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Acute cardiometabolic effects of brief active breaks in sitting for rheumatoid arthritis patients

**METHODS**

15 post-menopausal women with rheumatoid arthritis

- Random order: SIT, EX, BR
- Blood samples at 0, 0.5, 1.0, 2.0, 3.0, 4.0, 4.5, 5.0, 6.0, 7.0, and 8.0h
- Blood pressure at 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0h
- Skeletal muscle biopsy at 8.0h

**OUTCOMES**

- **Glucose**
  - Lower during BR vs. SIT (p=0.036)

- **Insulin**
  - Lower during BR vs. SIT (p=0.016)

- **C-peptide**
  - Lower during BR and EX vs. SIT (both p<0.05)

- **Triglycerides**
  - No differences between conditions (p=0.262)

- **Cytokines**
  - BR, but not EX, induced an overall reduction in the inflammatory milieu

- **Lipidomic**
  - EX, but not BR, promoted more pronounced changes in lipid classes (total of 6) vs. SIT

- **Blood pressure (BP)**
  - Systolic BP* (p=0.201)
  - Diastolic BP (p=0.120)
  - Mean arterial pressure (p=0.060)
    - Reduced during EX vs. BR and SIT in the morning (0h to 4h)

- **Protein expression**
  - pAS160 Thr642/AS160 (p=0.501)
  - GLUT 4 (p=0.578)
  - OXPHOS (all p>0.050)

- **Gene expression**
  - ACACα (p=0.174)
  - LPL (p=0.191)
  - PDK4 (p=0.299)

**CONCLUSION**

Frequent, brief active breaks in sitting and moderate-to-vigorous exercise promote beneficial, but differential cardiometabolic effects in patients with rheumatoid arthritis.
Acute cardiometabolic effects of brief active breaks in sitting for rheumatoid arthritis patients

Running title: Active breaks in sitting in rheumatoid arthritis

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Exercise is a treatment in rheumatoid arthritis but participation in moderate-to-vigorous exercise is challenging for some patients. Light-intensity breaks in sitting could be a promising alternative. We compared the acute effects of active breaks in sitting with those of moderate-to-vigorous exercise on cardiometabolic risk markers in patients with rheumatoid arthritis. In a cross-over fashion, 15 women with rheumatoid arthritis underwent three 8-h experimental conditions: prolonged sitting (SIT), 30-min bout of moderate-to-vigorous exercise followed by prolonged sitting (EX), and 3-min bout of light-intensity walking every 30 min of sitting (BR). Postprandial glucose, insulin, c-peptide, triglycerides, cytokines, lipid classes/subclasses (lipidomics), and blood pressure responses were assessed. Muscle biopsies were collected following each session to assess targeted proteins/genes. Glucose (-28% in area under the curve (AUC), p=0.036), insulin (-28% in AUC, p=0.016) and c-peptide (-27% in AUC, p=0.006) postprandial responses were attenuated in BR vs. SIT, whereas only c-peptide was lower in EX vs. SIT (-20% in AUC, p=0.002). IL-1β decreased during BR, but increased during EX and SIT (p=0.027 and p=0.085). IL-1ra was increased during EX vs. BR (p=0.002). TNF-α concentrations decreased during BR vs. EX (p=0.022). EX, but not BR, reduced systolic blood pressure (p=0.013). Lipidomic analysis showed that 7 of 36 lipid classes/subclasses were significantly different between conditions, with greater changes being observed in EX. No differences were observed for protein/gene expression. Brief active interruptions to sitting can offset markers of cardiometabolic disturbance, which may be particularly useful for patients who may find it difficult to adhere to exercise.

Keywords: sedentary behavior, active breaks, inflammatory arthritis, cardiovascular risk
NEW AND NOTEWORTHY

Exercise is a treatment in rheumatoid arthritis but is challenging for some patients. Light-intensity breaks in sitting could be a promising alternative. Our findings show beneficial, but differential cardiometabolic effects of active breaks in sitting and exercise in rheumatoid arthritis patients. Breaks in sitting mainly improved glycemic and inflammatory markers, whereas exercise improved lipidomic and hypotensive responses. Breaks in sitting show promise in offsetting aspects of cardiometabolic disturbance associated with prolonged sitting in rheumatoid arthritis.
INTRODUCTION

Rheumatoid arthritis is an autoimmune disease characterized by chronic inflammation, pain and physical disability (1). Patients with rheumatoid arthritis have a higher risk of morbidity and mortality from cardiovascular diseases (2), which can be partially explained by chronic inflammation and poor lifestyle habits (3, 4). Despite physical activity being advocated as an integral part of standard care (5), physical inactivity (too little exercise) and sedentary behavior (too much sitting) are highly prevalent among patients with rheumatoid arthritis (6). Importantly, both risk factors have been associated with worsened disease symptoms, poor health outcomes, and increased cardiovascular risk in this disease (6, 7).

Moderate-to-vigorous exercise is considered a cornerstone for prevention and treatment of chronic diseases (8). In rheumatoid arthritis, exercise improves disease symptoms, inflammatory markers, cardiometabolic risk factors, and physical capacity (8, 9). However, regular participation in moderate-to-vigorous physical activity may not be feasible for some patients, especially those with poor mobility or during disease flares. Recent evidence has shown that light-intensity physical activity is associated with lower disability, disease activity and cardiovascular risk in rheumatoid arthritis, in contrast to excessive sitting (6, 7).

Acute laboratory studies in which participants undergo frequent light-intensity breaks in sitting have shown cardiometabolic benefits in healthy and clinical populations (10). For instance, light- and moderate-intensity activity breaks in sitting have been shown to improve glucose, insulin, and triglycerides postprandial responses in healthy and clinical populations (11) and to reduce blood pressure in individuals at risk for type 2 diabetes (12). If these benefits are extended to patients with rheumatoid arthritis, active breaks in sitting could be
considered as a therapeutic tool in this disease, in which cardiometabolic disorders, such as insulin resistance, diabetes, dyslipidemia and hypertension, are highly prevalent comorbidities (4).

This study aimed to compare the acute effects of brief active breaks in sitting with those of a single bout of moderate-to-vigorous exercise followed by prolonged sitting, on postprandial glucose (primary outcome), insulin, c-peptide, triglycerides, blood pressure, inflammatory markers, and lipid classes and subclasses (secondary outcomes). Our working hypothesis was that breaks to sitting would be as effective as moderate-to-vigorous exercise to offset cardiometabolic disturbances induced by prolonged sitting.

METHODS

Ethical approval

This trial was approved by the local Ethical Committee (Commission for Analysis of Research Projects, CAPPesq; approval number: 1.958.321) and patients signed an informed consent before participation.

Study design

We performed a crossover study nested within a randomized controlled trial (clinicaltrials.org: NCT03186924). Data from this study is reported according to the recommendations by the CONSORT for randomized crossover trials (13).

Patients attended our laboratory in four different occasions interspaced by a 7-to-14-day-washout period (median [range]: 7 [7 to 14]). On the first visit, patients completed clinical assessments and underwent a maximal graded exercise test on a treadmill to
determine ventilatory thresholds (14), followed by a familiarization session to the experimental protocols. Thereafter, patients randomly completed three experimental sessions: (i) Prolonged sitting (SIT), in which patients engaged in prolonged sitting throughout an 8-h period; (ii) Exercise followed by prolonged sitting (EX), in which patients performed a 30-min bout of moderate-to-vigorous exercise (i.e., intensity corresponding to 10% below the heart rate at the respiratory compensation point; mean percentage of heart rate reserve [%HRR] was 55.4 ± 9.3) on a treadmill followed by prolonged sitting; (iii) Active breaks in sitting (BR), in which patients completed 3-min bouts of light-to-moderate-intensity walking (i.e., intensity corresponding to 10% below the HR at the anaerobic threshold; mean %HRR was 24.2 ± 10.4) every 30 min of sitting throughout the experimental period, corresponding to 42 min of activity in total. Seven days before each experimental session, sedentary behavior, standing, and stepping were assessed using activPAL micro™ accelerometers (Glasgow, UK), in line with current recommendations (15). Moderate-to-vigorous physical activity was objectively measured by actiGraph GT3X® accelerometers (Florida, USA), using Freedson cut-points to classify epochs (16). During the 48 h prior to each session (i.e., restrictive period), patients were required to fill a 2-day food diary and instructed to follow a similar dietary pattern and refrain from strenuous exercise, alcohol, and caffeine in all sessions (Fig. 1). Patients were also instructed to maintain their habitual physical activity level throughout the study.

On each experimental day, patients reported to the laboratory between 07:00 and 07:30 following a 12-hour overnight fast. After a 30-min rest, baseline measurements were performed. Thereafter, patients consumed a standardized meal and underwent the 8-h protocols for SIT, EX or BR, according to their allocation sequence. Standardized meals (~65% carbohydrate, 15% protein and 20% fat, ~500 kcal) were provided 15 min before and 4 h after the commencement of the session. Blood samples were collected from an antecubital
vein prior to the breakfast (baseline) and after 0.5, 1.0, 2.0, 3.0, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0-h.

Blood pressure was measured hourly. Skeletal muscle samples were collected 15 min after the 8.0-h time-point in all sessions (Fig. 1). Heart rate was continuously monitored to assess exercise and active breaks in sitting intensity during the 8-h protocols using a heart rate monitor (Polar RS800cx, Kempele, Finland; sampling rate: 1000 Hz). During all sessions, patients were transported in a wheelchair to avoid excessive movement in case they needed to use the restroom.

Allocation was performed according to the Latin-square procedure. Each possible sequence was written on a paper and placed into opaque envelopes by a research staff who was not involved in the study. Sequence was determined by random drawing (1:1:1:1:1:1). Allocation was then unmasked to the research team, but remained masked to patients until the day of each session.

Participants

Eighteen post-menopausal women diagnosed with rheumatoid arthritis (17) were recruited from the Outpatient Rheumatoid Arthritis Clinic (Clinical Hospital, University of Sao Paulo, Brazil). Patients were enrolled from March 2018 to April 2019. Final follow-up was May 2019. Exclusion criteria were any physical disabilities that could preclude physical exercise, participation in exercise training within the last 12 months, and unstable drug therapy in the last 3 months prior to the study.

Measurements

Blood sample processing and analysis
An intravenous catheter was inserted into an antecubital vein for blood sampling to analyze glucose (primary outcome), insulin, c-peptide, triglycerides, and pro- and anti-inflammatory cytokines (i.e., IFN-γ, IL-1β, IL-1ra, IL-4, IL-6, IL-8, IL-10, IL-17, and TNF-α; cytokines were only assessed at baseline and 8-h time-points, in a convenience sub-sample of 10 patients). Blood samples were not collected from one patient due to fail in cannulation. Blood samples were analyzed in an accredited laboratory from the Clinical Hospital or stored at -80°C for subsequent analysis. Glucose was assessed using a colorimetric enzymatic assay (Bioclin, Belo Horizonte, Brazil); in a solitary case of failed cannulation, glucose was assessed by finger prick test (3M, MN, USA). Insulin and c-peptide were assessed using an immunoassay technique (Cobas, Roche Diagnostics, Mannheim, Germany). Triglycerides was assessed using enzymatic colorimetric assays (CELM, Sao Paulo, Brazil). Cytokines were determined using MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel (Merck Millipore, MA, USA), according to manufacturer’s instructions.

Lipidomic analysis

Baseline and 8.0 h plasma samples (10 μL) from 11 patients were analyzed. The semiquantitative lipidomic analysis was performed as previously described (18). A total of 654 lipid species were measured and summed to calculate the concentration of 36 lipid classes and subclasses.

Blood pressure

Blood pressure was measured using the auscultatory technique using a non-mercury sphygmomanometer (19). All measurements were taken in the same arm by a trained
evaluator. During BR, blood pressure was assessed at least 25 min after the most recent activity break.

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193 Skeletal muscle biopsy and protein/gene expression

194 *Vastus lateralis* biopsies were performed 15 min after the 8-h time-point of each session in a convenience sub-sample of seven patients. Biopsies were obtained using the percutaneous needle biopsy technique with suction (20), and samples were snap frozen in liquid nitrogen and stored at -80°C.

198

199 Protein expression

200 Protein expression was determined by western blotting (21). In brief, 10μL of sample (25μg of protein) was loaded into 4-20% polyacrylamide gels and separated via SDS-polyacrylamide gel electrophoresis and transferred to PVDF membranes. Membranes were blocked for 1 h at room temperature with 5% nonfat dry milk in TBS-T and then incubated overnight with anti-AS160, anti-pAS160Thr642, anti-GLUT4, anti-oxidative phosphorylation complexes (OXPHOS), and anti-GADPH (Supplemental Table S1). Membranes were washed in TBS-T and incubated with species-specific peroxidase-conjugated secondary antibodies. Immunoreactive proteins were visualized by enhanced chemiluminescence reagent (Femto® SuperSignal, ThermoFischer Scientific®, USA) using a C-DiGit® Blot Scanner (LI-COR, USA) and quantified by densitometric analysis using ImageJ software, version 1.53. OXPHOS membranes were stripped and re-probed with GAPDH after removal of the first primary antibody by incubation in stripping buffer (RestoreTM PLUS Western Blot, ThermoFischer Scientific®, USA). Gel-to-gel variation and equal protein loading were controlled using a standardized sample on each gel and GAPDH expression, respectively.
**Gene expression**

Gene expression was determined by quantitative real-time PCR (qRT-PCR). Total RNA was extracted using the RNeasy Fibrous Tissue Mini Kit (Qiagen®), according to the manufacturer’s instructions. Gene expression was determined by quantitative real-time PCR (qRT-PCR) analyses using the Superscript Platinum One-Step kit (Invitrogen®, CA, USA) with incorporated Maxima SYBR Green/ROX qPCR Master Mix (ThermoFischer Scientific®, CA, USA). The mRNA levels of ACACα, LPL, and PDK4 were analysed (Supplemental Table S2). Fold changes from SIT were calculated using the $2^{-\Delta\Delta Cq}$ method (22). All mRNA levels were normalized using the beta-2-microglobulin ($\beta2M$) gene as a housekeeping.

**Statistical analysis**

Sample size calculation was performed using G-Power® software (Düsseldorf, Germany). Assuming an effect size of 0.44 (for glucose AUC) (23) and a correlation coefficient of 0.6 between repeated measures, 9 patients would be required to achieve a power ≥80% with a significance level of 5%. To increase power for secondary outcomes, we expanded our sample to 18 patients.

Net iAUC, positive iAUC, and total (tAUC) were calculated using the trapezoid method. Missing data were handled by repeated measures mixed models using restricted maximum likelihood; subsequently, the fitted values were used to calculate AUC.

Data normality was tested using the Shapiro-Wilk W-test. Between-condition differences for all dependent variables were tested using repeated measures mixed-model analyses, which consisted of experimental condition as fixed factor and patients as random
factor with an unstructured covariance matrix. All models were adjusted for baseline values.

For lipidomic analysis, p values obtained were corrected for multiple comparisons using the false discovery rate (FDR) method of Benjamini-Hochberg (24). *Post-hoc* tests with Tukey’s adjustment for multiple pairwise comparisons were performed. Sensitivity analyses for the meal-specific effect were conducted by isolating the 4-h period following both breakfast and lunch. Analyses were conducted according to the intention-to-treat principle, using SAS (Cary, USA).

Data are presented as mean ± standard deviation (SD) or mean, estimated mean difference (EMD) and 95% confidence intervals (95% CI), excepted otherwise stated. Non-parametric data were log-transformed and presented as back-transformed mean, EMD and 95% CI. Significance level was set at p≤0.050. P≤0.100 was interpreted as trend towards significance for secondary outcomes.

**RESULTS**

Eighteen patients were randomized; however, only 15 patients completed all experimental conditions and were included in the analysis (Supplemental Fig. S1). Mean age was 61.5±7.1 years, BMI was 26.9±3.7 kg/m², and disease activity ranged from remission to moderate activity (Table 1). Prescribed exercise and active breaks intensities are depicted in Table 1. Physical activity level and food consumption during the restrictive period did not differ between conditions (Table 2), nor there were between-condition differences for any outcomes at baseline (Table 2 and Supplemental Table S3).

**Postprandial metabolism**
Glucose net iAUC (p=0.019) and insulin net iAUC (p=0.021) were significantly lower in BR compared with SIT (EMD 95%CI: -37.1 mg/dL·h [-71.7, -2.4], p=0.036 and -59.0 µIU/mL·h [-122.4, -10.2], p=0.016; Fig. 2, panels A and B). C-peptide net iAUC were significantly lower in BR and EX compared with SIT (EMD: -7.6 ng/mL·h [-12.8, -2.4], p=0.006 and -5.8 ng/mL·h [-9.2, -2.4], p=0.002; Fig. 2, panel C). There were no differences between conditions for triglycerides net iAUC (p=0.262). tAUC and positive iAUC data were similar to those of net iAUC (Supplemental Table S4).

In the 4-h period after breakfast, glucose net iAUC was comparable between conditions (p=0.082; Supplemental Table S5). However, insulin net iAUC was lower in BR and EX compared with SIT (BR vs. SIT: p=0.014; EX vs. SIT: p<0.001) and c-peptide net iAUC was lower in EX compared with SIT (EX vs. SIT: p=0.002). Triglycerides tended to be lower in BR and EX vs. SIT (BR vs. SIT: p=0.067; EX vs. SIT: p=0.078). In the 4-h period following lunch, glucose net iAUC (p=0.023) was lower in BR than SIT (p=0.016). Insulin and c-peptide net iAUC were lower in BR vs. SIT and EX (insulin: p<0.001 and p=0.036; c-peptide: p=0.004 and p=0.003). There were no differences between conditions for triglycerides net iAUC (p=0.206).

**Inflammatory cytokines**

IL-1β decreased during BR, but increased during EX and SIT (BR vs. EX: p=0.027 and BR vs. SIT: p=0.085). IL-1ra increased during EX and decreased during SIT and BR (EX vs. BR: p=0.002 and EX vs. SIT: p=0.056). IL-10 concentrations decreased during BR and increased during EX and SIT (BR vs. SIT: p=0.088 and BR vs. EX: p=0.087). TNF-α concentrations decreased during BR and increased during EX (p=0.022), while it remained virtually unchanged during SIT. There were no differences between conditions for IFN-γ, IL-4, IL-6, IL-8 and IL-17 (all p>0.050; Fig. 3 and Supplemental Table S6).
Lipidomic analysis

Before Benjamini-Hochberg FDR correction, 9 out of 36 lipid classes and subclasses were significantly different between conditions. Seven lipid classes and subclasses remained different following correction: free fatty acids, lysophosphatidylethanolamine, lysoalkenylphosphatidylethanolamine, alkenylphosphatidylcholine, alkenylphosphatidylethanolamine, phosphatidylserine, and sphingosine.

BR had lower reduction in free fatty acids than SIT (p=0.009). Significant between-condition differences were found in lysophosphatidylethanolamine (p=0.006), lysoalkenylphosphatidylethanolamine (p=0.038), and phosphatidylserine (p=0.004). Greater percent changes were observed in EX vs. SIT and BR (all p<0.050). Sphingosine had a lower change in EX vs. SIT and BR (p=0.003 and p=0.001). Alkenylphosphatidylcholine and alkenylphosphatidylethanolamine had greater increases in EX vs. SIT (p=0.003 and p=0.001) (Fig. 4 and see Supplemental Table S7).

Blood pressure

Systolic, diastolic, and mean arterial pressure were not different between conditions (Fig. 5 and Supplemental Table S8). However, within the first 4 h after breakfast, there were greater reductions in systolic blood pressure and mean arterial pressure net iAUC (p=0.013 and p=0.007) in EX vs. BR (EMD: -14.4 mmHg·h [-25.0, -3.8], p=0.031 and -11.0 mmHg·h [-19.5, -2.6], p=0.038), with a tendency towards significance vs. SIT (EMD: -16.6 mmHg·h [-31.6, 1.6], p=0.080 and -10.7 mmHg·h [-19.5, -1.9], p=0.053). There were no differences between conditions for diastolic blood pressure. Following the 4-h period after lunch, no differences between conditions in blood pressure responses were observed (all p>0.050) (Supplemental Table S9).
**Protein and gene expression**

No significant between-condition differences were observed for pAS160 \textsubscript{Thr642}/AS160 (p=0.501; Fig. 6, panel A), GLUT4 (p=0.578; Fig. 6, panel B) and OXPHOS complexes I to V expression (all p>0.050; Fig. 6, panel C).

Similarly, there were no differences between conditions in ACAC\textsubscript{α} (p=0.174; Fig. 6, panel D), LPL (p=0.191; Fig. 6, panel E) and PDK4 (p=0.299; Fig. 6, panel F).

**DISCUSSION**

The main findings of this study were that (i) active breaks in sitting attenuated glucose (-28%), insulin (-28%) and c-peptide (-27%) postprandial concentrations, whereas exercise attenuated only c-peptide (-20%); (ii) metabolic benefits promoted by active breaks in sitting were observed throughout the 8-h assessment period, but exercise effects were lessened across the day; (iii) active breaks in sitting induced an overall reduction in the inflammatory milieu, which did not occur following exercise; (iv) exercise, but not active breaks in sitting, promoted hypotensive responses and changes in lipid classes and subclasses. These data reveal beneficial, but differential, effects of exercise and active breaks in sitting, with the latter being particularly useful for patients who may find it difficult to adhere to exercise.

Our findings align with others showing that frequent, light-intensity activity breaks in sitting improve glucose, insulin, and c-peptide, but not triglycerides, postprandial responses in healthy and clinical populations (e.g., obesity, type 2 diabetes) (10, 25). In contrast, although exercise has been shown to produce cardiometabolic effects throughout the day in healthy young and older adults (26, 27), its effects in rheumatoid arthritis were confined to the 4-h period succeeding breakfast, with prolonged sitting blunting the exercise effects in the
next 4 h after lunch. As the benefits promoted by active breaks in sitting appeared to persist across the day, rheumatoid patients should be advised to engage in regular breaks as much as they can to achieve better cardiometabolic outcomes, endorsing new public health guidelines suggesting that every move counts towards better health, including light-intensity ones (28).

Sustained high concentrations of inflammatory cytokines, such as IL-6 and TNF-α, are associated with insulin resistance, type 2 diabetes, and atherosclerosis not only in rheumatoid arthritis (29, 30) but also in healthy and other clinical populations (31). In fact, current literature consistently demonstrate the effectiveness of IL-6 and TNF blockers in treating the persistent inflammation observed in patients with rheumatoid arthritis (32-34). In turn, a single bout of exercise can induce a transitory secretion of selected cytokines by the skeletal muscle (so-called myokines), some of which are associated with anti-inflammatory and insulin sensitizing effects, a case in point being IL-6 (36). The role of IL-6 on exercise-induced adaptations has been further supported by studies demonstrating blunted adaptations to an exercise program in healthy individuals submitted to a pharmacological blockade of IL-6 receptor. Collectively, these data suggest that exercise-induced transient IL-6 secretion may, at least partially, mediate the chronic benefits of exercise (35). Interestingly, among adults with central adiposity, IL-6 concentrations increased over time with prolonged sitting, a response that was not attenuated with moderate-intensity breaks (36). In the current study, active breaks in sitting did not change IL-6 either, but reduced IL-1β, IL-1ra, IL-10, and TNF-α concentrations. As these cytokines may be markedly elevated in rheumatoid arthritis (37), active breaks in sitting emerges as a potential immunomodulatory tool able to attenuate the inflammatory milieu in this disease. However, whether these acute adjustments in inflammatory cytokines translate into chronic adaptations in inflammatory status in rheumatoid arthritis merits investigation. Conversely, exercise led to only minor changes in
cytokine levels, which, in fact, strengthens the notion that moderate-to-vigorous activities do not exacerbate inflammation in rheumatoid arthritis, at least acutely (8, 9).

Overall, improvements in glucose, insulin and inflammatory responses were more pronounced with light-intensity activity breaks in sitting than moderate-to-vigorous exercise. Assuming that these responses could be sustained chronically, this finding is of clinical relevance since some patients with rheumatoid arthritis may find it difficult to undergo exercise training programs due to physical limitations or other barriers, while breaking up sedentary time could be a more feasible alternative to implement on a daily basis. However, one should note that exercise was more effective than active breaks in sitting to promote blood pressure reduction, a well-described therapeutic effect experienced by hypertensive patients, known as post-exercise hypotension (38). This suggests that active breaks in sitting may have therapeutic value but do not replace all beneficial effects of more vigorous activities in rheumatoid arthritis.

Among adults with type 2 diabetes, breaks in sitting with light-intensity walk or simple resistance activities (e.g., squats, calf raises) changed concentrations of 4 lipid classes and 37 lipid species (39). In this study, active breaks in sitting only altered free fatty acids concentrations, whereas exercise modified 6 lipid classes and subclasses in a direction that suggests reduction in inflammation and platelet activation, and increase in antioxidant capacity, as presumed by the metabolic functions of these lipids (40-44). Of relevance, patients with rheumatoid arthritis were shown to have reduced alkenylphosphatidylethanolamine and phosphatidylserine, which are thought to contribute to higher cardiovascular risk and joint inflammation (45). Herein we showed that exercise induced increased concentrations of both lipid subclasses, which emerge as novel molecular candidates to partially explain the protective cardiometabolic role of exercise in this disease. We also used a targeted approach to explore transcriptional or translational changes that
could help explain the metabolic responses following the interventions; however, there were no changes in any of these. Although both exercise and active breaks in sitting have been shown to modulate genes and proteins involved in glucose and lipid metabolism, and cellular development, growth and proliferation (46, 47), it is possible that the absence of changes in this study may be related to the very-low intensity nature of the breaks and the timing of muscle biopsies (i.e., 7.5 h after the exercise bout), which may have not been ideal to detect differentially expressed proteins and genes due to the transient nature of their changes. Serial biopsies might be necessary to provide a broad view of the (differential) molecular adaptations to exercise and active breaks in sitting.

Current recommendations propose that physical activity should be considered as an integral part of standard care in rheumatoid arthritis (5). Our results extend this notion by showing that light-intensity activity breaks in sitting may also be a complementary strategy to mitigate cardiometabolic risk in this disease and should be incorporated in physical activity prescriptions. Given the differential effects between active breaks in sitting and exercise, rheumatologists and healthcare professionals may opt to prescribe them individually or in combination (for example, regularly interrupting sitting with slow walking and/or performing a 30-min bout of brisk walking), based on patients’ clinical symptoms, physical functioning, and individual preferences, bearing in mind that, among inactive/sedentary patients, engaging in light-intensity physical activity may represent a steppingstone to more intensive activities.

Strengths of this study include a cross-over design that mitigates inter-individual variability, the concomitant investigation of active breaks in sitting and exercise, and the comprehensive assessment of cardiometabolic responses to the interventions under well-controlled conditions. However, this study has limitations. Firstly, the acute nature of the interventions tested precludes determining whether the cardiometabolic changes seen herein could be sustained in the long-term. Secondly, the effects of active breaks in sitting and
exercise were tested separately; further studies should investigate potential additive effects of these strategies combined. Thirdly, this study might have been underpowered for some secondary outcomes. Fourthly, skeletal muscle biopsies were only performed at the end of each experimental condition to reduce the burden on the patients. Skeletal muscle samples were scarce in this study, precluding us from further exploring other pathways that may be underpinning metabolic responses showed herein, such as pathways associated with skeletal muscle remodeling and inflammation. Finally, data cannot be generalized to patients with different demographic and clinical features or to patients with other diseases.

In conclusion, light-to-moderate intensity activity breaks in sitting and moderate-to-vigorous exercise promote beneficial, but differential cardiometabolic effects in patients with rheumatoid arthritis. Active breaks in sitting attenuated glucose, insulin, c-peptide, and inflammatory markers postprandial concentrations, whereas exercise improved systolic blood pressure, mean arterial pressure and lipidomic responses. Whether the acute cardiometabolic adaptations observed herein can translate into durable clinical health benefits remains to be examined.

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Conflict of Interests

The authors declare no conflict of interests.

Ethics

This trial was approved by the local Ethical Committee (Commission for Analysis of Research Projects, CAPPesq; approval number: 1.958.321). All patients signed an informed consent form before participation.

Data sharing statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

REFERENCES


Table 1. Participant characteristics.

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<tbody>
<tr>
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<tr>
<td>Age (years)</td>
<td>61.5 ± 7.1</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>26.9 ± 3.7</td>
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<tr>
<td><strong>Disease parameters</strong></td>
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<tr>
<td>Disease duration (years)</td>
<td>16.1 ± 9.8</td>
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<td>DAS28</td>
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<tr>
<td>CDAI</td>
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<tr>
<td>HAQ</td>
<td>0.8 ± 0.6</td>
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<td>Rheumatoid factor positivity [n(%)]</td>
<td>11 (73.3%)</td>
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<td>Anticyclic citrullinated peptide positivity# [n(%)]</td>
<td>3 (27.3%)</td>
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<tr>
<td>Evidence of erosive disease [n(%)]</td>
<td>6 (40.0%)</td>
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<td><strong>Aerobic capacity and activity intensities</strong></td>
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<tr>
<td>HR at AT (bpm)</td>
<td>105 ± 19</td>
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<tr>
<td>HR at RCP (bpm)</td>
<td>125 ± 23</td>
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<tr>
<td>HRmax (bpm)</td>
<td>152 ± 24</td>
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<tr>
<td>Time-to-exhaustion (min)</td>
<td>10.4 ± 2.7</td>
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<tr>
<td>VO_{2peak} (ml/kg/min)</td>
<td>18.2 ± 4.1</td>
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<td>%HRR for EX</td>
<td>55.4 ± 9.3</td>
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<tr>
<td>%HRR for BR</td>
<td>24.2 ± 10.4</td>
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<td><strong>Comorbidities [n(%)]</strong></td>
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<td>7 (46.7%)</td>
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<tr>
<td>Dyslipidemias</td>
<td>7 (46.7%)</td>
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<tr>
<td>Type 2 diabetes</td>
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<tr>
<td>Fibromyalgia</td>
<td>5 (33.3%)</td>
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<tr>
<td>Other rheumatic diseases*</td>
<td>8 (53.3%)</td>
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<td>Depression</td>
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<td><strong>Medication [n(%)]</strong></td>
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<td>Prednisone</td>
<td>12 (80.0%)</td>
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<td>Current dose (mg/day)</td>
<td>4.5 ± 2.7</td>
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<td>DMARDs</td>
<td>13 (86.7%)</td>
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<td>Drug</td>
<td>Frequency</td>
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<td>-------------------------------------------</td>
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<td>Leflunomide</td>
<td>6 (40.0%)</td>
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<tr>
<td>Methotrexate</td>
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<td>Hydroxychloroquine diphosphate</td>
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<td>Sulfasalazine</td>
<td>1 (6.7%)</td>
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<tr>
<td>Tofacitinib</td>
<td>1 (6.7%)</td>
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<tr>
<td>Biological agents</td>
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<td>Abatacept</td>
<td>3 (20.0%)</td>
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<tr>
<td>Etanercept</td>
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<td>Rituximab</td>
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<tr>
<td>Non-steroidal anti-inflammatory drugs</td>
<td>7 (46.7%)</td>
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<tr>
<td>Pain killers</td>
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<tr>
<td>Antihypertensive drugs</td>
<td>7 (46.7%)</td>
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<tr>
<td>Antidyslipidemic drugs</td>
<td>7 (46.7%)</td>
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<tr>
<td>Antidiabetic drugs</td>
<td>2 (13.3%)</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>6 (40.0%)</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD or absolute and relative frequency (n [%]). Only 11 patients had information regarding anticyclic citrullinated peptide positivity. Other rheumatic diseases: osteoarthritis, osteoporosis, or Sjögren's syndrome. Abbreviations: AT, aerobic threshold; BMI, body mass index; CDAI, Clinical Disease Activity Index; DAS, Disease Activity Score; DMARDS, disease-modifying antirheumatic drug; HAQ, Health Assessment Questionnaire; HR, heart rate; HRR, heart rate reserve; RCP, respiratory compensation point; VO₂, oxygen consumption.


Table 2. Physical activity level and food intake during the restrictive period and baseline cardiometabolic markers.

<table>
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<tr>
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<th>EX</th>
<th>BR</th>
<th>p^a</th>
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<tr>
<td><strong>Restrictive period (n=15)</strong></td>
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<td>Physical activity level</td>
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<tr>
<td>Sedentary behavior (h/day)</td>
<td>8.1 ± 1.5</td>
<td>7.9 ± 1.5</td>
<td>8.0 ± 1.6</td>
<td>0.669</td>
</tr>
<tr>
<td>Standing (h/day)</td>
<td>5.9 ± 1.1</td>
<td>6.2 ± 1.2</td>
<td>6.0 ± 1.2</td>
<td>0.531</td>
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<tr>
<td>Stepping (h/day)</td>
<td>2.0 ± 0.6</td>
<td>2.0 ± 0.6</td>
<td>1.9 ± 0.6</td>
<td>0.833</td>
</tr>
<tr>
<td>MVPA (min/day)</td>
<td>13.7 ± 11.9</td>
<td>18.4 ± 16.8</td>
<td>17.6 ± 16.1</td>
<td>0.544</td>
</tr>
<tr>
<td>Food intake</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Total energy intake (kcal)</td>
<td>1244 ± 318</td>
<td>1267 ± 335</td>
<td>1249 ± 317</td>
<td>0.958</td>
</tr>
<tr>
<td>Carbohydrate (%TEI)</td>
<td>50.5 ± 8.3</td>
<td>49.8 ± 9.0</td>
<td>47.9 ± 8.0</td>
<td>0.606</td>
</tr>
<tr>
<td>Fat (%TEI)</td>
<td>31.3 ± 7.7</td>
<td>31.5 ± 6.3</td>
<td>33.4 ± 6.2</td>
<td>0.584</td>
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<tr>
<td>Protein (%TEI)</td>
<td>19.0 ± 4.3</td>
<td>18.2 ± 5.5</td>
<td>19.6 ± 4.9</td>
<td>0.625</td>
</tr>
<tr>
<td>Protein (g/kg)</td>
<td>0.91 ± 0.29</td>
<td>0.87 ± 0.27</td>
<td>0.91 ± 0.23</td>
<td>0.792</td>
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<tr>
<td><strong>Baseline metabolic markers (n=14)</strong></td>
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<tr>
<td>Glucose (mg/dL)*</td>
<td>90.3 ± 10.3</td>
<td>90.1 ± 13.9</td>
<td>87.1 ± 8.4</td>
<td>0.546</td>
</tr>
<tr>
<td>Insulin (µIU/mL)</td>
<td>9.6 ± 5.9</td>
<td>11.2 ± 14.5</td>
<td>7.9 ± 3.4</td>
<td>0.600</td>
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<tr>
<td>C-peptide (ng/mL)</td>
<td>2.37 ± 0.98</td>
<td>2.55 ± 1.85</td>
<td>2.32 ± 0.79</td>
<td>0.766</td>
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<tr>
<td>Triglycerides (mg/dL)</td>
<td>132.3 ± 47.5</td>
<td>133.2 ± 45.8</td>
<td>133.9 ± 55.5</td>
<td>0.983</td>
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<tr>
<td><strong>Baseline inflammatory markers (n=10)</strong></td>
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<tr>
<td>IFN-γ (pg/mL)</td>
<td>29.6 ± 29.1</td>
<td>25.7 ± 22.0</td>
<td>26.2 ± 18.3</td>
<td>0.784</td>
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<tr>
<td>IL-1β (pg/mL)</td>
<td>14.6 ± 8.4</td>
<td>14.6 ± 13.4</td>
<td>15.7 ± 10.3</td>
<td>0.862</td>
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<tr>
<td>IL-1ra (pg/mL)</td>
<td>63.5 ± 21.8</td>
<td>53.3 ± 15.8</td>
<td>62.8 ± 22.5</td>
<td>0.143</td>
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<tr>
<td>IL-4 (pg/mL)</td>
<td>31.2 ± 48.7</td>
<td>37.3 ± 71.2</td>
<td>35.0 ± 57.5</td>
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<td>IL-6 (pg/mL)</td>
<td>2.3 ± 3.4</td>
<td>2.3 ± 3.0</td>
<td>2.8 ± 4.2</td>
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<tr>
<td>IL-8 (pg/mL)</td>
<td>5.2 ± 1.5</td>
<td>5.2 ± 3.0</td>
<td>5.6 ± 3.4</td>
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<tr>
<td>IL-10 (pg/mL)</td>
<td>15.9 ± 14.5</td>
<td>16.9 ± 20.1</td>
<td>17.1 ± 15.3</td>
<td>0.885</td>
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<td>IL-17 (pg/mL)</td>
<td>15.0 ± 7.8</td>
<td>15.2 ± 11.0</td>
<td>16.7 ± 8.2</td>
<td>0.730</td>
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<tr>
<td>TNF-α (pg/mL)</td>
<td>50.9 ± 35.8</td>
<td>52.9 ± 52.6</td>
<td>53.9 ± 52.5</td>
<td>0.872</td>
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<tr>
<td><strong>Blood pressure (n=15)</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>122.6 ± 16.0</td>
<td>125.5 ± 12.4</td>
<td>124.4 ± 15.1</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75.2 ± 8.1</td>
<td>74.8 ± 7.5</td>
<td>74.0 ± 8.0</td>
<td>0.558</td>
</tr>
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</table>
Mean arterial pressure (mmHg)  

91.0 ± 9.6  
91.7 ± 8.2  
90.8 ± 9.3  
0.570

Data expressed as mean ± SD. *n=15 for glucose levels. a p value refers to main effect of condition, calculated by repeated measures mixed models. Abbreviations: MVPA, moderate-to-vigorous physical activity; TEI, total energy intake.
Patients completed three conditions in a random order, as follows: prolonged sitting (SIT), 30-min bout of moderate-to-vigorous exercise followed by prolonged sitting (EX) and 3-min bouts of light-intensity walking every 30 min of sitting (BR). Standardized meals were provided 15 min before and 4 h after the commencement of the experimental session. Blood samples were collected prior to the breakfast (baseline) and after 0.5, 1.0, 2.0, 3.0, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0-h time-points. Blood pressure was assessed hourly. Skeletal muscle samples were collected at the end of each experimental conditions. During the 7 to 14 days prior to each experimental condition, physical activity level was continuously monitored. During the 48 h prior to each experimental condition (restrictive period), patients were asked to follow the same diet and avoid caffeine, alcohol, and strenuous exercise. Legend: grey shade, sitting; white box + icon of a person running, moderate-to-vigorous physical activity; icon of a person walking, light-intensity breaks in sitting.

Panels A to D depict glucose (n=15), insulin (n=14), c-peptide (n=14), and triglycerides (n=14) concentrations as a time course over 8 h and as the 8-h net iAUC. Data are presented as mean (95%CI), calculated by repeated measures mixed models, and individual values. Shaded areas represent the timing of the moderate-to-vigorous exercise bout. Dashed lines represent the timing of breakfast and lunch. * significant between-condition difference (p<0.050) calculated by repeated measures mixed models. Net iAUC was defined as the area
above fasting concentration (positive iAUC) subtracted by the area below fasting
concentration, whereas tAUC was defined as the area above a concentration of zero.

**Figure 3. Pro- and anti-inflammatory cytokines delta change from baseline to 8 h.**
Data are presented as mean (95%CI), calculated by repeated measures mixed models. n=10
patients. p value refers to main effect of condition, calculated by repeated measures mixed
models. * significant estimated difference from SIT (p<0.050); # trend towards significance in
estimated difference from SIT (p<0.100); o significant estimated difference from EX
(p<0.050); ø trend towards significance in estimated difference from EX (p<0.100) calculated
by repeated measures mixed models. Abbreviations: IFN, interferon; IL, interleukin; TNF,
tumour necrosis factor.

**Figure 4. Postprandial plasma lipid classes and subclasses percentage change from
baseline to 8 h.**
Data are presented as mean (95%CI), calculated by repeated measures mixed models. n=11
patients. p value refers to main effect of condition, calculated by repeated measures mixed
models. * significant estimated difference from SIT (p<0.050); # trend towards significance in
estimated difference from SIT (p<0.100); o significant estimated difference from BR
(p<0.050); ø trend towards significance in estimated difference from BR (p<0.100) calculated
by repeated measures mixed models. Abbreviations: AC, acylcarnitine; C1P, ceramide-1-
phosphate; CE, cholesteryl ester; Cer(d), ceramide; COH, free cholesterol; DE,
dehydrocholestryl ester; DG, diacylglycerol; dhCer, dihydroceramide; FFA, free fatty acids;
G_{M1}, G_{M1} ganglioside; G_{M3}, G_{M3} ganglioside; HexCer, monohexosylceramide; Hex2Cer,
dihexosylceramide; Hex3Cer, trihexosylceramide; LPC, lysophosphatidylcholine; LPC(O),
Figure 5. Blood pressure responses.

Panels A and B depict systolic and diastolic blood pressure and panel C depicts mean arterial pressure (n=15) responses over 8 h and as the 8-h net iAUC. Data are presented as mean (95%CI), calculated by repeated measures mixed models, and individual values. Shaded areas represent the timing of the moderate-to-vigorous exercise bout. Dashed lines represent the timing of breakfast and lunch. Net iAUC was defined as the area above fasting concentration (positive iAUC) subtracted by the area below fasting concentration, whereas tAUC was defined as the area above a concentration of zero.

Figure 6. Fold change in protein and gene expression in the skeletal muscle.

Panels A to C depict fold change in pAS160Thr642/AS160, GLUT4 and OXPHOS complexes I to V protein expression (n=7). Representative blots are presented on the right side of the figure. Panels D to F depict fold change in ACACα, LPL and PDK4 gene expression (n=7). All the experiments have been run under exact same conditions. All fold changes were...
relative to the SIT condition. Data are presented as mean fold change (95%CI) and individual values.

SUPPLEMENTAL MATERIAL

### Overall design

<table>
<thead>
<tr>
<th>Baseline assessments</th>
<th>Physical activity level</th>
<th>Restrictive period</th>
<th>Condition 1</th>
<th>Physical activity level</th>
<th>Restrictive period</th>
<th>Condition 2</th>
<th>Physical activity level</th>
<th>Restrictive period</th>
<th>Condition 3</th>
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### Experimental Conditions

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- SIT
- EX
- BR

### Experimental Sessions

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<td><strong>Steady state</strong></td>
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