc pairwise comparisons, LIPA was shown to be significantly different to both SBF (p=0.024) and control (p=0.039). For intra-week variability individual variance, only PASTD% exhibited a significant difference between groups at baseline (p=0.037). Following post-hoc pairwise comparisons, LIPA was shown to be

significantly different to both SBF (p=0.003) and control (p=0.005). Regarding habitual diet, carbohydrate (p=0.049), relative carbohydrate (p=0.02) and protein (p=0.045) intake were all significantly different between groups at baseline. Post hoc testing revealed SBF exhibited a significantly (p=0.04) lower protein intake (66±11g) compared to control (84±15g). Similarly, post-hoc testing revealed SBF exhibited a significantly lower relative carbohydrate intake at baseline (2.01±1.00 g.kg) compared to both LIPA (2.85±0.71 g.kg, p=0.02), and control (2.93±0.67 g.kg, p=0.02) (please see table 3.6). Interestingly, a significant baseline difference between groups was observed regarding the starting intervention month (p=0.001). Accordingly, 43%, 29%, 21%, & 7% of SBF and LIPA participants began their intervention in months conventionally associated with winter [January: n=3 (21%), February: n=3 (21%)], Summer [July: n=4 (29%)], Autumn [October: n=3 (21%),], and spring [April: n=1, (7%)], respectively. In contrast, all control participants began their intervention during conventional spring months [April: n=2 (25%), May: n=6 (75%)]. Nevertheless, the length of each intervention in days was significantly matched between groups (p=0.13), or the days between instructions being given, and the final monitoring day.

Main Cohort Analysis (n=36)

Promisingly, 23% of participants positively shifted classification from sedentary to ambulator (SBF: n=3, LIPA: n=3, CON: n=2), with the remaining 77% remaining unchanged over time (please see table 3.1). In contrast, 83% of participants physically active classification was unchanged, 11% of participants negatively shifted classification from active to inactive, and only 6% of participants positively shifted from inactive to active. Despite such positive classification shifts no significant main effects or trends were observed for any absolute physical behaviour classification outcome in the main cohort (Please see table 3.2 and Figures 3.1-3.3).

Table 3.1- Baseline characteristics, intervention, and diary-based outcomes between different groups. **Boldened text** represents a significant baseline difference.

	Group		
	SBF(n=14)	LIPA (n=14)	Control (n=8)
Age (y)	75±7	72±12	68±4
Weight (kg)	69±11	66±9	65±10
BMI (kg/m^2)	26.9±3.6	25.3±3.6	26.2±3.7
Proportion classified as Obese/ Overweight (Normal)	14%/57% (29%)	14%/43% (43%)	14%/72% (14%)
Polypharmacy(n)	2±4	0±1	1±3
FRAT (number of positive responses)	1±1	1±1	0±1
Proportion who live alone (cohabitate)	36% (64%)	43% (57%)	71% (29%)
Weekly MVPA time (≥10min Bouts)	77±183	51±65	51±130
Proportion classified as Sedentary (Ambulator)	71% (29%)	79% (21%)	43% (57%)
Proportion classified as Active (Inactive)	29% (71%)	0% (100%)	14% (86%)

BMI; Body mass Index, FRAT, Falls risk assessment tool, LIPA; Light intensity physical activity; MVPA; Moderate to vigorous physical activity, SBF; Sedentary behaviour fragmentation.

Table 3.2 – Physical behaviour outcomes at baseline, week 8, and both the average absolute and relative change from baseline, for each group. **Boldened text** represents a significant baseline difference. * Represents a significant change over time in the sub-sample experimental analysis.

	SBF (n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (%)	Pre	Post	Change (%)	Pre	Post	Change (%)
Sleep (hours)	8.1±0.9	8.5±0.8	0.4±0.7 (6±9%)	8.4±0.7	8.5±0.6	0.2±0.0 (2±10%)	8.6±0.8	8.4±0.8	-0.3±0.7 (-3±8%)
SB (hours)	9.7±1.2	9.2±2.6	-0.4±1.1 (-4±12%) *	9.6±1.1	8.9±1.2	-0.7±0.9 (-7±10%) *	8.3±1.8	8.4±1.2	0.1±1.7 (6±29%)
STD (hours)	1.0±0.6	1.0±0.6	0.08±0.25 (6±26%)	1.4±1.1	1.5±0.7	0.12±0.44 (9±39%)	1.2±0.5	1.1±0.5	-0.05±0.28 (-4±18%)
LIPA (hours)	2.2±0.5	2.2±0.6	0.03±0.35 (2±18%) *	2.1±0.4	2.3±0.5	0.27±0.38 (13±20%) *	2.2±0.7	2.3±0.8	0.03±0.81 (7±47%)
MVPA (hours)	3.0±1.0	2.8±1.0	-0.15±0.64 (-5±22%)	2.5±0.8	2.8±0.7	0.26±0.58 (15±28%)	**3.6±1.1**	3.7±1.1	0.12±0.85 (6±25%)
SB (% of waking hours)	60±7	59±10	-1±6 (-2±11%) *	62±7	58±8	-4±6 (-7±9%) *	54±12	54±10	0±10 (4±24%)
STD (% of waking hours)	6±4	8±4	1±2 (8±25%)	9±7	10±4	1±3(13±31%)	8±3	7±3	0±1 (-5±9%)
LIPA (% of waking hours)	14±3	14±4	1±2 (4±17%) *	13±2	15±3	2±2 (14±18%) *	14±4	15±5	0±5 (6±46%)
MVPA (% of waking hours)	18±8	19±8	0±6 (-1±34%)	14±8	18±6	2±6 (10±46%)	**23±5**	23±12	0±10 (1±47%)
Average SB bout length (minutes)	31±8	27±9	-2.9±8.5 (-10±25%) *	32±14	29±11	-2.7±9.1 (-8±33%) *	28±12	22±29	1.0±19.8 (3±53%)
MVPA in bouts ≥10 minutes duration (minutes)	11±31	6±27	-3.45±20.37 (-45±59%)	9±10	9±16	1.59±10.73 (0±72%)	9±23	8±13	-3.32±23.73 (-18±277%)

LIPA; Light intensity physical activity, MVPA; Moderate to vigorous physical activity, SB; Sedentary behaviour, STD, Standing

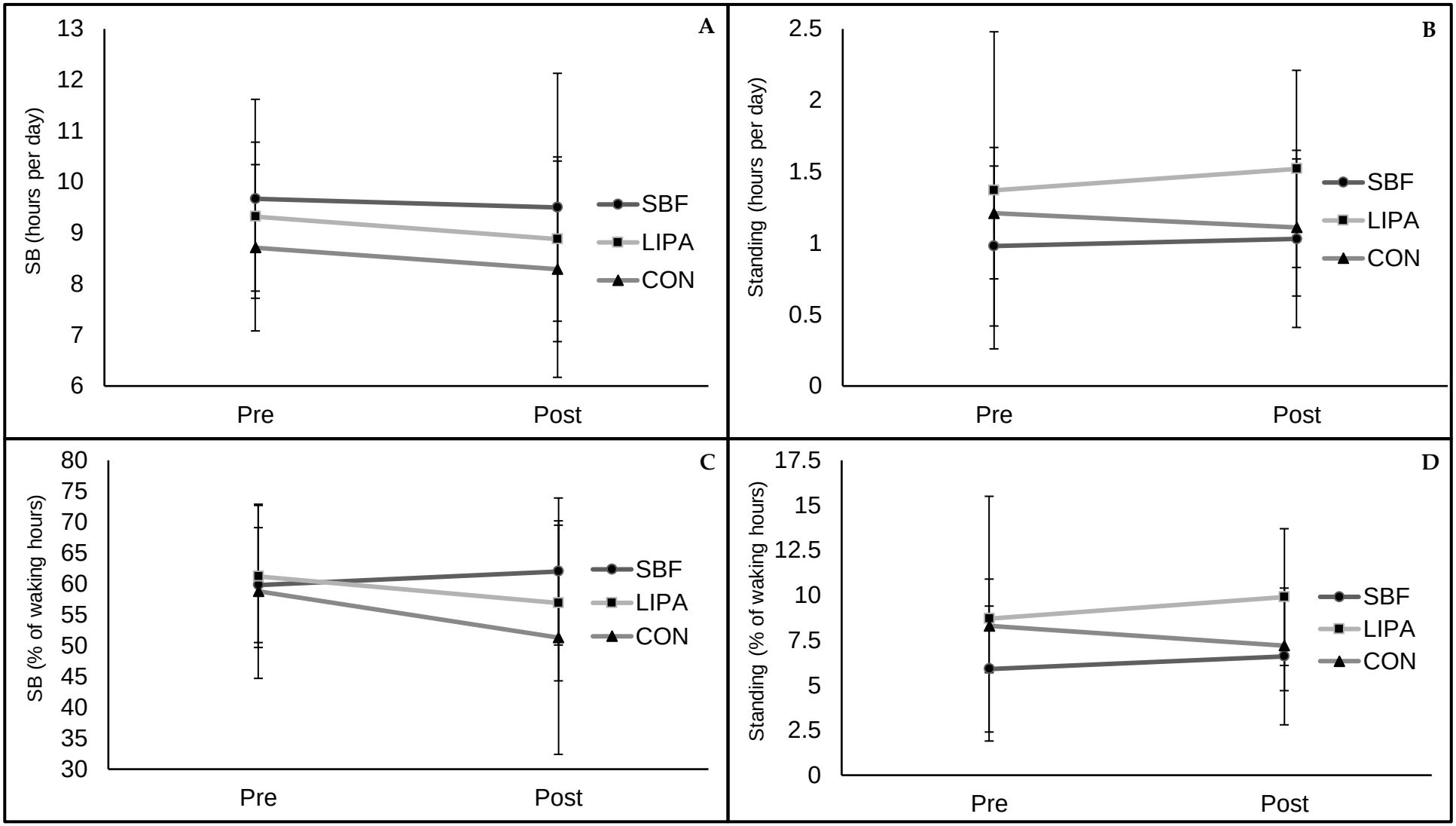

Figure 3.1- Group dependant pre and post values for four physical Behaviour variables. Panels A, B, C, & D represent SB time, standing time, proportional SB time, and proportional standing time. CON, control LIPA, light intensity physical activity, SB sedentary behaviour, SBF; sedentary behaviour fragmentation

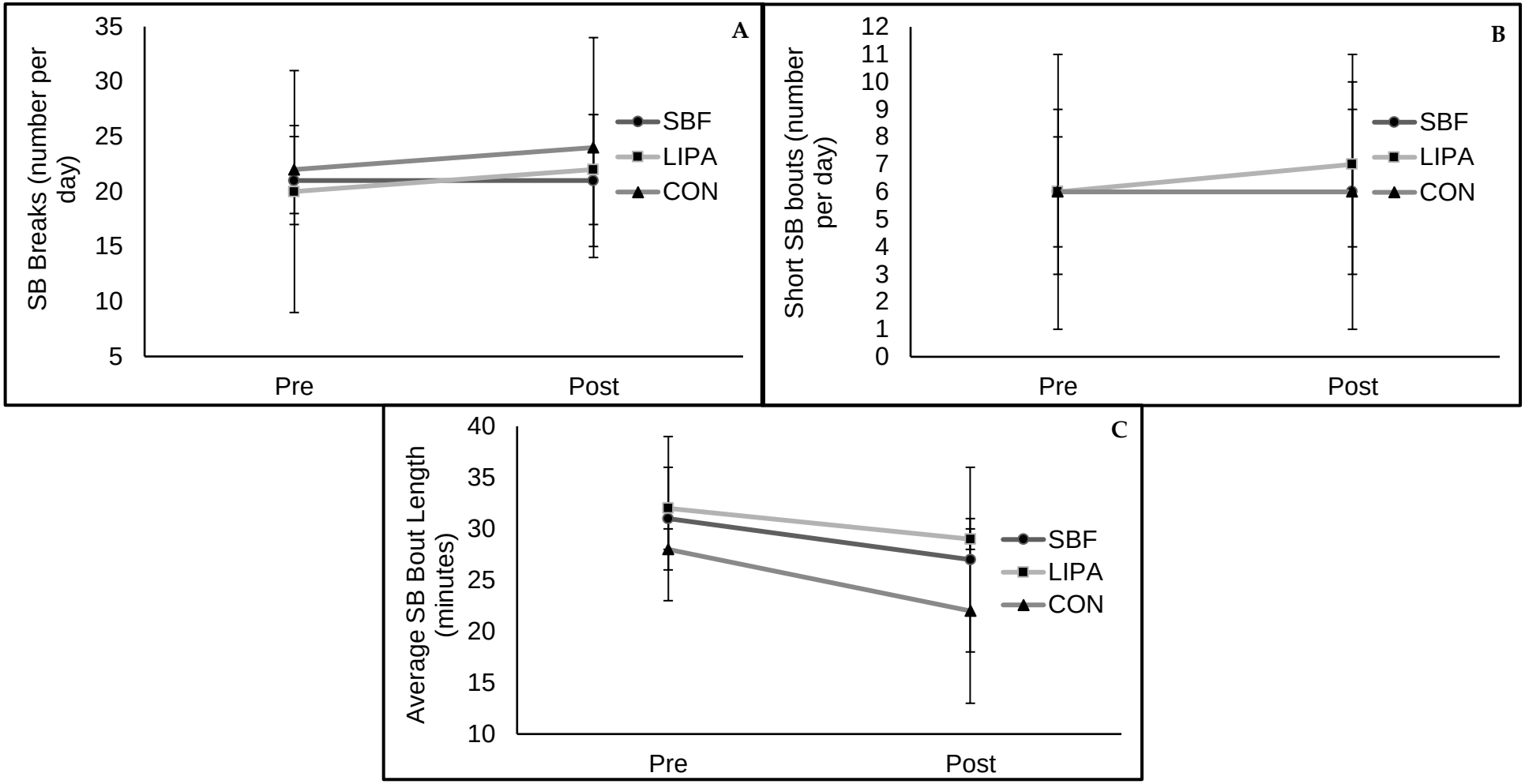

Figure 3.2-Group dependant relative pre and post values for four Physical Behaviour variables. Panels A, B, & C represent SB breaks, Short SB bouts, and average SB bout length respectively. CON, control LIPA, light intensity physical activity, SB; sedentary behaviour, SBF; sedentary behaviour fragmentation,

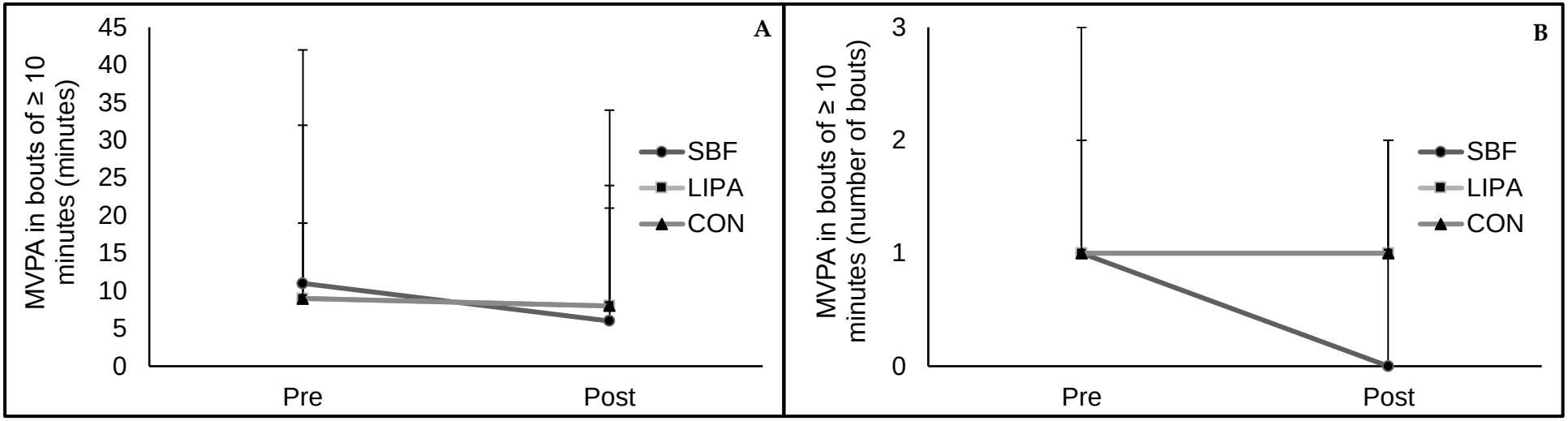

Figure 3.3- Group dependant relative changes (%) from baseline. Panels A and B represent 10minMVPA minutes, and 10minMVPA bouts, respectively. CON, control LIPA, light intensity physical activity, MVPA; moderate to vigorous physical activity, SBF; sedentary behaviour fragmentation

Regarding intra-week variability individual variance % of physical activity spent in SB exhibited a significant group×time interaction (p=0.029). Post-hoc analysis revealed significant differences between control (-73±28%), when compared to LIPA (1±149%], p=0.04, *r* = 0.58), and a trend in relation to SBF (5±283%, p=0.057). Similarly, % of physical activity time spent in LIPA exhibited a significant group×time interaction (p=0.047). Post-hoc analysis revealed significant differences between control (-39±75%), and SBF (127±250%, p=0.046, *r* = 0.48) (Please see appendices).

A trend towards a time effect (-23±245%, p=0.096) for sleep was also observed. Regarding intra-week variability co-efficient of variation no significant main effects were observed. Nevertheless, standing time (p=0.055), % of time spent standing (p=0.055), and % of physical activity time spent in LIPA (p=0.099) all exhibited trends towards a time effect. Specifically, standing time (Baseline: 27±9%, Week 8:22±10%) and % of time spent standing (Baseline: 27±10%, Week 8: 24±12%) trended towards becoming more homogenised over time. All the aforementioned effects were similar between groups (Please see appendices i). Accordingly, PALIPA% at baseline correlated with 3/8 following weeks for SBF, 0/8 for LIPA, and 0/8 for CON. Furthermore, sleep at baseline significantly correlated with 3/8 following weeks for SBF, 1/8 for LIPA, and 1/8 for control (Please see appendices).

Palatability Questionnaire

The only palatability question answered differently between groups was “Would you say you are more aware of the amount of light activity you perform daily following this intervention?” (p=0.047) (Please see tables 3.3, 3.4, & 3.5). Specifically, 93%, 43%, and 50% of LIPA, SBF and control participants responded with “Definitely”. In contrast all groups responded similarly when asked if they were more aware of their daily sitting behaviours following the intervention (p=0.14) (Please see Figure 3.4). SBF participants responded with definitely, fairly, and fairly not by 57%, 36%, and 7% respectively, when asked if the accelerometer prompt (vibration) was helpful. All participants (100%) responded with either “Definitely” or “Fairly”, when asked if they found the instructions easy to follow (p=0.45), and whether they were easy to implement at home (p=0.47). When asked if their muscles or joints felt sore during the intervention all groups responded similarly (p=0.39), with 3%, 6%, 22%, and 69%, responding with “Definitely”, “Fairly”, “Fairly Not”, and “Definitely Not” respectively.

Importantly, when asked if participants felt embarrassed, an overwhelming majority (94%) responded with either “Definitely Not” or “Fairly Not”, a response that was consistent across groups (p=0.69). Furthermore, when asked if the participants could see themselves continuing their intervention long-term, and if their intervention had motivated them to become more active, all groups responded similarly (p=0.25) (Please see Figure 3.4). Accordingly, when asked if their intervention had motivated them to make long-term changes to their health, 39%, 33%, 14%, and 14% responded with “Definitely”, “Probably”, “Undecided” and “Probably Not”, respectively. When asked if they felt more positive about their health all groups responded similarly (p=0.43), with 47%, 36%, 11%, 8%, and 8% of participants responding with “Definitely”, “Fairly”, “Undecided” “Fairly Not”, and “Definitely Not”, respectively.

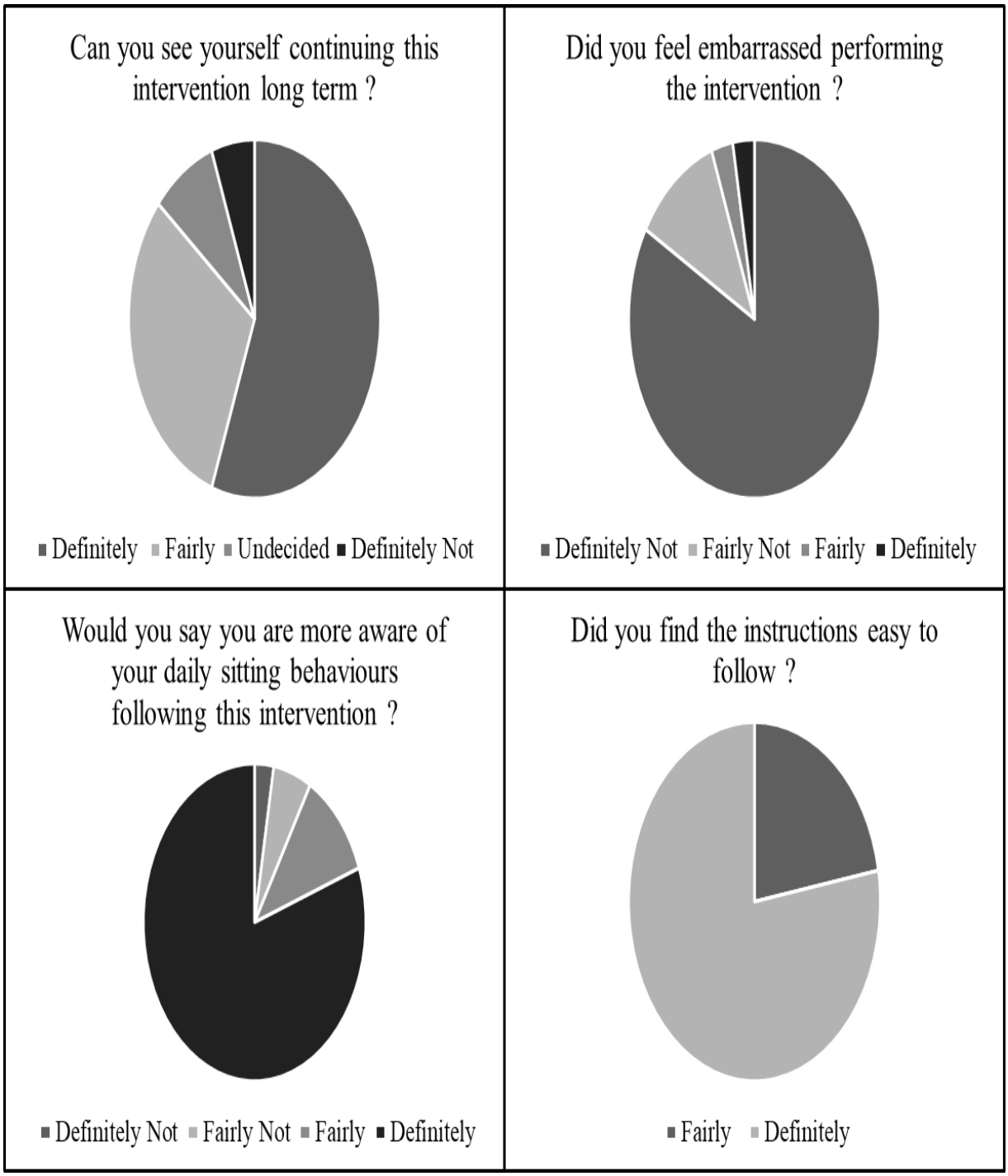

Figure 3.4– Proportions of responses for four Select palatability questions in the whole cohort.

Table 3.3- Palatability responses for the SBF group.

	SBF (n=14)				
	Definitely	**Fairly**	**Undecided**	**Fairly Not**	**Definitely Not**
Did you find the accelerometer prompt (vibration) helpful? (SBF only)	57%	36%	0%	7%	0%
Did you find the instructions easy to follow?	86%	14%	0%	0%	0%
Did you find the intervention easy to follow at home?	79%	21%	0%	0%	0%
Did you find the compliance diary easy to fill in?	72%	21%	7%	0%	0%
Did you find the compliance diary helpful?	43%	50%	0%	7%	0%
Do you think your balance has improved following the intervention?	22%	14%	50%	7%	7%
Did you feel short of breath during the intervention?	0%	0%	0%	14%	86%
Did your muscles or joints feel sore during the intervention?	0%	7%	0%	14%	79%
Did you feel embarrassed performing the intervention?	7%	7%	0%	14%	72%
Would you say you are more aware of the amount of light activity following this intervention?	43%	50%	7%	0%	0%
Would you say you are more aware of your daily sitting behaviours following this intervention?	79%	14%	0%	7%	0%
Would you say you feel more confident about performing household tasks following this intervention?	29%	29%	13%	%	29%
Would you say you feel more confident about your health following this intervention?	43%	43%	7%	7%	0%
	Definitely	**Probably**	**Undecided**	**Probably Not**	**Definitely Not**
Can you see yourself continuing this intervention long term?	43%	36%	7%	0%	14%
Has this intervention motivated you to become more active?	50%	29%	0%	21%	0%
Has this intervention motivated you to make long term changes to your health?	29%	43%	14%	14%	0%
Would you recommend this intervention to a friend?	58%	21%	21%	0%	0%

Table 3.4- Palatability responses for the LIPA group.

	LIPA (n=14)				
	Definitely	**Fairly**	**Undecided**	**Fairly Not**	**Definitely Not**
Did you find the instructions easy to follow?	79%	21%	0%	0%	0%
Did you find the intervention easy to follow at home?	57%	43%	0%	0%	0%
Did you find the compliance diary easy to fill in?	64%	29%	7%	0%	0%
Did you find the compliance diary helpful?	50%	43%	7%	0%	0%
Do you think your balance has improved following the intervention?	14%	7%	72%	0%	7%
Did you feel short of breath during the intervention?	0%	0%	0%	14%	86%
Did your muscles or joints feel sore during the intervention?	0%	0%	0%	29%	71%
Did you feel embarrassed performing the intervention?	0%	0%	0%	7%	93%
Would you say you are more aware of the amount of light activity following this intervention?	93%	7%	0%	0%	0%
Would you say you are more aware of your daily sitting behaviours following this intervention?	100%	0%	0%	0%	0%
Would you say you feel more confident about performing household tasks following this intervention?	21%	21%	21%	16%	21%
Would you say you feel more confident about your health following this intervention?	50%	29%	21%	0%	0%
	Definitely	**Probably**	**Undecided**	**Probably Not**	**Definitely Not**
Can you see yourself continuing this intervention long term?	79%	14%	7%	0%	0%
Has this intervention motivated you to become more active?	57%	36%	0%	7%	0%
Has this intervention motivated you to make long term changes to your health?	43%	29%	14%	14%	0%
Would you recommend this intervention to a friend?	50%	43%	7%	0%	0%

Table 3.5- Palatability responses for the Control group.

	Control (n=8)				
	Definitely	**Fairly**	**Undecided**	**Fairly Not**	**Definitely Not**
Did you find the instructions easy to follow?	62%	38%	0%	0%	0%
Did you find the intervention easy to follow at home?	62%	38%	0%	0%	0%
Did you find the compliance diary easy to fill in?	76%	7%	0%	7%	0%
Did you find the compliance diary helpful?	62%	25%	0%	13%	0%
Do you think your balance has improved following the intervention?	0%	13%	74%	0%	13%
Did you feel short of breath during the intervention?	0%	0%	0%	12%	88%
Did your muscles or joints feel sore during the intervention?	7%	7%	0%	25%	51%
Did you feel embarrassed performing the intervention?	0%	0%	0%	12%	88%
Would you say you are more aware of the amount of light activity following this intervention?	50%	26%	12%	0%	12%
Would you say you are more aware of your daily sitting behaviours following this intervention?	50%	26%	12%	0%	012%
Would you say you feel more confident about performing household tasks following this intervention?	13%	13%	37%	0%	37%
Would you say you feel more confident about your health following this intervention?	50%	38%	0%	0%	12%
	Definitely	**Probably**	**Undecided**	**Probably Not**	**Definitely Not**
Can you see yourself continuing this intervention long term?	38%	50%	12%	0%	0%
Has this intervention motivated you to become more active?	76%	7%	0%	0%	7%
Has this intervention motivated you to make long term changes to your health?	50%	26%	7%	7%	0%
Would you recommend this intervention to a friend?	88%	12%	0%	0%	0%

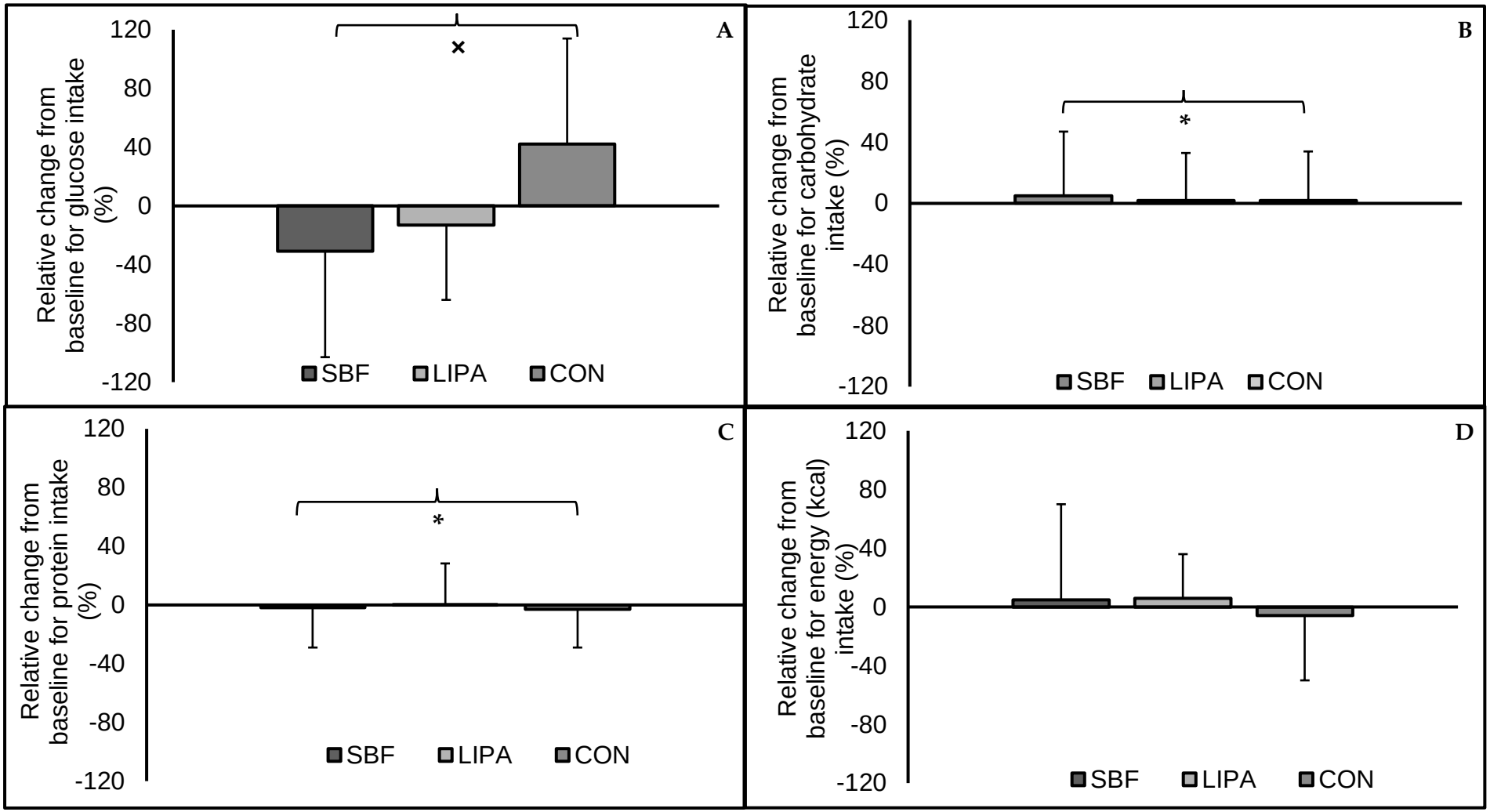

Figure 3.5– Group dependant relative change from baseline for four key habitual dietary outcomes. Panels A, B, C, & D represent relative changes from baseline in glucose, carbohydrate, protein, and energy intake respectively. × represents a signficant group×time interaction effect (significant post-hoc difference between SBF and CON for glucose, p=0.01). * represents a signficant effect for time (after controlling for baseline differences). CON; control, LIPA; light intensity physical activity, SBF; sedentary behaviour fragmentation.

Habitual Dietary Intake

Notably, 89% of participants consumed protein at or above the recommended level at baseline. Promisingly, 29%, 40%, and 100% of participants consumed below the recommended maximum daily intake of saturated, total, and trans fats, respectively. Furthermore, ≥94% of participants at baseline consumed at or above recommended daily intake of Vitamins C, E, as-well as phosphorous. Recommended daily consumption of Omega-3, calcium, zinc, and magnesium, was present in ≤60% of participants at baseline. Moreover, 100% of participants consumed at or above the recommended intake of vitamin B-12, and 86% consumed at or below the recommended intake of sodium. However, only ≤17% of participants consumed at or above the recommended daily levels of Potassium, omega-6, and vitamin-D (Table 3.6). Participants consumed ~3 portions of fruit, and ~2 portions of vegetables (Table 3.6) per day on average. Accordingly, 13 (37%) participants routinely consumed nutritional supplements (SBF: n=4, LIPA: n=5, Control: n=4), however there was no significant difference between groups at baseline regarding the number of supplements consumed (p=0.65) (Table 3.6).

Carbohydrate Intake as a factor of intervention

After accounting for baseline differences, a significant main of effect of time (p=0.001, n^2p= 0.3), but not a group×time interaction (p=0.36) was observed for carbohydrate intake (Please see figure 3.5, panel B). Furthermore, a significant group×time interaction effect (p=0.03), but not a time effect (p=0.48) was observed for glucose intake. Post-hoc tests revealed a significant difference between SBF and control (p=0.01, *r* = 0.48) (Please see figure 3.5, panel A). Despite no post-hoc effect between SBF and LIPA (p=0.37), a trend between LIPA and control (p=0.054) was observed. Thus, group dependant changes in glucose intake were primarily driven through experimental decreases [SBF: -2.8±6.7g (-31±72%), LIPA: -1.6±4.9g (-13±351%)], and a control increase [5.5±5.1g (42±72%)] (Please see table 3.6). A trend toward a group×time interaction was observed for Fructose intake (p=0.07), but no trend for time (p=0.61). Accordingly, SBF [-2.1±7.3g (0±66%)], and LIPA [-2.9±4.8g (-17±27%)], exhibited decreases, in contrast to control [3.4±4.3g (21±29%)].

Protein Intake as a factor of intervention

After accounting for baseline differences, a significant main of effect of time (p=0.004, n^2p= 0.24), but not a group×time interaction (p=0.59) was observed for protein intake. Accordingly, average daily protein intake decreased from pre to post (-2.6±18.2g, -1±26%) (Please see figure 3.5, panel C). However, within the sub-analysis of new ambulators, trends were observed for increased absolute (8.7±12.3g, 12±19%, p=0.09), and relative (0.2±0.2g.kg, 13±19%, p=0.08) protein intake.

Energy Balance as a factor of intervention

No significant main effects were observed for energy intake (p≥0.05), even after accounting for body mass (mass, BMI, etc) (Please see figure 3.5, panel D). Furthermore, basal metabolic rate (Harris-benedict) exhibited no significant time (p=0.34), nor group×time interaction (p=0.67) effects. Similarly, basal metabolic rate (Schofield) exhibited no significant time (p=0.58), nor group×time interaction (p=0.53) effects. Interestingly, total daily energy expenditure exhibited a significant increase over time effect when calculated with both Harris benedict (47±88kcal, 3±6%, p=0.006, n^2p= 0.41), and Schofield (5±37kcal, 0.3±2%, p=0.03, *r* = 0.25) equations. However, no main effects were observed for energy balance, when calculated with either the Harris-benedict equation (Time: p=0.64, group×time: p=0.99), or the Schofield equation (Time: p=0.51, group×time: p=0.054) (Please see appendices).

Micronutrient Intake as a factor of intervention

Vitamin B_{12} exhibited a trend toward a group dependant change over time (p=0.09), with control displaying the greatest increase [1.9±2.3µg, (45±45%)], followed by LIPA [0.9±2.1µg, (33±73%)], and SBF [-6.4±21.9µg, (-7±67%)]. Similarly, Vitamin B3 (Niacin) exhibited a time effect trend [-1.2±6.4mg, -1±47%p=0.09], but no group×time interaction (p=0.76). Interestingly no significant main effect for time or group×time interactions were observed for portions of fruit, portions of vegetables, or nutritional supplements consumed. However within the sub-analysis of new ambulators (n=8) zinc intake significantly increased (1.7±3.8mg, 29±63%, p=0.05, *r* = 0.49), as-well as a trend toward increased manganese intake (1.4±2.4mg, 32±48%, p=0.09) (please see table 3.6).

No other nutrient factor on its own, showed any main effects nor interactions. Thus, subsequent analysis grouped factors based upon their physiologic impact and differences by physical behaviour classification. This approach used radar graphs based on computed z-scores.

Dietary components promoting anabolism, as a factor of the two interventions

There were no differences between groups at baseline (p=0.88) regarding the amount of nutrients promoting anabolism each participant consumed at optimal levels (One= 9%, Two=57%, Three=14%, Four=17%, Five=3%) (please see appendices). Unit weighted composite Z-score analysis (Figure 2) exhibited that both SBF (Composite Z-score - Pre: 0.28, Post: 0.65), and LIPA (Composite Z-score - Pre: -0.73, Post: -0.62) increased intake of nutrients promoting anabolism from pre to post by 13% and 4% respectively. Control on the other hand decreased intake of nutrients promoting anabolism (Composite Z-scores- Pre: 0.91, Post: -0.06) by ~34% (please see figure 3.6).

Dietary components promoting Bone health as a factor of the two interventions

There were no differences between groups at baseline (p=0.78) regarding the number of bone health enhancing nutrients each participant consumed at optimal levels (One= 3%, Two=14%, Three=20%, Four=17%, Five=23%, Six=11%, Seven=9%, Eight=3%) (Please see appendices). Unit weighted composite Z-score analysis shows that SBF (Figure 3; Composite Z-score - Pre: -0.66, Post: -0.18), and control (Composite Z-scores - Pre: 0.48, Post: 1.26) increased intake of nutrients promoting bone health from pre to post by 17% and 21% respectively, whereas LIPA (Composite Z-scores - Pre: 0.42, Post: -0.45) decreased their intake of nutrients promoting bone health by ~34% (please see figure 3.7).

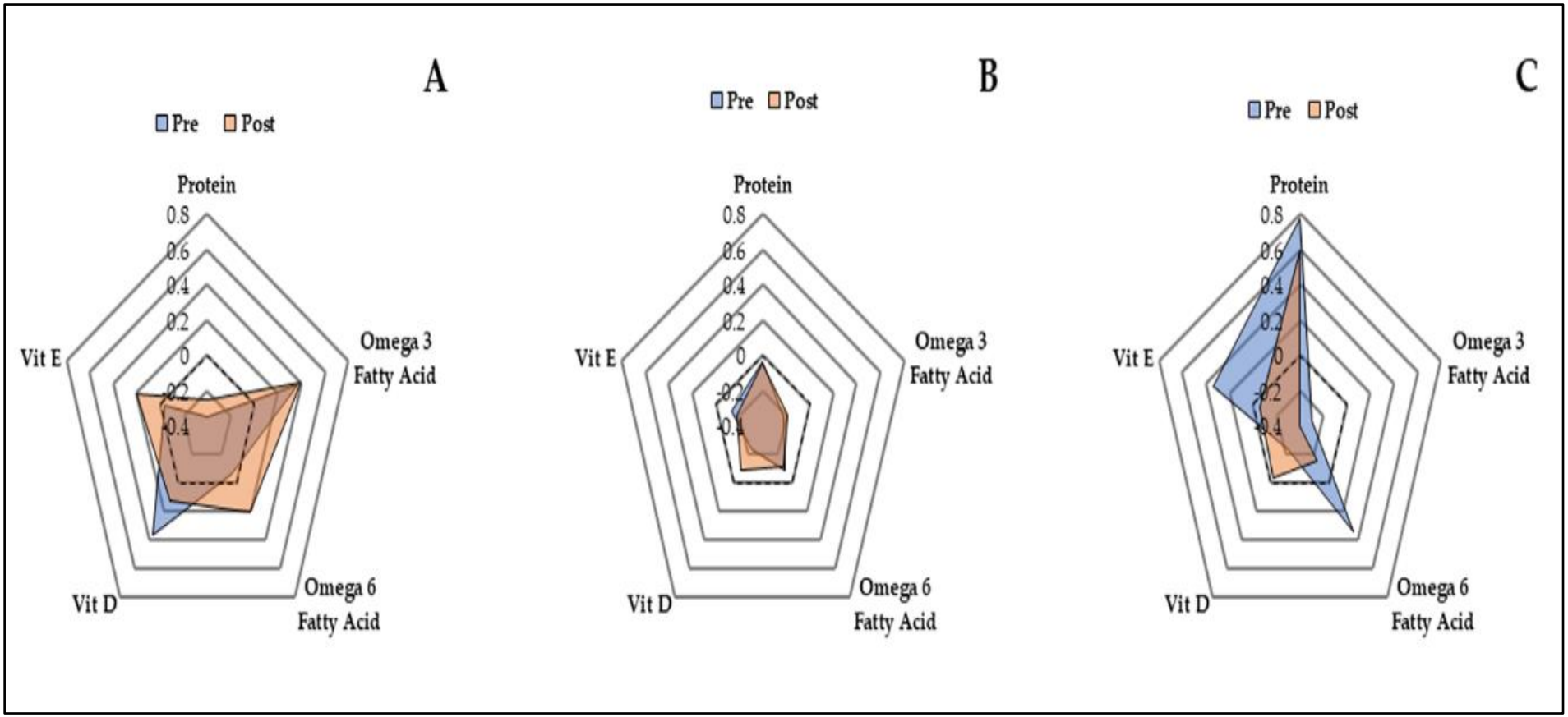

Figure 3.6– Radar graphs representing Z-scores for five nutrients promoting anabolism at baseline and post-intervention. Panels A, B, & C represent sedentary behaviour fragmentation, light intensity physical activity, and control respectively. The two colours represent a transparent overlap of the two dietary patterns.

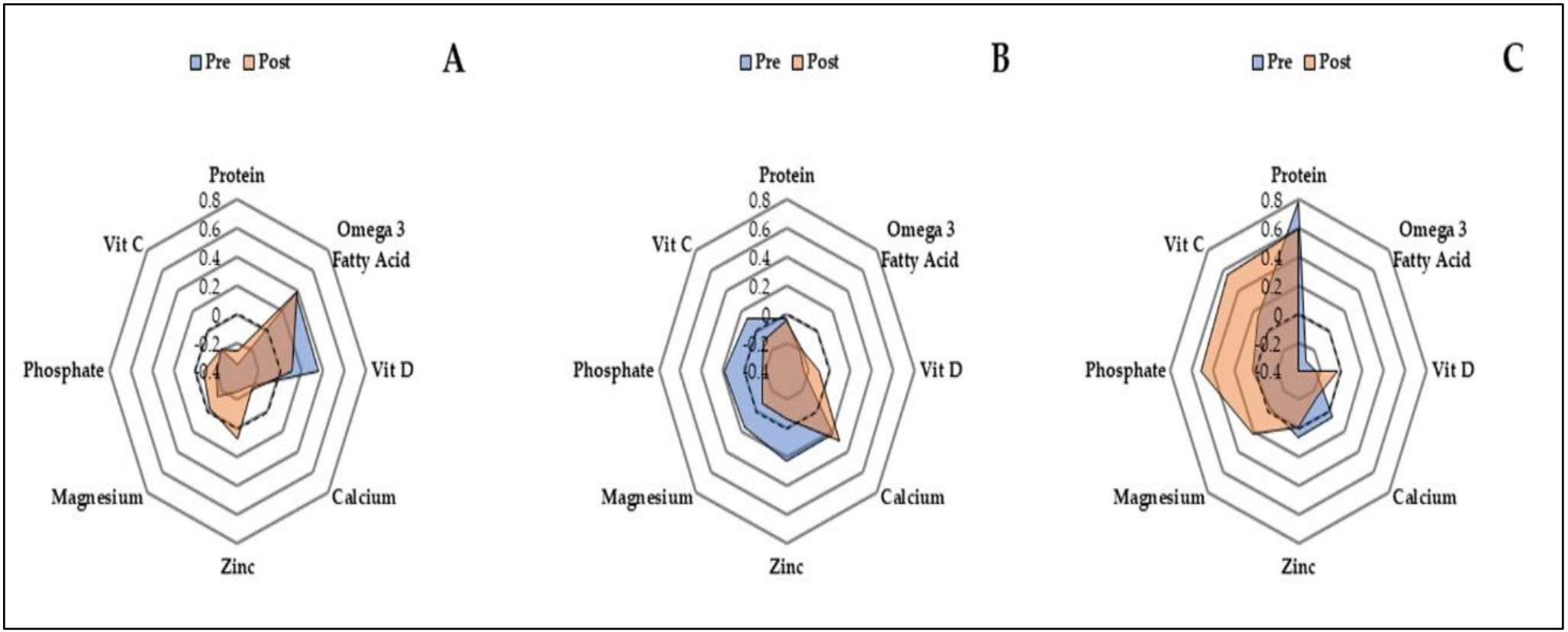

Figure 3.7– Radar graphs representing Z-scores for eight nutrients promoting bone health at baseline and post-intervention. Panels A, B, & C represent sedentary behaviour fragmentation, light intensity physical activity, and control respectively. The two colours represent a transparent overlap of the two dietary patterns.

Effect of physical behaviour classification change on habitual dietary outcomes

Following the sub-analysis regarding those who positively shifted from sedentary to ambulator physical behaviour classification (n=8), the overall nutrition Z-score radar graph highlighted the combined directional unit weighted score changed from 5.25 at baseline to 9.27 post intervention. Importantly, these participants also increased intake of nutrients promoting anabolism (Combined weighted unit scores- Pre: 1.01, Post: 3.33), and nutrients promoting bone health (Combined weighted unit scores- Pre: 2.08, Post: 3.72), by 2%, and 16% respectively (Please see figure 3.8).

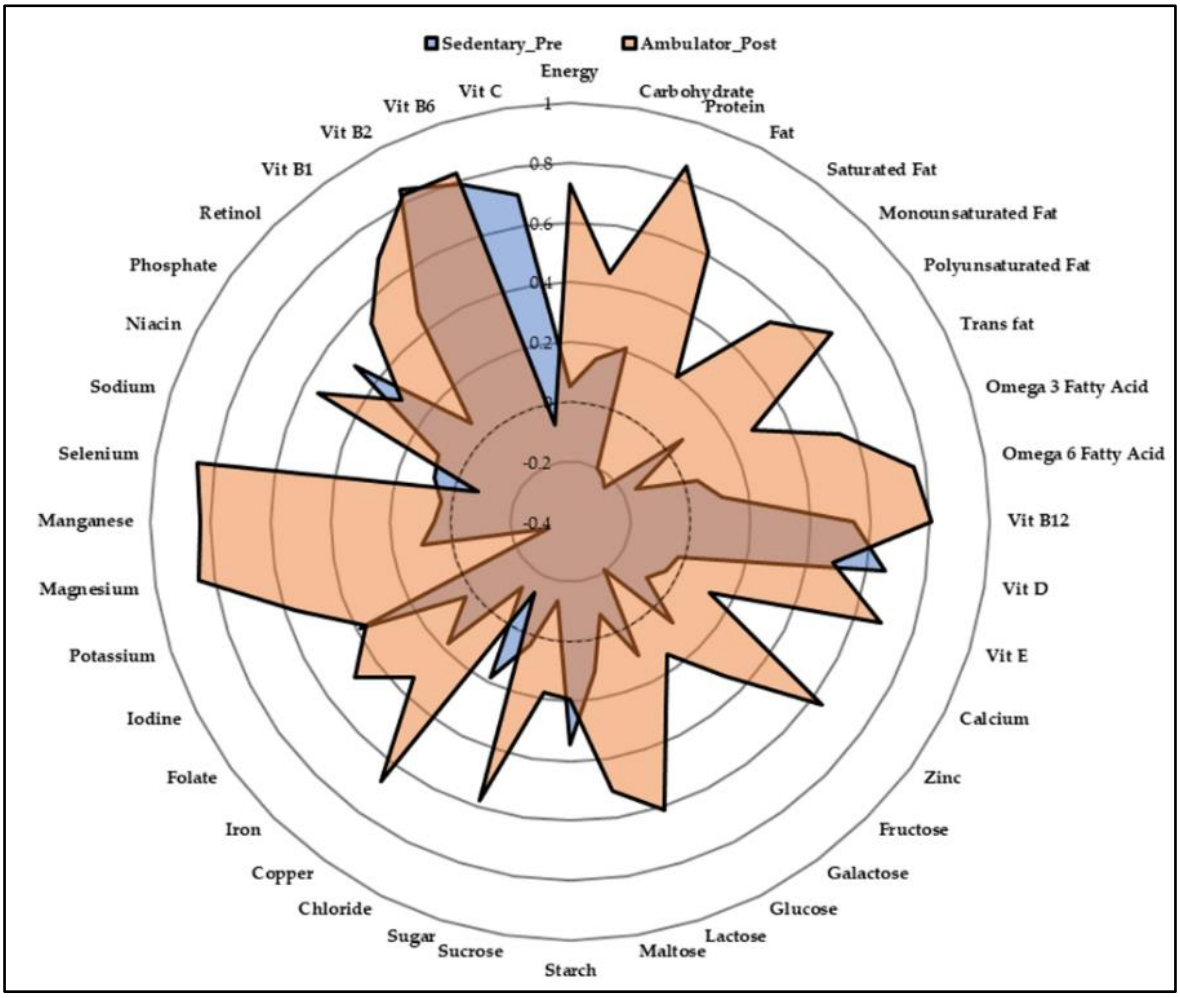

Figure 3.8– Radar graphs representing Z-scores at baseline and post-intervention for participants who shifted their physical behaviour classifciation from 'Sedentary' to 'Ambulator'. The two colours represent a transparent overlap of the two dietary patterns.

Table 3.6- Habitual dietary outcomes at baseline, and week 8, for each group. Boldened text represents a significant baseline difference. * represents a significant change over time. × represents a significant group×time interaction effect**.**

	SBF (*n*=14)		**LIPA (*n*=14)**		**Control (*n*=7)**	
	Baseline	**Week 8**	**Baseline**	**Week 8**	**Baseline**	**Week 8**
Energy (Kcal)	1371±616	1468±699	1543±509	1602±350	1825±679	1546±557
Energy (Kj)	5740±2566	6150±2911	6479±2118	6715±1478	7653±2799	6483±2351
Protein (g)	**66±11**	65±20*	71±18	69±16*	84±15	80±12*
Relative Protein intake (g/kg)	1.00±0.30	0.98±0.40	1.11±0.32	1.06±0.23	1.29±0.18	1.20±0.24
Portions of Fruit consumed (n)	2±1	2±2	3±2	3±2	3±1	3±2
Portions of Vegetables consumed (n)	2±5	2±1	2±1	2±1	2±1	2±1
Carbohydrate (g)	**144±38**	144±51*	177±48	174±45*	187±36	186±57*
Relative carbohydrate intake (g/kg)	**2.04±1.00**	2.03±0.97	2.85±0.71	2.78±0.60	2.93±0.67	2.56±1.51
Glucose (g)	13.4±6.4	10.5±5.1**×**	15.4±5.8	13.8±7.2**×**	14.3±4.5	19.7±7.3**×**
Total Fat (g)	64±32	69±31	66±19	67±23	75±27	67±23
Vitamin B3 (mg)	13.3±12.9	13.0±5.1	14.0±7.3	13.0±15.3	17.1±14.0	15.1±6.3
Vitamin B12 (µg)	5.0±3.0	4.4±3.6	4.1±2.8	4.7±4.0	4.5±2.5	5.0±4.3
Zinc (mg)	6.7±4.8	7.9±3.9	7.3±4.8	6.9±3.6	7.9±2.1	8.5±2.8

Subsample Analyses of experimental groups (n=28)

No baseline physical behaviour differences were observed between the two experimental groups in the sub-sample analysis. A significant main effect for time ($p=0.006$, $n^2p=0.26$), but no group×time interaction ($p=0.41$) was observed for SB. SB reduced by -4±12%, and -7±10%, for SBF and LIPA respectively. Furthermore, a significant main effect for time ($p=0.034$, $n^2p=0.16$), but no group×time interaction ($p=0.20$) was observed for proportional SB time (% of 24h) (Please see figure 3.9 panels A and B). Proportional SB time reduced by -2±11%, and -7±9% for SBF and LIPA, respectively. In contrast, a significant main effect for time ($p=0.04$, $n^2p=0.15$), but no group×time interaction ($p=0.11$) was observed for LIPA. Specifically, LIPA increased by 1±17%and 13±25% for SBF and LIPA respectively (Please see figure 3.9 panels C & D). Similarly, a significant main effect for time ($p=0.01$, $n^2p=0.23$), but no group×time interaction ($p=0.16$) was observed for proportional LIPA time (% of 24h). Proportional LIPA time increased by 2±18% and 13±20% for SBF and LIPA, respectively. Interestingly, a significant main effect for time ($p=0.045$, $r = 0.24$), but no group×time interaction ($p=0.96$) was observed for average SB bout length. Specifically, SBF and LIPA decreased average SB bout length by -10±25%, and -8±33% respectively. Sleep exhibited a trend toward a significant main effect for time ($p=0.054$) but no group×time interaction trend ($p=0.40$). SBF and LIPA increased sleep time by 0.4±0.7h, and 0.2±0.8h respectively. Finally, trends toward group×time interactions were observed for 10 min sporadic MVPA minutes ($p=0.10$), and % of physical activity time spent standing ($p=0.10$). Self-reported Prompts complied to in the SBF group significantly reduced ($p=0.008$, $d = 0.97$, 95% CI -8 to -2) from 9±12 to 6±8 per week. There was also a trend for Total prompts to decrease ($p=0.07$) from 15±9 to 10±9 per week. (please see appendices).

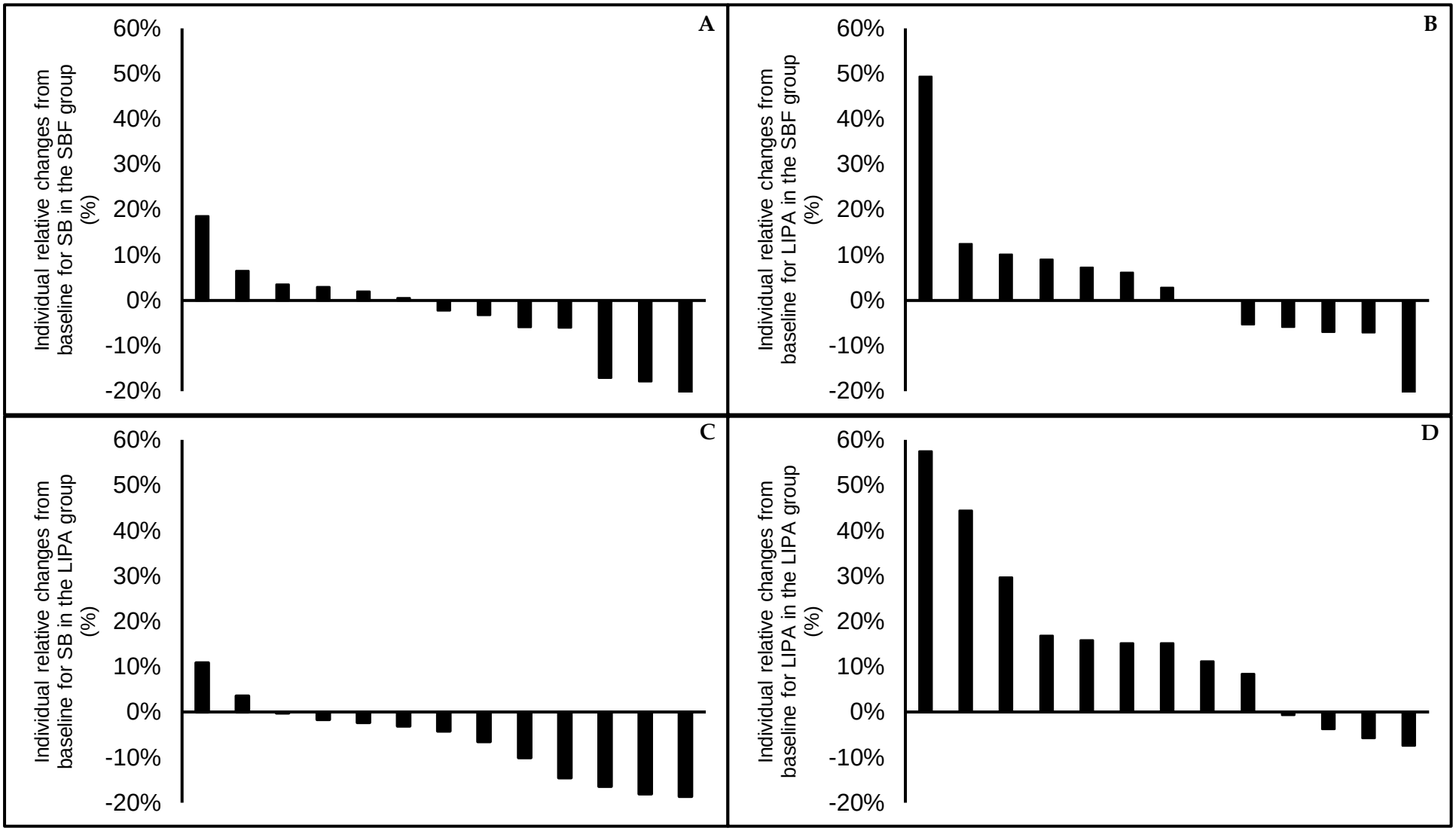

Figure 3.9- Individual relative changes from baseline (%). Panels A, B, C, & D, represent individual changes in SB for SBF, individual changes in LIPA for SBF, individual changes in SB for LIPA, and individual changes in LIPA for LIPA, respectively. LIPA, light intensity physical activity, SB; sedentary behaviour, SBF; sedentary behaviour fragmentation.

Discussion

The aim of this chapter was to examine the feasibility and palatability of displacing SB in older women with two distinct LIPA based interventions. A further aim was to examine and identify any potential compensatory lifestyle behaviours that accompany SB displacement.

The primary hypothesis was that 1) SB displacement would be implemented successfully irrespective of prescribed pattern and be perceived as a feasible alternative to structured exercise. Significant reductions were observed for SB, SB%, and mean SB bout length, with concurrent increases in LIPA, and LIPA% ($p \leq 0.05$), within the sub-sample analysis of experimental participants (n=28), but not the whole cohort (n=36). Furthermore, the palatability responses were overwhelmingly positive. Therefore, the primary hypothesis was upheld. It was further hypothesised that: 2) SB displacement would not cause spontaneous MVPA reductions. Accordingly, no significant main effects were observed for any MVPA parameters ($p \geq 0.05$). Therefore, the second hypothesis was upheld. 3) SB displacement would be accompanied by a spontaneous reduction in energy intake (thus managing energy balance more effectively), as-well as a relative improvement in dietary quality [improvements in macro (increased protein intake etc.)/ micro-nutrient profile]. Despite not observing any change in total energy intake, a significant reduction in daily protein intake (one of the most important anabolic nutrients) (318) was noted ($p=0.004$). Furthermore, carbohydrate intake exhibited a significant change over time ($p=0.004$). Z-score analysis for the entire dietary profile revealed both SBF and LIPA increased intake of nutrients promoting anabolism in contrast to control. Therefore the third hypothesis was only partially upheld. 4) SBF would be more successfully implemented and perceived as more palatable/ achievable. However, the only significant adherence/palatability difference, was a heightened awareness of daily light activity in the LIPA group. Consequently, the fourth hypothesis was rejected. 5) SBF would result in fewer compensatory lifestyle behaviours (MVPA, habitual diet etc) compared to continuous LIPA. A group dependant change in glucose intake ($p=0.03$) was observed driven by a reduction in SBF (-31%). Further Z-score analysis revealed SBF was the sole experimental group to increase nutrients promoting bone health. Therefore, the fifth and final SBF advantage hypothesis was partially upheld.

Within the sub-sample experimental analysis (n=28), significant reductions in SB parameters were observed (SB, SB%, and average SB bout length) together with increases in both LIPA, and LIPA%. Given that the primary experimental objective was to displace SB with LIPA, the specific alterations observed clearly indicates this objective was achieved. Specifically, average SB reductions of between 24 and 42 min/day were observed, in line with previous studies (284-287). LIPA increased by 2±21 mins (Range: -35 to 50 mins, 89 mins) in the SBF group, and 16±23 mins (Range: -16 to 60 mins, 76 mins), in the LIPA group. Interestingly, average LIPA time increase in the LIPA group (~16 mins), was considerably lower than the average increase LIPA participants self-reported (~49 mins). This suggests ~33 minutes was already being implemented at baseline, with only 16 minutes/day added during the intervention. This also supports overestimation of activity time in older adults (40, 281). Specifically, proportional SB time significantly reduced by ~2% (19 to -22%), and ~7% (11 to -22%), for SBF and LIPA, respectively. A similar study also reduced proportional SB time by ~3%, with a smaller range of inter-individual responses (13.6% to +8.0%) (287). SB displacement was prescribed in a quantitative fashion with specific targets. In contrast, previous studies prescribed a mere reduction in SB, and physical activity of varied intensity (LIPA, MVPA, Step count). This nonspecific approach holds merit, as with no target, successful behavioural alteration is more likely, and potentially more achievable (hence the smaller range of inter-individual responses). However, such an approach fails to consider both the behaviour that displaces SB, and at what level SB displacement begins to lose its perceived achievability/palatability. Accordingly, 10 participants reduced LIPA (Please see figure 13), with 50% of such participants also exhibiting increased SB. Interestingly, 40% stated they were either “undecided” or adamant the intervention had “probably not” motivated them to make long-term changes to their health. This suggests non-responders were not fully invested in the health promoting potential of the intervention which substantially limited behavioural alteration. Future studies should investigate further to determine which specific characteristics influence non-responders. Whilst the more specific method employed in the current study, has the limitation of imposing more rigid and potentially higher demands on participants (hence the larger range of inter-individual responses), the quantitative prescription of LIPA enables far more specific conclusions regarding the efficacy of specific SB displacement.

SB and LIPA intra-week variability was relatively stable in the study cohort (n=36). Specifically, SB co-efficient of variation remained between 10-20%, and 20-30% for LIPA. Furthermore, SB individual variance ranged from 0.9-1.5h, and within 0.2h for LIPA, irrespective of group or time point. Intra-week variability has only been investigated in elite athletes previously as means of monitoring training load (288-290). However the novel current results demonstrate both SB and LIPA exhibit stable day-to-day consistency in older women Considering, the promising displacement of SB with LIPA, stable co-efficient of variation and individual variance suggest such changes are consistently adopted in a similar intra-week pattern as baseline behaviours. Therefore, consistency in day-to-day SB at baseline, appears to spontaneously transition into consistency in day-to-day LIPA post intervention, in older women. Accordingly, individual variance, for proportion of physical activity time spent in both SB, and LIPA exhibited significant group dependant effects. with both experimental groups becoming more sporadic, and control becoming more homogenised. This suggests the day-to-day consistency in physical activity during SB and LIPA became more sporadic following the interventions. This is another promising finding, considering participants were attempting to manipulate a behaviour firmly imbedded into their routine (282). The probable everchanging context of daily life habits during SB manipulation, likely meant a greater daily variation in physical activity context/amount. SBF appeared to exhibit the greatest effect on PASB% and PALIPA%, possibly due to a greater variation in both the environment/ context in which SB was fragmented. This is reasonable considering SB is accumulated in many different contexts in older adults daily routine (319, 320). However, given that post-hoc testing revealed no significant differences between experimental groups, this suggests reduced behavioural consistency occurred irrespective of the prescribed LIPA pattern.

Co-efficient of variation for standing time and proportion of daily time spent standing, exhibited trends toward increased day-to-day consistency. Given that such an effect occurred irrespective of group, this points to the fact that all participants were aware they were being objectively monitored. Research participants tend to unconsciously alter behaviour when under observation (The Hawthorne Effect) (321, 322). Whilst this potentially facilitated compliance to the experimental interventions, unintentional side effects represent another novel finding. Given that older adults drastically under and over report SB and MVPA respectively, using self-report, this suggests a strong social

desirability bias (40). Due to the robustness of objective accelerometery, older adults do not have the option to misreport (whether deliberately or unintentionally), an increase in standing time consistency, meaning to fulfil the social desirability bias, the only available means is to perform the behaviour in question. Given that 4-7 days is considered sufficient to classify an individual's habitual physical behaviour (192) it may be rational to assume social desirability bias may not persist beyond a few days. However, no investigation has (to the author's knowledge) objectively assessed physical behaviour for 8-weeks. Therefore, prolonged accelerometer monitoring combined with the frequent visits from the principal investigator likely enhanced the effect social desirability bias had on standing time intra-week variability. Ultimately, this novel data shows that despite not changing the absolute amount of such behaviours the day-to-day consistency of such behaviours can alter with intervention. As such, future studies should investigate further to determine what effect this has on health outcomes.

Overwhelmingly positive responses regarding palatability were observed following SB displacement, with participants rating their respective intervention as acceptable, easy to implement, and not reporting any difficulties with tolerance or difficulty. Importantly, ≥79% of experimental participants stated they could either probably or definitely see themselves continuing their intervention long term, supporting previous findings that suggest older adults perceive SB displacement as acceptable and easy to incorporate (161). In contrast, older adults report a poor tolerance for intense physical activity (165) which generally results in poor long-term compliance (36, 166). SB is also negatively associated with self-rated health in older adults (323). Furthermore older adults perceive SB displacement as having a positive impact on their health (161, 324). The current results support such findings, given that 82% of experimental participants stated they either definitely or fairly felt more positive about their health. Given that self-rated health is associated with objective health status (325), such a finding is of benefit to both the mental and physical well-being of older adults. Given that most questions were answered in a similar fashion, this suggests both experimental groups perceived their intervention at least as tolerable as control. Furthermore, given that no major differences were observed between experimental groups regarding objective adherence, or self-reported palatability, this somewhat dispels the SBF advantage hypothesis. Nevertheless, future studies should carefully manipulate the frequency of

fragmentation, and monitor similar outcomes. Given that SB was displaced with LIPA in the current chapter this is a major strength, especially considering compliance was objectively determined. This promisingly indicates that specifically displacing SB time with LIPA is an achievable target for older women, which given the positive health effects of LIPA (269, 270), has broader implications for physical activity prescription in older adults.

A significant decrease in average SB bout length was also observed. In support, a previous study observed an increase of 4 additional SB breaks per day (287),following a generic prescription to decrease SB. In contrast, the current investigation prescribed SB displacement in two distinct patterns. However, both groups decreased average SB bout length by a similar magnitude of -10±25%, and -8±33%, for SBF and LIPA, respectively. This suggests that the intended SB fragmentation in the SBF group was largely successful. Given that 2 minutes of LIPA was prescribed for every 30 minutes seated, the clear reduction in average SB bout length from above 30 minutes at baseline (31±8) to below at week 8 (27±9), shows a very positive uptake of this message. Furthermore, a trend for reduced total self-reported weekly Activpal prompts was observed, as-well as a significant reduction for self-reported complied prompts (~3 per week). Out of context this may appear as reduced adherence to SBF, but combined with a reduction in total prompts, this alternatively suggests habitual behaviour change. Given reduced average SB bout length, this implies SBF participants were remaining sedentary for shorter periods, and thus not triggering the Activpal prompt as often. Anecdotally, several SBF participants reported they were attempting to “beat” the prompt through self-regulating SB (watching the clock, standing during TV adverts, etc). Combined with the reduction in Activpal prompts this self-regulation suggests behaviour change, considering participants were less reliant on accelerometer prompts, and more reliant on environmental cues. Such feedback aligns with the data retrieved from the GENEA monitors and self-report diaries. This is very promising, as decreased reliance on the fragmentation technology, aptly demonstrates independent behaviour change.

Despite prescribing LIPA in a continuous fashion (45-50 mins), the LIPA group similarly decreased average SB bout length from ~32 to ~29 minutes. Given that no specific instruction was provided on SB for this group, this suggests reduced average

SB bout length occurred spontaneously, and was similar in magnitude to SBF. Importantly, a significantly higher proportion of LIPA compared to SBF and control participants (LIPA:93%, SBF: 43%, CON: 50%), reported they “Definitely” felt more aware of the amount of light activity they engaged in each day following the intervention. This heightened awareness potentially resulted in additional spontaneous LIPA bouts beyond the prescribed continuous bout, which in turn reduced SB bout length. Promisingly, ≥79% of intervention participants stated they were definitely more aware of their daily sitting behaviours, suggesting a heightened awareness of SB. Given that older adults consistently underestimate SB time with self-report (40), such a finding is promising in the context of sustaining reduced SB long-term. Ultimately, greater SB awareness, combined with reduced SB bout length, is overall a very positive finding, considering SB accumulation pattern is an essential determinant of health outcomes (258, 273).

Given that all physical behaviours are carried out within a finite 24-h period, if one behaviour changes (SB, LIPA, etc) this spontaneously impacts another (sleep, MVPA, etc) (7, 326). Promisingly, no significant MVPA changes were observed in response to either intervention. Furthermore, MVPA time intra-week variability did not significantly deviate outside of 19-26%, and 0.2-0.5h, when expressed as co-efficient of variation and individual variance, respectively. Such findings promisingly indicate both the day-to-day and week-to-week variability of MVPA time was relatively stable. Concerns surrounding potential compensations in other physical behaviour domains following SB manipulation have previously been raised (71), highlighting the major limitation that a mere ~2% of waking hours is required to achieve recommended MVPA time (197), leaving 98% unaccounted (7). One such study showed older adults reduce LIPA by ~35 minutes per day following increased MVPA (296). Such compensatory behaviour, may restrict an individual’s progress towards overall health achievement (297). However, the current investigation suggests increased LIPA does not cause adverse MVPA reductions.

Sleep exhibited a trend to increase within experimental participants, with SBF and LIPA increasing sleep time by an average of ~0.4h, and ~0.2, respectively. Sleep is an essential daily health behaviour (327), and is associated with improved health outcomes (328, 329). Accordingly, ≥70% of older adults report experiencing insomnia

related symptoms (330). In-fact improvements in glucose regulation following SB displacement were previously noted only in those who were well-rested (331). Previous studies have noted improved sleep following reduced SB time (332), although both studies were conducted in younger adults, and merely convey promising acute findings. Nevertheless, a recent study in older adults found a similar association between reduced SB/ increased LIPA time with improved long-term sleep quality (333). However, intra-week variability expressed as both co-efficient of variation and individual variance, did not significantly change from pre to post intervention, and did not deviate beyond of 9%, and 0.5h respectively. This implies the observed experimental change of 0.2-0.4h is of marginal magnitude. Furthermore, the proportion of weeks that were significantly associated with baseline for sleep intra-week variability was ≤38%, suggesting the night-to-night variability in sleep time was generally sporadic across the intervention weeks. Future studies should attempt to replicate this finding and assess additional measures of sleep competency beyond mere duration (sleep architecture, circadian timing etc). Ultimately, both interventions did not result in significant changes in any physical behaviour parameters from pre to post intervention, indicating SB displacement with LIPA is an isolated intervention that does not appear to compromise other health promoting physical behaviours.

Most notably a group dependant change in glucose intake was observed, mediated by the exclusive difference between glucose intake reduction following SBF, and the increase in control. This implies an advantage of SBF. Given that no significant change in fruit/vegetable intake was observed, this suggests glucose reduction following SBF was from other dietary sources. This is supported by spontaneous reduced intake of sweets, soft drinks, breads, and pasta dishes following 15 weeks of moderate intensity exercise training in younger adults (334). The current results suggest such an improvement occurs following LIPA, and independent of nutritional counselling. Given that higher intake of free sugars is associated with increased incidence of type II diabetes (335, 336), reduced glucose intake is very encouraging. Combined with the aforementioned reduction in SB time/ bout length following SBF (see above), which are linked with acute glucose management improvements(317), such findings have promising implications for long-term glucose management.

A significant reduction in absolute protein intake was also observed across all groups. Older-adults typically present with protein-energy malnutrition (299, 301), which compromises bone mineral density (203), skeletal muscle quality (202, 337), and physical function (338). Daily protein intake in older adults is recommended in the range of 0.8-1.0g.kg.day (339, 340), but encouraged at greater intakes (1.2-1.6g.kg.day) for full benefits (337, 341-343), considering it is arguably the most important anabolic nutrient. Importantly, despite a reduction in absolute protein intake, potentially limiting the anabolic potential of the diet, all groups remained ≥0.98g.kg.day post intervention, and were thus still comfortably within the healthy range.

No significant change in energy intake was observed. Accordingly, 'the gravitostat' specifically mediates reduced energy intake following high loading through the lower limbs in rodents (314, 315). Furthermore, loading of the gravitostat (with heavy weighted vests for 3 weeks) has previously achieved a reduction in bodyweight (fat mass) been performed through utilising weighted vests for three weeks in younger adults (344). Moreover, SB displacement with LIPA reduces subsequent energy intake in younger adults (317). In contrast, the current investigation loaded the gravitostat only with bodyweight, whenever SB was replaced with standing/ light activity over eight weeks. In rodents, the energy intake reducing effect of the 'gravitostat' appears to be dependent on an osteocyte strain detection mechanism, that is activated in response to high loading through the lower limbs (314, 315). However, the current lack of observed change in energy intake persisted even after adjustment for baseline BMI. Given that all groups were on average classified as non-obese at baseline (<30kg/m^2), SB displacement with LIPA in older adults may simply have not produced high enough loading forces through the lower body bone structures, sufficient enough to activate the gravitostat.

The in-depth composite Z-score analyses showed that both SBF and LIPA increased overall intake of nutrients promoting anabolism, in contrast to control. This is a very promising finding considering intake of all five selected nutrients has previously been individually [protein (204), vitamin D (205, 206), vitamin E (207), as-well as omega-3 and omega-6 fatty acids (208, 209)], and collectively (202) positively associated with the observed quality of skeletal muscle in older adults, including higher muscle volume and greater specific force. Given that both experimental groups similarly increased,

this suggests such an enhancement occurs irrespective of the pattern of prescribed LIPA. Together with the effect LIPA has on stimulating skeletal muscle in older adults (91), secondary enhancements to dietary pro-anabolic potential may aid with perturbing the loss of skeletal muscle mass/function during ageing (sarcopenia) (202). Further Z-score analysis showed that only SBF increased overall intake of bone health enhancing nutrients, in contrast to LIPA who decreased intake of such nutrients. Similar to reduced glucose intake, this suggests an advantage of frequent SB displacement with LIPA. This is promising considering intake of all eight selected nutrients has previously been individually [Calcium (210), Zinc (203), Magnesium (211), Phosphorus (212), Vitamin C (203), Vitamin D (213), protein (214), omega 3 fatty acids (215)], and collectively (203) associated with bone health in older adults. Furthermore, a more fragmented SB pattern is specifically associated with enhanced BMD in older adults, due to the frequent exposure of bone structures to mechanical loading (112).

Within the sub-analysis of novel ambulators (n=8), several dietary trends conducive to optimal health emerged. Zinc intake significantly increased by ~29% which is promising considering Zinc deficiency is common amongst older adults (305), and can not only exacerbate the loss of bone mineral density (345)/ muscle mass (346), but also increase cardiovascular disease risk (347). Furthermore, new ambulators exhibited a trend toward increased manganese intake. Accordingly, increased serum Manganese levels have previously been associated with bone health in older adults (348, 349). Further trends were also noted for increased absolute (~12%) and relative (~13%) protein intake for new ambulators. Accordingly, Z-score analysis of the overall diet showed novel ambulators increased both intake of nutrients promoting anabolism (2%), and nutrients promoting bone health (16%). Such changes suggest shifting category from sedentary to ambulator, or put more simply, reducing average daily SB time to ≤8h/day may aid with maintaining musculoskeletal health during ageing.

Strengths and Limitations

The major strength of the current study was the objectively determined daily compliance to each 8-week intervention utilising gold-standard tri-axial accelerometery. According to a recent review (283), this has been a major limitation of the evidence base up to this point. Data was subsequently analysed with a peer

reviewed algorithm, rigorously validated in the target population (older adults) (192). This further permitted the measurement of physical behaviour consistency, with intra-week variability, which (to the author's knowledge) is completely novel. The intra-week variability descriptive statistics thus provide normative values on intra-week variability in older adults that can be used as a reference for future studies pursuing this promising avenue of research. Specifically, a randomised controlled trial was used to detect differences in novel physical behaviour outcomes, whilst broadly controlling for the pattern of prescribed LIPA. Data was also collected on compensatory health behaviours (habitual diet, MVPA time, self-perceived palatability), which previous intervention studies have failed to consider. In contrast to previous studies, weighted food diaries and rigorous nutritional analysis software were utilised (Nutritics / MyFitnessPal), to identify changes in specific macro and micronutrients.

The major limitation of the current study was the observed baseline differences in major outcome variables (MVPA, SB, Protein intake etc) for the control group compared to experimental. Given that control participants were recruited towards the end of data collection, a significant difference regarding the time of year participants began their intervention was observed. Accordingly, physical behaviour tends to vary seasonally across the year, with LIPA increasing during summer months, and MVPA declining throughout the winter (350, 351). The results of the current chapter support the notion that MVPA is higher in summer. Such a summer physical activity surge may have accounted for the lack of observed physical behaviour differences between control and experimental groups. Accordingly, control participants were advised to maintain their habitual routine, and not implement additional PA. However, the spontaneous increase in physical activity associated with the summer season was habitual and may have reduced differences between physical behaviour outcomes between experimental and control participants. However, it may have alternatively been the case that irrespective of season, this small cohort (n=8) of control participants were simply less sedentary, and more active compared to experimental. Even though only 2/35 (6%, SBF: n=1, Control: n=1) participants negatively shifted from active to inactive, both participants begun their intervention in months conventionally associated with spring. Conversely, previous evidence suggests MVPA time declines through winter months and peaks in summer in both middle-aged (350), and older (351) adults. This may suggest the negative shift toward inactive classification was

independent of season. Nevertheless, future studies should be carried out to confirm or otherwise refute such a conclusion of seasonal dependence. A recent meta-analysis concluded adults (irrespective of age) exhibit seasonal variations in energy, macro, and micronutrient intake (352), with the current results further suggesting protein and carbohydrate intake exhibit similar seasonal variation in older adults. Whilst controlling for the baseline values of such variables as co-variates during analysis is a straightforward statistical solution, such baseline differences would ideally not be present where possible. Nevertheless, future studies should exercise caution when collecting control participants data during separate times of year/seasons distinct from experimental, as this does seem to generate substantial differences in PB/ habitual diet outcomes. Data collection should instead occur simultaneously in all groups, irrespective of experimental/control condition, thus attempting to control for the clear confounding effect of seasonality on PB/ habitual diet. Furthermore, an additional limitation of the study is noted that the control group (n=8) was half the size of both experimental groups (SBF: n=14, LIPA: n=14), which may have contributed to greater Z-score effects for nutrients promoting anabolism/ bone health within the control group. Whilst this led to a more in-depth and ultimately more informative Z-score sub-analysis conducted on new ambulators, consistency between group sample sizes would also ideally be present where possible. Despite using two separate validated methods of basal metabolic rate estimation (Schofield & Harris-Benedict) (216, 218), direct assessment of basal metabolic rate with calorimetry would have also been more informative.

Conclusion

In conclusion, SB displacement with LIPA was successfully implemented in a cohort of 28 older adults, with a promising reduction in SB bout length, and stable MVPA over time. Participants also reported overwhelmingly positive feedback regarding perceived acceptability, and good likelihood of long-term compliance. In addition, LIPA implementation spontaneously increased intake of nutrients promoting anabolism. Furthermore, novel ambulators significantly increased Zinc intake, as-well intake of other high-quality nutrients. Additionally, SBF reduced habitual glucose intake, and increased intake of bone health promoting nutrients exclusively. Consequently, displacing SB with LIPA (irrespective of prescribed pattern) into the daily routine of older women, is achievable, palatable, and results in minimal deleterious lifestyle

compensations. In-fact SB displacement enhances habitual dietary quality, and potentially sleep time. Furthermore, despite not exhibiting superior palatability, SBF appears advantageous for various dietary outcomes.

Chapter 4 – The effects of displacing sedentary behaviour with light intensity physical activity on physical function in older women

Data from the current chapter are published in/ presented at (please see research outputs in appendices ii):

Minimising sedentary behaviour (without increasing medium-to-vigorous exercise) associated functional improvement in older females is somewhat dependant on a measurable adaptation in muscle size. *AGING (2020).* Dale Grant*, David Tomlinson, Kostas Tsintzas, Petra Kolić, Gladys L. Onambele-Pearson. https://dx.doi.org/10.18632%2Faging.202265

The Effects of Displacing Sedentary Behavior With Two Distinct Patterns of Light Activity on Health Outcomes in Older Adults (Implications for COVID-19 Quarantine). *Frontiers in Physiology* (2020). Dale Grant*, David Tomlinson, Kostas Tsintzas, Petra Kolić, Gladys L. Onambele-Pearson. https://doi.org/10.3389/fphys.2020.574595

Chapter take home message: Significant time effects were observed for peak gait speed, average gait speed, 30 second sit-to-stand count, 1 sit-to-stand time, and average hand grip strength (HGS). Unipedal stance time for left leg eyes closed exhibited a significant group×time interaction, with both experimental groups improving in contrast to control. Interestingly, peak handgrip strength exhibited a group×time interaction, with post-hoc testing revealing a trend towards a significant difference between the change in SBF and LIPA.

Abstract

The pattern of prescribed light intensity physical activity [fragmented vs continuous, (LIPA)] during Sedentary Behaviour (SB) has not previously been controlled for, following SB displacement in older adults. It is still undetermined how prescription pattern affects changes in physical function following SB displacement. Therefore, the aim of this chapter was to examine changes in physical function [gait speed, sit to stand ability, handgrip strength (HGS), & balance posturography] following 8-weeks of SB displacement with LIPA in older women. It was hypothesised SBF would induce greater functional improvement. Thirty-six older women were allocated to one of three groups: 1) sedentary behaviour fragmentation (SBF) (*n*=14), 2) continuous LIPA (*n*=14), or 3) control (*n*=8). Physical function was assessed at weeks 0 and 8. Significant time effects were observed for peak gait speed ($p<0.001$), average gait speed ($p=0.002$), 30 second sit-to-stand count ($p=0.003$), 1 sit-to-stand time ($p=0.011$), and average HGS ($p=0.04$), with both experimental groups exhibiting enhanced physical performance of similar magnitudes (3-11%). Despite no main effects observed for posturography, unipedal stance time for left leg eyes closed exhibited a significant group×time interaction effect ($p=0.02$), with both experimental groups increasing (SBF: 1±2s, LIPA: 1±4s) in contrast to control (-1±3s). Interestingly, peak HGS exhibited a group×time interaction ($p=0.001$), with post-hoc testing revealing a trend ($p=0.08$) towards a significant difference between the change in SBF (8%) and LIPA (2%). In conclusion, displacing SB with LIPA in older women induced clinically relevant improvements in gait speed, sit-to-stand ability, HGS, and unipedal stance duration. Accordingly, frequently displacing SB with LIPA appeared to induce greater peak HGS adaptation. The observed improvements are compellingly positive changes associated with an exercise intensity not customarily regarded as optimal. Furthermore, frequent SB displacement with LIPA appears more beneficial for certain physical function outcomes.

Introduction

Sedentary behaviour (SB) is strongly associated with compromised physical function (the ability to independently carry out tasks of daily living) in older adults (121-123, 131), increasing the subsequent risk of morbidity, mortality, and diminished quality of life (353, 354). Self-reported SB time is positively associated with self-reported functional impairment (58). Objectively assessed SB time is also associated with lower self-reported health (58, 59), and frailty (114, 115) in older adults. Specifically, frailty risk exponentially increases at >8.3 and >8.9 hours/day of self-reported SB in men and women respectively (116). Interestingly, the association between SB and compromised physical function in older adults (117), is exacerbated in frail individuals (116), persists following MVPA adjustment (118, 119), and appears worsened following prolonged SB engagement (longer sitting bouts) (21, 57, 120). Furthermore, women exhibit greater reductions in strength following disuse compared to men (152-154). Considering both sit-to-stand ability (125) and gait speed (126) are both independent predictors of mortality in older adults, consistent associations between SB and reduced sit-to-stand ability (122)/ gait sped (122) are concerning. Furthermore, SB mediates the association between obesity and falls risk in older adults (123), and is further associated with reduced balance/ increased risk of falls over a 1-2 year follow up (121, 122). Moreover, retrospective history of a fall, and prospective fear of experiencing a fall, are associated with an additional 22 and 45 mins of SB per day respectively (122, 124). Following an injurious fall older adults increase SB time when in hospital by around 15-25% (29, 355), highlighting an adverse event sequence. Ultimately, despite the evidence base being mostly epidemiological, clear associations exist between SB and geriatric health outcomes (48).

However, SB is not universally associated with compromised function in adults aged 36-80y (129), and only marginally associated (following MVPA adjustment) in adults aged 45-75y (130). Furthermore, SB is not independently associated with postural stability or lower extremity strength in women aged 50-65y old (128). Nevertheless, SB is associated with lower physical function in early old age (60-64y) (131). Such results suggest MVPA is potentially more beneficial to function in middle-aged adults. However older adults exhibit poor lifelong tolerance to MVPA (36, 165, 166, 356), which can be problematic as only excessive MVPA appears to offset the negative health effects of concurrent high SB time (268, 274). Therefore, older frailer

populations may benefit more from the SB reduction message. Light intensity physical activity (LIPA)] during SB displacement, is a pre-requisite for long-term health benefits (23, 67, 270), due to LIPA generating superior responses in both muscle activity (MA) (90, 91), and energy expenditure (91, 94, 313), compared to stationary standing. Whilst it may be rational to assume lower intensity activity may not produce a sufficient adaptation stimulus, low intensity training enhances both muscle strength (171, 357) and physical function (358) in older adults. Equally LIPA implementation improves physical function (359) in older adults generally, but especially in frail individuals (158, 357, 359, 360). Thus the potential for LIPA to generate comparable physiological responses relative to more conventional high intensity loading is a somewhat recent theorem, supported by previous observations whereby older adults engaging in low frequency stair climbing exhibit significantly reduced mortality (361). Therefore, due to the relative surge in intensity LIPA seems to generate in older adults closer to low physiological reserve, such activity may reach an appropriate loading threshold required for functional adaptation.

Accordingly, an 8-week SB reduction in older overweight adults significantly improved gait speed (156). Furthermore, a 12-week intervention assigned 38 older adults to either an MVPA, or an SB reduction group (157). Interestingly, only the SB reduction intervention significantly improved sit-to-stand ability, and only caused trends toward enhanced balance and increased gait speed. The authors speculated SB reduction caused a specificity of training effect, improving one's sit-to-stand ability. Interestingly, an increase in MVPA did not improve physical function (157). One sit-to-stand transition (followed by 10 minutes of sitting) results in a relatively low energy cost (1.49kcal/min) compared to structured lower body exercise [bodyweight squatting for 10 repetitions per minute (6kcal/min) (162), 5 minutes of parallel squatting, 40% of one repetition maximum (8-11kcal/min) (163)], but a comparable level of muscle activity (95). Comparable muscle activity may potentially be a reason for improvements in physical function following SB reduction (e.g. increased chair stand ability). Therefore, it can be anticipated that SB displacement with LIPA would be a safer, less effortful, and more sustainable alternative means of improving physical function, compared to structured MVPA in older adults. Moreover, a 10 week intervention in frail older adults, frequently prompted participants to fragment SB with a novel accelerometer prompt, which similarly resulted in enhanced sit-to-stand ability and increased gait speed (39).

However, despite a clearly established link with SB (52, 127), many functional markers like handgrip strength (HGS), have yet to be investigated with intervention studies. Furthermore, it is still unclear whether such promising results, are due to the specific displacement of SB or due to spontaneous increases in LIPA. Accordingly, general increases in light walking time over 6 (159), 10 (39), and 12 (160) weeks increases gait speed in older adults. Furthermore, general SB reduction does not appear to generate enhanced physical function in older adults (156). Instead SB fragmentation [regular sit-to-stand transitions, and frequent bouts of LIPA (SBF)] consistently stimulates functional improvement (39, 157). Increased LIPA time implementation may have still mediated such effects, albeit accumulated in micro-bouts throughout the day. Nevertheless, previous interventions have failed to control for the pattern of SB displacement with LIPA. Consequently, a longitudinal intervention trial is warranted to investigate what role the pattern (fragmentation vs. a single bout) of prescribed LIPA plays during SB displacement.

Therefore, the aim of this chapter was to quantify functional adaptation to two different LIPA interventions in older women. It was hypothesised displacing SB with LIPA would improve grip strength, balance posturography, and enhance the ability to mobilise from a seated position. It was also hypothesised SBF would induce greater functional improvement, compared to continuous LIPA.

Table 4.1- Baseline functional performance characteristics for each group.

	SBF (n=14)	LIPA (n=14)	Control (n=8)
Number of participants whose self-perceived dominant Leg was Left/ Right (%)	5/ 9 (36%/ 64%)	3 / 11 (21%/ 79%)	2/ 6 (33%/ 67%)
Number of participants classified as poor upper Body strength (<16kg handgrip strength)/ Normal (84) (%)	1/ 13 (7%/ 93%)	0/ 14 (0%/ 100%)	0/ 8 (0%/ 100%)
Number of participants classified as poor lower body strength (5 chair rises in >15s)/ Normal (84) (%)	0/ 14 (0%/ 100%)	0/ 14 (0%/ 100%)	0/ 8 (0%/ 100%)
Number of participants classified as poor functional performance (Gait speed <0.8 m/s)/ Normal (84) (%)	2/ 12 (17%/ 83%)	0/ 14 (0%/ 100%)	0/ 8 (0%/ 100%)

LIPA, light intensity physical activity, m/s; metres per second, SBF; sedentary behaviour fragmentation.

Table 4.2- Pre, Post, average change, and relative changes from baseline for functional performance measures expressed by group. **Boldened text** represents a significant baseline difference. * represents a significant time effect. ×represents a significant group×time interaction effect.

	SBF (n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
30s sit to stands (n)	14±5	17±5	2±4 (13±30%) *	16±4	18±6	1±5 (5±35%) *	16±3	16±1	0±2 (0±14%) *
1 sit to stand time (s)	2.5±1.0	2.2±0.7	-0.3±1.0 (-9±36%) *	2.0±0.5	1.9±0.5	-0.2±0.5 (-11±24%) *	2.0±0.6	1.9±0.5	0.0±0.7 (-1±29%) *
Average Gait Speed (m/s)	1.1±0.3	1.2±0.4	0.1±0.1 (5±6%) *	1.1±0.1	1.2±0.2	0.0±0.1 (3±8%) *	1.3±0.1	1.3±0.2	0.0±0.2 (1±18%) *
Peak Gait speed (m/s)	1.2±0.3	1.3±0.3	0.1±0.1 (6±11%) *	1.2±0.2	1.3±0.1	0.1±0.1 (4±10%) *	**1.4±0.1**	1.4±0.2	0.0±0.3 (-1±20%) *
Average Grip Strength (kg)	22.8±6.6	23.9±5.4	1.3±3.4 (1±15%) *×	22.9±5.7	23.8±7.2	1.1±2.0 (1±9%) *×	24.2±4.6	23.0±4.6	-1.2±2.1 (-5±9%) *×
Peak Grip Strength (kg)	26.2±8.5	26.8±6.1	1.8±2.6 (8±14%) ×	26.5±4.4	26.5±7.3	0.5±2.4 (2±10%) ×	27.0±5.0	24.3±4.6	-2.5±3.1 (-9±11%) ×
Left eyes open (s)	22±25	24±25	0±3 (0±31%)	30±5	27±10	0±4 (-1±17%)	29±6	30±9	0±4 (0±17%)
Left eyes closed (s)	2±3	3±2	1±2 (26±114%) ×	3±4	3±3	1±4 (79±164%) ×	3±5	2±3	-1±3 (-48±26%) ×
Right eyes open (s)	22±26	30±26	0±3 (0±31%)	29±10	29±5	0±9 (0±44%)	30±6	30±9	0±3 (0±13%)
Right eyes closed (s)	3±3	3±3	1±4 (67±143%)	3±3	3±1	0±2 (6±61%)	3±2	3±2	-1±3 (-18±111%)

LIPA, light intensity physical activity, m/s; metres per second, SBF; sedentary behaviour fragmentation.

Table 4.3- Pre, Post, average change, and relative changes from baseline for balance posturography outcomes measures expressed by group.

	SBF (n=12)			LIPA (n=9)			Control (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
Eyes Open									
Time (s)	27±18	27±20	0±0 (0±2%)	27±10	27±0	0±0 (0±0%)	27±0	27±0	0±1 (0±3%)
Total Displacement (mm)	34±18	26±16	-5±10 (-14±26%)	20±5	19±12	2±14 (13±74%)	20±12	20±4	0±4 (0±18%)
Sway Frequency (mm/s)	1.3±4.6	1.1±2.8	-0.2±0.33 (-12±26%)	0.8±0.7	0.7±0.5	-0.04±0.62 (-5±62%)	0.7±0.4	0.8±0.3	0.04±0.12 (5±17%)
Eyes Closed									
Time (s)	3±4	4±3	0±3 (0±77%)	4±5	4±2	0±3 (4±100%)	4±3	4±4	-1±6 (-16±121%)
Total Displacement (mm)	28±17	30±24	3±19 (14±89%)	28±12	27±14	-7±16 (-27±52%)	37±23	27±14	-12±19 (-33±53%)
Sway Frequency (mm/s)	8.4±7.2	8.0±10.4	-0.2±12.6 (-3±158%)	5.6±13.9	6.8±9.5	-0.2±7.2 (-2±70%)	10.5±8.3	7.0±9.5	2.1±11.1 (0±138%)

LIPA, light intensity physical activity, mm; millimetres; mm/s; millimetres per second, SBF; sedentary behaviour fragmentation.

Results

Baseline Differences

Groups were matched at baseline for physical function categories (please see table 4.1). The only variable that exhibited significant differences between groups at baseline was peak gait speed (p=0.03). A significant post-hoc difference (p=0.02) was exhibited between the control group (1.38±0.12m/s), and SBF (1.17±0.22m/s) but not LIPA (1.22±0.13m/s).

Gait Speed

After controlling for baseline differences, a significant effect for time was exhibited for peak gait speed (p<0.001, r = 0.31), but not a group×time interaction (p=0.93). Accordingly, both experimental groups increased peak gait speed post-intervention [SBF: 0.09±0.16m/s (10±20%), LIPA: 0.06±0.08m/s (5±7%), CON: -0.02±0.15m/s (1±11%)] (please see table 4.2). Similarly, average gait speed (of three trials) exhibited a significant main effect for time (p=0.002, r = 0.37). Interestingly, both experimental groups significantly increased to a similar extent [SBF:0.06±0.07m/s, 5±6%, LIPA: 0.03±0.09m/s, 3±8%], in contrast to control who decreased [0.02± 0.23m/s, 1± 18%] (please see figure 4.1).

Sit-to-Stand Ability

The number of sit-to-stand transitions performed in 30 seconds also exhibited a significant main effect of time (p=0.003, r = 0.35), but no group×time interaction (p=0.18). Nevertheless, SBF exhibited an increase in 30 second sit-to-stands (2±4, 10±30Ti%) (please see table 4.2 and figure 4.2). me taken to perform one sit to stand exhibited a significant main effect of time (p=0.011, r = 0.31), but no group×time interaction effect (p=0.62), with all groups decreasing post intervention [SBF: -0.3±1.0s (-9±36%), LIPA: -0.2±0.5s (-11±24%), CON: -0.04±0.7s (-1±29%)] (please see table 4.2 and figure 4.2).

Handgrip strength

Average HGS (of left and right arm) exhibited both a significant main effect for time (p=0.04, r = 0.24), and a significant group×time interaction (p=0.002). Post-hoc testing revealed significant differences between the changes in both SBF (1.3±3.4kg, 7±15%,

p=0.001, *r* = 0.65)/LIPA (1.1±2.0kg, 6±9%, p=0.008, *r* = 0.63) in relation to the change in the control group (-1.2±2.1kg, -5±9%,). No significant post-hoc difference was noted between the change in both experimental groups (p=0.37) %)] (please see table 4.2 and figure 4.3). Despite peak HGS not exhibiting a significant main effect of time (p=0.69), a significant group×time interaction was observed (p=0.001). Post-hoc testing revealed significant differences between the changes in both SBF (1.8±2.6kg, 8±14%, p<0.001, *r* = 0.65)/LIPA (0.5±2.4kg, 2±10%, p=0.03, *r* = 0.55) in relation to the change in the control group (-2.5±3.1kg, -9±11%). Interestingly here, a post-hoc trend was noted between the difference in both experimental groups (SBF vs LIPA, p=0.08) %)] (please see table 4.2 and figure 4.3).

Single leg stance/ Posturography

Regarding single leg stance time (Average of three trials manually assessed with stopwatch) in the whole cohort (n=36), only left leg eyes closed exhibited a significant group×time interaction (p=0.02). Post-hoc testing revealed significant differences between the changes in both SBF (1±2s, p=0.03, *r* = 0.44)/LIPA (1±4s, p=0.007, *r* = 0.58) in relation to the change in the control group (-1±3s) %)] (please see table 4.3 and figure 4.4). No significant post-hoc difference was noted between the change in both experimental groups (p=0.50). Furthermore, a trend toward a group×time interaction for average (of left and right leg) eyes closed time (p=0.054) was also observed. Regarding the sub-sample posturography analysis, no significant main effects nor group×time interactions were observed for any variable %)] (please see table 4.3).

For results and figures on associations between relative changes from baseline for physical behaviour outcomes (SB & LIPA) and relative changes from baseline for functional outcomes, please see appendices i. Notable outcomes with which SB/LIPA were associated included one sit-to-stand time, average HGS, peak HGS, and single leg stance duration.

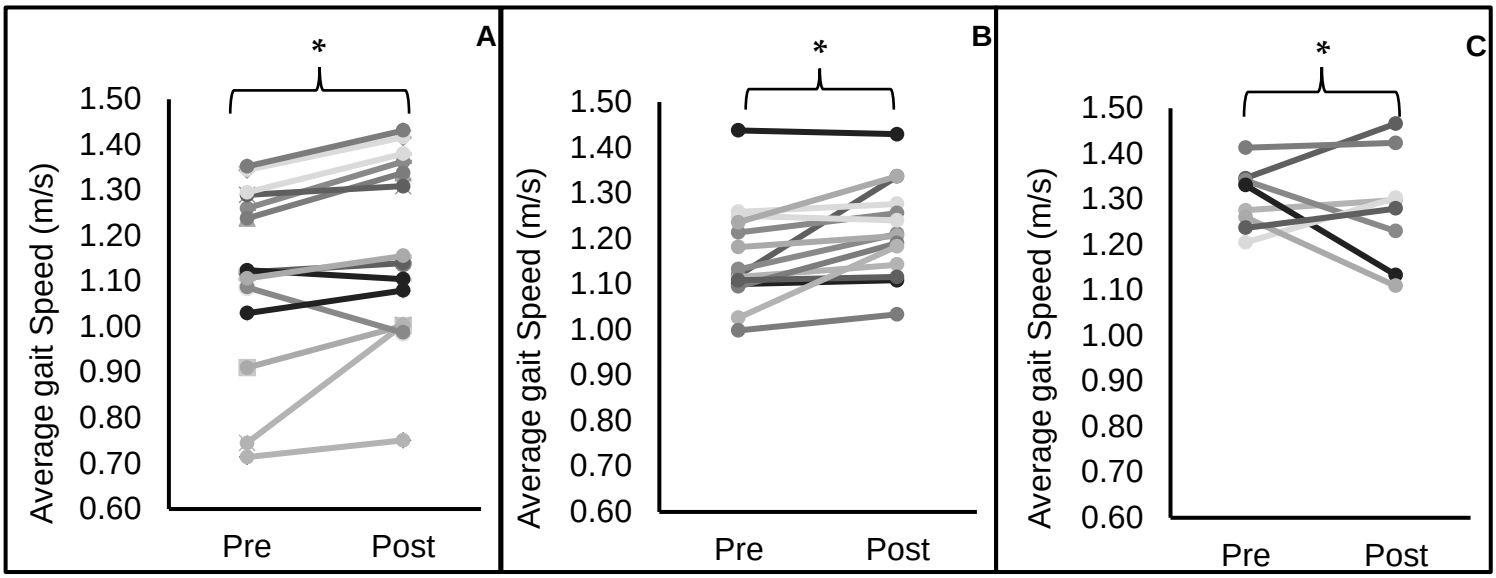

Figure 4.1- Individual changes in average gait speed. Panels A, B, & C represent SBF, LIPA, & Control, respectively. *represents a significant time effect. LIPA, light intensity physical activity, SBF; sedentary behaviour fragmentation.

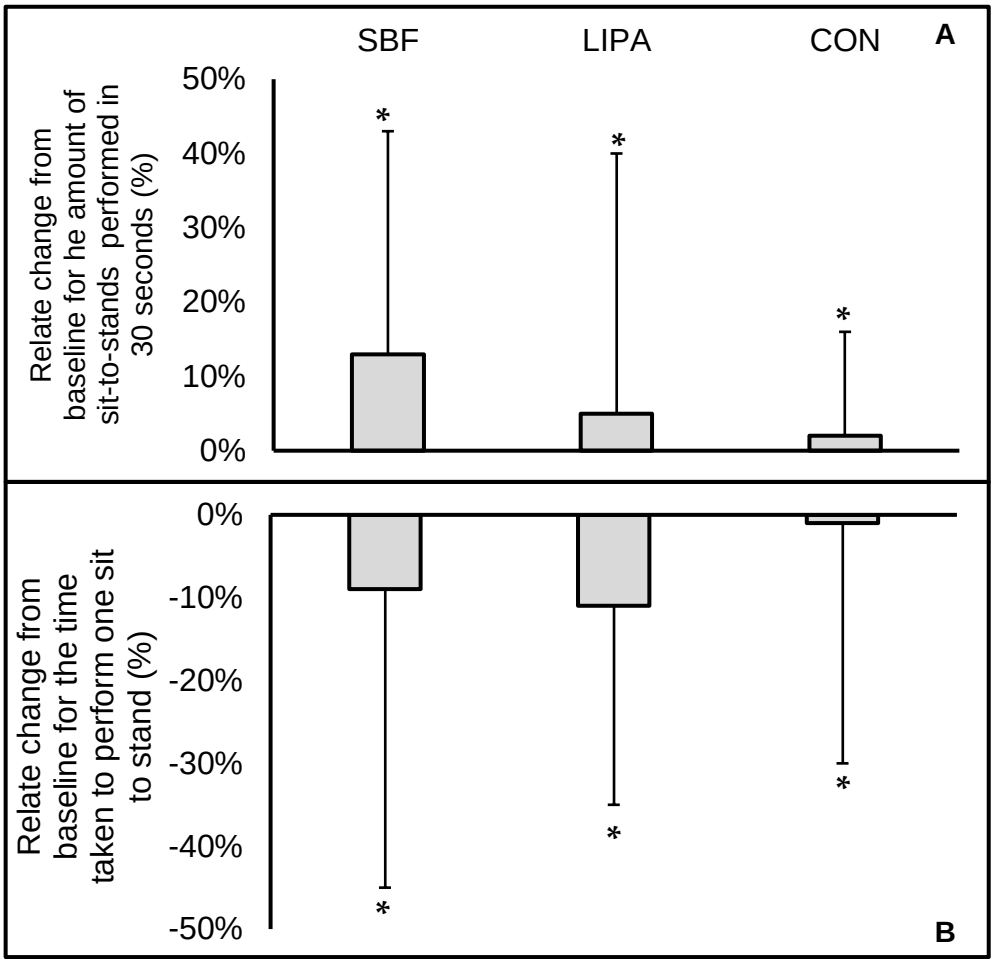

Figure 4.2- Relative changes from baseline for sit to stand ability. Panels A and B represent the change in 30 second sit-to-stand and one sit-to-stand ability, respectively. *represents a significant time effect. CON; control, LIPA, light intensity physical activity, SBF; sedentary behaviour fragmentation.

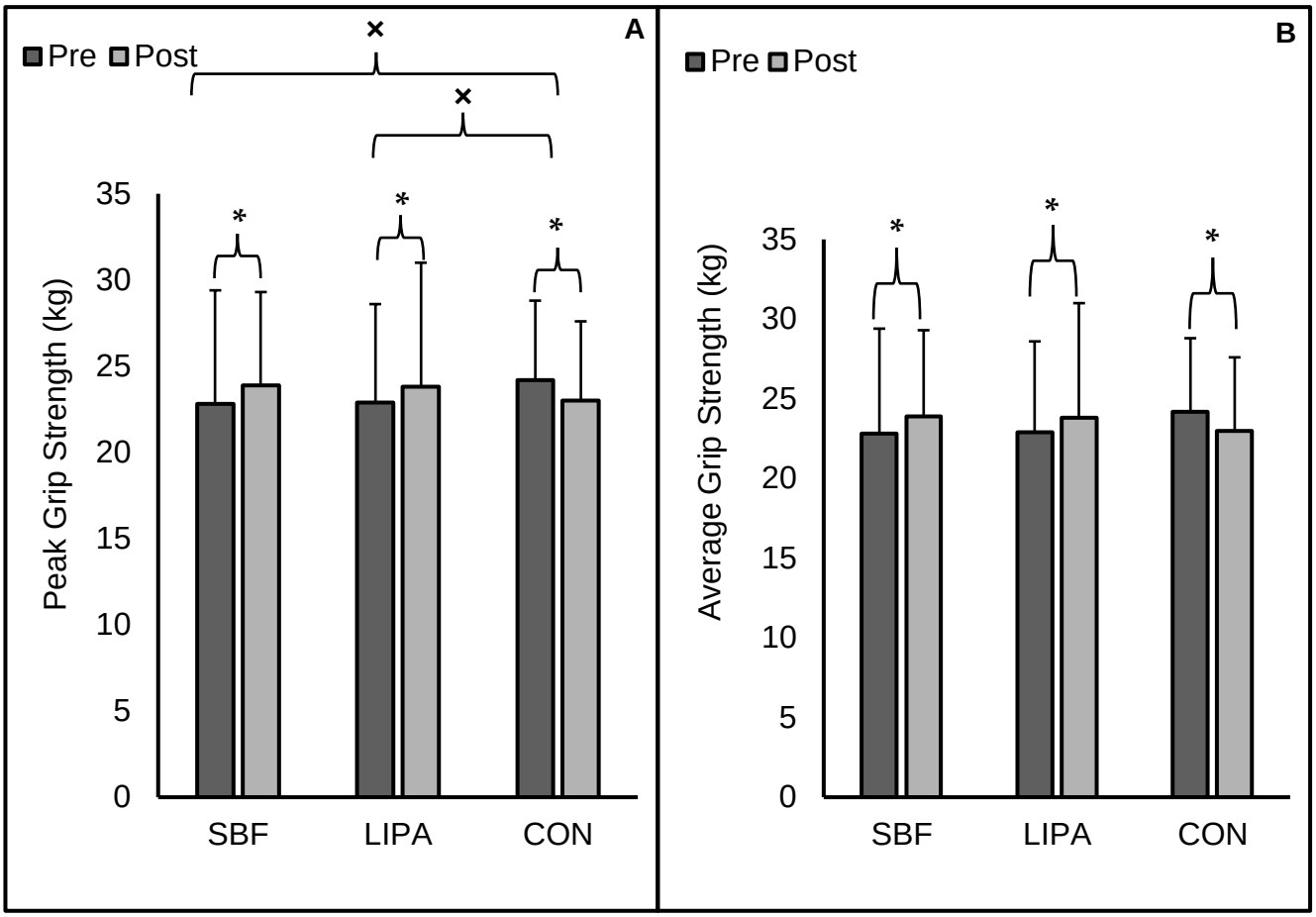

Figure 4.3- Changes for handgrip strength. Panels A and B represent the change in Peak and average grip strength, respectively. * represents a significant time effect. × represents a significant (SBF vs CON, p<0.001, LIPA vs CON, p=0.03). CON; control, LIPA, light intensity physical activity, SBF; sedentary behaviour fragmentation.

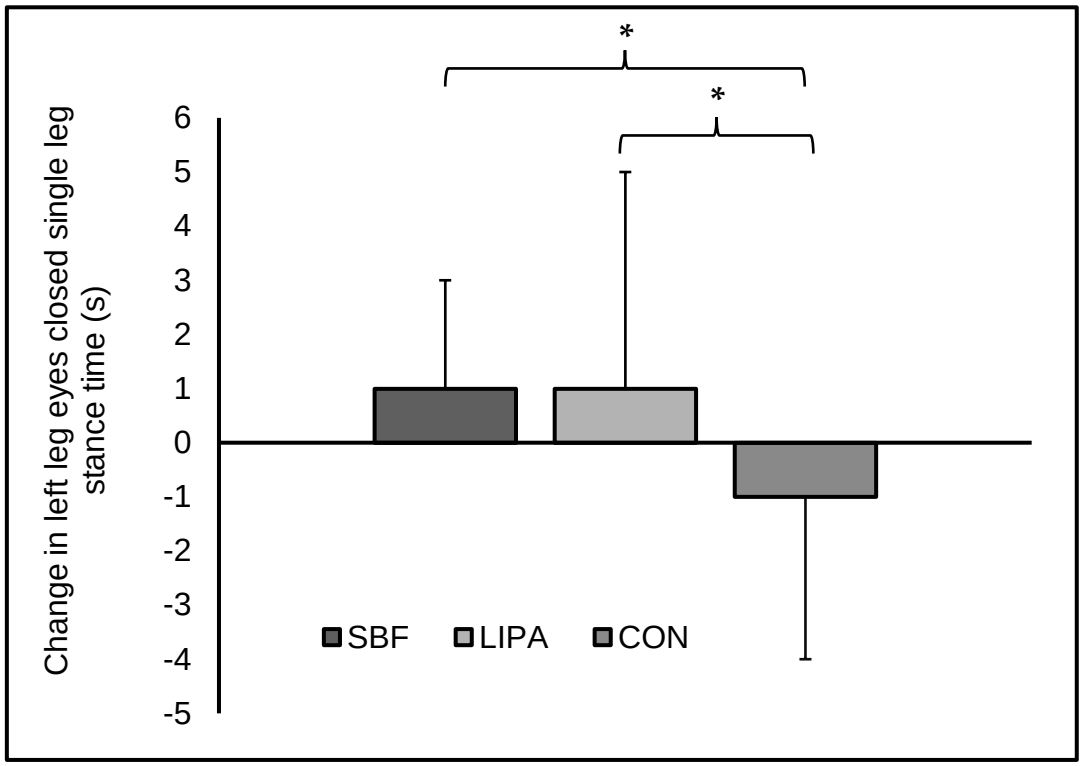

Figure 4.4- Absolute changes from baseline for left leg eyes closed unipedal stance duration (s). * represents a significant post hoc difference (SBF vs CON, p=0.03, LIPA vs CON p=0.007). CON; control, LIPA, light intensity physical activity, SBF; sedentary behaviour fragmentation.

Discussion

The aim of this chapter was to quantify functional adaptation to two different LIPA interventions in older women. It was hypothesised that displacing SB with LIPA would improve grip strength, balance posturography, and the ability to mobilise from a seated position. Both experimental groups exhibited similar improvements in gait speed, 30STS, one sit-to-stand time, average grip strength, and left leg eyes open single leg stance duration. Therefore, the first hypothesis was upheld. It was also hypothesised SBF would induce greater functional improvement, compared to continuous LIPA. Accordingly, SBF resulted in a trend toward greater peak grip strength improvements. Curiously, SB displacement with LIPA mediated increased one sit-to-stand time following SBF. Nevertheless, SB displacement with LIPA mediated enhanced eyes open single leg stance duration, and improved average/peak HGS following continuous LIPA implementation. Therefore, the second hypothesis was partially upheld dependant on outcome. SB is strongly associated compromised physical function (121-123, 131). Despite a proposed bi-directional relationship (116), it remains clear that SB is more detrimental to physical function in frailer and more elderly populations. The current chapter is therefore the first to examine functional adaptation to two distinct SB displacement interventions, which vary regarding the pattern of prescribed LIPA in older women.

Most notably, significant gait speed improvements were exhibited in both experimental groups, but not control. In support SB and LIPA time are associated with decreased (122, 129), and increased (132), gait speed during follow up in older adults respectively. Improved gait speed is also a consistent finding throughout SB displacement studies in older adults (156, 158, 293). Given that gait speed was assessed through the timed up and go test, such results similarly suggest LIPA can stimulate functional improvement, specifically an improved ability to efficiently mobilise from a seated position and ambulate. This is promising considering increased gait speed is associated with an increase in daily walking time (359), and time spent performing low-intensity resistance training (173). Accordingly, gait speed is used as a key diagnostic indicator of low functional performance and severe sarcopenia in older adults (84), however only one SBF participant positively shifted classification from poor functional performance (<0.8m/s) to non-sarcopenic (>0.8m/s).

However, it should be noted that the typical error for gait speed calculated during reliability analysis was 0.1m/s for both average and peak gait speed, suggesting the observed experimental improvements (0.1 m/s) are not of sufficient magnitude to be considered meaningful (1.5 to 2.0 times typical error (263). Nevertheless, given that the minimal clinically important difference in gait speed was recently identified as 0.1 m/s for multiple populations (255), this highlights the achieved gait speed improvements as not only notable, but also clinically relevant. For average gait speed, only SBF improved by 0.10 m/s in contrast to LIPA, suggesting an apparent advantage of frequent vs continuous LIPA. However, given that both experimental groups similarly improved, this suggests the act of displacing sedentary behaviour time with increased LIPA is the principal factor mediating gait speed improvements.

SB displacement with LIPA also improved sit-to-stand ability. Considering SB has previously been associated with diminished sit-to-stand ability and subsequent mortality in older adults (122), such a finding is both unsurprising and promising. In support, a previous intervention in older adults observed an identical 30 second sit-to-stand improvement (2 counts) following an SBF intervention of greater duration (10 weeks) (158). This suggests the current intervention was more successful, through achieving the same goal in less time. Nevertheless, a further SB reduction study in older adults similarly improved sit-to-stand ability, despite failing to control for the pattern of prescribed SB displacement (157). Previous authors speculated SBF specifically mediated such effects, inducing a specificity of training effect improving the ability to mobilise from a chair (39).This is in keeping with the aforementioned gait speed improvements. However, in contrast to the original SBF advantage hypothesis, displacing SB with LIPA irrespective of prescribed pattern enhanced sit-to-stand ability. However, it should be noted the observed changes in both experimental groups (1-2 sit to stands) were <1 times the typical error calculated during reliability analysis (2 sit to stands).

Both experimental groups exhibited reductions in the time taken to complete one sit-to-stand (an index of functional speed), further suggesting improved movement execution, and enhanced muscular power. This represents a novel finding of the current investigation. This is of notable impact given that inappropriate sit-to-stand transitions are responsible for up to 41% of falls in care home residents (362), with greater sit-to-stand time identified as a key predictor of all-cause mortality (125).

Nevertheless, considering both experimental groups improved by similar magnitudes (SBF: -9%, LIPA: -11%), this further devalues the SBF advantage hypothesis. Curiously, trading SB for LIPA appeared to mediate an increase in one sit-to-stand time following SBF (please see appendices i), thus impairing movement execution. Whilst this may seem counterintuitive, overall reduced one sit-to-stand time and improved 30 second sit-to-stand count, still represent enhanced sit-to-stand ability following SBF. Frequently displacing SB time with LIPA inherently replaces seated for an upright posture, thus reducing the frequency with which a given participant was in the starting position for performing sit-to-stand transitions. Future studies could test one sit-to-stand more frequently, to determine adaptation time course. Nevertheless, improved lower body muscular power/endurance is promising for maintaining older adult's independence. Again, it should be noted the observed changes in both experimental groups (-0.2-0.3 s) were < 1 times the typical error calculated during reliability analysis (0.44s).

Significant improvements in HGS were observed in both experimental groups but not control. This is promising considering HGS is associated with mortality in older adults (363, 364), and thus represents another promising novel finding of the current investigation. SB and LIPA time have previously been associated with reduced (52, 127) and enhanced (365) grip strength in older adults respectively. Furthermore, implementation of light upper body-based movements enhances HGS in older adults (366-368). Accordingly, the same upper body-based LIPA tasks were prescribed to both experimental groups (Sweeping up, etc). Considering average HGS increased by a similar magnitude in both SBF (7%), and LIPA (6%) this does not suggest an SBF advantage. In-fact, displacing SB in a continuous fashion significantly mediated observed improvements in average HGS (please see appendices i). In contrast, a trend ($p=0.08$) was observed between the change in experimental groups, whereby SBF (8%) enhanced peak HGS to a greater extent compared to LIPA (2%). This does point to an SBF advantage. Accordingly, gripping the arm of a chair, and pushing through one's arms are common cues given to older adults when performing sit-to-stand transitions (369). Therefore, it is proposed that increased sit-to-stand frequency increased the utilisation of the arm stabilisation tactic in SBF participants, subsequently enhancing functional adaptation in the upper body musculature. Nevertheless, within the LIPA group, displacing SB with LIPA significantly mediated

improvements in peak HGS (please see appendices i), possibly due to the continuous implementation of habitual upper body LIPA tasks. Furthermore, increased LIPA was negatively associated with the change in both peak and average HGS following control (please see appendices i). Given that control participants were not advised to implement upper body LIPA tasks (Sweeping up, etc), this distinguishing factor mediated maladaptation within the control group (Peak HGS: -9%, Average HGS: -1%). Interestingly, the only change in HGS that can be considered meaningful (1.8 times the typical error), was that of peak HGS following control (-2.5kg). Ultimately, such results highlight the importance of habitual light upper body-based tasks for sustaining/ improving HGS in older adults.

Eyes closed single leg stance duration (left leg only) exhibited significant improvements following SB displacement. Despite SB being associated with reduced balance/ increased risk of falls in older adults (121-123), previous SB reduction interventions in older adults have reported mixed results regarding balance improvements (122, 128). In-fact, a single study exhibited a trend towards improved eyes open balance following SB reduction (293). Accordingly, such studies failed to vary the pro-prioceptive feedback (eyes open vs closed). Considering functional characteristics of the *Triceps Surae* significantly account for ~69% of the variance in older adults balance ability (133), and SB displacement with LIPA stimulates the lower body musculature (90, 91), enhanced balance was originally hypothesised. Fortunately, both interventions enhanced eyes closed stance duration specifically, suggesting pro-prioceptive adaptation. Whilst general ambulation requires pro-prioceptive input during execution (238), the specific translation of this stimulus into enhanced eyes closed stance duration is both unexpected and promising. Furthermore, given similar improvements between experimental groups, this suggests enhanced eyes closed balance occurs irrespective of prescribed LIPA pattern. Curiously, increased LIPA time (LIPA group) mediates statistically non-significant reductions in eyes closed stance time (please see appendices i). However, eyes closed, and left leg trials specifically exhibited the poorest inter-day reliability, compared to right leg, and eyes open, respectively. Furthermore, the typical error calculated for eyes open single leg unipedal stance time (left) was 2.94s, suggesting the observed average change of between 1 and -1s, was merely 0.3 times the typical error and cannot be considered a meaningful change (263).

Nevertheless, displacing SB with LIPA (LIPA group) mediated statistically non-significant improvements in eyes open stance duration and thus balance ability (please see appendices i), despite not observing any significant main effects. Despite good reliability for eyes open single leg stance duration (stopwatch assessed) in both the current chapter and previous studies (370), caution should still be applied when interpreting a minimal stance duration improvement independent of posturography. Accordingly, despite no significant change in the posturography sub-sample analysis (n=28), the utilisation of such a measure is a major strength of the current chapter, especially considering previous interventions have failed to utilise posturography during balance assessment (122, 128). The current results, combined with previous evidence suggesting SB is not independently associated with postural stability in women aged 50-65y old (128), suggest SB displacement with LIPA fails to enhance posturography in older women. In fact, a minimum of 90 minutes/ week of specific balance training is suggested to be the minimum dose response threshold for balance improvement in older adults (371). Therefore, the results of the current chapter highlight the potential insufficiency of SB displacement as an appropriate modality for balance improvement. Together with longer intervention periods (> 8 weeks), it may be prudent for future SB displacement interventions to implement specific balance training (e.g. single leg challenges), whilst determining what effect a shift in modality away from LIPA has on perceived palatability and likelihood of long-term compliance. Consequently, SB displacement with specific activity modalities (e.g. single leg challenges) may still have a role to play in enhancing balance ability in older adults.

Strengths and Limitations

The major strength of the current chapter was investigating functional adaptation to SB displacement, whilst controlling for the pattern of prescribed LIPA. Furthermore, the change in HGS and one sit-to-stand time was examined, which to the author's knowledge is novel. Accordingly, gait speed, sit-to-stand ability, and balance were accurately assessed with highly sensitive equipment (pressure sensor and detailed posturography) in contrast to previous interventions. Nevertheless, certain discrepant findings do highlight the need for further research foci [Increased frequency (every 1-2 weeks) & follow up (> 8 weeks) testing]. Future studies should investigate the physiological mechanisms (e.g. muscle-tendon complex size/ neuromuscular adaptation) that underpin such positive functional adaptations.

Conclusion

In conclusion, displacing SB with LIPA in older women induced clinically relevant improvements in gait speed, 30STS, one sit-to-stand time, average HGS, and balance. Importantly, most enhancements occurred irrespective of the prescribed LIPA pattern. Nevertheless, SBF appears to enhance greater peak handgrip strength adaptations. The observed improvements are compelling positive changes associated with an exercise intensity not customarily regarded as optimal. Furthermore, frequent SB displacement with LIPA appears more beneficial for select outcomes.

Chapter 5 – The effects of displacing sedentary behaviour with light intensity physical activity on muscle strength and neuromuscular function in older women

Chapter take home message: Interestingly, both antagonist co-activation (AgCoA), and *Gastrocnemius medialis* agonist activation capacity increased following LIPA, whereas only AgCoA decreased following SBF. Accordingly, a significant time effect was observed for net plantar flexor maximum voluntary contraction, driven by increases in both experimental groups in contrast to control.

Abstract

The effects of displacing Sedentary Behaviour (SB) with light intensity physical activity (LIPA) on neuromuscular function and muscle strength in older adults are as yet undetermined. Therefore, the aim of this chapter was to examine changes in neuromuscular function [Antagonist co-activation (AgCoA), and *GM* agonist activation capacity% (AC%)], and muscle strength [Plantar flexion (PF) isometric maximum voluntary contraction (MVC)], following 8-weeks of SB displacement with LIPA in older women. It was hypothesised SB displacement would enhance both neuromuscular function and PF iMVC. Thirty older women were allocated to one of three groups: 1) sedentary behaviour fragmentation (SBF) (*n*=13), 2) continuous LIPA (*n*=11), or 3) control (*n*=6). Neuromuscular function and PF iMVC were assessed using isokinetic dynamometry, electromyography, and electrophysiological stimulation at weeks 0 and 8. Despite no significant main group×time interactions, trends were observed, including uncorrected PF MVC (p=0.09), AgCoA (p=0.05), and Agonist AC% (p=0.10). Only LIPA exhibited an increased in uncorrected PF iMVC (4%). Interestingly, both AgCoA (22%), and GM AC% (5%) increased following LIPA, whereas only AgCoA decreased following SBF (-21%). Accordingly, a significant time effect was observed for Net PF MVC (Corrected for AgCoA & agonist drive) (p=0.03), driven by increases in both experimental groups (SBF: 3%, LIPA: 2%), but not control (-4%). Muscle quality (net PF iMVC/ *GM* muscle volume) did not significantly change. In conclusion, SB displacement with LIPA in older women resulted in enhanced PF iMVC, once AgCoA and GM AC% were accounted for. Interestingly, SB displacement appears to increase net PF MVC through different neuromuscular pathways dependant on the pattern of prescribed LIPA. Accordingly, SBF appeared to enhance net PF iMVC via a reduction in AgCoA, whereas continuously implemented LIPA appeared to enhance net PF iMVC via enhanced GM AC%.

Introduction

Sedentary behaviour (SB) is higher among older adults (71) and is strongly associated with diminished physical function (121-123, 131). SB related functional diminishment may in turn be related to the age-related loss of muscular strength (138), otherwise known as Dynapenia (372). Interestingly, age-related reductions in muscle strength are considerably more rapid compared to muscle atrophy (373-375). Reduced agonist activation capacity (AC%), can partially account for Dynapenia (138, 142), due to changes in myocyte properties (Ca2+ dysregulation), motor cortex/ spinal cord alterations (372), as-well as motor-neuron loss (141)/denervation (138). Dynapenia can also be partially attributed to increased antagonist co-activation (AgCoA) (133, 139, 140). Despite the importance of AgCoA for sustaining joint integrity (376), disproportional co-activation reduces AC%/ agonist force output via a decline in Ia inhibitory interneuron mediated reciprocal inhibition (377). Nevertheless, increased AgCoA is proposed as a compensatory joint stability mechanism (activated in the response to relative agonist weakness) (378). Single fibre force generating capacity increases, thus compensating for decreased AC%/ AgCoA (379). However, this facilitates a shift toward reduced force generating capacity/contractile velocity (380), which combined with lower maximal motor unit discharges, leads to slower contractile properties and diminished function (353, 354, 381). Specifically, reductions in lower body muscular strength (e.g. *Triceps Surae* group) are associated with diminished stability during ambulation (137), reduced walking speed (382), and increased postural sway (133, 137) in older adults. Therefore, the association between SB and diminished functional performance in older adults may be due to deterioration in (neuro) muscular function. Lower levels of physical activity, especially at moderate to vigorous intensity (MVPA) are associated with greater age-related declines in muscle strength (142-144). Furthermore, physical activity is a modulator of neural activation (145), and fibre-specific tension (146) in older adults. However, infrequent muscle stimulation during SB is proposed to affect muscular function independent of concurrent MVPA time (10, 147). Whilst one study identified an association between self-reported TV viewing time and reduced knee extensor strength in older adults after accounting for MVPA (148), other studies have failed to observe such an effect (273, 383). Despite one study identifying a counterintuitive association between objectively assessed SB and enhanced ‘muscle quality’ in older adults (155), the authors failed to account for

neuromuscular function [AgCoA & AC%] (55). Accordingly, self-reported physical activity(adjusted for self-reported SB) is associated with enhanced specific force in older adults (202), which is a more robust muscle quality assessment technique (384). Furthermore, alternative disuse models (e.g. bed rest) reduce knee extensor AC% (~7%), and isometric strength (10-13%) in older adults (149-151), with strength losses greater in women (152). Therefore, despite the paucity of high-quality evidence, SB does appear to exhibit a detrimental impact on muscular function in older adults.

Displacing SB with light intensity physical activity (LIPA) improves physical function in older adults (156, 158, 293), which may be due to muscular functional adaptation. Accordingly, Standing and LIPA time have previously been associated with increased *Gastrocnemius Medialis (GM)* AC%, and Achilles tendon force in older adults respectively (385). Furthermore, LIPA has previously been positively associated with lower body strength in older adults in a dose dependant fashion (386), with total physical activity (including LIPA) further associated with lower limb muscle strength in middle aged women (~50y) (387). However, despite a significant increase in older adults LIPA over 6 months post knee arthroplasty, no corresponding increase in knee extensor strength was observed (388). Nevertheless, increases in general ambulation enhance lower body strength in older adults (389). Displacing SB with LIPA generates significant increases in lower body muscle activity in older adults (90, 91), thus highlighting a plausible mechanism for enhanced muscular function. Thus the potential for LIPA to generate comparable physiological responses relative to more conventional high intensity loading is a somewhat recent theorem, supported by previous observations whereby older adults engaging in low frequency stair climbing exhibit significantly reduced mortality (361). Therefore, due to the relative increment in physical activity intensity, LIPA would generate a loading stimulus in older adults who are closer to low physiological reserve, to a degree adequate to reach loading threshold for (neuro) muscular adaptation. Furthermore, it is generally accepted that rapid gains in strength following relatively short training periods (6-9 weeks), are mediated via neural adaptation (390). Nevertheless, due to relatively low mechanical loading following SB displacement with LIPA, any potential muscular functional adaptations are likely to be small in magnitude. Furthermore, functional disability is largely determined by lower limb function (354), suggesting a need for targeted investigation of the *Triceps Surae* muscle group.

Furthermore, SB accumulated in prolonged uninterrupted bouts appears to be more determinantal to physical function in older adults, compared with greater fragmentation (shorter sitting bouts, frequent standing breaks etc) (11, 21, 22). Improved function with greater fragmentation of sitting time may be due to the central nervous system being stimulated with greater frequency. This repeated contractile activity stimulus may potentially improve neuromuscular function specifically (147), especially considering SBF consistently stimulates the *Triceps Surae* muscle group (91). However, previous LIPA interventions have also failed to consider the pattern of LIPA prescription, and whether displacing SB in a more fragmented fashion enhances (neuro) muscular functional adaptation following LIPA prescription.

Therefore, the aim of the current chapter was to examine the effects of displacing SB with LIPA in two distinct patterns on muscle strength and neuromuscular function in older women. It was hypothesised that displacing SB with LIPA would induce functional adaptation (Increased agonist drive, reduced antagonist co-activation), subsequently resulting in enhanced muscle strength/quality. It was also hypothesised that SBF would induce greater adaptation compared to continuous LIPA, due to the frequent contractile activity stimulus.

Results

Baseline Differences

At baseline PF MVC (p=0.01), Agonist Drive (p=0.04), Net PF MVC (P=0.01), and *GM* muscle quality (p=0.02) were significantly different between groups. Post hoc testing revealed the control group exhibited significantly ($p<0.05$) higher values for all aforementioned variables compared to SBF and LIPA (please see table 5.1). Groups were significantly matched regarding dominant limb (Left: 28%, Right: 72%) (p=0.69), and proportion of participants who resided in homes with (75%) or without (25%) stairs (p=0.14).

Muscle Strength

After accounting for baseline differences, a trend toward a significant group×time interaction was observed for unadjusted PF iMVC (p=0.09), but no trend for time (p=0.96). Interestingly, such a trend was driven through a LIPA increase (3.8±26.9Nm, 4±31%), and decreases in both SBF (-3.8±21.5Nm, -3±24%), and control (-

1.5±26.1Nm, -2±20%). No significant main effects were observed for dorsiflexor iMVC %)] (please see table 5.1).

Neuromuscular Function

A trend toward a significant group×time interaction was observed for AgCoA (p=0.05), but no trend for time (p=0.78). Such a trend was driven through a LIPA increase (Relative change from baseline 22±109%) and decreases in both SBF (-21±112%), and control (-17±87%) %)] (please see table 5.1 and figure 5.1). After accounting for baseline differences, a trend toward a significant group×time interaction was observed for Agonist Drive (p=0.10), but no trend for time (p=0.21). Such a trend was driven through a LIPA increase (5±7%), in contrast to both SBF (0±8%), and control (-2±3%) %)] (please see table 5.1 and figure 5.2).

Corrected Muscle Strength and Muscle Quality

After accounting for baseline differences NET PF MVC (Corrected for AgCoA & agonist drive), exhibited a significant time effect (p=0.03, n^2p=0.16), but no significant group×time interaction (p=0.65). Despite this both experimental groups increased on average (SBF: 1.8±19.8Nm, 3±23%, LIPA: 2.5±14.6Nm, 2±15%), in contrast to control (-5.9±15.7Nm, -4±11%) %)] (please see table 5.1 and figure 5.3). No significant main effects were observed for *GM* muscle quality %)] (please see table 5.1).

For detailed results and figures on associations between relative changes from baseline for physical behaviour outcomes (SB & LIPA) and relative changes from baseline for neuromuscular functional outcomes, please see appendices i.

Table 5.1- Muscle function and neuromuscular outcomes for SBF, LIPA, and control, as-well as absolute and relative changes from baseline for each group.

Boldened text represents a significant baseline difference. * represents a significant time effect.

	SBF (n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
Number of participants whose dominant Leg was Right/ Left	9/ 5			11/ 4			2/ 6		
Number of participants who had stairs present in residence/ Not Present	12/ 2			8/ 6			7/ 1		
PF MVC (Nm)	97.1±33.0	90.2±20.3	-3.8±21.5 (-3±24%)	87.2±	98.7±36.1	3.8±26.9 (4±31%)	**125.9±36.1**	114.4±26.9	-1.5±26.1 (-2±20%)
DF MVC (Nm)	18.9±9.6	20.4±8.1	0.0±3.8 (0±22%)	19.6±3.1	19.6±2.3	0.8±3.1 (4±15%)	20.4±4.6	20.4±1.2	-0.8±3.5 (-4±15%)
	SBF (n=14)			LIPA (n=14)			Control (n=7)		
Antagonist Co-Activation (%)	9±8	8±4	-3±8 (-21±112%)	5±4	8±8	1±10 (22±109%)	11±14	9±5	-2±15 (-17±87%)
	SBF (n=13)			LIPA (n=11)			Control (n=7)		
GM Agonist Activation Capacity (%)	94±14	94±10	0±7 (0±8%)	90±10	93±5	4±5 (5±7%)	**96±5**	94±4	-2±3 (-2±3%)
	SBF (n=13)			LIPA (n=11)			Control (n=6)		
Net PF MVC (Nm)	99.6±22.8	101.5±29.5	1.8±19.8 (3±23%) *	103.3±19.5	105.8±22.8	2.5±14.6 (2±15%) *	**138.0±19.3**	132.0±21.7	-5.9±29.4 (-4±11%) *
GM muscle quality (Nm/cm^3)	0.14±0.05	0.14±0.05	0.002±0.03 (7±25%)	0.13±0.04	0.13±0.05	0.01±0.02 (4±18%)	**0.19±0.03**	0.17±0.04	-0.03±0.03 (-13±14%)

DF; Dorsi flexor, GM; Gastrocnemius medialis, LIPA; light intensity physical activity, MVC, Maximum voluntary contraction, PF; Plantar flexor; SBF; sedentary behaviour fragmentation.

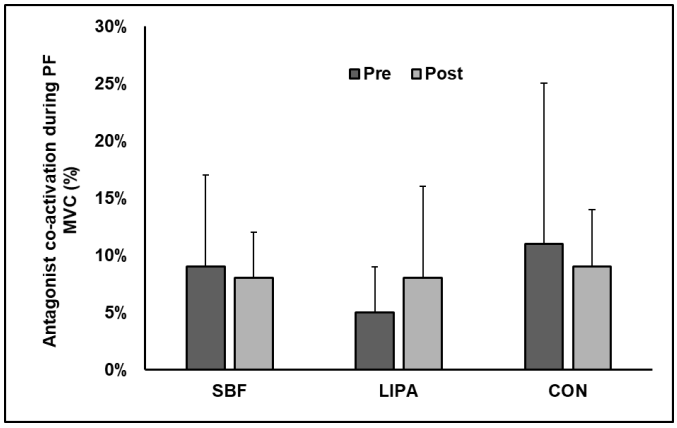

Figure 5.1- Group dependant values for *antago*nist co-activation during PF MVC for pre and post. CON; control,, LIPA, light intensity physical activity, MVC, maximum voluntary contraction, PF; plantar flexor, SBF; sedentary behaviour fragmentation.

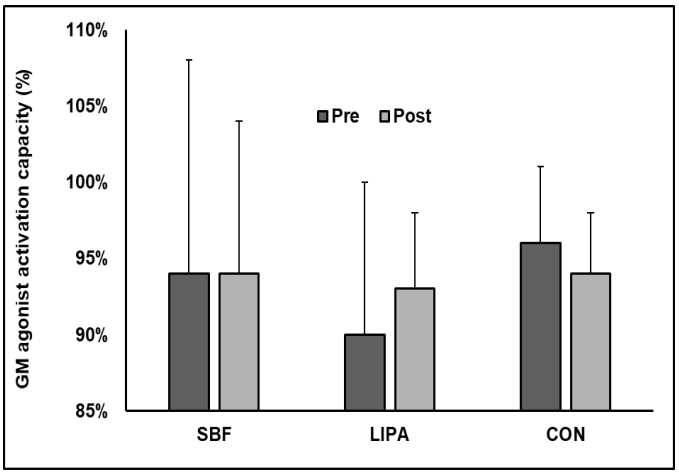

Figure 5.2- Group dependant values for *GM* agonist activation capacity for pre and post. CON; control, GM; Gastrocnemius medialis, LIPA, light intensity physical activity, SBF; sedentary behaviour fragmentation.

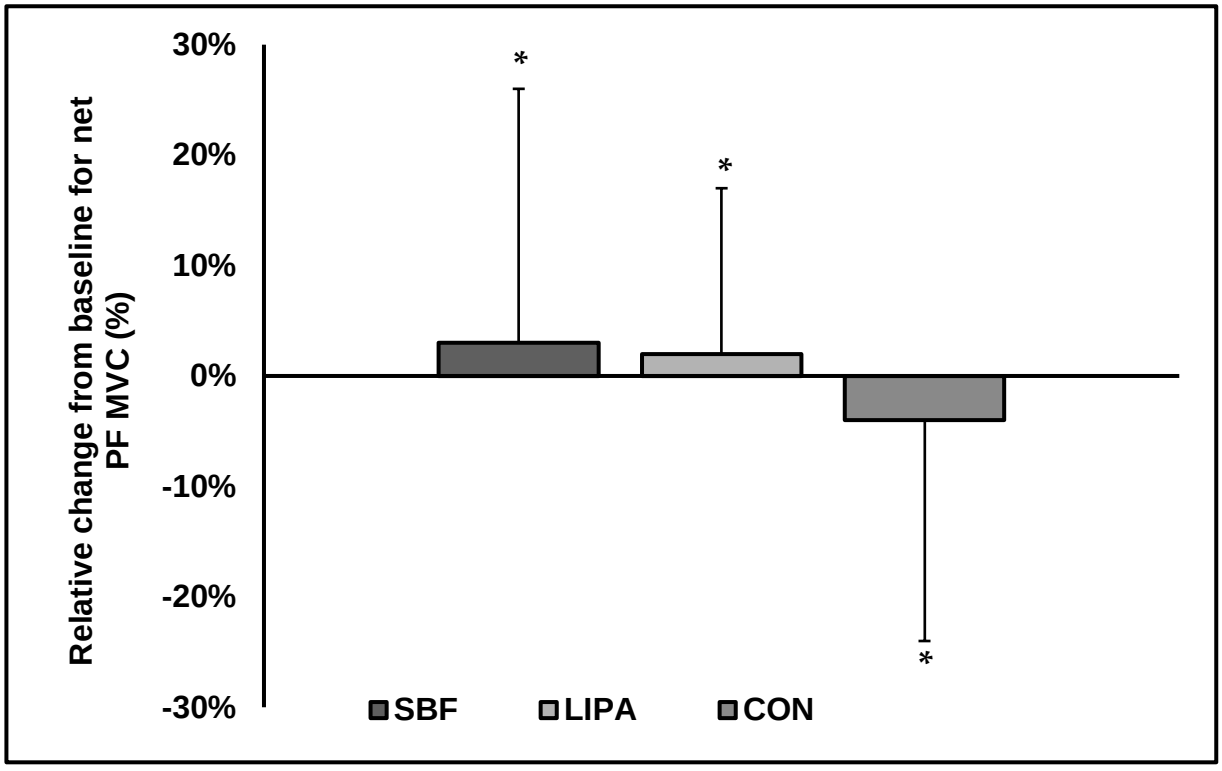

Figure 5.3- Group dependant relative changes from baseline for net PF MVC. *represents a significant time effect. CON; control, LIPA, light intensity physical activity, MVC, maximum voluntary contraction, PF; plantar flexor, SBF; sedentary behaviour fragmentation.

Discussion

The aim of the current chapter was to examine the effects of displacing SB with LIPA in two distinct patterns on muscle strength and neuromuscular function in older women. It was hypothesised that displacing SB with LIPA would induce neuro-muscular adaptations (Increased AC% & reduced AgCoA), subsequently resulting in enhanced muscle strength/ quality. Accordingly a significant time effect was observed for Net PF iMVC (Corrected for *GM* AC% & AgCoA) (p=0.03), driven by increases in both experimental groups (SBF: 3%, LIPA: 2%), but not control (-4%). The first hypothesis was therefore upheld. It was further hypothesised that SBF would induce greater adaptation compared to continuous LIPA. Despite no significant group×time interactions, trends were observed, including uncorrected PF iMVC (p=0.09), AgCoA (p=0.05), and *GM* AC% (p=0.10). However, all group dependant trends appear to have been driven by an exclusive increase in LIPA for uncorrected PF iMVC (4%), AgCoA (22%), and *GM* AC% (5%). The second hypothesis was therefore partially rejected. The current chapter represents the first formal investigation to examine the effects of displacing SB with LIPA on neuromuscular function in older women.

A trend toward a group×time interaction was observed for *GM* AC%, driven primarily by an increase following LIPA (~5%). Interestingly, a recent meta-analysis concluded resistance exercise [65-90% one repetition maximum (1-RM)] results in enhanced PF AC% of ~8% over an average of 36 weeks in older adults (390). Results from the current investigation further suggest enhanced *GM* AC% can be achieved by implementing activity of a considerably lower intensity, volume, and time. Early increases in agonist AC% following resistance training in older adults (< 6 weeks) are primarily mediated by increased motor unit firing rate (391), which reduces fast twitch motor unit activation threshold (392). Beyond 6-weeks further enhancements in agonist AC% are proposed to be mediated by axonal sprouting from type I motor units, re-innervating type II muscle fibres, that have undergone age related denervation (138), producing a larger motor unit with greater force producing capability (393). Similar mechanisms likely mediated the enhanced *GM* AC% following SB displacement.

However, only LIPA exhibited an increase in AC%. This may have been further related to the fact that LIPA accumulated in a single continuous bout sustained high *GM*

muscle activity causing neuromuscular adaptation, in contrast to SBF and control where muscle activity was likely more sporadic and absent respectively (91). In support, a previous investigation identified a positive association between standing and *GM* AC% in older adults (385), with the authors speculating longer durations of non SB activity increases *GM* AC%. Accordingly, LIPA implementation was primarily favoured over stationary standing as the former generates superior muscle activity response during SB displacement in older adults, likely due to dynamic muscular contraction (91). Lack of training induced neuromuscular adaptation has previously been attributed to inconsistency between training modality (dynamic contraction) and assessment specificity (isometric MVC) (394). However, enhanced *GM* AC% following LIPA was assessed via isometric PF MVC, whereas LIPA (dynamic contraction) and specifically not stationary standing (isometric contraction) was prescribed to displace SB. Considering no significant change in standing was observed (chapter 3), this alternatively suggests continuously implemented upright ambulatory activity further translated into enhanced AC% during isometric MVC. However it should be acknowledged that dynamic movement can result in isometric contractions via biarticulated muscles/ muscle-tendon interaction (e.g. PF during stair climbing), which may have also influenced neuromuscular adaptations during isometric assessments.

Continuous LIPA also likely involved a greater LIPA task variance, to achieve the prescribed 45-50-minute upright bout. Considering different household tasks cause different muscle activity responses (90), it remains plausible that a greater task variance during continuous LIPA, led to more varied and potentially greater muscular response. Accordingly, stair climbing generates the highest muscle activity of all habitual tasks (90), and also reduces mortality risk at relatively low frequencies (361). Whilst stair climbing was not explicitly prescribed as a LIPA task (due to safety concerns), it remains possible that prescribing “light self-paced walking”, may have been misinterpreted as stair climbing. Whilst all groups were matched regarding the number of participants whose home residence contained stairs (e.g. two-story house) and those that did not (e.g. bungalow, flat etc) it remains plausible that the LIPA group engaged in greater stair climbing outside of the home environment. Nevertheless, a previous training study in older adults concluded those with the lowest baseline AC% experienced the greatest relative increases following training (395). Accordingly, the group dependant trend for *GM* AC% was observed after accounting for baseline

differences. Furthermore, LIPA exhibited the lowest levels of baseline *GM* AC% at baseline, and thus had the greatest capacity for change. However, it should be noted that the typical error for GM AC% calculated during reliability analysis was 5%. This means the change following LIPA can only be considered 1 times the typical error and thus under the threshold considered meaningful (1.5 to 2.0) (263). Nevertheless, due to the relative surge in intensity LIPA seems to generate in older adults closer to low physiological reserve, such activity appears to reach an appropriate loading threshold required for enhanced AC%.

A further trend toward a significant group×time interaction was observed for AgCoA ($p=0.05$). This contrasts with the results of a recent meta-analysis which concluded resistance exercise [70-90% one repetition maximum (1-RM)], does not increase/decrease antagonist co-activation during PF in older adults (390). Aside from acknowledging the substantial heterogeneity in design, and conclusions reached by the studies analysed, the authors questioned the overall relevance of AgCoA during mechanically restricted isometric contractions (390). Accordingly, whilst adequate co-activation aids with joint integrity (376), increased co-activation can reduce AC%/ agonist force output (377). Therefore, it is still undetermined whether the nervous system prioritises joint stability (Increased AgCoA) or increased force production (Decreased AgCoA) following training (391), and to what extent changes in AgCoA influence changes in strength following training.

Nevertheless, an increase in AgCoA was observed following continuous LIPA implementation (~3%). In support, a 12-month training program [aerobic/ resistance training (80-100% one repetition maximum (1-RM)), 2-3 sessions per week] in older adults resulted in a significant increase in AgCoA during PF MVC of ~7.4% (396). High levels of voluntary force generation require substantial co-activation to maintain joint stability (377). Therefore, AgCoA likely increased following LIPA to match the increased force output being produced by the increase in *GM* AC%. This was likely mediated via Ia inhibitory interneuron mediated reciprocal inhibition (377), representing the nervous systems resultant attempt to uphold joint integrity/ stability, through matching agonist force output (376). Accordingly, previous studies have suggested increased AgCoA post-training represents a safety mechanism to aid with joint control during execution of various motor tasks (397). This is plausible as continuous LIPA implementation prescribed habitual motor tasks that stimulate the

lower body musculature (90, 91). Therefore, increased AgCoA following continuous LIPA implementation, highlights a secondary joint stability mechanism activated in response to higher AC%. Considering the reduction in *GM* AC% following control, this likely facilitated subsequent reductions in AgCoA, given reduced requirement for joint stability.

In contrast, a reduction in AgCoA was observed following SBF, despite no observed change in *GM* AC%. Considering reduced AgCoA was a specific neuromuscular effect, independent of AC%, this suggests SBF causes substantial adaptation through isolating the antagonist. Early phase (<6 weeks) reductions (398, 399) in AgCoA have been attributed to a learning effect as muscle contractions are set into a nervous system mediated pattern (400). Considering the similar 8-week length of the current investigation this supports the plausibility of early phase reductions in AgCoA. Nevertheless, continuous LIPA was also implemented over 8 weeks with matched LIPA implementation (prescribed movements and daily time), suggesting reduced AgCoA was a specific adaptation effect following the specific SBF stimulus. Considering SBF participants were frequently prompted to move (2 mins LIPA for every 30 mins SB), this likely meant greater environmental variation whilst performing LIPA, especially considering older adults accumulate SB time in various indoor community settings (Home, Cafes, Restaurants) (319, 320). Such variation also likely varied the type (e.g. carpet vs vinyl) and gradient (e.g. incline) of floor surfaces (e.g. carpeted vs. vinyl (401). Accordingly, both surface type (401), and incline (402) can affect ankle movement kinetics in older adults, due to variations in friction, and varied foot placement respectively. Interestingly, *Tibialis Anterior* muscle activity increases on an inclined surface with reduced friction, suggesting an attempt to enhance stability (403). Accordingly, environmental variation also likely generated greater variance in LIPA based tasks where ambulatory walking was not feasible. Interestingly, walking backwards increases *Tibialis Anterior* muscle activity (404, 405) due to reduced proprioceptive input (406). Whilst backwards walking was not implemented in the current study as a specific LIPA suggestion (due to safety concerns) habitual tasks were prescribed that varied movement direction (side to side shuffling, sweeping, tidying away objects etc). Ultimately, variations in movement direction and walking surface following SBF potentially increased *Tibialis Anterior muscle* activity during heel strike enhancing stability. Following heel strike, decreased *Tibialis Anterior* muscle

activity aids with PF torque production facilitating forward motion (407). This aligns with early phase learning related reductions in AgCoA (398-400), and highlights a plausible mechanism for reduced AgCoA following SBF. However, this does not suggest that the specific SBF stimulus (frequent bouts of LIPA) isolated the antagonist, but instead caused a specific adaptation through the adoption of different activities. Nevertheless, the average change in AgCoA following SBF (-3%) was 1.5 times the typical error calculated during reliability analysis (2%). This strengthens the argument that this was an exclusive and meaningful neuromuscular adaptation following SBF, considering other groups did not achieve a threshold of change considered meaningful (0.5-1.0 times typical error) (263).

A significant change over time was observed for net PF MVC. It has previously been well established that a dose-response relationship exists between progressive resistance training and strength gains in older adults (408), whereby 3x10 reps at ~70-80% of 1-RM 3 times per weeks for ~ 8weeks is considered optimal (409, 410). Therefore, whilst the length of the present investigation (8 weeks) can be considered optimal, LIPA implementation is conventionally considered sub-optimal regarding activity intensity/ volume for strength gains. Nevertheless, total activity time (including LIPA) is associated with lower limb muscle strength in middle aged women (~50y) (387).

However, despite no group×time interaction both experimental groups exhibited increased net PF MVC (SBF: 3%, LIPA: 2%, ~0.3% per week). In support, 10 weeks of light treadmill walking training enhanced knee extensor strength by ~5.9% i.e. ~0.3% per week (411). However, 20 weeks of moderate intensity continuous walking did not enhance knee extensor strength, in contrast to high intensity walking of the same duration which enhanced strength by ~12.5% i.e. ~0.6% per week (412). Considering the prioritisation of knee extensor strength investigation by previous studies, this does limit the comparison of the current results to previous interventions. However, functional disability is largely determined by lower limb function (133, 354). Nevertheless, the magnitude of change in strength per week (~0.3% per week) was similar to that of a previous study with a similar loading protocol (411). Displacing SB with LIPA generates significant increases in lower body muscle activity in older adults (90, 91). However, accumulation of habitual activities (walking, stair climbing, sit to stands etc) across the day in middle aged adults, results in a mere 118 seconds spent

at electromyography magnitudes corresponding to ≥70% of maximum MVC (thigh muscles) (90), the aforementioned optimal threshold for strength gains following training (409, 410). However, comparing habitual activities to maximal MVC of the thigh muscles is limited by potential gender and stature differences between participants, as well as intra-week variability (week vs weekend) and specific investigation of the thigh muscles (amplitudes may be different in other muscle groups) (90). Nevertheless, diminished strength gains following LIPA implementation appear intuitive when compared to implementation of walking at higher intensities, which unsurprisingly stimulates electromyography bursts of greater magnitude (90). Nevertheless, strength gains of smaller magnitude may persist over the long-term more effectively compared to higher intensity activity, especially considering the positive palatability and uptake of the SB displacement behaviour (see chapter 3). However, the lack of progressive overload following LIPA implementation could inhibit long-term gains in strength.

Interestingly, the significant time effect for net PF MVC was observed following correction for AgCoA and agonist activation. This suggests small gains in muscular strength following SB displacement with LIPA are dependent upon neuromuscular adaptation. This is unsurprising considering that the majority of early phase gains in strength following resistance training are primarily mediated by neuromuscular adaptation (390), and only partially mediated by muscle-tendon size, morphology, and tissue related quality adaptations. Muscle-tendon size, morphology, and tissue related quality adaptations, then become the primary mediator of strength gains following subsequent phases of resistance training (390). Furthermore, the significant effect for time was also observed after accounting for baseline differences. Similarly, increases in general ambulation enhance lower body strength in older adults with those displaying lower levels of strength at baseline exhibiting the greatest gains in strength (389). Interestingly, strength gains of similar magnitude in both experimental groups appear to have been generated through divergent neuro-muscular mechanisms dependant on the pattern of prescribed LIPA (intermittent vs continuous). Accordingly, increased net PF MVC following LIPA implemented in a continuous fashion appears to have been primarily driven through an increase in agonist AC%. In contrast increased PF MVC following SBF appears to have been mediated via reduced AgCoA. Perhaps future investigations could continue to investigate SB displacement to

determine which prescription pattern and associated neuromuscular adaptation mechanism sustains strength gains in the long term. Similarly, the observed reduction in net PF MVC following control was likely due to the reduction in agonist activation capacity. Finally, all of the data used to calculate Net PF MVC, exhibited acceptable inter-day reliability (PF MVC, dorsiflexion MVC, AgCoA, AC%), and the effect size can be considered large for the significant Net PF MVC time effect.

Furthermore, no change in muscle quality was observed. Only one previous cross-sectional study has noted that objectively assessed SB time is associated with enhanced muscle quality in older adults (413). However, the authors of the study defined 'muscle mechanical quality' as lower limb extensor power divided by lower limb fat free mass (obtained from a Dual X-Ray Absorptiometry scanner). However Dual X-Ray Absorptiometry underestimates thigh fat free mass compared to magnetic resonance imaging (414). The current investigation improved upon this by accounting for AgCoA and Agonist AC%, but also expressing normalised muscle strength relative to muscle volume of a primary PF muscle (in this case the *GM*), compared to lower limb fat free mass (413). Despite not observing a significant change in enhanced muscle quality assessment, this strengthens the original interpretation that gains in net PF MVC following SB displacement with LIPA are primarily due to neuromuscular adaptation. Future studies should investigate other factors known to affect muscular quality, including tendon mechanical properties, muscle architecture during MVC, and neuro-muscular efficiency (ratio of electromyography over torque). Specific force is one such comprehensive assessment of muscle quality that could be utilised for such a purpose (384). In support, a previous study identified an association between daily PA, and enhanced *GM* specific force in older adults (202), despite PA/SB being self-reported.

Strengths and Limitations

A major strength of the current investigation was the implementation of LIPA in two distinct patterns. This permitted the observation that SB displacement enhances net PF MVC through divergent neuromuscular pathways dependent on the prescribed LIPA pattern. Furthermore, MVC was assessed during PF MVC in contrast to previous studies that have focused on knee extensor strength primarily. This is important as functional disability is largely determined by lower limb function (133, 354). Moreover,

accounting for AgCoA and agonist AC% permitted the calculation of net PF MVC, and further improves upon previous studies as such key factors to force production can vary substantially between individuals and affect measures of force production (133, 138-140, 142). Furthermore, previous studies have stated the discrepancy between assessment contraction type (e.g. isometric) and contraction type during training (dynamic muscular contraction), may account for the lack of observed neuromuscular adaptation following training (394). Whilst this was the case in the current study, it remains extremely challenging to elicit electrophysiological stimulus to assess AC% during dynamic muscular contraction (390). Nevertheless previous studies have suggested increased AC% may be due to methodological issues rather than a lack of neural adaptation (64). Accordingly, using the interpolated twitch technique to assess AC% in older adults has previously been reported to not accurately assess AC% in older adults (415). This was reflected in the moderate inter-day reliability observed for *GM* AC% (intra-class coefficient: 0.79). However, baseline differences were present between groups, which would ideally not be present where possible. Furthermore, despite the inclusion of older women limiting the generalisability of such findings somewhat, this should ultimately be viewed as a strength considering functional adaptation to resistance training was recently demonstrated to be gender dependant (416). Finally, the lack of progressive overload is a general limitation of study design. Whilst this was done to increase palatability and compliance, implementing a non-progressive LIPA stimulus likely limited muscle strength and neuro muscular adaptation in response to the intervention.

Conclusion

In conclusion 8 weeks of SB displacement with LIPA in older women resulted in enhanced PF MVC, once AgCoA and AC% were accounted for. Interestingly, LIPA displacement increases net PF MVC through different neuromuscular pathways dependant on the pattern of prescribed LIPA. Accordingly, SBF enhances net PF MVC via a reduction in AgCoA, whereas continuously implemented LIPA enhances net PF MVC via enhanced AC%.

Chapter 6 – The effects of displacing sedentary behaviour with light intensity physical activity on muscle-tendon complex morphology, architecture, and tissue related quality in older women

Data from the current chapter are published in/ presented at (please see research outputs in appendices ii):

Minimising sedentary behaviour (without increasing medium-to-vigorous exercise) associated functional improvement in older females is somewhat dependant on a measurable adaptation in muscle size. *AGING (2020)*. Dale Grant*, David Tomlinson, Kostas Tsintzas, Petra Kolić, Gladys L. Onambele-Pearson. https://dx.doi.org/10.18632%2Faging.202265

Chapter take home message: A significant group×time interaction was observed for *Gastrocnemius Medialis* (*GM*) muscle volume, driven by experimental reductions, in contrast to control. Nevertheless, the relative change in sedentary behaviour (SB) was significantly negatively associated with the relative change in *GM* physiological cross-sectional area in the SBF group. Furthermore, significant time effects were observed for fascicle pennation angle, and normalised fascicle length in the *Vastus Lateralis muscle*.

Abstract

The effects of displacing Sedentary Behaviour (SB) with light intensity physical activity (LIPA) on muscle tendon complex (MTC) size (muscle volume), morphology (muscle architecture), and tissue related quality (echo intensity) in older adults is as yet undetermined. Therefore, the aim of this chapter was to examine changes in these MTC characteristics following 8-weeks of SB displacement with LIPA in older women. It was hypothesised that despite the relatively low load, SB displacement would induce MTC hypertrophy and enhance intrinsic muscle quality. Thirty-six older women (73±5 years) were allocated to one of three groups: 1) sedentary behaviour fragmentation (SBF) (*n*=14), 2) continuous LIPA (*n*=14), or 3) control (*n*=8). MTC parameters were assessed with ultrasonography at weeks 0 and 8, in the *Gastrocnemius Medialis* (*GM*)/ *Gastrocnemius Lateralis* (*GL*)*, Vastus Lateralis* (*VL)* muscle groups, as-well as the *Achilles tendon*. Significant group×time interactions were observed for *GM* MTC unit length (p=0.03) and *GM* muscle volume (p=0.012). *GM* volume changes were driven by experimental reductions (SBF: -4%, LIPA, -2%), in contrast to an unexpected, marked control increase (13%). The relative change in SB was negatively associated with the relative change in *GM* physiological cross-sectional area in the SBF group (R^2= 0.56, p=0.002). Furthermore, significant time effects were observed for fascicle pennation angle (p=0.007), and normalised fascicle length (p<0.001) in the *VL*. Interestingly, both *GM* & *GL* echo intensity exhibited a time × site × group interaction (p<0.001). Accordingly, the greatest reductions were observed in distal regions, and within the control group. In conclusion, SB displacement with LIPA has minimal effects on MTC size/morphology/ tissue related quality. In-fact significant improvements and changes were observed within the control group. Nevertheless, minor reductions in SB significantly mediated enhanced *GM* physiological cross-sectional area, following SBF suggesting an SBF advantage of SBF over continuous LIPA.

Introduction

Sedentary behaviour (SB) is higher among older adults (71) and is strongly associated with diminished physical function (121-123, 131). Considering SB is also associated with the age-related loss of muscle size [independent of concurrent moderate to vigorous physical activity (MVPA)] (273, 417), otherwise known as sarcopenia (138), reductions in muscle size may contribute to SB related functional diminishment. Accordingly, several markers of muscle tendon complex (MTC) status have been tracked in previous research to determine the response to loading/unloading. Severe disuse (e.g. bed rest (150, 418, 419) and limb immobilisation (420-423)) induces rapid muscle atrophy, with women exhibiting greater anabolic resistance compared to men (153, 154). Further alterations in muscle architecture may also contribute to SB related functional diminishment (233, 373, 424). SB is also associated with increased lower limb fat mass (155), and sarcopenic obesity [sarcopenia combined with a higher adiposity (SO)] (52) of which, a primary consequence is intramuscular fat accumulation, and reduced contractile tissue density (425). Accordingly, ultrasound echo intensity [a valid intramuscular fat quantification tool (EI)] (426)) has also been associated with diminished function in older adults (427). Despite only chronic unloading causing measurable tendon atrophy (428, 429), short-term disuse causes tenocyte mediated detection of force-induced deformations (430) triggering catabolism (431). Accordingly, 12-weeks detraining reduces tendon mechanical quality [tendon stiffness (*K*), & young's modulus (YM)] (428) which is strongly linked to echo intensity (232), and thus material alterations (432).

Interventions aiming to counteract MTC deterioration, have hitherto been MVPA based, which is reasonable considering MVPA is associated with reduced muscle atrophy (433), and improved muscle echo intensity (434), over time. Furthermore, high intensity resistance training (≥80% of 1-RM or MVC) is associated with enhanced tendon mechanical quality (168, 435) over time. However, older adults exhibit poor lifelong tolerance to intense activity (36, 165, 166, 356). Whilst it may be rational to assume lower intensity activity may not produce a sufficient MTC adaptation stimulus, evidence for this idea is scarce. Nevertheless, a body of work suggests that older women in particular would obtain various benefits from lower intensity loading including tendon material adaptation (172), muscle hypertrophy (173), and enhanced

physical function (171, 357, 358). Equally daily increases in light intensity physical activity (LIPA) enhance resting muscle architecture (436), increase muscle mass (360, 437), and improve physical function (359) in older adults generally, but especially in frail individuals (158, 357, 359, 360). Therefore, due to the relative surge in physical activity intensity LIPA seems to generate in older adults closer to low physiological reserve, such physical activity may reach an appropriate loading threshold required for MTC adaptation.

Similar functional improvements are also observed following SB reduction (156, 158, 293). However, it remains unclear whether such improvements are due to SB displacement or LIPA implementation. Furthermore, the potential role of MTC adaptation in functional adaptation following SB displacement also remains to be elucidated. Interestingly, acute muscle activity during SB displacement with LIPA appears higher in the *Triceps Surae* compared to the knee extensors (91), which is reasonable given the key role such muscles play in maintaining upright balance (133), and ambulating in general (438). This suggests the *Triceps Surae* may undergo greater adaptations than the knee extensors following SB displacement, and thus should be considered a primary investigation target. However, functional improvements are also observed following SB fragmentation [repeated interruption of prolonged sitting with longer standing and relatively more frequent sit-to-stand transitions and LIPA breaks (SBF)] (158). Considering previous interventions have failed to adequately control for the pattern of prescribed LIPA, SBF may have still induced measurable knee extensor adaptations. However MTC adaptation following SB displacement is likely to be relatively small in magnitude given that tendon has a relatively slow turnover rate (435, 439), and lower activity volumes generally stimulate less muscle hypertrophy (440). However, a recent study in a small sample of older adults (3 males, 3 females, 62±3yrs), actually suggested that human tendon tissue may have a higher, though non-significant, turnover rate (0.02% per hour) than skeletal muscle (441), meaning changes in both tissues may be equally small in magnitude due to an equally slow turnover rate. Nevertheless, despite muscle size not being a strong predictor of gait speed in older adults it still remains one of its significant predictors (442, 443). In other words, minor changes in MTC size may yet mediate functional improvement following SB displacement, especially in older adults.

The aim of this chapter was to quantify MTC hypertrophy and tissue quality related adaptations following SB displacement with LIPA in older women. It was hypothesised that both interventions would induce MTC hypertrophy and enhance intrinsic tissue quality (ultrasound EI). It was further hypothesised that muscular adaptations would be disproportionately observed in the *Triceps Surae* (*Gastrocnemius Medialis* (*GM*) and/or *Gastrocnemius Lateralis* (*GL*)*)* compared to the knee extensor muscle group [*Vastus Lateralis* (*VL)*]. Finally, it was hypothesised SBF would induce greater MTC adaptation compared to continuous LIPA.

Results

Baseline Differences

Participants were matched at baseline for most outcome variables of interest except *VL* Lf (p=0.03), *VL* normalized fascicle length (p=0.01), *VL* fascicle pennation angle (p<0.001), and *VL* physiological cross-sectional area (p=0.003) (please see table 6.3 and appendices).

GM intervention-induced changes

A significant group×time interaction effect was observed for *GM* volume (p=0.012, β=0.78). Despite no post-hoc differences, control exhibited an increase for *GM* volume (Please see figure 6.1). Trends toward group×time interactions were also observed for *GM* length (p=0.08), *GM* muscle-tendon unit length (p=0.08), *GM* Lf (p=0.09), and *GM* physiological cross-sectional area (p=0.06). Similarly, for *GM* EI, the 3×2×3 split plot ANOVA (3 sites, 2-time phases, 3 groups), showed a three-way time × site × group interaction (p<0.001). Accordingly, control exhibited marked reductions in average *GM echo intensity* (-18±5%) in contrast to both SBF (-1±8%) and LIPA (1±6%). Such reductions also appeared to be more pronounced in distal (25% of muscle length) compared to proximal (50%, & 75%) muscle regions (Please see table 6.1).

GL intervention-induced changes

No main effects were observed for *GL* length, ACSA, or volume. However, regarding *GL* echo intensity the 3×2×3 split plot ANOVA (3 sites, 2-time phases, 3 groups), exhibited a significant three-way time × site × group interaction (p<0.001). Accordingly, control exhibited marked reductions in average *GL echo intensity* (-15±4%) in contrast to both SBF (-0±7%) and LIPA (0±7%). Such reductions also appeared to be more pronounced in distal (25% of muscle length) compared to proximal (50%, & 75%) muscle regions (Please see table 6.2).

VL intervention-induced changes

After accounting for baseline differences, significant main effects for time were observed for *VL* normalised fascicle length (p<0.001, n^2p=0.36), and *VL* fascicle pennation angle (p=0.007, n^2p=0.21) (please see table 6.3 and figure 6.2). No main

effects were observed for *VL* length, ACSA, volume, physiological cross-sectional area, or EI.

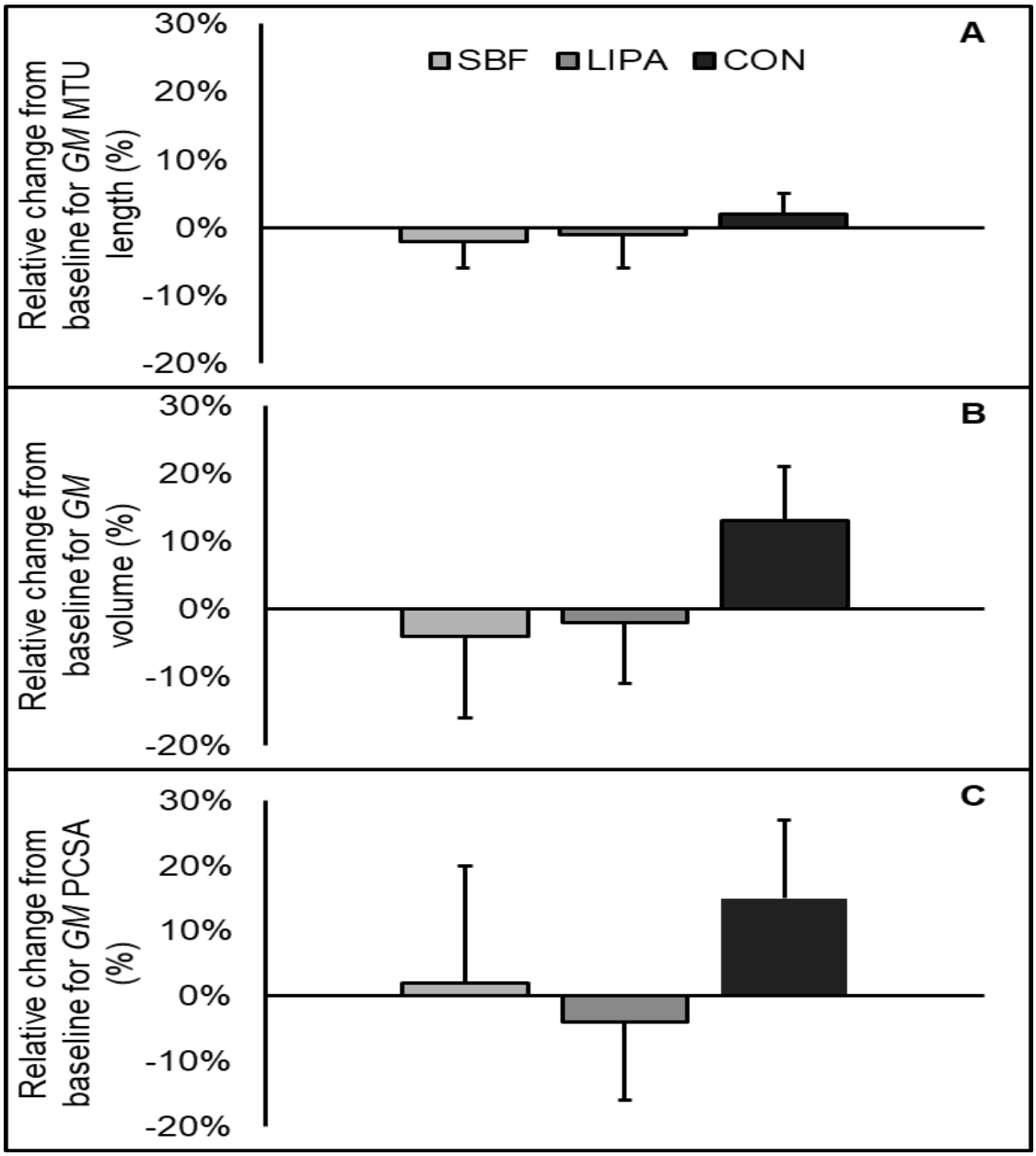

Figure 6.1 - Relative changes from baseline for GM parameters. Panels A, B, & C represent muscle-tendon unit length, volume, and physiological cross-sectional area, respectively. × represents a significant group×time interaction effect (no significant post-hoc differences for *GM* volume). CON, control, GM; Gastrocnemius Medialis, LIPA, light intensity physical activity, MTU; muscle tendon unit, PCSA, Physiological cross-sectional area, SBF; sedentary behaviour fragmentation.

Table 6.1- Pre, Post, and intervention related changes for all Gastrocnemius Medialis outcomes, categorised by group. * represents a significant time effect. ×represents a significant group×time interaction effect.

	(SBF n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
Length (cm)	24±2	25±3	0±2 (0±7%)	24±3	25±5	0±2 (0±6%)	24±1	25±1	1±2 (2±6%)
Average ACSA (cm^2)	28±5	28±6	-1±3 (0±11%)	30±7	29±6	-1±3 (-2±11%)	26±5	29±5	3±2 (12±8%)
Volume (cm^3)	193±33	185±40	-8±26 (-4±13%) ×	213±48	210±46	-3±20 (-1±9%) ×	178±33	199±31	21±14 (13±9%) ×
PCSA (cm^2)	35.2±7.2	35.4±8.0	-0.2±6.3 (2±18%)	37.1±8.1	35.2±7.7	-1.9±4.3 (-4±12%)	29.7±5.8	33.7±4.7	4.0±2.5 (15±12%)
Average echo intensity	118±19	117±20	-1±8 (-1±8%)	112±19	112±15	0±7 (1±6%)	118±13	101±9	-18±5 (-15±3%)
	(SBF n=14)			LIPA (n=13)			Control (n=7)		
FPA (°)	19±3	19±2	-1±4 (-1±21%)	18±3	18±2	-1±2 (-2±10%)	18±3	19±3	1±3 (8±16%)
Fascicle Length (cm)	5.6±1.0	5.3±0.4	-0.3±0.6 (-5±10%)	5.6±1.1	6.1±0.8	0.2±0.5 (4±8%)	6.2±1.3	6.1±1.4	-0.1±0.4 (-1±7%)
Lf-N (cm)	0.2±0.0	0.2±0.0	0.0±0.0(-3±10%)	0.2±0.0	0.2±0.0	0.0±0.0 (3±8%)	0.3±0.0	0.2±0.0	0.0±0.0 (-3±7%)

ACSA; anatomical cross-sectional area, FPA; fascicle pennation angle, Lf-N; normalised fascicle length, LIPA; Light intensity physical activity, MTU; muscle tendon unit, PCSA; physiological cross-sectional area, SBF; Sedentary behaviour fragmentation,

Table 6.2- Pre, Post, and intervention related changes for all *Gastrocnemius Lateralis* outcomes, categorised by group. * represents a significant time effect. ×represents a significant group×time interaction effect

	(SBF n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
Length (cm)	22±3	22±3	-1±1 (-2±5%)	22±3	22±3	0±1 (0±5%)	21±2	21±2	0±1 (0±5%)
Average ACSA (cm^2)	14±5	15±6	0±3 (2±19%)	14±3	16±5	1±3 (10±21%)	15±4	15±3	0±2 (3±12%)
Volume (cm^3)	125±62	133±58	7±27 (7±21%)	132±34	135±34	3±25 (5±20%)	129±33	134±35	5±22 (5±13%)
Average echo intensity	122±17	122±20	0±8 (0±7%)	125±18	124±14	0±8 (0±7%)	127±11	112±11	-15±4 (-12±4%)

ACSA; anatomical cross-sectional area, LIPA; Light intensity physical activity, SBF; Sedentary behaviour fragmentation,

.

Table 6.3- Pre, Post, and intervention related changes for all Vastus Lateralis outcomes, categorised by group. **Boldened text** represents a significant baseline difference. * represents a significant time effect. ×represents a significant group×time interaction effect.

	(SBF n=14)			LIPA (n=14)			CON (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
Length (cm)	32±4	32±4	0±1 (0±4%)	31±2	31±1	0±1 (1±3%)	30±2	31±2	1±1 (3±4%)
50% ACSA (cm^2)	41±9	41±9	-1±6 (-1±15%)	38±6	37±10	0±7 (-1±18%)	35±8	33±7	-1±3 (-4±9%)
Volume (cm^3)	476±185	440±194	-4±113 (0±20%)	431±91	424±106	-16±87 (-4±20%)	395±79	402±79	3±31 (1±7%)
PCSA (cm^2)	93±22	94±23	1±19 (4±21%)	80±16	77±23	-2±17 (-2±25%)	**62±17**	66±19	4±7 (7±11%)
FPA (°)	19±3	18±3	0±3 (0±19%) *	17±2	16±3	-1±3 (-4±22%) *	**14±3**	14±2	0±2 (3±13%) *
Fascicle Length (cm)	5±1	6±1	0±1 (-2±15%)	6±1	6±1	0±1 (5±12%)	**6±1**	6±2	-1±1 (-7±10%)
Lf-N (cm)	0.2±0.0	0.2±0.0	0.0±0.0 (-2±12%) *	0.2±0.0	0.2±0.0	0.0±0.0 (4±19%) *	**0.2±0.0**	0.2±0.0	0.0±0.0 (-10±8%) *
50% echo intensity	99±18	104±19	5±12 (5±12%)	110±15	111±15	1±6 (1±6%)	114±16	101±14	-13±6 (-12±5%)

ACSA; anatomical cross-sectional area, FPA; fascicle pennation angle, Lf-N; normalised fascicle length, LIPA; Light intensity physical activity, PCSA; physiological cross-sectional area, SBF; Sedentary behaviour fragmentation,

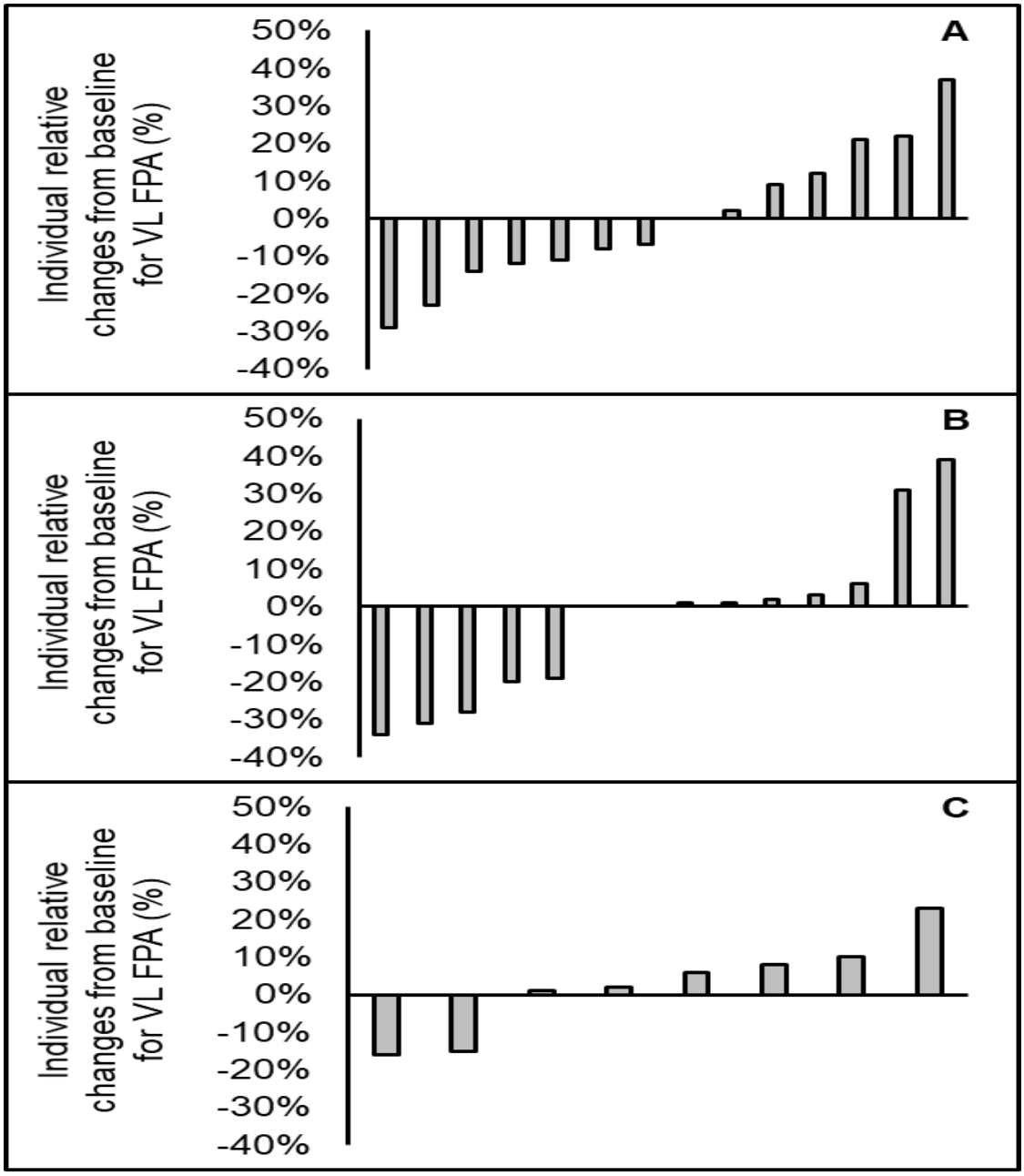

Figure 6.2- Relative changes from baseline for VL FPA. Panels A, B, & C represent SBF, LIPA, & control, respectively. CON; control, FPA; Fascicle pennation angle, LIPA; Light intensity physical activity, SBF; Sedentary behaviour fragmentation, VL; vastus lateralis.

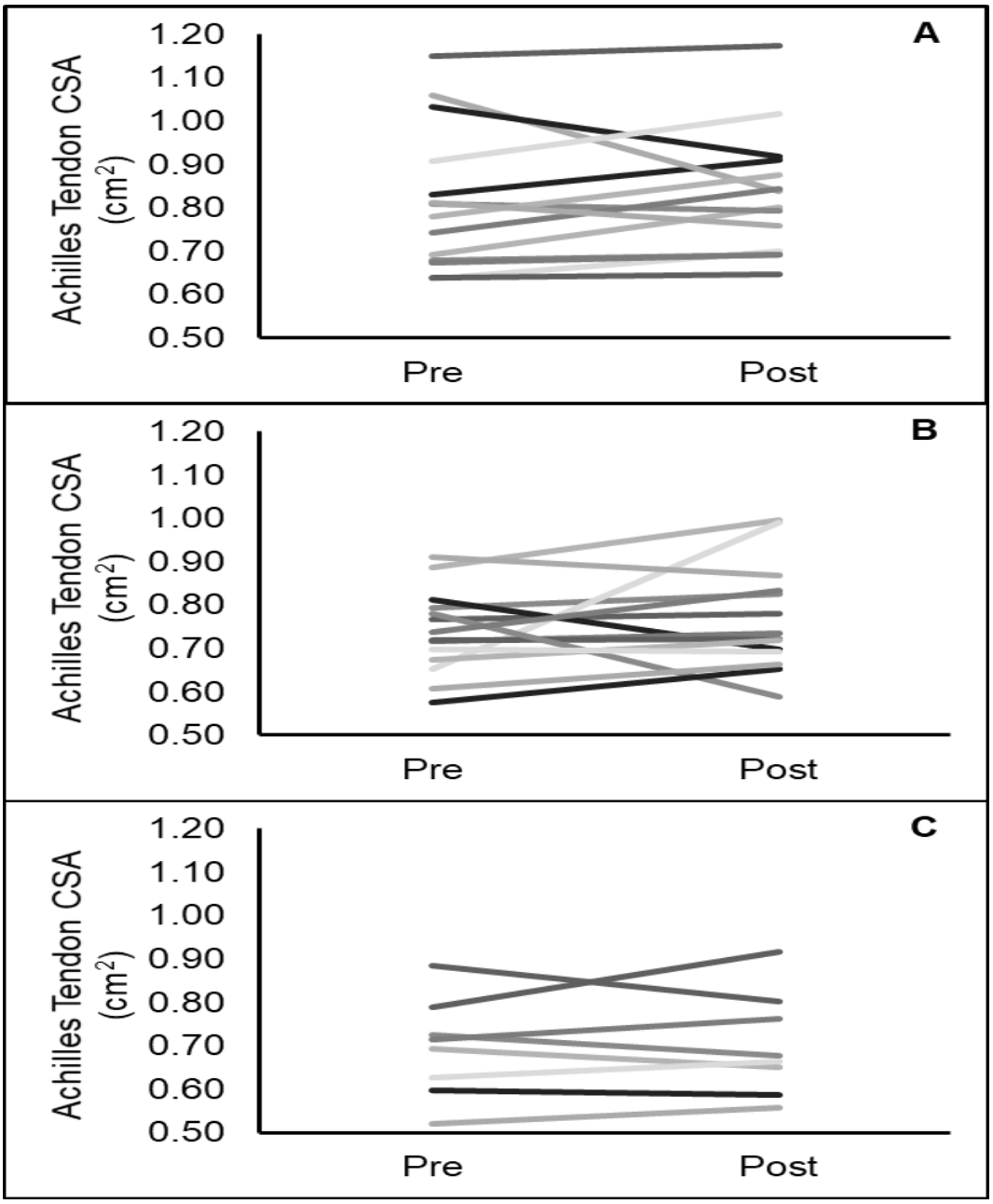

Figure 6.3- Individual changes for Achilles Tendon CSA. Panels A, B, & C represent SBF, LIPA, & control, respectively. CON; control, CSA; cross sectional area, LIPA; Light intensity physical activity, SBF; Sedentary behaviour fragmentation

Achilles tendon intervention-induced changes

Interestingly for both the analysis of the 4 discrete tendon ACSA sites, as-well as the average of all sites, there were no main effects nor interactions (please see table 6.4 and figure 6.3). However, the Achilles tendon echo intensity 4×2×3 split plot ANOVA (4 sites, 2-time phases, 3 groups), exhibited main effects for time ($p<0.001$), site ($p=0.03$), a site×group interaction ($p=0.002$, $\beta=0.95$, $\eta_p^2=0.22$), and a time×site interaction ($p<0.001$, $\beta=1.000$, $\eta_p^2=0.31$). However, this did not result in a significant three-way time × site × group interaction ($p=0.62$) (please see table 6.4 and appendices).

For results and figures on associations between relative changes from baseline for physical behaviour outcomes (SB & LIPA) and relative changes from baseline for MTC outcomes (please see appendices i). Most notably, a significant negative association was exhibited between the change in SB and *GM* physiological cross-sectional area following SBF.

Table 6.4- Pre, Post, and intervention related changes for all *Achilles Tendon* outcomes, categorised by group. **Boldened text** represents a significant baseline difference. * represents a significant time effect. ×represents a significant group×time interaction effect.

	(SBF n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
Length (cm)	17±2	17±2	0±1 (-1±3%)	16±3	15±3	0±1 (-2±5%)	16±2	17±2	1±1 (5±9%)
Average ACSA (cm^2)	0.82±0.16	0.83±0.14	0.00±0.00 (3±11%)	0.74±0.10	0.77±0.12	0.00±0.00 (5±17%)	0.69±0.12	0.71±0.13	0.00±0.00 (3±9%)
Average echo intensity	100±22	104±19	2±9 (2±8%)	100±22	110±24	0±9 (0±7%)	108±5	102±12	-12±5 (-11±4%)

ACSA; anatomical cross-sectional area, LIPA; Light intensity physical activity, SBF; Sedentary behaviour fragmentation,

Discussion

The current chapter is the first to examine MTC adaptation following SB displacement. The aim of this chapter was to quantify MTC hypertrophy and tissue quality related adaptations following SB displacement with LIPA in older women. It was hypothesised that both interventions would induce MTC hypertrophy and enhance tissue related quality. Significant effects for time were observed for *VL* fascicle pennation angle and normalised fascicle length, as-well as a group×time interaction for *GM* muscle volume. Furthermore, *GM*/ *GL echo intensity* exhibited significant time effects further dependant on group and site examined. Unexpectedly however, MTC improvements occurred in the control but not experimental groups. Consequently, the first hypothesis was rejected. It was further hypothesised that muscular adaptation would be disproportionately observed in the *Triceps Surae* (*GM*/ *GL*) group compared to the knee extensor group (*VL)*. Surprisingly, a localised maladaptation in *GM* volume was observed following both interventions, with echo intensity reductions occurring in the *GM*/*GL,* but not the VL. Thus, the second hypothesis was partially rejected. Finally, it was hypothesised SBF would induce greater MTC adaptation compared to LIPA. Despite no differences between experimental groups, changes in *GM* physiological cross-sectional area following SBF (2%), were mediated by measurably reduced SB. Consequently, the final hypothesis was partially upheld.

LIPA implementation (regardless of pattern) failed to elicit significant improvements in *GM*, *GL*, or *VL* muscle volume/ physiological cross-sectional area. Considering the novelty of these findings, low intensity resistance training resistance training [≤50% one repetition maximum (1RM)] (169, 444), offers the closest means of comparison. Accordingly, a single low intensity resistance training bout (40% 1RM) stimulates myofibrillar protein synthetic response (440). However, only slow tempo lifting through the entire range of motion, significantly improves quadriceps muscle thickness following 10 weeks of low intensity resistance training (30-50% 1RM) in older adults (169, 444). In parallel, utilising full range of motion during low intensity resistance training results in greater hypertrophy compared to partial range of motion (445). Consequently, LIPA should have theoretically provided enough intensity, but the lack of direct supervision likely led to variability in movement execution (range of motion/ contraction tempo). Furthermore, low volume (3 sets) low intensity resistance training appears inferior to high volume (6 sets), regarding the ability to stimulate myofibrillar

protein synthesis response in older adults (440), suggesting increasing training volume over time is essential for hypertrophy. In contrast, the current study prescribed a generic increase in LIPA, which was not progressed throughout the intervention period. Accordingly, increasing older adults walking time over 6 months, increases skeletal muscle mass (360, 437). Furthermore, considering the role body weight plays in MTC adaptation (446), variations in participants mass did not allow specific standardisation of training load for body weight based movements. LIPA interventions may therefore require to be carried out over longer periods to compensate for the lack of overload.

The group×time interaction for *GM* volume, was driven through an increase in the control group. Whilst experimental participants were instructed to maintain habitual MVPA, they were advised to avoid high-speed activities during SB displacement, ensuring LIPA replaced SB, which may have unintentionally reduced habitual gait. Accordingly, a 25% reduction from self-selected walking speed drastically reduces plantar flexor (PF) muscle activity (447), whereas faster walking speeds increase PF recruitment (448). Furthermore MVPA is associated with mid-calf muscle density in older adults (136). Accordingly, a significantly greater proportion of control participants commenced testing during summer months and exhibited significantly higher MVPA levels at baseline compared to experimental participants (chapter 3). Therefore, a maintained/or season-induced increased habitual gait in the control group, likely enhanced *GM* adaptation stimulus. However, the average change in *GM* volume following control (21cm^3) was < 1 times the typical error calculated during reliability analysis, suggesting this was not a meaningful change.

A trend toward a group×time interaction was observed for *GM* muscle-tendon unit length. Accordingly, MVPA is also associated with *GM* muscle-tendon unit length in older adults (385). Therefore, unintentional reductions in habitual gait also likely mediated the experimental reduction in *GM* muscle-tendon unit length. Older women exhibit smaller *GM* muscle-tendon unit lengths compared to younger women, which reduces maximal dorsiflexion range of motion (449), potentially compromising force-producing capability (381). However, a positive association has previously been identified between SB and angle of peak torque (i.e. closer to plantar flexion) in older adults (385), suggesting an increase in *GM* muscle-tendon unit length with decreased SB time, which supports previous evidence demonstrating angle of peak torque

shifting toward longer muscle lengths post-training (450). Alterations in *GM* muscle Lf may have also contributed to the proposed change in angle of peak toque. However, only trends toward change were observed for *GM* Lf. Furthermore, median SB bout length has also been associated with *GM* muscle-tendon unit length in older adults (385). Given that both experimental groups exhibited similar reductions in *GM* muscle-tendon unit length and muscle volume, this suggests *GM* maladaptation following LIPA implementation occurs independent of prescribed pattern.

Trends toward group×time interactions were observed for *GM* Lf and *GM* physiological cross-sectional area. Accordingly, LIPA increased *GM* Lf on average (4%), in contrast to both SBF (-5%) and control (-1%). In support eight weeks of light dancing increases *GM* Lf in older women by ~10% (436) and walking based SB displacement preferentially stimulates the *Triceps Surae* musculature in older adults (91). Therefore, greater time spent ambulating following LIPA may have generated the region-specific increase in *GM* Lf. Considering Lf represents the amount of sarcomeres in series, and is thus a major determinant of maximum shortening velocity (451), this may represent a shift toward greater *GM* contraction velocity capabilities. Accordingly 50% of the differences in maximum shortening velocity between young and old adults are explained by a reduction in *GM* Lf (452). However, the observed average change in *GM* Lf following LIPA (0.2cm) was not 1.5 to 2.0 times outside of the typical error calculated during reliability analysis (3.8cm), and thus cannot be considered a meaningful change (263).

Furthermore, the change in SB was also negatively associated with the change in *GM* physiological cross-sectional area (Mean change: 2%) following SBF (please see appendices i). In support, SB and standing time have previously been negatively and positively associated with *GM* physiological cross-sectional area in older adults respectively (385). Considering SBF participants were instructed to fragment SB this frequent standing stimulus appears to have generated small yet statistically insignificant improvements in *GM* physiological cross-sectional area. This is promising considering physiological cross-sectional area is directly linked to a muscle's maximum isometric force producing capabilities (424). Nevertheless, significant reductions in both *VL* fascicle pennation angle and *VL* normalised fascicle length were observed in all groups. In contrast, eight weeks of light dancing increases both *VL* fascicle pennation angle (~21%) and *VL* Lf (~11%) in older adults (436). Given that

increased fascicle pennation angle is associated with increased force transmission (233, 373), this also suggests adverse knee extensor maladaptation. However, 13/28 (46%) of experimental participants increased *VL* fascicle pennation angle post intervention, suggesting minor alterations in muscle architecture may still mediate a shift toward greater contraction velocity capabilities. Furthermore, the average change in *VL* fascicle pennation angle (~1°) was only 1 time the typical error calculated during reliability analysis.

SB displacement with LIPA similarly failed to elicit tendon hypertrophy. In contrast extreme low intensity resistance training (≤20% 1RM), has been shown to enhance strength in older adults (171, 357). However, human tendon mechano-sensitivity is less clear (435). Accordingly, following repeated mechanical loading, tenocytes sense loading induced deformations (430), triggering anabolic and catabolic pathways (431). However, previous studies implementing low intensity resistance training have concluded, tendon hypertrophy requires a training intensity threshold (≥40% 1RM) (432, 453), below which collagen synthesis is not initiated (454). In fact, 12 weeks of resistance training in older adults failed to induce patellar tendon (PT) hypertrophy, regardless of training intensity (453), further putting into question the likelihood of training-induced tendon hypertrophy. Nevertheless, such findings further question the universality of training-induced alterations in tendon size.

Significant improvements in tissue related quality were observed in the *GM/ GL*, but not *VL*. In support, 3 weeks of high-intensity interval training in middle-aged overweight/obese adults did not improve *VL* EI, determined through similar panoramic imaging (455). echo intensity is now recognised as a valid intramuscular fat quantification tool (426, 427). Intramuscular fat increases with age (irrespective of BMI) (456), with physical activity strongly associated with decreased intramuscular fat in older adults (457-459). Whilst such findings provide further support to the original disproportionate *Triceps Surae* adaptation hypothesis, greater improvements were observed in the control group. The aforementioned maintenance of habitual gait following control may have mediated this effect, considering higher intensities of activity mediate long term intramuscular fat infiltration in older adults (460)**.** Accordingly, increased step count (461), self-reported MVPA (434), and high force eccentric exercise (Ergometer) (458, 462) are all associated with reductions in intramuscular fat , and echo intensity over time, in older adults. In support, endurance

exercise stimulates lipolysis in humans, thus assisting with mobilisation and subsequent oxidation of intramuscular triglyceride stores (463). Consequently, in contrast to the current intervention study it seems clear that significant reductions in intramuscular fat, require longer time frames (≥12 weeks), as well as some combination of high training intensity and/ or eccentric muscular contraction. This may highlight a potential limitation of SB displacement with LIPA regarding the ability to mobilise intramuscular fat. In support, the average changes in GM and GL echo intensity following control were 2.4 and 3.5 times outside of the typical error calculated during reliability analysis. Therefore, only the change in echo intensity following control can be considered meaningful, in contrast to both experimental groups (1 times the typical error).

Interestingly, a significant time×site×group interaction was observed for *GM/GL* EI, with higher echo intensity observed in distal compared to proximal regions at baseline. Similarly to the current study for the VL, previous studies have merely assessed echo intensity in one site per muscle examined (227, 228), whereas differences in echo intensity have previously been observed across different *rectus femoris* regions (464). In support, intramuscular fat disproportionately displaces contractile tissue to a greater extent in distal compared to proximal muscle regions following specific types of muscle dystrophy (465, 466). Given greater intramuscular fat intramuscular fat stores at baseline, distal sites also likely had a greater relative capacity for echo intensity reduction, which may have mediated the greater distal echo intensity reduction. Nevertheless, distal specific muscle hypertrophy occurs following resistance training specific modalities, like eccentric (467), full range of motion (445), and constant load (468, 469) . However, eccentric resistance training has previously been linked to specific alterations in intramuscular fat in older adults (458, 462, 470). Such research supports a site-specific echo intensity change, whereby unaccustomed distal sarcomeres undergo greater adaptation, due to increased distal loading with greater exercise intensity/duration (471). Furthermore, site specific changes in GM/GL echo intensity over time, also differed by group. This suggests the reduction in *GM*/*GL echo intensity* was likely mediated by a maintained/or season-induced increased habitual gait in the control group, leading to pronounced echo intensity in distal regions via the in-series sarcomere mechanics described above.

Significant improvements in Achilles tendon tissue related quality were also observed. echo intensity is a reliable method to characterise internal tendon structure (472), and has been linked with tendon mechanical behaviour (232). However, there were no main interaction effects involving the intervention groups, suggesting displacing SB with LIPA had relatively little effect on tendon echo intensity compared to control. Material alterations following SB displacement with LIPA may require longer intervention periods to be observed. In the same way, 6 months of low intensity resistance training (bodyweight squats) (473), and progressive walking training (437) in older adults, failed to induce any change in tendon stiffness. Twelve weeks of low intensity resistance training also fails to affect young's modulus, and thus intrinsic tendon properties in older adults (474). Instead, 12 months of high intensity loading improves tendon mechanical properties in older adults (475). Consequently, only high intensity resistance training appears to improve older adults tendon mechanical quality (428), principally due to material alterations (432). Therefore, alterations in tendon mechanical quality also appear to depend on achieving a loading magnitude threshold (≥40% 1RM) (474) and/or longer-term intervention periods (476). Accordingly, compromised tendon tissue quality (adverse cross links), is observed when comparing age-matched inactive controls to lifelong runners (477), without differences in mechanical quality (431), supporting the need for longer intervention periods, to induce material alteration with low magnitudes of loading. Nevertheless, reductions in Achilles tendon echo intensity over time were greater in distal echo intensity regions. Accordingly, regional differences in tendon strain have been observed during isometric contractions (478) further dependant on joint angle and thus range of motion (479). Furthermore, region specific Achilles tendon hypertrophy has been linked to strength increases following resistance training in older adults, primarily due to the variability in tensile stress forces along the length of the tendon (480). Whilst this creates a plausible mechanism for region specific echo intensity changes, it is still yet undetermined whether distal specific reductions in Achilles tendon EI, affects the mechanical quality of tendon tissue, and thus warrants further investigation. Moreover, despite the reliability of echo intensity to characterise both internal tendon structure (472), and tendon mechanical behaviour (232), significant changes in tendon mechanical quality have not yet been clearly linked with changes in EI. Consequently, changes in tendon mechanical quality may have occurred following the intervention unrelated to the change in echo intensity and this requires further investigation.

Strengths and Limitations

Given the recruitment of older women, this does limit the generalisability of the current findings. However this can also be viewed as a strength given that MTC adaptation following low intensity resistance training may be gender dependent (481). The original hypothesis prioritized investigation of the *Triceps Surae* (2 muscles, 3 measurement sites) over the knee extensors (1 muscle, 1 measurement site). Thus being restricted to a single ACSA measurement site in the *VL* likely underestimated regional size differences (482-484). Together with a relatively small probe length (38mm) this may have also underestimated *VL* muscle architecture, and physiological cross-sectional area as a result.

Therefore, together with implementing longer intervention periods (>8 weeks) to compensate for the limited degree of overload, future studies should pursue more detailed investigation of the *Quadriceps Femoris* muscle group as a whole. A further limitation of this chapter was linked to the collection of echo intensity data. time gain compensation compensates for the attenuation of ultrasound energy with depth (485, 486), affecting image brightness, and allowing the technician to emphasise/de-emphasise a viewing region. However, it was discovered towards the end of data collection that this also affects EI, which is problematic considering there is no standardised descriptor reporting on the position of the time gain compensation sliders. Although great care was taken to ensure consistency of acquisition parameters, altering the position of the sliders is a rudimentary occurrence (accidental shifts, position from previous technician etc). Unfortunately, it was not feasible to retrospectively investigate what effect this had on the tissue quality data results. Alas, future studies should attempt to quantify the affect this previously unaccounted factor has on echo intensity values, whilst also determining what extent limited MTC adaptations mediate functional improvement following SB displacement with LIPA in older adults. Accordingly, *VL* muscle size has been identified as a small yet significant independent predictor of gait speed in older adults (443), whilst a large proportion of the variance in postural balance ability is mediated by characteristics of the *Triceps Surae* MTC (133). Despite MTC adaptations following SB displacement with LIPA potentially being small in magnitude and thus not reaching statistical significance, such changes may still hold clinical relevance through mediating functional adaptation in those adults closer to lower levels of physiological reserve. Finally, Achilles Tendon

ACSA may vary considerably across Achilles tendon length depending on participant size. This may have affected Achilles Tendon ACSA assessment in the current thesis, considering absolute increments were used (every 1cm along length), which may have led to an overfocus on the distal region. Perhaps future investigations could use relative increments across the length of each participants Achilles Tendon (e.g. every 1% of length).

Conclusion

In conclusion, displacing SB with LIPA (irrespective of prescribed pattern) fails to elicit significant MTC adaptation. In-fact significant improvements were counter-intuitively observed within the control group (though potentially owing to a seasonal effect on habitual physical behaviour). Interestingly, distal adaptations were observed for tissue related quality parameters, suggesting a region-specific effect. Future studies should determine to what extent such changes mediate functional improvement in older adults following SB displacement.

Chapter 7 – The effects of displacing sedentary time with light intensity physical activity on body composition in older women

Data from the current chapter are published in/ presented at (please see research outputs in appendices ii):

Minimising sedentary behaviour (without increasing medium-to-vigorous exercise) associated functional improvement in older females is somewhat dependant on a measurable adaptation in muscle size. *AGING (2020)*. Dale Grant*, David Tomlinson, Kostas Tsintzas, Petra Kolić, Gladys L. Onambele-Pearson. https://dx.doi.org/10.18632%2Faging.202265

The Effects of Displacing Sedentary Behavior With Two Distinct Patterns of Light Activity on Health Outcomes in Older Adults (Implications for COVID-19 Quarantine). *Frontiers in Physiology* (2020). Dale Grant*, David Tomlinson, Kostas Tsintzas, Petra Kolić, Gladys L. Onambele-Pearson. https://doi.org/10.3389/fphys.2020.574595

Poster Title: *Displacing Sedentary Behaviour with light intensity activity improves bone mineral density in older females*. Presented online at the Bone Research Society Annual Meeting (06/07/2020-08/07/2020) https://boneresearchsociety.org/meeting/brs2020online/

Chapter take home message: A significant increase over time was observed for total-spine bone mineral density (BMD), and thoracic spine BMD, driven by increases in both experimental groups. Interestingly, leg BMD exhibited a group×time interaction driven by a significant difference between sedentary behaviour fragmentation (SBF) and control. Significant reductions in body fat percentage (BFP%) were observed within the control group.

Abstract

The effects of displacing Sedentary Behaviour (SB) with light intensity physical activity (LIPA) on body composition [Lean body mass, bone mineral density (BMD), body fat percentage (BFP%)] in older adults is still unclear. Therefore, the aim of this chapter was to examine changes in body composition following 8-weeks of SB displacement with LIPA in older women. It was hypothesised SB displacement would cause beneficial body composition changes (reduced adiposity, improved lean body mass, and enhanced BMD). Thirty-six older women (73±5 years) were allocated to one of three groups: 1) sedentary behaviour fragmentation (SBF) (*n*=14), 2) continuous LIPA (*n*=14), or 3) control (*n*=8). Body composition was assessed using a whole-body Dual X-Ray Absorptiometry scanner at weeks 0 and 8. A significant increase over time was observed for total-spine BMD (p=0.048), and thoracic spine BMD (p=0.003), driven by increases in both experimental groups (SBF: 5%, LIPA: 4%). Interestingly, leg BMD exhibited a group×time interaction (p=0.04) driven by a significant post-hoc difference between SBF (1%) (p=0.04) and control (-1%), but not LIPA against any other group. Significant reductions in BFP% were observed within the control group, despite all groups being classified as obese at baseline [BFP% ≥35%]. Lean body mass was unaltered. In conclusion, displacing SB with LIPA in older women leads to overall improved body composition. General displacement of SB with LIPA (irrespective of prescribed pattern) increased Thoracic-spine BMD, whilst counterintuitively increasing BFP%. Nevertheless, displacing SB with LIPA in a more fragmented pattern, generated region-specific changes including enhanced leg BMD, and increased android fat tissue content. Greater SB fragmentation also appears to mediate this enhanced body composition effect, producing marked beneficial/detrimental effects dependant on the tissue/ region examined.

Introduction

Sedentary behaviour (SB) is higher among older adults (71) and is strongly associated with diminished physical function (121-123, 131). Accordingly, lower lean body mass than predicted to be required for physical independence [Relative appendicular skeletal muscle mass, < 7.0kg/m^2 for men, and <5.5kg/m^2 for women], defined as pre-sarcopenia (487, 488), increases the risk of both compromised function (489), and cardio-metabolic morbidity (490). Although, chronological ageing is associated with a substantial rapid decline in lean body mass of ~0.5-1.0% per year (82, 375, 491, 492), both self-reported (51, 417) and objectively assessed (53) SB are associated with accelerated pre-sarcopenia in older adults independent of moderate to vigorous physical activity (MVPA). Accordingly, total lean body mass is lost at a rate of ~0.5-0.6% per day during ~10-42 days of bed rest in older adults (85, 86). Interestingly, women tend to exhibit greater anabolic resistance compared to men (153, 154), suggesting a greater susceptibility to muscle atrophy during disuse. Furthermore, ~14 days of step reduction (↓~76%), decreases leg lean body mass by ~1.5-4.0% (87-89). Chronological ageing is also associated with altered bone tissue mechanical properties (493), causing a shift towards porosity (494), and a substantial rapid decline in bone mineral density (BMD) of ~3% per year (80, 495-497). Irrespective of MVPA, SB engagement also accelerates age related BMD decline (111, 112), which in turn increases frailty risk (80, 81) in older adults. Whole body BMD is typically used to broadly classify individuals into one of three BMD health categories, normal [T-score <1.0 standard deviation (SD) below sex-matched reference population (Average 30-year old)], Osteopenic (T-score >1.0 - <2.5 SD below), and Osteoporotic (T-score >2.5 SD below) (498). Accordingly, physical activity is associated with a maintenance of BMD over time (98-101), primarily due to the frequent mechanical loading stimulus (102, 103). Such loading increases lower body BMD (104-106), and reduces fracture risk (107, 108) in older adults. Conversely, activity cessation results in decreased lower body (104, 105) and spine (109, 110) BMD in older adults.

Obesity is conventionally defined as a BMI of ≥30kg/m^2 (499), whereas the gold standard for obesity determination is the world health organisation's (WHO) criterion reference standard, based on total BFP% (≥25% for men, & ≥35% for women) (499, 500). Both self-reported (92, 501) and objectively assessed (93) SB time are associated with increased age related upturns in obesity (increases in both BFP%, &

Waist to hip Ratio) (83, 502), even when adjusted for MVPA. Accordingly, visceral abdominal adiposity [waist circumference ≥ 102cm for men, & ≥ 88cm for women or a Waist to hip Ratio ≥ 0.90 for men, & 0.85 for women (503-505)], is also a proxy measure of ectopic fat deposition (506-510). In support further studies have also demonstrated a strong association between SB and visceral-abdominal adiposity in older adults (46, 52, 511, 512). Interestingly, reduced physical activity and increased SB are both associated with sarcopenic obesity (pre sarcopenia combined with excess adiposity accumulation) (502) in older adults (52, 513). Accordingly, sarcopenic obesity is associated with diminished skeletal muscle quality (55, 202, 514) compromised functional ability (52, 515) and mortality (77-79) in older adults Ultimately, SB appears to exacerbate the risk of all three adverse body composition states (reduced BMD/ lean body mass, & excess adiposity accumulation), posing a significant challenge to the long-term health and vitality of older adults.

Fortunately and in parallel, light intensity physical activity (LIPA) is a promising SB displacement option, and has previously been associated with reduced BMI (365), enhanced BMD (112), and reduced fracture risk (107) in older adults. However, the association between LIPA and enhanced BMD in older adults, was exhibited after accounting for body weight metrics (112), suggesting body weight influences mechanical loading of bone during LIPA, and thus bone tissue remodelling (203). Nevertheless, older adults at greater risk of osteopenia/osteoporosis obtain greater BMD enhancing utility from LIPA, as was shown in a recent longitudinal study (516). Considering age related lean body mass declines, older adults may also have greater capacity to gain lean tissue following physical activity (373).

SB that is accumulated in a prolonged uninterrupted pattern is linked with greater obesity (83, 502), accelerated pre-sarcopenia (51, 53), greater age related BMD decline (111, 112), and increased frailty risk (80, 81) in older adults. This suggests prolonged SB may be more detrimental to body composition outcomes in older adults, compared to a more fragmented SB pattern. Accordingly, interrupting SB every 20 minutes with 2 minutes of LIPA, increases net energy expenditure by ~0.33 kcal/min, compared to continuous sitting in younger adults (91, 94, 95). Frequent LIPA interruptions during bed rest also perturbs BMD loss in younger adults (109, 113). In older adults specifically, frequent LIPA interruptions to prolonged SB, significantly stimulates whole body skeletal musculature, compared with prolonged sitting (90, 91).

Together these findings suggest frequently displacing SB with frequent bouts of LIPA (SBF) may further enhance body composition outcomes in older adults. However, whether SB displacement with LIPA (SBF or continuous), produces a sufficient hypertrophic, or osteogenic stimulus over a chronic intervention period is as yet undetermined.

Therefore, the aim of this chapter was to examine the effects of displacing SB with LIPA on lean body mass, BMD, and adiposity in older adults. It was hypothesised that SB displacement with LIPA, would cause significant reductions in adiposity, improve lean body mass, and enhance BMD. It was further hypothesised that the fragmentation group would undergo greater region-specific enhancements in BMD, lean body mass, and adiposity outcomes.

Results

Baseline Differences

During baseline Dual X-Ray Absorptiometry scanning no significant between group differences (p=0.12) were identified, regarding those who had to be placed in a prone arm orientation (SBF: 50%, LIPA: 79%, CON: 88%) and those who were placed in mid prone (SBF: 50%, LIPA: 21%, CON:12%) (please see table 7.1). Similarly, groups were significantly matched at baseline for all variables of interest. No significant baseline differences existed between groups regarding the proportion of participants classified as pre-sarcopenic (p=0.30), obese (p=0.30), or osteoporotic/ osteopenic (p=0.65). Accordingly, 44%, 83%, and 5% (25%) of participants, were classified as pre-sarcopenic, obese, and osteoporotic (osteopenic) at baseline, respectively. Interestingly, when defined by waist circumference and WHR, obesity prevalence ranged from 75% to 92% respectively (please see table 7.1). Similarly, no significant differences existed between groups at baseline for either waist circumference (p=0.91), or Waist to hip Ratio (p=0.12) defined obesity.

Bone Mineral Density Changes

Following accounting for the aforementioned co-variates (android:gynoid ratio, total body fat tissue, body mass index), total spine BMD significantly increased over time (p=0.048, n^2p=0.12), an effect which was similar for all groups (p=0.69) (please see table 7.3). Sub-analysis of the spine revealed this was driven through a significant time

effect in the thoracic (p=0.003, n^2p=0.14), but not the lumbar (p=0.45) region. Despite thoracic spine changes over time being statistically similar between groups (p=0.20), both experimental groups exhibited marked increases (SBF:0.04±0.12g/cm^3, LIPA:0.04±0.09g/cm^3), in contrast to control (-0.03±0.10g/cm^3) (please see table 7.3 and figure 7.1 panel A). Leg BMD exhibited a significant group×time interaction (p=0.04), where the primary mediator was the difference between SBF and control (p=0.04, *r* = 0.45) (please see table 7.3 and figure 7.1 panel B). Accordingly, the average change in SBF, LIPA, and control was 0.01±0.01g/cm^3, 0.004±0.02g/cm^3, and -0.01±0.01g/cm^3, respectively.

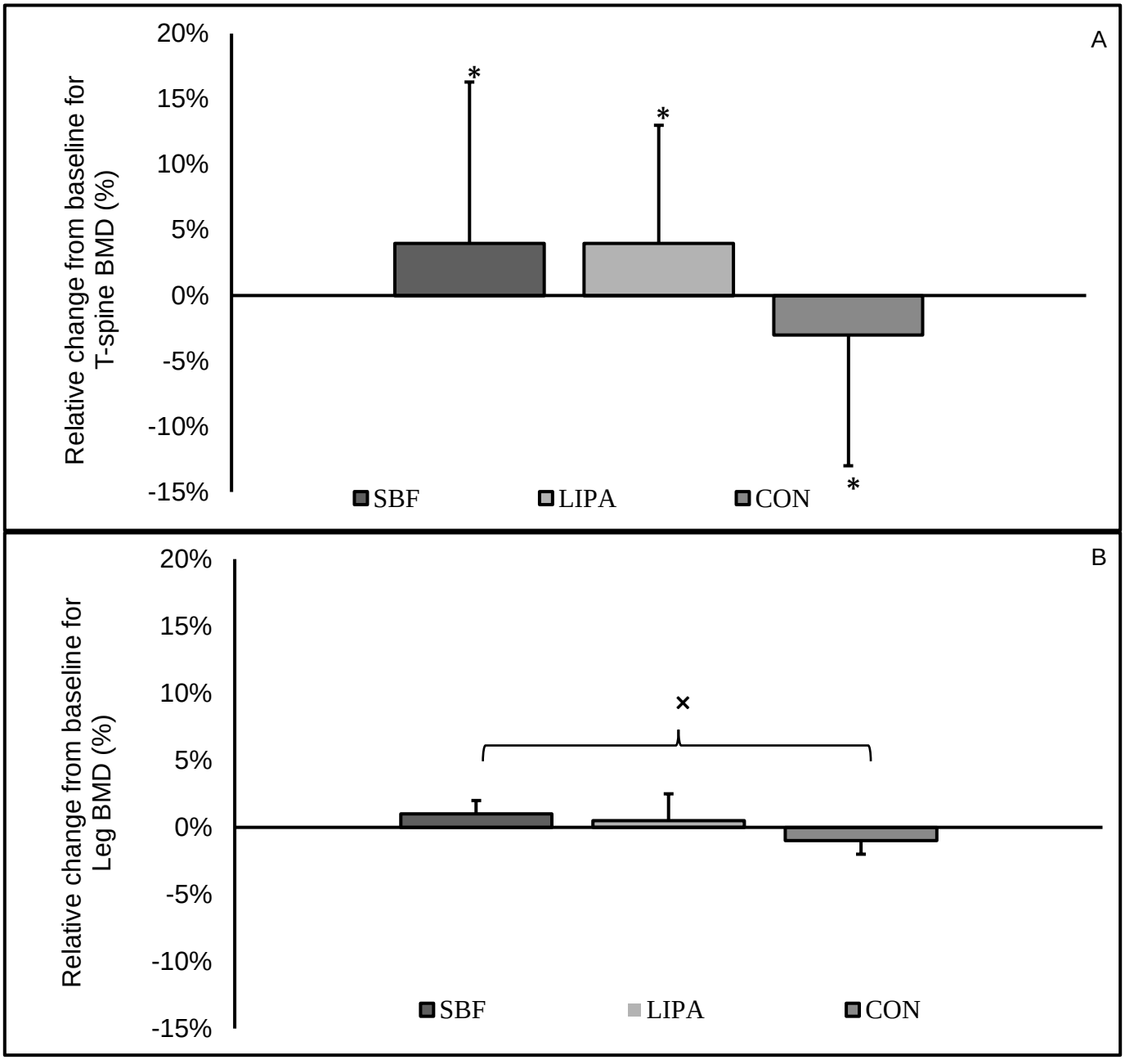

Figure 7.1- Relative changes from baseline for T spine (panel A) and Leg (panel B) bone mineral density. *represents a significant time effect. × represents a significant group×time interaction effect (significant post-hoc difference between SBF and CON, p=0.004, as well as LIPA and CON, p=0.01 for Leg BMD). BMD; bone mineral density, CON; control, LIPA; light intensity physical activity, SBF; sedentary behaviour fragmentation. Post-hoc testing revealed SBF and control (p=0.04) for leg BMD.

Total Fat and Body Fat % Changes

Significant group×time interaction effects were also observed, for right leg fat tissue (p=0.05), Gynoid fat tissue (p=0.007), and total body fat tissue (p=0.004) (please see table 7.2). Further significant group×time interactions were observed for right arm %fat (p=0.03), average arm %fat (p=0.009), trunk %fat (p=0.03), average leg %fat (p=0.03) (please see table 7.5), Gynoid %fat (p=0.007), and total BFP% (p=0.004) (please see figure 7.2). Despite observing multiple group×time interactions, only right arm %fat, average arm %fat, and total BFP% exhibited significant post-hoc differences. Interestingly, both Gynoid fat tissue (β=0.84), & total body fat tissue (β=0.88), were adequately powered to detect a group×time interaction and thus post-hoc differences, whereas average leg %fat (β=0.65), and trunk %fat (β=0.69) were not. Accordingly, post-hoc testing for total BFP% revealed the difference between the change in control (-1.7±1.2, -5±3%), in comparison to both SBF (p=0.004, *r* = 0.71, 0.4±2.8, 1±8%) and LIPA (p=0.01, *r* = 0.6, 0.7±2.8, 2±7%), was the primary mediator for the observed group×time interaction (please see table 7.5 and figure 7.2). This was similar for right arm %fat, and average arm %fat.

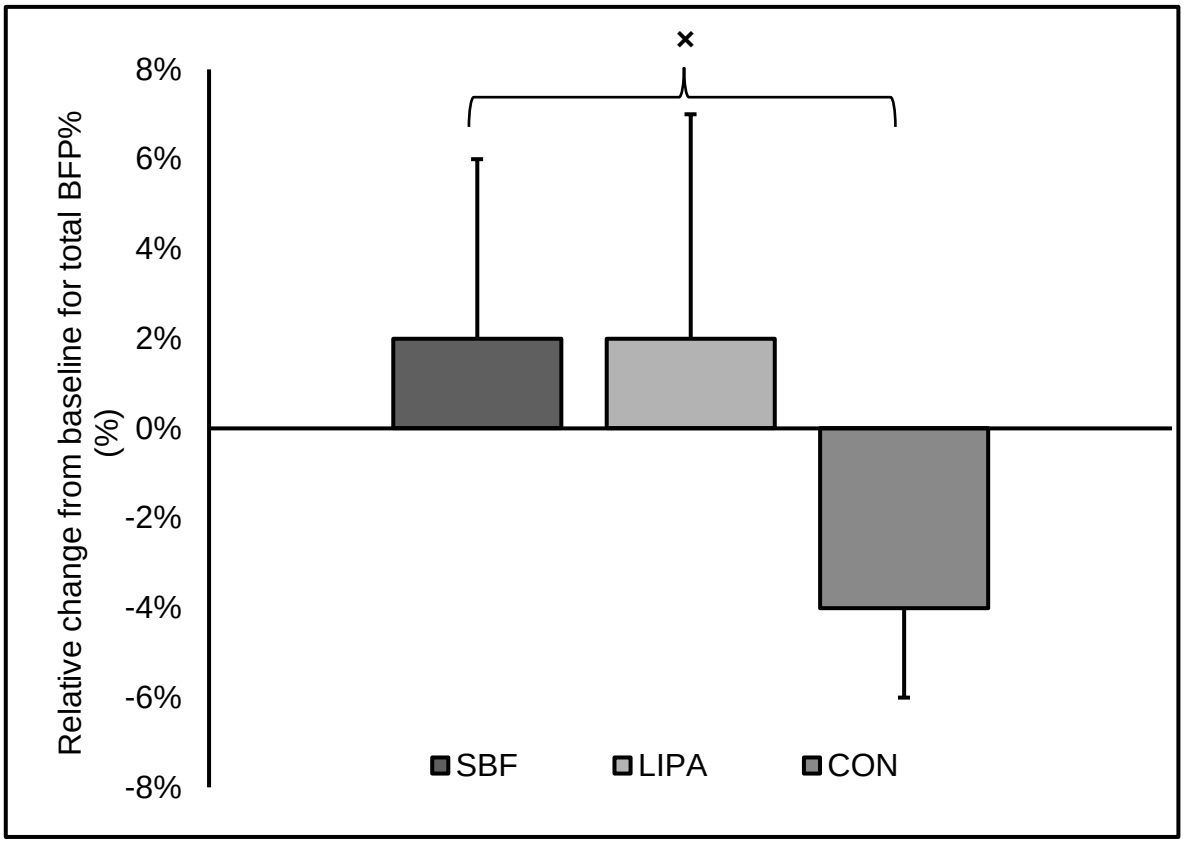

Figure 7.2- Relative changes from baseline for total BFP%. × represents a significant group×time interaction effect. BFP%; body fat percentage, CON; control, LIPA; light intensity physical activity, SBF; sedentary behaviour fragmentation. Post-hoc testing for total BFP% revealed the difference between the change in control in comparison to both SBF (p=0.004) and LIPA (p=0.01).

Similar trends toward a group×time interaction were observed for hip circumference (p=0.07), total lean body mass (p=0.08), average arm fat tissue (p=0.051), trunk fat tissue (p=0.07), left leg fat tissue (p=0.08), and average leg fat tissue (p=0.06). Finally, an isolated trend toward a main effect of time for android: gynoid fat percentage ratio (p=0.08), was observed.

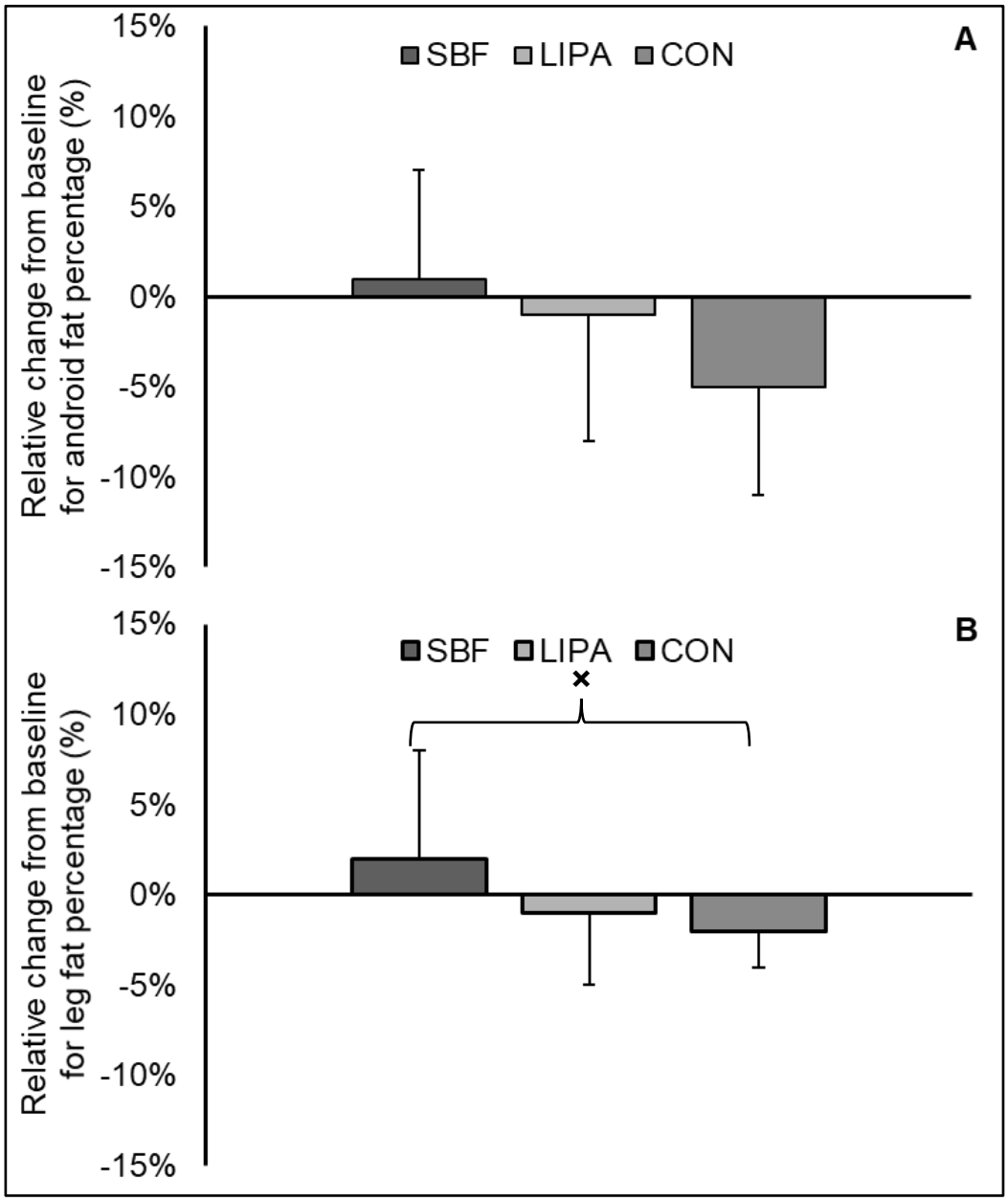

Figure 7.3- Relative changes from baseline for Android (Panel A), and Leg (Panel B) fat percentage. × represents a significant group×time interaction (no significant post-hoc difference for leg fat percentage). CON; control, LIPA; light intensity physical activity, SBF; sedentary behaviour fragmentation.

For results and figures on associations between relative changes from baseline for physical behaviour outcomes (SB & LIPA) and relative changes from baseline for body

composition outcomes, please see appendices i. Notable outcomes with which SB/LIPA were associated included android fat percentage, total fat tissue, and T-spine BMD.

Table 7.1- Baseline characteristics and body composition parameters.

		SBF (n=14)	LIPA (n=14)	Control (n=8)
Age (y)		74±5	73±6	70±3
Height (m)		1.6±0 1	1.6±0.1	1.6±0.1
Weight (kg)		68.6±11.3	65.5±8.9	65.4±9.7
BMI (kg.m^2)		26.9±3.6	25.3±3.6	26.2±3.7
Dual X-Ray Absorptiometry hand position	Prone/ Mid-Prone (%)	50% / 50%	79% / 21%	88% / 12%
Proportion classified as Pre-sarcopenic (Non-Sarcopenic) (%)		29% (71%)	57% (43%)	50% (50%)
Proportion classified as Obese (Total body fat ≥35%)/ Non-Obese (%)		71%/ 29%	93%/ 7%	88%/ 12%
Proportion classified as Sarcopenic-Obese (%)		14%	50%	50%
Proportion classified as Obese (Waist circumference ≥88cm)/ Non-Obese (%)		71%/ 29%	79%/ 21%	75%/ 25%
Proportion classified as Obese (Waist to hip ratio ≥0.85) / Non-Obese (%)		100%/ 0%	93%/ 7%	75%/ 25%
Proportion classified as Osteoporotic/ Osteopenic (Normal) (%)		7% / 21% (72%)	7% / 36% (57%)	0% / 13% (87%)
Proportion classified as Osteo-Sarcopenic-Obese (%)		0%	21%	13%

BMI; body mass index, LIPA; light intensity physical activity, SBF; sedentary behaviour fragmentation.

Table 7.2- Adiposity based outcomes as a factor of the intervention. Stratified by group. * represents a significant time effect. ×represents a significant group×time interaction effect.

	SBF (n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
Waist to Hip Ratio	0.95±0.08	0.96±0.09	0.01±0.04 (1±4%)	0.91±0.06	0.92±0.05	0.002±0.03 (0.4±3%)	0.94±0.08	0.94±0.07	-0.001±0.02 (-0.03±2%)
Android:Gynoid Ratio	0.94±0.15	0.93±0.14	-0.01±0.06 (-1±6%)	0.93±0.18	0.91±0.17	-0.03±0.05 (-3±6%)	0.87±0.10	0.85±0.12	-0.01±0.03 (-1±4%)
Total fat tissue content									
Average of both Arms (kg)	1.61±0.48	1.67±0.51	0.06±0.11 (4±7%)	1.51±0.37	1.53±0.36	0.02±0.11 (2±8%)	1.58±0.44	1.51±0.39	-0.07±0.13 (-4±7%)
Trunk (kg)	12.29±3.08	12.46±3.18	0.18±1.13 (2±9%)	11.91±2.90	12.17±3.12	0.27±0.86 (2±8%)	11.43±2.99	10.75±2.84	-0.68±0.58 (-6±5%)
Average of both Legs (kg)	4.84±1.58	4.91±1.60	0.07±0.29 (2±6%)	4.60±1.33	4.60±1.34	0.001±0.29 (0.1±6%)	5.08±1.38	4.87±1.28	-0.20±0.14 (-4±2%)
Android (kg)	2.10±0.70	2.06±0.61	0.07±0.26 (3±14%)	2.00±1.24	2.03±1.00	-0.04±0.19 (-2±11%)	1.97±0.35	1.85±0.47	-0.12±0.35 (-9±16%)
Gynoid (kg)	4.37±0.93	4.45±0.90	0.82±0.29 (2±8%) ×	4.44±1.09	4.54±1.15	0.10±0.25 (2±6%) ×	4.71±1.00	4.44±0.98	-0.27±0.21 (-6±5%) ×
Total (kg)	26.09±6.60	26.51±6.29	0.42±0.96 (2±4%) ×	25.06±5.47	25.35±5.78	0.29±1.26 (1±5%) ×	25.65±6.17	24.44±5.68	-1.21±0.94 (-5±3%) ×

LIPA; light intensity physical activity, SBF; sedentary behaviour fragmentation.

Table 7.3- Bone Mineral Density outcomes as a factor of the intervention. Stratified by group. * represents a significant time effect. ×represents a significant group×time interaction effect

	SBF (n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
Bone Mineral Density									
Arms (g/cm^3)	0.76±0.25	0.72±0.13	-0.04±0.13 (-3±8%)	0.67±0.05	0.67±0.04	-0.01±0.03 (-1±5%)	0.68±0.05	0.68±0.05	-0.003±0.01 (-0.3±2%)
Thoracic Spine	0.90±0.09	0.94±0.13	0.04±0.12 (5±14%) *	0.90±0.14	0.94±0.15	0.03±0.09 (4±10%) *	0.97±0.09	0.95±0.10	-0.03±0.10 (-2±10%) *
Lumbar Spine (g/cm^3)	0.98±0.16	0.96±0.15	-0.01±0.06 (-1±6%)	0.97±0.16	0.98±0.16	0.01±0.08 (1±8%)	1.04±0.15	1.04±0.13	0.01±0.07 (1±7%)
Legs (g/cm^3)	1.05±0.17	1.06±0.17	0.01±0.01 (1±1%) ×	1.06±0.16	1.05±0.16	0.01±0.02 (1±2%) ×	1.11±0.09	1.11±0.08	-0.01±0.01 (-1±1%) ×
Total (g/cm^3)	1.10±0.11	1.09±0.11	-0.01±0.03 (-1±3%)	1.11±0.15	1.11±0.13	0.00±0.02 (0±2%)	1.13±0.06	1.13±0.07	-0.01±0.01 (-1±1%)

LIPA; light intensity physical activity, SBF; sedentary behaviour fragmentation.

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Table 7.4- Lean Body Mass outcomes as a factor of the intervention. Stratified by group. * represents a significant time effect. ×represents a significant group×time interaction effect.

	SBF (n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
Lean Body Mass									
Average of both Arms (kg)	1.72±0.28	1.72±0.26	0.003±0.15 (1±8%)	1.66±0.20	1.65±0.22	-0.14±0.10 (-1±7%)	1.57±0.23	1.62±0.22	0.06±0.07 (4±4%)
Average of both Legs (kg)	5.84±1.13	5.70±0.89	-0.14±0.44 (-2±6%)	5.54±0.71	5.49±0.67	-0.04±0.21 (-1±3%)	5.28±0.71	5.29±0.80	0.01±0.20 (0±4%)
Total (kg)	38.02±4.67	37.87±4.44	-0.29±1.56 (-1±3%)	37.70±4.94	37.82±6.59	-0.04±1.57 (-0.1±3%)	37.18±5.06	37.67±11.67	0.56±1.35 (2±4%)
Appendicular skeletal muscle mass (kg)	14.51±2.95	14.39±2.43	-0.11±0.98 (-1±7%)	14.34±2.57	14.49±2.87	-0.02±0.47 (-0.1±4%)	14.11±2.29	14.00±2.69	0.11±0.54 (1±4%)
Relative appendicular skeletal muscle mass ($kg.m^2$)	5.61±1.05	5.60±0.66	-0.06±0.37 (-2±6%)	5.29±0.75	5.54±0.81	0.02±0.27 (0.3±5%)	5.57±0.90	5.50±0.84	0.04±0.25 (1±4%)

LIPA; light intensity physical activity, SBF; sedentary behaviour fragmentation.

Table 7.5- Body fat percentage (%) outcomes as a factor of the intervention. Stratified by group. * represents a significant time effect. ×represents a significant group×time interaction effect.

	SBF (n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
Average of both Arms (%)	45±8	46±8	1±2 (2±4%) ×	45±6	46±5	1±2 (2±5%) ×	47±9	46±9	-2±2 (-4±4%) ×
Trunk (%)	36±5	36±4	0.4±2 (2±7%) ×	36±5	37±5	1±2 (2±7%) ×	35±5	33±5	-2±1 (-6±4%) ×
Average of both Legs (%)	43±8	44±8	1±2 (2±4%) ×	43±6	43±6	0.2±2 (1±4%) ×	47±6	46±6	-1±1 (-2±2%) ×
Android (%)	39±10	39±9	0.3±2 (1±6%)	39±12	40±9	-0.2±2 (-1±7%)	40±4	38±7	-2±2 (-5±6%)
Gynoid (%)	41±5	42±5	1±2 (2±5%) ×	42±8	43±9	1±2 (2±4%) ×	46±7	42±7	-2±1 (-4±3%) ×
Total (%)	39±7	39±5	1±1 (2±4%) ×	38±7	38±7	1±2 (2±5%) ×	40±5	40±5	-2±1 (-4±2%) ×

LIPA; light intensity physical activity, SBF; sedentary behaviour fragmentation.

Discussion

Changes in body composition in response to chronic SB displacement, have not previously been investigated (irrespective of methodology quality) to the author's knowledge. The aim of this chapter was to examine the effects of displacing SB with LIPA (in two patterns) on the three principal aspects of body composition in older adults, lean body mass, adiposity, and BMD. It was hypothesised that SB displacement with LIPA, would cause significant reductions in adiposity, improved lean body mass, and enhanced BMD. Accordingly, significant increases in both spine and T-spine BMD were observed in both experimental groups after accounting for adiposity indices. Similarly, an increase in leg BMD was also seen with LIPA implementation. However, significant reductions in both total adiposity (arm average, right leg, gynoid region, and total body), and localised fat percentage (right arm, arm average, trunk, leg average, gynoid region, and total body) outcomes, were observed in the control group. Therefore, the first hypothesis was partially upheld. It was further hypothesised that the fragmentation group would experience greater region-specific enhancements in BMD, lean body mass, as-well as region specific reductions in adiposity outcomes. The primary mediator for the significant Leg BMD group×time interaction, was the difference between SBF and CON, but not LIPA. Therefore, the second hypothesis was partially upheld. This chapter represents the first investigation to examine the effects of SB displacement with LIPA on adverse body composition states in older adults.

Significant increases in spine BMD were observed in both experimental groups, only after accounting for adiposity indices. Such findings support a previous study whereby significant associations between reduced SB/ increased LIPA and higher spine BMD (112), were only observed after accounting for adiposity indices. Accordingly, BMI and total fat tissue are significantly positively associated with BMD in older adults (112, 203), most likely due to the high mechanical load additional body weight places on the skeleton (102, 203). The effect on spine BMD was discrete and region specific, given that this effect was present in the thoracic but not the lumbar region. In support, a previous study failed to detect a significant association between walking activity and lumbar spine BMD in postmenopausal women (106). Interestingly, the association between BMI and higher BMD in older adults persists uniformly across loaded (e.g. lumbar spine, pelvis) and unloaded bone regions (thoracic spine, ribs, & arms),

suggesting BMI has a mechanical loading independent effect on enhancing BMD (203). However, the absence of significant change in other loaded (lumbar spine) and unloaded regions (ribs & arms) in the current investigation, points to specific effect of SB displacement in the thoracic spine. A previous study similarly observed an association between decreased SB/ increased LIPA and increased T-spine BMD specifically (112). The authors speculated that excessive kyphotic curvature, which is common amongst many older adults (517), likely increases shear between thoracic vertebrae whilst walking (112). The results of the current investigation further support this interpretation, suggesting displacing SB with LIPA in older adults enhances T-spine BMD, due to higher mechanical loading at the thoracic vertebrae exclusively, most likely due to habitual forward stooped posture. Curiously, the change in LIPA (within the LIPA group) was negatively associated with the change in T-spine BMD, suggesting continuous LIPA implementation mediated adverse T-spine BMD losses following such an intervention (please see appendices i). Whilst this may point toward an advantage of SBF, no group×time interaction effect was observed with the magnitude of enhanced T-spine BMD similar in both experimental groups (SBF: 4±12%, LIPA: 4±9%). This alternatively suggests that implementing LIPA (irrespective of prescribed pattern), is the key factor mediating enhanced spine BMD following SB displacement, further supporting recent conclusions drawn from the UK physical activity guidelines (112, 280).

In contrast, a significant group×time interaction was observed for leg BMD in the current study, whereby the difference between the increase in SBF (1±1%) and the decrease in control (-1±1%) significantly mediated such an effect. The increase in leg BMD of ~1% over 8 weeks following SBF roughly translates to ~0.13% per week. Interestingly, previous resistance training studies in older adults have exhibited mere increases of between 0.02-0.05% and 0.02-0.09% per week for total hip and trochanter BMD respectively (518-520). As such this firstly highlights the impressive magnitude of enhanced leg BMD following SBF. Frequent LIPA similarly perturbs BMD loss in the lower limbs during bed rest (109, 113). Accordingly, a more fragmented SB pattern, has previously been associated with enhanced leg BMD in older women (111, 112). Therefore, such results suggest a clear advantage of frequent vs continuous LIPA for enhancing Leg BMD, most likely due to increased frequency of exposure to mechanical loading. General increases in walking time are associated with increased

calcaneal (104, 105), and femoral neck BMD (106), as-well as reduced fracture risk during follow-up (107), in older adults. However, previous studies have only noted an association between increased LIPA and enhanced Leg BMD in older adults after accounting for adiposity indices (112, 203). Thus, whilst such results support the positive region-specific effect LIPA has on Leg BMD, this effect appeared independent of controlling for adiposity indices. This suggests older women may benefit from displacing SB with frequent LIPA, independent of their weight status. Furthermore whilst the magnitude of reduced spine (-1±7%) and leg BMD (-1±1%) in the control group, is not as extreme as the loss following 4-12 weeks of extreme bed rest in young adults (spine: ~3%, hip 2-4%) (109, 110), such a loss does support the notion of uninhibited SB accelerating the age-related loss of bone tissue. Despite observing increased BMD of small magnitude in specific regions (1-4%), select participants improved bone health endpoints, one participant positively shifted from osteoporotic to osteopenic in response to the LIPA intervention. This supports the notion that the benefits of LIPA implementation may be greater for older adults at increased risk of osteopenia/osteoporosis (516). Ultimately, such results suggest LIPA implementation improves spine, and T-spine BMD specifically, in older women irrespective of prescribed pattern. In contrast, regular displacement of SB with frequent LIPA, conveys an advantage of improving Leg BMD (independent of weight status) to a greater extent than continuous LIPA. This gives another potential intervention option to those seeking to improve bone health in older adults.

Curiously, significant reductions in total adiposity, and fat percentage outcomes, were observed following the control condition. This was typified through the reduction in total BFP% in the control (-4±2%), in contrast to both experimental groups (SBF: 2±4%, LIPA: 2±5%). An experimental increase in BFP% is in direct contrast to the original hypothesis, where it was predicted SB displacement with LIPA would facilitate a reduction in adiposity. Furthermore, the relative change in SB/LIPA time within the SBF group was negatively and positively associated with android fat percentage respectively (please see appendices i), suggesting shifting to a more fragmented SB pattern is associated with an adverse increment in visceral-abdominal adiposity. This contrasts with previous findings whereby, a more fragmented SB pattern is associated with decreased waist circumference (52), BFP% (413), and BMI (92), in older adults. Given that, aside from specific factors (Genetics, hormonal disorders), excess

adiposity accumulation is generally caused by positive energy balance (300, 521), this suggests experimental participants shifted into positive energy balance during the intervention. However, this is unlikely to have been due to reduced EE, given that breaking up SB (every 20 mins) with frequent bouts of LIPA (~2 mins), significantly increases net energy expenditure by ~0.33 and 0.29 kcal/min, compared to continuous sitting and standing breaks respectively (91, 94, 95). Furthermore, increased non-exercise activity thermogenesis drastically increases total daily energy expenditure (522). Introducing work breaks in office situations has previously generated concern regarding the association between activity breaks and snacking behaviours (523, 524). Therefore, it is possible that frequently interrupting SB with LIPA inadvertently increased snacking behaviour, potentially due to increased exposure to adverse environmental food cues similarly present within the home (525, 526). Accordingly, a higher meal frequency (snacking) has previously been associated with increased visceral abdominal adiposity in older adults (527), but only in overweight/obese adults who tend to frequently snack on relatively poor quality foods (crisps, sweets, chocolates etc) (528). Regardless of definition employed (BFP%, WC, WHR) 75-92% of participants were classified as obese at baseline, suggesting their snacking options were likely of greater energy density/ reduced nutritional quality. Increased snacking is thus a reasonable potential rationale for the increased visceral-abdominal adiposity following reductions in mean SB bout length.

Within the LIPA group, the relative change in SB/ LIPA was positively for the latter, and negatively for the former, associated with the change in leg adiposity respectively (please see appendices i). This suggests an advantage of continuously implemented LIPA, mediating reductions in leg fat tissue content. In support, lower SB in older adults has previously been associated with reduced lower body total fat tissue (413). The change in LIPA was also positively associated with gynoid fat percentage. In-fact, LIPA exhibited the greatest reduction in android:gynoid fat percentage (SBF: -1±6%, LIPA: -3±6%, CON: -1±4%). This somewhat supports previous studies that have identified a strong positive association between SB time and visceral abdominal adiposity in older adults (46, 52, 511, 512). Overall, this suggests that LIPA implementation mediates statistically insignificant reductions in lower body fat tissue content. However, the current results ultimately suggest LIPA implementation increases adiposity, in contrast to the control condition. Interestingly, associations between SB and adiposity in older

adults have been suggested to be primarily mediated by reverse causality, whereby adiposity has a detrimental effect on skeletal muscle function (55, 220), thus diminishing one's functional ability leading to greater SB time (529). Considering, intentional SB displacement with LIPA failed to reduce adiposity, such results support bi-directional causality between SB and adiposity. However, all groups were already classified as obese at baseline (SBF: 38±5%, LIPA: 39±4%, CON: 40±5%), with only 2 experimental participants unfavourably shifting from non-obese to obese, in response to LIPA implementation. Therefore, relatively minor increases in total BFP% following SB displacement (2-4%), must be viewed in the context of pre-existing obesity, and thus of arguably minor consequence.

Aside from a trend toward a group×time interaction for total lean body mass, no other lean body mass variables significantly changed in the main analysis. Accordingly, only three participants (SBF: n=1, LIPA: n=1, CON: n=1) positively shifted from pre-sarcopenic to non-sarcopenic. In support, light homebased body weight resistance training failed to induce changes in fat-free mass over 9 months (530). This demonstrates an apparent insufficiency of SB displacement with LIPA to enhance lean body mass in older adults. In contrast, various studies have exhibited a negative association between SB and lean body mass in older adults (51, 273). Such observations are speculated to be mediated by the reduction in muscle activity that accompanies SB engagement (91). Considering LIPA significantly stimulates the whole body musculature, this is speculated to provide a sufficiently intense hypertrophic stimulus (90, 91). Accordingly, a single bout of low-intensity resistance training (40% 1RM) is sufficient to stimulate myofibrillar protein synthetic response (440). However, only slow tempo lifting through the entire range of motion, induced significant muscle hypertrophy following 10 weeks of low intensity resistance training (30-50% 1RM) in older adults (169, 444). Therefore, the lack of direct supervision likely led to variability in movement execution (range of motion/training tempo). Furthermore, a conservative increase in LIPA was prescribed (45-50mins), with no increase in training volume over time. Low volume (3 sets) low-intensity resistance training appears inferior to high volume (6 sets), regarding the ability to stimulate myofibrillar protein synthetic response in older adults (440), suggesting increasing training volume over time is essential for hypertrophy. LIPA interventions may therefore require to be carried out over longer periods to compensate for the lack of overload. In support,

moderate term low-intensity resistance training (10-20 weeks, ≤40% 1RM) does not significantly alter lean body mass in older adults (165, 531), whereas increasing walking time over 6-months, increases lean body mass (360, 437).

Interestingly, the relative change in SB was negatively associated with the relative change in right arm adiposity following the control condition (-3±12%) but not the experimental conditions (SBF: 5±10%, LIPA: 3±10%) (please see appendices i). Continued SB engagement in the control condition may have inadvertently increased engagement in tasks that preferentially stimulate the upper body musculature, compared to both experimental groups. Accordingly, ~35% of SB bouts in older adults are comprised of upper body muscularly demanding tasks such as self-care (hairstyling, dressing), and taking care of others (caring for grand-children etc) (319, 320). Furthermore, performing computer typing in a standing posture is associated with significantly less activation of the upper body musculature (Wrist extensors, trapezius), compared to typing whilst seated (532), suggesting the seated posture is more upper body muscularly demanding. In support, muscular contraction stimulates lipolysis in humans, thus assisting with mobilisation and subsequent oxidation of intramuscular triglyceride stores (463). Therefore, continued engagement in upper body demanding SB tasks, may have facilitated a region-specific reduction in arm fat tissue. For alternative assessments it was determined 72% of participants favoured their right foot for balance assessments (chapters 4 and 5), which combined with the observation that ~90% of humans are right handed (533), strongly suggests that for the majority of control participants their dominant hand was their right. Accordingly, professional tennis players exhibit significantly lower arm fat percentage in their dominant (racket) hand due to disproportionate activation of such musculature (534), and region specific increases in lipolysis as a result (463, 535). Given that the majority of operational tasks are performed with the dominant hand in older adults (536), continued engagement in upper body demanding SB tasks, likely preferentially activated lipolysis in the dominant arm. Whilst it is unknown if a localised reduction in fat tissue influences functional outcomes (increased grip strength etc), such a marginal reduction is unlikely to result in such consequences. Nevertheless, these results highlight the region-specific effects SB displacement has on body composition.

Strengths and Limitations

The major strength of the current chapter is the utilisation of a gold standard body composition assessment tool like Dual X-Ray Absorptiometry (537). The results of the current chapter are not only steadfast, but extremely novel. Furthermore, the pattern of prescribed LIPA was also controlled for within the overall study design, permitting conclusions to be drawn on SB accumulation pattern. However, Dual X-Ray Absorptiometry tends to underestimate the age-related loss of muscle mass compared with magnetic resonance imaging (414), which may have contributed to the lack of observed lean body mass change. Therefore, future SB displacement interventions should explore changes in body composition, in concert with more robust assessments of muscle size/ quality (e.g. magnetic resonance imaging), as-well as examine what specific role (if any) alterations in body composition have on changes in functional ability. Nevertheless, most significant effects observed were of relatively small magnitude (1-5%). Whilst this was not unexpected, it may have been the reason why the majority of participants were stable in their body composition (pre-sarcopenia, obesity, bone health) categorisations over time, as small changes shifted select participants categorisation due to their close proximity to a conventional threshold at baseline. It may be prudent for future studies to repeat such interventions with longer time frames, and follow-up periods to examine whether the magnitude of these effects is maximised after 8 weeks, or whether longer time frames stimulate greater adaptation.

Conclusion

In conclusion, such results show that displacing sedentary behaviour with LIPA in older women leads to overall improved body composition. General displacement of SB with LIPA (irrespective of prescribed pattern), increased T-spine BMD. In contrast, displacing SB with LIPA in a more fragmented pattern, generated region-specific changes including enhanced leg BMD. Collectively the results of this chapter show displacing SB with LIPA in older women results in multiple tissue/region-specific changes in body composition. The pattern of SB displacement also mediates this effect, with greater fragmentation appearing more beneficial/detrimental depending on the target tissue.

Chapter 8 – General Discussion

Most promisingly, the results from chapter 3 suggest a very positive uptake of the SB displacement with LIPA message, including most notably good likelihood of long-term adherence. This is promising considering older adults display poor long term tolerance to exercise (166). Interestingly, SB and frailty are proposed to share a bidirectional relationship, whereby frailty also strongly predicts SB (538). This suggests that age-related reductions in muscle strength and physical function, may reduce one's tolerance for everyday tasks (ambulating, stair climbing, sit-to-stand ability), and consequently lead to greater amounts of SB. Furthermore, retrospective fall history, and prospective fear of falling are associated with an additional 22 and 45 minutes of SB per day respectively in older adults (122, 124). The current investigation improves upon the limited cross-sectional design employed by such studies through directly manipulating one such independent variable (SB) and observing potential changes in health-related outcomes. Considering the observed improvements in physical function [gait speed, sit-to-stand ability, and single leg stance time (chapter 4)], this primarily suggests SB displacement with LIPA benefits physical functioning. Given that gold standard tri-axial accelerometery was used to classify the change in physical behaviour, this also strengthens the validity of such findings.

However, it should be noted the observed changes in physical function (gait speed, and sit-to-stand ability), were consistently <1 times the typical error calculated during reliability analysis, and thus below the threshold at which a meaningful change is considered to have occurred (1.5-2.0 times typical error) (263). Nevertheless, the minimal clinically important difference in gait speed was recently identified as 0.1 m/s for multiple populations (255). This suggests the experimental improvements in gait speed (0.1m/s) can still be considered clinically relevant, and thus highlights how SB displacement with LIPA enhances one's ability to mobilise from a seated position. The prospect of bi-directional causality also remains present, especially pertaining to the time course of changes. It was reasoned that the wider range of inter individual responses observed for SB reduction (Range: 19 to -22%), compared to previous studies (8% to -14%) (287), was primarily due to the specificity of the current intervention. However, experimental participants that experienced reductions in SB time of substantial magnitude, also experienced the greatest enhancements in muscle strength/ physical function, which in turn may have led to greater reductions in SB time.

Furthermore, due to the relative surge in physical demands that LIPA seems to generate in older adults closer to the lower limits of their physiological reserve (361), this may further translate into greater physical behaviour profile enhancements following an increase in function. Accordingly, enhanced *GM* AC% following LIPA (chapter 5) was attributed to the fact that LIPA exhibited the lowest levels of *GM* AC% at baseline, and thus had the greatest capacity for change in response to LIPA implementation (395). However, the average change in *GM* AC% following LIPA cannot be considered meaningful considering it was less than 1.5 to 2.0 times the typical error calculated during reliability analysis (263). Nevertheless, an increase in *GM* AC%, is a reasonable explanation as to why LIPA exhibited a greater average reduction in SB time (-7±10%) compared to SBF (-4±12%), through enhancing reduced neuromuscular function.

Considering only minor improvements in unipedal stance duration were observed (Chapter 4), combined with the fact that the majority (~65%) of participants reported no improvement in self perceived balance ability, it is unlikely that improved balance ability mediated improved physical behaviour profile. Furthermore, the change in single leg stance time observed in chapter 4, cannot be considered meaningful considering it was less than 1.5 to 2.0 times the typical error calculated during reliability analysis (263). Nevertheless, future studies could specifically examine fear of falling to determine if SB displacement causes any changes in such a parameter, and secondly whether this influences the effectiveness of SB displacement. Regarding the time course of physical behaviour alterations, enhanced muscle strength/function following SB displacement may have also influenced secondary enhancements in physical behaviour profile. Increased PF MVC observed in chapter 5 following SB displacement with LIPA, appeared to be dependent on neuromuscular adaptation. This is reasonable as rapid strength gains following relatively short training periods (<6 weeks), are primarily mediated via neural adaptation (390, 398, 399). Therefore, the current study's duration of 8 weeks appears to be sufficient to observe neuromuscular adaptation. Rapid onset neuromuscular adaptation and strength gains may have in turn led to further reductions in SB time at the latter stages of the intervention (> 6 weeks) due to higher functioning. However, the time course of such events remains to be elucidated. Perhaps future studies could examine the temporal course of neuromuscular strength gains and enhancements in physical function in

isolated time frames (every 1-2 weeks), to determine what effect this has on the time course of physical behaviour alterations.

Considering the neuromuscular adaptations observed, the lack of observed significant changes for Dual X-Ray Absorptiometry derived lean body mass, and ultrasound determined muscle size is unsurprising. As discussed in chapters 6 and 7 the lack of progressive overload and variability in movement execution (range of motion/training tempo), likely limited mechanical tension on muscle tissue during LIPA implementation. Therefore, LIPA interventions may require to be carried out over longer periods to compensate for the lack of overload. However, previous cross-sectional associations have been observed between SB and pre-sarcopenia in older adults (273, 383). Such studies likely detected an association between SB and compromised muscle mass that developed over longer time frames (months to years). Perhaps future studies wishing to induce muscular hypertrophy could implement LIPA based tasks that execute full range of motion (bodyweight squats), utilise slow training tempos (slow chair rises/ descents), and gradually overload prescribed LIPA volume over time to >45-50 minutes per day. It may also be prudent to use longer time frames (> 8 weeks) to observe changes in lean body mass, and ultrasound determined muscle size. However, it is unclear how this would in turn affect the palatability of the intervention.

Both SB displacement interventions did increase intake of nutrients promoting anabolism (SBF: 13%, LIPA: 4%). Furthermore, those participants who positively shifted classification from sedentary to ambulatory (reduced average SB time to <8h/day) similarly increased intake of nutrients promoting anabolism (2%). Given that older adults consistently under consume protein (299, 301), and other nutrients promoting anabolism (202), this represents a promising secondary lifestyle enhancing effect of SB displacement. However, increased intake of anabolic nutrients failed to mitigate the insufficient mechanical overload stimulus being generated by LIPA to induce significant muscle hypertrophy. This is in line with previous evidence suggesting a combination of adequate dietary anabolic stimuli combined with an activity stimulus of sufficient magnitude, optimally mitigates against age related muscle wasting (539). Perhaps future studies could employ SB displacement as an indirect means of dietary improvement alongside a sufficient hypertrophic resistance-training stimulus and examine whether muscle hypertrophy is enhanced. However,

considering older adults exhibit a poor tolerance for intense activity (166) the potential for SB displacement to produce isolated improvements in dietary anabolic stimuli is still highly relevant for older adults health. Accordingly, increased intake of nutrients promoting anabolism may have influenced the aforementioned functional improvements, considering greater intakes of such nutrients are associated with enhanced skeletal muscle function (202, 338). This is strengthened by the fact that control reduced intake of such important nutrients (-34%) and did not exhibit increased function. Reduced activity can also further blunt the muscle protein synthetic response to feeding in older adults (87-89), making dietary anabolic stimuli of paramount importance during such conditions. In support, iso-calorically enhancing dietary protein quality can counteract the negative effects of bed rest in older adults, including partially protecting lean body mass and fully recovering strength with rehabilitation (540). Therefore, aside from the inadequacy of SB displacement with LIPA to induce significant muscle hypertrophy, the potential for enhanced anabolic dietary quality is clinically relevant.

Interestingly, SB displacement with LIPA enhanced thoracic spine BMD in older women to similar magnitudes in both experimental groups (SBF: 5%, LIPA: 4%). As discussed in chapter 7 this was likely mediated by increased region specific loading of the thoracic spine during LIPA, due to a habitual kyphotic posture (112), as-well as body weight related overload in such a region (203). However, those participants who positively shifted classification from sedentary to ambulator (reduced average SB time to <8h/day) similarly increased intake of nutrients promoting bone health (16%), including significantly increased zinc intake (29%). This likely enhanced the osteogenic potential of SB displacement. Furthermore, the increase in leg BMD following SBF (1%), was originally attributed to the superior osteogenic stimulus of frequent vs continuous SB displacement (112). Whilst frequent activity prescription still likely mediated large parts of this effect, the dietary data from chapter 3 also reveals that SBF was the sole experimental group to increase nutrients promoting bone health (SBF: 17%, LIPA: -34%). This suggests that enhanced leg BMD following SBF, was due to a combination of frequent mechanical loading of the lower body through SBF (112), combined with spontaneous increased intake of osteogenic nutrients that enhanced this region specific loading effect.

Furthermore, it was hypothesised in chapter 3 that SB displacement with LIPA would cause a reduction in energy intake, due to recent evidence demonstrating 'the gravitostat' mediates reduced energy intake following high loading in rodents (314, 315). However, in rodents, the energy intake reducing effect of the 'gravitostat' appears to be dependent on an osteocyte strain detection mechanism, that is activated in response to high loading through the lower limbs (314, 315). Therefore, the lack of change in energy intake, was attributed to the fact that all groups were on average classified as non-obese at baseline (BMI ≤30kg/m^2). Thus, SB displacement with LIPA in such individuals may simply have not produced high enough loading forces through the lower body bone structures, sufficient to activate the gravitostat. Yet, when obesity was defined with the WHOs gold standard reference criterion (Total BFP%: ≥35%) (499, 500) from chapter 7, all groups were classified as obese at baseline (SBF: 39±7%, LIPA: 38±7%, Control: 40±5%). Further results from chapter 7 demonstrate frequent SB displacement enhanced leg BMD. Therefore, frequent LIPA implementation may induce a mechanical loading stimulus sufficient for leg BMD enhancement, but not gravitostat activation in older overweight (BMI 25-30kg/m^2) women, with excess adiposity accumulation (Total BFP%: ≥35%). This further suggests gravitostat activation with SB displacement may be dependent on high body weight, and not necessarily high adiposity in older women.

Nevertheless, a significant group×time interaction was observed for total BFP%, whereby both experimental groups increased (SBF 1%, LIPA: 2%), in contrast to control (-5%). This suggests that both experimental groups shifted into positive energy balance across the course of the intervention which facilitated adiposity accumulation. Accordingly, the dietary data from chapter 3 supports this as both experimental groups as-well as novel ambulators increased intake of most nutrients. An increase in total BFP% must however be placed into context as all groups were already classified as obese on average at baseline (SBF: 39±7%, LIPA: 38±7%, Control: 40±5%). Furthermore, the relative experimental increase in total BFP% was small in magnitude (1-2%). The control group likely did not receive the spontaneous increased nutrient intake effect both experimental groups received, accounting for a relative reduction in total BFP%. Accordingly, minor increases in adiposity are an acceptable trade off considering increased energy intake following SB displacement involved both anabolism and bone health promoting nutrients, promoting the enhancements in

region specific BMD/ muscle function. Furthermore, minor increases in adiposity may have beneficially enhanced BMD through increased body mass related mechanical loading during upright activity (203). Changes in total body adiposity may also help provide an explanation for anomalous echo intensity findings in chapter 6. Specifically, average *GM* and *GL* echo intensity reduced by 15% and 12% respectively following control (both >2.0 times the typical error and thus meaningful (263)), whereas both experimental groups exhibited minor changes (-1 to 2%). The reduction in control total BFP% of ~5% may partially explain these findings as, echo intensity is considered a valid proxy indicator for intra muscular fat deposition (426), and increased adiposity is linked to intramuscular fat accumulation in older adults (541).

The relative change in SB and LIPA time was negatively and positively associated with android fat content respectively, within the SBF group (Chapter 7). Despite the rigorous habitual dietary analysis employed in chapter 3 such methods failed to account for nutrient timing. Introducing work breaks in office situations has previously generated concern regarding the association between activity breaks and snacking behaviours (523, 524). Therefore, it is possible that frequently interrupting SB with LIPA inadvertently increased meal frequency without significantly affecting energy, macro, or micronutrient intake. Accordingly, increased meal frequency in older adults is associated with increased weight gain (527, 542), primarily due to increased exposure to adverse environmental food cues (525, 526), and higher perceived hunger (543). A higher meal frequency (snacking) has also previously been associated with increased visceral abdominal adiposity specifically in older adults (527), but only in overweight/obese adults who tend to frequently snack on relatively poor quality foods (crisps, sweets, chocolates etc) (528). Accordingly, dietary data from chapter 3 suggested 71%, and 60% of participants consumed above the recommended maximum daily intake of saturated, and total fat intake at baseline, respectively. Increased snacking frequency on poorer quality foods is thus a reasonable explanation for observing increased visceral-abdominal adiposity following reductions in mean SB bout length. In fact one such SBF participant observed in their post-intervention questionnaire "*When the buzzer went off whilst watching TV in the evenings I tended to grab a snack when I got up, whereas If I wasn't wearing the device I would have just continued to Watch TV and would not have thought about food*". Nevertheless, despite the fact that increased meal frequency is associated with exacerbated type 2

diabetes mellitus risk in older adults (544) due to increased sugar intake (545), significant reductions in glucose intake were observed following SBF (-31%).

The observed gains in PF MVC in chapter 5 are promising considering LIPA is not an exercise intensity conventionally regarded as optimal. Promisingly, all of the data used to calculate Net PF MVC, exhibited acceptable inter-day reliability (PF MVC, dorsiflexion MVC, AgCoA, AC%), and the effect size can be considered large for the significant Net PF MVC time effect. Despite both experimental groups increasing PF MVC by similar magnitudes (SBF: 3%, LIPA: 2%), such strength gains were mediated via different neuromuscular pathways. However, as mentioned the change in *GM* AC% following LIPA cannot be considered meaningful considering it was less than 1.5 to 2.0 times the typical error calculated during reliability analysis (263). In contrast, the change in AgCoA following SBF was 1.5 times the typical error calculated during reliability analysis and can thus be considered meaningful. As such, the exclusive reduction in AgCoA following SBF, can be considered the only meaningful neuromuscular adaptation following the intervention. Rapid strength gains following relatively short training periods (<6 weeks), are mediated via neural adaptation (390, 398, 399). In-fact, such strength gains occurred despite reduced *GM* muscle volume following both experimental conditions in chapter 6, in contrast to control who increased. However, the average change in *GM* volume following control (21cm^3) was < 1 times the typical error calculated during reliability analysis, suggesting this was not a meaningful change.

Nevertheless, a significant negative association was observed between SB time and *GM* physiological cross-sectional area. In support, reduced SB and greater standing time have both been associated with increased *GM* physiological cross-sectional area in older adults (385), suggesting frequent standing performed by SBF participants, appears to have generated small yet statistically insignificant improvements in *GM* physiological cross-sectional area. Considering physiological cross-sectional area is directly linked to the maximum isometric force producing capabilities of a muscle (424), increased net isometric PF MVC following SBF may have also been somewhat dependent on a non-significant increase in *GM* physiological cross-sectional area. This is reasonable considering the most plausible mechanism was increased stationary standing time (isometric contraction), and PF MVC was assessed isometrically at 0°. Accordingly, previous studies have stated that consistency

between assessment contraction type (e.g. isometric MVC) and contraction type during training, increases the likelihood of observing training induced adaptation (394). However it should be acknowledged that dynamic movement can result in isometric contractions via biarticulated muscles/ muscle-tendon interaction (e.g. PF during stair climbing), which may have also influenced neuromuscular adaptations during isometric assessments.

The different neuromuscular pathways by which SB displacement enhances strength dependant on the prescribed LIPA pattern is a very interesting insight. Perhaps future studies could try and link pattern dependant neuromuscular adaptations following SB displacement to changes in physical function. Furthermore, future SB displacement interventions wishing to enhance muscle strength by a greater magnitude than the current investigation (2-3%), should try to implement LIPA based movements that generate the required higher levels of muscle activity. This has previously been dubbed 'Exercise by stealth' (158), with specific habitual tasks like sit-to-stand transitions, stair climbing, and faster walking speeds generating greater muscle activity relative to other habitual tasks (90). Future intervention studies could also implement progressive overload with LIPA based tasks as a means of enhancing the magnitude of strength gains. However, it is again unclear how this would in turn affect the palatability of the intervention.

Experimental participants were prescribed SB displacement with a specific amount of daily LIPA implementation (45-50 minutes), in contrast to previous studies (156, 287, 293), where participants were merely prescribed a nonspecific SB reduction with non-specific displacement behaviours (standing, LIPA, MVPA). Therefore enhanced physical function following both experimental trials was likely mediated via enhanced muscle activity stimulating subsequent muscle adaptation, following specific SB displacement with LIPA (90, 91). Despite only observing minimal change in muscle hypertrophy/morphology it remains plausible that small and statistically marginal changes in muscle volume/architecture may have mediated some of the enhanced physical function effects observed. Accordingly, despite thigh muscle size only accounting for a small amount of the explained variance in older adults gait speed (~33%) it still remains a significant predictor (442, 443). Furthermore, considering improvements in sit-to-stand ability, and gait speed (assessed through the TUG), this further points to such improvements being partially mediated through small

enhancements in *VL* muscle volume, considering the key role the knee extensors play in sit-to-stand transitional performance and ambulation in general (90). Furthermore, LIPA exhibited a trend towards increased *GM* Lf (4%). Specifically, 50% of the differences in maximum shortening velocity between young and old adults are explained by a reduction in *GM* Lf (452), highlighting another potential mechanism by which SB displacement may have enhanced physical function. However, the average change in Lf following LIPA (-1cm) was <1.5 times the typical error calculated during reliability analysis (3.8cm), suggesting the change was not meaningful (263). Such potential associations are more likely to be detected, as significant alterations in muscle volume/ architecture likely follow neuromuscular adaptation (> 6 weeks). Future studies should therefore investigate associations between small alterations in muscle volume/ architecture and enhanced physical function following SB displacement.

The results of the current investigation suggest the pattern of prescribed SB displacement does not appear to be of greater relative importance compared to simply accumulating more LIPA time across the day, at least with regards to physical function. Such results are in line with previous interventions (156, 158, 293) suggesting a specificity of training effect following SB displacement, with enhanced sit-to-stand ability and gait speed highlighting improvements in an individual's ability to mobilise from a seated position. Even improved handgrip strength was partially attributed to frequently utilising ones arm muscles to grip a surface facilitating mobilisation from a seated position (369). However, the only change in HGS that can be considered meaningful (1.8 times the typical error), was that of peak HGS following control (-2.5kg). Furthermore, the significant difference between both SBF/LIPA in relation to control both exhibited large effect sizes. This highlights the importance of habitual light upper body-based tasks for sustaining/ improving HGS in older adults.

As discussed in chapter 7, it was unknown whether the observed association between increased SB and the localised reduction in right arm fat tissue following control, would lead to any functional adaptation. It was reasoned that continued engagement in upper body demanding SB tasks (319, 320, 532), likely preferentially activated lipolysis in the dominant arm following control. However, in contrast to both experimental groups both peak and average handgrip strength reduced following control (chapter 4). Therefore, reduced right arm fat tissue following SB engagement did not counteract

the reduction in grip strength following a lack of LIPA implementation. Furthermore, considering the detrimental effect obesity has on skeletal muscle function (446, 514) it is promising that functional adaptation still occurred irrespective of the minor experimental increase in BFP% observed in chapter 7.

In contrast no effects on balance posturography were observed with minimal effects observed on single leg stance duration. This highlights the potential inadequacy of SB displacement as an appropriate physical activity modality for balance improvement. In fact, a minimum of 90 minutes/ week of specific balance training is suggested to be the minimum dose response threshold for balance improvement in older adults (371). In contrast to potent resistance exercise training interventions in older adults the improvement in PF isometric MVC of relatively small magnitude observed (2-3%) likely did not translate into comprehensive functional improvements, beyond the specific activity assessed, in this case mobilising from a seated position. Therefore, the results of the current investigation do suggest the desired functional improvement following SB displacement should be considered during experimental design and tailored to such an outcome. For example, if improvements in balance are desired, one should consider implementing single leg challenges during LIPA implementation. Nevertheless, the improvements in function observed still hold great clinical relevance. Specifically, SB displacement with LIPA can be considered an alternative option to those older adults who struggle to implement MVPA under habitual conditions (166), as-well as during conditions when MVPA is especially challenging (e.g. COVID-19 self-isolation) (546-549). Nevertheless, the lowest risk of adverse health outcomes are observed in those performing regular MVPA, and minimising time spent in SB (Active-ambulator) (12, 13). Therefore, enhanced function following SB displacement should still be viewed as the first progress step on the physical activity spectrum aiming towards achieving 150 minutes/ week MVPA (197), whilst simultaneously engaging in LIPA, and minimising SB time.

Strengths, Limitations, and Future Directions

The major strength of the thesis was the implementation of two distinct SB displacement interventions with specific daily targets and goals. Whilst previous interventions have observed chronic health related improvements following generic SB reduction (156, 158, 293) such studies did not consider specific displacement of SB with LIPA. Furthermore, the current thesis investigation controlled for the pattern of prescribed LIPA (fragmented vs continuous). This is important as recent epidemiological evidence has suggested longer sitting bouts are more detrimental to health than shorter sitting bouts (258). However aside from select outcomes (glucose intake, Bone health enhancing nutrients, Leg BMD, & peak grip strength), the results from the current thesis do not suggest an overt advantage of frequent vs continuous LIPA implementation. Instead LIPA implementation irrespective of the prescribed pattern appears to enhance older women's health and physical function, in keeping with the conclusions of a recent review (23).

The original sample size calculation suggested 120-150 participants were required to identify a significant moderate change in gait speed. Considering only 24-30% of this target sample was recruited (n=36), this suggests the study was severely underpowered and not sufficiently powered to detect changes in key outcome measures (type 2 error). An insufficient sample size was likely the reason why many significant effects observed were main effects for time without a significant group×time interaction, as the study was not sufficiently powered to detect changes between groups. In-fact, for certain outcomes [e.g. muscle tendon complex morphology (architecture and tissue related quality) in chapter 6] group×time interaction effects were observed without post-hoc differences.

Despite the study being evidently underpowered, significant changes were still observed for most outcome measures (physical function, neuromuscular function, and even bone mineral density). Accordingly, the original sample size calculation was based upon studies that had observed improvements in gait speed following non-specific SB displacement. In other words participants in previous studies may have simply traded SB time for standing time, leading to a moderate change in gait speed. Despite the current study appearing to be statistically underpowered, specifically displacing SB with LIPA is a considerable strength in design, meaning significant

improvements in physical function, neuromuscular function, and even bone mineral density were still observed. Therefore the specific displacement of SB with LIPA is a major strength of the current study's design. Future SB reduction intervention studies should continue to displace SB time with LIPA specifically to observe positive changes in health markers. This further highlights the importance of LIPA during SB displacement to achieve health benefits in older adults. Furthermore, no participants were lost to follow up during the intervention suggesting the intervention was highly palatable despite this key design moderation.

Considering the primary recruitment strategy was recruiting older women from a pre-existing research database this likely had an impact on the characteristics of the participants. First of all, all participants recruited were older females. Ultimately this was justified considering the majority of previous studies had used a high proportion of female participants, and there was a specific rationale for investigating female participants. However, this does prevent the generalisability of such results to younger adults and men. Furthermore, participants were recruited from a pre-existing research database, which meant participants had previously taken part in a sport and exercise science research study. This likely meant participants were somewhat engaged with being a research participant prior to recruitment, which may have increased their motivation to take part, and comply to the intervention.

All recruited participants were White women, meaning findings cannot be generalised to older women from other ethnic backgrounds. However this is also a strength considering all participants were English speaking, meaning there was no language barrier during recruitment. Furthermore, all participants were from the same local community (Cheshire) as the primary researcher. The principal investigator was also a physically active fitness professional (personal trainer), with an enthusiasm for sport and exercise. These personal biases may have motivated participants to become more active and increase compliance to the intervention. Whilst the sample size limitation has been discussed, the recruitment team and experimental process involved one researcher overseeing the entire process with limited time. In contrast, recruitment of large numbers of participants, is typically done with a large academic team, with shared responsibilities (174). A larger team, may have recruited a greater sample size, tested more participants, and uncovered more physiological mechanisms.

The use of only one familiarisation session may also be considered a limitation. However, participants were given a minimum of three attempts at each assessment during familiarisation as this is the point at which functional performance begins to stabilise in older adults (178). Participants were also asked if they felt comfortable with each assessment before continuing. Nevertheless future studies may wish to use additional familiarisation sessions to ensure participants are fully familiarised to outcome measure assessments. This is especially the case for neuromuscular and muscle strength assessments considering previous studies have noted difficulty in achieving accurate data in older adults (415). Accordingly, only 5/17 (29%) of significant effects observed were between 1.5 to 2.0 times the typical error calculated during reliability analysis, and could thus be considered meaningful changes (263). This supports the need for additional familiarisation sessions (1-3 sessions) to reduce the impact of systematic error.

The use of multiple outcome measures may also be considered a limitation as with more outcome measures the chances of observing a significant change purely by chance (type 1 error) is increased. The choice to investigate multiple outcome measures is ultimately defended considering this was the first study (to the authors knowledge) to specifically displace SB time with LIPA in older adults and observe health changes. Whilst previous studies provided a rationale to investigate certain broad health related outcomes [e.g. muscle strength, neuromuscular function, muscle tendon complex morphology (architecture and tissue related quality), and body composition], it was necessary to investigate a comprehensive range of specific outcomes within these broad categories. In this way, the current study also investigated the physiological mechanisms that underpin such health improvements. Therefore, the use of many outcome measures is ultimately a strength of the current investigation. Nevertheless, future studies could use the results of the current study to isolate select outcomes that are more likely to change in response to SB displacement with LIPA in older adults like gait speed, handgrip strength, neuromuscular function, and bone mineral density. This would give greater confidence that results have not occurred purely by chance. Perhaps future studies should endeavour to recruit an evenly distributed sample, that permits the use of parametric statistics, and thus a 95% confidence interval calculation. Furthermore, whilst the use of Fishers least significant difference test to examine post hoc comparisons is defended in the current thesis (due

to a 3-group design), future studies could consider using a Bonferroni adjustment instead (worst case scenario based on independent comparisons).

The major limitation observed from the thesis was the lack of matched experimental phases between the experimental groups and the control group. Whilst this has been repeatedly acknowledged, controlled for statistically where appropriate, and the implications discussed at length, such differences would ideally not be present where possible. Ultimately, the decision to prioritise testing of experimental participants (SBF & LIPA) was justified, given that such interventions were the most logistically challenging. Nevertheless, future studies should control for the timing of experimental phases between groups and give equal priority to control as-well as experimental participants. It should also be acknowledged that there is a growing movement within the scientific literature to not publish randomised controlled trials that have assessed baseline differences with significance testing (550). This is due to an argument that the prognostic strength of a variable only becomes relevant when there is a significant baseline difference. Whilst the use of baseline comparisons is defended in the current thesis, this practice may soon become obsolete. Future studies should screen participants more carefully before randomisation occurs to avoid this issue. Blinding of participants to their intervention group was not possible considering, participants needed to receive specific instructions on how to perform their respective intervention. However, a strength of the current study was that participants in one group (e.g. SBF) were not made aware of participants in the concurrently operating groups (LIPA and control), thus reducing performance bias. However, considering only one researcher oversaw the entire process it was not possible to blind the researcher to a participants group allocation during data collection or analysis. Perhaps future studies could use multiple research personnel to blind researchers to participant group allocation during data collection as a minimum, thus helping to alleviate the potential for performance bias even more.

Based upon the Grading of recommendations, Assessment, Development and Evaluations (GRADE) scoring system framework (551), the author would score the current trial as moderate. A moderate score was chosen considering the high levels of reliability (moderate to excellent) observed for all outcomes assessed, suggesting a low risk of imprecision. Furthermore, the results are mostly in line with previous studies, suggesting a low risk of inconsistency. Next, the study also directly observed

changes in health markers following an intervention trial conducted with older adults from the local community, suggesting a low risk of indirectness. Finally, the relatively large effect sizes observed for most outcome measures, without the need to correct for co-variates also suggests a large magnitude of effect without a high risk of residual confounding. Nevertheless, there is still the risk of substantial bias within the current study as discussed (group differences at baseline, different control group recruitment, lack of blinding etc), preventing a high score. Ultimately, the author is confident that the effects observed following SB displacement in this group of older adults is probably close to the true effect.

Future studies should employ additional testing points to determine the time course of such positive adaptations (e.g. every 2 weeks), as-well as follow up tests to determine whether changes in physical behaviour/ function are sustained in the long term (>8 weeks). Based on the findings observed future investigations could also investigate the effects of SB displacement with LIPA on endocrine markers, more in-depth muscle function tests (neuromuscular efficiency, muscle fatigability, length-tension, and force velocity relationships), tendon mechanical quality (tendon stiffness and young's modulus), and a specific focus on additional muscle groups (e.g. knee extensors) with more in depth assessments. It may also be prudent for future investigations to manipulate the SB displacement stimulus. Direct manipulation of the LIPA modality could be employed to increase the likelihood of achieving a specific outcome (e.g. single leg balance challenges during breaks to enhance balance posturography). Furthermore, interventions could assess the effects of directly manipulating habitual tasks, such as performing LIPA tasks with a slow tempo, or implementing LIPA tasks with higher muscular demands (stair climbing) to increase the likelihood of observing significant changes in motor control, muscle hypertrophy, neuromuscular adaptation, or muscle strength changes of greater magnitude respectively. Finally, future interventions could overload the SB displacement stimulus specifically, through gradual progressions in LIPA time (>45-50 minutes), and further titration of the fragmentation stimulus (fragmenting SB with LIPA every 10, 15 or 20 minutes). However, potential alterations to the design of future SB displacement interventions should always consider what effect the alteration will have on the intervention palatability, and likelihood of long-term adherence. On this note, the results from the partially validated questionnaire should be viewed as good pilot data highlighting the

barriers to SB displacement in older adults. Future studies could further validate the custom designed to questionnaire and use it to assess the palatability of future SB displacement interventions in older adults.

Conclusion

In conclusion SB displacement with LIPA is achievable, palatable, and results in good likelihood of long-term adherence. Combined with minimal alterations in other important lifestyle behaviours (Sleep, MVPA), SB displacement promisingly enhanced overall dietary quality. Despite no significant effect of SB displacement with LIPA on muscle hypertrophy, thoracic spine BMD was enhanced following both interventions, as-well as increased PF maximum voluntary contraction (2-3%). Interestingly, such an effect was mediated through divergent neuromuscular adaptation pathways dependant on the pattern of prescribed LIPA. Pattern dependant alterations in muscle architecture were also observed. Enhancements in physical function following SB displacement with LIPA (improved sit-to-stand ability, gait speed, and grip strength) likely represent a specificity of training effect improving one's ability to mobilise from a seated position. However aside from increases in bone health enhancing nutrients (habitual diet), greater enhancements in leg BMD, and greater improvements in peak handgrip strength, the results from the current thesis do not suggest an overt advantage of frequent vs continuous LIPA implementation. Instead most functional improvements observed were equal in magnitude irrespective of whether LIPA was prescribed in a fragmented or continuous fashion. Ultimately, despite its perceived designation as a suboptimal physical activity prescription, LIPA implementation appears to enhance overall health and function in older women. Notably, statistically non-significant muscular adaptation following LIPA implementation may still hold clinical benefit in older adults.

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Appendix i

Table Ai.1- Baseline characteristics, intervention, and diary-based outcomes between different groups. **Boldened text** represents a significant baseline difference (additional information)

	Group				
	SBF(n=14)		LIPA (n=14)		Control (n=8)
Nutritional supplements(n)	0±1		0±1		1±1 (n=7)
Basal Metabolic Rate (kcal) (Harris-Benedict)	1252±125		1230±77		1256±95 (n=7)
Metabolic Balance (kcal) (Harris-Benedict)	-98±626		72±546		243±419 (n=7)
Basal Metabolic Rate (kcal) (Schofield)	1281±102		1253±78		1270±79 (n=7)
Metabolic Balance (kcal) (Schofield)	-311±607		-80±528		74±489 (n=7)
Proportion consuming optimal levels of ≥3/5 pro anabolic nutrients	43%		29%		29% (n=7)
Proportion consuming optimal levels of ≥5/8 bone health enhancing nutrients	36%		50%		57% (n=7)
Intervention Outcomes					
Intervention Length (Days)	57±2		56±1		54±5
Proportion who begun intervention in Spring/Summer (Autumn/Winter)	36% (64%)		36% (64%)		**100% (0%)**
Proportion who shifted classification from sedentary to ambulator (stable)	21% (79%)		21% (79%)		29% (71%)
Diary Based Outcomes					
	Week 1	Week 8	Week 1	Week 8	
Self-reported prompts complied (n)	9±12	6±8*			
Self-reported prompts non-complied (n)	5±5	4±4			
Self-reported total Prompts (n)	15±9	10±9			
Self-reported days complied to LIPA (n)			7±1	7±1	

Self-reported days non-complied to LIPA(n)	[redacted]	1.0±1.5	0.0±0.6	[redacted]
Self-reported daily LIPA (mins)		49±14	49±11	

LIPA; light intensity physical activity.

Table Ai.2 – Physical behaviour outcomes at baseline, week 8, and both the average absolute and relative change from baseline, for each group. **Boldened text** represents a significant baseline difference. * Represents a significant change over time in the sub-sample experimental analysis. Additional information

	SBF (n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (%)	Pre	Post	Change (%)	Pre	Post	Change (%)
Breaks in SB time (number)	21±4	21±6	1±3 (2±18%)	20±5	22±5	1±3 (3±16%)	22±4	24±10	2±6 (8±27%)
Bouts of SB time <5 minutes (number)	6.1±2.0	6.0±2.6	-0.07±2.44 (-1±14%)	5.9±3.0	6.7±3.3	-0.02±3.09 (-1±47%)	6.1±4.8	6.2±5.3	-0.38±5.02 (-4±91%)
True mean bout of SB (minutes)	15.5±4.1	16.0±4.8	0.6±3.2 (4±21%)	15.6±2.6	16.7±4.7	1.01±3.61 (6±25%)	16.5±5.5	17.8±5.7	0.98±3.06 (6±18%)
Power law exponent used to describe SB accumulation	1.5±0.1	1.5±0.0	0.02±0.06 (1±4%)	1.5±0.1	1.5±0.1	0.01±0.04 (1±2%)	1.5±0.0	1.5±0.1	-0.02±0.09 (-1±6%)
The bout duration above and below which half of all SB is accrued (minutes)	61±29	51±21	-6±11 (-10±24%)	53±22	49±15	-6±18 (-10±24%)	45±25	36±36	-1±27 (-3±55%)
Bouts of PA (number)	21±4	21±6	0.53±3.37 (2±18%)	20±5	22±4	0.51±3.03 (3±16%)	22±4	24±10	1.84±5.65 (8±27%)
Daily sum of PA bout time (minutes)	383±104	353±100	-1.1±62.4 (-0.3±17%)	345±120	404±125	35.0±65.5 (12±24%)	356±170	454±202	5.22±90.1 (4±26%)
True mean PA bout (minutes)	18.7±4.7	17.6±5.2	-0.2±4.8 (-1±33%)	17.1±3.6	19.0±6.3	1.25±7.03 (8±47%)	20.8±6.7	19.2±4.4	-1.53±6.60 (-0.3±13%)
Proportion of PA time spent in SB	2±1	1±1	0±1 (-2±36%)	1±1	1±1	0±0 (16±35%)	1±1	1±1	0±1 (-14±37%)
Proportion of PA time spent in STD	15±13	17±18	2±5 (9±33%)	22±12	23±10	-1±3 (-4±14%)	18±4	19±6	-1±5 (-3±32%)
Proportion of PA time spent in LIPA	37±5	35±9	0±5 (1±15%)	35±4	36±10	1±3 (2±9%)	29±7	31±10	0±6 (1±22%)

Proportion of PA time spent in MVPA	46±17	45±23	-3±6 (-8±16%)	41±11	42±9	0±5 (2±14%)	**48±7**	50±6	0±6 (2±12%)
Bouts of MVPA ≥10 minutes (number)	1±2	0±2	-0.17±1.07 (-36±60%)	1±1	1±1	0.00±0.80 (0±81%)	1±1	1±1	-0.19±1.27 (-21±334%)
Sporadic MVPA in bouts of <10 minutes (minutes)	155.8 ±42.5	155.2 ±49.6	-0.62±28.87 (-1±20%)	142.4 ±46.3	154.2 ±41.3	11.8±28.31 (12±24%)	**202.6 ±71.5**	209.6 ±54.7	7.05±50.2 (10±34%)

LIPA; Light intensity physical activity, MVPA; Moderate to vigorous physical activity, PA; Physical activity, SB; Sedentary behaviour, STD, Standing

Table *Ai*.3- Significant associations between physical behaviour outcomes at baseline, and subsequent intervention weeks for each group.

	SBF (n=14)		LIPA (n=14)		Control (n=8)	
	Weeks associated with baseline (n)	Proportion	Weeks associated with baseline (n)	Proportion	Weeks associated with baseline (n)	Proportion
Sleep (hours)	4/8	50%	5/8	63%	1/8	13%
SB (hours)	8/8	100%	0/8	0%	0/8	0%
STD (hours)	8/8	100%	0/8	0%	0/8	0%
LIPA (hours)	1/8	13%	5/8	63%	2/8	25%
MVPA (hours)	8/8	100%	5/8	63%	7/8	88%
SB (% of waking hours)	7/8	88%	6/8	75%	1/8	13%
STD (% of waking hours)	7/8	88%	7/8	88%	4/8	50%
LIPA (% of waking hours)	7/8	88%	5/8	63%	3/8	38%
MVPA (% of waking hours)	8/8	100%	5/8	63%	7/8	88%
Breaks in SB time (number)	8/8	100%	7/8	88%	3/8	38%
Bouts of SB time <5 minutes (number)	8/8	100%	8/8	100%	5/8	63%
True mean bout of SB (minutes)	6/8	75%	4/8	50%	4/8	50%
Average SB bout length (minutes)	7/8	88%	4/8	50%	5/8	63%
Power law exponent used to describe SB accumulation	0/8	0%	1/8	13%	3/8	38%
The bout duration above and below which half of all SB is accrued (minutes)	8/8	100%	7/8	88%	1/8	13%
Bouts of PA (number)	8/8	100%	7/8	88%	3/8	38%

Daily sum of PA bout time (minutes)	6/8	75%	4/8	50%	0/8	0%
True mean PA bout (minutes)	6/8	75%	1/8	13%	1/8	13%
Proportion of PA time spent in SB	8/8	100 %	8/8	100 %	3/8	38%
Proportion of PA time spent in STD	8/8	100 %	8/8	100 %	3/8	38%
Proportion of PA time spent in LIPA	6/8	75%	4/8	50%	3/8	38%
Proportion of PA time spent in MVPA	8/8	100 %	7/8	88%	1/8	13%
MVPA in bouts ≥10 minutes duration (minutes)	8/8	100 %	1/8	13%	0/8	0%
Bouts of MVPA ≥10 minutes (number)	6/8	75%	0/8	0%	0/8	0%
Sporadic MVPA in bouts of <10 minutes (minutes)	7/8	88%	6/8	75%	3/8	38%

LIPA; Light intensity physical activity, MVPA; Moderate to vigorous physical activity, PA; Physical activity, SB; Sedentary behaviour, STD, Standing

Table Ai.4- Physical behaviour intra-week variability expressed as co-efficient of variation at baseline, and week 8, for each group. **Boldened text** represents a significant baseline difference. * represents a significant change over time in the whole cohort analysis.

	SBF (n=14)		LIPA (n=14)		Control (n=8)	
	Baseline	Week 8	Baseline	Week 8	Baseline	Week 8
Sleep (hours)	6±13%	9±6%	9±8%	7±4%	6±8%	7±8%
SB (hours)	11±13%	13±15%	13±7%	14±6%	15±9%	15±9%
STD (hours)	26±9%	23±16%	27±11%	22±6%	28±6%	21±14%
LIPA (hours)	20±14%	20±10%	25±10%	21±15%	22±15%	25±15%
MVPA (hours)	20±8%	19±14%	22±11%	26±17%	18±6%	21±14%
SB (% of waking hours)	10±6%	10±11%	12±5%	13±7%	13±6%	14±7%
STD (% of waking hours)	28±11%	23±14%	28±11%	23±10%	28±6%	24±16%
LIPA (% of waking hours)	20±11%	19±13%	25±12%	24±14%	23±16%	26±18%
MVPA (% of waking hours)	20±9%	19±13%	21±11%	25±17%	19±3%	19±15%
Breaks in SB time (number)	17±12%	20±11%	18±9%	18±16%	21±11%	23±20%
Bouts of SB time <5 minutes (number)	40±26%	42±33%	45±25%	48±28%	44±30%	46±20%
True mean bout of SB (minutes)	19±9%	16±17%	20±11%	19±7%	22±5%	26±15%
Average SB bout length (minutes)	29±18%	26±24%	34±21%	32±18%	23±12%	39±28%
Power law exponent used to describe SB accumulation	5±2%	6±4%	6±3%	5±3%	4±1%	5±4%
The bout duration above and below which half of all SB is accrued (minutes)	28±20%	31±9%	35±12%	32±27%	34±18%	45±52%
Bouts of PA (number)	17±12%	20±11%	18±9%	18±16%	21±11%	23±20%
Daily sum of PA bout time (minutes)	18±7%	23±24%	21±7%	19±8%	16±3%	19±12%
True mean PA bout (minutes)	28±14%	28±16%	25±11%	24±11%	35±9%	29±13%
Proportion of PA time spent in SB	35±27%	48±28%	44±19%	49±12%	37±22%	32±30%
Proportion of PA time spent in STD	20±10%	19±11%	22±8%	21±8%	19±5%	18±8%
Proportion of PA time spent in LIPA	9±8%	13±10%	12±6%	13±6%	13±11%	12±6%

Proportion of PA time spent in MVPA	12±6%	10±11%	**15±7%**	18±7%	11±6%	9±6%
MVPA in bouts ≥10 minutes duration (minutes)	77±191 %	10±143 %	158±123 %	181±99 %	166±139 %	133±155 %
Bouts of MVPA ≥10 minutes (number)	79±194 %	12±140 %	155±119 %	175±113 %	169±135 %	131±162 %
Sporadic MVPA in bouts of <10 minutes (minutes)	20±5%	17±9%	23±9%	24±17%	19±9%	22±14%

LIPA; Light intensity physical activity, MVPA; Moderate to vigorous physical activity, PA; Physical activity, SB; Sedentary behaviour, STD, Standing

Table *Ai.*5- Significant associations between physical behaviour intra-week variability co-efficient of variation outcomes at baseline, and subsequent intervention weeks for each group.

	SBF (n=14)		LIPA (n=14)		Control (n=8)	
	Weeks associated with baseline (n)	Proportion	Weeks associated with baseline (n)	Proportion	Weeks associated with baseline (n)	Proportion
Sleep (hours)	3/8	38%	1/8	13%	1/8	13%
SB (hours)	1/8	13%	0/8	0%	2/8	25%
STD (hours)	0/8	0%	0/8	0%	0/8	0%
LIPA (hours)	0/8	0%	0/8	0%	0/8	0%
MVPA (hours)	1/8	13%	1/8	13%	0/8	0%
SB (% of waking hours)	0/8	0%	0/8	0%	0/8	0%
STD (% of waking hours)	0/8	0%	0/8	0%	0/8	0%
LIPA (% of waking hours)	0/8	0%	0/8	0%	0/8	0%
MVPA (% of waking hours)	2/8	25%	0/8	0%	0/8	0%
Breaks in SB time (number)	1/8	13%	0/8	0%	2/8	25%
Bouts of SB time <5 minutes (number)	1/8	13%	2/8	25%	2/8	25%
True mean bout of SB (minutes)	1/8	13%	1/8	13%	1/8	13%
Average SB bout length (minutes)	0/8	0%	0/8	0%	0/8	0%
Power law exponent used to describe SB accumulation	2/8	25%	0/8	0%	1/8	13%
The bout duration above and below which half of all SB is accrued (minutes)	0/8	0%	0/8	0%	0/8	0%
Bouts of PA (number)	1/8	13%	0/8	0%	2/8	25%
Daily sum of PA bout time (minutes)	1/8	13%	0/8	0%	0/8	0%
True mean PA bout (minutes)	1/8	13%	1/8	13%	1/8	13%
Proportion of PA time spent in SB	0/8	0%	2/8	25%	1/8	13%
Proportion of PA time spent in STD	2/8	25%	1/8	13%	0/8	0%
Proportion of PA time spent in LIPA	3/8	38%	0/8	0%	0/8	0%

Proportion of PA time spent in MVPA	1/8	13%	0/8	0%	0/8	0%
MVPA in bouts ≥10 minutes duration (minutes)	0/8	0%	0/8	0%	1/8	13%
Bouts of MVPA ≥10 minutes (number)	0/8	0%	0/8	0%	0/8	0%
Sporadic MVPA in bouts of <10 minutes (minutes)	1/8	13%	0/8	0%	0/8	0%

LIPA; Light intensity physical activity, MVPA; Moderate to vigorous physical activity, PA; Physical activity, SB; Sedentary behaviour, STD, Standing

Table Ai.6- Physical behaviour intra-week variability expressed as individual variance at baseline, and week 8, for each group. **Boldened text** represents a significant baseline difference. × represents a significant group×time interaction effect in the whole cohort analysis.

	SBF (n=14)		LIPA (n=14)		Control (n=8)	
	Baseline	Week 8	Baseline	Week 8	Baseline	Week 8
Sleep (hours)	0.2±1.78	0.4±0.6	0.5±1.0	0.4±0.3	0.2±0.8	0.3±0.8
SB (hours)	0.9±2.2	0.9±1.4	1.2±1.2	1.2±1.0	1.5±0.9	1.5±1.8
STD (hours)	0.1±0.1	0.1±0.2	0.1±0.2	0.1±0.2	0.1±0.1	0.04±0.1
LIPA (hours)	0.2±0.3	0.2±0.3	0.2±0.1	0.2±0.2	0.2±0.1	0.2±0.1
MVPA (hours)	0.2±0.5	0.2±0.3	0.2±0.5	0.5±0.5	0.3±0.4	0.4±0.7
SB (% of waking hours)	32±46	31±47	47±28	39±49	43±33	47±71
STD (% of waking hours)	2±2	2±9	4±8	4±6	4±4	2±3
LIPA (% of waking hours)	5±9	5±12	9±8	9±8	8±6	8±6
MVPA (% of waking hours)	7±12	10±16	9±14	21±13	13±13	16±26
Breaks in SB time (number)	14±16	14±16	13±11	13±33	16±14	17±18
Bouts of SB time <5 minutes (number)	5±5	4±7	6±7	5±12	5±5	4±6
True mean bout of SB (minutes)	7±8	7±12	8±8	7±8	9±9	11±9
Average SB bout length (minutes)	72±133	50±110	97±175	107±107	33±46	56±372
Power law exponent used to describe SB accumulation	0.004±0.01	0.004±0.01	0.004±0.01	0.003±0.01	0.003±0.0	0.001±0.01
The bout duration above and below which half of all SB is accrued (minutes)	242±305	151±230	328±350	138±317	181±285	450±1356
Bouts of PA (number)	14±16	14±16	13±11	13±33	16±14	17±18
Daily sum of PA bout time (minutes)	4386±3393	5099±6305	4551±2033	5075±4487	3798±1827	4995±4511
True mean PA bout (minutes)	16±46	9±41	15±27	12±43	38±80	13±65
Proportion of PA time spent in SB	20±98	32±46×	21±42	27±44×	22±60	10±11×

Proportion of PA time spent in STD	10±11	6±26	**23±12**	16±11	9±8	7±14
Proportion of PA time spent in LIPA	10±13	16±14×	14±12	16±14×	14±20	7±10×
Proportion of PA time spent in MVPA	25±19	15±14	27±30	38±34	21±29	17±30
MVPA in bouts ≥10 minutes duration (minutes)	142±515	30±369	142±220	135±623	169±519	141±414
Bouts of MVPA ≥10 minutes (number)	0±1	0±1	1±1	1±1	1±2	1±1
Sporadic MVPA in bouts of <10 minutes (minutes)	654±856	809±842	604±1453	1277±1800	1056±1057	1186±1679

LIPA; Light intensity physical activity, MVPA; Moderate to vigorous physical activity, PA; Physical activity, SB; Sedentary behaviour, STD, Standing

Table *Ai.*7- Significant associations between physical behaviour intra-week variability individual variance outcomes at baseline, and subsequent intervention weeks for each group.

	SBF (n=14)		LIPA (n=14)		Control (n=8)	
	Weeks associated with baseline (n)	Proportion	Weeks associated with baseline (n)	Proportion	Weeks associated with baseline (n)	Proportion
Sleep (hours)	1/8	13%	1/8	13%	0/8	0%
SB (hours)	0/8	0%	1/8	13%	0/8	0%
STD (hours)	2/8	25%	0/8	0%	0/8	0%
LIPA (hours)	0/8	0%	1/8	13%	0/8	0%
MVPA (hours)	1/8	13%	0/8	0%	2/8	25%
SB (% of waking hours)	1/8	13%	0/8	0%	0/8	0%
STD (% of waking hours)	1/8	13%	0/8	0%	1/8	13%
LIPA (% of waking hours)	0/8	0%	0/8	0%	0/8	0%
MVPA (% of waking hours)	1/8	13%	0/8	0%	1/8	13%
Breaks in SB time (number)	2/8	25%	0/8	0%	0/8	0%
Bouts of SB time <5 minutes (number)	0/8	0%	3/8	38%	0/8	0%
True mean bout of SB (minutes)	2/8	25%	0/8	0%	0/8	0%
Average SB bout length (minutes)	1/8	13%	0/8	0%	2/8	25%
Power law exponent used to describe SB accumulation	2/8	25%	1/8	13%	2/8	25%

The bout duration above and below which half of all SB is accrued (minutes)	0/8	0%	0/8	0%	0/8	0%
Bouts of PA (number)	2/8	25%	0/8	0%	0/8	0%
Daily sum of PA bout time (minutes)	1/8	13%	0/8	0%	0/8	0%
True mean PA bout (minutes)	2/8	25%	1/8	13%	0/8	0%
Proportion of PA time spent in SB	1/8	13%	2/8	25%	1/8	13%
Proportion of PA time spent in STD	1/8	13%	1/8	13%	0/8	0%
Proportion of PA time spent in LIPA	1/8	13%	1/8	13%	0/8	0%
Proportion of PA time spent in MVPA	0/8	0%	0/8	0%	1/8	13%
MVPA in bouts ≥10 minutes duration (minutes)	5/8	63%	0/8	0%	0/8	0%
Bouts of MVPA ≥10 minutes (number)	3/8	38%	1/8	13%	2/8	25%
Sporadic MVPA in bouts of <10 minutes (minutes)	0/8	0%	0/8	0%	0/8	0%

LIPA; Light intensity physical activity, MVPA; Moderate to vigorous physical activity, PA; Physical activity, SB; Sedentary behaviour, STD, Standing

Table Ai8- Habitual dietary outcomes at baseline, and week 8, for each group. Boldened text represents a significant baseline difference. * represents a significant change over time. × represents a significant group×time interaction effect**.**

	SBF (*n*=14)		**LIPA (*n*=14)**		**Control (*n*=7)**	
	Baseline	**Week 8**	**Baseline**	**Week 8**	**Baseline**	**Week 8**
Fructose (g)	17±8	15±7	19±7	16±9	17±5	20±7
Maltose (g)	1.2±0.8	1.3±1.0	1.7±0.7	1.8±0.9	2.0±1.2	2.0±2.6
Sucrose (g)	18.5±10.3	16.2±6.9	23.5±12.5	25.1±13.1	18.5±11.5	16.7±8.3
Galactose (g)	1.1±1.3	0.4±0.6	1.2±2.2	0.3±0.4	0.8±1.0	0.9±1.7
Lactose (g)	12.7±8.4	10.2±5.4	15.5±8.4	13.1±6.7	10.7±2.2	11.0±5.3
Starch (g)	66±35	72±51	70±31	79±43	80±29	87±57
Total Sugars (g)	71.2±38.4	65.6±47.9	96.9±47.9	75.8±25.6	83.4±35.4	86.7±44.2
Non-starch Polysaccharides (g)	15.0±3.0	15.2±4.7	15.7±6.0	15.6±6.7	18.4±5.1	15.8±4.1
Saturated Fatty Acids (g)	21±22	20±13	23±19	23±10	26±8	21±17
Mono-Unsaturated Fatty Acids (g)	21±17	20±21	21±14	20±9	22±12	20±11
Poly-Unsaturated Fatty Acids (g)	8±8	11±7	8±4	10±8	13±5	9±3
Trans Fatty Acids (g)	0.5±0.6	0.6±0.4	0.6±0.6	0.8±0.5	0.7±0.2	0.5±1.0
Omega-3 Fatty Acids (g)	2.6±2.5	1.6±3.5	1.3±1.4	1.9±1.8	1.2±1.3	0.8±1.2
Omega-6 Fatty Acids (g)	5.8±5.5	7.9±10.4	5.6±4.5	5.6±4.6	7.9±5.4	5.3±3.5
Vitamin A (µg)	908±812	989±792	836±429	1052±1139	582±139	754±528
Vitamin B1 (mg)	1.2±0.3	1.2±0.9	1.5±0.7	1.4±0.8	1.3±0.3	1.2±0.7

Vitamin B2 (mg)	1.6±0.9	1.6±0.8	1.6±0.4	1.5±0.4	1.8±0.7	1.4±0.9
Vitamin B6 (mg)	1.7±0.4	1.4±0.5	1.8±.9	1.3±0.6	1.7±0.8	1.7±0.4
Vitamin B9 (µg)	243±103	232±73	267±141	219±108	206±156	256±82
Vitamin C (mg)	101±49	95±61	116±54	100±55	117±51	139±69
Vitamin D (µg)	4.9±4.2	3.8±5.3	3.6±4.3	3.6±4.1	3.1±2.0	4.2±3.8
Vitamin E (mg)	7.4±4.1	7.7±5.8	7.3±7.1	6.7±4.2	10.7±4.2	7.4±3.9
Calcium (mg)	727±295	702±251	867±350	882±466	817±194	724±249
Chloride (mg)	2400±1233	3033±2506	2739±1294	2472±873	2646±852	3105±1109
Copper (mg)	1.2±0.5	1.4±1.0	1.4±0.6	1.1±0.3	1.3±0.5	1.5±0.5
Iodine (ug)	183±168	149±98	154±67	137±47	138±70	186±89
Iron (mg)	8.9±2.5	9.4±5.7	9.9±3.3	9.1±3.6	16.6±17.5	10.3±1.8
Magnesium (mg)	297±93	285±120	325±102	280±60	307±82	306±75
Manganese (mg)	3.3±0.8	3.8±2.5	4.3±2.7	3.6±1.2	4.0±0.8	4.0±1.4
Phosphorous (mg)	1159±332	1084±337	1285±367	1055±374	1234±182	1283±208
Potassium (mg)	2882±544	2551±724	3250±786	2798±588	2819±644	3000±455
Selenium (µg)	55.8±22.9	51.7±48.8	45.8±21.1	43.8±22.3	53.3±20.3	58.2±22.8
Sodium (mg)	1459±804	1775±1518	1833±1030	1447±536	1672±679	1914±720
Alcohol (g)	0±11	0±8	4±10	4±24	7±9	16±18

Table Ai9: Habitual dietary outcomes expressed relative to recommended daily amounts (RDA).

	Recommended daily amount (RDA)	Whole sample at baseline (n=35)	SBF (n=14)		LIPA (n=14)		CONTROL (n=7)	
			Group average expressed as %RDA					
		Proportion meeting RDA	Pre	Post	Pre	Post	Pre	Post
Protein (g/kg)	≥0.8 g/kg/day	31/35	125 %	123 %	139 %	133 %	161%	150%
Carbohydrate (g)	Within 45-65% Daily caloric intake	10/35	93%	87%	102 %	96%	91%	107%
Total Fat (g)	≤35% Daily caloric intake	14/35	120 %	121 %	110 %	108 %	106%	111%
Saturated Fatty Acids (g)	<11% of Daily caloric intake	10/35	125 %	111 %	122 %	118 %	117%	111%
Trans Fatty Acids (g)	<2% of Daily caloric intake	35/35	16%	18%	17%	22%	17%	14%
Mono-Unsaturated Fatty Acids (g)	≥28g/day	10/35	75%	71%	75%	71%	79%	71%
Poly-Unsaturated Fatty Acids (g)	≥14g/day	6/35	57%	79%	57%	71%	93%	64%
Omega-3 Fatty Acids (g)	≥1.6 g/day	12/35	163 %	100 %	81%	119 %	75%	50%
Omega-6 Fatty Acids (g)	≥10 g/day	6/35	58%	79%	56%	56%	79%	53%
Vitamin A (µg)	≥600 µg/day	23/35	151 %	165 %	139 %	175 %	97%	126%
Vitamin B1 (mg)	≥0.8 mg/day	33/35	150 %	150 %	188 %	175 %	163%	150%
Vitamin B2 (mg)	≥1.1 mg/day	32/35	145 %	145 %	145 %	136 %	164%	127%
Vitamin B3 (mg)	≥12.6 mg/day	23/35	106 %	103 %	111 %	103 %	136%	120%

Vitamin B6 (mg)	≥1.2 mg/day	31/35	142 %	117 %	150 %	108 %	142%	142%
Vitamin B9 (µg)	≥200 µg/day	25/35	122 %	116 %	134 %	110 %	103%	128%
Vitamin B12 (µg)	≥1.5 µg/day	35/35	333 %	293 %	273 %	313 %	300%	333%
Vitamin C (mg)	≥40 mg/day	33/35	253 %	238 %	290 %	250 %	293%	348%
Vitamin D (µg)	≥10 µg/day	3/35	49%	38%	36%	36%	31%	42%
Vitamin E (mg)	≥3 mg/day	35/35	247 %	257 %	243 %	223 %	357%	247%
Calcium (mg)	≥700 mg/day	19/35	104 %	100 %	124 %	126 %	117%	103%
Chloride (mg)	≥ 2500mg/day	15/35	96%	121 %	110 %	99%	106%	124%
Iodine (ug)	≥140 µg/day	16/35	131 %	106 %	110 %	98%	99%	133%
Iron (mg)	≥8.7 mg/day	20/35	102 %	108 %	114 %	105 %	191%	118%
Magnesium (mg)	≥ 270mg/day	21/35	110 %	106 %	120 %	104 %	114%	113%
Phosphorous (mg)	≥ 550mg/day	34/35	211 %	197 %	234 %	192 %	224%	233%
Potassium (mg)	≥ 3500mg/day	3/35	82%	73%	93%	80%	81%	86%
Selenium (µg)	≥60 µg/day	7/35	93%	86%	76%	73%	89%	97%
Sodium (mg)	<2.4 g/day	30/35	61%	74%	76%	60%	70%	80%
Zinc (mg)	≥7 mg/day	18/35	96%	113 %	104 %	99%	113%	121%

Chapter 3

The following outcome variables exhibited non-normal distributions and unequal variances:

Absolute physical behaviour variables: Regarding the absolute physical behaviour variables, those that possessed non-normally distributed data sets included, SB_BL, STD_BL, STD_W8, SB%_BL, STD%_BL, SB%_W8, MVPA%_BL, SBBREAKS_BL, <5SB_W8, >=SB_BL, >=SB_W8, MSBBoutMins_BL, MeanSBBoutMins_W8, Alfa_BL, The bout duration above and below which half of all sedentary time is accrued_W8, Arousable, PABoutsMins_BL, PASTD%_W8, PALIPA%_BL, >=10MVPAMins_BL, >=10MVPAMins_W8, and >=10MVPABouts_W8. Furthermore, those that possessed heterogenous data sets included LIPA_BL, Alfa_W8, PASB%_W8, PASTD%_BL, PASTD%_W8, PAMVPA%_W8, and >=10MVPABouts_BL. Finally, data that were both non-normally distributed and heterogeneously variant were PASTD%_W8, >=10MVPAMins_BL.

Regarding the absolute physical behaviour variables for the subsample analysis (n=28) of experimental participants that excluded the control participants data sets that were non-normally distributed included STD_BL, STD_W8, STD%_BL, STD%_W8, SBBREAKS_BL, <5SB_W8, >=SB_W8, MEANSBBoutMins_BL, MEANSBBoutMins_W8, The bout duration above and below which half of all sedentary time is accrued_W8, PABouts_BL, PALIPA%_BL, >=10MVPAMins_W8, & >=10MVPABouts_W8. Those that possessed heterogenous data sets were PASB%, & PA_MVPA%_W8. Finally, data that were both non-normally distributed and heterogeneously variant were PASTD%_W8, >=10MVPAMins_BL, & >=10MVPABouts_BL.

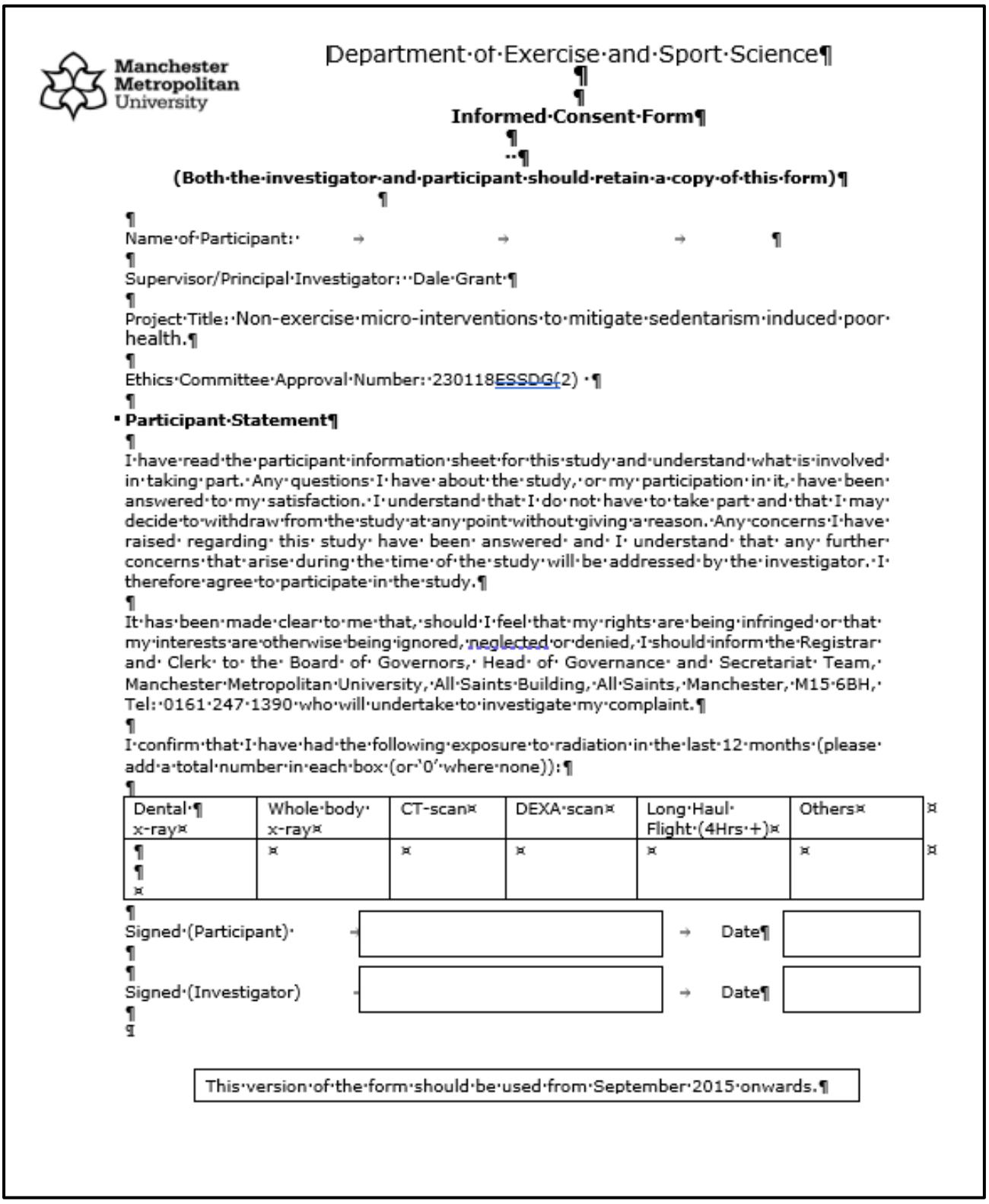
Intra-week co-efficient of variation: Regarding the calculated co-efficient of variation, those that possessed non-normally distributed data sets included, Sleep_BL, SB_BL, SB_W8, STD_BL, STD_W8, LIPA_W8, MVPA_W8, SB%_BL, SB%_W8, STD%_BL, STD%_W8, LIPA%_W8, MVPA%_W8, SBBREAKS_W8, <5SB_W8, >=SB_W8, MSBBoutMins_BL, MeanSBBoutMins_W8, The bout duration above and below which half of all sedentary time is accrued_W8, PABouts_W8, MeanPABouts_W8, PASB%_BL, PASB%_W8, PASTD%_W8, PALIPA%_W8, PAMVPA%_BL,

PAMVPA%_W8, >=10MVPAMins_W8, >=10MVPABouts_W8, & SPMVPA_W8. Only MVPA%_BL was both non-normally distributed and heterogeneously variant.

Intra-week individual variance: Regarding the calculated individualised variance the variables that were non-normally distributed were as follows: Sleep_BL, Sleep_W8, SB_BL, SB_W8, STD_W8, LIPA_W8, MVPA_BL, MVPA_W8, SB%_W8, Std%_W8, LIPA%_W8, MVPA%_BL, MVPA%_W8, SBBREAKS_BL, SBBREAKS_W8, <5SB_BL, >=SB_BL, >=SB_W8, MSBBoutMins_BL, Alfa_W8, The bout duration above and below which half of all sedentary time is accrued_W8, PABouts_BL, PABouts_W8, , MeanPABouts_W8, PASB%_BL, PASB%_W8, PASTD%_BL, PASTD%_W8, PALIPA%_BL, PALIPA%_W8, PAMVPA%_BL, PAMVPA%_W8, >=10MVPAMins_BL, >=10MVPAMins_W8, >=10MVPABouts_BL, >=10MVPABouts_W8, SPMVPAMins_BL, and SPMVPA_W8.

Habitual Diet: The habitual dietary variables that were non-normally distributed were kilocalories_post, kilojoules_post, relative carbohydrate intake_post, relative protein intake_post, saturated fat_post, mono-unsaturated fat_pre, polyunsaturated fat_pre, polyunsaturated fat_post, omega 6 fatty acids_pre, omega 6 fatty acids_post, vitamin B12_pre, vitamin B12_post, vitamin E_pre, vitamin E_post, Calcium_post, Zinc_pre, Zinc_post, Galactose_pre, Glucose_post, Lactose_pre, Maltose_pre, Starch_pre, Starch_post, Sugar_pre, Non-starch polysaccharides_post, Chloride_pre, Chloride_post, Copper_pre, Iron_post, Folate_post, Iodine_pre, Iodine_post, Maganese_pre, Manganese_pre, Manganese_post, Selenium_pre, Selenium_post, Sodium_pre, Sodium_post, Vitamin B3_pre, Phosphorous_post, Retinol_pre, Retionol_post, Retinol equivalents_pre, Retionol equivalents_post, Vitamin B1_pre, Vitamin B2_pre, Vitamin B2_post, Vitamin B6_pre, and Alcohol_pre. The variables with unequal variances were Trans fatty acids_post, and Vitamin B3_post. Thus the variables that were both non-normally distributed and had unequal variances were omega 3 fatty acids _pre, omega 3 fatty acids _post, Vitamin D_pre, Vitamin D_post, Galactose_post, Maltose_post, Copper_post, Iron_pre, Vitamin B1_post, Vitamin B6_post, and Alcohol_post.

Informed Consent Form



Manchester Metropolitan University

Department of Exercise and Sport Science

Informed Consent Form

(Both the investigator and participant should retain a copy of this form)

Name of Participant: → → → ¶

Supervisor/Principal Investigator: Dale Grant

Project Title: Non-exercise micro-interventions to mitigate sedentarism induced poor health.

Ethics Committee Approval Number: 230118ESSDG(2)

Participant Statement

I have read the participant information sheet for this study and understand what is involved in taking part. Any questions I have about the study, or my participation in it, have been answered to my satisfaction. I understand that I do not have to take part and that I may decide to withdraw from the study at any point without giving a reason. Any concerns I have raised regarding this study have been answered and I understand that any further concerns that arise during the time of the study will be addressed by the investigator. I therefore agree to participate in the study.

It has been made clear to me that, should I feel that my rights are being infringed or that my interests are otherwise being ignored, neglected or denied, I should inform the Registrar and Clerk to the Board of Governors, Head of Governance and Secretariat Team, Manchester Metropolitan University, All Saints Building, All Saints, Manchester, M15 6BH, Tel: 0161 247 1390 who will undertake to investigate my complaint.

I confirm that I have had the following exposure to radiation in the last 12 months (please add a total number in each box (or '0' where none)):

Dental x-ray	Whole body x-ray	CT-scan	DEXA scan	Long Haul Flight (4Hrs +)	Others

Signed (Participant) → [ ] → Date [ ]

Signed (Investigator) → [ ] → Date [ ]

This version of the form should be used from September 2015 onwards.

Participant Information Sheet

MANCHESTER METROPOLITAN UNIVERSITY

MMU Cheshire

Department of Exercise and Sport Science

Information Sheet for Participants

Title of Study: Non-exercise micro-interventions to mitigate sedentarism-induced poor health

Ethics Approval Number: 230118ESSDG(2)

Participant Information Sheet

1) This is an invitation to take part in a piece of research.

You are being invited to take part in a research study. Before you decide whether to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Please take time to decide whether you wish to take part.

2) What is the purpose of the research?

The overall purpose of this research is to figure out how all of the movement you perform across a single day (physical behaviours) may affect your long-term health. We are also interested in whether the manipulation of these behaviours (i.e. less sitting and more standing), can improve markers of health, such as your blood sugar or your ability to stand on one leg (balance). Lastly, we want to identify and find solutions to any barriers that stop you from becoming more physically active.

3) Why is the study being performed?

Older adults (65y+) spend a lot of their time in a seated position. Increased time spent sitting increases the risk for poor health conditions such as cardiovascular disease, and diabetes. Furthermore, increased time spent sitting increases the risk for a fall, leading to injury, and potentially frailty in older adults. Consequently, reducing sitting time with non-exercise micro-interventions is one such viable option. However, the effectiveness of such interventions is still undetermined. Therefore, the current study aims to figure out, whether manipulating physical behavior, is an effective strategy to maintain vitality and improve health during aging.

4) Why am I being asked to take part?

You are being asked to take part because you meet the desired inclusion criteria.

- ⇒Female aged 65-85y
- ⇒From the Cheshire area
- ⇒No history of lower limb muscle/tendon/joint disorders that affect movement or strength through the ankle joint in the past 6 months.
- ⇒Not suffering from chronic health conditions likely to affect your ability to safely and independently undertake a program of increased physical activity (cardiovascular disease, uncontrolled diabetes, active cancer, etc.).
- ⇒Not currently using prescribed medication likely to affect your ability to perform movement.

Furthermore, you may have previously demonstrated an interest in taking part in the research we are undertaking at Manchester Metropolitan University.

5) Do I have to take part?

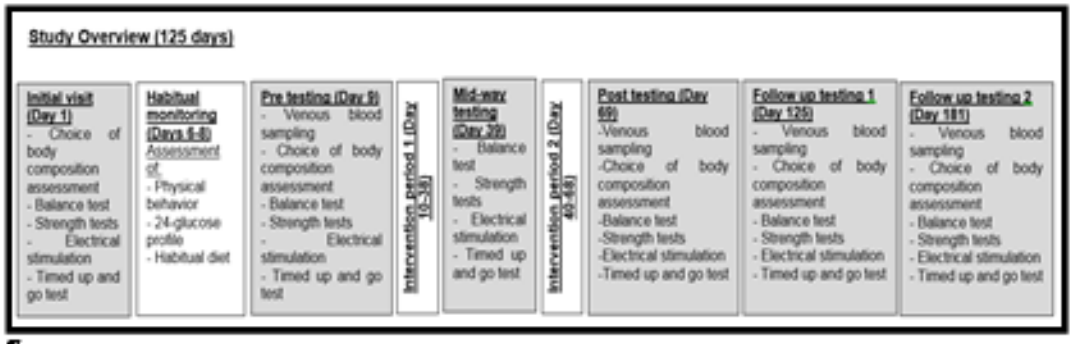
You are under no obligation to take part in this study. If, after reading this information sheet and asking any additional questions, you do not feel comfortable taking part in the study you do not have to. If you do decide to take part you are free to withdraw from the study at any point, without having to give a reason. If you do withdraw from the study you are free to take any personal data with you by written request to me, the principal investigator, and this will not be included when the research is reported. If you decide not to take part or withdraw from the study it will not affect your relationship with any of the staff at the Manchester Metropolitan University.

If you do decide to take part, you will be asked to sign an informed consent form stating your agreement to take part and you will be given a copy together with this information sheet to keep.

6) What will happen to me if I agree to take part?

Time commitment

You will be asked to attend the laboratories at Manchester Metropolitan University a total of four times over the initial eight week study period (please see study overview below). Each laboratory visit will take no longer than 2 hours at a time. In addition, you may also be asked to undergo, a seven-day free-living physical behaviour monitoring, and two four-week interventions. Therefore, the initial study will require a total of up to 9-weeks, time commitment (though only a maximum of 8 hours of this will be for laboratory-based assessments). Finally, should you agree to, you will be invited back to the laboratories following the completion of the initial study period, to conduct follow up tests (56 and 112 days after completion) (please see study overview below).



You will be coached through all of these test and given instructions during each procedure. Briefly about each test:

Fasted venous blood sample

Upon first arriving at the lab you will be asked to provide a fasted venous blood sample. Following a brief rest period a trained phlebotomist will disinfect the extraction site (mid arm) with an alcohol based wipe, and make a small puncture into the vein using a needle. The phlebotomist will extract a small amount of blood (approximately 15-20 ml), which will be labelled and stored for later analysis. The puncture site will be protected with a breathable and waterproof dressing.

Choice of body composition assessment between the following three tests:

Appropriate clothing is required for each body composition assessment. This is to ensure your safety and comfort. Overall, it is important that no metal clothing item (zips, heavy buttons, fasteners, or belts) impede the quality of our assessments. Therefore, please try to arrive at the laboratory wearing trousers without a zip (tracksuit trousers). Further assessments (Ultrasound scans) will require access to the upper leg. In this case, a pair of shorts in your bag ready to change into, would be ideal. Alternatively, a hospital style gown will be provided which you can change into once you have arrived at the laboratory. The appropriate clothing required for each specific assessment is further detailed in the individual assessment descriptions below. Prior to each assessment you will be asked to lie and rest for approximately 15-20 minutes.

1. Bioelectrical Impedance (BIA)

Appropriate clothing: *Tracksuit trousers, a pair of shorts.*

You will then be asked to lie on your back on a bed. Once lying comfortably, four body sites will be prepared with an alcohol based disinfectant wipe, (wrist, middle finger, ankle and middle toe) on one side of your body. You will then have four electrodes placed on these sites, and attached to a bioelectrical impedance analysis machine via cables. A small electrical signal, which you will not even feel, will be passed through your body to measure body composition.

2. Ultrasonography

Appropriate clothing: *A pair of shorts, Hospital style gown.*

You will then be asked to lie on your front on the bed. Ultrasound gel will be applied to the skin, and then using the probe non-invasive images of the calf and thigh muscles will be obtained. Images of the Achilles tendon (bottom of the leg attaching to the back of the foot), will also be obtained whilst you are applying gradual force.

3. Dual x-ray absorptiometry (DEXA)

Appropriate clothing: *Tracksuit trousers, a pair of shorts, Hospital style gown.*

The investigator will take you into the private scanning room. After changing into appropriate scan clothing if need be, answering to a very short health questionnaire, and your height and weight being assessed. You will then be asked to lie on your back on a scanner table, avoiding any contact between your arms/legs and your body. We will position your legs so that your toes point inwards, which gives us a clearer picture of your hip. The slow moving 'arm' of the DEXA scanner will pass over your body over the course of 7 minutes, and take some pictures of your body. If you have a hearing aid, you will be asked to wear it so that you can hear the practitioner carrying out your scan.

Balance ability test

You will be asked to undergo a standing balance test. This involves standing one legged on a force plate with your eyes closed and hands by your side, for up to 30 seconds. This involves three attempts on your preferred leg.

Muscle Strength and tendon tests

You will be asked to perform a muscle strength test, specifically focusing on the lower limb muscles (ankle joint). You will be strapped into a specialised chair for strength testing in a seated upright position, and asked to perform three tests by pushing and pulling against the machine as hard as possible for a few seconds at a time, whilst the instructor performs ultrasound imaging. Assessment of the tendon involves you staying completely relaxed whilst your test ankle is slowly being moved, at the same time as the researcher performs ultrasound imaging.

Electromyography

Prior to the balance ability test you will have electrodes (small medical patches that help record the activity of the muscle) attached to the skin on four body sites (calf and shin muscles, and 2 reference points either side of the knee) following preparation with an alcohol based disinfectant wipe. These electrodes will also be used during the muscle strength test.

Percutaneous Electrostimulation

Whilst still strapped into the strength testing chair, two rubber stimulation pads will be placed on your lower leg. These will be used to electrically stimulate your calf muscle, through the skin. This will be done at specific times whilst you are relaxed and whilst you are applying a push effort with your foot.

Timed up and Go test

You will be asked to rise from a seated position (sat in a chair), and walk as fast as possible (without running) around a marker approximately 6m away from the chair, before returning to the seated position you started in. This process will be timed by the instructor, and performed three times.

Out of Lab, continuous monitoring

Prior to leaving the lab after the tests, you will be fitted/provided with the following to wear/record over 5-7 continuous days with a request that all equipment is returned in good condition at your next lab visit (note that associated mounting methods are secure, waterproof, and should not interfere with daily activities):

1. Assessment of habitual physical behaviour

In order to avoid skin irritation the watch-sized piece of equipment (movement monitor) will be taped to a foam dressing, before being mounted on the mid-thigh with two adhesive patches.

2. Assessment of 24h glucose profile.

The continuous glucose monitor (e.g. Freestyle®Libre™) will be placed at the back of the arm, through making a small incision into the skin.

3. Assessment of habitual dietary intake

You will be provided with a food diary, and a portable food scale. For 4 days (3 week days and 1 weekend day), you will be asked to keep track of your food and drink intake, through utilising the provided scales to weigh food amount, and record in the diary.

Additional (elective) assessments

You will be invited to undertake two additional assessments, should you be available:

1. Assessment of resting energy expenditure (EE).

Prior to the test you will be fitted with a heart rate monitor and asked to rest in seated position, before breathing into a mouth piece connected to a tube for a brief time period. This tube will be linked up to a Douglas bag (large plastic bag), to collect your expired air.

2. Magnetic resonance imaging (MRI)

You will be asked to undergo an MRI scan of the calf and thigh muscles at our Manchester laboratory (note: you will be unable to partake in this assessment if you have a metallic implanted device, such as a pacemaker). Prior to the test, you will be asked to remove all metallic objects, and be given a pair of ear defenders. Transport to and from the Manchester site will be provided.

3. Follow Up Testing

You will be asked to return to the laboratory once the initial study period is over (day 1-69), at two (day 125) and four (day 181) months post-intervention, to repeat the main battery of tests aforementioned in this document.

7) Are there any disadvantages or risks in taking part?

There will be no cost of participating within this study, other than making yourself available during the study period making your way to Manchester Metropolitan University Cheshire campus on four separate occasions. Most procedures are non-invasive, but some will be performed in a fasted state (no food for ≥12h prior to lab visit), such as venous blood sampling, and body composition assessment. In this case, a mixed meal will be provided following fasting procedures.

A venous blood sample will be taken, which if you are uncomfortable with the venipuncture procedure, may cause some discomfort. However, the phlebotomist performing the procedure will be well trained, and endeavor to make the procedure as comfortable as possible.

Furthermore, the muscular function assessments involve electrical stimulation. The discharge of current through the skin and involuntary movement of the foot can feel uncomfortable at first but most participants become accustomed to the sensation and do not find the procedure distressing.

Should you agree to take undergo an additional MRI scan, in very rare cases this assessment is associated with some sensations of discomfort, such as sensitivity to loud acoustic noise, mild pain, and nausea. Should you experience any of these uncomfortable sensations to a point where you feel unable to proceed, the testing will be stopped immediately, and you will have the right to withdraw from the study should you choose to.

The x-ray dose from a DEXA scan is very low and much lower than other medical x-ray procedures. It is so low the practitioner can safely stay in the scan room with you and it is about the same as a day's worth of background radiation. Background radiation is the dose we get each day just by living on planet Earth. A few people find it difficult to lie on their back, and remain completely still for the 7 minute scanning procedure. °If you do have discomfort please tell the practitioner carrying out your scan. °They will then try to make you as comfortable as possible. Should you experience any uncomfortable sensations to a point where you feel unable to proceed, the testing will be stopped immediately, and you will have the right to withdraw from the study should you choose to.

As part of our testing procedure, we familiarize all of our participants to all of the tests they will undertake during the study, in order to identify any tests that may cause

distress. It is once again important to stress that you have the right at any time to refuse any given procedure, and may continue with whichever aspects of the study you find suitable. Alternatively, you also have the right to withdraw from the study altogether at any time, with no questions asked. This will not affect your relationship with any of the staff at the Manchester Metropolitan University.

8) What are the possible benefits of taking part?

As a study participant, you will have supervised access to the state of the art laboratory facilities we have here at Manchester Metropolitan University. The study involves a comprehensive analysis of your overall health and physical capabilities. This information will be presented back to you in an informative manner, which can then be used to make guided lifestyle alterations that enhance vitality and wellbeing.

9) Who are the members of the research team?

I Dale Grant (PhD candidate) am the Principal investigator being supervised by my Director of Studies Dr. Gladys Pearson. My other supervisors include Dr. David Tomlinson, Dr. Emma Bostock and, Dr. Kostas Tsintzas. Please find below the contact details of the principal investigator and the project supervisors, if you have any additional questions or require any further information regarding the study that has not already been answered within this document.

Dale Grant (Principal Investigator):
University Phone Number: 0161 247 5170
University Email Address: 1105574@stu.mmu.ac.uk

Dr. Gladys Pearson (Project Supervisor, Director of studies):
University Phone Number: 0161 247 5594
University Email Address: g.pearson@mmu.ac.uk

Dr. David Tomlinson (2nd Supervisor):
University Phone Number: 0161 247 5590
University Email Address: david.tomlinson@mmu.ac.uk

Dr. Emma Bostock (3rd Supervisor):
University Phone Number: 0161 247 5539
University Email Address: emma.bostock@mmu.ac.uk

Dr. Kostas Tsintzas (4th Supervisor):
University Phone Number: 0115 82 30127
University Email Address: kostas.tsintzas@nottingham.ac.uk

10) Who is funding the research?

The research is being funded through the Health Exercise and Active Living (HEAL) Research Centre, at the Manchester Metropolitan University.

11) Who will have access to the data?

All information collected during the duration of the research project will remain strictly confidential and the data collected will only be used for the purpose of the study. The

data collected will be sorted within an anonymous and coded form. The data will not be accessible by any other person other than the named investigators. All information and data collected will be kept for the duration of the study (dates for the duration of the study are: 18/09/2017 -- 18/09/2020 dates inclusive) and will be destroyed approximately 5 years after the research project terminates.

The results gathered throughout this research project are likely to be communicated at conferences and potentially be published within scientific journals within the near future for the purposes of furthering current research. These publications will not allow any individuals identity to be determined. If the findings from the study are published within the future, as a participant you retain the right to obtain a copy of any publication, on written request to the principal investigator, that display the results from the research project.

If you wish to obtain any publication from the resultant study please feel free to contact either myself or my project supervisor with the contact details below:

Dale Grant (Principal Investigator):
University Phone Number: 0161 247 5170
University Email Address: 1105574@stu.mmu.ac.uk

Dr. Gladys Pearson (Project Supervisor):
University Phone Number: 0161 247 5594
University Email Address: g.pearson@mmu.ac.uk

12) Who do I contact if I feel my rights have been violated?

Registrar & Clerk to the Board of Governors
Head of Governance and Secretariat Team
Manchester Metropolitan University,
All Saints Building, All Saints
Manchester, M15 6BH
Tel: 0161 247 1390.

I confirm that the insurance policies in place at Manchester Metropolitan University will cover claims for negligence arising from the conduct of the University's normal business, which includes research carried out by staff and by undergraduate and postgraduate students as part of their course. This does not extend to clinical negligence.

13) Thankyou

Thank you for taking the time to read this participant information sheet, and considering taking part in my research study. Despite the testing protocol being complex, I believe this is important research with profound implications for health and wellbeing. Once again, if you desire any additional information or have any further questions please do not hesitate to contact me.

Cover Letter

Research study and Data protection (21/05/2018)

Manchester Metropolitan University

Dear Miss/ Mrs xxx,

The Musculoskeletal Sciences and Sport Medicine (MSSM) at Manchester Metropolitan University Crewe is recruiting participants to take part in our latest research study. Due to the imminent closure of the MMU Crewe campus in July 2019, sadly this will be our final research study in the Cheshire area.

We are looking for people like you to help us uncover how everyday activities like sitting or standing affect health and vitality in later life. In brief, you would visit the campus on only four occasions, over a 9 week period. During the visits, state of the art equipment will be used to assess your current body composition, muscular function, and metabolic health. The beneficial feedback from your testing sessions will be returned to you in an informative manner weeks later, and used to give lifestyle alteration recommendations.

I have enclosed various forms along with this letter:

1. **An information booklet detailing what the experiments entail.**
2. **A health questionnaire**
3. **A business reply envelope (note that you DO NOT need to put a stamp on this prior to posting it back to us with the necessary forms)**

If you are interested in taking part in the latest research study, please read form 1. Then complete/ return form 2 , and (see below), in the prepaid envelope.

You are being contacted because you live in the Cheshire area and we have retained your contact details on file. Due to the new General Data Protection Regulations ("GDPR") coming into force on the 25th of May, we are required by law to offer you the choice of what happens to your personal data.

At the bottom of this letter you will find a return slip ( ), indicating whether or not you wish for us to keep a record of your contact details data. If you select No, we will remove your contact details from our database and will not contact you regarding research studies. Please complete with appropriate response, sign, and return the slip in the prepaid envelope.

On behalf of the MSSM team, I would like to thank you for your time, and look forward to hearing from you at your earliest convenience.

Kind regards,

Dale Grant

PhD Candidate (MMU Cheshire)
MMU Cheshire Campus
Seeley Building (1-06)
Crewe Green Road, Crewe, Cheshire
CW1 5DU
Tel: 0161 247 5170
dale.grant@stu.mmu.ac.uk

Section Break (Next Page)

Please indicate below if you would like us to keep your contact details on file?

Name: xxx

Signature: .....

YES ☐ NO ☐

Health Questionnaire

HEALTH QUESTIONNAIRE

Name:-·····Date:-

Date-of-Birth:-_____·····

Age:- ····Gender-(Please-circle):-M-/-F

Contact-Telephone-Number:-_____

Home-Address:

Email-Address:-_____·····

GENERAL-HEALTH:

Please-check····· [x] to-indicate-if-you-have-recently-been-diagnosed-with-or-are-receiving-treatment-for-any-of-the-following- chronic-health-conditions:

Diabetes [ ]

Cardiovascular-Disease [ ]

High-blood-pressure [ ]

Cancer·····[ ]type:-_____

Other,-please-explain:-_____

Please-list-any-medications,-which-you-are-currently-taking.-Please-note-the-dosage-if-possible.

Medication-Name·····Dosage

_____ _____

_____ _____

_____ _____

_____ _____

_____ _____

Have-you-experienced-any-lower-limb-muscle/-tendon/-joint-disorders-within-the-past-6-months?

YES [ ] NO [ ]

If-YES-Please-provide-details-below:

_____

_____

_____

SBF Intervention Packages

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Manchester Metropolitan University

INTERVENTION·¶

PACKAGE¶

Non-exercise·micro-interventions·to·mitigate·sedentarism·induced·poor·health¶

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Participant·code:¶

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Purpose of the Intervention

The purpose of the intervention is to break up the amount of time you spend performing sedentary behaviour (SB).

This includes activities like:

- Sitting
  - Watching television
  - Computer use

In order to help you, the research team will have fitted you with two accelerometers.

The orange ACTIVPal unit will be prompting you to move over the next 8 weeks. If you have been sitting down for more than 30 minutes the device will gently vibrate against the skin.

This prompt is your cue to stand up, and perform your 2 minutes of LIPA. The device will not prompt you to move if you are already stood up and moving.

This document also contains a ***Compliance Diary***. For each intervention week (1-8), there is a column for each day of the week, and rows in 30-minute intervals (09:00-21:00pm). This is your diary to keep during the intervention period.

Let's say for example you are seated at 09:30am and the accelerometer vibrates. If you comply to the prompt, (i.e. if you stand up and perform 2 minutes of LIPA) simply place an **X** in the space next to 09:30 for that particular day.

If you do not comply with the prompt for whatever reason (tiredness, social situation etc), i.e. you remain seated, simply place an **O** in the box. Obviously, we would like you to comply to the majority of prompts but understand this cannot always be the case.

If you are already stood up and moving please leave the box blank.

Example Week

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake Up	07:40	07:50	07:45	07:40	07:40	07:45	07:50
09:00	X		X		X	X	X
09:30	X		X		X	X	X
10:00	X		X		X	X	X
10:30	X	X	X	X	X	X	X
11:00		X	0	X		0	
11:30	0	X	0	X		0	
12:00	0	X	0	X		0	
12:30	0		0			0	
13:00	0			X		0	
13:30	X			X		0	
14:00	X			X		0	
14:30		X		X		0	
15:00		X					
15:30	X	X	X	X	X		X
16:00	X	X	X	X	X		X
16:30	0		0	X	0		0
17:00	0		0	X	0	X	0
17:30	0		0	X	0	X	0
18;00	0	X	0	X	0	X	0
18:30	0	X	0	X	0	X	0
19:00	X	X	X		X	0	X
19:30	X	X	X		X	0	X
20:00	X		X		X	0	X
20:30	X		X		X		X
21:00	0		0		0		0
Sleep Time	21:50	21:30	22:00	21:40	21:40	22:10	21:40
Non-wear Time	:-:	:-:	:-:	:-:	:-:	:-:	:-:

In order to help us identify when you are performing specific behaviours, this document contains a diary including entry spaces for wake and sleep times. These times do not have to be exact, but please try and be as accurate as possible.

For the wake time please make a note of the time you get out of your bed in the morning.

For the sleep time please note the time you switch the lights off to go to sleep at night time.

When you lie down in bed to go to sleep it is important that you flex your hip for 10 seconds (shown below).

Following 10 seconds you will feel the device vibrate. This will turn off the vibrational prompt whilst you remain still and sleeping. If you get up and use the toilet during the night please repeat the same process to switch off the vibrational prompt.

The prompt will switch back on automatically the next morning when you get out of bed and resume movement.

Light Intensity Physical Activity — Intervention examples

Below we have provided you with a list of LIPA examples, to provide you with ideas of the kinds of behaviours we would like you to be performing when you stand up. This list contains simple activities, which are merely designed to get you moving. This list is not strict, so you may perform as many or as few of these activities as pleases you, in whatever combination. Alternatively, you may perform your own movements, bearing in mind we want you to keep the intensity **light**.

Therefore, please try to **avoid** tasks that:

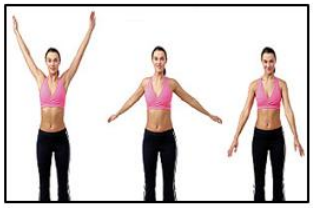
- Cause you to get out of breath
- Cause you to sweat
- Are technically complex (Bodyweight exercises like squats, etc.).
- Are performed at a high speed

1. Any light self-paced walking

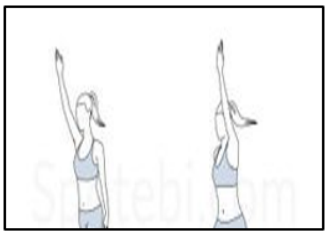
Non-exercise micro-interventions to mitigate sedentarism induced poor health. Ethics Code: 230118ESSDG(2)

2. Side-to-side shuffling

3. Standing Shoulder Circles



4. Stood Alternating Arm Raises



5. On the spot stepping

Non-exercise micro-interventions to mitigate sedentarism-induced poor health. Ethics Code: 230118ESSDG(2)

6. Washing Dishes/ putting away (stood)

7. Hanging out washing (stood)

8.→Ironing·¶

9.→Sweeping·¶

Non-exercise·micro-interventions·to·mitigate·sedentarism·induced·poor·health.·Ethics·Code:·230118ESSDG(2)¶

10. → Tidying·Away·out-of-place·objects·(stood)¶

11. → Stood·Up·Dusting¶

12. → Stood watering plants (weather permitting)

13. → Preparing meals in the kitchen (stood)

14. → Stood Hairstyling/ Applying make up

15. → Stood Cleaning windows

The black GENEACtiv unit will be tracking how much activity you perform over the next 8 weeks.

In the rare event that either device completely detaches from the skin, please note the times on the date you noticed the device had detached (Non wear time on intervention diary), and contact one of the researchers with the information at the end of this document.

You will be provided with three spare adhesive patches, in case the film begins to peel away from the skin. Should this occur, please do not remove the existing film, instead place the spare film over the part of the existing film that is beginning to peel away.

If fluid begins to accumulate within the air bubble around the GENEA, please cut a small hole in the bottom of the air bubble allowing the fluid to drain and then once again place the spare film over the top to seal the air bubble once again.

Home Visits

Every 2 weeks you will receive a visit from one of the investigators performing the study. These visits will be to check your wellbeing, and whether you have any issues/queries with the intervention up to this point. Furthermore, the researcher will replace the accelerometer devices.

Obviously, if you have any issues/queries with any aspects of performing the above intervention, and cannot wait for your fortnightly visit please do not hesitate to make contact with one of the investigators (contact details listed below).

Non-exercise micro-interventions to mitigate sedentarism induced poor health. Ethics Code: 230118ESSDG(2)

1. Dale Grant (Principal Investigator):

University Phone Number: 0161 247 5170

University Email Address: **11055744@stu.mmu.ac.uk**

2. Dr. Gladys Pearson (Project Supervisor):

University Phone Number: 0161 247 5594

University Email Address: **g.pearson@mmu.ac.uk**

Manchester Metropolitan University

Tracking Diary

Participant Code:

Week One

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake Up Time	:	:	:	:	:	:	:
09:00							
09:30							
10:00							
10:30							
11:00							
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12:30							
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20:00							
20:30							
21:00							
Sleep Time	:	:	:	:	:	:	:
Non-wear Time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Two

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake Up Time	:	:	:	:	:	:	:
09:00							
09:30							
10:00							
10:30							
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20:30							
21:00							
Sleep Time	:	:	:	:	:	:	:
Non-wear Time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Three

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake Up Time	:	:	:	:	:	:	:
09:00							
09:30							
10:00							
10:30							
11:00							
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21:00							
Sleep Time	:	:	:	:	:	:	:
Non-wear Time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Four

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake Up Time	:	:	:	:	:	:	:
09:00							
09:30							
10:00							
10:30							
11:00							
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20:00							
20:30							
21:00							
Sleep Time	:	:	:	:	:	:	:
Non-wear Time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Five

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake Up Time	:	:	:	:	:	:	:
09:00							
09:30							
10:00							
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20:30							
21:00							
Sleep Time	:	:	:	:	:	:	:
Non-wear Time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Six

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake Up Time	:	:	:	:	:	:	:
09:00							
09:30							
10:00							
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20:30							
21:00							
Sleep Time	:	:	:	:	:	:	:
Non-wear Time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Seven

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake Up Time	:	:	:	:	:	:	:
09:00							
09:30							
10:00							
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20:30							
21:00							
Sleep Time	:	:	:	:	:	:	:
Non-wear Time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Eight

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake Up Time	:	:	:	:	:	:	:
09:00							
09:30							
10:00							
10:30							
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20:00							
20:30							
21:00							
Sleep Time	:	:	:	:	:	:	:
Non-wear Time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

LIPA intervention Packages

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Manchester Metropolitan University

INTERVEVTION·¶

PACKAGE¶

Non-exercise·micro-interventions·to·mitigate·sedentarism·induced·poor·health¶

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Participant·code:¶

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Purpose·of·the·Intervention¶

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The·purpose·of·the·intervention·is·to·increase· the· amount· of· time· you·spend· performing· light· intensity·physical·activity·(LIPA).·This·means·you·can·still·continue·what·you·are·doing·every·day.·We·just·want·to·you·to·perform·some·light·activities·every·morning.·Therefore,·we·would·like·to·request·you·perform·45-50·minutes·of· continuous·LIPA·every·morning,·over·the·next·8·weeks.·¶

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Following·this·intervention·our·hope· is· that· it· will· make· you·healthier·and·more·able·to·be·active·each·day.·Over·the·long·term· this· will· lead· to· better·health· outcomes,· and· allow·you·to·maintain·vitality·and·a·high·quality·of·life.¶

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In order to help you, we request you perform your LIPA bout first thing in the morning. Therefore, once this has be completed you may return to your regular habitual routine and not have to concern yourself with performing any more activity.

Every morning over the next 8 weeks we would like you to **avoid sitting down for around 45-50 minutes, and perform some LIPA.** Once this LIPA bout has been completed, you may resume your normal habitual routine for the remainder of the day/evening.

This package also contains a ***Compliance diary***. For each intervention week (1-8), there is a column for each day of the week, and rows for compliance check ins (Compliance question and time). This is your diary to keep during the intervention period.

Let's say you wake up on Monday, and complete 45 minutes of LIPA that morning without sitting. You would place a Y, for yes, in the adjacent column for Monday, to indicate you completed the LIPA bout. You would also place the number 45 next to the time-completed section.

If you do not complete your LIPA bout for whatever reason (tiredness, social situation, etc), simply place an N for No, in the Complied box. Obviously, we would like you to comply every day, but understand this cannot always be the case.

Non-exercise micro-interventions to mitigate sedentarism induced poor health. Ethics Code: 230118ESSDG(2)

Example Week

¤	Day 1¤	Day 2¤	Day 3¤	Day 4¤	Day 5¤	Day 6¤	Day 7¤
Wake up time¤	07:40¤	07:50¤	07:45¤	07:40¤	07:40¤	07:45¤	07:50¤
Complied (Y/N)?¤	Y¤	Y¤	Y¤	Y¤	N¤	Y¤	Y¤
Time Completed (Minutes)¤	45¤	42¤	45¤	50¤	-¤	50¤	45¤
Sleep time¤	21:50¤	21:30¤	22:00¤	21:40¤	21:40¤	22:10¤	21:40¤
Non wear time¤	:--:--:¤	:--:--:¤	:--:--:¤	:--:--:¤	:--:--:¤	:--:--:¤	:--:--:¤

In order to help us identify when you are performing these specific behaviours, the diary includes entry spaces for wake and sleep times. These times do not have to be exact, but please try and be as accurate as possible.

For the wake time please make a note of the time you get out of your bed in the morning.

For the sleep time please note the time you switch the lights off to go to sleep at night time.

Light Intensity Physical Activity — Intervention examples

Below we have provided you with a list of LIPA examples, to provide you with ideas of the kinds of behaviours we would like you to be performing when you perform your 45-50 minute LIPA bout, every morning. This list contains simple activities, which are simply designed to get you moving. This list is not strict, so you may perform as many or as few of these activities, as pleases you, in whatever combination. Alternatively, you may perform your own movements, bearing in mind we want you to keep the intensity **light**.

Therefore, try and avoid movements/ tasks that:

- Cause you to get out of breath
- Cause you to sweat
- Are technically complex (Bodyweight exercises like squats, etc.).
- Are performed at a high speed

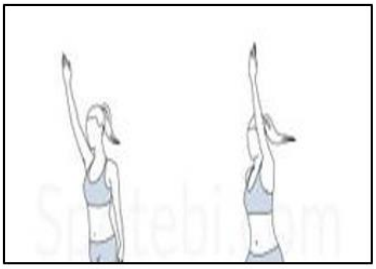
1. **Any light self-paced walking**

Non-exercise micro-interventions to mitigate sedentarism induced poor health. Ethics Code: 230118ESSDG(2)

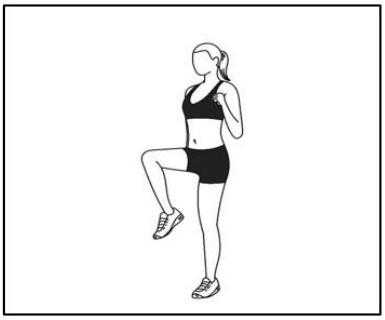
2. **Side-to-side shuffling**

3. **Standing Shoulder Circles**

4. Stood Alternating Arm Raises



5. On the spot stepping



Snow Zoom
Non-exercise micro-interventions to mitigate sedentarism induced poor health. Ethics Code: 230118ESSDAI(2)

6. Washing Dishes/ putting away (stood)

7. Hanging out washing (stood)

8. Ironing

9. Sweeping

Non-exercise micro-interventions to mitigate sedentarism induced poor health. Ethics Code: 230118ESSDG(2)

10. → Tidying Away out of place objects (stood)

11. → Stood Up Dusting

12. → Stood watering plants (weather permitting)

13. → Preparing meals in the kitchen (stood)

14. → Stood Hairstyling/ Applying make up

15. → Stood Cleaning windows

The black GENEACtiv unit will be tracking how much activity you perform over the next 8 weeks.

In the rare event the device does completely detach from the skin, please note the time on the date you notice the device had detached (Non wear time on intervention diary).

You will be provided with two spare adhesive patches, in case the film begins to peel away from the skin. Should this occur, please do not remove the existing film, instead place the spare film over the part of the existing film that is beginning to peel away.

If fluid begins to accumulate within the air bubble around the GENEA, please cut a small hole in the bottom of the air bubble allowing the fluid to drain and then once again place the spare film over the top to seal the air bubble once again.

Home Visits

Every 2 weeks you will receive a visit from one of the investigators performing the study. These visits will be to check your wellbeing, and whether you have any issues/ queries with the intervention up to this point. Furthermore, the researcher will replace the accelerometer device.

Obviously, if you have any issues/ queries with any aspects of performing the above intervention, and cannot wait for your fortnightly visit please do not hesitate to make contact with one of the investigators (contact details listed below).

Non-exercise micro-interventions to mitigate sedentarism induced poor health. Ethics Code: 230118ESSDG(2)

1. Dale Grant (Principal Investigator):

University Phone Number: 0161 247 5170

University Email Address: **11055744@stu.mmu.ac.uk**

2. Dr. Gladys Pearson (Project Supervisor):

University Phone Number: 0161 247 5594

University Email Address: **g.pearson@mmu.ac.uk**

Compliance Diary

Participant Code:

Week One

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake up time	:	:	:	:	:	:	:
Compiled (Y/N)?							
Time completed (minutes)							
Sleep Time	:	:	:	:	:	:	:
Non-wear time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Two

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake up time	:	:	:	:	:	:	:
Compiled (Y/N)?							
Time completed (minutes)							
Sleep Time	:	:	:	:	:	:	:
Non-wear time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Three

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake up time	:	:	:	:	:	:	:
Compiled (Y/N)?							
Time completed (minutes)							
Sleep Time	:	:	:	:	:	:	:
Non-wear time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Four

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake up time	:	:	:	:	:	:	:
Compliied (Y/N)?							
Time completed (minutes)							
Sleep Time	:	:	:	:	:	:	:
Non-wear time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Five

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake up time	:	:	:	:	:	:	:
Compliied (Y/N)?							
Time completed (minutes)							
Sleep Time	:	:	:	:	:	:	:
Non-wear time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Six

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake up time	:	:	:	:	:	:	:
Compliied (Y/N)?							
Time completed (minutes)							
Sleep Time	:	:	:	:	:	:	:
Non-wear time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Seven

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake up time	:	:	:	:	:	:	:
Compliied (Y/N)?							
Time completed (minutes)							
Sleep Time	:	:	:	:	:	:	:
Non-wear time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Eight

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake up time	:	:	:	:	:	:	:
Compliied (Y/N)?							
Time completed (minutes)							
Sleep Time	:	:	:	:	:	:	:
Non-wear time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Control Intervention Package

Manchester Metropolitan University

STUDY PACKAGE

Non-exercise micro-interventions to mitigate sedentarism induced poor health

Participant code:

Purpose of the Intervention

The purpose of the intervention is to track your habitual activity and monitor the corresponding changes in health markers. The purpose is to see how the habits and behaviours you engage within everyday are affecting your health.

It is therefore imperative that you maintain your normal routine over the next 8 weeks and do not try to change anything, as this could affect the results. You can continue your day as you normally would and the carefree tools we use to track your behaviour will take care of the rest. At the end of the study this information will be presented back to you in an informative manner and used to give feedback and recommendations. The more honest you are with your daily routine the more honest our feedback can be.

Following this tracking period our hope is that, based on the information we give back to you, will make you healthier and more able to be active each day. Over the long term this will lead to better health outcomes, and allow you to maintain vitality and a high quality of life.

In order to help us identify when you are performing these specific behaviours, this package includes a tracking diary which includes entry spaces for wake and sleep times. These times do not have to be exact, but please try and be as accurate as possible.

For the wake time please make a note of the time you get out of your bed in the morning.

For the sleep time please note the time you switch the lights off to go to sleep at night time.

Example

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Wake up time	07:40	07:50	07:45	07:40	07:40	07:45	07:50
Sleep time	21:50	21:30	22:00	21:40	21:40	22:10	21:40
Non wear time	:--:	:--:	:--:	:--:	:--:	:--:	:--:

The black GENEActiv unit will be tracking how much activity you perform over the next 8 weeks.

In the rare event the device does completely detach from the skin, please note the time on the date you notice the device had detached (Non wear time on intervention diary).

Non-exercise micro-interventions to mitigate sedentarism induced poor health. Ethics Code: 230118ESSDG(2)

You will be provided with two spare adhesive patches, in case the film begins to peel away from the skin. Should this occur, please do not remove the existing film, instead place the spare film over the part of the existing film that is beginning to peel away.

If fluid begins to accumulate within the air bubble around the GENEA, please cut a small hole in the bottom of the air bubble allowing the fluid to drain and then once again place the spare film over the top to seal the air bubble once again.

Home Visits

Every 2 weeks you will receive a visit from one of the investigators performing the study. These visits will be to check your wellbeing, and whether you have any issues/ queries with the intervention up to this point. Furthermore, the researcher will replace the accelerometer device.

Obviously, if you have any issues/ queries with any aspects of performing the above intervention, and cannot wait for your fortnightly visit please do not hesitate to make contact with one of the investigators (contact details listed below).

1. Dale Grant (Principal Investigator):

University Phone Number: 0161 247 5170

University Email Address: 11055744@stu.mmu.ac.uk

2. Dr. Gladys Pearson (Project Supervisor):

University Phone Number: 0161 247 5594

University Email Address: g.pearson@mmu.ac.uk

Tracking Diary

Participant Code:

Week One

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake Up Time	:	:	:	:	:	:	:
Sleep Time	:	:	:	:	:	:	:
Non-wear Time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Two

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake Up Time	:	:	:	:	:	:	:
Sleep Time	:	:	:	:	:	:	:
Non-wear Time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Three

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake Up Time	:	:	:	:	:	:	:
Sleep Time	:	:	:	:	:	:	:
Non-wear Time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Four

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake Up Time	:	:	:	:	:	:	:
Sleep Time	:	:	:	:	:	:	:
Non-wear Time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Five

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake Up Time	:	:	:	:	:	:	:
Sleep Time	:	:	:	:	:	:	:
Non-wear Time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Six

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake Up Time	:	:	:	:	:	:	:
Sleep Time	:	:	:	:	:	:	:
Non-wear Time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Seven

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake Up Time	:	:	:	:	:	:	:
Sleep Time	:	:	:	:	:	:	:
Non-wear Time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Week Eight

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Wake Up Time	:	:	:	:	:	:	:
Sleep Time	:	:	:	:	:	:	:
Non-wear Time	: - :	: - :	: - :	: - :	: - :	: - :	: - :

Palatability questionnaire

PALATABILITY·AND· COMPLIANCE· QEUSTIONNAIRE¶

Non-exercise·micro-interventions·to·mitigate· sedentarism·induced·poor·health.¶

Name:·¶

Date·of·Birth:¶

Please·circle·your·answer·where·appropriate:·¶

Section·1:·Intervention·¶

Did·you·find·the·instructions·easy·to·follow:?·¶

Definitely·not·····Fairly·not·····Undecided·····Fairly·····Definitely·¶

Did·you·find·the·intervention·easy·to·follow·at·home:?·¶

Definitely·not·····Fairly·not·····Undecided·····Fairly·····Definitely·¶

Did·you·find·the·compliance·diary·easy·to·fill·in:?·¶

Definitely·not·····Fairly·not·····Undecided·····Fairly·····Definitely·¶

Did·you·find·the·compliance·diary·helpful:?·¶

Definitely·not·····Fairly·not·····Undecided·····Fairly·····Definitely·¶

Do·you·think·your·balance·has·improved·following·the·intervention:?·¶

Definitely·not·····Fairly·not·····Undecided·····Fairly·····Definitely·¶

Did·you·feel·tiered·or·short·of·breath·during·the·intervention:?·¶

Definitely·not·····Fairly·not·····Undecided·····Fairly·····Definitely·¶

Did·your·muscles·or·joints·feel·sore·during·the·intervention:?·¶

Definitely·not·····Fairly·not·····Undecided·····Fairly·····Definitely·¶

If·so·were·there·any·particularly·sore·areas:?·¶

1·-·¶

2·-·¶

3·-·¶

Did you feel embarrassed performing the intervention?

Definitely not ····· Fairly not ····· Undecided ····· Fairly ····· Definitely

Would you say you are more aware of the amount of light activity you perform daily following this intervention?

Definitely not ····· Fairly not ····· Undecided ····· Fairly ····· Definitely

Would you say you are more aware of your daily sitting behaviours following this intervention?

Definitely not ····· Fairly not ····· Undecided ····· Fairly ····· Definitely

Would you say you feel more confident about performing household tasks following this intervention?

Definitely not ····· Fairly not ····· Undecided ····· Fairly ····· Definitely

Would you say you feel more positive about your health following this intervention?

Definitely not ····· Fairly not ····· Undecided ····· Fairly ····· Definitely

Were there any places/ social environments you struggled to implement the intervention (please list 3):

1 -

2 -

3 -

Were there any times of day you struggled to implement the intervention (please tick appropriate):

Morning ☐

Afternoon ☐

Evening ☐

Section 2: Future Implications

Can you see yourself continuing this intervention long term?

Definitely not ····· Probably not ····· I'm Not sure ····· Probably ····· Definitely

Has this intervention motivated you to become more active?

Definitely not ····· Probably not ····· I'm Not sure ····· Probably ····· Definitely

Has this intervention motivated you to make long-term changes to your health?

Definitely not ····· Probably not ····· I'm Not sure ····· Probably ····· Definitely

Would you recommend this intervention to a friend?

Definitely not ····· Probably not ····· I'm Not sure ····· Probably ····· Definitely

Would you consider returning for follow up tests in 4 months' time?

YES ☐ ····· *NO* ☐

Are there any other thoughts you wish to share regarding your experience, or even constructive feedback as to how we can improve the intervention for future studies?

PALATABILITY·AND· COMPLIANCE· QEUSTIONNAIRE¶

Non-exercise·micro-interventions·to·mitigate· sedentarism·induced·poor·health.¶

Name:¶

Date·of·Birth:¶

Please·circle·your·answer·where·appropriate:·¶

Section·1:·Intervention·¶

Did·you·find·the·instructions·easy·to·follow·?·¶

Definitely·not·····Fairly·not·····Undecided·····Fairly·····Definitely·¶

Did·you·find·the·intervention·easy·to·follow·at·home·?·¶

Definitely·not·····Fairly·not·····Undecided·····Fairly·····Definitely·¶

Did·you·find·the·accelerometer·prompt·(vibration)·helpful·?·¶

Definitely·not·····Fairly·not·····Undecided·····Fairly·····Definitely·¶

Did·you·find·the·compliance·diary·easy·to·fill·in·?·¶

Definitely·not·····Fairly·not·····Undecided·····Fairly·····Definitely·¶

Did·you·find·the·compliance·diary·helpful·?·¶

Definitely·not·····Fairly·not·····Undecided·····Fairly·····Definitely·¶

Do·you·think·your·balance·has·improved·following·the·intervention·?·¶

Definitely·not·····Fairly·not·····Undecided·····Fairly·····Definitely·¶

Did·you·feel·tiered·or·short·of·breath·during·the·intervention·?·¶

Definitely·not·····Fairly·not·····Undecided·····Fairly·····Definitely·¶

Did·your·muscles·or·joints·feel·sore·during·the·intervention·?·¶

Definitely·not·····Fairly·not·····Undecided·····Fairly·····Definitely·¶

If·so·were·there·any·particularly·sore·areas·?·¶

1 .·¶

2 .·¶

3 .·¶

Did you feel embarrassed performing the intervention? ¶

Definitely not ..... Fairly not ..... Undecided ..... Fairly ..... Definitely ¶

¶

Would you say you are more aware of the amount of light activity you perform daily following this intervention? ¶

Definitely not ..... Fairly not ..... Undecided ..... Fairly ..... Definitely ¶

¶

Would you say you are more aware of your daily sitting behaviours following this intervention? ¶

Definitely not ..... Fairly not ..... Undecided ..... Fairly ..... Definitely ¶

¶

Would you say you feel more confident about performing household tasks following this intervention? ¶

Definitely not ..... Fairly not ..... Undecided ..... Fairly ..... Definitely ¶

¶

Would you say you feel more positive about your health following this intervention? ¶

Definitely not ..... Fairly not ..... Undecided ..... Fairly ..... Definitely ¶

¶

Were there any places/ environments you struggled to implement the intervention (please list 3): ¶

¶

1. . ¶

2. . ¶

3. . ¶

¶

Were there any times of day you struggled to implement the intervention (please tick appropriate): ¶

¶

Morning ¶ ☐

Afternoon ¶ ☐

Evening ¶ ☐

¶

¶

¶

Section 2: Future Implications ¶

¶

Can you see yourself continuing this intervention long term? ¶

Definitely not ..... Probably not ..... I'm Not sure ..... Probably ..... Definitely ¶

¶

Has this intervention motivated you to become more active? ¶

Definitely not ..... Probably not ..... I'm Not sure ..... Probably ..... Definitely ¶

¶

Has this intervention motivated you to make long-term changes to your health? ¶

Definitely not ..... Probably not ..... I'm Not sure ..... Probably ..... Definitely ¶

¶

Would you recommend this intervention to a friend? ¶

Definitely not ..... Probably not ..... I'm Not sure ..... Probably ..... Definitely ¶

¶

Would you consider returning for follow up tests in 4 months' time? ¶

¶

YES ☐ ..... *NO* ¶ ☐

¶

Are there any other thoughts you wish to share regarding your experience, or even constructive feedback as to how we can improve the intervention for future studies? ¶

¶

¶ _____

_____

_____

_____

FOOD·AND·DRINK·DIARY

Non-exercise·micro-interventions·to·mitigate·sedentarism·induced·poor·health.

Participant·Code:·

Page Break

How·to·complete·the·food·record·booklet

1. The·purpose·of·asking·you·to·record·your·dietary·intake·is·to·assess·the·relationship·between·habitual·diet·and·body·composition.
2. Please·record·everything·you·eat·and·drink·each·day·for·4·consecutive·days·(e.g.·Thursday,·Friday·and·Saturday).·It's·important·that·you·do·not·change·your·eating·habits·in·any·way·due·to·the·dietary·analysis,·so·please·be·as·honest·as·you·can·and·eat·the·foods·that·you·normally·eat.
3. Please·give·us·as·much·detail·as·possible·about·what·you·eat·and·drink,·i.e.·description·(e.g.·wholemeal·or·white·bread),·portion·size,·packaging,·etc.,·and·what·time·you·eat·and·drink.
4. Please·state·the·method·of·cooking·e.g.·boiled,·grilled,·fried.
5. Please·state·the·amount·of·food·eaten·(small,·medium·and·large·portion)·and·drink·consumed,·e.g.·300·mL·mug·of·tea,·½·or·full·pint·beer,·small/large·(175/250·mL)·glass·wine.
6. Please·state·the·brand·of·food·wherever·possible,·e.g.·Heinz·Cream·of·Tomato·Soup,·McVities·Digestive·Biscuits.
7. If·two·items·are·eaten·together,·please·state·the·individual·amounts,·e.g.·apple·and·custard:·fist-size·helping·of·stewed·apple·and·half·a·350·mL·can·Ambrosia·Custard.
8. For·items·regularly·consumed·(e.g.·cup·of·tea),·please·state·the·components,·(i.e.·water,·milk,·sugar)·once.··We·will·then·assume·all·mugs·of·tea·are·the·same·thereafter.
9. For·the·milk-·please·also·specify·skimmed,·semi-skimmed,·full-fat,·and·whether·cow,·soya,·goat,·rice·source
10. For·the·sugar-·please·also·specify·whether·brown,·white,·cane·etc.
11. For·the·tea·itself,·please·specify·which·brand·and·leaf-type.
12. Please·remember·to·record·all·snacks·and·drinks.
13. Once·you·have·completed·the·4-day·diary,·the·researcher·will·either·collect·during·your·home·visit,·or·bring·it·back·to·the·laboratory·with·you·on·your·final·visit.

Page Break

Food weighing

The researcher will have provided you with a set of digital scales.

Please try to measure and accurately record the majority of your food intake for the 4 days.

Be sure to place a bowl or plate onto the scales first, then zero the scales. Following this weigh food as close to the method of cooking as possible (e.g. pasta once cooked).

If you do not know the exact quantity of food (in grams) or drink (in mL), please use the following tips to help you estimate these quantities:

1. **Household measures** use cups, teaspoons, tablespoons.
2. **Visually**
   - The Palm of your hand = 85g of meat or to describe the size of potatoes

   - Your fist = the same size as a cup. Use it to indicate amounts of Pasta, rice, cereal and fruit/veg

3. The tip of your thumb is a teaspoon – indicate sugar, marg, oils etc.

Please retain any packaging and return to the researcher in a plastic bag in an effort to more accurately estimate your food intake.

Especially for foods your regularly consume

For example:

- Breakfast cereal boxes
- Packaged food like rice
- Canned foods like Beans (Please rinse the can).
- **Please do not retain raw meat or poultry packaging**

An example of a daily record of nutritional intake:

DAY ONE		DATE 16.06.2011			OFFICIAL USE ONLY	
Meal	**Time**	**Food & Drink**	**Amount**	**Left-overs?**	**Food Code**	**Amount (g)**
Early am	7am	Mug of tea, strong, milky, made with skimmed milk	300ml (30ml milk)			
Breakfast	7.30am	Bowl of Kellogg's cornflakes Semi-skimmed milk Sliced banana White sugar Orange juice Mug tea, made as above	Medium bowl ½ pt milk Large 2 teaspoons ½ pt 300ml			
Mid am	10am	Tesco finest white toast Flora margarine 2 glasses water Mug of filter coffee, black	2 slices Thinly applied ½ pt in total 300ml			
Midday Meal	1pm	2 crusty rolls Flora marg Mature Cheddar cheese 2 Jordan's cereal bar fruit and nut 1 can of Coca-cola 1 glass water	2 medium-sized rolls Thinly applied Thick chunks 2 x 60g bar 330ml ½ pt glass			
Mid pm	3pm	1 mug of filter coffee, as above 3 custard cream biscuits (nutritional info attached)	300ml 3 biscuits			
Evening Meal	6.30pm	New potatoes steamed in skins, Steamed broccoli, Grilled fillet of salmon with paprika 2 low fat Ski strawberry yoghurt 2 large glasses of white wine	7 small potatoes Handful 130g (uncooked) Pinch 2 x 120g 2 x 250ml	3 potatoes, Salmon skin		
Evening Snack	9pm to 10.30pm	2 mugs of tea 1 chocolate brownie (nutritional info attached) 1 large glass of Tesco's blackcurrant squash (no added sugar)	2 x 300ml large 1 pk			
Extras		Mars Bar	1 standard size			

Page Break

DAY ONE e.g. Wednesday		DATE			OFFICIAL USE ONLY	
Meal	**Time**	**Food & Drink**	**Amount**	**Left-overs?**	**Food Code**	**Amount (g)**
Early am						
Breakfast						
Mid am						
Midday Meal						
Mid pm						
Evening Meal						
Evening Snack						
Extras						

Name:

NOTES:

Please record anything else which you may feel is relevant, e.g. illness

Page Break

DAY TWO e.g. *Thursday*		DATE			OFFICIAL USE ONLY	
Meal	Time	Food & Drink	Amount	Left-overs?	Food Code	Amount (g)
Early am						
Breakfast						
Mid am						
Midday Meal						
Mid pm						
Evening Meal						
Evening Snack						
Extras						

Name……

NOTES:

Please record anything else which you may feel is relevant, e.g. illness

…… Page Break ……

DAY THREE e.g. *Friday*		DATE			OFFICIAL USE ONLY	
Meal	Time	Food & Drink	Amount	Left-overs?	Food Code	Amount (g)
Early am						
Breakfast						
Mid am						
Midday Meal						
Mid pm						
Evening Meal						
Evening Snack						
Extras						

Name……

NOTES:

Please record anything else which you may feel is relevant, e.g. illness

…… Page Break ……

DAY FOUR e.g. Saturday	DATE			OFFICIAL USE ONLY	
Meal	Time	Food & Drink	Amount	Left-overs?	Food Code	Amount (g)
Early am						
Breakfast						
Mid am						
Midday Meal						
Mid pm						
Evening Meal						
Evening Snack						
Extras						

Name .....

NOTES:

Please record anything else which you may feel is relevant, e.g. illness

Chapter 4

Parametricity Tests

Data that were non-normally distributed were, one sit to stand time_PRE, Left/Right average grip strength_POST, Left/Right Peak grip strength_POST, Left eyes closed time PRE, Left eyes closed time POST, Right eyes closed PRE, and Right eyes closed POST. Those date with unequal variances were 30 seconds Sit to Stands_Post, Average gait speed_PRE, and Average gait speed_POST. Those data that were both non-normally distributed and had unequal variances were Left eyes open time PRE, left eyes open time POST, Right eyes open PRE, and right eyes open POST.

Associations between the relative change from baseline in SB/ LIPA, and the relative change from baseline for physical function outcomes

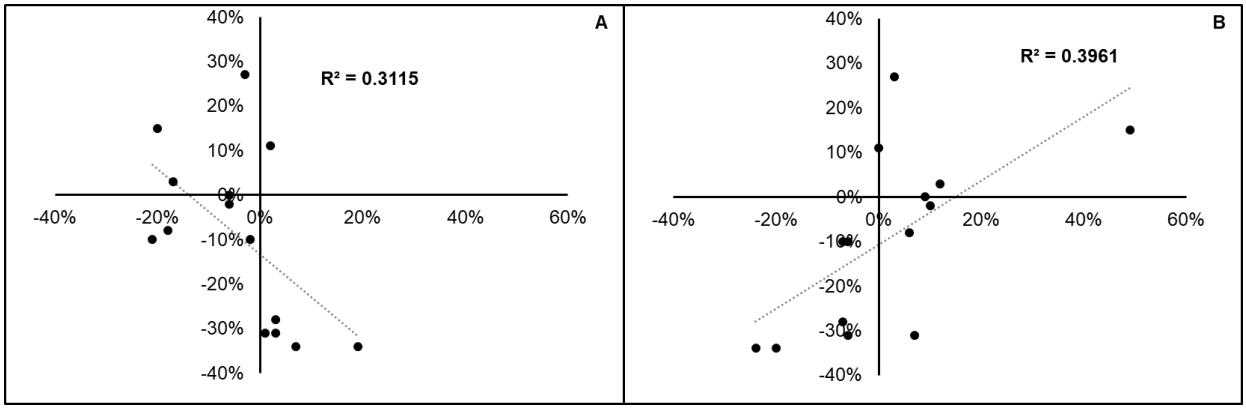


Figure Ai1- The association between the relative change in physical behaviour and peak handgrip strength (Y axis) in the LIPA group. Panels A and B represent the association between SB and LIPA (X axis), respectively. LIPA; light intensity physical activity, SBF; sedentary behaviour fragmentation.

Within the whole cohort (n=36), only the change in SB time was significantly negatively associated with the change in one sit-to-stand time (R^2=0.22, p=0.004). When sub-analysed by group such a negative association persisted within the SBF group (R^2=0.36, p=0.023), accounting for 36% of the explained variance. Accordingly, the change in LIPA was also positively associated with the change in one sit-to-stand time within the SBF group (R^2=0.46, p=0.007). Within the LIPA group, the relative change in SB was significantly negatively associated with the change in average HGS (R^2=0.59, p=0.001), whereas the relative change in LIPA was significantly positively associated with the change in average HGS (R^2=0.66, p=0.015). Furthermore, the relative change in SB (R^2=0.30, p=0.04) and LIPA (R^2=0.31, p=0.04) were negatively and positively associated with the change in peak HGS respectively, within the LIPA group. Within the LIPA group the relative change in SB, was significantly negatively

associated with eyes open single leg stance time for the left (R^2=0.53, p=0.003), right (R^2=0.36, p=0.02) and average of both legs (R^2=0.33, p=0.03). Accordingly, the relative change in LIPA was significantly positively associated the relative change in left eyes open single leg stance time for the left leg (R^2=0.29, p=0.047) only within the LIPA group.

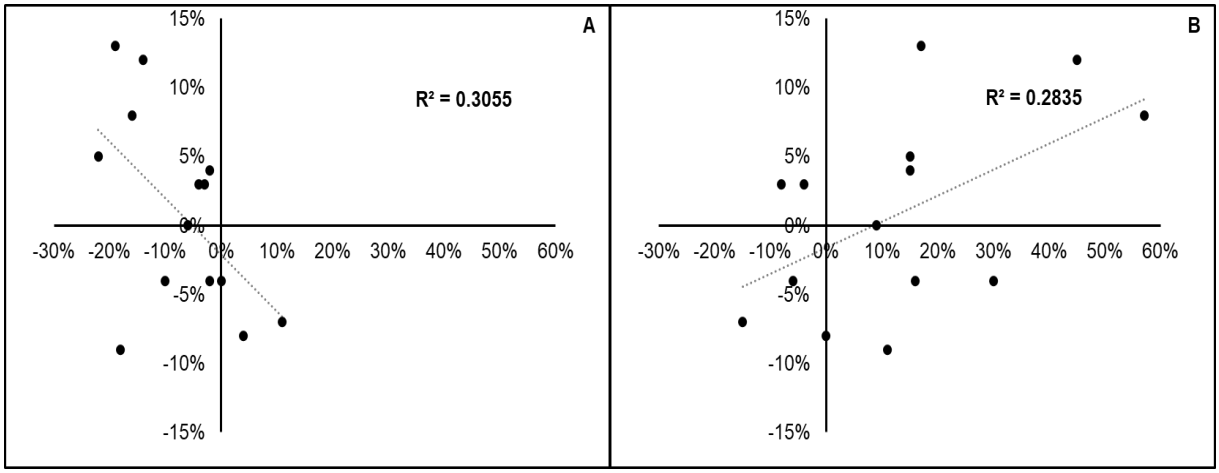


Figure Ai2- The association between the relative change in physical behaviour and one sit-to-stand time (Y axis) in the SBF group. Panels A and B represent the association between SB and LIPA (X axis), respectively. LIPA; light intensity physical activity, SBF; sedentary behaviour fragmentation.

Chapter 5

Data sets that were non-normally distributed included PF MVC, AgCoA, Net PF MVC, and muscle quality. Data sets that were both non-normally distributed and exhibited unequal variances were Agonist Drive, and dorsiflexor MVC.

Associations between the change in physical behaviour and the change in muscle function

Despite no significant effects for associations, notable trends were observed. In the main cohort (n=31) a trend toward a significant association between the change in SB and the change in AgCoA was observed (R^2=0.10, p=0.09), whereby SB accounted for 10% of the explained variance. Interestingly within the control group (n=7), a further trend toward a significant association between the change in SB and the change in Agonist drive was observed (R^2=0.50, p=0.07), whereby SB accounted for 50% of the explained variance.

Chapter 6

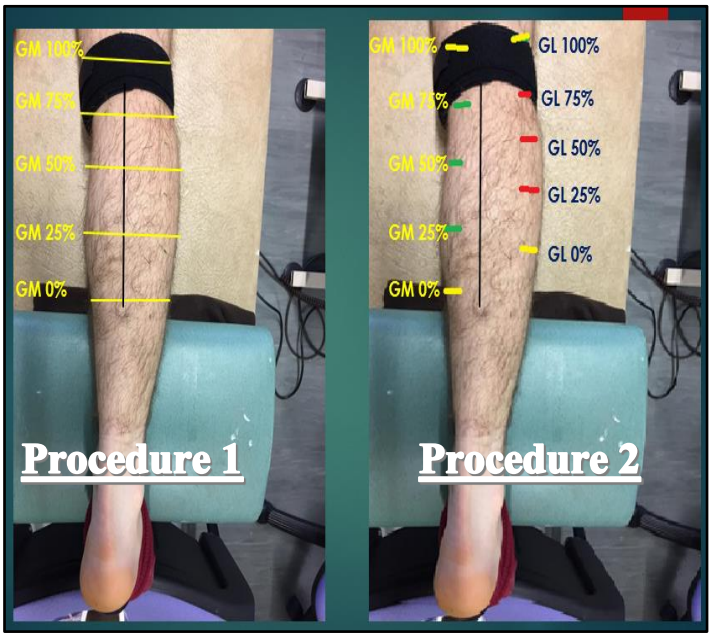


Figure Ai3- Representative image of setup and scanning zones used in Procedures 1 and 2. Note: Thin black line represents GM/GL medial borders. Thick black strap near top of the picture, used to guide the probe round the leg. GL, gastrocnemius lateralis, GM; Gastrocnemius medialis.

VPAN reliability

Ultrasound panoramic imaging (VPAN) has previously been established as a reliable and valid when compared against magnetic resonance imaging [55, 56], and is sensitive to detect muscle hypertrophy and atrophy [57]. Nevertheless, considering the techniques novelty an initial reliability cohort of young healthy adults (n=6, 26±2y, 72.2±9.2kg, 22.8±1.2 kg/m^2), was recruited and visited the lab on two separate occasions. Several measures were monitored and maintained between scans to ensure image quality [61, 66]. Firstly, care was taken to ensure consistency of scanning speed as this can affect image quality [64]. A Velcro strap was loosely attached (to avoid compression) at each length marker, maintaining probe tilt/angle during scanning. Lastly, all acquisition parameters (Depth: 67mm; Frequency: 27Hz; Focal Points: 2; Fixed position for time gain compensation sliders) were held constant. Briefly, panoramic imaging was selected and the probe (7.5MHz linear array probe, 38 mm wide), held perpendicular to the muscles lateral border [Gastrocnemius Medialis (GM), Gastrocnemius Lateralis (GL)]. Transmission gel was applied axially,

to ensure an adequate transmission signal. Once processing, the probe was moved in a sweeping fashion along the designated pathway (pen and Velcro). When the medial border had been reached the imaging process was stopped, and the resultant image displayed on screen (See figure Ai4). This procedure was repeated three times for each site.

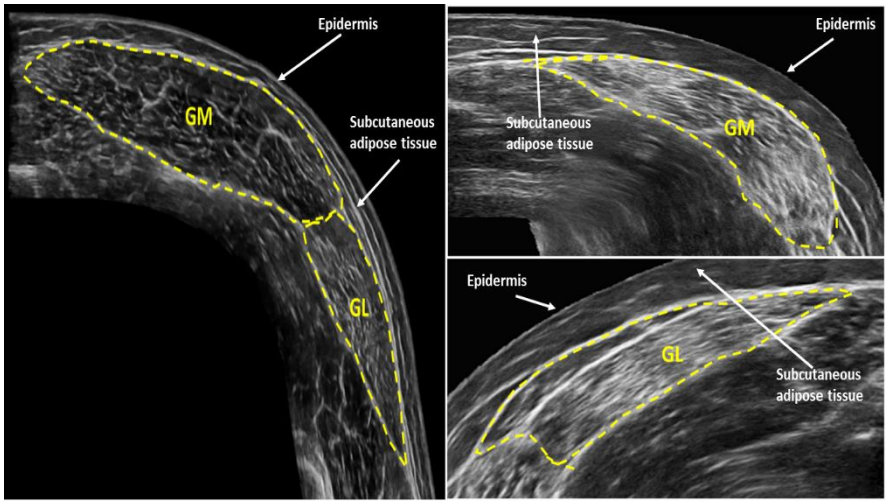


Figure Ai4- Representative ultrasound-VPAN image of GM/GL at 25% of muscle length from procedures one (left) and two (right, upper, and lower). Note procedure one captures both GM and GL in one single muscle sweep, whereas procedure two utilises two single sweeps for each individual muscle. The outer most dermal layer (epidermis), and subcutaneous adipose tissue is annotated on each image. GL, gastrocnemius lateralis, GM; Gastrocnemius medialis, VPAN; panoramic imaging.

Procedure 1 involved 3 single images captured at 75, 50, and 25% of *GM* muscle length, repeated 3 times per site (9 scans in total, please see figure Ai3). Muscle ACSA, was measured offline using IMAGEJ software (1.45 s; National Institutes of Health, Bethesda, MD, USA). Briefly, muscle border connective tissue was manually outlined, and the resulting polygon area calculated. Intra-day reliability was determined through calculation of the intraclass correlation co-efficient (ICC), and Co-efficient of variation % for the 3 repeated scans at each muscle site (75, 50 and 25% of *GM* length, for *GM* and *GL*). For inter-day reliability, ICC, co-efficient of variation, and a paired sample T test was conducted between the averages for Trials 1 and 2. Systematic Bias was also calculated through dividing the typical error [standard deviation of the differences/ $\sqrt{2}$] by the mean of grand mean (average of trials 1 and 2) and multiplying by 100. Alas, inter-day reliability was not deemed of appropriate standard following procedure 1 (Please see Tables Ai 10 and Ai 11). The ultrasound-VPAN method was repeated with a second cohort of young healthy adults using procedure 2 (n=6, 29±4y,

78.7±19.6kg, 25.4±5.2 kg/m^2). All the laboratory and statistical procedures remained constant, except the muscle sites, as a single scan (repeated 3 times) was performed at 75,50 and 25% of the GM and GL length individually (18 scans in total, Please see figure Ai3). Furthermore, to improve Inter-day reliability, acetate sheets were used to record muscle scanning zones during trial 1 in relation to anatomical landmarks (Popliteal crease, or pigmented skin), and manually drawn back onto the skin during trial 2. This alternative procedure drastically improved both the Intra-day and Inter-day reliability for the ultrasound-VPAN method (Please see tables Ai12 and Ai13), with an average ICC of around 0.96 for trial 1, and 0.98 for trial 2, and a co-efficient of variation of ~4%. Furthermore, the inter-day reliability following procedure 2, resulted in a very small average typical error of 0.39cm^2.

Table Ai10 – Intra-day reliability of ULTRASOUND-VPAN using procedure 1.

	Trial 1					**Trial 2**				
Scan Site	**Scan 1 (Mean ± SD)**	**Scan 2 (Mean ± SD)**	**Scan 3 (Mean ± SD)**	**CV (%)**	**ICC**	**Scan 1 (Mean ± SD)**	**Scan 2 (Mean ± SD)**	**Scan 3 (Mean ± SD)**	**CV (%)**	**ICC**
GM 75%	7. 42 ± 1.85 cm^2	8.07 ± 2.41 cm^2	7.52 ± 1.69 cm^2	7	0.90	7.09 ± 1.88 cm^2	7.11 ± 1.66 cm^2	7.12 ± 1.46 cm^2	7	0.86
GM 50%	11.96 ± 1.73 cm^2	11.96 ± 1.93 cm^2	11.80 ± 1.90 cm^2	2	0.96	11.77 ± 1.40 cm^2	11.67 ± 1.78 cm^2	12.15 ± 1.56 cm^2	3	0.97
GM 25%	10. 38 ± 2.46 cm^2	10.35 ± 2.37 cm^2	10.44 ± 2.97 cm^2	4	0.96	10.11 ± 3.10 cm^2	9.85 ± 2.99 cm^2	10.04 ± 3.31 cm^2	3	0.99
GL 75%	4.79 ± 1.93 cm^2	4.67 ± 1.67 cm^2	4.80 ± 1.56 cm^2	5	0.98	3.71 ± 1.45 cm^2	3.67 ± 1.27 cm^2	3.87 ± 1.84 cm^2	8	0.93

GL 50%	7.86 ± 1.17 cm^2	7.67 ± 0.95 cm^2	7.72 ± 1.28 cm^2	3	0.95	7.96 ± 0.72 cm^2	7.91 ± 0.94 cm^2	7.95 ± 0.94 cm^2	4	0.93
GL 25%	5.89 ± 1.30 cm^2	5.78 ± 1.64 cm^2	5.64 ± 1.27 cm^2	6	0.93	6.29 ± 1.40 cm^2	6.04 ± 1.41 cm^2	6.11 ± 1.44 cm^2	4	0.97
AVERAGE				5	0.95	**AVERAGE**			5	0.94

CV; co-efficient of variation, ICC; Intra-class correlation co-efficient, GL, gastrocnemius lateralis, GM; Gastrocnemius medialis, SD; standard deviation

Table Ai11 – Inter-day reliability of ULTRASOUND-VPAN using procedure 1.

	Trial 1 to Trial 2						
Scan Site	**Trial 1 (Mean ± SD) (cm^2)**	**Trial 2 (Mean ± SD) (cm^2)**	**Mean diff (cm^2)**	**Mean % diff**	**Typical Error (cm^2)**	**Systematic Bias (%)**	**ICC**
GM 75%	7.67 ± 1.94	7.11 ± 1.59	-0.56	-6	0.70	9	0.70
GM 50%	11.90 ± 2.31	11.86 ± 1.89	-0.04	0.1	0.46	4	0.93
GM 25%	10.39 ± 2.77	10.00 ± 3.88	-0.39	-4	0.87	9	0.91
GL 75%	4.72 ± 2.10	3.75 ± 1.81	-0.97	-21	0.36	8	0.95
GL 50%	7.75 ± 1.30	7.94 ± 0.96	0.19	5	1.04	13	-0.11
GL 25%	5.77 ± 0.82	6.15 ± 1.30	0.38	9	0.98	17	0.50

AVERAGE	-0.23	-3	0.74	10	0.67

ICC; Intra-class correlation co-efficient, GL, gastrocnemius lateralis, GM; Gastrocnemius medialis, SD; standard deviation

Table Ai12 – Intra-day reliability of ULTRASOUND-VPAN using procedure 2.

	Trial 1					**Trial 2**				
Scan Site	**Scan 1 (Mean ± SD)**	**Scan 2 (Mean ± SD)**	**Scan 3 (Mean ± SD)**	**CV (%)**	**ICC**	**Scan 1 (Mean ± SD)**	**Scan 2 (Mean ± SD)**	**Scan 3 (Mean ± SD)**	**CV (%)**	**ICC**
GL 75%	4.36 ± 1.71 cm^2	4.51 ± 2.02 cm^2	4.30 ± 1.67 cm^2	6	0.97	4.01 ± 1.68 cm^2	4.28 ± 1.82 cm^2	4.40 ± 1.82 cm^2	6	0.98
GL 50%	8.46 ± 2.54 cm^2	8.42 ± 2.60 cm^2	8.18 ± 2.43 cm^2	4	0.98	7.69 ± 2.21 cm^2	8.26 ± 2.22 cm^2	7.97 ± 2.33 cm^2	4	0.99
GL 25%	7.83 ± 2.37 cm^2	7.94 ± 2.58 cm^2	8.05 ± 2.56 cm^2	2	0.99	7.43 ± 2.18 cm^2	7.54 ± 2.48 cm^2	7.55 ± 2.52 cm^2	4	0.98
GM 75%	7.24 ± 1.38 cm^2	7.62 ± 1.73 cm^2	7.46 ± 2.05 cm^2	5	0.93	7.64 ± 1.97 cm^2	7.81 ± 1.93 cm^2	7.58 ± 2.03 cm^2	4	0.97

GM 50%	13.18 ± 2.00 cm^2	13.53 ± 2.50 cm^2	13.67 ± 3.11 cm^2	5	0.88	13.40 ± 2.98 cm^2	13.60 ± 3.07 cm^2	13.58 ± 2.90 cm^2	2	0.99
GM 25%	11. 74 ± 3.75 cm^2	11.35 ± 3.71 cm^2	11.21 ± 3.57 cm^2	3	0.99	12.16 ± 3.29 cm^2	11.89 ± 3.32 cm^2	12.03 ± 3.53 cm^2	3	0.99
AVERAGE				4	0.96	**AVERAGE**			4	0.98

CV; co-efficient of variation, ICC; Intra-class correlation co-efficient, GL, gastrocnemius lateralis, GM; Gastrocnemius medialis, SD; standard deviation

Table Ai13– Inter-day reliability of ULTRASOUND-VPAN using procedure 2.

			Trial 1 to Trial 2				
Scan Site	**Trial 1 (Mean ± SD) (cm^2)**	**Trial 2 (Mean ± SD) (cm^2)**	**Mean diff (cm^2)**	**Mean % diff**	**Typical Error (cm^2)**	**Systematic Bias CV (%)**	**ICC**
GL 75%	4.39 ± 1.79	4.23 ± 1.77	-0.16	-4	0.20	5	0.99
GL 50%	8.36 ± 2.50	7.97 ± 2.25	-0.38	-3	0.43	5	0.97
GL 25%	7.94 ± 2.49	7.51 ± 2.38	-0.43	-5	0.26	3	0.99
GM 75%	7.44 ± 1.70	7.68 ± 1.96	0.24	3	0.35	5	0.97
GM 50%	13.46 ± 2.47	13.53 ± 2.98	0.07	0.1	0.55	4	0.96

GM 25%	11.43 ± 3.67	12.03 ± 3.37	0.59	6	0.54	5	0.98
AVERAGE			-0.01	-0.5	0.39	4	0.98

ICC; Intra-class correlation co-efficient, GL, gastrocnemius lateralis, GM; Gastrocnemius medialis, SD; standard deviation

Table Ai.14- Pre, Post, and intervention related changes for all Gastrocnemius Medialis outcomes, categorised by group. * represents a significant time effect. ×represents a significant group×time interaction effect.

	(SBF n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
MTU Length (cm)	42±3	41±3	-1±2 (-2±3%)	41±3	42±3	0±1 (1±3%)	40±2	41±1	1±2 (2±5%)
75% ACSA (cm^2)	19±7	18±6	-1±4 (0±27%)	24±4	26±6	2±5 (11±30%)	20±6	21±6	2±3 (9±16%)
50% ACSA (cm^2)	38±7	38±10	0±6 (-1±16%)	39±9	36±9	-3±6 (-6±15%)	32±7	34±3	2±4 (7±16%)
25% ACSA (cm^2)	29±7	28±7	-1±3 (-4±11%)	28±9	26±8	-2±4 (-3±18%)	27±7	32±10	6±6 (23±23%)
75% echo intensity	110±32	108±32	-4±11 (-3±12%)	97±33	90±30	2±11 (3±10%)	104±26	92±10	-13±6 (-12±4%)
50% echo intensity	108±33	109±25	0±8 (0±8%)	100±42	108±24	0±11 (2±10%)	120±18	96±13	-21±8 (-18±5%)
25% echo intensity	131±18	131±22	-1±9 (-1±7%)	128±19	125±16	-2±8 (-1±6%)	135±19	115±16	-20±7 (-15±5%)

ACSA; anatomical cross-sectional area, FPA; fascicle pennation angle, Lf-N; normalised fascicle length, LIPA; Light intensity physical activity, MTU; muscle tendon unit, PCSA; physiological cross-sectional area, SBF; Sedentary behaviour fragmentation,

Table Ai.15- Pre, Post, and intervention related changes for all *Gastrocnemius Lateralis* outcomes, categorised by group. * represents a significant time effect. ×represents a significant group×time interaction effect

	(SBF n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
75% ACSA (cm^2)	13±2	13±3	1±4 (9±32%)	16±3	17±5	2±3 (8±21%)	16±4	15±3	-1±4 (0±22%)
50% ACSA (cm^2)	30±11	32±13	0±7 (0±23%)	27±10	29±10	2±7 (6±34%)	26±12	30±10	1±10(5±34%)
25% ACSA (cm^2)	21±8	25±11	4±6 (20±27%)	22±7	20±6	-2±5 (-6±26%)	26±10	28±8	2±5 (10±20%)
75% echo intensity	118±16	120±18	2±10 (2±9%)	112±30	112±17	1±10 (1±10%)	118±17	100±15	-17±11 (-14±10%)
50% echo intensity	114±18	114±20	0±9 (0±9%)	123±18	122±17	0±7 (0±6%)	124±12	111±11	-12±7 (-11±6%)
25% echo intensity	134±23	133±25	-1±8 (0±6%)	139±21	139±16	-1±15 (0±11%)	141±12	125±11	-16±4 (-11±3%)

ACSA; anatomical cross-sectional area, LIPA; Light intensity physical activity, SBF; Sedentary behaviour fragmentation,

Table Ai16- Pre, Post, and intervention related changes for all *Achilles Tendon* outcomes, categorised by group. **Boldened text** represents a significant baseline difference. * represents a significant time effect. ×represents a significant group×time interaction effect.

	(SBF n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
0cm ACSA (cm^2)	0.85±0.23	0.86±0.22	0.01±0.20 (6±27%)	0.74±0.16	0.82±0.17	0.08±0.25 (16±33%)	0.72±0.16	0.73±0.15	0.02±0.22 (6±30%)
1cm ACSA (cm^2)	0.78±0.21	0.82±0.31	0.03±0.16 (5±19%)	0.82±0.18	0.80±0.23	0.04±0.20 (8±27%)	0.75±0.33	0.82±0.25	0.05±0.10 (9±16%)
2cm ACSA (cm^2)	0.80±0.17	0.83±0.17	0.03±0.10 (5±14%)	0.77±0.13	0.75±0.16	-0.02±0.10 (-2±12%)	0.72±0.22	0.69±0.26	-0.03±0.10 (-2±14%)
3cm ACSA (cm^2)	0.73±0.25	0.76±0.20	-0.02±0.14 (-1±15%)	0.64±0.18	0.63±0.20	0.02±0.11 (6±25%)	0.55±0.30	0.69±0.29	0.02±0.10 (4±17%)
0cm echo intensity	103±17	106±15	2±13 (3±12%)	107±16	109±12	2±17 (4±18%)	118±14	101±8	-16±10 (-14±8%)
1cm echo intensity	109±33	111±61	-2±15 (-1±13%)	115±25	117±25	-1±11 (0±9%)	119±11	111±17	-10±5 (-9±5%)
2cm echo intensity	102±14	106±16	-4±13 (4±14%)	109±17	111±18	1±7 (1±7%)	104±9	95±7	-10±10 (-9±10%)
3cm echo intensity	89±19	92±21	2±11 (3±12%)	107±38	97±41	-4±10 (-3±8%)	95±16	88±6	-10±8 (-10±8%)

ACSA; anatomical cross-sectional area, LIPA; Light intensity physical activity, SBF; Sedentary behaviour fragmentation,

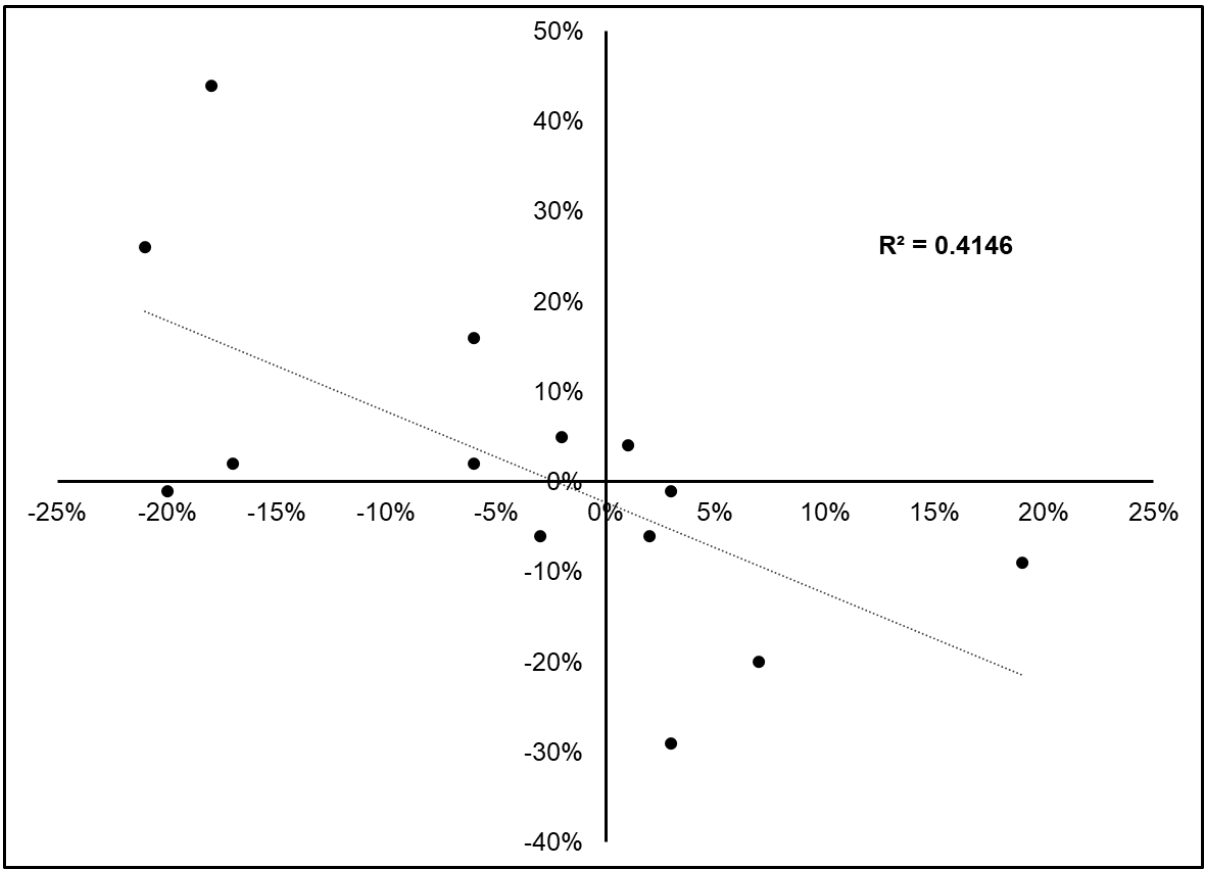


Figure Ai5- Association between the relative change from baseline for sedentary behaviour (X axis) and the relative change from baseline for GM physiological cross-sectional area (Y axis), for SBF exclusively. GM; Gastrocnemius Medialis, SBF; sedentary behaviour fragmentation.

Data sets that were non-normally distributed were *GM* muscle-tendon unit Length, *GM* Lf, echo intensity at 50% of *GM* length, ACSA at 50% of *GL* length, average *GL* ACSA, *GL* muscle volume, Echo intensity at 50% of *GL* length, *VL* muscle length, *VL* muscle volume, *VL* Lf, Achilles tendon Length, Achilles tendon ACSA at 1cm length, & Achilles tendon ACSA at 3cm length. Data sets that possessed unequal variances included Echo intensity at 75% of *GM* muscle length, Echo intensity at 25% of *GL* muscle length, Echo intensity at 1cm of Achilles tendon length, echo intensity, at 3cm of Achilles tendon length. Data that was both non-normally distributed and exhibited unequal variances were: *GM* muscle Length, & average echo intensity for the Achilles tendon.

Associations between the relative change from baseline in SB/ LIPA, and the relative change from baseline for muscle-tendon parameters.

Within the whole cohort (n=36) the relative change from baseline in SB was negatively associated with *GM* physiological cross-sectional area (R^2=0.11, p=0.049), accounting for 11% of the explained variance. Following sub-analysis by group, such an association persisted within SBF (R^2=0.56, p=0.002), accounting for 56% of the explained variance but not LIPA or control (p>0.05). The change in SB was also significantly positively associated with the change in *GL* echo intensity at 50% of muscle length (R^2=0.29, p=0.048). following LIPA, accounting for 29% of the explained variance. Within the control group the change in SB was negatively and positively associated with the change in *GM* ACSA at 25% of muscle length (R^2=0.51, p=0.047), and echo intensity at the same site (R^2=0.59, p=0.025), respectively. The change in SB accounted for similar amounts of the explained variance in both outcomes (ACSA: 51%, Echo intensity: 59%). The relative change from baseline for LIPA, was significantly negatively associated with the change in Achilles' tendon echo intensity at 3cm (R^2=0.31, p=0.038) within the LIPA group accounting for 31% of the explained variance. Accordingly, the change in LIPA was significantly positively associated with the change in Achilles tendon echo intensity at 2cm (R^2=0.59, p=0.027), within the control group, accounting for 59% of the explained variance.

Chapter 7

The body composition parameters that were non normally distributed included LegLBM_ Pre, TotalLBM_Pre, Appendicular skeletal muscle mass-_Pre, lean body mass -Relative appendicular skeletal muscle mass_Pre, TotalFatAndroid_Pre, TotalFat_Pre, LeftArmFP%_Pre, RightArmFP%_Pre, AveragearmFP%_Pre, Android FP%_Pre, TotalBFP%_Pre, TrunkFP%/Leg FP%_Pre, ARMBMD_PRE, PELVISBMD_PRE, LEGBMD_PRE, T_SCOREBMD_PRE.

Body Composition Definitions

Pre-sarcopenia: Pre-sarcopenia was defined through using relative appendicular skeletal muscle mass. Given that all participants were older women the previously validated threshold of <5.5kg/m^2 was adopted.

Obesity: Obesity was principally defined using the world health organisation criterion reference standard based upon BFP%. Participants were defined as obese if they presented with a BFP% of ≥35% (32). Secondary visceral obesity definitions were also investigated, determined by participants who presented with a waist circumference and/or WHR, of ≥ 88cm and 0.85, respectively.

Bone Health Status: Bone health status was classified by each participants T-score. Hologic software was used to calculate the number of standard deviations (SD) a participant differed from a sex-matched reference population (Source: 2008 NHANES White Female). Participants were classified as normal, osteopenic, and osteoporotic, with a T-score of <1.0, >1.0 - <2.5, and >2.5, respectively.

Comprehensive definitions: Given that there is no consensus on comprehensive body composition states in older adults, the definitions of all three major parameters (BMD t-score, pre-sarcopenia relative appendicular skeletal muscle mass, and body fat percentage) were pragmatically combined. Firstly, participants were classified as either bone compromised (>1.0 SD) or normal (<1.0 SD) based upon their BMD t-score. This was then combined with the relative appendicular skeletal muscle mass determined pre-sarcopenia definition (≤5.5kg.m^2), and total BFP% determined obesity definition (≥35%). Participants were thus given one of eight classifications ranging from Normal-NonSarcopenic-NonObesity, to Osteo-sarcopenic-obesity.

Regarding bone health classification, only one LIPA participant positively shifted from osteoporotic to osteopenic in response to the intervention. Furthermore, two LIPA participants and one SBF participant positively shifted from pre-sarcopenic to non-sarcopenic in response to their respective interventions. Unexpectedly two SBF participants negatively shifted category from non-obese to obese, with LIPA participants remaining stable over time. Regarding obesity defined by waist circumference, two SBF participants positively shifted from obese to non-obese, with one LIPA participant shifting from non-obese to obese. Furthermore, for waist to hip ratio defined obesity, one SBF and one LIPA participant positively shifted from obese to non-obese. Unsurprisingly, no control participants shifted classification for bone health, sarcopenia, primary or secondary obesity definitions. Promisingly, only 11% of participants were classified as Osteo-Sarcopenic-Obese at post-test, the same as baseline. Two LIPA participants exchanged the bone health aspect of their classification from Normal-Sarcopenic-Obese to Osteo-Sarcopenic-Obese and vice versa. Furthermore, two SBF participants shifted the obesity aspect of their classification from non-obese to obese. Positively, one SBF participant shifted from Osteo-Sarcopenic-NonObese to Osteo-NonSarcopenic-NonObese post-intervention. Finally, two control participants shifted to be classified as Osteo-NonSarcopenic-Obese, and Normal-Sarcopenic-NonObese at post intervention. However, most participants remained stable in their comprehensive body composition classification state over time (78%) with no difference between groups regarding classification shift ($p=0.38$).

Table Ai17- Adiposity based outcomes as a factor of the intervention. Stratified by group. * represents a significant time effect. ×represents a significant group×time interaction effect.

	SBF (n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
Waist Circumference (cm)	95±12	95±12	-0.2±4 (-0±5%)	91±6	91±7	0.1±3 (0.1±3%)	93±13	94±12	0.4±1 (1±1%)
Hip Circumference (cm)	100±7	99±6	-2±2 (-0.02±2%)	100±8	99±7	-0.3±2 (±%)	99±9	100±8	0.3±2 (±%)
%Fat Trunk/ %Fat Legs	0.81±0.24	0.79±0.21	-0.01±0.07 (-1±8%)	0.84±0.13	0.86±0.19	0.02±0.08 (2±9%)	0.76±0.11	0.74±0.15	-0.04±0.06 (-5±7%)
Trunk/ Limb Fat mass ratio	0.98±0.19	0.98±0.25	0.004±0.12 (-0.01±13%)	1.00±0.22	1.02±0.22	0.02±0.07 (2±8%)	0.87±0.12	0.85±0.13	-0.02±0.03 (-2±4%)
Total fat tissue content									
Left Arm (kg)	1.60±0.50	1.65±0.53	0.05±0.19 (4±12%)	1.51±0.34	1.53±0.36	0.02±0.14 (2±9%)	1.56±0.38	1.48±0.35	-0.08±0.09 (-5±5%)
Right Arm (kg)	1.61±0.48	1.69±0.50	0.08±0.14 (5±10%)	1.51±0.40	1.54±0.37	0.03±0.12 (3±10%)	1.59±0.49	1.54±0.45	-0.05±0.21 (-3±12%)
Trunk (kg)	12.29±3.08	12.46±3.18	0.18±1.13 (2±9%)	11.91±2.90	12.17±3.12	0.27±0.86 (2±8%)	11.43±2.99	10.75±2.84	-0.68±0.58 (-6±5%)
Left Leg (kg)	4.78±1.57	4.81±1.61	0.02±0.28 (1±6%)	4.54±1.28	4.59±1.28	0.04±0.32 (1±8%)	4.99±1.30	4.77±1.20	-0.22±0.16 (-5±3%)
Right Leg (kg)	4.88±1.60	5.01±1.59	0.12±0.28 (3±6%) ×	4.65±1.39	4.61±1.41	-0.04±0.31 (-1±6%) ×	5.15±1.47	4.97±1.37	-0.19±0.17 (-3±3%) ×

LIPA; light intensity physical activity, SBF; sedentary behaviour fragmentation.

Table Ai18- Bone Mineral Density outcomes as a factor of the intervention. Stratified by group. * represents a significant time effect. ×represents a significant group×time interaction effect

	SBF (n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
Bone Mineral Density									
Ribs (g/cm^3)	0.63±0.05	0.64±0.06	0.01±0.03 (1±5%)	0.62±0.05	0.62±0.04	-0.01±0.03 (-1±5%)	0.66±0.05	0.67±0.05	0.004±0.02 (1±4%)
Spine (g/cm^3)	0.94±0.12	0.95±0.13	0.01±0.07 (2±8%) *	0.94±0.14	0.96±0.14	0.02±0.07 (2±7%) *	1.00±0.11	0.99±0.11	-0.01±0.07 (-1±7%) *
Pelvis (g/cm^3)	1.10±0.16	1.09±0.8	-0.01±0.05 (-1±4%)	1.10±0.20	1.10±0.23	-0.01±0.05 (-1±5%)	1.14±0.21	1.14±0.21	-0.01±0.04 (-0.3±4%)
T-score	-0.14±1.36	-0.27±1.41	-0.13±0.37 (-3±3%)	-0.01±1.75	-0.02±1.63	0.01±0.21 (-5±2%)	0.30±0.72	0.25±0.82	-0.05±0.20 (-3±84%)
Z-score	1.05±1.25	1.05±0.98	-0.05±0.30 (0±30%)	0.80±2.25	0.70±2.10	0.00±0.20 (0±12%)	1.30±0.80	1.25±1.13	-0.05±0.25 (-3±21%)

LIPA; light intensity physical activity, SBF; sedentary behaviour fragmentation.

Table Ai19- Lean Body Mass outcomes as a factor of the intervention. Stratified by group. * represents a significant time effect. ×represents a significant group×time interaction effect.

	SBF (n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
Lean Body Mass									
Left Arm (kg)	1.67±0.33	1.65±0.25	-0.02±0.19 (0±10%)	1.56±0.22	1.57±0.23	0.01±0.12 (1±8%)	1.50±0.24	1.51±0.19	0.02±0.10 (2±7%)
Right Arm (kg)	1.78±0.25	1.80±0.28	0.02±0.18 (2±10%)	1.76±0.20	1.72±0.24	-0.04±0.14 (-2±8%)	1.65±0.22	1.74±0.27	0.09±0.11 (5±6%)
Sum of both Arms (kg)	3.45±0.56	3.46±0.52	0.01±0.29 (1±8%)	3.32±0.40	3.29±0.45	-0.03±0.20 (-1±7%)	3.15±0.45	3.25±0.45	0.10±0.13 (3±4%)
Trunk (kg)	21.42±2.98	21.37±3.12	-0.06±0.82 (-0.3±4%)	20.14±2.25	19.90±2.15	-0.23±0.88 (-1±4%)	20.28±2.40	20.80±2.07	0.52±0.59 (3±3%)
Left Leg (kg)	5.54±1.46	5.22±1.23	-0.23±0.55 (-3±8%)	5.23±0.81	5.32±0.93	-0.02±0.27 (-0.1±5%)	5.32±0.83	5.30±0.85	-0.04±0.23 (-1±4%)
Right Leg (kg)	5.97±1.09	5.92±0.92	-0.05±0.35 (-0.4±5%)	5.73±0.75	5.66±0.71	-0.08±0.26 (-1±4%)	5.44±0.82	5.50±0.91	0.06±0.23 (1±4%)
Sum of both Legs (kg)	11.69±2.26	11.41±1.78	-0.28±0.88 (-2±6%)	11.08±1.41	10.99±1.33	-0.09±0.41 (-1±3%)	10.55±1.43	10.57±1.59	0.02±0.38 (0.03±4%)
Appendicular skeletal muscle mass (kg)	14.51±2.95	14.39±2.43	-0.11±0.98 (-1±7%)	14.34±2.57	14.49±2.87	-0.02±0.47 (-0.1±4%)	14.11±2.29	14.00±2.69	0.11±0.54 (1±4%)
Relative appendicular skeletal muscle mass ($kg.m^2$)	5.61±1.05	5.60±0.66	-0.06±0.37 (-2±6%)	5.29±0.75	5.54±0.81	0.02±0.27 (0.3±5%)	5.57±0.90	5.50±0.84	0.04±0.25 (1±4%)

LIPA; light intensity physical activity, SBF; sedentary behaviour fragmentation.

Table Ai20- Body fat percentage (%) outcomes as a factor of the intervention. Stratified by group. * represents a significant time effect. ×represents a significant group×time interaction effect.

	SBF (n=14)			LIPA (n=14)			Control (n=8)		
	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)	Pre	Post	Change (% Change)
Left Arm (%)	46±9	47±8	1±3 (3±7%)	47±6	47±5	0.1±3 (1±6%)	49±8	47±8	-2±2 (-3±4%)
Right Arm (%)	45±7	46±8	1±3 (2±7%) ×	44±7	45±7	1±2 (3±6%) ×	46±9	44±10	-2±3 (-5±7%) ×
Left Leg (%)	43±8	44±8	1±2 (2±4%)	44±6	44±6	0.3±2 (1±6%)	47±6	46±5	-1±1 (-2±2%)
Right Leg (%)	43±8	44±8	1±2 (2±4%)	43±6	43±7	0.2±2 (0.3±4%)	46±6	45±6	-1±2 (-2±3%)
Average of both Legs (%)	43±8	44±8	1±2 (2±4%) ×	43±6	43±6	0.2±2 (1±4%) ×	47±6	46±6	-1±1 (-2±2%) ×

LIPA; light intensity physical activity, SBF; sedentary behaviour fragmentation.

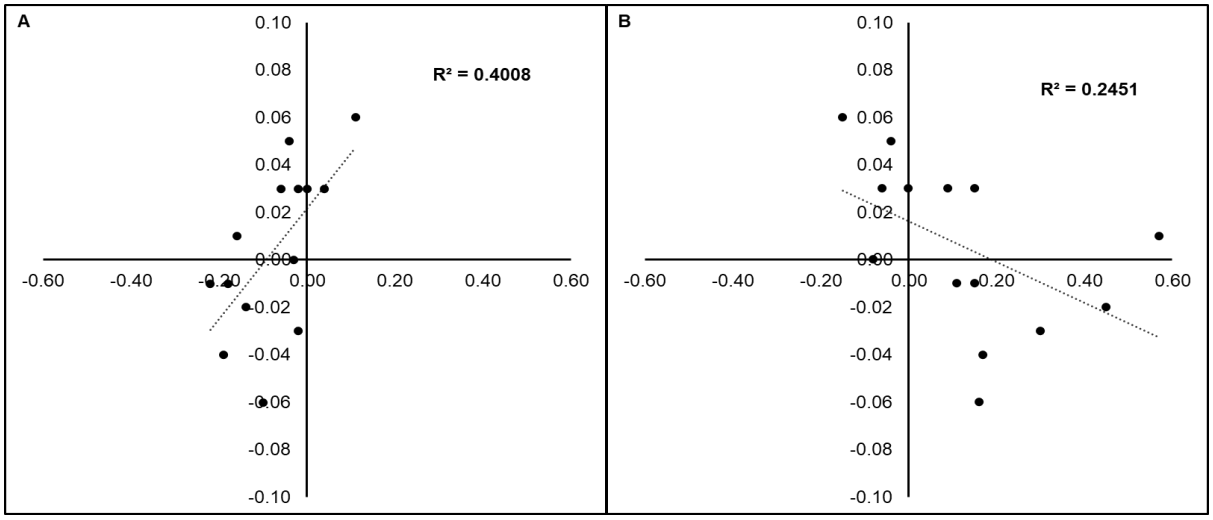


Figure Ai6- Association between the relative change in SB (Panel A)/ LIPA (Panel B) (X axis) and the relative change from baseline in leg fat percentage (Y axis). Within the LIPA group. LIPA; light intensity physical activity group, SB; sedentary behaviour.

Association between the relative change from baseline for both SB and LIPA, and body composition outcomes

Within the whole cohort the relative change from baseline for sedentary behaviour was negatively associated with android: gynoid ratio (R^2=0.12, p=0.036), accounting for 12% of the explained variance. Such a negative association persisted within the SBF group (R^2=0.59, p=0.001), whereby SB accounted for 59% of the explained variance for the relative change in android:gynoid ratio. Accordingly, the relative change from baseline for SB was significantly negatively associated with the relative change from baseline in android fat percentage (R^2=0.66, p<0.001), within the SBF group accounting for 66% of the explained variance. Similarly, within the SBF group, the relative change from baseline for LIPA was significantly positively associated with the relative change in android fat percentage (R^2=0.42, p=0.012), accounting for 42% of the explained variance. Within the whole cohort analysis, the relative change in LIPA was significantly positively associated with the relative change from baseline for trunk fat: leg fat ratio (R^2=0.15, p=0.018), accounting for 15% of the explained variance. Similarly, within the whole cohort analysis the relative change in LIPA was significantly positively associated with the relative change from baseline for trunk fat: limb fat ratio (R^2=0.16, p=0.016), accounting for 16% of the explained variance. Both associations persisted within the SBF group whereby LIPA was positively associated with both trunk fat: leg fat ratio (R^2=0.32, p=0.035), and trunk fat: limb fat ratio (R^2=0.31, p=0.037),

accounting for 32% and 31% of the explained variance respectively. The relative change from baseline in LIPA was also negatively associated with the relative change form baseline in leg fat percentage (R^2=0.12, p=0.039), accounting for 12% of the explained variance. Such an association persisted within the LIPA group whereby the relative change in LIPA was negatively associated with the relative change in leg fat percentage (R^2=0.44, p=0.009), accounting for 44% of the explained variance. A positive association was observed between the relative change from baseline for SB, and the relative change in leg fat percentage within the LIPA group (R^2=0.34, p=0.027), accounting for 34% of the explained variance. Similar associations were observed between SB/LIPA and total leg fat tissue, left leg fat tissue, and left leg fat percentage. Similarly, the relative change in LIPA was significantly negatively associated with gynoid fat percentage (R^2=0.31, p=0.039), accounting for 31% of the explained variance. Furthermore, the relative change in LIPA was negatively associated with the relative change from baseline for T-spine BMD (R^2=0.33, p=0.03), within the LIPA group accounting for 33% of the explained variance. Finally within the control group, the relative change in SB was significantly positively associated with right arm total fat (R^2=0.58, p=0.028) right arm fat percentage (R^2=0.73, p=0.007) and total fat tissue (R^2=0.51, p=0.047), accounting for 58%, 73%, and 51% of the explained variance respectively.

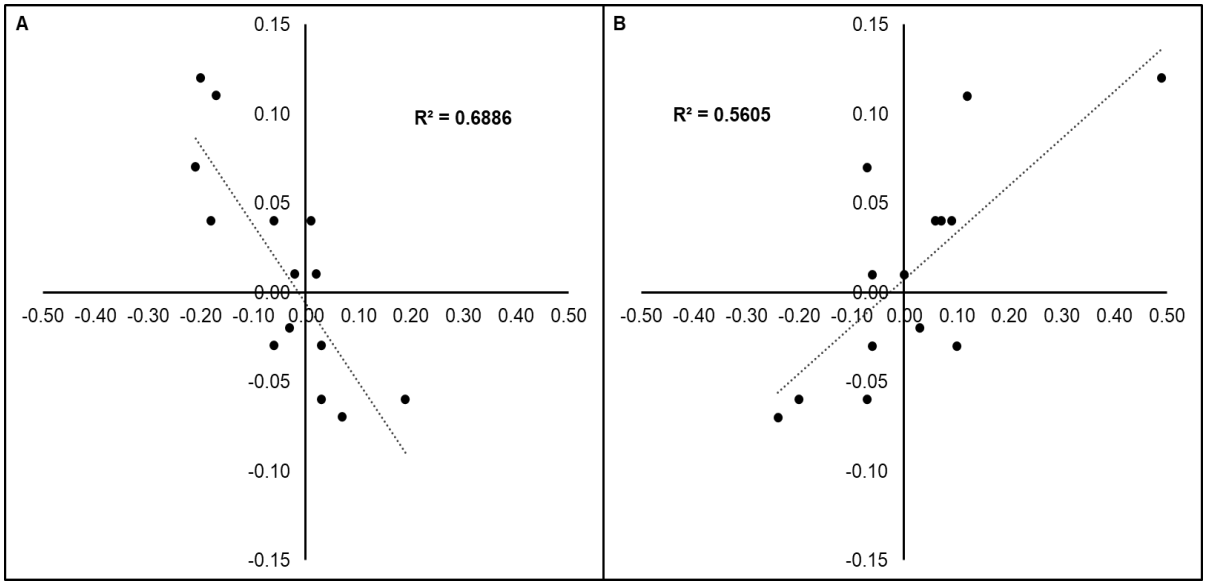


Figure Ai7- Association between the relative change in SB (Panel A)/ LIPA (Panel B) (X axis) and the relative change from baseline in Android fat percentage (Y axis). within the SBF group. LIPA; light intensity physical activity group, SB; sedentary behaviour, SBF; sedentary behaviour fragmentation.

Appendix ii

Published Articles

Grant, D., Tomlinson, D., Tsintzas, K., Kolic, P. and Onambele-Pearson, G., 2020. Displacing Sedentary Behaviour with Light Intensity Physical Activity Spontaneously Alters Habitual Macronutrient Intake and Enhances Dietary Quality in Older Females. *Nutrients*, *12*(8), p.2431. https://doi.org/10.3390/nu12082431

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Displacing Sedentary Behaviour with Light Intensity Physical Activity Spontaneously Alters Habitual Macronutrient Intake and Enhances Dietary Quality in Older Females

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Abstract: Displacing Sedentary Behaviour (SB) with light intensity physical activity (LIPA) is increasingly viewed as a viable means of health enhancement. It is, however, unclear whether any behavioural compensations accompany such an intervention. Therefore, the aim of this study was to identify any dietary changes that accompany SB displacement. We hypothesised that SB displacement would improve dietary quality. Thirty-five elderly females (73 ± 5 years) were randomly allocated to one of three groups: (1) sedentary behaviour fragmentation (SBF) ($n = 14$), (2) continuous LIPA ($n = 14$), or (3) control ($n = 7$). Habitual diet (four-day food diary) and physical behaviour (accelerometery) were assessed at weeks 0 and 8. Out of 45 nutrients examined, only glucose exhibited a group × time interaction ($p = 0.03$), mediated by an exclusive reduction following SBF (−31%). SBF was also the sole experimental group to increase nutrients promoting bone health (SBF: 17%, LIPA: −34%, control: 21%), whereas both experimental groups consumed more nutrients promoting anabolism (SBF: 13%, LIPA: 4%, control: −34%) (z-scores). New ambulators ($n = 8$) also consumed more nutrients promoting bone health (16%)/anabolism (2%) (z-scores), including significantly increased Zinc intake ($p = 0.05$, 29%). Displacing SB with LIPA improves dietary quality in older females. Furthermore, SB fragmentation appears advantageous for various dietary outcomes.

Keywords: anabolism; bone health; energy intake; LIPA; older adults; sedentary behaviour fragmentation

1. Introduction

Older adults (herein defined as ≥65 years) are recommended to perform 150 min of moderate to vigorous (MVPA) physical activity (PA) per week [1]. However, performing high amounts of sedentary behaviour (defined as sitting or being in an reclined posture for >8 h/day) [2,3] is now recognised as an independent determinant of health [3,4], distinct from other physical behaviours, such as a lack of PA. Accordingly, recommended MVPA engagement does not fully mitigate the health risks of concurrent high sedentary time [2,5]. Furthermore, light intensity PA (LIPA) [6,7] is associated with positive health outcomes [8,9]. With older adults reported to be the most sedentary population [10], spending ~65–80% of their waking hours performing sedentary behaviour [11], it is clear that the distinct health impact of sedentary behaviour, in this population especially, needs to be elucidated. Indeed, sedentary behaviour accumulated in a prolonged vs. fragmented pattern appears to be more detrimental to health [12,13],

especially in older adults [4,14,15]. However, the few SB reduction intervention studies that have been conducted have reported mixed efficacy regarding the ability to both alter an individual's behaviour, as well as improve health outcomes [11]. Furthermore, SB reduction intervention studies have seldom considered the potential for spontaneous compensations in habitual nutrition. This is a limitation given that it is unknown whether a change in sedentary behaviour time worsens/enhances its overall health-promoting potential through concurrent alterations in important healthy diet-related practices. This is of high importance, given that older adults present with various habitual dietary practices not conducive to optimal health. As a case in point, older adults typically reduce their energy intake over time [16], primarily driven by a lack of hunger, which is termed "the anorexia of aging" [17]. Conversely, positive energy balance (energy intake exceeding expenditure), could potentially facilitate adiposity accumulation [18,19]. Furthermore, older adults consistently under-consume protein [17,20], and exhibit a higher saturated fatty acid to polyunsaturated fatty acid intake ratio, as well as a specific deficiency in omega-3 fatty acids like alpha-linolenic acid [16,20,21], with both dietary patterns strongly associated with cardiovascular disease mortality [22,23]. Various micronutrient deficiencies are also exhibited in ageing, including vitamins B, C, and D, as well as key minerals such as calcium, magnesium, and zinc [20,21,24,25]. Reductions in dietary quality over time are highlighted by the fact that older adults exhibit serving size reductions in foods of a high dietary quality (i.e., foods consisting of a good balance of starchy root vegetables, proteins, and dairy products, as well as a variety of fruit/vegetables) [16], whereas correcting such deficiencies can improve vitality and longevity [26].

Promisingly, PA has previously been identified as a gateway behaviour for the adoption of further healthy behaviours [27], with those consistently adhering to adequate levels of PA more likely to exhibit healthier dietary practices [28]. Accordingly, metabolic balance is defined as "The extent to which one's physical behaviour profile influences nutritional intake and vice versa" [29]. Various subtypes of sedentary behaviour are consistently linked with unhealthy eating behaviours, including a) a long driving time (≥3 h/day) associated with a reduced fruit/vegetable intake [30] and b) adults who engage in ≥2 h/day TV viewing time, consuming ~137 kcal/day more than adults who engage in <1 h/day [31].

However, it remains to be determined whether displacing sedentary behaviour leads a person to a more beneficial or an adverse habitual nutritional profile. Interestingly, high self-reported standing time has been associated with a reduced risk of obesity in middle-aged women (55–65 years) [32]. Acutely displacing sedentary time in younger adults with standing marginally increases energy expenditure [18,33], suggesting that a reduced obesity risk with a high standing time may primarily be due to a reduced energy intake. Accordingly, sustained postural transition in rodents results in a reduced energy intake [34], which appears to be exclusively dependent on an osteocyte strain detection mechanism (termed "the gravitostat"), activated as an effector in response to increased loading through the lower limbs [35]. In support of this notion, relative energy intake during a subsequent meal was 39% lower following a LIPA breaks protocol compared to continuous sedentary behaviour in young adults [36]. Therefore, despite a lack of direct replication in human intervention studies [37], "the gravitostat" provides the first plausible mechanism for reduced energy intake following reduced sedentary time. Whilst promising, such acute experimental studies do not quantify changes in diet quality following sedentary behaviour displacement, since such changes are generally implemented in the long-term. PA intervention studies may therefore offer an insight into the changeability of dietary quality. In line with this theorem, compensatory health beliefs are based on the idea that the health-promoting effects of a positive lifestyle behaviour (e.g. improved physical behaviour profile) can counteract the negative effects of an unhealthy behaviour (e.g. reduced dietary quality) [38]. Therefore, whilst previous findings must be interpreted carefully, they do identify a promising trend of improved dietary quality with improvements in physical behaviour profile (generally more activity compared to inactivity), that should be investigated further, and identify what potential role (if any) sedentary behaviour displacement (of varied prescribed patterns) plays in such an effect.

Therefore, the aim of this study was to examine and identify any compensatory dietary behaviours that accompany sedentary behaviour displacement. We hypothesized that sedentary behaviour displacement in older adult females would be accompanied by a spontaneous reduction in energy intake (thus managing the energy balance more effectively), as well as a relative improvement in dietary quality (improvements in macro (increased protein intake etc.)/micro-nutrient profile).

2. Materials and Methods

2.1. Participants and Experimental Design

Thirty-five community-dwelling elderly females (age: 73 ± 5 years, height: 1.6 ± 0.1 m, weight: 67.1 ± 9.6 kg, BMI: 26.3 ± 3.6 kg/m^2) voluntarily participated in the study. Participants were all from the local community and were recruited from a pre-existing research database. The study was approved by the ethical committee at the Manchester Metropolitan University (approval code: 230118-ESS-DG- [2]), and written informed consent was obtained prior to any procedures being performed, in line with the declaration of Helsinki. Exclusion criteria included suffering from chronic health conditions likely to affect an ability to safely and independently undertake a program of decreased sedentary behaviour (e.g., cardiovascular disease, uncontrolled diabetes, active cancer). Participants visited the lab to undergo test familiarisation, during which time they were fitted with physical behaviour monitoring equipment. After seven days of habitual physical behaviour and nutrition monitoring, participants returned the physical behaviour monitoring equipment to the laboratory and received intervention instructions. All participants were then randomly allocated in a single blind fashion to one of three groups: (1) sedentary behaviour fragmentation (SBF) (n = 14), (2) single bout light intensity physical activity (LIPA) (n = 14), or (3) control, i.e., no lifestyle change (n = 7). Habitual nutrition assessment was conducted at weeks 0 and 8.

2.2. Anthropometric Assessments

Participant height was measured in meters (m), using a stadiometer (Seca model 213 portable stadiometer, Seca, Germany). Participant mass was then measured with digital scales (Seca model 873, Seca, Germany) to the nearest 0.1 kg.

2.3. Assessment of Habitual Dietary Intake

Participants were provided with comprehensive written and verbal instructions to complete a 4-day weighed food diary. Participants were instructed to record their habitual dietary intake on 3 weekdays and 1 weekend day during the baseline data collection period. Participants were also encouraged to record any nutritional supplements they consumed habitually. The potential limitations of self-reported dietary intake, for estimating energy intake and macronutrient composition have been well documented [39], with ~35 days needed to estimate true average energy intake in women [40]. Therefore, steps were taken to maximise accuracy from the self-report method. Accordingly, standard sized digital weighing scales (Salter, Kent, United Kingdom) were provided to each participant to allow all food and drink consumed to be weighed to the nearest gram. Each food diary was checked by the principal investigator with any uncertainties clarified by the participant. In the event that the participant was unavailable to provide clarification, food/drink quantity was estimated from previous diary entries. Diaries were analysed by the same investigator with the use of Nutritics software (Version 5.0, Nutritics Ltd., Dublin, Ireland) to produce a comprehensive report of energy, as well as macro- and micronutrient intakes. If a consumed food item was missing from the database, the nutritional data was located from the manufacturer's database and was manually entered into the database. Furthermore, participants were asked to retain and return any packaging from foods they regularly consumed (breakfast cereals, canned foods, etc.), to estimate intake as close to the manufacturer's information as possible. Where available, food packaging barcodes were scanned and specific nutritional information was digitally logged using MyFitnessPal software (MyFitnessPal,

San Fransisco, CA, USA). Such specific nutritional information was cross-referenced against the habitual nutrition outcomes calculated with Nutritics to more accurately determine the type/amount of the reported consumed nutrients. Where a discrepancy was found, more specific values were obtained from MyFitnessPal, were added to the Nutritics database, and were inputted into the 4-day analysis. In the course of the current study protocol, participants did not undertake nutritional counselling.

2.4. Recommended Daily Intake and Health-Enhancing Nutrients

The nutrient intake thresholds recommended for older females' (65–74 years) health [41,42] were used to compare against all nutrients with the criteria available. Furthermore, previous research has identified specific nutrients as principal mediators of musculoskeletal health in older adults [29,43]. Accordingly, skeletal muscle health in older adults is specifically modulated by a habitual intake of protein [44], vitamin D [45,46], and vitamin E [47] as well as omega-3 and omega-6 fatty acids [48,49]. Therefore, all five nutrients were grouped as key nutrients promoting anabolism. Furthermore, bone health in older adults is specifically modulated by the habitual intake of calcium [50], zinc [43], magnesium [51], phosphorus [52], vitamin C [43], vitamin D [53], protein [54] and omega-3 fatty acids [55]. Therefore, all eight nutrients were grouped as key dietary components promoting bone health. Key pro-anabolic and bone health-enhancing nutrients were compared against recommended levels as a means of comparing the specific health-enhancing properties of each intervention through habitual nutrition.

2.5. Physical Behaviour Profile

Individual participant physical behaviour profile was objectively determined at baseline and week 8 of the intervention, using a thigh mounted GENEActiv original triaxial accelerometer for 4–7 days (Activinsights Ltd., UK). Data were extracted using GENEA software. We then used a previously validated algorithm for baseline and post-intervention data analysis [56]. Briefly, the aforementioned validation study calculated the incremental metabolic cost of ten everyday tasks in 40 healthy older adults (~74 years) (e.g., lying down, brisk treadmill walking etc), and used regression analysis to identify specific physical activity intensity ranges [utilising Metabolic equivalent of task (METS) thresholds (SB: <1.5 METS, LIPA: 1.5–3.0 METS, MVPA: >3.0 METS)] mapped against the concurrently recorded GENEActiv gravitational pull and acceleration data. The robustly derived data on SB, LIPA, and MVPA in older adults were used for further analyses. Following physical behaviour analysis, participants were classified as either being sedentary (≥8 h/day) or an ambulator (<8 h/day) depending on their average daily sedentary behaviour time. Participants were also further classified as physically active (≥150 min/week MVPA ≥ 10 min bouts), or non-physically active (<150 min/week MVPA ≥ 10 min bouts). Such limits were selected as classification thresholds given that sedentary time appears to be exponentially hazardous above 8 h/day [2,3], and the World Health Organisation (WHO) recommends a weekly MVPA engagement time of ≥150 min/week [1].

2.6. Energy Balance

The Harris–Benedict formula [57] was first used to calculate basal metabolic rate of all participants pre and post intervention, given that this method has previously been shown to be valid in older adults [58]. The basal metabolic rate was then multiplied by an activity factor to give the total daily energy expenditure. The activity factor was determined based upon each participant's objectively determined physical behaviour profile as opposed to using physical activity classification, given that intense activity contributes minimally to total daily energy expenditure [59]. Specifically, the basal metabolic rate was multiplied by an activity factor of 1.2 and 1.375 when a participant was classified as sedentary or ambulatory, respectively. Secondly, we also used the Schofield equation [60] to calculate basal metabolic rate. Basal metabolic rate calculated from the Schofield equation was then multiplied by activity factors of 1.3 and 1.5, depending on whether a participant was classified as sedentary or ambulatory, respectively. This gave a secondary estimate of total daily energy expenditure.

Both methods of total daily energy expenditure estimation were then subtracted from total daily energy intake to give estimates of energy balance. Both Harris–Benedict and Schofield have previously been used by the WHO as reference standards for energy intake [61].

2.7. Physical Behaviour Interventions

The purpose of the two intervention groups was to manipulate the protocol for displacing sedentary behaviour time with added daily LIPA (45–50 min in total). Both intervention groups were provided with an illustrated booklet, which contained examples of LIPA compiled from the compendium of physical activities [62]. Importantly, such activities were intentionally selected due to their simplicity, safety, and ease of implementation within the home environment. Individual participant compliance was objectively monitored at baseline and at week 8 of the intervention for 4–7 days, as outlined above.

SBF group: Participants were told that the purpose of their intervention was to reduce the amount of time spent performing sedentary behaviour (sitting, lying, or reclining) especially in prolonged uninterrupted bouts. Participants were instructed not to perform sedentary behaviour for more than 30 min at a time, and that for every 30 min of sedentary behaviour performed the participant should stand up and perform 2 min of upright LIPA (general ambulatory walking, side-to-side shuffling, washing dishes, etc.)

LIPA group: Participants were informed that the purpose of their intervention was to increase the amount of time spent performing LIPA whilst maintaining habitual routines. Participants were instructed to perform a continuous single bout of 45–50 min LIPA (general ambulatory walking, side-to-side shuffling, washing dishes etc.), every day for the duration of the 8-week intervention.

Control group: Participants who were randomly allocated to the control group were specifically instructed to maintain their habitual routine. Control participants were told that the overall purpose of the study was to investigate the link between health and habitual physical behaviour profiles.

2.8. Statistical Analysis

Analyses were carried out using SPSS (Version 26, SPSS Inc., Chicago, IL, USA). Baseline group differences were examined with a one-way analysis of variance (ANOVA) or a Kruskal–Wallis ANOVA as appropriate. Accordingly, the effects of the interventions were determined using a 2×3 split plot ANOVA [2 time phases (pre and post intervention) and 3 intervention groups (SBF, LIPA, and control)]. In cases where groups were unmatched at baseline, the baseline values were added into the statistical analysis model as a co-variate. Furthermore, in cases of non-normally distributed data, within-group comparisons were made using the Wilcoxon-Sign Rank test, whilst between-group differences were analysed through a Kruskal–Wallis ANOVA test on the relative changes from baseline. A chi-squared test was used to compare between group differences for ordinal/nominal data. In addition, a sub-analysis was run on participants who positively shifted sedentary classification from sedentary to ambulatory post intervention ($n = 8$), using a paired samples T-test or a Wilcoxon signed-rank test as appropriate. Finally, z-scores were calculated for each nutrient, and unit-weighted composite z-scores for groups of nutrients to enable a) the nutrients grouping comparisons at baseline versus post intervention for diet promoting anabolism, and diet promoting bone health data reduction analysis; b) comparison of the diet composition change in those participants classified as sedentary pre-intervention, who changed to ambulatory post intervention. Data are reported as Mean ± SD (or Median, IQR for non-parametric data). Statistical trends were accepted as p values between 0.1 and 0.05, whereas statistical significance was accepted when $p \leq 0.05$.

3. Results

3.1. Baseline Group Differences

All groups were matched for the majority of baseline values, including physical behaviour profile (Table 1). However, participants were different at baseline regarding the month, and therefore season the intervention commenced. Accordingly, 12 participants began their intervention on months conventionally associated with winter, 9 on months conventionally associated with spring, 8 on months conventionally associated with summer, and 6 on months conventionally associated with autumn. Specifically, the control group had a significantly higher proportion (100%) ($p = 0.02$) of participants who had begun their intervention during spring/summer (April: $n = 2$, May: $n = 5$), in contrast to both SBF (January: $n = 3$, February: $n = 3$, April: $n = 1$, July: $n = 4$, October: $n = 3$), and LIPA (January: $n = 3$, February: $n = 3$, March: $n = 1$, July: $n = 4$, October: $n = 3$) (Please see Table 1). Furthermore, carbohydrate ($p = 0.049$), relative carbohydrate intake ($p = 0.02$), and protein ($p = 0.045$) were the only food-related outcomes to exhibit differences between groups at baseline. For protein, post hoc testing revealed between group differences were significant between SBF and control ($p = 0.04$), where SBF had a much lower protein intake (66 ± 11 g) at baseline compared to control (84 ± 15 g). A similar post-hoc trend was exhibited for carbohydrate where SBF tended to be lower at baseline (144 ± 38 g) compared to control (187 ± 36 g) ($p = 0.08$). Relative carbohydrate intake, on the other hand, exhibited significant differences between SBF and LIPA ($p = 0.02$), as well as SBF and control ($p = 0.02$), whereas SBF was lower at baseline (2.01 ± 1.00 g.kg) compared to both LIPA (2.85 ± 0.71 g.kg) and control (2.93 ± 0.67 g.kg) (Table 2).

Table 1. Baseline measures based on group.

	Group		
	SBF(n = 14)	**LIPA (n = 14)**	**CONTROL (n = 7)**
Age (years)	75 ± 7	72 ± 12	68 ± 4
Weight (kg)	69 ± 11	66 ± 9	67 ± 9
Body Mass Index (kg/m^2)	26.9 ± 3.6	25.3 ± 3.6	26.9 ± 3.4
Proportion classified as Obese/Overweight (Normal)	14%/57% (29%)	14%/43% (43%)	14%/72% (14%)
Polypharmacy(#)	2 ± 4	0 ± 1	1 ± 3
Nutritional supplements(#)	0 ± 1	0 ± 1	1 ± 1
FRAT (number of positive responses)	1 ± 1	1 ± 1	0 ± 1
Proportion who live alone (cohabitate)	36% (64%)	43% (57%)	71% (29%)
Sedentary Behaviour (hrs/24 h)	9.7 ± 2.0	9.3 ± 1.5	8.9 ± 2.0
LIPA (hrs/24 h)	2.1 ± 0.7	2.2 ± 0.6	2.0 ± 1.5
Sedentary Behaviour (% of 24 h time)	60 ± 7	62 ± 7	53 ± 13
LIPA (% of 24 h time)	14 ± 3	13 ± 2	15 ± 4
LIPA (% of PA time)	37 ± 5	35 ± 4	29 ± 7
Weekly MVPA time (≥10 min Bouts)	77 ± 183	51 ± 65	51 ± 130
Proportion classified as Sedentary (Ambulatory)	71% (29%)	79% (21%)	43% (57%)
Proportion classified as Active (Inactive)	29% (71%)	0% (100%)	14% (86%)
Basal Metabolic Rate (kcal) (Harris–Benedict)	1252 ± 125	1230 ± 77	1256 ± 95
Metabolic Balance (kcal) (Harris-Benedict)	−98 ± 626	72 ± 546	243 ± 419
Basal Metabolic Rate (kcal) (Schofield)	1281 ± 102	1253 ± 78	1270 ± 79
Metabolic Balance (kcal) (Schofield)	−311 ± 607	−80 ± 528	74 ± 489
Proportion consuming optimal levels of ≥3/5 pro anabolic nutrients	43%	29%	29%
Proportion consuming optimal levels of ≥5/8 bone health enhancing nutrients	36%	50%	57%
Intervention Outcomes			
Intervention Length (Days)	57 ± 2	56 ± 1	54 ± 5
Proportion who began intervention in Spring/Summer (Autumn/Winter)	36% (64%)	36% (64%)	**100% (0%)**
Proportion who shifted classification from sedentary to ambulatory (stable)	21% (79%)	21% (79%)	29% (71%)

Boldened font represents a significant baseline difference. FRAT: Falls Risk Assessment Tool, LIPA: light intensity physical activity, MVPA: moderate to vigorous physical activity.

Table 2. Habitual dietary outcomes at baseline, and week 8, for each group.

	SBF (*n* = 14)		*LIPA* (*n* = 14)		*CONTROL* (*n* = 7)	
	Baseline	**Week 8**	**Baseline**	**Week 8**	**Baseline**	**Week 8**
Energy (Kcal)	1371 ± 616	1468 ± 699	1543 ± 509	1602 ± 350	1825 ± 679	1546 ± 557
Energy (Kj)	5740 ± 2566	6150 ± 2911	6479 ± 2118	6715 ± 1478	7653 ± 2799	6483 ± 2351
Protein (g)	**66 ± 11**	65 ± 20 *	71 ± 18	69 ± 16 *	84 ± 15	80 ± 12 *
Relative Protein intake (g/kg)	1.00 ± 0.30	0.98 ± 0.40	1.11 ± 0.32	1.06 ± 0.23	1.29 ± 0.18	1.20 ± 0.24
Portions of Fruit consumed (#)	2 ± 1	2 ± 2	3 ± 2	3 ± 2	3 ± 1	3 ± 2
Portions of Vegetables consumed (#)	2 ± 5	2 ± 1	2 ± 1	2 ± 1	2 ± 1	2 ± 1
Carbohydrate (g)	**144 ± 38**	144 ± 51 *	177 ± 48	174 ± 45 *	187 ± 36	186 ± 57 *
Relative carbohydrate intake (g/kg)	**2.04 ± 1.00**	2.03 ± 0.97	2.85 ± 0.71	2.78 ± 0.60	2.93 ± 0.67	2.56 ± 1.51
Glucose (g)	13.4 ± 6.4	10.5 ± 5.1×	15.4 ± 5.8	13.8 ± 7.2×	14.3 ± 4.5	19.7 ± 7.3×
Fructose (g)	17 ± 8	15 ± 7	19 ± 7	16 ± 9	17 ± 5	20 ± 7
Maltose (g)	1.2 ± 0.8	1.3 ± 1.0	1.7 ± 0.7	1.8 ± 0.9	2.0 ± 1.2	2.0 ± 2.6
Sucrose (g)	18.5 ± 10.3	16.2 ± 6.9	23.5 ± 12.5	25.1 ± 13.1	18.5 ± 11.5	16.7 ± 8.3
Galactose (g)	1.1 ± 1.3	0.4 ± 0.6	1.2 ± 2.2	0.3 ± 0.4	0.8 ± 1.0	0.9 ± 1.7
Lactose (g)	12.7 ± 8.4	10.2 ± 5.4	15.5 ± 8.4	13.1 ± 6.7	10.7 ± 2.2	11.0 ± 5.3
Starch (g)	66 ± 35	72 ± 51	70 ± 31	79 ± 43	80 ± 29	87 ± 57
Total Sugars (g)	71.2 ± 38.4	65.6 ± 47.9	96.9 ± 47.9	75.8 ± 25.6	83.4 ± 35.4	86.7 ± 44.2
Non-starch Polysaccharides (g)	15.0 ± 3.0	15.2 ± 4.7	15.7 ± 6.0	15.6 ± 6.7	18.4 ± 5.1	15.8 ± 4.1
Total Fat (g)	64 ± 32	69 ± 31	66 ± 19	67 ± 23	75 ± 27	67 ± 23
Saturated Fatty Acids (g)	21 ± 22	20 ± 13	23 ± 19	23 ± 10	26 ± 8	21 ± 17
Mono-Unsaturated Fatty Acids (g)	21 ± 17	20 ± 21	21 ± 14	20 ± 9	22 ± 12	20 ± 11
Poly-Unsaturated Fatty Acids (g)	8 ± 8	11 ± 7	8 ± 4	10 ± 8	13 ± 5	9 ± 3
Trans Fatty Acids (g)	0.5 ± 0.6	0.6 ± 0.4	0.6 ± 0.6	0.8 ± 0.5	0.7 ± 0.2	0.5 ± 1.0
Omega-3 Fatty Acids (g)	2.6 ± 2.5	1.6 ± 3.5	1.3 ± 1.4	1.9 ± 1.8	1.2 ± 1.3	0.8 ± 1.2
Omega-6 Fatty Acids (g)	5.8 ± 5.5	7.9 ± 10.4	5.6 ± 4.5	5.6 ± 4.6	7.9 ± 5.4	5.3 ± 3.5
Vitamin A (µg)	908 ± 812	989 ± 792	836 ± 429	1052 ± 1139	582 ± 139	754 ± 528
Vitamin B1 (mg)	1.2 ± 0.3	1.2 ± 0.9	1.5 ± 0.7	1.4 ± 0.8	1.3 ± 0.3	1.2 ± 0.7
Vitamin B2 (mg)	1.6 ± 0.9	1.6 ± 0.8	1.6 ± 0.4	1.5 ± 0.4	1.8 ± 0.7	1.4 ± 0.9
Vitamin B3 (mg)	13.3 ± 12.9	13.0 ± 5.1	14.0 ± 7.3	13.0 ± 15.3	17.1 ± 14.0	15.1 ± 6.3
Vitamin B6 (mg)	1.7 ± 0.4	1.4 ± 0.5	1.8 ± 0.9	1.3 ± 0.6	1.7 ± 0.8	1.7 ± 0.4
Vitamin B9 (µg)	243 ± 103	232 ± 73	267 ± 141	219 ± 108	206 ± 156	256 ± 82
Vitamin B12 (µg)	5.0 ± 3.0	4.4 ± 3.6	4.1 ± 2.8	4.7 ± 4.0	4.5 ± 2.5	5.0 ± 4.3
Vitamin C (mg)	101 ± 49	95 ± 61	116 ± 54	100 ± 55	117 ± 51	139 ± 69
Vitamin D (µg)	4.9 ± 4.2	3.8 ± 5.3	3.6 ± 4.3	3.6 ± 4.1	3.1 ± 2.0	4.2 ± 3.8
Vitamin E (mg)	7.4 ± 4.1	7.7 ± 5.8	7.3 ± 7.1	6.7 ± 4.2	10.7 ± 4.2	7.4 ± 3.9
Calcium (mg)	727 ± 295	702 ± 251	867 ± 350	882 ± 466	817 ± 194	724 ± 249
Chloride (mg)	2400 ± 1233	3003 ± 2506	2799 ± 1294	2472 ± 873	2646 ± 852	3105 ± 1109
Copper (mg)	1.2 ± 0.5	1.4 ± 1.0	1.4 ± 0.6	1.1 ± 0.3	1.3 ± 0.5	1.5 ± 0.5
Iodine (µg)	183 ± 168	149 ± 98	154 ± 67	137 ± 47	138 ± 70	186 ± 89
Iron (mg)	8.9 ± 2.5	9.4 ± 5.7	9.9 ± 3.3	9.1 ± 3.6	16.6 ± 17.5	10.3 ± 1.8
Magnesium (mg)	297 ± 93	285 ± 120	325 ± 102	280 ± 60	307 ± 82	306 ± 75
Manganese (mg)	3.3 ± 0.8	3.8 ± 2.5	4.3 ± 2.7	3.6 ± 1.2	4.0 ± 0.8	4.0 ± 1.4
Phosphorous (mg)	1159 ± 332	1084 ± 337	1285 ± 367	1055 ± 374	1234 ± 182	1283 ± 208
Potassium (mg)	2882 ± 544	2551 ± 724	3250 ± 786	2798 ± 588	2819 ± 644	3000 ± 455
Selenium (µg)	55.8 ± 22.9	51.7 ± 48.8	45.8 ± 21.1	43.8 ± 22.3	53.3 ± 20.3	58.2 ± 22.8
Sodium (mg)	1459 ± 804	1775 ± 1518	1833 ± 1050	1447 ± 536	1672 ± 679	1914 ± 720
Zinc (mg)	6.7 ± 4.8	7.9 ± 3.9	7.3 ± 4.8	6.9 ± 3.6	7.9 ± 2.1	8.5 ± 2.8
Alcohol (g)	0 ± 11	0 ± 8	4 ± 10	4 ± 24	7 ± 9	16 ± 18

Boldened font represents a significant baseline difference. * represents a significant change over time; × represents a significant group× time interaction effect.

3.2. Habitual Dietary Intake

Notably, 89% of participants consumed protein at or above the recommended level at baseline. Promisingly, 29%, 40%, and 100% of participants consumed below the recommended maximum daily intake of saturated, total, and trans-fats, respectively. Furthermore, ≥94% of participants at baseline consumed at or above the recommended daily intake of vitamins C and E, as well as phosphorous. Recommended daily consumption of omega-3, calcium, zinc, and magnesium, was present in ≤60% of participants at baseline. Moreover, 100% of participants consumed at or above the recommended intake of vitamin B-12, and 86% consumed at or below the recommended intake of sodium. However, only ≤17% of participants consumed at or above the recommended daily levels of potassium, omega-6, and vitamin D (Table 3). Participants consumed ~3 portions of fruit, and ~2 portions of vegetables (Table 2) per day on average. Accordingly, 13 (37%) participants routinely consumed nutritional supplements (SBF: $n = 4$, LIPA: $n = 5$, Control: $n = 4$), however there was no significant difference between groups at baseline regarding the amount of supplements consumed ($p = 0.65$) (Table 1).

Table 3. Habitual dietary outcomes expressed relative to recommended daily amounts (RDA).

	Recommended Daily Amount (RDA)	Whole Sample at Baseline (n = 35)	SBF (n = 14)		LIPA (n = 14)		CONTROL (n = 7)	
			Group Average Expressed as %RDA					
		Proportion Meeting RDA	Pre	Post	Pre	Post	Pre	Post
Protein (g/kg)	≥0.8 g/kg/day	31/35	125%	123%	136%	133%	161%	150%
Carbohydrate (g)	Within 45–65% Daily caloric intake	10/35	93%	87%	102%	96%	93%	105%
Total Fat (g)	≤35% Daily caloric intake	14/35	126%	121%	132%	126%	106%	111%
Saturated Fatty Acids (g)	<11% of Daily caloric intake	10/35	125%	111%	122%	116%	117%	111%
Trans Fatty Acids (g)	<2% of Daily caloric intake	35/35	16%	16%	17%	22%	17%	14%
Mono-Unsaturated Fatty Acids (g)	≥28 g/day	10/35	75%	71%	75%	71%	79%	71%
Poly-Unsaturated Fatty Acids (g)	≥14 g/day	4/35	57%	79%	57%	71%	93%	64%
Omega-3 Fatty Acids (g)	≥1.6 g/day	12/35	163%	100%	81%	119%	75%	50%
Omega-6 Fatty Acids (g)	≥10 g/day	4/35	58%	79%	56%	56%	79%	53%
Vitamin A (µg)	≥600 µg/day	23/35	151%	165%	139%	175%	95%	126%
Vitamin B1 (mg)	≥0.8 mg/day	33/35	156%	156%	188%	175%	163%	156%
Vitamin B2 (mg)	≥1.1 mg/day	32/35	145%	145%	145%	136%	164%	127%
Vitamin B3 (mg)	≥12.6 mg/day	23/35	106%	103%	111%	103%	136%	126%
Vitamin B6 (mg)	≥1.2 mg/day	31/35	142%	117%	150%	108%	142%	142%
Vitamin B9 (µg)	≥200 µg/day	25/35	122%	116%	134%	116%	103%	128%
Vitamin B12 (µg)	≥1.5 µg/day	35/35	333%	293%	273%	313%	300%	333%
Vitamin C (mg)	≥40 mg/day	33/35	253%	236%	290%	250%	293%	346%
Vitamin D (µg)	≥10 µg/day	3/35	49%	36%	36%	36%	21%	42%
Vitamin E (mg)	≥3 mg/day	35/35	247%	257%	243%	223%	157%	247%
Calcium (mg)	≥700 mg/day	19/35	104%	100%	124%	126%	117%	103%
Chloride (mg)	≥2500 mg/day	15/35	96%	121%	110%	99%	106%	124%
Iodine (ug)	≥140 µg/day	14/35	131%	106%	110%	98%	99%	133%
Iron (mg)	≥8.7 mg/day	20/35	102%	108%	134%	105%	191%	118%
Magnesium (mg)	≥270 mg/day	21/35	110%	106%	120%	104%	114%	113%
Phosphorous (mg)	≥550 mg/day	34/35	211%	197%	234%	192%	224%	233%
Potassium (mg)	≥3500 mg/day	3/35	82%	73%	93%	80%	81%	86%
Selenium (µg)	≥60 µg/day	7/35	93%	86%	76%	73%	89%	97%
Sodium (mg)	<2.4 g/day	30/35	61%	74%	76%	60%	70%	80%
Zinc (mg)	≥7 mg/day	18/35	96%	113%	104%	99%	113%	121%

3.3. Physical Behaviour Profile

There were no group differences at baseline in the proportion of participants classified as either sedentary ($p = 0.09$) or physically active ($p = 0.10$). Thus, 91% were sedentary, and 86% physically inactive at baseline. Absolute sedentary behaviour time exhibited a significant reduction over time ($p = 0.02$, -0.5 ± 1.2 h, $-3 \pm 19\%$), but no group×time interaction ($p = 0.58$). Furthermore, at week 8, 23% of participants positively shifted classification from sedentary to ambulatory (SBF: $n = 3$, LIPA: $n = 3$, control: $n = 2$), with the remaining 77% remaining unchanged in terms of classification over time (please see Table 1). Importantly, no participants shifted classification from ambulatory to sedentary post intervention. No significant effects were observed for percentage of 24 h time spent in sedentary behaviour or LIPA, nor percentage of PA time spent in LIPA ($p > 0.05$). In contrast, in 83% of participants, the physically active classification remained unchanged, 11% of participants negatively shifted classification from active to inactive, and only 6% of participants positively shifted from inactive to active.

3.4. Carbohydrate Intake as a Factor of Intervention

After accounting for baseline values as a co-variate within the analysis, we observed a significant main of effect of time for carbohydrate intake ($p = 0.001$), but not a group×time interaction ($p = 0.36$). Furthermore, we observed a significant group×time interaction effect for glucose intake ($p = 0.03$), but not a main effect for time ($p = 0.48$). Post-hoc testing revealed a significant difference exclusively between the change in SBF and the change in control ($p = 0.01$) (Please see Figure 1A). Whilst no post-hoc effect was observed between SBF and LIPA ($p = 0.37$), a trend was observed for the difference between the change in LIPA and the change in control ($p = 0.054$). In short, the group-dependant change in glucose intake was primarily driven through the decreases in the experimental groups [SBF: -2.8 ± 6.7 g ($-31 \pm 72\%$), LIPA: -1.6 ± 4.9 g ($-13 \pm 351\%$)], and the increase in the control group (5.5 ± 5.1 g ($42 \pm 72\%$)) (please see Table 2). Similarly, we observed a trend towards a group×time interaction effect for fructose intake ($p = 0.07$), but not a main effect for time ($p = 0.61$). Accordingly,

SBF [−2.1 ± 7.3(0 ± 66%)], and LIPA [−2.9 ± 4.8(−17 ± 27%)] exhibited decreases, in contrast to the control group whose fructose intake increased [3.4 ± 4.3(21 ± 29%)].

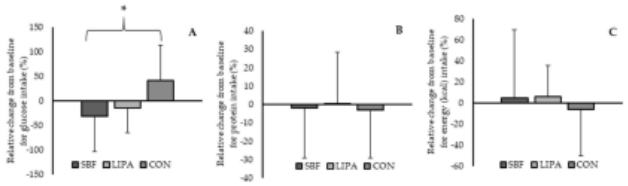

Figure 1. Group-dependent relative changes from baseline for three key nutrients. Panels A, B, and C represent changes in glucose, protein, and energy intake (kcal) respectively. Note: Regarding panel (**A**), glucose exhibited a group×time interaction effect for glucose intake ($p = 0.03$), but not a main effect for time ($p = 0.48$). Thus, * represents the significant post-hoc difference between the decrease in glucose for SBF, and the increase for control (CON) ($p = 0.01$). Regarding panel (**B**), daily protein intake exhibited a main effect of time ($p = 0.004$), but not a group×time interaction ($p = 0.59$). Regarding panel (**C**), no significant main effect of time nor time×group interaction was observed for energy intake ($p \geq 0.05$).

3.5. Protein Intake as a Factor of Intervention

After accounting for baseline values as a co-variate, we observed a significant main of effect of time for daily protein intake ($p = 0.004$), but not a group×time interaction ($p = 0.59$). Accordingly, average daily protein intake decreased from pre to post by 2.6 ± 18.2 g (−1 ± 26%) (Please see Figure 1B). However, within the sub-analysis of participants who positively shifted from sedentary to ambulatory, trends were observed for increased intake of absolute (8.7 ± 12.3 g, 12 ± 19%, $p = 0.09$), and relative (0.2 ± 0.2 g.kg, 13 ± 19%, $p = 0.08$) protein intake.

3.6. Energy Balance as a Factor of Intervention

We observed no significant main effect of time nor time×group interaction for energy intake ($p \geq 0.05$), even after accounting for body mass metrics (mass, BMI, etc) (Please see Figure 1C). Accordingly, given stable anthropometrics over time, it is unsurprising that calculated BMR calculated with the Harris–Benedict formula did not significantly change over time ($p = 0.34$), nor exhibit a time×group interaction ($p = 0.67$). In addition, no main effect for time ($p = 0.58$), nor time×group interaction ($p = 0.53$) was observed when BMR was calculated with the Schofield equation. We observed a significant increase over time for total daily energy expenditure (TDEE) (47 ± 88 kcal, 3 ± 6%, $p = 0.006$), but not a group×time interaction ($p = 0.97$), when calculated with the Harris–Benedict formula. A significant increase over time for TDEE (5 ± 37 kcal, 0.3 ± 2%, $p = 0.03$), but not a group×time interaction ($p = 0.98$), was also observed when TDEE was calculated with the Schofield equation. However, no main effects were observed for energy balance, when calculated with either the Harris–Benedict equation (Time: $p = 0.64$, group×time: $p = 0.99$), or the Schofield equation (time: $p = 0.51$, group × time: $p = 0.054$) (please see Table 1).

3.7. Micronutrient Intake as a Factor of Intervention

Vitamin B12 exhibited a trend toward a group-dependant change over time ($p = 0.09$), with control displaying the greatest increase (1.9 ± 2.3 µg, (45 ± 45%)), followed by LIPA (0.9 ± 2.1 µg, (33 ± 73%)) and SBF (−6.4 ± 21.9 µg, (−7 ± 67%)), which decreased on average. A similar pattern was noted when comparing the group averages to recommended B12 levels (Table 3), despite all groups far exceeding recommended daily intakes. We also observed a trend toward a decrease over time for vitamin B3 (Niacin) (−1.2 ± 6.4 mg, −1 ± 47% $p = 0.09$), and unsurprisingly no group×time interaction

($p = 0.76$). A similar pattern was noted when comparing the group averages to recommended B3 levels (Tables 2 and 3). However, no significant main effect for time ($p = 0.82$), or group×time interaction ($p = 0.96$) was observed for portions of fruit consumed. Similarly, no significant main effect for time ($p = 0.12$), or group×time interaction ($p = 0.92$) was observed for portions of vegetables consumed. Finally, no significant main effect for time ($p = 0.18$), or group×time interaction ($p = 0.21$) was observed for habitual daily consumption of nutritional supplements. Following the sub-analysis regarding those who positively shifted from sedentary to ambulatory ($n = 8$), a significant increase in zinc intake was observed (1.7 ± 3.8 mg, 29 ± 63%, $p = 0.05$), as well as a trend towards increased manganese intake (1.4 ± 2.4 mg, 32 ± 48%, $p = 0.09$).

No other nutrient factor on its own showed any group, time, or interaction effect. Thus, the subsequent analysis grouped factors by their physiologic impact of the musculoskeletal system, and changes with intervention and differences by physical behaviour classification. This approach used radar graphs based on computed z-scores.

RDA criteria for each nutrient is expressed in the second column. The number of participants who meet each criterion at baseline is expressed in the third column. Each subsequent column represents each group average for pre and post, expressed relative to the RDA. Four pro-anabolic nutrients remained stable around recommended levels in response to each intervention, omega-3 fatty acids exhibit the greatest adaptability. Specifically, average omega-3 intake for SBF and control remained within and outside recommended intakes post intervention respectively, despite both groups exhibiting marked decreases. Interestingly, average omega-3 intake started below recommended levels for LIPA, but increased to above recommended levels post intervention. Average omega-6 intake remained at sub-optimal levels for all groups post intervention, whereas average intake for SBF increased closer to recommended levels, LIPA remained similar, and control tended to decrease further away from recommended levels. Despite a reduction in vitamin E intake for control, average post-intervention intake was still twice the recommended amount. Unsurprisingly, all groups remained under the recommended levels of vitamin D intake post intervention. Despite average intake of phosphorous remaining at twice the recommended levels for SBF and LIPA, both groups exhibited decreases in contrast to control post intervention. Furthermore, average intake of zinc increased to above the recommended levels post intervention for SBF, whereas average intake of zinc for LIPA decreased to below recommended levels, post intervention. Average intake of magnesium, calcium, and vitamin C remained within the recommended levels for all groups pre and post intervention.

3.8. Dietary Components Promoting Anabolism, as a Factor of the Two Interventions

There were no differences between groups at baseline ($p = 0.88$) regarding the amount of nutrients promoting anabolism each participant consumed at optimal levels (One = 9%, Two = 57%, Three = 14%, Four = 17%, Five = 3%) (Table 1). Unit-weighted composite z-score analysis (Figure 2) shows that both SBF (composite z-score—pre: 0.28, post: 0.65), and LIPA (composite z-score—pre: −0.73, post: −0.62) increased intake of nutrients, promoting anabolism from pre to post by 13% and 4% respectively. Control on the other hand decreased intake of nutrients, promoting anabolism (composite z-scores—pre: 0.91, post: −0.06) by ~34%.

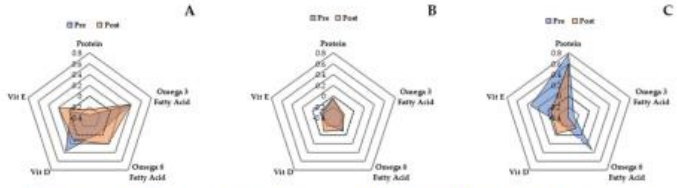

Figure 2. Radar graphs representing z-scores for five nutrients promoting anabolism at baseline and post intervention. Panels (**A**–**C**) represent SBF, LIPA, and control respectively.

3.9. Dietary Components Promoting Bone Health as a Factor of the Two Interventions

There were no differences between groups at baseline ($p = 0.78$) regarding the amount of bone health-enhancing nutrients each participant consumed at optimal levels (One= 3%, Two = 14%, Three = 20%, Four = 17%, Five = 23%, Six = 11%, Seven = 9%, Eight = 3%) (please see Table 1). Unit-weighted composite z-score analysis shows that SBF (Figure 3; composite s-score–pre: −0.66, post: −0.18) and control (composite s-scores—pre: 0.48, post: 1.26) increased intake of nutrients, promoting bone health from pre to post by 17% and 21% respectively, whereas LIPA (composite s-scores–pre: 0.42, post: −0.45) decreased their intake of nutrients, promoting bone health by ~34%.

Figure 3. Radar graphs representing z-scores for eight nutrients promoting bone health at baseline and post intervention. Panels (**A**–**C**) represent SBF, LIPA, and control respectively.

3.10. Effect of Physical Behaviour Classification Change on Habitual Dietary Outcomes

Pre intervention, 91% and 9% of participants were classified as sedentary and ambulatory, respectively. Post intervention, 69% and 31% of participants were classified as sedentary and ambulatory respectively. Following the sub-analysis regarding those who positively shifted from a sedentary to an ambulatory physical behaviour classification ($n = 8$), the overall nutrition z-score radar graph highlighted the combined directional unit-weighted scores changed from 5.25 at baseline to 9.27 post intervention. Specifically, such participants also increased their intake of nutrients promoting anabolism (combined weighted unit scores—pre: 1.01, post: 3.33), and nutrients promoting bone health (combined weighted unit scores—pre: 2.08, post: 3.72), by 2%, and 16% respectively (please see Figure 4).

Figure 4. Radar Graphs representing z-scores at baseline and post-intervention for participants who shifted their physical behaviour classification from 'Sedentary' to 'Ambulator'.

4. Discussion

The aim of this study was to examine and identify any compensatory dietary behaviours that accompany sedentary behaviour displacement. We hypothesized that sedentary behaviour displacement in older adult females would be accompanied by a spontaneous reduction in energy intake (thus managing energy balance more effectively), as well as a relative improvement in dietary quality (improvements in macro (increased protein intake etc.)/micro-nutrient profile). Despite not observing any change in energy intake ($p \geq 0.05$), we noted a significant reduction in daily protein intake, after accounting for baseline differences ($p = 0.004$). Following similar adjustment for baseline differences, carbohydrate exhibited a significant change over time ($p = 0.004$), driven by a significant group-dependant change in glucose intake ($p = 0.03$). Furthermore, z-score analysis for the entire dietary profile shows that both SBF and LIPA increased the intake of nutrients promoting anabolism in contrast to control. However, LIPA decreased their intake of nutrients promoting bone health in contrast to both SBF and control who increased theirs. Therefore, our hypothesis was partially upheld. In terms of individual nutrient changes as a factor of the intervention, only 2/45 nutrients examined showed a time effect and 1/45 nutrients exhibited a time×group interaction.

We observed a group-dependant change in glucose intake. Such an effect was mediated by the difference between the exclusive reduction in glucose in SBF, and an increase in controls, but not LIPA. This implies an apparent advantage of frequent LIPA vs. continuous. Given that we observed no significant change in fruit/vegetable intake, this suggests the reduction in glucose following SBF was from other dietary sources. Such a promising finding is supported by spontaneous reduced intake of sweets, soft drinks, breads, and pasta dishes following 15 weeks of moderate intensity exercise training in younger adults [63]. Our results further suggest that such an improvement also occurs in older adults, following a much lower intensity/volume of PA implementation, and independent of concurrent nutritional counselling. Certainly, reduced glucose intake is promising in the context of metabolic morbidity, given that a higher intake of free sugars is associated with increased incidence of type II diabetes [64,65]. Combined with the inherent physical benefits of sedentary behaviour displacement [36], such a finding also has promising implications for long-term glucose management.

Our results indicate a significant reduction in absolute protein intake in all groups. Such a finding is of particular concern, given that older adults typically present with protein-energy malnutrition habitually [17,20], which compromises bone mineral density [43], skeletal muscle mass [66], physical function [67], and the quality of skeletal muscle [29]. Daily protein intake in older adults is minimally recommended in the range of 0.8–1.0 g.kg.day [68,69], but is encouraged at even higher intakes (1.2–1.6 g.kg.day) to gain the full benefits [66,70–72]. Therefore, the observed decrease in absolute protein intake must be placed into context, as all groups remained ≥0.98 g.kg.day post intervention, and were thus still comfortably within the minimal daily intake range.

We failed to observe any significant change in energy intake. Accordingly, "the gravitostat" exclusively mediates reduced energy intake following sustained postural transition in rodents [34,35]. Furthermore sedentary behaviour displacement reduces subsequent energy intake in younger adults [36]. However, we failed to observe any significant reduction in energy intake. Loading of the gravitostat has previously been performed through utilising weighted vests for three weeks in humans [37]. In contrast, the current study will have loaded the gravitostat only with bodyweight whenever sitting was replaced with standing/light activity over eight weeks. In rodents, the energy intake reducing effect of the "gravitostat" appears to be dependent on an osteocyte strain detection mechanism, which is activated in response to high loading through the lower limbs [34,35]. However, the current lack of observed change in energy intake persisted even after adjustment for baseline BMI. Given that all groups were on average classified as non-obese at baseline (≤30 kg/m^2), sedentary behaviour displacement with LIPA in older individuals may simply have not produced high enough loading forces through the lower body bone structures sufficient to activate the gravitostat.

Our in-depth composite z-score analysis showed that both SBF and LIPA increased overall intake of nutrients promoting anabolism, in contrast to control. This is a very promising finding considering intake of all five selected nutrients has previously been individually (protein [44], vitamin D [45,46], vitamin E [47], as well as omega-3 and omega-6 fatty acids [48,49]), and collectively [29] positively associated with the observed quality of skeletal muscle in older adults, including higher muscle volume and greater specific force. Given that both experimental groups similarly increased, this suggests that such an enhancement occurs irrespective of the pattern of prescribed LIPA. Together with the effect that LIPA has on stimulating skeletal muscle in older adults [73], secondary enhancements to dietary pro-anabolic potential may aid with perturbing the loss of skeletal muscle mass/function during aging (sarcopenia) [29].

Our in-depth composite z-score analysis showed that only SBF increased overall intake of bone health enhancing nutrients, in contrast to LIPA, which decreased intake of such nutrients. Similar to reduced glucose intake, this suggests an advantage of frequent sedentary behaviour displacement with LIPA. This is promising considering intake of all eight selected nutrients has previously been individually (calcium [50], zinc [43], magnesium [51], phosphorus [52], vitamin C [43], vitamin D [53], protein [54], omega-3 fatty acids [55]), and collectively [43] associated with bone health in older adults. Furthermore, a more fragmented sedentary behaviour pattern is specifically associated with enhanced BMD in older adults, due to frequent exposure of bone structures to mechanical loading [74].

We further hypothesized that shifting towards being classified as ambulatory post intervention would also be associated with enhanced dietary quality. Within the sub-analysis of novel ambulators (n = 8), several dietary trends conducive to optimal health emerged. Zinc intake significantly increased from pre to post by 29 ± 63%, which is promising considering zinc deficiency is common amongst older adults [24], and can not only exacerbate the loss of bone mineral density [75]/muscle mass [76], but can also increase CVD risk [77]. Furthermore, new ambulators exhibited a trend toward increased manganese intake. Accordingly, increased serum manganese levels have previously been associated with bone health in older adults [78,79]. Further trends were also noted for increased absolute (~12%) and relative (~13%) protein intake for new ambulators. Accordingly, z-score analysis of the overall diet showed novel ambulators increased both intake of nutrients promoting anabolism (2%), and nutrients

promoting bone health (16%). Such changes suggest shifting category from sedentary to ambulatory may aid with maintaining musculoskeletal health during ageing.

The major strength of the current study was investigating novel changes in habitual diet in response to sedentary behaviour displacement in older adults. In contrast to previous studies, we used weighted food diaries and rigorous nutritional analysis software (Nutritics/MyFitnessPal) to identify changes in specific macro and micronutrients. Furthermore, we specifically designed a randomised controlled trial to detect differences in outcomes concerning the pattern of prescribed LIPA during sedentary behaviour displacement. However, a potential limitation may have been the timing of the experimental phases during the summer or winter season for the different sub-groups, which may have been the cause for the baseline differences in two key habitual dietary outcomes (protein and carbohydrate intake). Accordingly, 100% of control participants began their intervention in months conventionally associated with spring, in contrast to 36% of experimental participants. A recent meta-analysis concluded adults (irrespective of age) exhibit seasonal variations in energy, macro, and micronutrient intake [80], with our results further suggesting that protein and carbohydrate intake exhibits similar seasonal variation in older adults. Whilst controlling for the baseline values of such variables as co-variates during analysis is a straightforward statistical solution, such baseline differences would ideally not be present where possible. Despite the fact that only 2/35 (6%, SBF: $n = 1$, control: $n = 1$) participants negatively shifted from active to inactive, both participants began their intervention in months conventionally associated with spring. Conversely, previous evidence suggests MVPA time declines throughout the winter months and peaks in summer in both middle aged [81], and older [82] adults. This suggests that the negative shift towards inactive classification was independent of season. Nevertheless, future studies should be carried out to confirm or otherwise refute our conclusion of no-seasonal independence. Furthermore, as an additional limitation of the study, it is noted that the control group ($n = 7$) was half the size of both the experimental groups (SBF: $n = 14$, LIPA: $n = 14$), which may have contributed to greater z-score effects for nutrients promoting anabolism/bone health within the control group. Whilst this led us to conduct a more in-depth and ultimately more informative z-score sub-analysis on new ambulators, consistency between group sample sizes would also ideally not be present where possible. Despite the fact we used two separate validated methods of basal metabolic rate estimation (Schofield and Harris–Benedict) [57,60], direct assessment of basal metabolic rate with calorimetry would have been more informative. Accordingly, robust assessments of body composition (lean body mass etc.) are recommended in future studies looking to accurately quantify basal metabolic rate (e.g., Katch-Mcardle) [83].

5. Conclusions

In conclusion, our results suggest that frequent sedentary behaviour displacement with LIPA can spontaneously reduce habitual glucose intake and can exclusively increase intake of bone health promoting nutrients. In addition, LIPA implementation (irrespective of prescribed pattern) spontaneously causes an increased intake of nutrients promoting anabolism. Furthermore, those participants who positively shift classification from sedentary to ambulatory also significantly increased zinc intake, as well as increased intake of other high-quality nutrients. Displacing sedentary behaviour with LIPA in older females results in enhanced nutritional quality, and as such can be viewed as a comprehensive means of lifestyle improvement beyond just a mere increase in physical activity. Future studies should further investigate any link between physical behaviour profile, habitual nutrition, and health outcomes including those in frail older adults.

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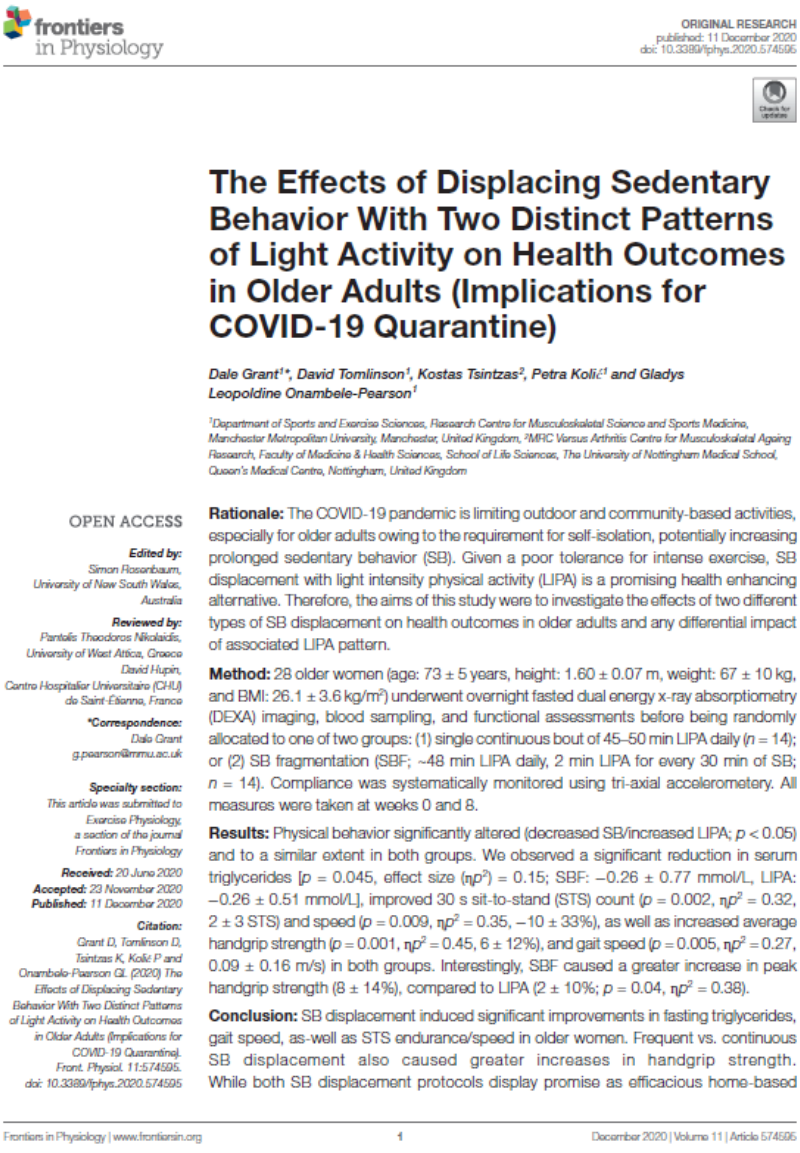

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The Effects of Displacing Sedentary Behavior With Two Distinct Patterns of Light Activity on Health Outcomes in Older Adults (Implications for COVID-19 Quarantine)

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Rationale: The COVID-19 pandemic is limiting outdoor and community-based activities, especially for older adults owing to the requirement for self-isolation, potentially increasing prolonged sedentary behavior (SB). Given a poor tolerance for intense exercise, SB displacement with light intensity physical activity (LIPA) is a promising health enhancing alternative. Therefore, the aims of this study were to investigate the effects of two different types of SB displacement on health outcomes in older adults and any differential impact of associated LIPA pattern.

Method: 28 older women (age: 73 ± 5 years, height: 1.60 ± 0.07 m, weight: 67 ± 10 kg, and BMI: 26.1 ± 3.6 kg/m^2) underwent overnight fasted dual energy x-ray absorptiometry (DEXA) imaging, blood sampling, and functional assessments before being randomly allocated to one of two groups: (1) single continuous bout of 45–50 min LIPA daily (n = 14); or (2) SB fragmentation (SBF; ~48 min LIPA daily, 2 min LIPA for every 30 min of SB; n = 14). Compliance was systematically monitored using tri-axial accelerometery. All measures were taken at weeks 0 and 8.

Results: Physical behavior significantly altered (decreased SB/increased LIPA; $p < 0.05$) and to a similar extent in both groups. We observed a significant reduction in serum triglycerides [p = 0.045, effect size (ηp^2) = 0.15; SBF: −0.26 ± 0.77 mmol/L, LIPA: −0.26 ± 0.51 mmol/L], improved 30 s sit-to-stand (STS) count (p = 0.002, ηp^2 = 0.32, 2 ± 3 STS) and speed (p = 0.009, ηp^2 = 0.35, −10 ± 33%), as well as increased average handgrip strength (p = 0.001, ηp^2 = 0.45, 6 ± 12%), and gait speed (p = 0.005, ηp^2 = 0.27, 0.09 ± 0.16 m/s) in both groups. Interestingly, SBF caused a greater increase in peak handgrip strength (8 ± 14%), compared to LIPA (2 ± 10%; p = 0.04, ηp^2 = 0.38).

Conclusion: SB displacement induced significant improvements in fasting triglycerides, gait speed, as-well as STS endurance/speed in older women. Frequent vs. continuous SB displacement also caused greater increases in handgrip strength. While both SB displacement protocols display promise as efficacious home-based

interventions for self-isolating older adults, our results would suggest a physical functioning advantage of the SBF protocol for certain outcomes.

Keywords: COVID-19, physical functioning, sedentary behaviour, sit-to-stand, triglyceride, light intensity physical activity

INTRODUCTION

The rapid spread of Coronavirus disease 2019 (COVID-19) has prompted many nationwide lockdowns (Lu et al., 2020; Sohrabi et al., 2020). In most cases, it is understood that patients requiring intensive care, are more likely to be older (Wang et al., 2020), prompting the call for all older adults (herein defined as ≥65y), to shield themselves, by proceeding to immediately begin prolonged and strict self-isolation (Armitage and Nellums, 2020). Habitually, older adults spend ~65–80% of their waking hours performing sedentary behavior (SB; Wullems et al., 2016; Loyen et al., 2017). SB is associated with sarcopenic obesity (Henson et al., 2018; Reid et al., 2018), reduced bone mineral density (BMD; Onambele-Pearson et al., 2019), heightened cardio-metabolic risk profile (Biswas et al., 2015; Hadgraft et al., 2020), frailty (da Silva et al., 2019), and premature mortality (Ekelund et al., 2019), in older adults. Furthermore, women tend to exhibit greater anabolic resistance and larger reductions in strength following disuse compared to men (Smith et al., 2008, 2012). Self-isolation is likely to exacerbate SB, given that habitual SB is primarily accumulated at home, during social isolation (Leask et al., 2015; Dontje et al., 2018). Despite acknowledgement of their limited efficacy/palatability (Chen et al., 2020; Jiménez-Pavón et al., 2020; Lippi et al., 2020), the default solution is simply recommending that older adults engage in moderate to vigorous physical activity [structured exercise (moderate to vigorous physical activity, MVPA)] with no clear directives *vis-a-vis* breaking up sitting time. However, many barriers inhibit long-term adherence to conventional MVPA recommendations (≥150 min/week, ~21 min/day; World Health Organization, 2010) in older adults (Hansen et al., 2019), including a lack confidence (Forkan et al., 2006) and appropriate equipment (Rhodes et al., 1999; Forkan et al., 2006; Bell et al., 2007). Given such barriers, older adults report a poor tolerance for intense physical activity, including greater perceived difficulty, and greater dropout rate (Onambélé-Pearson et al., 2010; Brawner et al., 2016). This can be problematic in the long term as only supramaximal MVPA engagement (≥ 420 min/week, ~60 min/day), appears to offset the negative health effects of concurrent high SB time (Ekelund et al., 2016; Manas et al., 2019). Furthermore, given that sudden surges in exercise can compromise immune response (Siedlik et al., 2016; Nieman and Wentz, 2019), reduced protection from infections like COVID-19, is a further concern. Such limitations create scope for safer alternative home-based interventions to mitigate the potential for further compromised health during self-isolation.

Displacing or breaking up SB time is one such viable option. Promisingly, older adults perceive SB displacement as acceptable and easy to incorporate in their daily routine (Matson et al., 2018). Light intensity physical activity (LIPA) during SB displacement, is a pre-requisite for long-term health benefits (Dohrn et al., 2018; Chastin et al., 2019; Stamatakis et al., 2019), due to LIPA generating superior responses in both muscle activity (MA; Tikkanen et al., 2013; Lerma et al., 2016), and energy expenditure (Carter et al., 2015; Lerma et al., 2016; Saeidifard et al., 2018), compared to stationary standing. Acute reductions in both postprandial glucose (Bailey and Locke, 2015; Welch et al., 2019) and triglycerides (TGs; Miyashita et al., 2016; Kashiwabara et al., 2017), as-well as chronic functional improvement (Barone Gibbs et al., 2017; Harvey et al., 2018), following SB displacement further highlights its potential to enhance cardio-metabolic health and physical function in older adults. However, despite a clearly established link with SB (Hamer and Stamatakis, 2013; Aggio et al., 2016), many functional markers like handgrip strength (HGS), have yet to be investigated. Furthermore, previous studies have merely displaced SB in arbitrary fashion without controlling for the prescribed pattern of LIPA. SB tends to be accumulated in prolonged uninterrupted bouts (Schlaff et al., 2017), which are associated with worse health outcomes (Gennuso et al., 2013, 2016; Diaz et al., 2017), compared with a more fragmented pattern. Therefore, a longitudinal intervention trial is warranted to investigate the chronic effects of SB displacement on health in older adults, while elucidating what role the pattern (fragmentation vs. a single bout) of prescribed LIPA plays in benefiting anyone but especially self-isolated frail older adults such as during the COVID-19 pandemic.

Therefore, the aims of this study were to (1) compare the chronic effects of two distinct SB displacement interventions on commonly assessed markers of health in older adults and (2) examine the impact prescribed patterns of activity have on the aforementioned outcomes. Given the clearly established link between SB and poor health outcomes in older adults (da Silva et al., 2019; Ekelund et al., 2019; Hadgraft et al., 2020), especially when SB is accumulated in a prolonged pattern (Gennuso et al., 2013, 2016; Diaz et al., 2017), it was hypothesized that (1) SB displacement would have small yet positive effects on markers of health and physical functioning in older adults and (2) SB fragmentation (SBF) throughout the day would induce greater health benefits compared to a single continuous bout of LIPA.

MATERIALS AND METHODS

Participants and Experimental Design

Twenty-eight elderly women voluntarily participated in the study. Ethical approval was obtained [230118-ESS-DG-(2)], and written informed consent obtained prior to any procedures being performed, in line with the Declaration of Helsinki.

Participants were recruited from the local community (Cheshire East) through advertising (posters, speaking engagements, etc.) and from a research volunteer database (local participants). Prior to the general data protection regulation deadline on May 25, 2018, recruitment packages (which included "General Data Protection Regulation" opt in/out permission slips, health questionnaires, participant information sheets, informed consent forms, and a pre-paid return envelope), were sent to all contacts aged 65–85 years. Returned questionnaires were screened for potential eligibility. Exclusion criteria included recent history of lower limb disorders, or current chronic health conditions [e.g., cardiovascular disease (CVD), uncontrolled diabetes, active cancer, etc.], likely to affect their ability to safely and independently undertake a program of decreased SB. Estimation of required sample size to detect significant changes in the desired outcomes was based upon the fact that previous SB interventions in older adults that have observed improvements to physical function, utilized total sample sizes of ~25–38 (Rosenberg et al., 2015; Barone Gibbs et al., 2017; Harvey et al., 2018). The current achieved sample size of 28 older women, falls within this range. Participants underwent familiarization and, after 7 days, returned to the laboratories to undergo body composition analysis, blood sampling, and functional assessments. Participants were then randomly allocated in a 1:1 fashion to one of two groups: (1) SBF (n = 14) or (2) single bout LIPA (n = 14). All measures were taken at weeks 0 (baseline) and 8 (post intervention).

Body Composition

A dual energy x-ray absorptiometry (DEXA) scanner (Hologic Discovery: Vertec Scientific Ltd., United Kingdom) was used (whole body procedure, EF 8.4 lSv; Tomlinson et al., 2014), to ascertain BMD, lean body mass (LBM), and body fat percentage (BFP%) metrics.

Blood Sampling

A 20 ml blood sample was drawn using a 0.5 Inch 23 g BD Needle (Mistry Medical Supplies, England). Whole blood analyses of fasting plasma glucose, total cholesterol, and TGs were performed using an Accutrend Plus (Roche Diagnostics Limited, United Kingdom), while glycated hemoglobin (HbA1C%) was analyzed using a 501 device (HemoCue, Sweden). Accordingly, both Accutrend and Hemocue have shown good reliability (Luley et al., 2000; Newman and Turner, 2005; Phillips et al., 2014) and validity (Luley et al., 2000; Coquetro et al., 2014; Hirst et al., 2017), when compared to laboratory testing.

Physical Function Assessment: Gait Speed, Sit-to-Stand Ability, and Handgrip Strength

A modified pressure sensor (Tekescan, United States) and height adjustable stool were used to reduce testing variability (Demura and Yamada, 2007). Gait speed was assessed through the timed up and go test (TUG; Podsiadlo and Richardson, 1991). Participants rose from the chair and walked at maximum speed to a marker 6 m away before returning to the seated position. Gait speed was defined as the quickest speed recorded over three trials [meters per second (m/s)]. Participants were then instructed to rise from the chair until the knee was fully extended and then return to a seated position. This was performed once as quickly as possible in the case of the one sit-to-stand (1STS, functional speed), and as many times as the participant could perform in an exact 30 s time frame for the 30STS (30 s STSs, functional endurance). A handgrip dynamometer (Takei, Japan), was used to assess HGS. Dynamometry is both a reliable and valid measure of strength in the elderly (Bohannon and Schaubert, 2005; Abizanda et al., 2012). Briefly, participants were instructed to maximally squeeze the handle and discontinue grasping at self-perceived maximum voluntary effort. Three trials were performed on each hand, with peak HGS defined as the maximum value achieved across both hands, and the average of three trials used to provide an average of both arms. Importantly, gait speed (Studenski et al., 2011), STS ability (Cooper et al., 2010), and grip strength (Sasaki et al., 2007) are all significant predictors of mortality in older adults.

Physical Behavior Interventions

The purpose of the two intervention groups was to manipulate the protocol for displacing SB time with added daily LIPA (45–50 min in total). The interventions were confined to a 12-h period between 09:00 and 21:00. The prescribed amount of LIPA (45–50 min) was based upon two key points. First, the WHO's MVPA recommendation (World Health Organization, 2010) gives a theoretical starting point for what activity amount may be beneficial. Utilizing metabolic equivalent of task (MET) thresholds (SB: <1.5 METs, LIPA: 1.5–3.0 METs, MVPA: >3.0 METs), 150 min/week translates into ~21 min/day moderate activity (~64 MET·min/day), meaning the same amount of MET·min/day, performed in LIPA (with a minimum intensity of 1.6 METs), would theoretically total ~40 min/day. Furthermore, the SBF group was instructed to fragment sitting time every 30 min over a 12-h period (09:00-21:00), based on recent epidemiological evidence linking a more prolonged sedentary accumulation pattern (≥30 min bouts) with greater all-cause mortality (Diaz et al., 2017). Consequently, this totaled a maximum of 24 2-min LIPA bouts throughout the day (48 min). Envisaging a varied compliance response, the LIPA group was prescribed a range for their single continuous bout. Accordingly, the prescribed amount of LIPA (an additional 45–50 min per day), was equally matched between the two groups, whereas the prescribed pattern (intermittent micro-bouts vs. single continuous bout) was different. Both intervention groups were provided with an illustrated booklet, which contained LIPA suggestions compiled from the compendium of physical activities (Ainsworth et al., 2011). Importantly such activities were intentionally selected due to their simplicity, safety, and ease of implementation within the home environment.

Individual participant compliance was objectively monitored at weeks 0 and 8, using a thigh mounted GENEActiv original triaxial accelerometer (Activinsights Ltd., United Kingdom). Data were subsequently extracted using GENEA software, and a previously validated algorithm (Wullems et al., 2017) used

for baseline and post-intervention data analysis. Briefly, the aforementioned validation study calculated the incremental metabolic cost of 10 everyday tasks in 40 healthy older adults (~74 years; e.g., lying down, brisk treadmill walking, etc.), and used regression analysis to identify specific physical activity intensity ranges [utilizing MET thresholds (SB: <1.5 METs, LIPA: 1.5–3.0 METs, MVPA: >3.0 METs)] mapped against the concurrently recorded GENEActiv gravitational pull and acceleration data. The robustly derived data on SB, standing, LIPA, and MVPA in older adults were used for further analyses. Participants were also further classified as physically active (≥150 min/week $MVPA_{\geq 10\ min\ bouts}$), or non-physically active (<150 min/week $MVPA_{\geq 10\ min\ bouts}$), given that the World Health Organization (WHO) recommends a weekly MVPA engagement time of 150 min/week (World Health Organization, 2010).

SBF Group

Participants were told that the purpose of their intervention was to reduce the amount of time spent performing SB (sitting, lying, or reclining) especially in prolonged uninterrupted bouts. Participants were instructed not to perform SB for more than 30 min at a time, and that for every 30 min of SB performed the participant should stand up and perform 2 min of upright LIPA (general ambulatory walking, side to side shuffling, washing dishes, etc.).

LIPA Group

Participants were informed that the purpose of their intervention was to increase the amount of time spent performing LIPA while maintaining habitual routines. Participants were instructed to perform a continuous single bout of 45–50 min LIPA (general ambulatory walking, side to side shuffling, washing dishes, etc.), every day for the duration of the 8-week intervention.

Palatability Assessment

During the post-test visit, participants were asked to complete a palatability questionnaire. Each question was designed to rate an aspect of the participants experience and gain insight on perceived quality of life (QoL).

Statistical Analyses

Statistical analyses were carried out using SPSS (Version 26, SPSS Inc., Chicago, IL, United States). Normal distribution and equality of variances between groups were checked using the Shapiro-Wilk and Levene's tests, respectively. Baseline group differences were subsequently examined with an independent sample's T-test or Mann-Whitney U test (SBF vs. LIPA) as appropriate. The effects of the interventions were determined using 2 × 2 split plot ANOVA [two time phases (pre and post intervention) and two intervention groups]. In cases of non-normally distributed data, within group comparisons were made using the Wilcoxon-Sign Rank test, while, between group differences were analyzed through a Mann-Whitney U test on the relative changes from baseline. A Chi squared test was used to compare between group differences for ordinal/nominal data from the palatability questionnaire. Furthermore, Spearman bivariate correlations were utilized to investigate associations between the relative changes in LBM metrics and the relative changes in functional assessments. Data are reported as mean ± SD [or median ± interquartile range (IQR) for non-parametric data]. Statistical significance was accepted when $p \leq 0.05$. Furthermore, a statistical trend was deemed to be present when p was in the range of between 0.05 and 0.10. Effect size (η_p^2) was also reported, where p is significant.

RESULTS

Descriptive Characteristics of Participants at Baseline

The 28 older women (age: 73 ± 5 years, height: 1.60 ± 0.07 m, weight: 67 ± 10 kg, and BMI: 26.1 ± 3.6 kg.m²) were matched at baseline for all outcomes of interest ($p > 0.05$), denoting a well-matched study sample (see **Table 1**).

Intervention, Compliance, and Palatability

No differences existed between groups regarding the number of intervention days (days from pre-lab visit to post) that the participants undertook (SBF: 56 ± 2 days, LIPA:56 ± 1 days; $p = 0.37$). Regarding 3D-accelerometer-based compliance data, both groups were matched for all variables at baseline. SB significantly decreased over time in both groups ($p = 0.006$, $\eta_p^2 = 0.26$), but did not exhibit a group × time interaction ($p = 0.41$; **Figure 1**). Similarly, mean SB bout time significantly decreased over time in both groups ($p = 0.045$, $\eta_p^2 = 0.27$), but did not exhibit a group × time interaction ($p = 0.96$; **Table 2**). LIPA significantly increased over time in both groups ($p = 0.04$, $\eta_p^2 = 0.15$), but did not exhibit a group × time interaction ($p = 0.11$; **Figure 1**). Standing and MVPA time, however, did not significantly change (**Table 2**). Concerning intervention palatability, promisingly, all participants agreed the instructions were easy to follow at home, with 89% reporting

TABLE 1 | Participant characteristics at baseline.

Participants characteristics	SBF (n = 14)	LIPA (n = 14)
Age (y)	74 ± 5	73 ± 6
Weight (kg)	68.6 ± 11.3	65.5 ± 8.6
BMI (kg.m²)	26.9 ± 3.6	25.3 ± 3.6
Total lean body mass (LBM; kg)	39.3 ± 5.7	37.2 ± 3.9
Proportional T-score classification as osteoporotic/osteopenic (normal)	29% (71%)	43% (57%)
Proportion who live alone/ (cohabitate)	36% (64%)	43% (57%)
Polypharmacy (n)	2 ± 2	1 ± 1
FRAT (number of positive responses)	1 ± 1	1 ± 1

FRAT, falls risk assessment tool; LIPA, light intensity physical activity; and SBF, sedentary behavior fragmentation.

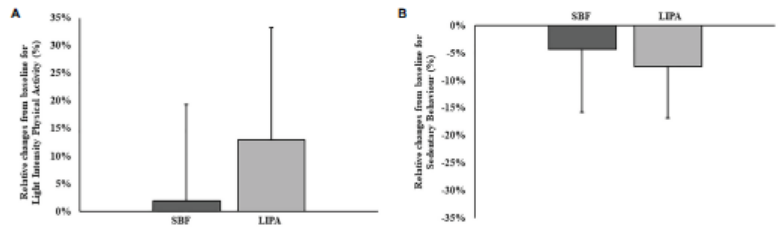

FIGURE 1 | Group dependent changes relative to baseline for physical behaviour outcomes. Panel **A** represents changes in light intensity physical activity (LIPA), while panel **B** represents changes in sedentary behaviour.

increased awareness of their daily sedentarism. Accordingly, 82% of participants reported feeling more positive about their health, and most importantly, 61% of participants stated they could definitely continue following this intervention long term. Furthermore, 54% of participants stated their intervention had motivated them to become more active. However, only 25% of participants stated they definitely felt more confident about performing household tasks following their respective interventions. There was no difference in self-reported satisfaction or continued adherence between groups ($p \geq 0.05$).

Bone Mineral Density

After accounting for previously identified co-variates [total body fat (TFAT), Android:Gynoid fat ratio (AGR), and BMI; Onambele-Pearson et al., 2019], thoracic ($p = 0.09$, $\eta_p^2 = 0.12$), but not lumbar spine mineral density ($p = 0.70$), exhibited a trend to change over time. Importantly, thoracic spine did not exhibit a significant group × time interaction effect ($p = 0.71$) with changes similar in both groups (SBF: 5 ± 14%, LIPA: 4 ± 9%).

Body Composition

Neither arm ($p = 0.73$), leg ($p = 0.17$), nor total ($p = 0.20$) LBM, significantly changed over time. Despite no change in BFP% ($p = 0.12$), we did observe trends for AGR ($p = 0.08$, $\eta_p^2 = 0.11$), and TFAT ($p = 0.10$, $\eta_p^2 = 0.004$), to change over time (**Table 2**). We also observed a significant reduction in hip circumference over time ($p = 0.02$, $\eta_p^2 = 0.19$), as-well as a trend ($p = 0.07$, $\eta_p^2 = 0.12$), toward a group × time interaction effect (**Table 2**) primarily driven through apparent greater reductions in SBF (−1.79 ± 2.34 cm), compared to LIPA (−0.25 ± 1.94 cm; **Figure 2**).

Cardio-Metabolic Biomarkers

We observed a significant main effect of time for fasting blood TG ($p = 0.045$, $\eta_p^2 = 0.15$), which was similar in the two groups, given no significant group × time interaction ($p = 0.98$; SBF: −0.26 ± 0.77 mmol/L, LIPA: −0.26 ± 0.51 mmol/L; **Figure 3**). No other cardio-metabolic serum biomarkers exhibited main effects for group, time, or group × time interactions.

Physical Function

A significant main effect for time was exhibited for gait speed ($p = 0.005$, $\eta_p^2 = 0.27$, 0.09 ± 0.16 m/s), but not a group× time interaction effect ($p = 0.44$). There was also a significant main effect of time for 30STS ($p = 0.002$, $\eta_p^2 = 0.32$, 2 ± 3 STS), 1STS ($p = 0.009$, $\eta_p^2 = 0.35$, −10 ± 33%; see **Figure 4**), and average HGS ($p = 0.001$, $\eta_p^2 = 0.45$, 6 ± 12%). Furthermore, peak HGS was the only functional outcome to exhibit both a significant main effect of time ($p = 0.044$, $\eta_p^2 = 0.27$), and a group × time interaction ($p = 0.04$, $\eta_p^2 = 0.38$), with a greater increase in SBF than LIPA (SBF: 8 ± 14% and LIPA: 2 ± 10%; **Figure 4**). Interestingly, the relative change from baseline in arm LBM, was significantly associated with the relative change from baseline in peak HGS ($R^2 = 0.17$ $p = 0.03$), accounting for ~17% of the explained variance when both groups were pooled. Furthermore, when sub-analyzed by group, such an association persisted in LIPA ($R^2 = 0.53$, $p = 0.004$) but not SBF, accounting for 53% of the explained variance.

DISCUSSION

This is the first study to investigate the chronic effects of SB displacement on health outcomes in older women and provide recommendations to mitigate any negative health consequences. We hypothesized that SB displacement would have measurable and positive effects on markers of health and physical functioning in older adults. We observed significant improvements over time for circulating TG, hip circumference, gait speed, 30STS, 1STS time, average HGS, and peak HGS, thereby upholding our first hypothesis. We further hypothesized that SBF would induce greater benefits compared to continuous LIPA. Here, we observed a trend for hip circumference ($p = 0.07$) and a significant effect for peak HGS ($p = 0.04$) to exhibit the predicted SBF advantage. Consequently, the second hypothesis was partially upheld.

TABLE 2 | Pre and post values for health outcomes.

		SBF (n = 14)		LIPA (n = 14)	
		Pre	Post	Pre	Post
LBM (dual energy x-ray absorptiometry, DEXA)	Arms (kg)	1.86 ± 0.32	1.85 ± 0.28	1.78 ± 0.21	1.76 ± 0.23
	Legs (kg)	6.18 ± 1.19	6.04 ± 0.95	5.89 ± 0.75	5.84 ± 0.68
Bone mineral density	Thoracic spine (g/cm^2)	0.90 ± 0.09	0.94 ± 0.13	0.90 ± 0.14	0.94 ± 0.15
	Lumbar spine (g/cm^2)	0.98 ± 0.16	0.96 ± 0.15	0.97 ± 0.16	0.98 ± 0.16
	Total (g/cm^2)	1.10 ± 0.11	1.09 ± 0.11	1.11 ± 0.15	1.11 ± 0.14
Adiposity indices	Total (kg)	26.1 ± 6.6	26.5 ± 6.3	25.1 ± 5.5	25.3 ± 5.8
	Android: Gynoid ratio	0.94 ± 0.15	0.93 ± 0.14	0.93 ± 0.18	0.90 ± 0.17
	Waist (cm)	92 ± 18	92 ± 24	91 ± 5	92 ± 8
	Hip (cm)	100 ± 7	99 ± 6*	100 ± 8	99 ± 8*
	WHR	0.95 ± 0.11	0.95 ± 0.18	0.91 ± 0.10	0.92 ± 0.10
	Body fat percentage (BFP, %)	39 ± 7	38 ± 5	38 ± 7	38 ± 7
Cardio-metabolic biomarkers	HBA1C (%)	5 ± 1	6 ± 0	6 ± 1	6 ± 1
	Glucose (mmol/L)	5.34 ± 0.98	5.01 ± 1.73	4.94 ± 0.86	4.73 ± 0.88
	Triglycerides (mmol/L)	2.19 ± 0.82	1.94 ± 0.50*	1.94 ± 0.52	1.68 ± 0.40*
	Total cholesterol (mmol/L)	5.53 ± 1.47	5.80 ± 1.86	5.33 ± 1.58	5.97 ± 1.25
Physical function	Peak HGS (kg)	26.3 ± 8.5	26.8 ± 6.1*^	26.5 ± 4.4	26.5 ± 7.3*^
	Average HGS (kg)	22.8 ± 6.6	23.9 ± 5.4*	22.9 ± 5.7	23.8 ± 7.2*
	30STS	14 ± 3	17 ± 3*	17 ± 3	18 ± 4*
	1STS (s)	2.49 ± 1.02	2.15 ± 0.70*	1.98 ± 0.52	1.86 ± 0.52*
	Max gait speed (m/s)	1.17 ± 0.22	1.26 ± 0.19*	1.22 ± 0.13	1.28 ± 0.11*
Daily physical behavior	SB time (hr/24 h)	9.6 ± 1.2	9.2 ± 1.6*	9.6 ± 1.1	8.9 ± 1.2*
	Standing time (hrs/24 h)	1.0 ± 0.6	1.0 ± 0.6	1.4 ± 1.1	1.5 ± 0.7
	LIPA time (hrs/24 h)	2.2 ± 0.5	2.2 ± 0.6*	2.1 ± 0.4	2.3 ± 0.5*
	MVPA time (hrs/24 h)	3.0 ± 1.0	2.8 ± 1.0	2.5 ± 0.8	2.8 ± 0.7
	Mean SB bout time (min)	31 ± 8	27 ± 9*	32 ± 14	29 ± 11*
	Proportion meeting recommended MVPA time [≥150 min/week MVPA (≥10 min bouts; World Health Organization, 2010)]/ below recommended MVPA	29%/71%	7%/93%	0%/100%	7%/93%

*Significant change from baseline.
^Significant group dependent effect.
HGS, handgrip strength; LIPA, light intensity physical activity; MVPA, moderate to vigorous physical activity; SB, sedentary behavior; SBF, sedentary behavior fragmentation; STS, sit-to-stands; and WHR, waist to hip ratio.

We observed significant functional improvements post-intervention. Firstly, increased muscular endurance (30STS) and enhanced gait speed are consistent findings across previous SB studies (Barone Gibbs et al., 2017; Harvey et al., 2018), potentially highlighting a specificity of training effect, improving one's ability to mobilize from a seated position. We also observed for the first time a decrease in the time taken to complete 1STS (an index of functional speed), further suggesting improved movement execution and enhanced muscular power. This positive effect is of notable impact given that inappropriate STS transitions are responsible for up to 41% of falls in care home residents (Rapp et al., 2012). Importantly, peak HGS improved to a greater extent in SBF compared to LIPA. Holding onto the arm of a chair and pushing through one's arms are common cues given to older adults when performing STS (Kindblom-Rising et al., 2010). Therefore, we propose that the increased STS frequency may have also increased the frequency with which the SBF participants utilized the arm stabilization tactic, subsequently, causing gradual functional adaptation in the upper body (including arm) musculature. Nevertheless, we advised all participants to implement many upright upper body tasks (sweeping up, etc.), and improvements in HGS have been reported following implementation of light upper body based movements (Nicholson et al., 1997; Anthony et al., 2013; Sexton and Taylor, 2019). Interestingly, arm LBM did not significantly change from pre to post, yet the relative change in arm LBM significantly accounted for 17–53% of the explained variance for the change in peak HGS. The greater association between muscle tissue content and peak HGS in the LIPA group may be linked to the fact that these participants were requested to perform various operational tasks in a continuous fashion, which appears to have caused a statistically insignificant yet clinically meaningful hypertrophic response leading to enhanced peak HGS. The observed improvements in lower body muscular endurance/power, in both intervention groups, as-well as HGS are compelling positive changes associated with an exercise intensity not customarily regarded as optimal.

We observed significant reductions in fasting circulating TG. Acutely interrupting sitting time with brief bouts of LIPA attenuates postprandial TG concentrations (Miyashita et al., 2016; Kashiwabara et al., 2017), and habitual LIPA is associated with reduced TG in older adults (Ryan et al., 2015), which

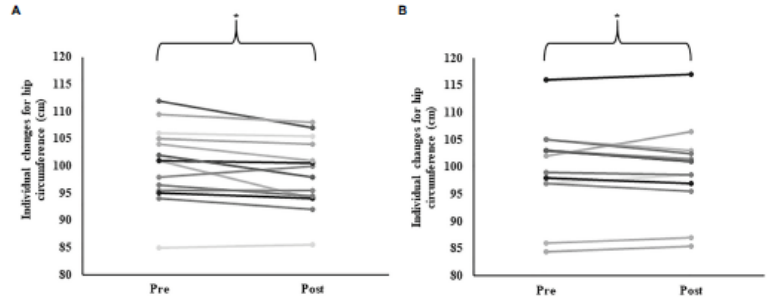

FIGURE 2 | Group dependent individual changes for hip circumference. Panel **A** represents individual changes following sedentary behaviour fragmentation (SBF); while panel **B** represents individual changes following continuous light intensity physical activity (LIPA). *Represents a significant change over time.

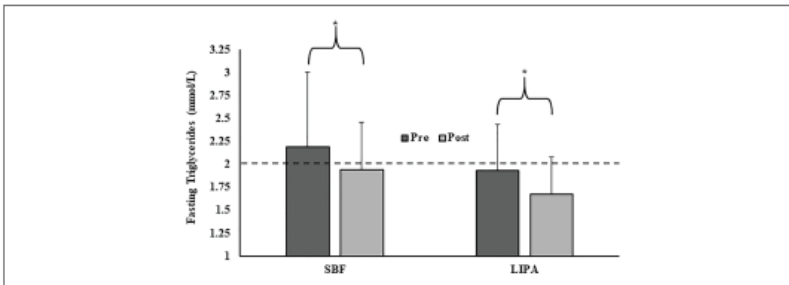

FIGURE 3 | Group changes in fasting triglycerides from pre to post intervention. *Represents a significant change over time. The dashed line (- - -) represents the fasting triglycerides threshold (2 mmol/L) below which significantly reduced CVD risk is observed (Iso et al., 2014; Nordestgaard and Varbo, 2014).

in turn is linked to reduced CVD risk (Baigent et al., 2010). Our data demonstrate such acute effects, persist into accumulated long-term benefits. Importantly, fasting TG levels below 2 mmol/L, confer significantly reduced risk of CVD (Iso et al., 2014; Nordestgaard and Varbo, 2014), a level that was beneficially achieved by both groups, post intervention (SBF: 1.94 ± 0.50 mmol/L and LIPA: 1.68 ± 0.40 mmol/L). Increased lipoprotein lipase (LPL) is a probable underlying mechanism, given the significant role it plays in reduced CVD risk (Hamilton et al., 2007). We thus propose persistent increases in the energy demand of contracting muscle facilitated enhanced substrate uptake. In contrast to previous evidence showing a more fragmented SB pattern is associated with decreased TG (Carson et al., 2014; Brocklebank et al., 2015), our data suggest the prescribed LIPA pattern is not of such relative importance, given that both groups decreased TG to a similar extent over time. Elucidating alterations in peripheral insulin sensitivity, requires a glucose tolerance test (Davies et al., 2000; Petersen and McGuire, 2005; Tabák et al., 2012), which we recommend future studies investigate. As they currently stand, our data simply suggest that chronic SB displacement in older women causes beneficial reductions in fasting circulating TG, irrespective of prescribed pattern.

Our data show reduced hip circumference following both interventions. Given that LIPA raises energy expenditure (Carter et al., 2015; Lerma et al., 2016), a chronically sustained LIPA increase likely created a negative energy balance (Levine et al., 2005), beneficially reducing what we will assume were fat deposits around the hips. Furthermore, AGR also exhibited a trend to decrease over time. Together, these changes can

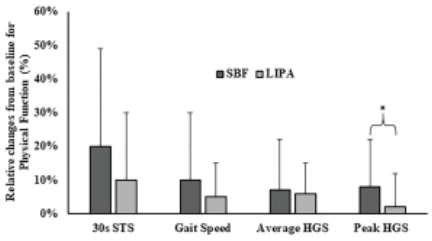

FIGURE 4 | Group dependent changes relative to baseline for physical function parameters. *Represents a significant group × time interaction effect. HGS, handgrip strength and STS, sit-to-stands.

be viewed as positive given that abdominal adiposity (Android) is more detrimental to health compared to lower body accumulation (Bastien et al., 2014; Chrysant and Chrysant, 2019). In support of our findings, a previous exercise intervention has noted a reduction in waist to hip ratio (de Mendonça et al., 2014). We also observed trends toward improved thoracic spine BMD. Accordingly, LIPA is associated with increased thoracic spine BMD in older adults (Onambele-Pearson et al., 2019), where the authors speculated excessive kyphotic curvature likely increases (forward) shear forces between thoracic vertebrae while walking (Kohrt et al., 1997), and thus places stress/strain on the bone structures, sufficient to cause adaptation. Our findings therefore support the notion of beneficial body composition changes (statistically significant and trends) following SB displacement in older women.

Together with the successful implementation of a randomized chronic SB intervention study in older adults, the novel manipulation of the prescribed LIPA pattern, makes the current study's design one of its primary strengths. Despite the lack of a control group limiting our design, the different patterns of LIPA prescription took priority. Furthermore, while the exclusive inclusion of older women somewhat limits the generalizability of our findings, we ultimately see this as a strength, given that muscle-tendon adaptation to resistance training appears to be gender dependent (McMahon et al., 2018). Moreover, we collected data on a range of health and physical functioning markers, 3D-accelerometer-based compliance, and self-reported adherence following SB displacement. Given that we successfully altered objectively measured SB, LIPA, and SB bout length in our participants, this reinforces our conclusion, that SB displacement specifically with LIPA mediated the health improvements observed. Furthermore, both interventions, were similarly rated as easy to implement at home, increased awareness of habitual SB, self-perceived health, and marked likelihood to integrate into lifestyle in the long term. Our findings add to the knowledge-base in the topic of SB effects (Wu et al., 2013; Matson et al., 2018, 2019; Wilson et al., 2019). Perhaps future studies could implement a similar intervention strategy, while elucidating the physiological mechanisms that underpin such positive changes, including muscle-tendon complex adaptation (e.g., neuromuscular adaptation), serum lipid transporters (e.g., LPL), and biological markers of inflammation (interleukin 6, tumor necrosis factor alpha, and C-reactive protein). Future studies should also investigate the effects of SB displacement on validated QoL assessments in older adults (e.g., SF-36 and EQ-5D; Bohannon and DePasquale, 2010), as-well as comprehensive physical capacity assessments [e.g., 6 min walk test (6MWT; Agarwala and Salzman, 2020)].

Given that older adults are being requested to "shield" and engage in prolonged and strict self-isolation (Armitage and Nellums, 2020), this makes the results of the current study very applicable. Accordingly, we recruited community dwelling older women from the local community, the population in need of targeted activity interventions during COVID-19 related quarantine. Our results suggest displacing SB with LIPA enhances various markers of health status. Such an intervention can be carried out from the home environment, with minimal effort/support, and displays good likelihood of long-term compliance. However, it must be noted, participants received a fortnightly home visit from the principal investigator to facilitate compliance and troubleshoot issues, which under quarantine conditions is simply not permitted. Such a limitation could be somewhat mitigated through indirect means of contact (telephone calls, emails, video conferencing software, etc.). Nevertheless, our results suggest SB displacement with LIPA is an efficacious home-based intervention for self-isolating community dwelling older adults to mitigate the detrimental health consequences of prolonged sedentarism during quarantine.

CONCLUSION

Due to the unrelenting global spread of pandemics such as COVID-19, further quarantine periods are looking increasingly

likely for the general population but especially for frail older adults (Lu et al., 2020; Sohrabi et al., 2020). Following 8 weeks of SB displacement with LIPA, we observed significant improvements in blood biomarkers (fasting TGs), and markers of physical function (gait speed, STS endurance/speed, and hand grip strength) in older women. Frequent vs. continuous SB displacement also caused greater increases in peak HGS. Therefore, based on our results, we propose SB displacement is an efficacious home-based intervention for self-isolating older adults, where MVPA is especially challenging. Our data suggest that MVPA engagement is not always necessary for mitigating the detrimental health consequences of prolonged SB. We propose that the positive palatability and high adherence results from our LIPA interventions are testament to the potential for long-term and wide adoption of this type of exercise interventions by key end-users. Furthermore, certain outcomes may be enhanced favorably with fragmented physical activity throughout the day rather than a single bout of exercise, even though both do enhance markers of health and physical functioning.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical committee of the Manchester Metropolitan University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GO-P, DT, and KT designed the research. DG conducted the research. DG and GO-P analyzed data, wrote the paper, and had primary responsibility for final content. All authors contributed to the article and approved the submitted version.

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Research Paper

Minimizing sedentary behavior (without increasing medium-to-vigorous exercise) associated functional improvement in older women is somewhat dependent on a measurable increase in muscle size

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ABSTRACT

The optimal pattern of sedentarism displacement and mechanisms underlying its health effects are poorly understood. Therefore, the aim of this study was to quantify muscle-tendon adaptation in response to two different sedentarism displacement interventions and relate any adaptations to functional outcomes. Thirty-four older women (73±5yrs) underwent skeletal muscle-tendon size and functional assessments. Participants were randomly allocated to: Sedentary behavior fragmentation (SBF), Light intensity physical activity (LIPA), or Control groups. Measures were taken at weeks 0 and 8. Gait speed significantly increased (p=0.003), in both experimental groups (SBF: 0.06 ± 0.08m/s, 6±10%, LIPA: 0.06 ± 0.07m/s, 6±6%), but not control (-0.02 ± 0.12m/s, -2±9%). Accordingly, the relative change in Vastus Lateralis muscle volume, accounted for 30% (p=0.027), and 45% (p=0.0006) of the explained variance in the relative change in gait speed, for SBF and LIPA respectively. Gastrocnemius Medialis fascicle length changes were positively associated with gait speed changes, following LIPA exclusively (R^2= 0.50, p=0.009). This is the first study to show SBF and LIPA are adequate loading in older women, with related muscle adaptation and clinically relevant gait speed improvements. Such adaptations appear similar irrespective of whether sedentarism displacement is prescribed in a single bout (LIPA) or in frequent micro-bouts (SBF).

INTRODUCTION

Sedentary behavior is characterized by low energy expenditure, and a seated/ reclined posture during waking hours [1]. Sedentary time appears hazardous above 8h/day [2, 3] with the achievement of current moderate-to-vigorous physical activity (MVPA) recommendations [4] being insufficient to offset high sedentary time [5]. Accordingly, light intensity physical activity (LIPA) displays a strong inverse correlation with sedentary behavior [6], suggesting LIPA displacement may contribute to the detrimental effects of sedentary time. Furthermore, a prolonged sedentarism accumulation pattern (longer sitting bouts) is associated with worse health outcomes compared to a more fragmented pattern (shorter sitting bouts) [7].

Sedentary time is higher among older adults [8] and is strongly associated with a myriad of poor health outcomes [9–14], most notably compromised physical

function [15–18]. The association between sedentary time and compromised function is exacerbated in frail individuals [13], and those accumulating sedentary time in a prolonged pattern [19], which also appears independent of concurrent MVPA time [20]. One mechanism potentially mediating such detriments is muscle tendon complex deterioration. Accordingly, severe disuse induces rapid muscle atrophy [21–23], with sedentary behavior specifically associated with the accelerated age-related loss of muscle mass (pre-sarcopenia) [24]. Furthermore, women tend to exhibit greater anabolic resistance and larger reductions in strength following disuse compared to men [25, 26]. However, the mechano-sensitivity of the human tendon is less clear [27], with only chronic unloading causing tendon atrophy [28]. Nevertheless, short term disuse causes tenocyte mediated detection of force-induced deformations [29] that subsequently trigger catabolic pathways in tendon [30]. Furthermore, alterations in muscle architecture and the force producing capabilities of muscle may also play a role [31–33]. Therefore, sedentary behavior could contribute towards age-related muscle-tendon complex deterioration.

Despite the positive effects of high intensity activity on both muscle [34] and tendon [27, 35, 36] older adults exhibit poor prolonged adherence to MVPA regimens [37–40]. Whilst it may be rational to assume lower intensity activity may not produce a sufficient muscle-tendon adaptation stimulus, evidence for/against this idea is scarce. Indeed, a body of work suggests the necessity for high intensity loading [35, 39], whilst another suggests that older women in particular would benefit from lower intensity loading [41]. Nevertheless, low intensity training has still been shown to stimulate muscle hypertrophy [42], contributing to enhanced strength [43, 44] and physical function [45]. Equally, increases in daily LIPA have been shown to change muscle architecture at rest [46], increase muscle mass [47, 48] and improve physical function [49] in older adults generally, but especially in frail individuals [44, 48–50]. Thus, the potential for LIPA to generate comparable physiological responses relative to more conventional high intensity loading is a somewhat recent theorem, supported by previous observations whereby older adults engaging in low frequency stair climbing exhibit significantly reduced mortality [51]. Therefore, due to the relative surge in physical demands that LIPA seems to generate in older adults closer to the lower limits of their physiological reserve, such activity may reach an appropriate loading threshold required for muscle-tendon complex hypertrophy.

Specifically, displacing sedentary behavior with LIPA improves balance [52] and enhances both gait speed [53] and sit-to stand ability [50, 52] in older adults. Interestingly, acute muscle activity during LIPA appears higher in the *Triceps Surae* compared to the knee extensors [54], which is reasonable given the key role such muscles play in maintaining upright balance [55] and ambulation [56]. Considering gait speed improvements, this ultimately suggests that the *Triceps Surae* may undergo greater adaptation following SB displacement, and thus should be considered a primary target for investigation. Nevertheless, previous interventions have failed to adequately control for the pattern of prescribed LIPA, meaning sedentary behavior fragmentation [repeated interruption of prolonged sitting with frequent sit-to-stand transitions and LIPA breaks (SBF)] may have still caused sufficient knee extensor adaptation. However, muscle-tendon complex hypertrophy following LIPA is likely to be small in magnitude given that tendon has a relatively slow turnover rate [27, 57], and lower activity volumes generally stimulate less muscle hypertrophy [58]. Nevertheless, despite muscle size not being a strong predictor of gait speed in older adults, it remains a significant predictor [59, 60], which may ultimately indicate that minor changes in muscle-tendon complex size can still mediate functional improvement following sedentary behavior displacement with light activity in older adults.

Therefore, the aim of the current study was to quantify muscle-tendon complex hypertrophy in response to two different LIPA interventions in older females and relate any such adaptations to functional outcomes. The first intervention would emulate traditional exercise through a single daily LIPA bout, whereas the second would implement the same amount of LIPA as in the first group but be spread throughout the day (SBF). It was hypothesized that both interventions would induce muscle-tendon complex hypertrophy and improve overall lean body mass, thus translating to improved function (such as gait speed). It was further hypothesized that muscular adaptation would be disproportionately observed in the *Triceps Surae* [(*Gastrocnemius Medialis* (*GM*)/ *Gastrocnemius Lateralis* (*GL*)] group compared to the knee extensor group [*Vastus Lateralis* (*VL*)]. Finally, we hypothesized SBF would induce comparable muscle-tendon complex hypertrophy and functional improvement to those attained through continuous LIPA.

RESULTS

Descriptive characteristics of participants at baseline

The 34 older women were matched at baseline for all outcome variables of interest (Table 1). Briefly, there was no statistically significant difference between the three groups at study onset for either *GM*, *GL*, or *VL*

Table 1. Baseline characterstics of the study sample.

		SBF (n=13)	LIPA (n=13)	Control (n=8)	Whole sample (n=34)
Age (years)		74 ± 5	74 ± 6	70 ± 3	73 ± 5
Height (m)		1.59 ± 0.07	1.61 ± 0.07	1.58 ± 0.1	1.60 ± 0.06
Mass (Kg)		68.8 ± 11.7	65.6 ± 8.9	65.4 ± 9.7	66.8 ± 10.1
Dual X-Ray absorptiometry derived data	Sarcopenic index	6.32 ± 0.81	5.97 ± 0.80	5.92 ± 0.81	6.08 ± 0.80
Sarcopenic Categorization	Proportion classified as Non-sarcopenic (Pre-sarcopenic/Low functional performance)	85% (0%/15%)	77% 23%/0%)	88% 12%/0%)	83% (12%/5%)
Physical Behavior classification	Proportion classified as Sedentary (Non-sedentary)	92% (8%)	100% (0%	75% (25%)	91% (9%)
Physical Behavior	Sedentary Behavior (h/24h)	9.6 ± 1.3	9.5 ± 1.0	8.3 ± 1.8	9.3 ± 1.4
	Light intensity physical activity (h/24h)	2.0 ± 0.8	2.1 ± 0.4	2.2 ± 0.7	2.1 ± 0.5
	Moderate to vigrous physical activity (h/24h)	3.0 ± 1.0	2.5 ± 0.8	3.6 ± 1.1	3.0 ± 1.0

Participant characteristics values are means ± SD.

regional anatomical cross sectional area (CSA), total muscle volumes, or fascicle length (Lf). Furthermore no baseline differences were observed for *Achilles Tendon* average or regional CSA, nor any of the segmental DEXA-derived body composition outcome variables (Table 2). Only *VL* fascicle pennation angle (FPA) ($p \geq 0.001$) and *VL* physiological cross-sectional area (PCSA) ($p=0.005$) exhibted signficant differences between groups at baseline.

Physical behavior

All groups were significantly matched for sedentary behavior, LIPA, and MVPA at baseline ($p \geq 0.05$). There was no group×time interaction for sedentary behavior ($p=0.41$). However, a trend for an effect over time was observed ($p=0.08$, $\eta_p^2=0.21$) driven primarily by a decrease in both experimental groups (SBF: -2±15%, LIPA: -4±14%) in contrast to control (4±30%). Promisingly, 8 participants (24%) positively shifted classification from sedentary to non-sedentary (SBF: n=3, LIPA: n=3, CON: n=2) in response to the intervention, with the other 26 participants (74%) remaining stable in their category over time. Furthermore no significant effects were observed for MVPA ($p \geq 0.05$) (Please see table 1).

Sarcopenia categories

All groups were significantly matched at baseline for categories of sarcopenia status ($p=0.18$), where 82%, 12%, and 6% were categorized as non-sarcopenic, pre-sarcopenic, and low functional performance respectively (Please see table 1). Only one participant positively shifted sarcopenia classification from low-functional performance to non-sarcopenic in response to the SBF intervention, with all other participants remaining stable in their category over time.

GM, GL, VL volume and PCSA intervention-induced changes

GM volume showed no effect of time ($p=0.47$), no effect of group ($p=0.22$) but a group×time interaction ($p=0.014$, $\beta=0.77$, $\eta_p^2=0.24$), with an increase in the control group *GM* volume being the driver for this interaction (Figure 1A). The associated changes for each group are illustrated in Figure 1B–1D. Similarly, a group×time interaction trend was observed for GM PCSA ($p=0.06$, $\beta=0.56$, $\eta_p^2=0.18$), with the control group increasing on average (15±12%) in contrast to both SBF (0±17%), and LIPA (-5±12%). However, there were no time, group, or group×time interactions observed for CSA, volume, or PCSA, in the *GL* and *VL* (Please see Table 2 and Figure 2).

GM and VL resting muscle architecture

GM fascicle length (Lf) exhibited a significant group×time interaction effect ($p=0.04$). The primary driver for this effect was the significant difference between SBF, in which Lf decreased (-0.16±0.55cm, -4±10%), and LIPA, in which it increased (0.35±0.40cm, 5±8%) ($p=0.04$). Furthermore, once corrected for

Table 2. Changes in skeletal muscle-tendon size, muscle architecture, lean body mass, and functional performance.

		SBF (n=13)			LIPA (n=13)			Control (n=8)		
		Pre	Post	Absolute change (Δ% change)	Pre	Post	Absolute change (Δ% change)	Pre	Post	Absolute change (Δ% change)
Gastrocnemius Medialis	Total Volume (cm^3)	195.1 ± 33.3	186.2 ± 41.6	-8.9 ± 27 (-5 ± 13%)×	215.0 ± 49.0	211.2 ± 46.0	-3.8 ± 21.2 (-1 ± 9%)×	175.0 ± 31.6	196.3 ± 30.5	21.3 ± 12.6 (13 ± 8%)×
	FPA (°)	20 ± 3	19 ± 2	-1 ± 4 (-2 ± 22%)	18 ± 3	18 ± 2	0 ± 2 (-1 ± 9%)	18 ± 3	19 ± 3	1 ± 3 (0 ± 16%)
	Fascicle Length (cm)	5.5 ± 0.7	5.2 ± 0.6	-0.2 ± 0.6 (-4 ± 10%)×	5.7 ± 0.7	5.9 ± 0.5	0.3 ± 0.4 (5 ± 8%)×	6.0 ± 0.7	6.0 ± 0.7	-0.1 ± 0.4 (-1 ± 7%)×
	PCSA (cm^2)	36.0 ± 6.7	35.7 ± 8.2	-0.2 ± 6.3 (0 ± 17%)	37.5 ± 8.3	35.5 ± 8.0	-2.0 ± 4.5 (-5 ± 12%)	29.7 ± 5.8	33.4 ± 4.7	4.0 ± 2.5 (15 ± 12%)
Gastrocnemius Lateralis	Total Volume (cm^3)	130.8 ± 30.7	134.9 ± 39.1	4.2 ± 25.3 (4 ± 19%)	135.2 ± 29.5	137.5 ± 25.4	2.4 ± 26.2 (4 ± 21%)	133.5 ± 28.6	138.3 ± 21.0	4.8 ± 22.0 (5 ± 13%)
Vatus Lateralis	Cross sectional area at 50% muscle length (cm^2)	41.3 ± 9.1	41.7 ± 9.7	0.4 ± 5.3 (1 ± 14%)	37.8 ± 6.0	37.8 ± 10.5	-0.1 ± 7.2 (-1 ± 18%)	34.8 ± 8.2	33.4 ± 7.4	-1.4 ± 2.8 (-4 ± 9%)
	Total Volume (cm^3)	464.2 ±191.55	446.6 ± 201.8	0.5 ± 88.9 (0 ± 18%)	448.1 ± 95.2	435.6 ± 128.8	-17.4± 100.0 (-4± 23%)	395.2 ± 79.21	402.2 ± 78.45	2.7 ± 31.0 (1 ± 7%)
	FPA (°)	**19 ± 3**	18 ± 3*	2 ± 3 (11 ± 21 %)	**16 ± 2**	16 ± 3*	-1 ± 3 (-4 ± 18 %)	**14 ± 3**	14 ± 2*	-3 ± 2 (-18 ± 9 %)
	Fascicle Length (cm)	5.4 ± 1.3	5.6 ± 1.3	-0.1 ± 0.9 (0 ± 16 %)	5.2 ± 1.1	5.4 ± 1.4	0.2 ± 0.5 (5 ± 9%)	6.3 ± 1.2	6.3 ± 1.8	-0.4 ± 1.0 (-8 ± 12%)
	PCSA (cm^2)	**89.8 ± 21.9**	92.4 ± 24.1	4.2 ± 24.7 (5 ± 30%)	**79.2 ± 16.1**	73.6 ± 22.4	-7.9 ± 20.3 (-11±23 %)	**58.3 ± 16.5**	58.8 ± 30.5	3.8 ± 11.3 (4 ± 22 %)
Achilles Tendon	Average cross sectional area (cm^2)	0.78 ± 0.19	0.80 ± 0.20	0.03 ± 0.10 (3 ± 13%)	0.74 ± 0.14	0.74 ± 0.17	0.03 ± 0.11 (4 ± 15%)	0.70 ± 0.17	0.67 ± 0.19	0.01 ± 0.07 (1 ± 9%)
	Cross sectional area at 0cm (cm^2)	0.86±0.34	0.90±0.31	0.03±0.19 (11±40%)	0.77±0.22	0.79±0.18	0.02±0.31 (13±48%)	0.74±0.20	0.69±0.08	-0.05±0.26 (-7±45%)
	Cross sectional area at 1cm (cm^2)	0.78±0.19	0.81±0.33	0.03±0.17 (4±24%)	0.82±0.19	0.82±0.25	0.02±0.11 (3±19%)	0.74±0.28	0.78±0.23	0.04±0.17 (3±24%)
	Cross sectional area at 2cm (cm^2)	0.76±0.14	0.78±0.15	0.02±0.12 (4±17%)	0.81±0.16	0.79±0.29	-0.02±0.11 (-2±15%)	0.70±0.36	0.67±0.25	-0.02±0.15 (-1±21%)
	Cross sectional area at 3cm (cm^2)	0.72±0.22	0.71±0.11	-0.01±0.12 (-1±19%)	0.65±0.18	0.65±0.21	0.01±0.07 (1±9%)	0.59±0.24	0.65±0.25	0.03±0.15 (3±26%)

Dual X-Ray absorptiometry derived data	Arms Lean Tissue (Kg)	1.86 ± 0.33	1.85 ± 0.29	-0.01 ± 0.15 (0 ± 8%)	1.80 ± 0.22	1.76 ± 0.24	-0.03 ± 0.08 (-2 ± 5%)	1.71 ± 0.24	1.76 ± 0.24	0.05 ± 0.07 (3 ± 4%)
	Legs Lean Tissue (Kg)	6.17 ± 1.24	6.06 ± 0.99	-0.11 ± 0.45 (-1 ± 6%)	5.93 ± 0.77	5.85 ± 0.71	-0.07 ± 0.20 (-1 ± 3%)	5.66 ± 0.83	5.66 ± 0.83	0.01 ± 0.19 (0 ± 3%)
	Total Lean Tissue (Kg)	39.14 ± 5.92	38.91 ± 5.59	-0.23 ± 1.46 (-1 ± 3%)	37.10 ± 4.07	36.70 ± 3.88	-0.0 ± 1.13 (-1 ± 3%)	36.75± 4.05	37.44 ± 4.00	0.68 ± 0.79 (2 ± 2%)
Functional Performance Measures	Gait Speed (m/s)	1.12 ± 0.29	1.16 ± 0.37*	0.06 ± 0.08 (6 ± 10%)	1.12 ± 0.13	1.21 ± 0.18*	0.06 ± 0.07 (6 ± 6%)	1.30 ± 0.10	1.29 ± 0.24*	-0.02 ± 0.12 (-2 ± 9%)
	Eyes open balance (s)	19 ± 11	20 ± 12	1 ± 6 (11 ± 46%)	27 ± 5	27 ± 5	0 ± 4 (6 ± 30%)	26 ± 5	25 ± 8	-1 ± 4 (-7 ± 20%)
	Eyes closed balance (s)	3 ± 2	3 ± 3	0 ± 3 (29 ± 85%)	4 ± 3	5 ± 5	1 ± 2 (33 ± 63%)	3 ± 2	3 ± 1	0 ± 2 (2 ± 56%)

Participant Characteristics values are means ± SD.
Boldened baseline values represent significant baseline differences. * represents a significant time effect. × represents a significant group×time interaction effect.

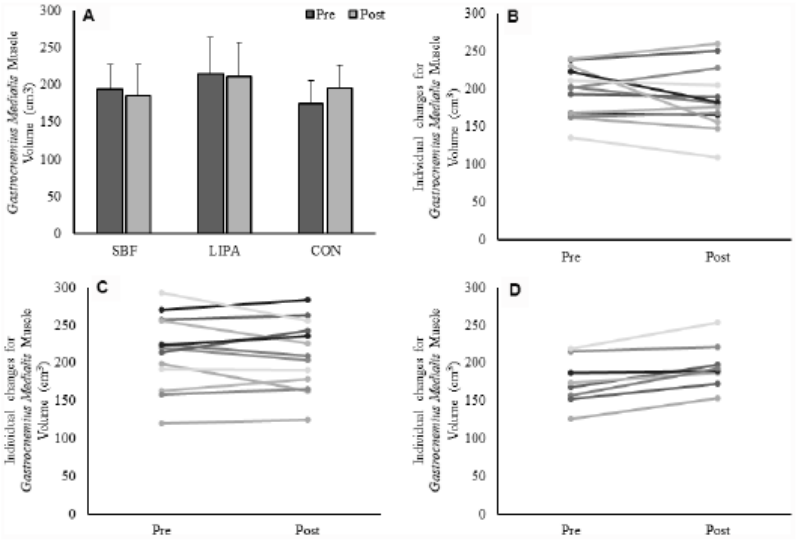

Figure 1. Changes in *GM* muscle volume from baseline to post-intervention. Panel (A) Group-dependent *GM* muscle volume (Mean ± SD) at pre (week 0) and post intervention (week 8). There was a significant group×time interaction (p = 0.014) for GM volume. Panels (B–D) represent individual participants changes from baseline to post-intervention, for the SBF, LIPA, and control groups, respectively.

baseline differences, *VL* FPA exhibited a significant time effect (p=0.010, β=0.75, η_p^2=0.20), but not a group×time interaction (p=0.20) (Please see table 2).

Achilles Tendon dimensions intervention-induced changes

Interestingly for the analysis of the 4 discrete tendon CSA sites, a single trend was observed toward a significant main effect for time at 1cm of *AT* length (p=0.08, η_p^2=0.22), but no group×time interaction effect (p=0.99), with all groups increasing to a similar extent (SBF: 4±24%, LIPA: 3±19%, Control: 3±24%). Similarly, average tendon CSA (average of 4 discrete sites) showed no time, group, or group×time interaction (Please see table 2).

DEXA derived body composition intervention-induced changes

None of the DEXA-derived outcome variables (total lean tissue, arms, legs, and sarcopenic index) exhibited main effects of group, time, nor group×time interactions.

Functional performance measures intervention-induced changes

Gait speed exhibited a significant main effect for time (p=0.003, η_p^2=0.36). Despite no significant group×time interaction effect (p=0.24), both SBF (0.06 ± 0.08m/s, 6±10%) (Please see Figure 3, Panel A), and LIPA (0.06 ± 0.07m/s, 6±6%) increased from pre to post (Please see Figure 3, Panel B), in contrast to control (-0.02 ± 0.12m/s, -2±9%) (Please see Figure 3, Panel C). However, no significant main effects were observed for postural balance ability.

Associations between relative changes in muscle-tendon complex size and relative changes in gait speed

There was a significant positive association between % change in *VL* volume and % change in gait speed (p=0.006). Specifically, within the pooled analysis of all participants, the percent change from baseline in *VL* volume significantly (R^2=0.18, p=0.006), accounted for 18% of the explained variance in relative change from baseline in gait speed. Following sub-analysis by group, the explained variance in gait speed significantly (R^2=0.31, p=0.027) rose to 31% in the SBF group (Figure 3, Panel D), 45% in the LIPA group (R^2=0.45, p=0.0006) (Figure 3, Panel E) with no significant variance in the control group (Figure 3, Panel F). Furthermore, there was a significant positive association between the % change in *GM* Lf and % change in gait speed (R^2=0.24, p=0.004), accounting for 24% of the explained variance. Interestingly, when sub-analyzed by group such an association only persisted for LIPA (R^2=0.50, p=0.009) and Control (R^2=0.64, p=0.014), with both groups accounting for similar amounts of the explained variance (LIPA: 50%, CON: 64%). Finally, a significant negative association was observed between the % change in *GM* PCSA and % change in gait speed (R^2=0.33, p=0.001), accounting for 33% of the explained variance. Following sub-analysis by group, the explained variance in gait speed only persisted for SBF (R^2=0.46, p=0.010), and LIPA (R^2=0.37, p=0.014). Both experimental groups accounted for similar amounts of the explained variance (SBF: 46%, LIPA: 37%). No other significant correlations were observed between relative changes from baseline in muscle-tendon complex or DEXA outcomes and relative changes from baseline in functional performance measures.

DISCUSSION

The aim of the current study was to quantify muscle-tendon complex hypertrophy in response to two LIPA interventions in older women and relate any changes to functional outcomes. Firstly, it was hypothesized that both interventions would induce measurable muscle-tendon complex hypertrophy, improve overall lean body mass, translating into enhanced function. Accordingly, we observed a significant change over time for *VL* FPA, and group-dependent changes over time for *GM* Lf and *GM* muscle volume. Furthermore, gait speed significantly improved in both experimental groups but not control. The % change in gait speed was significantly associated with the % change in *VL* volume (R^2=18%), and *GM* Lf (R^2=24%) thereby partially upholding the primary hypotheses. It was further hypothesized that muscular adaptation would be disproportionately observed in the *Triceps Surae* group. We observed localized maladaptation in *GM* volume following both LIPA interventions, with the relative change in *GM* PCSA negatively associated with the percent change in gait speed (R^2= -33%). Thus, the third hypothesis was partially refuted. Finally, we hypothesized that SBF would induce comparable muscle-tendon complex hypertrophy, and functional improvement, to those of continuous LIPA. *GM* Lf significantly increased in LIPA only, whereas a decrease was observed in SBF. Accordingly, the % change in *GM* Lf was significantly associated with the % change in gait speed for LIPA but not SBF. Nevertheless, gait speed improved by similar magnitudes in both experimental groups, with the relative change in *VL* volume accounting for similar amounts of the explained variance for the relative change in gait speed (SBF:30%, LIPA:45%), thereby upholding the final hypothesis.

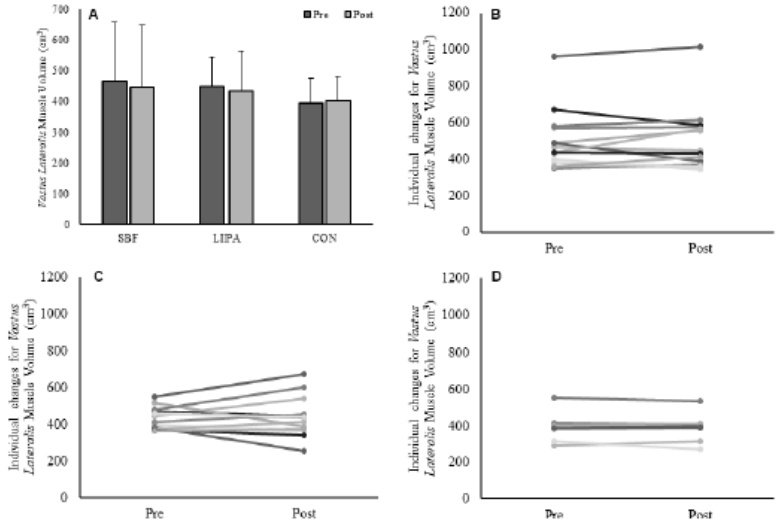

Figure 2. Changes in *VL* muscle volume from baseline to post-intervention. Panels (A) Group-dependent *VL* muscle volume (Mean ± SD) at pre (week 0) and post intervention (week 8). Panels (B–D) individual participant changes from baseline to post-intervention for the SBF, LIPA, and control groups respectively.

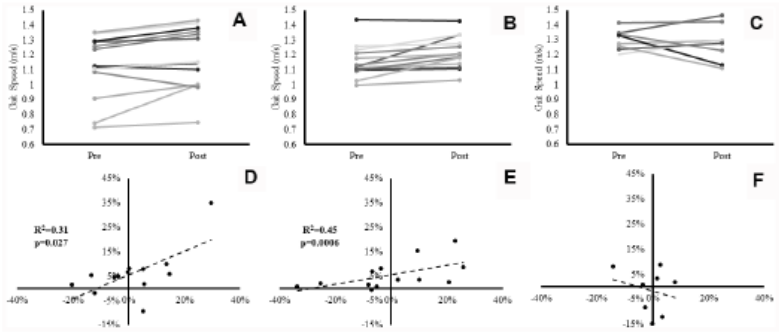

Figure 3. Individual participants' changes in gait speed from baseline to post-intervention. Panels (A–C) represent individual changes for the SBF, LIPA, and control groups respectively. Panels (D–F) represent the associations between the relative changes in VL Volume (X axis), and the relative changes in gait speed (Y axis) for the SBF, LIPA, and control groups respectively.

Despite the abundance of health benefits that exercise induces [61], older adults exhibit poor long-term adherence to exercise [40]. Furthermore, recommended exercise does not offset the negative effects of high sedentary time [5]. Such limitations create scope for alternative interventions that potentially yield greater long-term efficacy. Accordingly, displacing sedentary behavior with LIPA in older adults, consistently improves physical function [50, 52, 53], however the physiological mechanisms remain undetermined. The current study is the first to examine muscle-tendon complex hypertrophy following sedentary behavior displacement in older females, and link adaptations to functional improvements.

We found that LIPA implementation failed to elicit statistically significant improvements in *GM*, *GL*, or *VL* muscle volume/ PCSA. In contrast, a single bout of low-intensity resistance training (40% 1RM) is sufficient to stimulate myofibrillar protein synthetic response [58]. However, only slow tempo lifting through the entire range of motion, significantly improved quadriceps muscle thickness following 10 weeks of low-intensity resistance training (30-50% 1RM) in older adults [62, 63]. Consequently, prescribed LIPA should have theoretically provided enough intensity, but the lack of direct supervision may have led to variability in movement execution (range of motion/training tempo). Furthermore, low volume (3 sets) low-intensity resistance training appears inferior to high volume (6 sets), regarding the ability to stimulate myofibrillar protein synthetic response in older adults [58], suggesting increasing training volume over time is essential for hypertrophy. LIPA interventions may therefore require to be carried out over longer periods to compensate for the lack of overload. Furthermore, considering the role body weight plays in muscle-tendon complex adaptation [64], variations in participants mass did not allow specific standardization of training load for body weight-based movements. Nevertheless, increasing older adults walking time over 6 months, increases skeletal muscle mass [47, 48]. However, previous LIPA interventions of similar durations similarly did not increase training volume, or manipulate range of motion/training tempo, yet still observed improved function [50]. This suggests improved physical function following sedentary behavior displacement, may occur independent of significant muscle hypertrophy.

Despite observing a group-dependent effect for *GM* volume, this effect was driven through a marked increase in the control group only. Whilst we instructed intervention participants to maintain habitual MVPA, we did prescribe specific instructions to avoid high-speed activities when displacing sedentary behavior, which may have unintentionally reduced habitual gait. Accordingly, plantar flexor muscle activity is affected by alterations in walking speed [65], and increases during faster walking speeds [66]. The control group received no such instruction and thus may have continued receiving the habitual walking stimulus required to elicit GM hypertrophy. Accordingly, MVPA but not LIPA is associated with mid-calf muscle density in older adults [67]. This points to a localized muscular effect following alterations in ambulation (specifically in the *GM*) that was not generalized across the whole leg. Whilst this supports our original hypothesis that muscular adaptation would be disproportionately observed in the *Triceps Surae* group, we failed to anticipate a maladaptation. Nevertheless, we still observed significant gait speed improvements following both interventions, despite an apparently compromised GM volume.

We failed to observe any significant main effects for *Achilles Tendon* size. Extreme low-intensity resistance training (≤20% 1RM), has been shown to enhance strength in older adults [43, 44]. Given the relative surge in intensity such activity likely stimulates in populations close to the lower end of the physiological reserve spectrum, this suggests light activity may reach an appropriate tendon adaptation loading threshold. Accordingly, tenocytes sense loading induced deformations [29], triggering anabolic and catabolic pathways [30]. However, tendon hypertrophy seems dependent upon reaching an intensity threshold (≥40% 1RM) [27, 35, 36]. Therefore, the lack of significant main effects, further questions the likelihood of training-induced alterations in tendon size. However, this does suggest functional adaptation following sedentary behavior displacement, occurs independent of changes in *Achilles Tendon* size.

Displacing sedentary behavior similarly did not alter lean body mass, with no participants shifting pre-sarcopenia categorization post-intervention. In support, moderate term low-intensity resistance training (10-20 weeks, ≤40% 1RM) does not significantly alter lean body mass in older adults [39, 68]. Furthermore, light homebased body weight resistance training failed to induce changes in fat-free mass over 9 months [69]. However, DEXA tends to underestimate the age-related loss of muscle mass compared with MRI [70]. Nevertheless, given minimal alterations in *GM*, *GL*, and *VL* muscle volume, it is unsurprising that lean body mass similarly did not exhibit significant change post intervention. Furthermore, this points to a localized muscular effect of increased ambulation (specifically in the *GM/VL*), that was not generalized across the whole leg. Similar deficiencies (lack of mechanical impulse/overload), that do not appear to be

compensated for through longer time frames, likely inhibited lean body mass gains following sedentary behavior displacement.

We did not observe significant changes in postural balance ability. Previous studies examining the association between SB and postural balance in older adults have reported mixed results [18, 71]. Furthermore, only one study exhibited a trend towards improved balance following SB displacement in older adults [52]. However, the latter study utilized a slightly longer intervention period (12 weeks), and only assessed balance through timing single leg stance [52]. The current study also varied the proprioceptive feedback through adding an eyes closed balance assessment [72], although this did not alter the results. Therefore, our findings potentially highlight the insufficiency of SB displacement as an appropriate PA modality for balance improvement. In fact, a minimum of 90 minutes a week of specific balance training is suggested to be the minimum dose response threshold for balance improvement in older adults [73]. Future studies could therefore implement single leg challenges during SB displacement, utilize more nuanced balance assessments (posturography), and employ longer intervention times (≥12 weeks) to further determine if balance/postural sway is impacted through displacing older adults SB time.

Most notably, we did observe significant improvements in gait speed. Gait speed is used as a key diagnostic indicator of low functional performance and severe sarcopenia in older adults [74]. Improvements in gait speed are frequently associated with an increase in daily walking time [49], and time spent performing low-intensity resistance training [42] in older adults. Improved gait speed is also a consistent finding throughout sedentary behavior displacement studies in older adults [50, 52, 53]. Therefore, our results in line with previous research suggest, LIPA can stimulate functional improvement in older adults. Furthermore, given that both experimental groups improved their gait speed to a similar extent, this suggests the act of displacing sedentary behavior time with increased LIPA is the principal factor mediating gait speed improvements, irrespective of the prescribed pattern.

Interestingly, our results do reveal the relative change in gait speed was significantly associated with changes in *VL* volume, accounting for ~18-45% of the observed variance. This further suggests improvements in gait speed may be dependent on 'small' changes in *VL* muscle size. Accordingly, *VL* muscle size has been identified as a small yet significant independent predictor of fast gait speed in older adults [60]. This is reasonably expected, given that we assessed gait speed through the TUG test, and the knee extensors play a key role in sit-to-stand transitional performance and ambulation in general [75], with previous authors speculating sedentary behavior displacement was specifically improving the ability to mobilize from a seated position [50]. Accordingly, we observed a significant increase in *VL* FPA following SBF (~11%). In support, eight weeks of light dancing similarly increases *VL* FPA in older women (~21%) [46]. Given that increased FPA is associated with increased force transmission [31, 33], this supports positive knee extensor adaptation following SBF. However, only *VL* volume significantly correlated with the change in gait speed, suggesting an exclusive role. Accordingly, muscle volume appears superior to CSA regarding the ability to evaluate age-related differences in muscle strength [76]. Furthermore, thigh muscle volume has specifically been associated with muscle power, sit-to-stand ability, and fast gait speed in older adults [59]. The significant negative association between % change in *GM* PCSA and % change in gait speed in both experimental groups also supports this finding. Whereas PCSA represents the amount of sarcomeres in parallel, and thus a muscles maximal force production capabilities [77, 78], gait speed appears more dependent on contraction velocity and the adequate production of muscular power [59].

Our results also revealed that the % change in gait speed was significantly associated with % changes in *GM* Lf. In contrast to FPA and PCSA, Lf accurately represents the amount of sarcomeres in series, and is thus a major determinant of maximum shortening velocity [77]. Specifically, 50% of the difference in maximum shortening velocity between young and old adults is explained by a reduction in *GM* Lf [79]. Our results support this finding given that 50% of the variance for % change in gait speed, was accounted for through changes in *GM* Lf, following the LIPA intervention exclusively. Accordingly, *GM* Lf significantly increased in LIPA (5%), but not SBF (-4%). In support of this finding, eight weeks of light dancing increases *GM* Lf in older women by a similar magnitude (~10%) [46], suggesting a specific mechanism by which continuous LIPA increases gait speed. Walking preferentially stimulates the *Triceps Surae* musculature in older adults [54], suggesting continuous LIPA may have involved greater time spent ambulating in contrast to SBF. Consequently, greater time spent ambulating may have generated the region-specific effect on *GM* Lf. Therefore, together with reduced *GM* PCSA, increased *GM* Lf may represent a shift toward greater contraction velocity capabilities in the *GM*. Ultimately, gait speed improvements following LIPA implementation in older women, appear to be comprehensively mediated

through small changes in *VL* muscle volume, as-well as a pattern dependent shift in *GM* Lf. Despite identifying such important mediators, ~76-82% of the variance remains unexplained, suggesting other physiologic mechanisms further mediate gait speed improvements following sedentary behavior displacement. These additional mechanisms likely include alterations in fiber type composition, tendon mechanical properties, as-well as neuromuscular adaptations, which we recommend future studies investigate.

Given that we exclusively recruited older females, this does limit the generalisability of our findings to other populations. However, we see this as a strength given that it was recently shown muscle-tendon complex response to resistance training may be gender dependent [80]. Whilst we acknowledge splitting our small sample into three groups likely reduced our statistical power, we view this as a necessary trade off given the strong study design we employed (accounting for prescribed LIPA pattern, utilizing a control group). Indeed, our achieved sample size (n=34) is in line with previous interventions. Furthermore, given that DEXA underestimates the age-related loss of muscle tissue [70], we view the simultaneous utilization of DEXA and ultrasound muscle assessment as a key strength. Nevertheless, we recommend future interventions utilize longer time frames (>8 weeks), in order to compensate for the limited degree of overload. Our original hypothesis led us to prioritize investigation of the *Triceps Surae* (2 muscles, 3 regional measurement sites per muscle) over the knee extensors (1 muscle, 1 regional measurement site). Thus being restricted to a single CSA measurement site in the *VL* meant we may have underestimated regional differences in size along the entire length of the muscle [81–83]. Therefore, given the relevance of *VL* volume within our results, we strongly encourage future studies place greater importance on investigating the knee extensors following sedentary behavior displacement, using multiple measurement sites, and further investigation of the *Quadriceps Femoris* as a whole.

In conclusion, displacing sedentary behavior with LIPA (irrespective of prescribed pattern) produces limited muscle-tendon complex adaptations and significant gait speed improvements in older adults. Furthermore, small alterations in *VL* muscle volume explained a large part of the variance in gait speed changes, which were also associated with changes in *GM* fascicle length. Collectively, these findings suggest that LIPA reaches an appropriate loading threshold required to induce clinically impactful functional adaptations. Future studies should investigate other physiologic mechanisms underlying such observed improvements.

MATERIALS AND METHODS

Thirty-four community dwelling elderly women voluntarily participated in the study (See table 1). Intervention studies manipulating sedentary behavior in older adults are few, and to the authors' knowledge no published interventions have examined changes in muscle-tendon complex size or lean body mass. Therefore, estimation of required sample size to detect significant changes in the desired outcomes was based upon two points: (a) previous sedentary behavior interventions in older adults that have observed improvements to physical function, have utilized total sample sizes of ~25-38 [50, 52, 53] (b) previous low-intensity resistance training studies in older adults, deemed total sample sizes of 17 [35], and 18 [63], adequate to detect changes in tendon and muscle size respectively. The current achieved sample size of 34 older females, falls within this range. Participants were all recruited from the local community. The study was approved by the local university ethics committee [approval code: 230118-ESS-DG-(2)], and written informed consent obtained prior to any procedures being performed, in line with the declaration of Helsinki. Exclusion criteria included history of lower limb muscle/ tendon/ joint disorders in the past six months, or current suffering from any chronic health condition which could affect the participants ability to independently perform an intervention of increased activity (e.g. cardiovascular disease, uncontrolled diabetes, active cancer, current diagnosis of stroke, Parkinson's disease, etc). Furthermore, participants who partook in structured progressive resistance training (free weights etc) were also excluded at baseline. Nevertheless, inclusion criteria comprised all habitual physical activity profiles (regardless of meeting recommended MVPA levels). Participants initially visited the laboratories to complete screening/ questionnaires, as-well as undergo familiarization to the gait speed and balance (functional performance) assessments. After seven days, participants returned to the laboratories and underwent overnight rested and fasted, ultrasonographic assessment of the *(GM), Gastrocnemius Lateralis (GL), Vastus Lateralis (VL)* and *Achilles Tendon (AT)*. Segmental analysis of body composition using DEXA imaging, and functional performance assessments were also conducted. Participants were randomly allocated in a single blind fashion to one of three groups: 1) Sedentary behavior fragmentation (SBF) (n = 13), 2) Single bout continuous light activity (LIPA) (n = 13), or 3) Control i.e. no lifestyle change (n = 8). All measures were taken at weeks 0 and 8.

Dual X-ray absorptiometry scan

Participants arrived at the laboratory in a fasted state (10-h to 12-h overnight) and were taken into a private scanning room. Participants changed into a hospital style gown and had their height (to the nearest 0.01m) and body mass (to the nearest 0.1kg) measured using a stadiometer (Seca model 213 stadiometer, Seca, Germany), and digital scales (Seca model 873, Seca, Germany) respectively. A dual energy x-ray absorptiometry (DEXA) scanner (Hologic Discovery: Vertec Scientific Ltd, UK) was used to ascertain lean body mass. Briefly, participants were asked to lie in a supine position, avoiding any contact between the trunk and the appendicular mass [84] (whole body procedure, EF 8.4 lSv). The slow moving 'arm' of the DEXA scanner passed over the body over the course of 7 minutes. Hologic software was then used to draw segmental analysis lines through the skeleton along regions of interest (Arms, Legs, Total) [85, 86].

Ultrasonography

Participants lay in a prone position, and rested for ~20 minutes to avoid fluid shifts [87, 88]. The ankle joint was then secured in neutral angle (0°) against a footplate. Participants were asked to remain still and relaxed, as Brightness-mode ultrasound (MyLab Twice, Esaote Biomedica, Genoa, Italy) was performed. Discrete muscle sites were marked by drawing a line from the medial to the lateral border of the *GM* and *GL*, at 25, 50, and 75% of each muscle's respective length. Proximal and Distal endpoints of the *AT* were also marked, and length markers drawn in 1cm proximal increments from the calcaneal tuberosity. A novel panoramic imaging technique (panoramic view) granted an image of the *GM/GL* heads and thus anatomical CSA. Ultrasound panoramic imaging has previously been established as a reliable and valid method of CSA assessment when compared against magnetic resonance imaging [89, 90], and is sensitive to detect hypertrophic and atrophic alterations [83]. Briefly, the probe was moved with a constant speed and light pressure across the leg, to avoid compression during scanning. A Velcro strap was loosely attached (again to avoid compression) around the lower leg at each length marker to ensure the probe maintained the appropriate path and angle during each scan. Lastly, all ultrasound acquisition parameters were monitored, and consistency reproduced between scans. The ultrasound probe (7.5MHz linear array probe, 38 mm wide), was held perpendicular to the muscle. Once processing, the ultrasound probe was moved along the marked pathway, from the lateral to the medial border of the muscle (for representative images, please see Figure 4A, 4B) This procedure was repeated three times at each muscle site. The ultrasound probe was then positioned along the mid-sagittal line, at 50% of the GM muscle length, in order to record resting muscle architecture. Images of both resting fascicle pennation angle (FPA) and resting fascicle length (Lf), were then analysed using ImageJ (1.45s; National Institutes of Health). Three fascicles (defined from the deep to the superficial aponeurosis) of the GM were recorded and the mean value of both FPA and Lf determined. Linear extrapolation of fascicles was carried out where fascicles extended beyond the reach of the probe, as described previously [84]. This method has previously demonstrated good validity and reliability [33, 91].

AT length was measured as the distance from the distal gastrocnemius myotendinous junction to the calcaneal insertion. Subsequently, *AT* CSA was obtained from representative transverse images (Depth: 30mm; Frequency: 27Hz; Focal Points: 1) at 0, 1, 2 and 3cm, of *AT* length. Offline ultrasound analysis was performed using IMAGEJ (1.45 s; National Institutes of Health, Bethesda, MD, USA) in a non-blind fashion. Determination of tendon CSA using this method has previously demonstrated good validity and reliability [92, 93]. Participants then switched to a supine position, with the knee fully extended and the hip angle raised to 45°, on top of a 30cm platform. The proximal and distal insertions of the *VL* were identified and 50% of *VL* length marked on the skin. Three more panoramic images of the VL head and thus VL CSA were then obtained as described previously (for representative image, please see figure 4C).. *VL* muscle architecture (FPA and Lf) was then determined, as previously described for the *GM*.

Ultrasound reliability

The same sonographer performed all scans and demonstrated excellent intra and inter day reliability. Specifically for the panoramic CSA imaging of the *GM*, *GL*, and *VL* the Intraclass Correlation Coefficient (ICC) was ~0.98, and the Coefficient of variation (CV%) ~4%, when reliability assessments were carried out on a subset of participants (n=8, 24% of total sample), comparing familiarization values to pre-test. Good inter day reliability was also observed for *VL* muscle architecture, specifically Lf (ICC = 0.96, CV% = 5%), and FPA (ICC = 0.87, CV% = 5%). For the *Achilles Tendon*, good inter day reliability was observed when CSA was examined at 0cm (ICC = 0.87, CV% = 7%), 1cm (ICC = 0.93, CV% = 6%), 2cm (ICC = 0.92, CV% = 6%), and 3cm (ICC = 0.76, CV% = 8%), and average of all sites (ICC = 0.97, CV% = 3%), when comparing tendon CSA at familiarization with pre-test values. Finally, good inter day reliability was also observed for GM

muscle architecture, specifically FPA (ICC = 0.80, CV% = 4%), and Lf (ICC = 0.91, CV% = 67%), in a sub sample of participants (n=7, 21% of total sample).

Calculation of muscle volume and physiological cross-sectional area

GM and *GL* muscles volumes were calculated by treating the muscles as a series of truncated cones [94, 95], through the construction of several CSAs taken at discrete muscle sites (25, 50 and 75% of *GM* and *GL* length). Each of the four truncated cones was calculated using the following equation:

$$muscle\ volume = \frac{1}{3} \cdot d \cdot [a + \sqrt{(a.b)} + b]$$

Where:
d is the distance between the two CSA's (*a* and *b*)

The sum of the four cones provided *muscle volume* for *GM* and *GL*. *VL* muscle volume was calculated from a

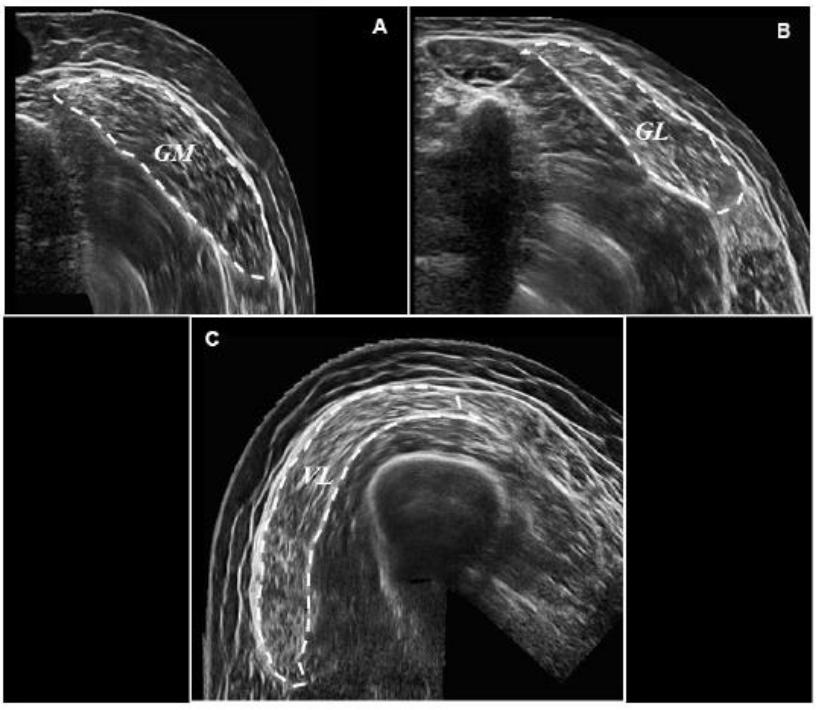

Figure 4. Representative ultrasound images following panoramic ultrasound imaging. Panel (A) represents a transverse image of *GM* CSA (outlined for effect) at 50% of muscle length, Panel (B) represents a transverse image of *GL* CSA (outlined for effect) at 50% of muscle length, and Panel (C) represents a transverse image of *VL* CSA (outlined for effect) also at 50% of muscle length.

single CSA re-construction at 50% of *VL* length and extrapolated to calculate overall muscle volume. This method of calculating muscle volume from a single CSA has been validated previously [96]. Physiological cross-sectional area (PCSA) was then calculated for both *GM* and *VL* using the following equation, as described previously [84].

$$PCSA = muscle\ volume / Lf$$

Postural balance assessment

The single balance postural test is well established in research using older persons, with documented reliability [55, 97]. Participants performed a single leg balance test with their eyes either open or with visual feedback removed through utilizing blacked out goggles to isolate proprioceptive feedback [72]. Each participant's postural balance was tested on the leg they self-perceived to be their strongest. A number of measures were in place during assessment: (i) the researcher was present during all balance assessments; (ii) a soft chair was positioned behind the participants as a safety measure; (iii) participants hovered their hands above a height adjustable physiotherapy bed to begin the test before placing their hands by their side; (iv) in the event they felt they were going to lose their balance they would immediately place their hands back on the bed. This also marked the end of a particular trial along with raising the arms above head height or putting the non-balancing leg on the floor. Trial duration (up to a maximum of 30s) was recorded using a stopwatch. Three trials were performed with ~60s rest in-between. The average of the three trials for eyes open and eyes closed was then reported for each participant. Inter-day reliability was excellent for eyes open trials [Intraclass correlation co-efficient (ICC): 0.97%], and good for eyes closed trials (ICC: 0.75).

Gait speed assessment

Gait speed was assessed through the timed "Up and Go" test (TUG) [98, 99]. In an attempt to reduce TUG variability [100], the height of an adjustable stool was standardized to the length of each participants lower leg (distance in cm from the tibio-femoral junction, to the bottom of the footwear). The time taken between rising from and returning to the seated position was accurately monitored with a modified pressure sensor (Tekescan, South Boston, USA), and corresponding software. The sensor was attached to the surface of the chair in a manner that allowed accurate timing (0.01s) but did not impede the participants comfort whilst seated. Once instructed, participants rose from the chair, and walked at a maximum self-selected pace up to a box marked out on the floor with masking tape (approximately 6m away), before returning to the seated position. Total time was divided by the total course distance (12m) in order to calculate average gait speed [metres per second (m/s)]. The test was repeated 3 times with 60s rest in-between, and the average of three trials reported. Gait speed assessment exhibited excellent inter-day reliability (ICC: 0.91).

Comprehensive sarcopenia definition

DEXA derived appendicular lean body mass was divided by body height to provide a relative indicator of muscle quantity, termed sarcopenic index. Previously determined cut off points for both sarcopenic index and gait speed [74], were then used to classify participants into one of four categories, 1. Non-sarcopenic (sarcopenic index $\geq$5.5kg/m^2 and gait speed >0.8 m/s), 2. Pre-sarcopenic (sarcopenic index <5.5kg/m^2 and gait speed >0.8 m/s), 3. Low functional performance (sarcopenic index $\geq$5.5kg/m^2 and gait speed $\leq$0.8 m/s), and 4. Sarcopenic (sarcopenic index <5.5kg/m^2 and gait speed $\leq$0.8 m/s).

Physical behavior interventions

The purpose of the two intervention groups was to manipulate the method for displacing sedentary behavior time with added daily LIPA (45-50 mins). Both intervention groups were provided with a booklet, which contained simple LIPA suggestions compiled from the compendium of physical activities [101]. Participants were explicitly told to continue performing any pre-existing MVPA routines (e.g., exercise classes, etc). Throughout the 8-week intervention period all participants received fortnightly home visits from a member of the research team, to check on the progress of the intervention. Participants daily sedentary behavior, LIPA, and MVPA were assessed at baseline and the final intervention week, with a thigh mounted GENEActiv original triaxial accelerometer (GENEA, Activinsights Ltd, Kimbolton, UK), and a previously validated algorithm [102]. Participants were classified as sedentary if average daily sedentary time was $\geq$8h/day, as sedentary time appears to be exponentially hazardous above this threshold [2, 3].

SBF group: Participants were told that the purpose of their intervention was to reduce the amount of time spent performing sedentary behavior (sitting, lying, or reclining) especially in prolonged uninterrupted bouts. Participants were instructed not to perform sedentary behavior for more than 30 minutes at a time, and that for every 30 minutes of sedentary behavior performed the participant should stand up and perform 2 minutes of upright LIPA (general ambulatory walking, side to side shuffling, washing dishes etc).

LIPA group: Participants were informed that the purpose of their intervention was to increase the amount of time spent performing LIPA whilst maintaining habitual routines. Participants were instructed to perform a continuous single bout of 45-50 minutes LIPA (general ambulatory walking, side to side shuffling, washing dishes etc), every day for the duration of the 8-week intervention.

Control group: Participants who were randomly allocated to the control group were specifically instructed to maintain their habitual routine. Control participants were told that the overall purpose of the study was to study the link between health and habitual activity profiles.

Statistical analyses

Statistical analyses were carried out using SPSS (Version 25, SPSS Inc., Chicago, IL, USA). Parametricity was checked through the Shapiro–Wilk test to determine data normal distribution and the Levene's test to determine equality of variances between groups. If parametric assumptions were met, baseline group differences were examined by a one-factor analysis of variance (ANOVA) (SBF, LIPA, CON) with post-hoc pairwise comparisons conducted using the Least Significant Difference. The effects of the interventions were determined using 2×3 split plot ANOVA (2 phases and 3 groups) or 2×4×3 (2 phases, 4 anatomical sites and 3 groups) split plot ANOVA depending on the outcome variable. Furthermore, linear regression analysis was performed on the relative changes from baseline for each muscle-tendon complex/ lean body mass outcome, and the relative changes from baseline for each functional performance outcome. GM muscle architecture data was not collected for 2 participants, meaning such analyses were carried out on a sub-sample (n=32). In cases of heteroscedasticity in variances, the Greenhouse Geisser correction was applied. In cases of non-normal distribution within group comparisons were made using the Wilcoxon-Sign Rank test, whilst, between group differences utilized a Kruskal-Wallis non-parametric equivalent of ANOVA (SBF, LIPA, CON) with post-hoc pairwise comparisons examined by Mann-Whitney U test. Chi-squared analysis was used to investigate nominal variables. Data are reported as Mean±SD (or Median, IQR for non-parametric data). Statistical significance was accepted when $P<0.05$. Furthermore, a statistical trend was deemed to be present when P was in the range of between 0.05 to 0.10. Study power (β) and effect size (η_p^2) are also reported where P is significant.

AUTHOR CONTRIBUTIONS

GOP, DT and KT designed the research; DG conducted the research; DG and GOP analyzed data; and DG and GOP wrote the paper. DG and GOP had primary responsibility for final content. All authors read and approved the final manuscript.

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CONFLICTS OF INTEREST

D Grant - no conflicts of interest. DJ Tomlinson - no conflicts of interest. P Kolic - no conflicts of interest. K Tsintzas - no conflicts of interest. GL Onambélé-Pearson – no conflicts of interest.

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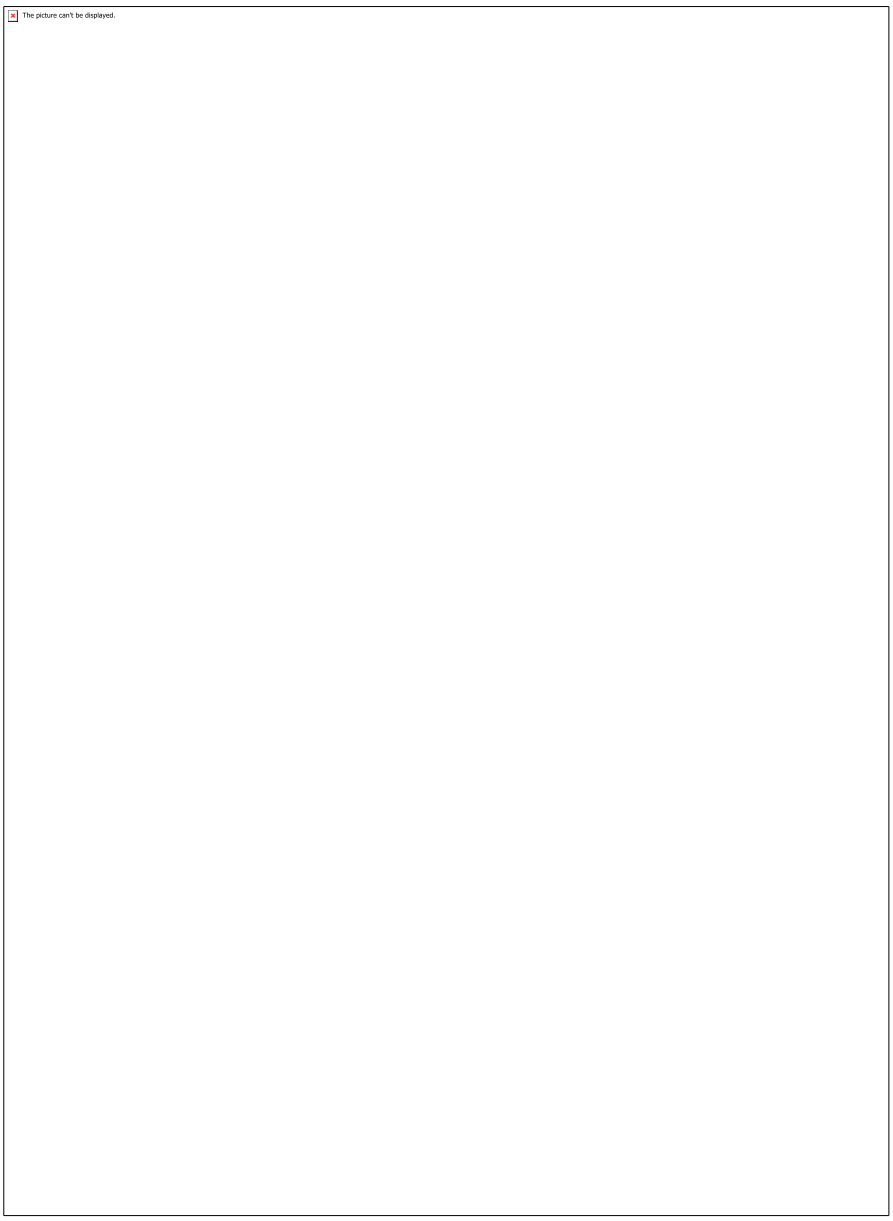
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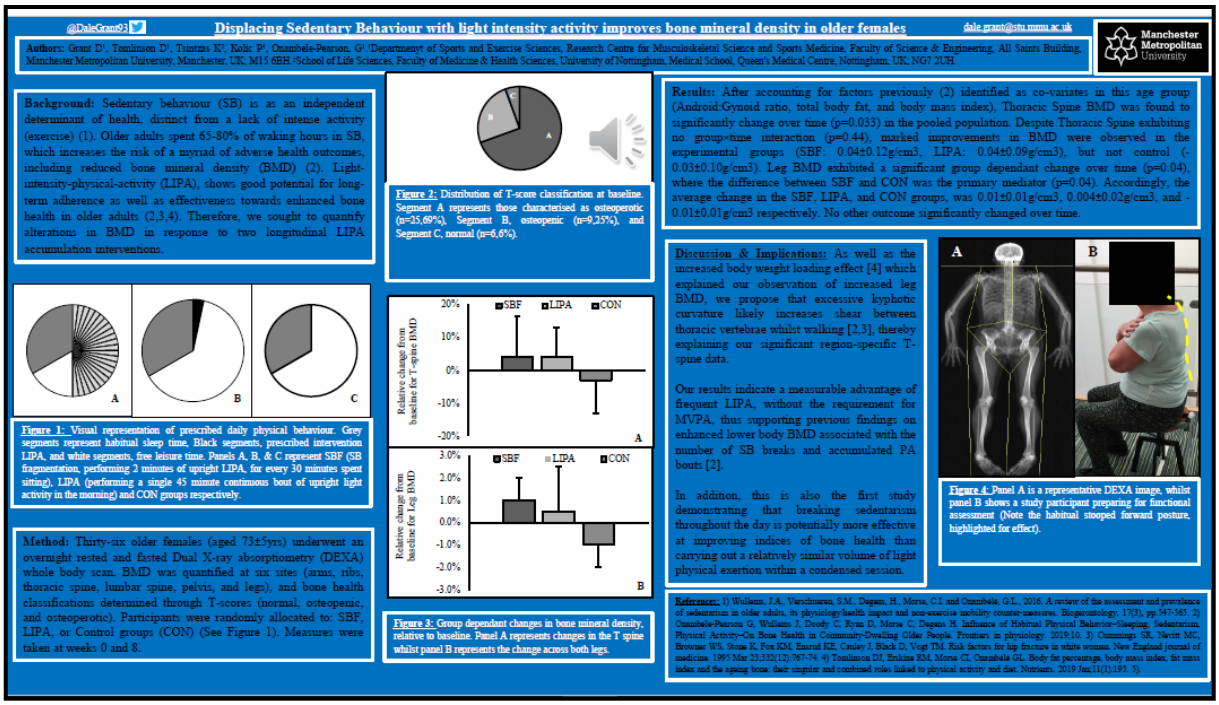
Research Outputs



Poster Title: ***Displacing Sedentary Behaviour with light intensity activity improves bone mineral density in older females***

Presented online at the **Bone Research Society Annual Meeting** (06/07/2020-08/07/2020) **https://boneresearchsociety.org/meeting/brs2020online/**