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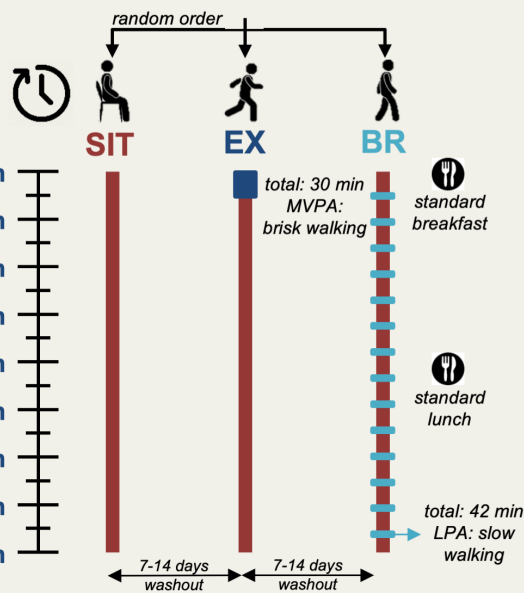
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Acute cardiometabolic effects of brief active breaks in sitting for rheumatoid arthritis patients

METHODS

15 post-menopausal women with rheumatoid arthritis



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

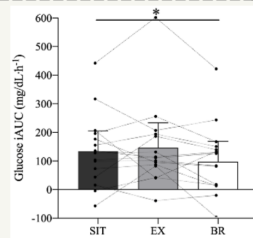
Skeletal muscle biopsy at 8.0h

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

ACAC α ($p=0.174$)
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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32

33

34 Supplemental Material available at

35 URL: <https://figshare.com/s/c733b62a13928197731d>

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37 **ABSTRACT**

38 Exercise is a treatment in rheumatoid arthritis but participation in moderate-to-vigorous
39 exercise is challenging for some patients. Light-intensity breaks in sitting could be a
40 promising alternative. We compared the acute effects of active breaks in sitting with those of
41 moderate-to-vigorous exercise on cardiometabolic risk markers in patients with rheumatoid
42 arthritis. In a cross-over fashion, 15 women with rheumatoid arthritis underwent three 8-h
43 experimental conditions: prolonged sitting (SIT), 30-min bout of moderate-to-vigorous
44 exercise followed by prolonged sitting (EX), and 3-min bout of light-intensity walking every
45 30 min of sitting (BR). Postprandial glucose, insulin, c-peptide, triglycerides, cytokines, lipid
46 classes/subclasses (lipidomics), and blood pressure responses were assessed. Muscle biopsies
47 were collected following each session to assess targeted proteins/genes. Glucose (-28% in
48 area under the curve (AUC), $p=0.036$), insulin (-28% in AUC, $p=0.016$) and c-peptide (-27%
49 in AUC, $p=0.006$) postprandial responses were attenuated in BR *vs.* SIT, whereas only c-
50 peptide was lower in EX *vs.* SIT (-20% in AUC, $p=0.002$). IL-1 β decreased during BR, but
51 increased during EX and SIT ($p=0.027$ and $p=0.085$). IL-1 α was increased during EX *vs.* BR
52 ($p=0.002$). TNF- α concentrations decreased during BR *vs.* EX ($p=0.022$). EX, but not BR,
53 reduced systolic blood pressure ($p=0.013$). Lipidomic analysis showed that 7 of 36 lipid
54 classes/subclasses were significantly different between conditions, with greater changes being
55 observed in EX. No differences were observed for protein/gene expression. Brief active
56 interruptions to sitting can offset markers of cardiometabolic disturbance, which may be
57 particularly useful for patients who may find it difficult to adhere to exercise.

58

59 **Keywords:** sedentary behavior, active breaks, inflammatory arthritis, cardiovascular risk

60 **NEW AND NOTEWORTHY**

61

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

69 INTRODUCTION

70

71 Rheumatoid arthritis is an autoimmune disease characterized by chronic
72 inflammation, pain and physical disability (1). Patients with rheumatoid arthritis have a
73 higher risk of morbidity and mortality from cardiovascular diseases (2), which can be
74 partially explained by chronic inflammation and poor lifestyle habits (3, 4). Despite physical
75 activity being advocated as an integral part of standard care (5), physical inactivity (too little
76 exercise) and sedentary behavior (too much sitting) are highly prevalent among patients with
77 rheumatoid arthritis (6). Importantly, both risk factors have been associated with worsened
78 disease symptoms, poor health outcomes, and increased cardiovascular risk in this disease (6,
79 7).

80 Moderate-to-vigorous exercise is considered a cornerstone for prevention and
81 treatment of chronic diseases (8). In rheumatoid arthritis, exercise improves disease
82 symptoms, inflammatory markers, cardiometabolic risk factors, and physical capacity (8, 9).
83 However, regular participation in moderate-to-vigorous physical activity may not be feasible
84 for some patients, especially those with poor mobility or during disease flares. Recent
85 evidence has shown that light-intensity physical activity is associated with lower disability,
86 disease activity and cardiovascular risk in rheumatoid arthritis, in contrast to excessive sitting
87 (6, 7).

88 Acute laboratory studies in which participants undergo frequent light-intensity breaks
89 in sitting have shown cardiometabolic benefits in healthy and clinical populations (10). For
90 instance, light- and moderate-intensity activity breaks in sitting have been shown to improve
91 glucose, insulin, and triglycerides postprandial responses in healthy and clinical populations
92 (11) and to reduce blood pressure in individuals at risk for type 2 diabetes (12). If these
93 benefits are extended to patients with rheumatoid arthritis, active breaks in sitting could be

94 considered as a therapeutic tool in this disease, in which cardiometabolic disorders, such as
95 insulin resistance, diabetes, dyslipidemia and hypertension, are highly prevalent
96 comorbidities (4).

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

103

104 **METHODS**

105

106 **Ethical approval**

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

110

111 **Study design**

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

115 Patients attended our laboratory in four different occasions interspaced by a 7-to-14-
116 day-washout period (median [range]: 7 [7 to 14]). On the first visit, patients completed
117 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-1 β , 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 μ L) 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 (Restore™ 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.

214

215 *Gene expression*

216 Gene expression was determined by quantitative real-time PCR (qRT-PCR). Total
217 RNA was extracted using the RNeasy Fibrous Tissue Mini Kit (Qiagen®), according to the
218 manufacturer's instructions. Gene expression was determined by quantitative real-time PCR
219 (qRT-PCR) analyses using the Superscript Platinum One-Step kit (Invitrogen®, CA, USA)
220 with incorporated Maxima SYBR Green/ROX qPCR Master Mix (ThermoFischer
221 Scientific®, CA, USA). The mRNA levels of *ACACa*, *LPL*, and *PDK4* were analysed
222 (Supplemental Table S2). Fold changes from SIT were calculated using the $2^{-\Delta\Delta C_q}$ method
223 (22). All mRNA levels were normalized using the beta-2-microglobulin (*β2M*) gene as a
224 housekeeping.

225

226 **Statistical analysis**

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

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

235 Data normality was tested using the Shapiro-Wilk W-test. Between-condition
236 differences for all dependent variables were tested using repeated measures mixed-model
237 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 \pm 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/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: $-37.1 \text{ mg/dL}\cdot\text{h}$ $[-71.7, -2.4]$, $p=0.036$ and $-59.0 \text{ }\mu\text{IU/mL}\cdot\text{h}$ $[-122.4, -10.2]$, $p=0.016$; Fig. 2, panels A and B). C-peptide net iAUC were significantly lower in BR and EX compared with SIT (EMD: $-7.6 \text{ ng/mL}\cdot\text{h}$ $[-12.8, -2.4]$, $p=0.006$ and $-5.8 \text{ ng/mL}\cdot\text{h}$ $[-9.2, -2.4]$, $p=0.002$; 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-1 β 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).

287

288 **Lipidomic analysis**

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

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

301

302 **Blood pressure**

303 Systolic, diastolic, and mean arterial pressure were not different between conditions
304 (Fig. 5 and Supplemental Table S8). However, within the first 4 h after breakfast, there were
305 greater reductions in systolic blood pressure and mean arterial pressure net iAUC ($p=0.013$
306 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
307 $[-19.5, -2.6]$, $p=0.038$), with a tendency towards significance vs. SIT (EMD: -16.6 mmHg·h $[-$
308 $31.6, 1.6]$, $p=0.080$ and -10.7 mmHg·h $[-19.5, -1.9]$, $p=0.053$). There were no differences
309 between conditions for diastolic blood pressure. Following the 4-h period after lunch, no
310 differences between conditions in blood pressure responses were observed (all $p>0.050$)
311 (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-1 β , 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.

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590

591 **TABLE**

592

593 **Table 1.** Participant characteristics.

	n=15
Age (years)	61.5 ± 7.1
BMI (kg/m ²)	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%)

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

601 **Table 2.** Physical activity level and food intake during the restrictive period and baseline
602 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
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603 Data expressed as mean ± SD. ^{*}n=15 for glucose levels. ^a p value refers to main effect of
604 condition, calculated by repeated measures mixed models. Abbreviations: MVPA, moderate-
605 to-vigorous physical activity; TEI, total energy intake.

FIGURES CAPTIONS

Figure 1. Experimental design.

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.

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

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 ($p<0.100$); ° significant estimated difference from EX ($p<0.050$); ° 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 ($p<0.100$); ° significant estimated difference from BR ($p<0.050$); ° 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),

653 lysoalkylphosphatidylcholine; LPC(P), lysoalkenylphosphatidylcholine; LPE,
 654 lysophosphatidylethanolamine; LPE(P), lysoalkenylphosphatidylethanolamine; LPI,
 655 lysophosphatidylinositol; PC, phosphatidylcholine; PC(O), alkylphosphatidylcholine; PC(P),
 656 alkenylphosphatidylcholine; PE, phosphatidylethanolamine; PE(O),
 657 alkylphosphatidylethanolamine; PE(P), alkenylphosphatidylethanolamine; PG,
 658 phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; S1P, sphingosine-1-
 659 phosphate; SM, sphingomyelin; Sph, sphingosine; TG(O), alkyl diacylglycerol; TG(SIM),
 660 triacylglycerol (total).

661

662 **Figure 5. Blood pressure responses.**

663 Panels A and B depict systolic and diastolic blood pressure and panel C depicts mean arterial
 664 pressure (n=15) responses over 8 h and as the 8-h net iAUC. Data are presented as mean
 665 (95%CI), calculated by repeated measures mixed models, and individual values. Shaded areas
 666 represent the timing of the moderate-to-vigorous exercise bout. Dashed lines represent the
 667 timing of breakfast and lunch. Net iAUC was defined as the area above fasting concentration
 668 (positive iAUC) subtracted by the area below fasting concentration, whereas tAUC was
 669 defined as the area above a concentration of zero.

670

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

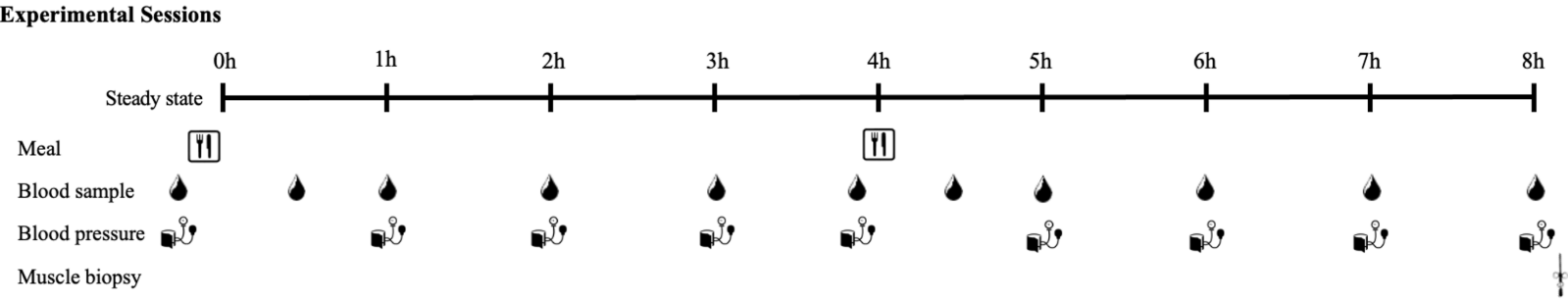
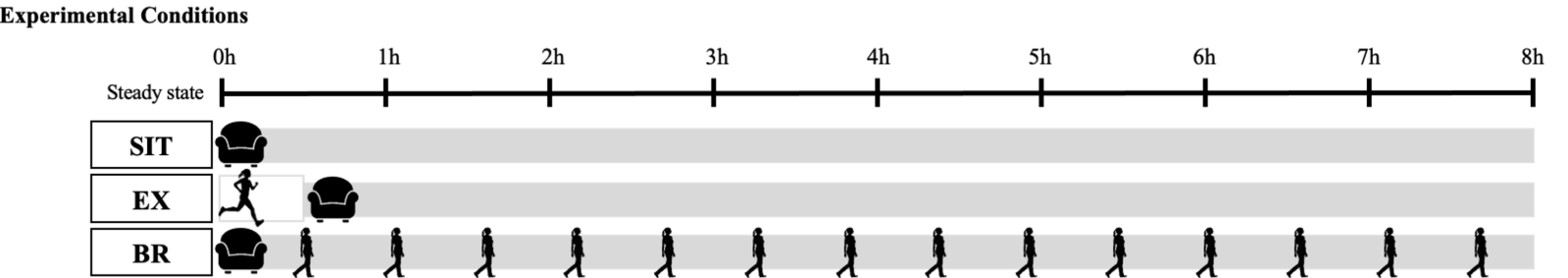
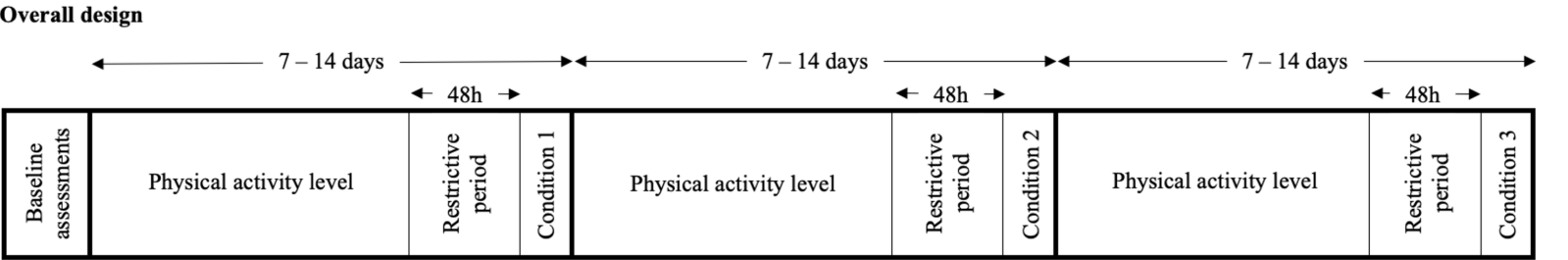
672 Panels A to C depict fold change in pAS160^{Thr642}/AS160, GLUT4 and OXPHOS complexes I
 673 to V protein expression (n=7). Representative blots are presented on the right side of the
 674 figure. Panels D to F depict fold change in ACAC α , LPL and PDK4 gene expression (n=7).
 675 All the experiments have been run under exact same conditions. All fold changes were

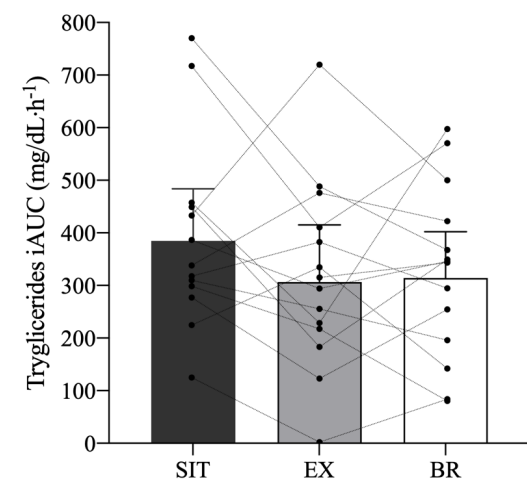
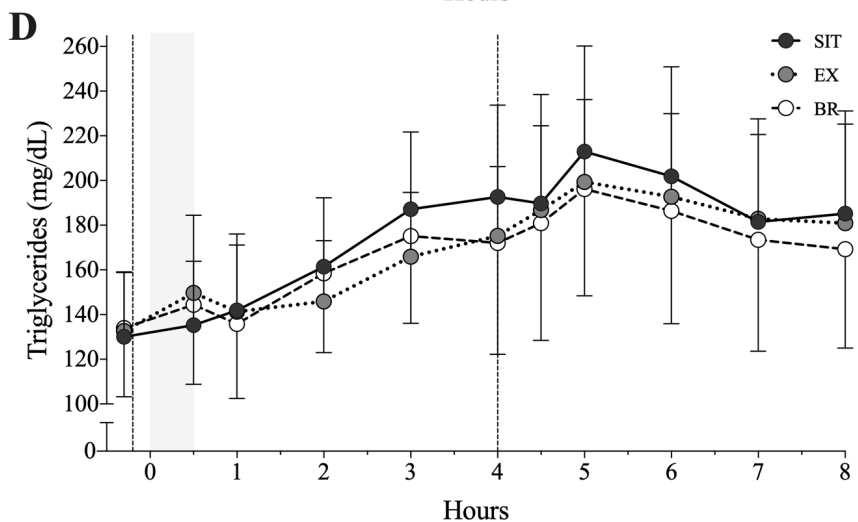
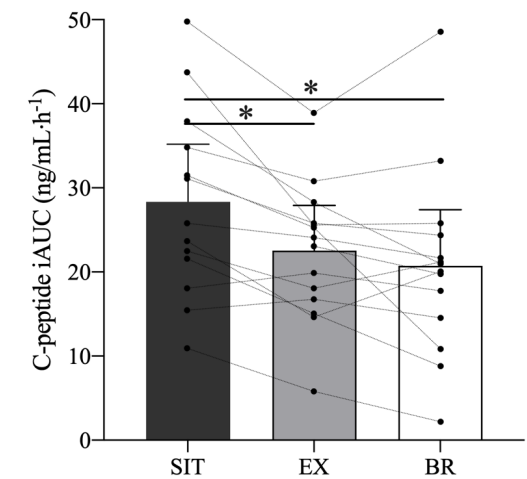
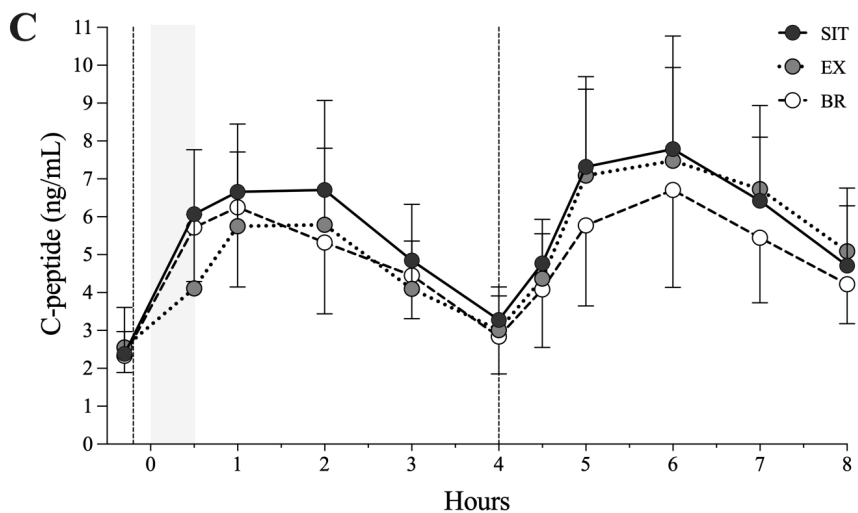
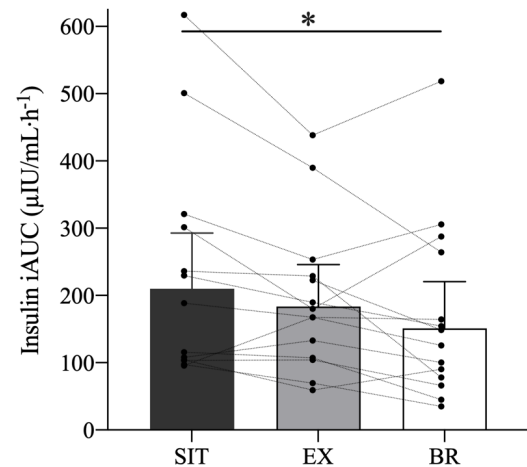
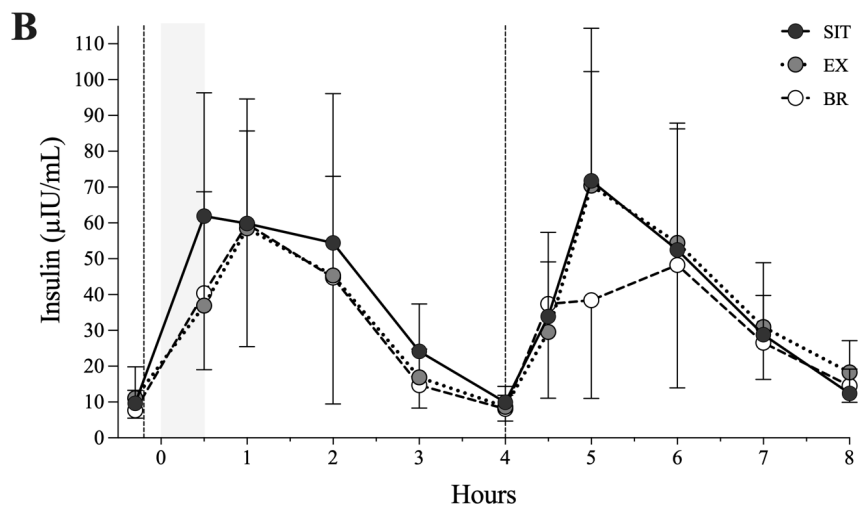
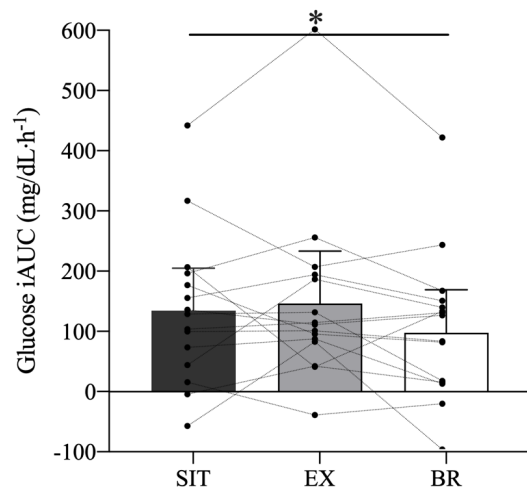
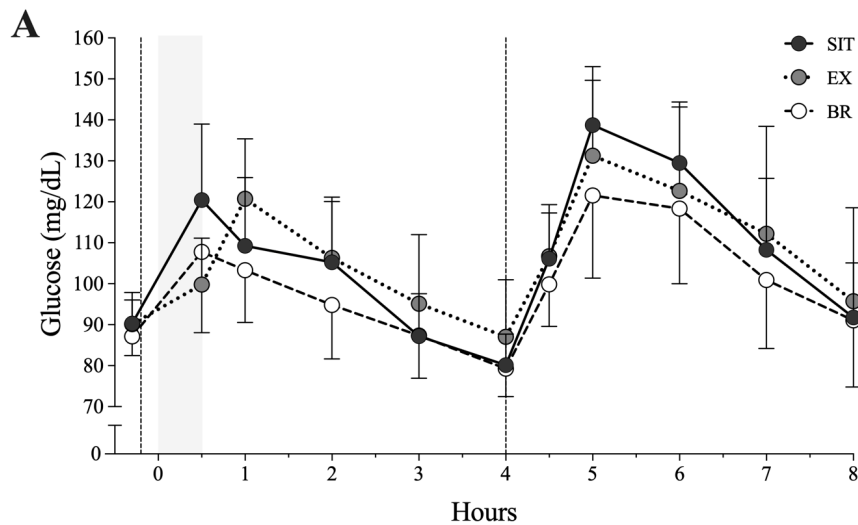
676 relative to the SIT condition. Data are presented as mean fold change (95%CI) and individual
677 values.

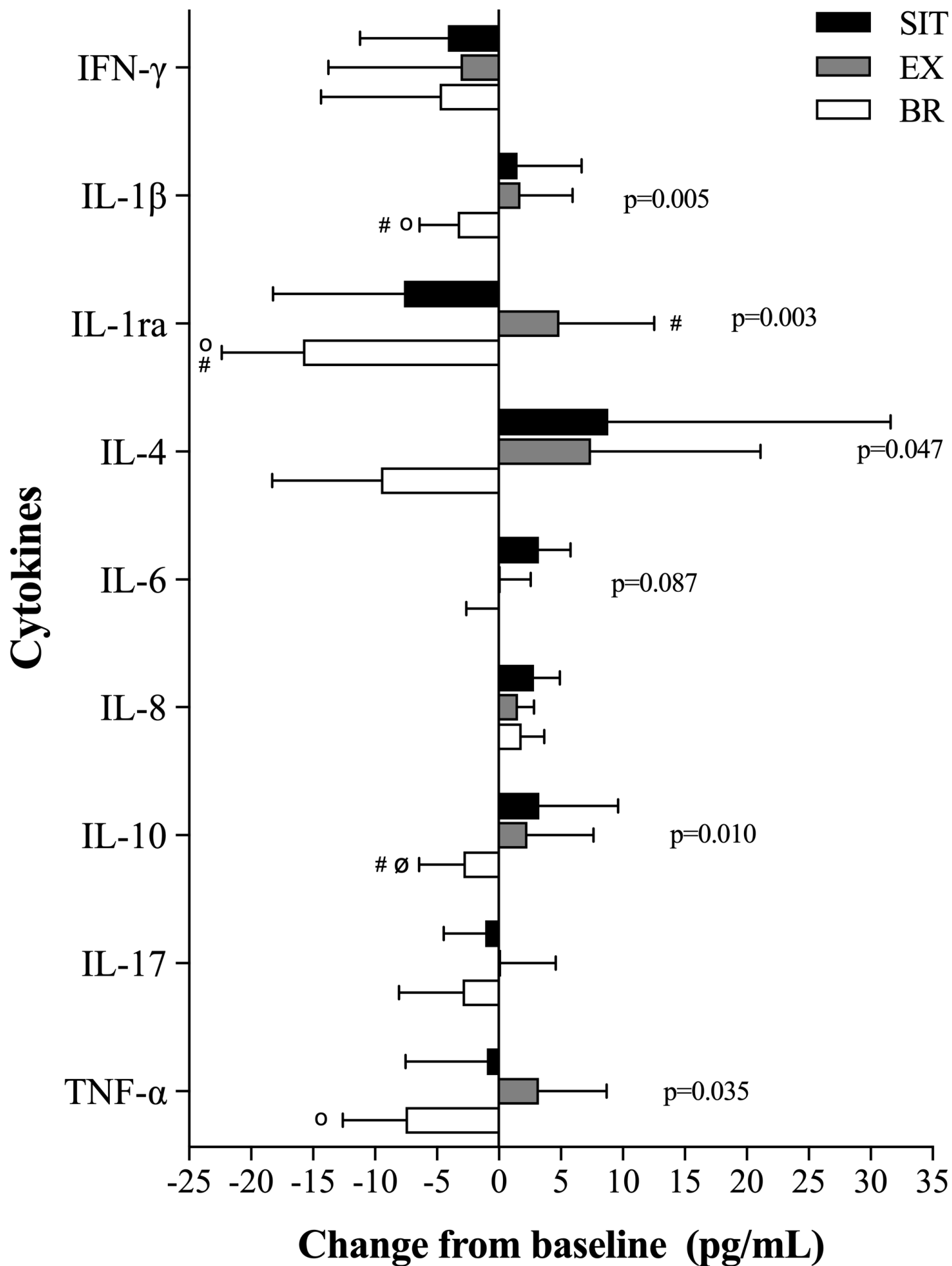
678 **SUPPLEMENTAL MATERIAL**

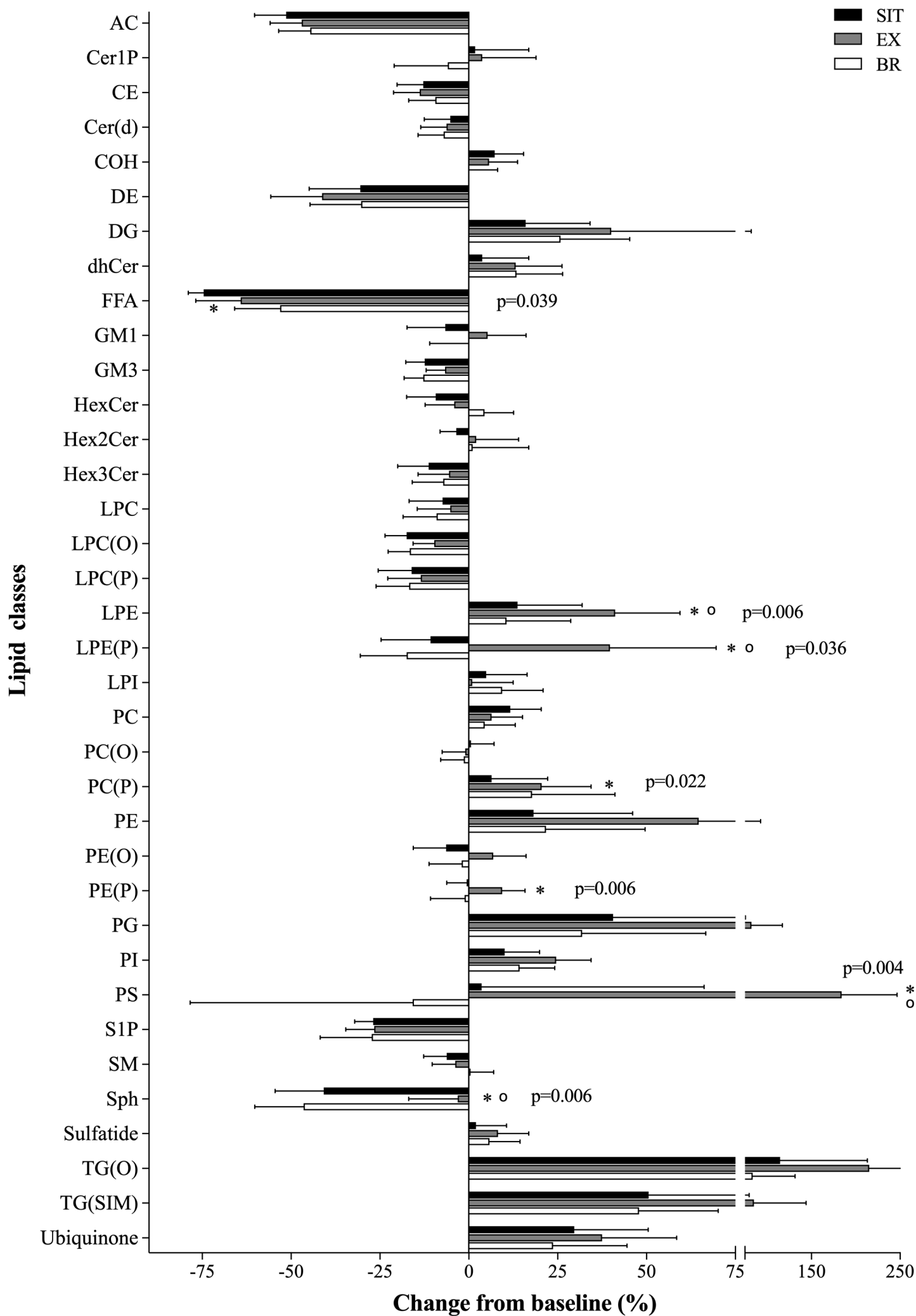
679

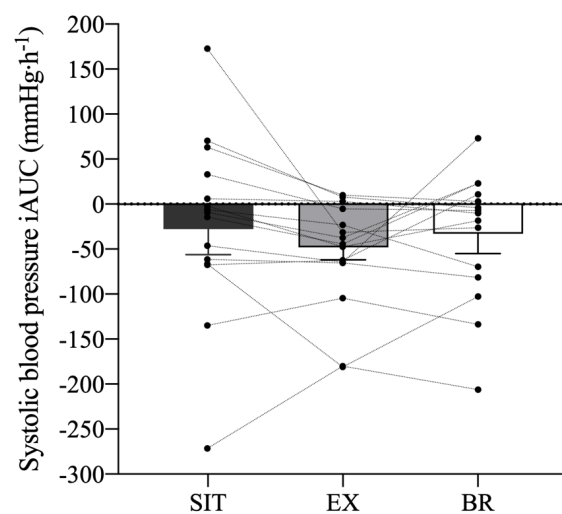
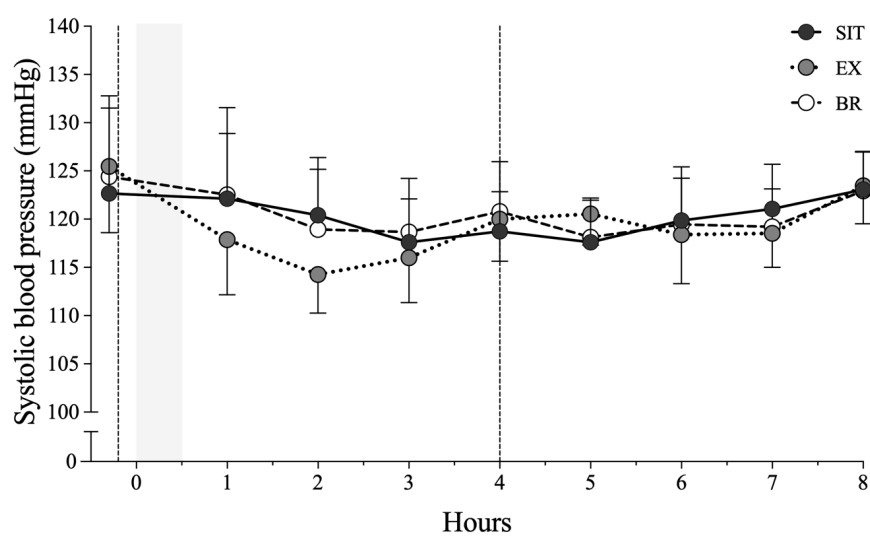
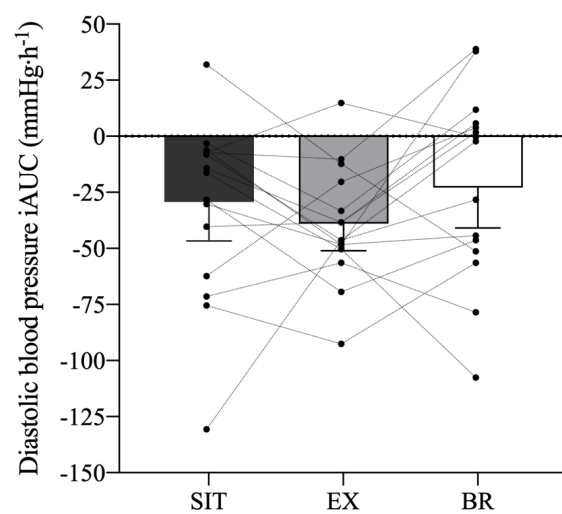
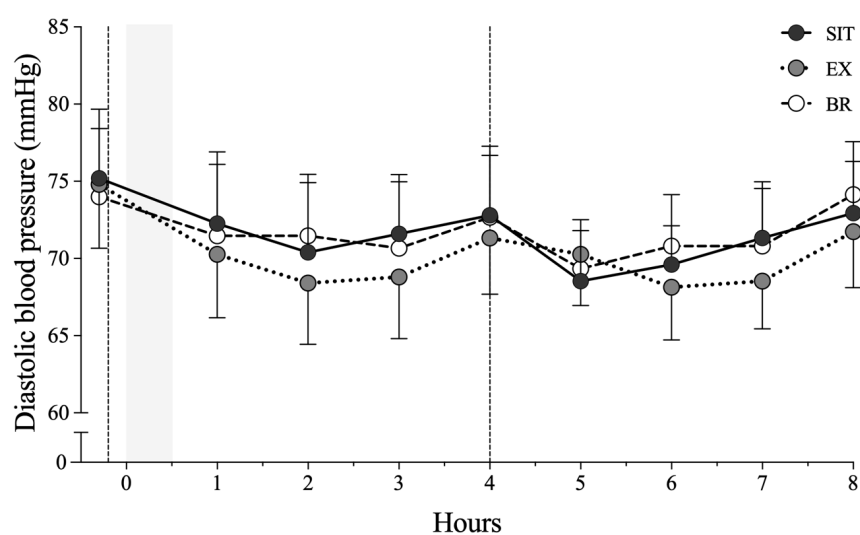
680 Can be downloaded at <https://figshare.com/s/c733b62a13928197731d> (doi:
681 <https://doi.org/10.6084/m9.figshare.14839701.v2>).









A**B****C**