Influences of Carbohydrate plus Amino-acid supplementation on Differing Exercise Intensity Adaptations in Older Persons: Skeletal Muscle and Endocrine responses

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Short title: Adaptations to exercise and nutrition in the elderly

Abstract

Background: Losses in physiological function in ageing occur partly as a consequence of inadequate diet and inappropriate physical activity regimes. **Purpose:** The current study aimed to compare the effects of two different intensities of resistance training in healthy older adults, whose habitual dietary intake was supplemented with carbohydrate and amino-acid preparations. We hypothesized that the less intense exercise prescription would be similarly effective as the more intensive exercise prescription when both are combined with carbohydrate and amino acid supplementation, in terms of *in vivo* markers of healthy physiologic and endocrine functions in previously sedentary older individuals. **Methods:** Twenty-nine older adults (out of 32) completed the study after being randomly assigned to low (SUP_LowR, i.e. ~40% 1RM; n=16) versus high resistance training (SUP_HighR, i.e. ~80% 1RM; n=13) for 12 weeks. A carbohydrate supplement was ingested immediately before and during every exercise session. The post-exercise supplement was an amino-acids cocktail. **Results:** Neither intervention significantly impacted upon body composition markers including, BMI, waist:hip ratio and bioelectric impedance. Muscle strength data showed an advantage for the SUP_HighR protocol with 46 ± 8%, 10.8 ± 4.4% and 26.9% ($P<0.05$) improvements in 1-RM strength, unilateral and bilateral knee extension torque respectively, compared with 39 ± 2%, 9.4 ± 3.7% and 29.5% ($P<0.05$) increments in the same measures in the SUP_LowR group. Lean tissue data however showed a greater benefit of the SUP_LowR protocol (8.7 ± 3.9% increase, $P < 0.05$) compared with the SUP_HighR protocol (no significant change). In terms of functional abilities, only the Standing-from-lying (SFL) test exhibited an improvement in the SUP_HighR group (–11.4%, $P<0.05$). The SUP_LowR group on the other hand showed significant improvements in the get-up-and-go (8.7 ± 3.6%, $P<0.05$), the SFL (–4.7% change, $P = 0.05$) and the six-minute walk (7.2 ± 2.2%, $P < 0.01$) tests. Following overnight fasting serum levels of glucose changed significantly (~ 13 % decrease, $P < 0.01$) in SUP_LowR. Serum levels of insulin (~ 25 % decrease, $P = 0.05$), NPY (~ 35 % decrease, $P = 0.02$), and IGFBP-3 (~ 15 % decrease, $P = 0.03$), changed significantly in SUP_HighR. Circulating levels of IL-6, TNF-α and IGF-I did not alter significantly in either intervention group. **Conclusion**: These data suggest that whilst both interventions were beneficial in older persons, the end targets as well as possibly the mechanisms for the improvements are different. The supplementation plus low exercise regimen tended to impact on muscle hypertrophy combined with increased habitual function. Supplementation plus high intensity exercise regimen improved markers of strength, but not to a significantly greater extent than supplementation plus low intensity exercise.

Key words: Elderly, Endocrinology, Cytokines, Physiology, Resistance Exercise Intensity, Nutritional Supplementation.

Introduction

The evidence is that many of the biological changes, risks of, and occurrences in chronic diseases which have been attributed to ageing, are in fact caused by less than optimal nutritional intake (Blumberg, 1994; Vellas et al., 1997; Volkert et al., 1992). Indeed, ingested protein accounts for 30% of whole-body protein turnover, which is thought to decline by ~20% by 70-years of age (2001). As a consequence of a lack of dietary protein a loss in physiological function can occur, with the body seeking to re-establish a steady metabolic state. Castaneda et al. (1995) found that elderly women who consumed insufficient protein (56% RDA) over a 9-week period lost ~9% lean muscle mass (sarcopenia), thus compromising their skeletal muscle functional capacity, as a direct consequence of diminished protein consumption. Thus, optimal hypertrophy of skeletal muscle after resistance training requires the maximal stimulation of protein synthesis; this may particularly be the case in the elderly (Esmarck et al., 2001). In a study by (Balagopal et al., 1997) ageing-related decrement in myosin heavy chain (crucial in muscle contractile function) synthesis rate, were reported in 24 participants aged 20-92 years. These results imply a decreased ability to remodel this essential protein with age, which may contribute directly to decreases in muscle mass and muscle contractile function. Hence, attempts to improve the quality of life and to prevent/delay age-related sarcopenia in populations aged 60 and above, should involve tackling the issue of less than optimal nutritional intake or 'sub-nutrition' in this group. Indeed the evidence for increased health benefits with protein only (Jensen & Hessov, 1997), or protein plus carbohydrate (Gray-Donald et al., 1995) supplementation for instance, has been shown.

Amino acids, even above the RDA (1-1.3 versus 0.8 kg/day) are potent and safe stimulators of muscle protein synthesis (Bohe et al., 2003) and hence muscle mass increases in the young, the elderly and the frail elderly (Jensen & Hessov, 1997); (Lauque et al., 2000), provided the person's health status (Tobin & Spector, 1986), as well as calcium intake (Lucas & Heiss, 2005), are both adequate. However, due to impacts on satiety, ingestion of nutritionally balanced supplements has often been found to reduce the caloric intake of the rest of the food consumed in a day, by an amount equivalent to the calories supplied in the supplement (Fiatarone et al., 1994). Therefore such dietary supplements in the elderly end up being dietary substitutes and do not fulfil their desired goal. On the other hand, the ingestion of only essential amino acids (EAAs) has been found to be enough to stimulate muscle protein synthesis in this age group (Paddon-Jones et al., 2006; Volpi et al., 2003; Volpi et al., 1999), without the negative impact on habitual food intake seen with bulkier

preparations. In summary, following adequate health check ups, it would appear that improved nutritional balance in otherwise undernourished older persons will have beneficial consequences in terms of muscle protein adaptations and hence sarcopenia.

That resistance exercise is also important to combat age-related sarcopenia has been demonstrated (Frontera et al., 1988; Klitgaard et al., 1990). Indeed, provided the stimulus is sufficient and exceeds the rate of protein degradation, net muscle protein synthesis and hence positive balance (i.e. when synthesis outweighs breakdown) is increased after a bout of exercise (Short et al., 2004). Despite these compelling studies, any added benefit of combining exercise and supplementation regimens is yet to be fully assessed, particularly for older populations. Nevertheless, in support of the potential of combining these two interventions, a study by Levenhagen et al. (2002) showed that amino acid availability is more important than the availability of energy *per se* for post-exercise repair and synthesis of muscle proteins. Furthermore, studies also exist, which show that supplements alone have little or no effect on muscle strength and mass without concomitant exercise interventions in frail elderly (Bonnefoy et al., 2003; Fiatarone et al., 1994; Rosendahl et al., 2006) and otherwise undernourished sedentary individuals (Bonnefoy et al., 2003; Fiatarone et al., 1994; Rosendahl et al., 2006).

The current study therefore aimed to determine the impact of nutrition and exercise in an otherwise healthy older population, and to assess whether combining nutritional supplementation (i.e. carbohydrate plus amino-acids) with high vs. low resistance exercise, would have comparable effects on several factors associated with physical wellbeing.

Methods

1-Participants

Thirty-six older adults volunteered to participate in the current study having responded to advertisements posted locally. Four of these prospective volunteers were subsequently excluded from this study as they had a known history of, either kidney, liver, cardiovascular, neurological, inflammatory or myopathic disease. The remainder of the participants gave written informed consent to take part in the study after they had obtained medical clearance to participate from their general practitioner. Thus the current study included relatively healthy, community dwelling, and

habitually active individuals, with no recent history of structured resistance training. The local Human Ethics Committee approved all experimental procedures.

Of the 32 healthy older adults who started the study, 29 completed the 12-week 'combined exercise + supplementation' intervention. As a result of adjustments owing to one participant's own preference, as well as drop out rates, the study was completed with 13 'combined supplementation + low' (SUP_LowR) and 16 'combined supplementation + high' (SUP_HighR) resistance-training groups: The characteristics of the completing participants are presented in table 1.

→ *[Table 1 near Here]*

2-Muscle Strength Measurements

2a. One Repeated Maximum Measurement- During a familiarization session no more than 7-days prior to the 12-week intervention, participants' 1 repetition maximum (1RM) was determined for all exercises employed in the training programme. Participants first performed a standardized warm-up on the leg press (6 × 50% perceived 1RM; 4 ×70% perceived 1RM with three min recovery). After warming up, the load was set at 90 % of the estimated 1RM, and increased after each successful lift by 5kg until failure. Each participant was given six lifting attempts in order to achieve their 1RM and a maximum of two attempts to lift the chosen weight, once it had been established. The greatest amount of weight lifted successfully was recorded to determine the training load. Between successive attempts, 3 min rest periods were allowed. A repetition was valid if the participant used correct form and was able to complete the entire lift in a controlled manner without assistance. Participants 1RM for each exercise was reviewed every 2-weeks during training, if 1RM had increased the training load was adjusted accordingly. Additionally if any participants felt that in-between 1RM assessments the training load was not providing adequate resistance, the load was increased so they were always lifting at the desired percentage of their maximum. Participants were familiarized with the resistance exercise training protocol on a subsequent visit to the laboratory.

2b. Isometric Knee extensors Muscle Strength Measurements- Participants were familiarized with the experimental procedures on a separate occasion no more than 7 days prior to the baseline test sessions. In one single testing session quadriceps isometric strength measures were taken on the right leg and isokinetic measures on the left leg, using a Cybex Dynamometer (Cybex Norm, Cybex International Inc., NY, USA). The centre of rotation of the lever arm of the dynamometer was

aligned with the axis of rotation of the knee. Participants were positioned with the hip joint at 85° (supine = 0°). In order to minimise any extraneous movement of the hip joint or the trunk, participants were strapped over the shoulders, pelvis and thighs. Settings of chair height and positioning relative to the dynamometer were adjusted individually with all settings recorded and replicated at the post-intervention testing phase. Gravity corrections were then made following the manufacturers' own procedure, having adjusted the attachment of the lever arm cuff relative to the length of the participant's shank. Previous work from within our laboratory has utilized similar methods of measurement, finding them to be both valid and reliable (Pearson & Onambele, 2005, 2006).

2b-I- Maximal Unilateral Isometric Torque: Maximal isometric knee-extension torque was measured with the knee at 70° angle (full knee extension = 0°) on the right leg of all participants. After a series of warm-up trials consisting of 10 isokinetic contractions at $60°s^{-1}$ at 50-75% maximal effort, participants were instructed to rapidly exert maximal isometric force against the Cybex lever arm over a 3-4 s period. Participants were given both verbal and visual encouragement/feedback throughout their effort. Joint torque data were displayed on the screen of a Macintosh G4 computer (Apple Computer, Cupertino, CA), which was interfaced with an A/D system (Acknowledge, Biopac Systems, Santa Barbara, CA) with a sample frequency of 500 Hz. Isometric contractions were held for ~2 s at the plateau, with a 90 s rest period between contractions. Peak torque was averaged over a 500 ms period at the plateau phase. The mean peak torque of three extensions was used as the measure of strength in each participant.

2b-II- Maximal Bilateral Isometric Torque- Maximal bilateral isometric knee-extension torque was measured with the two knees at 70° angle (full knee extension = 0°) pushing against a customized lever arm. Similar precautions to record time of day were taken. After a series of warm-up trials consisting of 3 isokinetic contractions at $60°s\text{-}^{1}$ at 50% maximal effort, participants were instructed to rapidly exert maximal isometric force against the Cybex lever arm over a 3-4 s period. Participants were given both verbal and visual encouragement/feedback throughout their effort. Joint torque data were acquired and processed as during the unilateral efforts, with the only difference that here the best of the three efforts was used as the measure of strength for each participant.

3-Mid-thigh Lean Tissue Thickness

Real-time B mode ultrasonography with a 7.5 MHz linear-array probe (AU5, Esaote, Genoa, Italy) was used to study mid-thigh muscle thickness in the area of the Vastus Intermedius (VI) and the Vastus Lateralis (VL) muscles. Muscle thickness was taken at rest with the knee at 70° angle. Scans were acquired in the mid-sagittal plane, at approximately 50% length of the VL muscle as measured from the origin at the linea aspera & lateral femur to insertion at the tibial tuberosity via patella tendon on the anterior surface. Medio-lateral width of the VL was determined over the skin surface and the position of one half of the width was used as the measurement site. The ultrasound probe was coated with water-soluble transmission gel to provide acoustic contact and was held in place without depressing the dermal surface by the experimenter. Three separate recordings of mid-thigh muscle thickness were made with 90 s rest between each recording. Ultrasound images were acquired using a digital recorder and frames exported to capture software (iMovie HD6, Apple computer Inc, USA). The VL thickness was measured as the distance from the top of the peripheral muscle aponeurosis to the deep aponeurosis. VI thickness was from the deep aponeuroses to the surface of the femur. The three points of interest were measured at 3 standardised points on each ultrasound frame to obtain an average tissue thickness using ImageJ analysis software (ImageJ 1.37, Maryland, USA). Total muscle thickness was computed as the sum of VI+VL muscle thickness measured.

4-Body Composition

4a. Waist:Hip Ratio- A measuring tape was used to measure the circumference of the hips at the widest part of the buttocks, and the waist at the smaller circumference of the individual's natural waist (just above the navel). The waist:hip ratio was used as the individual's score of central adiposity.

4b. Bioelectrical Impedance Analysis (BIA) (BODYSTAT, Isle of Man, British Isles) was used to estimate body composition based on the difference in electric conductive properties of various tissues. The BODYSTAT applied 500 micro Amps at a single frequency of 50 kHz through self adhesive electrodes placed on the right hand and foot, of a participant lying flat on their back with their arms away from the trunk, thighs not touching and ankles at least 20cm apart for at least 5 min. prior to any measures being recorded. Although BIA has acknowledged limitations when applied to

non-standard or elderly populations due to the built in equation utilised to calculate body composition, it is important to state that the BIA in this study was used to assess within participant adaptations as a consequence of the intervention, and as such carries more validity. What is more, to lend greater external validity to data from this instrument, raw values were corrected for the inherent 15% overestimation in body fat content (when comparing pilot participants BIA versus DEXA outputs in our laboratories – others have drawn similar conclusions regarding the differences in body fat content values depending on the methodology used (Jorgensen et al., 1996); (Lintsi et al., 2004)and have tended to consider DEXA readings as gold standard since it yields values similar to those obtained from the hydrostatic weighing method (Prior et al., 1997).

4c. Body Mass Index (BMI) was defined as the individual's body mass (Kg) divided by the height in m^2.

5- Functional Ability Measures

The tests of functional ability were all performed on one day at the outset and completion of the study and were similar to a battery of functional tests performed by participants in previous research (Chandler et al., 1998; Skelton et al., 1995).

5a. Get-Up-and-Go- Three cones were placed 1m apart on the floor in front of a rigid chair of adjustable height. The participant's knee-to-floor height (i.e. the distance from the knee joint axis to the floor) was recorded prior to testing to determine the appropriate chair height for each test. Once knee height was determined the chair height was set at 100, 80 and 60% of each individual knee-to-floor height for the tests. The test started with the participant seated on the chair (at the appropriate height), with feet flat on the floor and arms folded across the chest. They were then asked to rise unaided as quickly as possible, walk around the furthest cone (3m away) and back to the initial seated position on the chair. The elapsed time between the chair rise to sitting back down was recorded. The quickest of three trials was used as the participant's score.

5b. Standing from Lying (SFL) - Participants were asked to lie flat on the floor on a gym mat and on their preferred side, with their arm on the floor outstretched and their head resting on the outstretched arm. Participants were then instructed to rise as quickly as possible using their preferred technique. The time elapsed between the instruction to 'Go' and the participant standing

upright and steadily with both feet firmly on the ground, was recorded. The fastest of 3 trials was utilised as their score.

5c. Six-minute Walk- A 10m course was set with cones 1m apart in a straight line. Participants were instructed to walk around this course using their fastest, non-running, walking pace, with the aim of completing as many revolutions of the circuit as possible in 6 minutes. The score was calculated as the total distance covered in the allocated time.

6- Endocrine profiling

At the onset and end of the intervention and following an overnight fasting period, participants reported to the laboratory. A 21-gauge 1-inch ultra thin wall needle (Terumo medical corporation, New Jersey, USA) was inserted into the anticubital vein of the forearm. Using a vacutainer assembly and serum separator tubes (Monovette, Sarstedt, Numbrecht, Germany), 10mL blood samples were collected. Blood glucose was analysed immediately using a single drop of freshly sampled blood using the AccuChek Advantage System (Roche Diagnostics Ltd, Lewes, UK. Sensitivity of <10 mg/dL (i.e. minimum detectable concentration); Intra-assay variability of 2% (i.e. coefficient of variation)). The remainder of the sample was centrifuged at 2-5°C for 5 minutes at 4000 rpm, with the supernatant being removed and stored in eppendorfs at -70° Celsius for later analyses. Insulin (Biosource, Nivelles, Belgium. Sensitivity of 0.15 µlU/ml; Intra-assay variability of 4.2%). IGF-I (Biocode-Hycel, Liege, Belgium. Sensitivity of 4.9 ng/ml; Intra-assay variability of 8.0%). TNF-α (Diaclone, Besancon Cedex, France. Sensitivity <8 pg/ml; Intra-assay variability of 3.3%). NPY (Phoenix Europe GmbH, Karlsruhe, Germany. Sensitivity of 0.13ng/ml; Intra-assay variability <5%). IGFBP-3 (Biocode-Hycel, Liege, Belgium. Sensitivity of 10.5ng/ml; Intra-assay variability of 6.5%). Finally IL-6 (Diaclone, Besancon Cedex, France. Sensitivity <0.8 pg/ml; Intra-assay variability of 3.3%) were analysed using standard enzyme-linked immuno-sorbent assay (ELISA) procedures.

7- Training Programme

The training programme was 12-weeks in duration and consisted of one supervised gym-based class and two home-based sessions per week in LowR. In HighR the programme was for two supervised gym-based classes and one home-based session per week. All exercise sessions were 1 hr in duration.

Briefly, the supervised exercise classes consisted of a warm-up (stretching, aerobic and coordination work), resistance exercises (using therabands for all major muscle groups, Leg Press, Leg Extension, Calf Rotator and Glute conditioner (Technogym, Gambettola, Italy), with a progression from 8-11 reps in 2-4 sets at 40 or 80% 1RM), and a cool-down (i.e. stretches, pilates, Tai Chi). The unsupervised home-based exercises were similar in design to the supervised classes with the exception that all the resistance work was carried out using therabands, and a 20-min brisk walk was also included. An exercise booklet illustrated, using photographic and/or cartoons, all the exercises in detail. Home-based exercise was not to be performed the day preceding or following the supervised class exercise.

8- Nutritional Supplementation

All participants received the supplement, and all were administered the same dosage, regardless of the exercise intensity they had been allocated to carry out. Administration of the combined treatments is illustrated below in figure 1.

→ *[Figure 1 near Here]*

8a. Carbohydrate Supplementation- This was provided in the form of a 500mL (containing 26g of carbohydrates), orally administered isotonic Lucozade drink (Kindly donated by GlaxoSmithKline, Basildon, Essex, England). Participants were instructed to ingest 250mL of the drink upon arrival for each training session prior to participating in any exercise, and to consume the rest of the beverage during the exercise session. For home exercise sessions participants were given a personal stock to keep at home.

8b. Protein Supplementation- Supplementation of protein came in the form of an orally administered mixed amino acid supplement (Bodyfortress, Holland & Barret, Warwickshire, UK). Participants were instructed to ingest the supplement within 15 mins post-training mixed with water. Each participant ingested a two-table spoon dose of supplement containing 22g of essential amino-acids. As with the carbohydrate supplement, for the home-based exercise sessions participants received a personal stock of the amino acid supplement and were given clear instructions regarding the dosage and mixture of the formula. Demonstrations of the correct dose measurement were given at all face-to-face sessions.

Statistical analyses

T-tests were carried out to compare data at baseline. Two-way factorial ANOVAs were carried out with group as one factor (two levels: SUP_HighR vs. SUP_LowR) and phase as the second factor (two levels: baseline vs. post-intervention) to determine any main effects of the group or interventions. Data are expressed as mean ± S.E (standard error) unless otherwise stated. Significance was set at $P \leq 0.05$.

Results

The two intervention groups did not differ in age, habitual physical activity levels, height or weight at the onset of the current programme. Post-intervention, both groups showed slight tendencies towards increased body mass (SUP_HighR = ~ 2.6% vs. SUP_LowR group = ~ 0.9%), though this effect was not significant.

Body composition changes

As a consequence of unaltered body weight, no significant difference in BMI was observed in the two groups after the 12-week interventions (26.5 ± 0.9 to 26.7 ± 1 in SUP_LowR and 26.4 ± 1.3 to 26.5 ± 1.2 in SUP_HighR). What is more, there was no change in waist:hip ratio in either the SUP_LowR population (0.91 ± 0.02 to 0.87 ± 0.02) or the SUP_HighR population (0.91 ± 0.03 to 0.89 ± 0.03). In terms of body-fat percentage, neither SUP_LowR (28.3 ± 2.0% to 27.5 ± 2.0%), nor SUP_HighR populations (26.0 ± 2.4% to 27.2 ± 2.5%) showed any changes.

Knee extensors muscle strength changes

a) 1-RM - Prior to training there were no significant differences in mean 1RM strength of the leg press, leg extension or glute conditioner between groups. Ankle rotation was different between the groups at baseline. At 12 weeks, the average strength increase for each exercise was significant for both intervention groups ($P < 0.05$). However the increase for SUP_LowR was less (though not significantly so) pronounced in all exercises compared to SUP_HighR (see table 2). The average 12-week increase in strength was 39 ± 2% (i.e. 20.3 ± 4.2 Kg) for SUP_LowR and 46 ± 8% (i.e. 20.3 ± 5.0 Kg) for SUP_HighR. This effect was present even after accounting for baseline difference in ankle rotator measures.

→ *[Table 2 near Here]*

b) Isometric: Unilateral and Bilateral torque- Prior to training there was no significant difference in either the mean isometric unilateral (MVCuni-ext) or bilateral knee extensor strength (MVCbilat-ext) between the two intervention groups. Table 2 shows the mean changes in torque after the 12-week interventions in each group. Briefly, unilateral isometric torque increased significantly post interventions. Indeed the SUP_LowR group exhibited an increase in MVCext of 9.4 ± 3.7% (124.8 ± 8.6Nm to 135.3 ± 9.3Nm; $P = 0.04$). Similarly, SUP_HighR showed an increase in MVCuni-ext of 10.8 ± 4.4% (121.6 ± 13.8Nm to 137.6 ± 18.8Nm; $P = 0.04$). The 12-week interventions also resulted in similar and significant increases in bilateral torque in both SUP_HighR (26.9% $P \leq 0.05$) and SUP_LowR (29.5% $P \leq 0.05$) groups.

Mid-thigh lean tissue thickness

Total muscle thickness (VL+VI) was significantly increased in the SUP_LowR participants by 8.7 ± 3.9% (33.6 ± 1.7mm to 36.1 ± 1.4mm; $P < 0.05$). SUP_HighR exhibited no change in total muscle thickness (36.1 ± 2.2mm to 35 ± 2.2mm; NS).

Functional measures

a- Get-Up-and-Go- At baseline there was no significant difference in reaction time (as determined by the get-up-and-go (GUG) test) between the two groups. GUG time significantly improved by 8.7 ± 3.6% (5.7 ± 0.52 sec to 5.1 ± 0.2 sec) in the SUP_LowR population. However, SUP_HighR did not significantly change their performance in this test (5.1 ± 0.4 sec to 5.1 ± 0.4 sec).

b- Standing from lying (SFL) - Functional power determined by SFL time showed significant improvements in both SUP_HighR (3.33 ± 0.41 to 2.67 ± 0.29, i.e –11.4% change, $P<0.05$) and SUP_LowR (3.26 ± 0.32 to 3.00 ± 0.23, i.e –4.7% change, $P = 0.05$) populations.

c- Six-minute walk- The distance covered during the six-minute walk test was increased by 7.2 ± 2.2% (27 ± 1m to 28.7 ± 0.7m) in the SUP_LowR population ($P < 0.003$). The SUP_HighR participants exhibited a non-significant 3.1± 2.7% (26.3 ± 1.8m to 26.8 ± 1.7m) change in distance covered.

Endocrine characteristics changes

Summary of the findings in terms of endocrine changes (i.e. post-intervention values normalised for baseline values) are summarised in figure 2.

→ *[Figure 2 near Here]*

Metabolic profile – Plasma levels of Glucose and Insulin

At baseline, there was a significant difference in the mean plasma glucose levels between intervention groups. The 12-week intervention resulted in no change in plasma glucose in the SUP_HighR group (4.77 ± 0.16 mmol/L to 4.79 ± 0.18 mmol/L). Interestingly, the SUP_LowR group exhibited significantly lower post-intervention plasma glucose of -13 ± 4.7% (5.45 ± 0.18 mmol/L to 4.71 ± 0.18 mmol/L). Even after accounting for the baseline differences, the difference in intervention-induced changes in glucose levels was significant ($P = 0.031$).

Prior to training there was no significant difference in the mean plasma insulin levels between the two intervention groups. The 12-week intervention resulted in a trend for decreased plasma insulin level in SUP_LowR (17 ± 6.7% i.e. 8.8 ± 1.2 mmol/L to 7.2 ± 0.9 mmol/L; $P = 0.08$). SUP_HighR group on the other hand exhibited a significant 25 ± 5.3% decline (i.e. 11.68 ± 0.42 mmol/L to 8.79 ± 0.83 mmol/L; $P = 0.05$) in plasma insulin levels.

Control of energy homeostasis in relation to metabolic status- Plasma levels of NPY

Prior to training there was no significant difference in the mean plasma NPY levels between the two intervention groups. The 12-week interventions resulted in a significant decrease in plasma NPY

levels for SUP_HighR (-35.0% change (i.e. 24.7 ± 4.26 ng/ml to 16.1 ± 1.90 ng/ml), P = 0.016) but no change in SUP_LowR groups (+1.3% change (i.e. 19.0 ± 1.72 ng/ml to 19.3 ± 1.8 ng/ml).

Protein synthesis potential - Plasma levels of IGF-I and IGFBP-3

Serum levels of IGF-I did not differ significantly between the two groups at baseline. Post intervention, values did not change significantly in either group (318.3 ± 39.9 ng/ml at baseline to 282.0 ± 26.5 ng/ml post training in the SUP_LowR group, and 309.9 ± 35.1 ng/ml at baseline to 322.8 ± 25.1 ng/ml post training in the SUP_HighR group).

Serum levels of IGFBP-3 were similar in the two groups at baseline. Post intervention, in the SUP_HighR group, values changed significantly from 3716.6± 507.5 ng/ml at baseline to 3171.1 ± 370.2 ng/ml post training, a –14.7% (P = 0.03) decrease with the intervention. However in the SUP_LowR group, values did not change significantly with the intervention whilst increasing from 4252.6 ± 314.0 ng/ml at baseline to 4506.1 ± 273.7 ng/ml post training, a 6.0% but non-significant change. The difference in changes seen in the two groups was significant (P=0.049).

Cellular degradation/inflammation signal - Plasma levels of TNF-α and IL-6

Serum levels of TNF-α did not differ significantly between the two groups at baseline. Post intervention, values did not change significantly in either group (29.3 ± 8.2 pg/ml at baseline to 31.4 ± 7.1 pg/ml post training in the SUP_LowR group, and 35.4 ± 13.7 pg/ml at baseline to 27.6 ± 11.8 pg/ml post training in the SUP_HighR group).

Similar to the observations on TNF-α, serum levels of IL-6 did not differ significantly between the two groups at baseline, nor did the interventions have any significant effects on the amount of circulating IL-6. Indeed values changed from 3.4 ± 0.6 pg/ml at baseline to 2.6 ± 0.3 pg/ml post training in the SUP_LowR group, and 2.31 ± 0.32 pg/ml at baseline to 2.08 ± 0.26 pg/ml post training in the SUP_HighR group.

Discussion

Protocol design

The current study proposed to systematically quantify the changes associated with two degrees of lifestyle changes in an otherwise healthy older population, comparing 1) a high intensity protocol which combined nutritional supplementation (i.e. carbohydrate plus amino-acids) with high intensity resistance exercise, twice supervised and once self-directed each week, and b) a lower intensity protocol which combined nutritional supplementation (also carbohydrate plus amino-acids) with low intensity resistance exercise, once supervised and twice self-directed each week. Notably here, we used amino-acid rather than proteins to a) diminish the likelihood of any deleterious impact on amount of habitual food intake (Fiatarone Singh et al., 2000), but also b) as amino-acids have been shown to be more effective for required end results (e.g. increased net muscle protein synthesis) than protein preparations in this age group (Fiatarone Singh et al., 2000; Paddon-Jones et al., 2005). Crucial also to the study design, was the issue of timing of supplementation. In a recent study of older persons aged 70-80 years (Esmarck et al., 2001), it was demonstrated that the timing of a carbohydrate + protein supplement ingestion affected the degree of response to the treatment in that increments in the CSA and strength of the quadriceps femoris as well as the mean fibre area in this muscle, were greater where ingestion was immediately after exercise. Arguably the timing of the protein supplement is key, hence the current study required for participants to adhere to a 15mins post-exercise window.

Summary of findings in view of the study hypotheses

The principal hypothesis of the current study was that replacing high with low resistance activity in the combined therapy groups may not impact on outcome and indeed several factors associated with physical wellbeing may be comparably improved. We also aimed to discuss our findings in the context of recent publications on the benefits of exercise-only and/or combined-exercise-supplementation therapies in older persons. Our findings show that whilst muscle strength data (two of three strength parameters) showed a slight (but not significant) advantage for the SUP_HighR protocol, functional abilities tests showed a significant advantage in following the SUP_LowR protocol. Linked to these observations, lean tissue data in fact showed a greater benefit of a SUP_LowR protocol, as did the endocrine measures. In terms of the latter, metabolic (a combination of glucose, insulin and NPY effects), pro-protein synthetic (a snapshot of IGF-I and IGFBP-3), and pro-protein degradative (as shown through IL-6 and TNF-α trends) effects did not tend to favour either protocols in particular.

Muscle size and strength changes

Cuthbertson and colleagues (2005) have previously demonstrated an anabolic signalling deficit to EAA ingestion in older adults and stated that a deficit in protein synthesis in the basal state is unlikely. These authors attributed the loss of muscle mass with age to decreased sensitivity and responsiveness of muscle protein synthesis to EAAs. It was also proposed that this lack of responsiveness may be associated with decrements in the expression and activation of components of anabolic signalling pathways, and hence, this may be a major contributor to the failure of muscle maintenance in older adults. Previous studies in older adults have shown that provision of dietary supplements has not been effective in improving lean body mass (Campbell et al., 1995; Welle & Thornton, 1998). What our data seem to suggest is that different adaptations may occur when combining exercise (intensity) and EAA ingestion. We propose that a) in the presence of relatively low exercise levels, there is no impairment in protein synthesis in the older age group, b) if exercise intensity is high, muscle strengthening routes appear to favour other, non-hypertrophic (at least in the first few weeks) as yet unidentified factors (likely neural related (Onambélé et al., 2008; Onambele et al., 2006). Our data in the SUP_HighR group in fact mirror observations made by Borsheim and colleagues (2008) who found that 16-weeks of EAA supplementation (total 22g per/day) – with no exercise, increased 1RM in the order of 22% in older adults, but did not significantly increase muscle mass.

Molecular background of the observed in vivo muscle characteristics

A key intracellular pathway coordinating signals in the regulation of protein synthesis is the mammalian target of rapamycin or mTOR (Bodine et al., 2001), the signalling of which to its downstream effectors, ribosomal S6 kinase 1 (S6K1) and 4E binding protein 1 (4E-BP1), plays a key regulatory role in the regulation of translation initiation, an initial step in protein synthesis (Wang et al., 2006). Following resistance exercise in animals and humans, components of the mTOR pathway are rapidly up-regulated (Bolster et al., 2003). Whilst the acute muscle protein synthesis (MPS) response after resistance exercise and EAA ingestion is similar between the young and the old, the response is in fact delayed in aging (Dreyer et al., 2008). It is thought that the lack of specificity in older muscle signalling may be the reason for the delay relative to younger persons' MPS signalling. Others have proposed 'potential mechanisms through which mechanical signals and insulin-like growth factor (IGF)-I-derived signals may be mediated through overlapping

pathways' (Tidball, 2005). However, our data in terms of changes in IGFBP-3 (which showed a trend for increase (+6%) in SUP_LowR and a significant decrease (-14.7%) in SUP_HighR) would tend to support the above theorem in that overall ageing would seem to be associated with a lessened response in this system. Furthermore, total IGF-I levels were unchanged, therefore questioning the mechanisms culminating in the small but significant (in SUP_LowR) and non-existent (in SUP_HighR) hypertrophic responses in the presence of exercise, when this is supplemented with EAAs. The changed ratios of IGFBP-3 to IGF-I, however, may favour IGF stability in the SUP_LowR group, thus underpinning the small hypertrophy response. However, the, the pathways accounting for improved functional adaptations in the absence of extensively altered size warrants further investigations. Critically, we must not forget that hormonal measures were made at the beginning and end of the trial and therefore do not report early adaptations which may or may not have occurred.

Body composition and metabolism at the whole body as well as endocrine level

(Meredith et al., 1992), in a study of the effects of a protein + carbohydrate supplement cocktail with heavy resistance training, found that the supplemented group demonstrated significantly increased weight, skin fold thickness and subcutaneous mid-thigh adiposity. Our results show no effect on BMI, waist:hip ratio or bioelectric impedance measures, although the trend towards increased body mass in SUP_HighR, combined with no change in thigh lean tissue content tended in fact to indicate increased adiposity with this intervention. Positively for the SUP_LowR group however, the combination of observed trends for increased body mass, decreased waist:hip ratio, decreased bioelectric impedance fat tissue measurement, and significantly increased thigh lean tissue content, is suggestive of improved body composition.

In previous studies, both increased carbohydrate and increased protein supplementation have individually demonstrated positive effects on of insulin sensitivity (Baba et al., 1999; Solerte et al., 2004). Similarly exercise, and in particular cardiovascular workout (Tonino, 1989), has been linked to improved insulin sensitivity. Therefore, the potential for a positive outcome in terms of insulin sensitivity through the combined interventions was investigated. Our data were encouraging in that both levels of exercise were associated with decreased insulin levels (significantly so for SUP_HighR) and these were coupled with glucose effects which also had a potential to indicate increased health (no change in glucose for SUP_HighR, significant decrement for SUP_HighR).

Some have suggested the loss of muscle mass commonly associated with the aging process is a relative resistance to insulin stimulated amino acid uptake and stimulation of muscle protein synthesis (Volpi et al., 2000). Insulin itself is a known regulator of protein metabolism. However, the manner by which insulin promotes anabolism in human skeletal muscle is unresolved. Available data shows that insulin activates several proteins (e.g., phosphotidylinositide-3- kinase), that cause downstream phosphorylation of factors known to play key roles in regulating protein and glycogen synthesis (Kimball & Jefferson, 1994). Moreover, insulin has been shown to attenuate ubiquitin proteolysis (Roberts et al., 2003), which is believed to be responsible for the degradation of the bulk of muscle proteins (Ventadour & Attaix, 2006). Following resistance type exercise insulin appears to have little effect on protein synthesis. This may be due to a reduction of amino acids in the intracellular pool, if insulin does indeed reduce muscle protein breakdown. In addition, post-exercise insulin concentrations elevated independently of amino acid availability, through carbohydrate ingestion alone, have been found to reduce proteolysis and promote muscle protein accretion (Borsheim et al., 2004). Thus, the present study findings of improved functional ability and gains in strength may be due to improvements in glucose and insulin profiles promoted through amino acid -carbohydrate feeding and resistance training programme.

Functional abilities changes with exercise training

The energy required to build new tissues appears to increase with age (Shizgal et al., 1992). Yet the direct impact of supplementation-only interventions on habitual functional ability tends to vary from limited (Gray-Donald et al., 1995) to non-existent (Payette et al., 2002). In fact, prior to the current study, any validation of the use of EAAs (supplements in combination with resistance training) in improving functional abilities in older adults was scarce, and tended to be limited to frail older persons (Fiatarone et al., 1994), rather than the comparatively healthy group in the current study. In fact, where it existed in healthy populations, earlier evidence was as contradictory as in frail persons, with some studies showing no additional benefit (Rosendahl et al., 2006) and other highlighting clear benefits (Bonnefoy et al., 2003) of supplementation on improvements in functional abilities. Further than this, our data would suggest that background physical activity levels need to be high (to stimulate protein synthesis response) but not so high (as to impede the potential of the combined, or indeed each separate therapy through inducing a competition), to improve markers of increased independence in old age.

Conclusion

The current data suggest that whilst both levels of lifestyle changes are beneficial to older person, the end target as well as possibly the mechanisms for the improvements is different. The supplementation plus low exercise regiment effects tend to be on muscle hypertrophy combined with increased habitual function. With a supplementation plus high intensity exercise regiment, markers of strength are greatly increased (but not significantly higher compared with low intensity exercise), suggesting, together with the endocrine data, a preferred metabolic and/or possibly neural route for its effect, which warrant further investigation.

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Tables, Figures and Legends

Table 1: Completing participants' baseline characteristics. Means ± S.D.

Group	Gender (N)	Age (Yrs)	Activity (mins/wk)	Height (m)	Weight (Kg)
SUP_LowR	Male (9)	71.8 ± 3.7	360.0 ± 167.4	1.77 ± 0.08	82.2 ± 11.5
	Female (9)	72.6 ± 5.9	319.6 ± 202.4	1.61 ± 0.07	70.8 ± 14.9
	Both (18)	**71.8 ± 4.8**	**339.8 ± 184.9**	**1.69 ± 0.08**	**76.5 ± 13.2**
SUP_HighR	Male (7)	68.9 ± 5.2	277.5 ± 89.3	1.80 ± 0.07	85.6 ± 13.9
	Female (6)	67.2 ± 5.0	227.5 ± 145.0	1.58 ± 0.08	65.7 ± 13.1
	Both (13)	**68.1 ± 5.1**	**252.5 ± 117.2**	**1.69 ± 0.08**	**75.5 ± 13.5**

Table 2: Change in 1RM load lifted (Kg), and maximum isometric torque (Nm) from pre- to post 12-week intervention. *Indicates significantly lower baseline value in SUP_HighR compared to SUP_LowR ($P < 0.05$). Values are Mean ± S.E.

	SUP_LowR			SUP_HighR		
	PRE	POST	Δ (%)	PRE	POST	Δ (%)
Leg Press (Kg)	85.2 ± 8.1	121.1 ± 7.7	**42**	86.1 ± 6.3	124.4 ± 6.8	**44**
Leg Extensor (Kg)	31.4 ± 2.3	42.1 ± 2.3	**34**	26.3 ± 2.4	40.1 ± 3.2	**52**
Calf Rotator (Kg)	37.2 ± 3 *	53.5 ± 2.8	**44**	29.8 ± 1.8	50.1 ± 2.6	**68**
Glute Conditioner (Kg)	48.5 ± 4.8	66.8 ± 3.5	**38**	50.5 ± 1.5	59.4 ± 2.4	**18**
Unilateral MVC (Nm)	124.8 ± 8.6	135.3 ± 9.3	**9.4**	121.6 ± 13.8	137.6 ±18.8	**10.8**
Bilateral MVC (Nm)	136.5 ±13.8	166.8 ±13.8	**29.5**	149.5 ± 19.5	188.7 ± 24.5	**26.9**

Figure 1: Illustration of the weekly schedule for training and supplementation during the study programme. A) Scheduling of exercise sessions. B) Timing of supplement ingestion during the exercise sessions (both Gym- and Home-based).

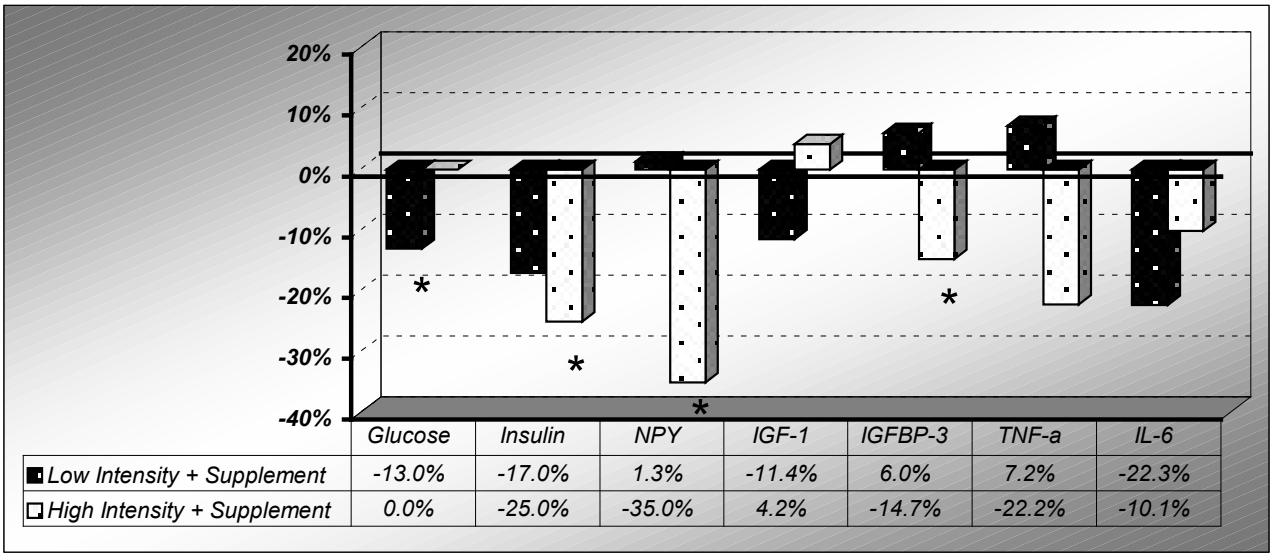
A)

Day of week	1	2	3	4	5	6	7
SUP_LowR	Home-Based		Gym-Based			Home-Based	
SUP_HighR	Gym-Based		Home-Based		Gym-Based		

B)

ACTIVITY	15mins Pre-Exercise	60 mins Exercise Session	15mins Post-Exercise
INGESTION	½ of Carbohydrate solution 	½ of Carbohydrate solution 	Amino-Acid Solution 

Figure 2: Summary of endocrine characteristics changes with the two levels of exercise (high versus low intensity) in the presence of nutritional supplementation. The * indicates where post-intervention data were significantly different to baseline values, with P≤0.05.



	Glucose	Insulin	NPY	IGF-1	IGFBP-3	TNF-a	IL-6
Low Intensity + Supplement	-13.0%	-17.0%	1.3%	-11.4%	6.0%	7.2%	-22.3%
High Intensity + Supplement	0.0%	-25.0%	-35.0%	4.2%	-14.7%	-22.2%	-10.1%