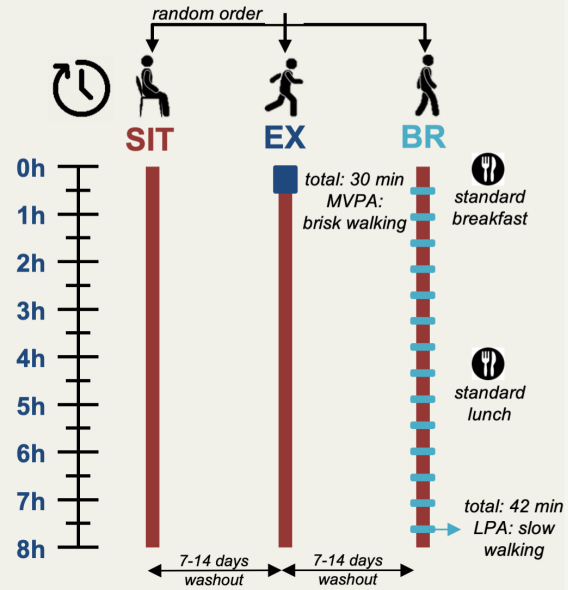
Acute cardiometabolic effects of brief active breaks in sitting for rheumatoid arthritis patients

METHODS

15 post-menopausal women with rheumatoid arthritis



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

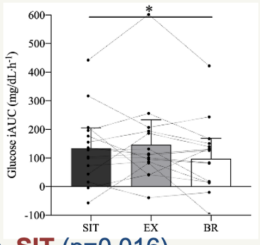
Blood pressure at 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0,and 8.0h

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

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)

Lipidomic

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

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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ABSTRACT

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

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

137 On each experimental day, patients reported to the laboratory between 07:00 and
138 07:30 following a 12-hour overnight fast. After a 30-min rest, baseline measurements were
139 performed. Thereafter, patients consumed a standardized meal and underwent the 8-h
140 protocols for SIT, EX or BR, according to their allocation sequence. Standardized meals
141 (~65% carbohydrate, 15% protein and 20% fat, ~500 kcal) were provided 15 min before and
142 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 -80ºC 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.

Skeletal muscle biopsy and protein/gene expression

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.

Protein expression

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-pAS160$_{Thr642}$, 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 (*β2M*) 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 $p \leq 0.050$. $P \leq 0.100$ 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/m^2, 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).

312

313 **Protein and gene expression**

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

317 Similarly, there were no differences between conditions in *ACACα* (p=0.174; Fig. 6,
318 panel D), *LPL* (p=0.191; Fig. 6, panel E) and *PDK4* (p=0.299; Fig. 6, panel F).

319

320 **DISCUSSION**

321

322 The main findings of this study were that (i) active breaks in sitting attenuated glucose
323 (-28%), insulin (-28%) and c-peptide (-27%) postprandial concentrations, whereas exercise
324 attenuated only c-peptide (-20%); (ii) metabolic benefits promoted by active breaks in sitting
325 were observed throughout the 8-h assessment period, but exercise effects were lessened
326 across the day; (iii) active breaks in sitting induced an overall reduction in the inflammatory
327 milieu, which did not occur following exercise; (iv) exercise, but not active breaks in sitting,
328 promoted hypotensive responses and changes in lipid classes and subclasses. These data
329 reveal beneficial, but differential, effects of exercise and active breaks in sitting, with the
330 latter being particularly useful for patients who may find it difficult to adhere to exercise.

331 Our findings align with others showing that frequent, light-intensity activity breaks in
332 sitting improve glucose, insulin, and c-peptide, but not triglycerides, postprandial responses
333 in healthy and clinical populations (e.g., obesity, type 2 diabetes) (10, 25). In contrast,
334 although exercise has been shown to produce cardiometabolic effects throughout the day in
335 healthy young and older adults (26, 27), its effects in rheumatoid arthritis were confined to
336 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 siting 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

386 could help explain the metabolic responses following the interventions; however, there were
387 no changes in any of these. Although both exercise and active breaks in sitting have been
388 shown to modulate genes and proteins involved in glucose and lipid metabolism, and cellular
389 development, growth and proliferation (46, 47), it is possible that the absence of changes in
390 this study may be related to the very-low intensity nature of the breaks and the timing of
391 muscle biopsies (i.e., 7.5 h after the exercise bout), which may have not been ideal to detect
392 differentially expressed proteins and genes due to the transient nature of their changes. Serial
393 biopsies might be necessary to provide a broad view of the (differential) molecular
394 adaptations to exercise and active breaks in sitting.

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

405 Strengths of this study include a cross-over design that mitigates inter-individual
406 variability, the concomitant investigation of active breaks in sitting and exercise, and the
407 comprehensive assessment of cardiometabolic responses to the interventions under well-
408 controlled conditions. However, this study has limitations. Firstly, the acute nature of the
409 interventions tested precludes determining whether the cardiometabolic changes seen herein
410 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.

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590

591 **TABLE**

592

593 **Table 1.** Participant characteristics.

	n=15
Age (years)	61.5 ± 7.1
BMI (kg/m²)	26.9 ± 3.7
Disease parameters	
Disease duration (years)	16.1 ± 9.8
DAS28	2.8 ± 1.2
CDAI	7.6 ± 6.1
HAQ	0.8 ± 0.6
Rheumatoid factor positivity [n(%)]	11 (73.3%)
Anticyclic citrullinated peptide positivity[#] [n(%)]	3 (27.3%)
Evidence of erosive disease [n(%)]	6 (40.0%)
Aerobic capacity and activity intensities	
HR at AT (bpm)	105 ± 19
HR at RCP (bpm)	125 ± 23
HR_{max} (bpm)	152 ± 24
Time-to-exhaustion (min)	10.4 ± 2.7
VO_{2peak} (ml/kg/min)	18.2 ± 4.1
%HRR for EX	55.4 ± 9.3
%HRR for BR	24.2 ± 10.4
Comorbidities [n(%)]	
Hypertension	7 (46.7%)
Dyslipidemias	7 (46.7%)
Type 2 diabetes	2 (13.3%)
Fibromyalgia	5 (33.3%)
Other rheumatic diseases[*]	8 (53.3%)
Depression	1 (6.7%)
Medication [n(%)]	
Prednisone	12 (80.0%)
Current dose (mg/day)	4.5 ± 2.7
DMARDs	13 (86.7%)

Leflunomide	6 (40.0%)
Methotrexate	9 (60.0%)
Hydroxychloroquine diphosphate	3 (20.0%)
Sulfasalazine	1 (6.7%)
Tofacitinib	1 (6.7%)
Biological agents	6 (40.0%)
Abatacept	3 (20.0%)
Etanercept	2 (13.3%)
Rituximab	1 (6.7%)
Non-steroidal anti-inflammatory drugs	7 (46.7%)
Pain killers	10 (66.7%)
Antihypertensive drugs	7 (46.7%)
Antidyslipidemic drugs	7 (46.7%)
Antidiabetic drugs	2 (13.3%)
Antidepressants	6 (40.0%)

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; VO_2, oxygen consumption.

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

	SIT	EX	BR	p[a]
Restrictive period (n=15)				
Physical activity level				
Sedentary behavior (h/day)	8.1 ± 1.5	7.9 ± 1.5	8.0 ± 1.6	0.669
Standing (h/day)	5.9 ± 1.1	6.2 ± 1.2	6.0 ± 1.2	0.531
Stepping (h/day)	2.0 ± 0.6	2.0 ± 0.6	1.9 ± 0.6	0.833
MVPA (min/day)	13.7 ± 11.9	18.4 ± 16.8	17.6 ± 16.1	0.544
Food intake				
Total energy intake (kcal)	1244 ± 318	1267 ± 335	1249 ± 317	0.958
Carbohydrate (%TEI)	50.5 ± 8.3	49.8 ± 9.0	47.9 ± 8.0	0.606
Fat (%TEI)	31.3 ± 7.7	31.5±6.3	33.4 ± 6.2	0.584
Protein (%TEI)	19.0 ± 4.3	18.2 ± 5.5	19.6 ± 4.9	0.625
Protein (g/kg)	0.91 ± 0.29	0.87 ± 0.27	0.91 ± 0.23	0.792
Baseline metabolic markers (n=14)				
Glucose (mg/dL)[*]	90.3 ± 10.3	90.1 ± 13.9	87.1 ± 8.4	0.546
Insulin (μIU/mL)	9.6 ± 5.9	11.2 ± 14.5	7.9 ± 3.4	0.600
C-peptide (ng/mL)	2.37 ± 0.98	2.55 ± 1.85	2.32 ± 0.79	0.766
Triglycerides (mg/dL)	132.3 ± 47.5	133.2 ± 45.8	133.9 ± 55.5	0.983
Baseline inflammatory markers (n=10)				
IFN-γ (pg/mL)	29.6 ± 29.1	25.7 ± 22.0	26.2 ± 18.3	0.784
IL-1β (pg/mL)	14.6 ± 8.4	14.6 ± 13.4	15.7 ± 10.3	0.862
IL-1ra (pg/mL)	63.5 ± 21.8	53.3 ± 15.8	62.8 ± 22.5	0.143
IL-4 (pg/mL)	31.2 ± 48.7	37.3 ± 71.2	35.0 ± 57.5	0.758
IL-6 (pg/mL)	2.3 ± 3.4	2.3 ± 3.0	2.8 ± 4.2	0.778
IL-8 (pg/mL)	5.2 ± 1.5	5.2 ± 3.0	5.6 ± 3.4	0.849
IL-10 (pg/mL)	15.9 ± 14.5	16.9 ± 20.1	17.1 ± 15.3	0.885
IL-17 (pg/mL)	15.0 ± 7.8	15.2 ± 11.0	16.7 ± 8.2	0.730
TNF-α (pg/mL)	50.9 ± 35.8	52.9 ± 52.6	53.9 ± 52.5	0.872
Blood pressure (n=15)				
Systolic blood pressure (mmHg)	122.6 ± 16.0	125.5 ± 12.4	124.4 ± 15.1	0.226
Diastolic blood pressure (mmHg)	75.2 ± 8.1	74.8 ± 7.5	74.0 ± 8.0	0.558

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.

606 **FIGURES CAPTIONS**

607

608 **Figure 1. Experimental design.**

609 Patients completed three conditions in a random order, as follows: prolonged sitting (SIT),
610 30-min bout of moderate-to-vigorous exercise followed by prolonged sitting (EX) and 3-min
611 bouts of light-intensity walking every 30 min of sitting (BR). Standardized meals were
612 provided 15 min before and 4 h after the commencement of the experimental session. Blood
613 samples were collected prior to the breakfast (baseline) and after 0.5, 1.0, 2.0, 3.0, 4.0, 4.5,
614 5.0, 6.0, 7.0, 8.0-h time-points. Blood pressure was assessed hourly. Skeletal muscle samples
615 were collected at the end of each experimental conditions. During the 7 to 14 days prior to
616 each experimental condition, physical activity level was continuously monitored. During the
617 48 h prior to each experimental condition (restrictive period), patients were asked to follow
618 the same diet and avoid caffeine, alcohol, and strenuous exercise. Legend: grey shade, sitting;
619 white box + icon of a person running, moderate-to-vigorous physical activity; icon of a
620 person walking, light-intensity breaks in sitting.

621

622 **Figure 2. Postprandial glucose, insulin, c-peptide, and triglycerides concentrations.**

623 Panels A to D depict glucose (n=15), insulin (n=14), c-peptide (n=14), and triglycerides
624 (n=14) concentrations as a time course over 8 h and as the 8-h net iAUC. Data are presented
625 as mean (95%CI), calculated by repeated measures mixed models, and individual values.
626 Shaded areas represent the timing of the moderate-to-vigorous exercise bout. Dashed lines
627 represent the timing of breakfast and lunch. * significant between-condition difference
628 ($p<0.050$) 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),

lysoalkylphosphatidylcholine; LPC(P), lysoalkenylphosphatidylcholine; LPE, lysophosphatidylethanolamine; LPE(P), lysoalkenylphosphatidylethanolamine; LPI, lysophospha-tidylinositol; PC, phosphatidylcholine; PC(O), alkylphosphatidylcholine; PC(P), alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE(O), alkylphosphatidylethanolamine; PE(P), alkenylphosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; S1P, sphingosine-1-phosphate; SM, sphingomyelin; Sph, sphingosine; TG(O), alkyldiacylglycerol; TG(SIM), triacylglycerol (total).

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.

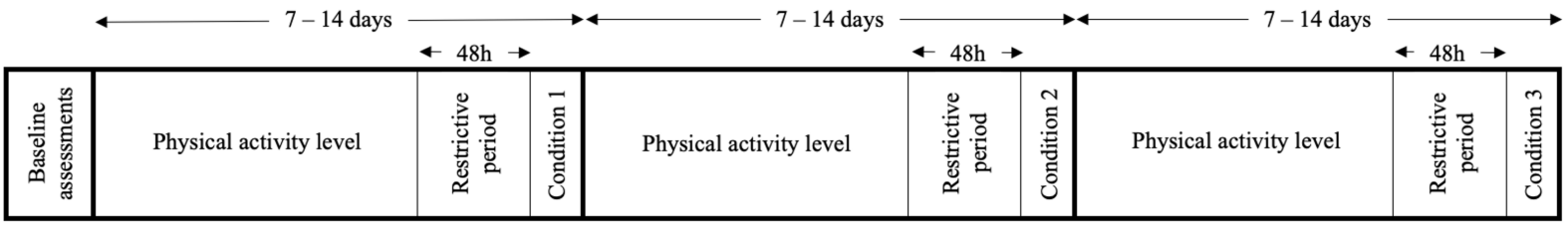
Panels A to C depict fold change in $pAS160_{Thr642}$/AS160, GLUT4 and OXPHOS complexes I to V protein expression (n=7). Representative blots are presented on the right side of the figure. Panels D to F depict fold change in ACACα, LPL and PDK4 gene expression (n=7). All the experiments have been run 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.

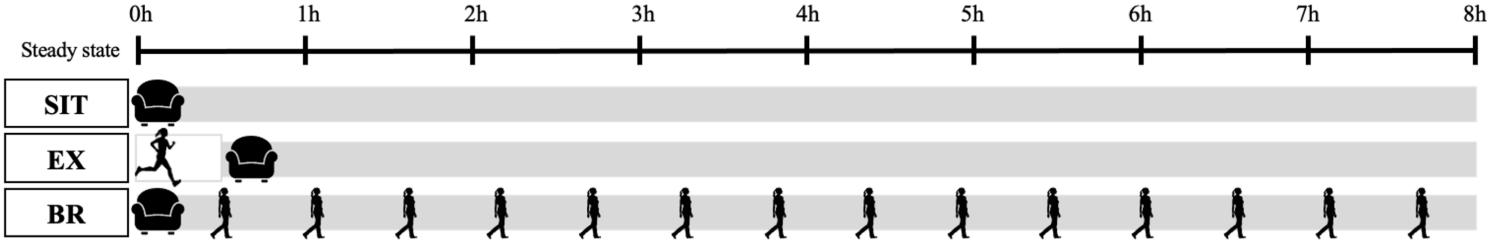
SUPPLEMENTAL MATERIAL

Can be downloaded at https://figshare.com/s/c733b62a13928197731d (doi: https://doi.org/10.6084/m9.figshare.14839701.v2).

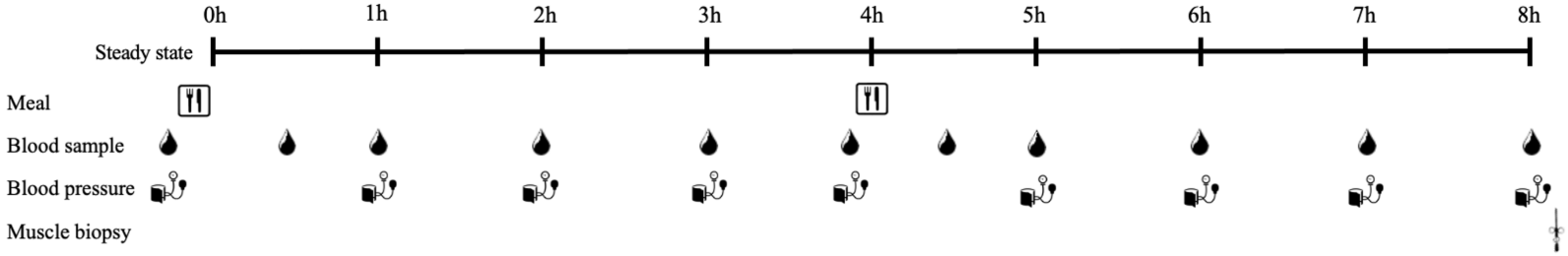
Overall design

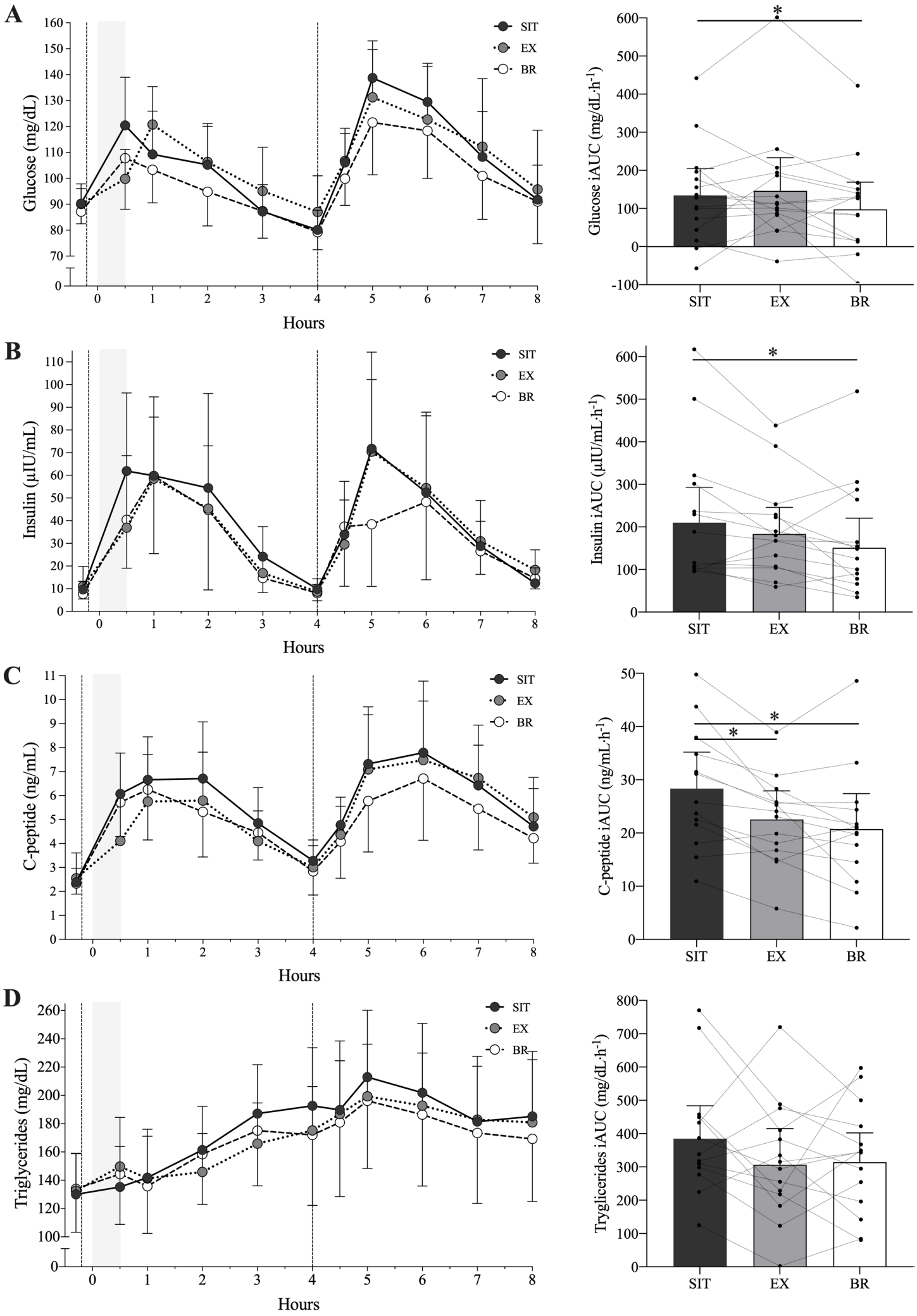


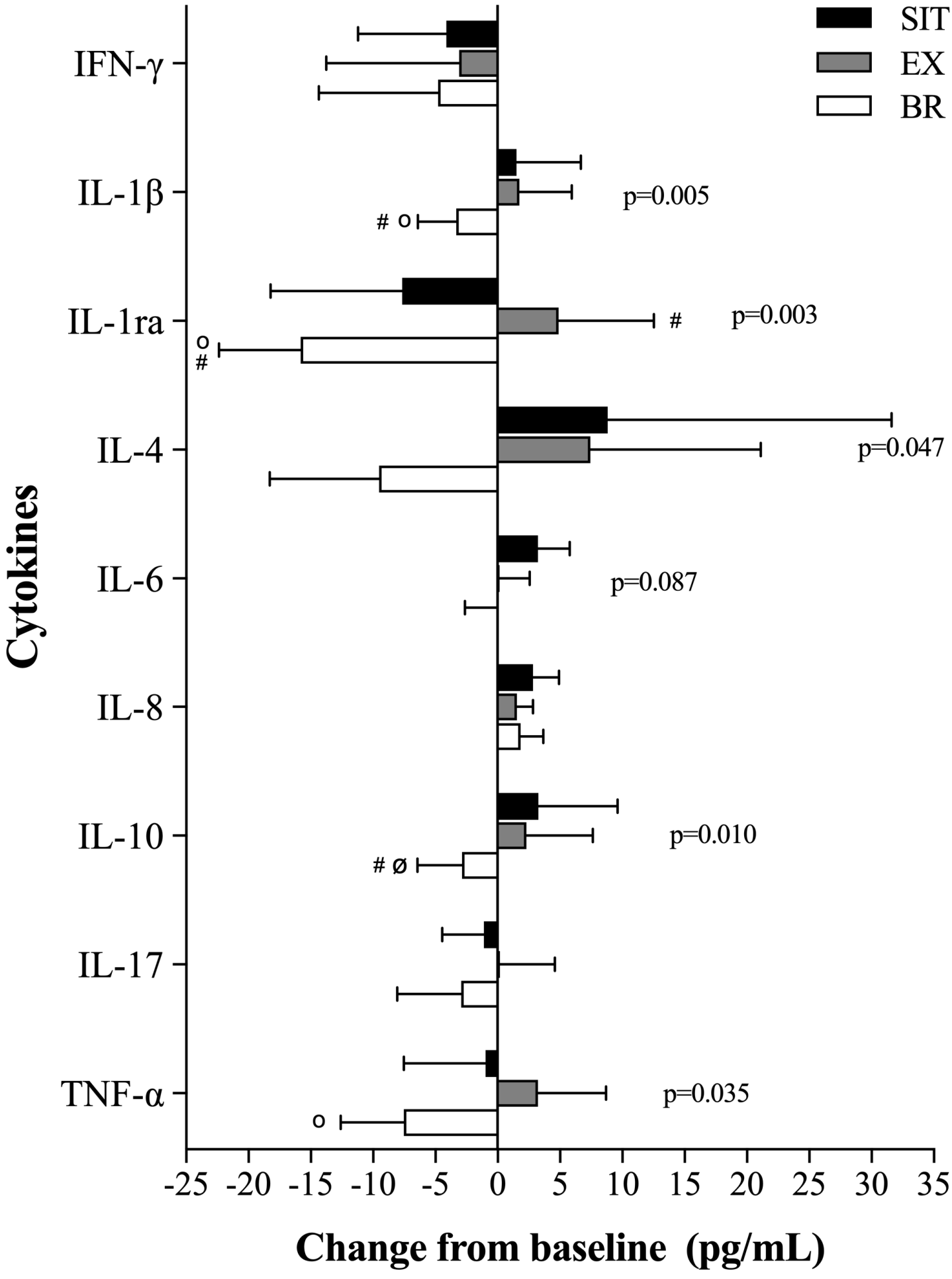
Experimental Conditions

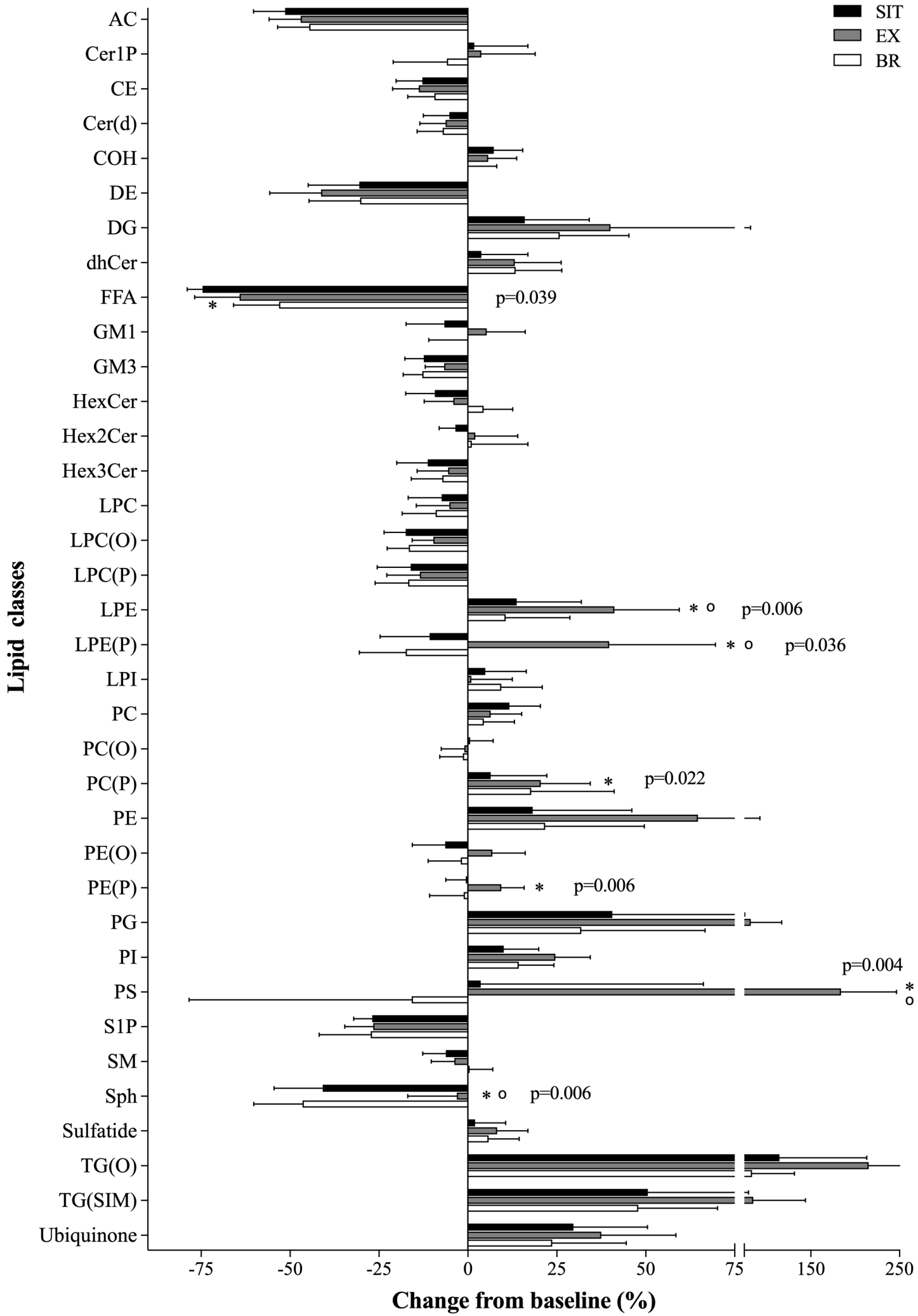


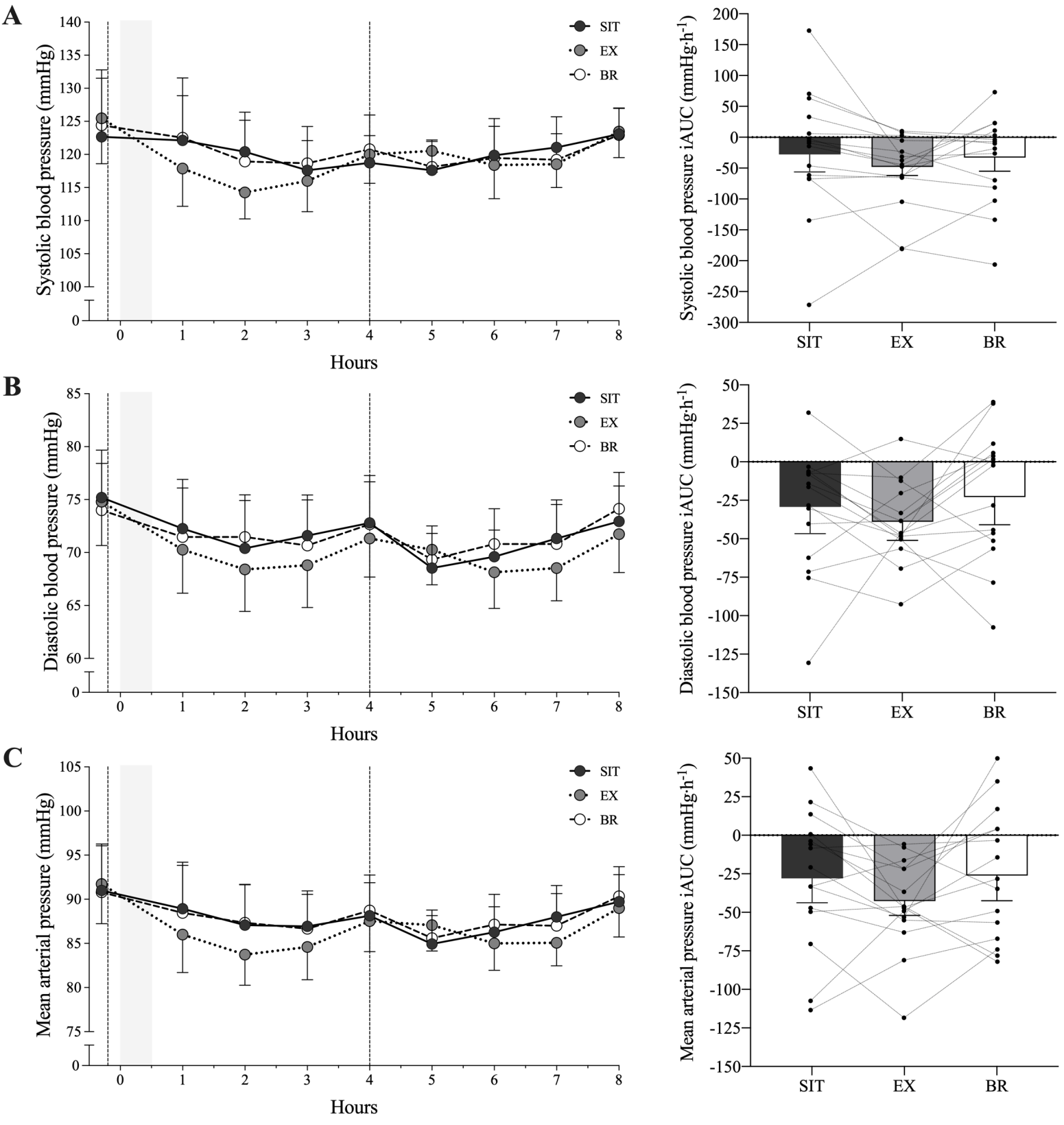
Experimental Sessions

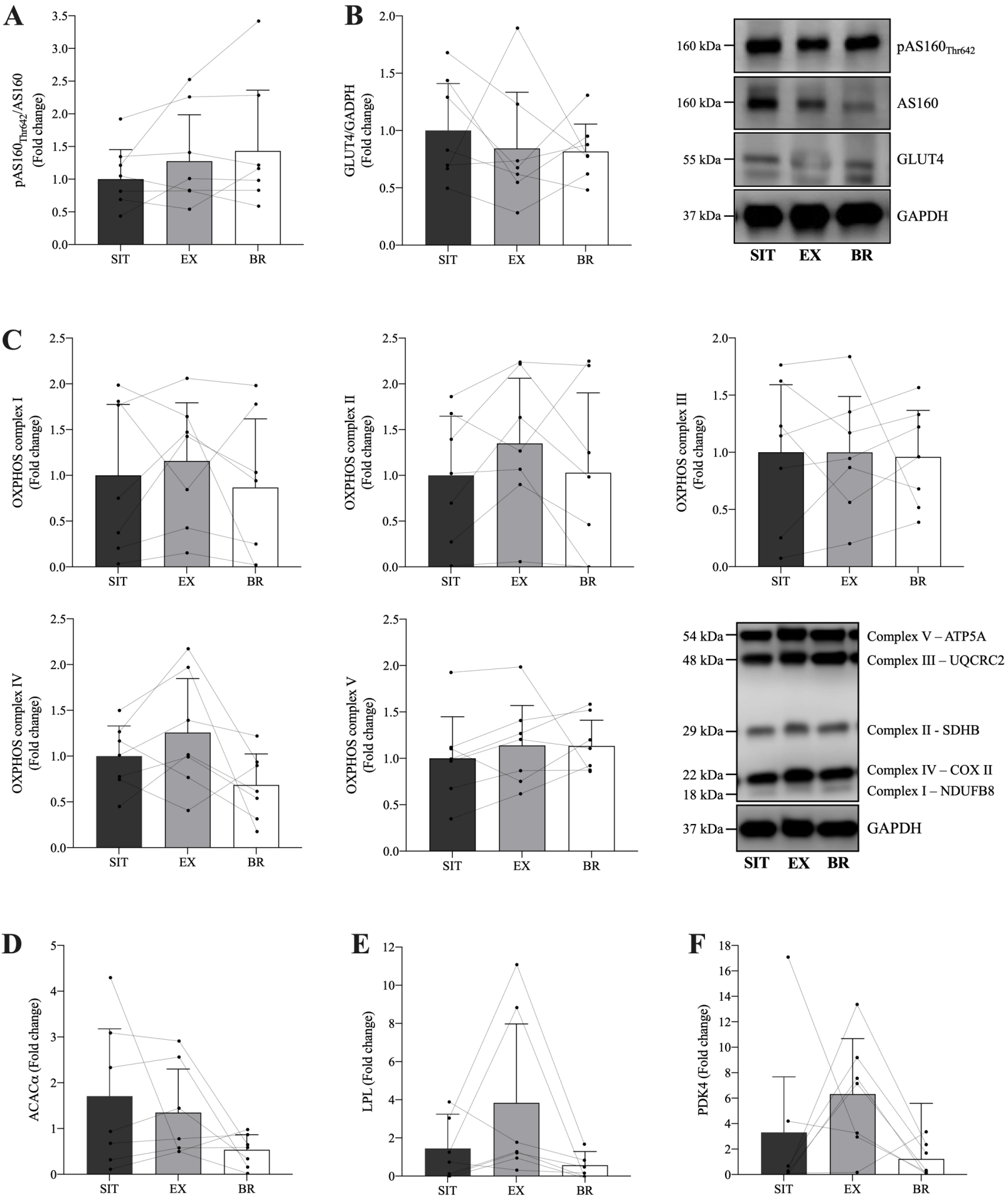



A
Glucose (mg/dL)
Hours
SIT
EX
BR
Glucose iAUC (mg/dL·h-1)
*
B
Insulin (µIU/mL)
Insulin iAUC (µIU/mL·h-1)
C
C-peptide (ng/mL)
C-peptide iAUC (ng/mL·h-1)
D
Triglycerides (mg/dL)
Tryglicerides iAUC (mg/dL·h-1)


SIT
EX
BR
IFN-γ
IL-1β
IL-1ra
IL-4
IL-6
IL-8
IL-10
IL-17
TNF-α
Cytokines
p=0.005
p=0.003
p=0.047
p=0.087
p=0.010
p=0.035
-25 -20 -15 -10 -5 0 5 10 15 20 25 30 35
Change from baseline (pg/mL)


SIT
EX
BR
AC
Cer1P
CE
Cer(d)
COH
DE
DG
dhCer
FFA
GM1
GM3
HexCer
Hex2Cer
Hex3Cer
LPC
LPC(O)
LPC(P)
LPE
LPE(P)
LPI
PC
PC(O)
PC(P)
PE
PE(O)
PE(P)
PG
PI
PS
S1P
SM
Sph
Sulfatide
TG(O)
TG(SIM)
Ubiquinone
p=0.039
p=0.006
p=0.036
p=0.022
p=0.006
p=0.004
p=0.006
Lipid classes
-75
-50
-25
0
25
50
75
150
250
Change from baseline (%)


A
Systolic blood pressure (mmHg)
Hours
SIT
EX
BR
Systolic blood pressure iAUC (mmHg·h-1)
B
Diastolic blood pressure (mmHg)
Diastolic blood pressure iAUC (mmHg·h-1)
C
Mean arterial pressure (mmHg)
Mean arterial pressure iAUC (mmHg·h-1)


A
pAS160Thr642/AS160 (Fold change)
SIT
EX
BR
B
GLUT4/GADPH (Fold change)
160 kDa
pAS160Thr642
160 kDa
AS160
55 kDa
GLUT4
37 kDa
GAPDH
C
OXPHOS complex I (Fold change)
OXPHOS complex II (Fold change)
OXPHOS complex III (Fold change)
OXPHOS complex IV (Fold change)
OXPHOS complex V (Fold change)
54 kDa
Complex V – ATP5A
48 kDa
Complex III – UQCRC2
29 kDa
Complex II - SDHB
22 kDa
Complex IV – COX II
18 kDa
Complex I – NDUFB8
37 kDa
GAPDH
D
ACACα (Fold change)
E
LPL (Fold change)
F
PDK4 (Fold change)