Frequently interrupting prolonged sitting with light body-weighted resistance activity alters psychobiological responses to acute psychological stress: A randomised crossover trial

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All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Lay Summary:

Sitting for long periods without interruption and the way in which we physically respond to short-term psychological stress are linked to heart disease risk. Breaking up sitting with short, frequent bouts of light activity can lower heart disease risk but how this may improve how we respond to stress is unknown. Our study investigated if interruptions to prolonged sitting with body-weighted resistance activity lowered changes seen under stress such as changes in blood pressure and inflammation. 17 participants undertook 2 testing sessions. One session interrupted 4 hours of sitting with 4-min of light activity every 30-min, and the other session was 4 hours of uninterrupted sitting. After each session, participants did two stress tasks: one math-based task and one where feet were submerged in cold water. The changes in blood pressure and inflammation to stress were measured. We found when breaking up sitting time with activity, blood pressure was lower after the cold-water task compared to when people didn't break up their sitting. In summary, breaking up sitting with frequent bouts of light activity may influence how we respond to short-term stress, but future research needs to explore what these short-term changes mean for the longer-term risks of heart disease.

Abstract

Background: Uninterrupted prolonged sitting and exaggerated psychobiological reactivity to acute psychological stress are associated with increased risk of cardiovascular disease (CVD). Breaking up prolonged sitting with frequent, short bouts of light intensity physical activity acutely lowers CVD risk markers under resting conditions.

Purpose: To examine whether frequent interruptions to prolonged sitting with body-weighted resistance activity can acutely lower SBP (primary outcome) and other cardiovascular, inflammatory and cortisol (secondary outcomes) responses to acute psychological stress.

Methods: This randomised crossover trial included 17 sedentary participants (9 men; mean ± SD age; 24.0 ± 0.5 years) who completed two conditions: (1) interrupting 4h of sitting with 4-min of light body-weighted resistance activity every 30-min (BREAK), and (2) 4h of uninterrupted sitting (SIT). Following the BREAK and SIT intervention windows, cardiovascular, inflammatory and cortisol markers were measured at rest, during stress tasks (8-min Paced Auditory Serial Addition Test [PASAT] and 3-min Cold Pressor [CP]) and during 45-min recovery periods.

Results: There were main effects of time for cardiovascular parameters (SBP, DBP, HR, cardiac output, and total peripheral resistance [all $p<.001$]), inflammatory markers (interleukin-6) and cortisol ($p<.05$) in response to stress. Time-by-condition interaction effects revealed that in the BREAK condition there was lower SBP during immediate recovery from the CP (mean [95% CI]:127.2 [121.3, 133.4] vs 133.4 [125.5, 141.7] mmHg; $p=.020$), higher concentrations of plasma interleukin-6 45-min post-PASAT (2.70 [1.97, 3.70] vs 1.71 [1.32, 2.22] pg/ml; $p=.010$), and larger (non-significant) salivary cortisol concentrations 8-min post-CP (6.29 [4.60, 8.58] vs 3.97 [3.16, 4.99] nmol/l; $p=.079$).

Conclusions: Interrupting prolonged sitting with frequent bouts of light intensity body-weighted resistance activity alters psychobiological responses to acute psychological stress. Further research should explore the longer-term implications for CVD risk.

Introduction

Cardiovascular disease (CVD) remains the leading cause of global disease burden, with the number of disability adjusted life years lost due to CVD in 2019 approaching 393 million [1]. An emerging risk factor for CVD is sedentary behaviour [2], which is defined as "any waking behavior characterized by an energy expenditure ≤1.5 metabolic equivalents (METs), while in a sitting, reclining or lying posture" (p. 9) [3]. High volumes of habitual sedentary behaviour increase CVD risk [2], and one potential mechanism is through exaggerated cardiovascular (CV), inflammatory and cortisol reactivity to acute psychological stress [4]. Exaggerated psychobiological (i.e., CV, inflammatory, cortisol) responses to stress, and impaired recovery post-stress, are associated with the acute triggering of adverse CV events [5] and can temporally augment the risk of experiencing CVD outcomes (e.g., mortality, hypertension, atherosclerosis) if ignited regularly over time [6]. For example, exaggerated systolic blood pressure (SBP)/diastolic blood pressure (DBP) [7], interleukin-6 (IL-6) [8], and cortisol reactivity [9] are longitudinally associated with higher future resting blood pressure (BP). Although a higher total volume of sedentary behaviour is detrimentally associated with CVD risk, emerging evidence suggests that sedentary behaviour accrued in prolonged, uninterrupted bouts is especially harmful [2]. Frequently interrupting prolonged sitting with light walking acutely lowers psychobiological parameters under resting conditions, including BP [10], inflammation [11] and cortisol [12]. Breaking up sitting with body-weighted resistance activity is also beneficial for resting psychobiological parameters [2], may even be superior to light walking interruptions for lowering BP and sympathetic activity at rest [13], and is highly tolerable in a real-world environment [14].

Lower resting psychobiological parameters are associated with healthier psychobiological responses to (and recovery from) acute psychological stress [7,15]. Psychobiological stress testing is a paradigm that could be used to glean unique insights into the effects of breaking up sitting on CVD risk, by exacerbating psychobiological changes that might not be observable under resting conditions [16]. There are two major categories of psychological stress task: active and passive. Active stress paradigms require participant engagement, and individuals can change task outcomes via alterations in behaviour/performance [17]. Active stress tasks primarily stimulate beta-adrenergic pathways, which is reflected by immediate increases in BP, heart rate (HR) and cardiac output (CO) [17]. Contrastingly, passive stress paradigms (e.g., the cold pressor) involve participants undertaking a task where they cannot change the outcome via behavioural or performance-related adjustments [17]. Passive stressors tend to elicit immediate alpha-adrenergic activation, which is associated with increases in BP and total peripheral resistance (TPR) [17]. Importantly, using both active and passive stress tasks allow examination into whether the effects of interrupting prolonged sitting impact on different physiological (alpha- vs. beta-adrenergic) mechanisms, which is not yet fully understood in the literature.

Studies have considered cardiorespiratory fitness and physical activity (PA) in the context of stress reactivity, with a recent review finding fitter and more active individuals produce smaller CV and cortisol responses to acute psychological stress [18]. However, findings from highly-controlled exercise training trials are inconsistent, as some show exercise training can lower psychobiological responses to stress [19] and others report null results [20]. To our knowledge, only one study has experimentally manipulated sedentary behaviour in the context of psychobiological stress reactivity, where a 14-day intervention increased ActiGraph-determined sedentary time, but did not alter CV (SBP, DBP and HR), interleukin-6 (IL-6), or cortisol responses to stress [21]. Additional research is needed to

build on this study by exploring a physically inactive population, considering postural components of sedentary behaviour, and accounting for sedentary behaviour accumulation patterns, as these factors might be important in the context of psychobiological reactivity to stress.

The aim of this study was to examine the acute effects of interrupting prolonged sitting with short, frequent bouts of light intensity body-weighted resistance activity on psychobiological responses to acute psychological stress. SBP stress changes was selected as our primary outcome, due to the known effects of interruptions to sitting time on resting SBP. Secondary measures included stress-induced changes in other CV markers, inflammatory measures, and salivary cortisol. We hypothesised that a healthier pattern of psychobiological changes to stress (i.e., smaller magnitude changes from baseline to stress, and more efficient recovery post-stress) would emerge after a prolonged bout of sitting was frequently broken-up with body-weighted resistance activity, compared to when psychobiological changes to stress were measured after a prolonged bout of uninterrupted sitting.

Methods

Participants

Seventeen healthy participants aged between 18-30 years were recruited from Loughborough University and the surrounding area between May 2021 and August 2021. Required sample size was calculated for our primary outcome measure (SBP) using G*Power (Dusseldorf, Germany), with effect size estimates derived from research testing the effects of a similar intervention to the present study on resting SBP [13]. With $\alpha=0.05$, $\beta=0.80$, Cohen's f=0.39, number of groups=2, number of measurements=5, correlation amount repeated measures=0.3 and nonsphericity correction E=1.0, a minimum sample size of 14 was computed. Exclusion criteria were any individual who reported: meeting United Kingdom PA guidelines (>150 min/week of moderate PA or >75 min/week of vigorous PA), sitting for <8

hours/day, any current or previous non-communicable chronic disease, acute or chronic illness, taking prescription medication (excluding oral contraceptives), or being current smokers/vapers. Individuals with hypertensive levels of resting BP and obese levels of body fat percentage (>32% [male] or >45% [female]) were also excluded. Ethical approval was granted by Loughborough University's Human Participants Ethics Sub-committee (2020-1256-1299) and informed consent was obtained (during each study visit) from all participants included in the study. This project adhered to the ethical standards laid down in the 1964 Declaration of Helsinki.

Study design and procedure

This crossover trial involved three sessions (one screening condition, and two experimental conditions separated by a ≥7-day washout). For the experimental conditions, intervention and stress task order were randomised, but each participant completed the same stress task first during both experimental conditions.

Screening session: The screening session checked participant eligibility. Brachial BP (Omron M6 comfort, Omron Healthcare, Milton Keynes, UK) was assessed, questionnaires (e.g., sociodemographics) were administered, and anthropometric measurements (height [274 stadiometer, Seca, Hamburg, Germany]; weight and body fat percentage [mBCA 515 bioimpedance scales, Seca, Hamburg, Germany]) were taken. Familiarisation to the body-weighted activities were also conducted. Participants were fitted with an activPAL3 micro (PAL Technologies Ltd, Glasgow, UK) and ActiGraph GT3X BT+ (ActiGraph, Florida, USA) to wear for seven days so that habitual levels of sedentary behaviour and PA could be assessed (see below).

Intervention window: Each participant started the experimental conditions at the same time (between 8am-9am) in a fasted state (>10 hours), after having abstained from vigorous PA for 24h, alcohol for 12h and any over-the-counter medication for 7d. First, there was a 30-min rest period, during which a cannula was inserted into an antecubital vein and the first

blood sample was taken. An activPAL3 micro was attached to the thigh as an intervention-related manipulation check (detailed below). The 4h intervention window then started (0h), and a low-fat breakfast was provided (cereal + skimmed milk, cereal bars, orange juice) as this has been shown to have a minimal effect on stress reactivity [22]. A cereal bar was also provided at 2h. Participants were allowed to drink water *ad libitum* in the first experimental condition*,* with volume of water consumption matched in the second condition. Individuals were asked to avoid unnecessary movement but were allowed to use the toilet *ad hoc* and complete unstimulating activities (e.g., reading) when measurements were not being taken, which were also matched across condition. Hourly BP measurements (starting 20-min after the previous bout of resistance activity) and perceived exertion ratings [23] were collected throughout the intervention window, and a blood sample was taken post-intervention (called "pre-stress" sample).

The intervention to interrupt sitting (BREAK) involved breaking up prolonged sitting with 4-min bouts of body-weighted resistance activity every 30-min [13] (Electronic Supplementary Material Figure 1). The resistance activities were 20s of half squats, 20s of calf raises, and 20s of gluteal contractions with knee raises, which were completed in sequence and repeated four times per bout. This intervention was selected because it engages all of the major muscles of the lower body and attenuates resting BP and sympathetic activity with impressive magnitude [13]. Due to the addition of a stress reactivity protocol in this study, 4-min activity bouts (rather than 3-min bouts) were selected, which ensured that the total volume of resistance activity (36 min) was matched to previous work [13]. The sitting intervention (SIT) involved 4h of uninterrupted sitting.

Stress protocol: After the intervention window of each condition, participants completed a seated stress protocol (Electronic Supplementary Material, Figure 2) in a light and temperature (20–22°C) controlled laboratory. This consisted of a 20-min "pre-stress" baseline

period, followed by two stress tasks delivered in a randomised order across participants: an 8-min mental arithmetic task and a 3-min cold pressor task. After each stress task there were 45-min recovery periods. Participants self-reported (Likert scale; 0-7) levels of stress, engagement, difficulty, arousal, and perceived performance after each period of the stress protocol, with higher scores reflecting greater levels. Participants quietly watched a nature documentary (Planet Earth 1/2 or Blue Planet 1/2; BBC, UK) when data were not being recorded.

Assessment of sedentary behaviour (activPAL)

A thigh-mounted activPAL3 micro (PAL Technologies Ltd, Glasgow, UK) was deployed in the experimental sessions as an intervention-related manipulation check. Data were recorded during the 4h intervention windows and analysed by Processing PAL software (version 1.3, Leicester, UK), with time spent sitting during each intervention summed, after the removal of any standing/moving time (e.g., performing activities in the BREAK-condition). An activPAL3 micro was also used to measure habitual levels of sedentary behaviour, with the device attached to the middle-anterior line of the non-dominant thigh. Data started recording from the first midnight after deployment during the screening session, for seven continuous days (24 hours/day). Again, data were analysed using Processing PAL (version 1.3, Leicester, UK), and each participant required at least four days of valid data, with a valid day defined as >10 hours of wear time, >499 steps and <95% of time in any one posture [24]. Processing PAL data were cross-referenced with daily log diaries that measured sleep and wake times, any clear errors were manually corrected [24].

Assessment of physical activity (ActiGraph)

An ActiGraph GT3X BT+ triaxial accelerometer (ActiGraph, Florida, USA) was initialized (100hz; using ActiLife version 6) and deployed on the non-dominant wrist, with recording starting from the first midnight after the screening session. All incidental PA was

measured across seven full days and nights. As described elsewhere [4,25], the R-package

GGIR (version 2.0) was used to process and analyse this data, using the triaxial signal and

raw (gravitational) acceleration, rather than accelerometer "brand specific" count per minute

cut points. Validated algorithms were used to identify and remove any sleep and non-wear

periods [26]. Moderate-to-vigorous PA (MVPA) was defined as raw acceleration >100 milli-

g and light PA was defined as >30 milli-g [27].

Psychological stress tasks

Participants completed an active psychological stress task, which was an 8-min

version of the Paced Auditory Serial Addition Test (PASAT) [28] with socio-evaluative

elements of scoring, competition, and video-recording [29]. This task involves remembering

and adding sequential numbers, and is effective in perturbing multisystem physiology with

good test re-test reliability across multiple days [30]. Participants provided answers by

pointing to a number on a sheet of paper in front of them. One point was awarded for every

correct answer.

The passive Cold Pressor (CP) task required participants to put both feet (up to their

ankles) in a box of cold water (4°C) for up to three minutes and is efficacious at acutely

increasing CV and cortisol activity [31] with good temporal reproducibility [32]. Participants

were told that they could remove their feet before the 3-min limit, but none exercised this

option.

Psychobiological measures

Resting cardiovascular activity during the intervention

Brachial resting BP and heart rate (HR) measurements were taken during the

intervention windows (Omron M6 comfort, Omron Healthcare, Milton Keynes, UK), with the

first measurement preceded by five minutes of quiet rest and the others by two minutes; the

average of the two final readings was used [33].

Cardiovascular activity during the stress protocol

The Human Non-Invasive Blood Pressure (NIBP) system (ADInstruments, Oxford, UK) with Modelflow algorithms [34] gathered beat-to-beat CV data, via a photoplethysmographic cuff attached the middle phalanx, of the middle finger, of the arm without the indwelling cannula. This yielded measures of SBP (primary outcome) alongside DBP, mean arterial pressure (MAP), stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR) [secondary outcomes]. The arm was positioned at heart level and wrapped in a heated blanket to ensure consistency in the waveform. Data were collected in second-by-second format, cleaned for erroneous values, and then averaged into the following periods: the final 8-min of the pre-stress baseline period (pre-stress), the 8-min PASAT (PASAT), the first 8-min of PASAT recovery (PASAT recovery), the 3-min CP (CP) and the first 8-min of CP recovery (CP recovery). Photoplethysmography is well validated and frequently used in stress reactivity research [29]. In addition, three-lead electrocardiography was used to measure HR, with bipolar silver-silver chloride electrodes attached to the left and right clavicle and lower left rib. Data were sampled at 1000hz (PowerLab, ADInstruments, Oxford, UK) and analysed by LabChart version 8 (ADInstruments, Oxford, UK), where R waves were automatically detected and manually checked.

Blood sampling and analysis

A 20-gauge BD Nexiva cannula (BD, New Jersey, USA) was inserted into the most suitable antecubital vein before the intervention windows began, with blood samples collected at the following time points: pre-intervention, pre-stress baseline (pre-stress), immediately post-PASAT (PASAT), 45-min post-PASAT (PASAT recovery), immediately post-CP (CP) and 45-min post-CP (CP recovery). During each draw, the first 2ml of collected blood were discarded before 7.6ml were withdrawn into potassium ethylene diaminetetraacetic acid [K_3EDTA] tubes (Starstedt, Leicester, UK). The cannula was then

flushed with 5cc infusion saline (0.9% NaCl). To determine total and differential leukocyte counts, a 20μl volume of whole blood was analysed by an automated cell counter (Yumizen H500, Horiba Medical, Montpellier, France). The remains of each sample were stored in ice (maximum of 20 min), centrifuged (2500rpm, 10 min at 4ºC), aliquoted, and then frozen (-80ºC). IL-6 was assayed in duplicate using a high-sensitivity ELISA (R&D systems, Minneapolis, USA) following the manufacturer's instructions. The intra- and inter-assay coefficients of variation were 3.17% and 8.22%. Inflammatory data were adjusted for plasma volume changes, relative to pre-intervention concentrations [35].

Saliva sampling and analysis

Whole saliva samples were collected via passive drool (into a polypropylene vial using a SalivaBio Collection Aid [Salimetrics, California, USA]) at the end of the pre-stress baseline period (pre-stress), 8-min post-PASAT (PASAT), 30-min post-PASAT (PASAT recovery), 8-min post-CP (CP) and 30-min post-CP (CP recovery). All samples were temporarily placed in ice before being frozen (80ºC) and were later assayed in duplicate using a high sensitivity cortisol immunoassay kit (Salimetrics, California, USA). The intra- and inter-assay coefficients of variation were 1.85% and 4.10%, respectively.

Statistical analyses

Data were analysed using SPSS version 27 (IBM, Chicago, USA), with $\alpha < .05$. One-way ANOVAs investigated any significant sex differences across participant characteristics. After data were screened for outliers and erroneous values, generalized estimating equation models (GEEs) were used for our analyses. Briefly, GEEs were used because they take all available data into account in an unbalanced design when data might be missing completely at random (i.e., these models are highly appropriate for handing missing data) and therefore lead to more efficient effect estimates (e.g., treatment effects) [36]. GEEs also account for dependency within repeated measure designs through residuals and their correlation structure

and can handle non-normally distributed data. [37]. Firstly, GEEs compared activPAL-derived sedentary time across conditions (as a manipulation check). GEEs also investigated time-by-condition interaction effects regarding the effect of the intervention on resting BP and HR (-20 min, 50min, 1h50min, 2h50min and 3h50min), IL-6 and leukocyte concentrations (pre- and post-intervention [called "pre-stress"]), and perceived exertion ratings (0min, 60min, 120min, 180min and 240min). Intervention allocation order was added as a covariate.

Our stress data were also analysed using GEEs, where stress task and intervention allocation order was added to each model as one covariate, to adjust for possible order effects. Within-subject effects of time (mean of each period in the stress protocol) and time-by-condition interaction effects were assessed separately for each parameter. An autoregressive [AR(1)] correlation structure and appropriate choice of distribution and link were selected. Cramér's V (V) is reported to represent effect size; $V=0.1$ (small), $V=0.3$ (medium), $V=0.5$ (large). Any outcomes showing significant time-by-condition interaction effects were interrogated with post-hoc pairwise comparisons that were integrated within the GEE models, such that psychobiological data (e.g., mean SBP) were compared (across SIT vs BREAK conditions) for the different phases of the stress protocol (e.g., during baseline, PASAT, CP recovery). Holm-Bonferroni corrections [38] were applied to adjust for an inflated type I error rate that occurs during multiple comparison analyses.

Results

Sample characteristics

All (n=17) participants completed the three sessions that form this study. Sample characteristics are summarised in Table 1. Participants were highly sedentary, based on data from UK adults [39], and although daily MVPA levels appear high, they are lower than national averages derived from similar methodological approaches, where approximately 1,000 min/week of MVPA can be expected [40]. Participants were normotensive with resting

concentrations of IL-6 and leukocytes within the healthy range (Table 2). There were no differences across condition for PASAT score, CP engagement time, or self-reported appraisal (stress, engagement, difficulty, arousal, or perceived performance) during our stress tasks (all $p > .05$). The only participant characteristic variable which significantly differed ($p < .05$) across males and females was body fat percentage, which was higher in females (mean $\pm$ SD = 31.23 $\pm$ 5.35%) compared to males (22.73 $\pm$ 10.71%).

Intervention-related manipulation check

ActivPAL-measured sedentary behaviour time was higher ($p<.001$) in the SIT-condition (mean {95% confidence interval [CI]}; 3.94 [3.86, 4.00] hours) versus the BREAK-condition (3.39 [3.33, 3.49] hours). For ratings of perceived exertion, there was no effect of time ($p>.05$), but there was a significant time-by-condition interaction effect (Wald χ^2=9.81, p=.044, V=.12). The average (main effect of condition) BORG score in the SIT condition was 6.3 [6.1, 6.6] (i.e., reflecting "rest"), versus 10.0 [9.3, 10.7] (i.e., reflecting "light exertion") in the BREAK-condition ($p<.001$).

Interrupting prolonged sitting and resting cardiovascular changes during the intervention window

There were significant main effects of time for SBP (Wald χ^2=17.66, p=.001, V=.16) and HR (Wald χ^2=259.17, $p<.001$, V=.62), but not DBP ($p>.05$). There were no significant time-by-condition interaction effects observed for SBP, DBP or HR (all $p>.05$; Electronic Supplementary Table 1), suggesting that resting CV activity changed similarly during the intervention window of both conditions.

Interrupting prolonged sitting and resting inflammatory changes during the intervention window

The was a significant main effect of time for IL-6 (Wald χ^2=4.09, p=.043, V=.26), which increased from 1.37 [1.15, 1.65] pg/ml at pre-intervention to 2.10 [1.50, 2.96] pg/ml at post-intervention. The time-by-condition interaction effect for IL-6 was non-significant

(p>.05; Figure 1). There were no time or time-by-condition interaction effects observed for total or differential leukocyte counts (all p>.05).

Interrupting prolonged sitting and cardiovascular responses to stress

There were main effects of time for all of our CV variables, which suggests CV perturbation (all p<.001, Figure 2). A significant time-by-condition interaction effect was found for our primary outcome of SBP (Wald χ^2=13.42, p=.009, V=.14), with post-hoc pairwise comparison revealing that SBP was higher in the SIT-condition versus the BREAK-condition during CP recovery only (133.4 [125.5, 141.7] vs 127.2 [121.3, 133.4] mmHg; unadjusted p=.004, adjusted p=.020). As shown in Figure 2, there was also a time-by-condition interaction effect for HR (Wald χ^2=15.17, p=.004, V=.15). Pairwise comparisons revealed that HR was statistically higher in the BREAK-condition (relative to the SIT-condition) during the pre-stress baseline (65.8 [61.1, 70.8] vs 73.1 [67.2, 79.5] bpm; unadjusted p<.001, adjusted p<.001), the PASAT (77.7 [73.0, 82.8] vs 84.5 [78.8, 90.5] bpm; unadjusted p=.012, adjusted p=.024), PASAT recovery (68.9 [64.0, 74] vs 73.6 [68.1, 79.5] bpm; unadjusted p=.001, adjusted p=.003), and CP recovery (66.8 [62.4, 71.6] vs 72.6 [66.6, 79.1] bpm; unadjusted p<.001, adjusted p<.001). There were no time-by-condition interaction effects observed for DBP (Wald χ^2=6.80, p=.147, V=.10), MAP (Wald χ^2=6.00, p=.199, V=.09), TPR (Wald χ^2=5.33, p=.255, V=.06), or CO (Wald χ^2=3.79, p=.435, V=.07)

Interrupting prolonged sitting and inflammatory responses to stress

There were significant main effects of time for all the inflammatory markers in this study, which increased in concentration in response to our stress protocol (all p<.05; Figure 3). There was a significant time-by-condition interaction effect for IL-6 (Wald χ^2=10.75, p=.030, V=.14). Post-hoc pairwise comparison revealed that there was a higher concentration of IL-6 in the PASAT recovery sample (45-min post-PASAT) during the BREAK-condition (2.70 [1.97, 3.70] pg/ml), relative to the SIT-condition (1.71 [1.32, 2.22] pg/ml) (unadjusted p=.002, adjusted p=.010). A significant time-by-condition interaction effect also emerged for

total leukocyte count (Wald χ^2=13.83, p=.008, V=.15), with a higher number of leukocyte

cells in the CP sample (i.e., immediately post-CP) during the BREAK-condition (7.01 [6.07,

8.09] x 10^9/l) versus the SIT-condition (6.39 [5.51, 7.43] x 10^9/l) (unadjusted p<.001,

adjusted p<.001). The time-by-condition interaction effects for our differential leukocyte

counts were all non-significant (all p>.05).

Interrupting prolonged sitting and salivary cortisol responses to stress

There was a significant main effect of time (p<.05) and time-by-condition interaction

effect (Wald χ^2=17.31, p=.002, V=.16; Figure 3) found for salivary cortisol. Post-hoc

analyses revealed that there was a higher concentration of cortisol in the CP sample (8-min

post-CP) during the BREAK (3.97 [3.16, 4.99] nmol/l) relative to the SIT (6.29 [4.60, 8.58]

nmol/l) condition, but this was non-significant after Holm-Bonferroni correction (unadjusted

p=.017, adjusted p=.079). No significant pairwise differences in cortisol across condition

emerged with respect to any of the other stress protocol phases (all unadjusted and adjusted

p>.05).

Discussion

This is the first study to examine whether frequent interruptions to prolonged sitting

can impact psychobiological responses to acute psychological stress. Relative to 4h of

uninterrupted sitting (SIT-condition), breaking up 4h of sitting with 4-min of light body-

weighted resistance activity every 30-min (BREAK-condition) yielded lower SBP during

cold pressor (CP) recovery, higher HR during the PASAT, PASAT recovery and CP

recovery, larger IL-6 concentrations during PASAT recovery, and higher total leukocyte and

cortisol concentrations during the CP task.

Interrupting prolonged sitting and cardiovascular responses to stress

In the BREAK- compared to SIT-condition, a lower mean SBP of approximately 5-

mmHg was found during CP recovery, which suggests that frequent sitting interruptions

encouraged a more complete and prompt return of SBP back towards baseline (i.e., pre-

stress) levels post-stress (see Figure 2). The passive and vasoconstrictory effects of the CP task may help explain why significant findings only emerged during CP (and not PASAT) elements of our stress protocol, potentially because acutely interrupting sitting impacts on alpha-adrenergic mechanisms, rather than beta-adrenergic mechanisms [17].

Healthier patterns of post-stress SBP recovery (i.e., quicker recovery post-stress) are linked with healthier recovery patterns for procoagulatory haemostatic and rheostatic markers (again, more efficient recovery after stress) [41]. Therefore, if interrupting prolonged sitting can promote healthier SBP recovery, it may also beneficially impact procoagulatory mechanisms, which have been implicated in the stress-induced triggering of acute CV events [5]. However, as procoagulatory parameters were not measured in this study, this must remain speculative at this time. Improved SBP recovery after psychological stress is also longitudinally associated with an attenuated CVD risk status, including that healthier recovery of SBP after the CP task predicts lower resting BP across three years in young healthy adults [42]. Consequently, frequently breaking up sitting may also exert clinically relevant cardioprotective effects over time, if healthier patterns of SBP stress recovery are adopted regularly. Finally, incomplete or slow recovery of SBP back towards resting levels after stress might signal early dysregulation of BP control, that is not yet measurable under conditions of rest [6,16]. Overall, these three considerations highlighted above might represent novel mechanisms to help explain the lowered CVD risk found in individuals who regularly break up sedentary behaviour, versus those who predominantly engage with prolonged sedentary behaviour [2].

There are several physiological pathways that could underpin our SBP findings, including exercise-induced reductions in sympathetic activity, vagal rebound, and vascular resistance under rest and stress [2,43]. For example, acutely interrupting sitting is associated with improvements in resting plasma noradrenaline [13] and vascular function (flow

mediated dilation) [44], which may encourage a more favourable SBP recovery profile during recovery from stress. Future research must directly test these potential mechanisms. A positive relationship was found between pre-stress to PASAT changes in TPR and MAP in the SIT condition, and a positive association emerged between pre-stress to PASAT changes in CO and MAP in the BREAK-condition (data not reported). Interestingly, our earlier work revealed that habitual sedentary behaviour was positively associated with BP and TPR (and negatively associated with CO) stress reactivity [4]. Taken together, this suggests that acute bouts of prolonged sitting and habitual sedentary behaviour activate vascular (i.e., TPR) rather than cardiac (i.e., CO) pathways of BP reactivity. This is important as BP changes under stress appear most harmful for CVD when driven by vascular mechanisms [6]. We found evidence that increases in IL-6 from pre-stress to PASAT were positively associated with increases in TPR from pre-stress to PASAT, but only in the SIT-condition. This indicates that prolonged sitting may also encourage interactions between vascular and inflammatory responses to stress, which might contribute to mechanisms underlying the acute triggering of adverse CV events [45]. Further work needs to explore additional and more intricate vascular markers under stress.

There were no differences in resting brachial BP during the intervention windows of the SIT and BREAK conditions, which is in contrast to previous work [10]. This is likely to reflect methodological variability in intervention durations and/or populations. For example, sitting interruption interventions have been shown to provoke a larger attenuation of resting BP in pre-hypertensive and hypertensive populations [10], whereas our sample were normotensive and perhaps less likely to benefit. As significant differences (across condition) in BP, inflammatory markers and cortisol emerged during psychobiological stress testing, this may highlight the prognostic importance of sympathetically challenging the physiological systems to exacerbate pathophysiological changes that are unobservable at rest, but might

relate to future CVD risk [16]. Our study demonstrates a novel method of exploring risk factors associated with prolonged sitting, which might be particularly relevant when studying populations who are less likely to show psychobiological changes at rest (e.g., young, healthy individuals).

Regularly interrupting sitting induced a higher mean HR during baseline, the PASAT, PASAT recovery and CP recovery, when compared to uninterrupted sitting. This is important as lower stress-induced HR is linked to CVD risk factors, including elevated future resting DBP [6]. However, given large HR responses to stress are also associated with a poor CVD risk status [6], further research should confirm the consequences of our HR findings. Others have shown hourly interruptions to prolonged sitting with light resistance activities increased resting HR by approx. 3bpm [46]. The magnitude of condition-related differences in HR were greater in our study, which might be due to augmented metabolic and sympathetic activity induced by breaking up sitting more frequently [47].

Interrupting prolonged sitting and inflammatory responses to stress

IL-6 concentrations were higher in the PASAT recovery sample (45-min post-PASAT) in the BREAK-condition, when compared to the SIT-condition. This is the first study we are aware of to examine the effects of resistance-based interruptions to prolonged sitting on IL-6 concentrations. However, it is important to note IL-6 concentrations at pre-intervention, and in response to stress, are comparable to other crossover studies (including a study which adopted a lifestyle-based intervention to increase sedentariness, rather than an acute laboratory-based study to interrupt sitting) using similar populations [21]. Others have shown interrupting sitting with light walking every 30 min did not impact resting levels of plasma IL-6 [48], but lower resting salivary IL-8 concentrations were found after breaking up sitting with high intensity cycling every 60 min [11]. This may highlight that higher intensity PA is necessary to acutely lower inflammatory markers, perhaps due to the anti-inflammatory

window that is observed in the hours following vigorous PA [49]. Importantly, lower

intensity resistance activities can stimulate IL-6 release from muscle fibres, in a manner

proportional to the intensity of the contractions [50]. When released by muscle, IL-6 can

induce potent anti-inflammatory effects [51]. Thus, the higher concentration of IL-6 that was

found during PASAT recovery in the BREAK-condition might reflect anti-inflammatory

cytokine release as a counter action to the pro-inflammatory environment that is induced

during acute psychological stress. However, this must remain speculative because the present

study did not measure sources of IL-6 release. Conversely, because large IL-6 responses to

stress can be harmful for CVD risk [52], it is possible that inflammatory stress pathways are

not a mechanism linking interrupted sitting patterns to lowered CVD risk. Further work

should extend our findings by measuring anti-inflammatory cytokines such as IL-10, and

other markers in the pro-inflammatory cascade, including acute phase proteins and

endothelial adhesion molecules. Finally, to highlight other potential mechanisms that might

help explain our IL-6 findings, exercise-induced shear stress and elevated sympathetic

activity might have stimulated IL-6 release from vascular endothelial cells [53], and

leukocytes [54]. In partial support for this, we found a significant increase in total leukocytes

in response to stress (neutrophils appeared to be the driving force), including a higher

leukocyte count in the CP sample during the BREAK-condition, when compared to SIT-

condition.

Interrupting prolonged sitting and cortisol responses to stress

In the BREAK relative to SIT-condition, there was a higher concentration of salivary

cortisol in the CP sample (8-min post-CP), although it should be noted that this difference

across condition was non-significant (p=.079). Exercise-induced increases in heightened

sympathetic nervous system activity [55] and elevated IL-6 [56] may help explain our

cortisol findings. Reduced cortisol output during stress is prospectively linked with CVD risk

factors, including obesity and depression [57], perhaps due to the potent anti-inflammatory effects of cortisol [58]. Consequently, elevated cortisol output could be a novel cardioprotective mechanism that contributes to associations found between breaking-up sitting and lower CVD risk. However, because larger cortisol reactivity to stress also has maladaptive correlates, including hypertension [6], longitudinal research must examine the health related consequences of our cortisol findings. Relative to uninterrupted sitting, interrupting sitting with walking leads to a larger reduction in the diurnal salivary cortisol change [12] or does not influence resting plasma cortisol concentrations [48]. Inconsistences across these studies, and to our own, might be explained by methodological variability, including the effects of resistance vs endurance activity breaks, time of cortisol sampling, and plasma vs saliva collection techniques. More homogenous research is needed before conclusions can be drawn when considering the effect of breaking up sitting on cortisol, particularly when considering cortisol responses to acute psychological stress.

Methodological considerations

Strengths of this study include the randomised crossover design and the generalised statistical approach to analysis (which accounts for random missing data without deleting cases, and non-normally distributed variables without the need for log transformation). The intervention we tested is also feasible and tolerable to perform in a real-world environment [14]. As mentioned, this study was powered to detect changes in our primary (stress-induced SBP changes), but not secondary (stress-induced changes in other CV outcomes, inflammatory markers, and cortisol) outcome measures. This should be recognised as a limitation, but our sample size is comparable to similar studies examining psychobiological parameters under resting conditions [44,47] and our data should be considered hypothesis generating for future studies. Our findings may have been confounded by habitual PA/sedentary behaviour levels, or the acute effects of exercise (rather than the act of breaking

up sitting). However, we adjusted for habitual volumes of sedentary behaviour/MVPA (data not reported), and this made no difference to our findings. In addition, we selected low intensity resistance activities, as higher intensity exercise can impact on stress reactivity measures [18,19]. It is possible that the timing of eating as part of the study design may have impacted on some of the biomarker responses assessed, by inadvertently inducing a state of fasting. However, there was a 2h window between the final consumption of food and the start of the "pre-stress" baseline period, which is consistent with other studies [29]. It is also possible that menstrual cycle phase influenced certain markers in this study (e.g., cortisol) [59], however, others have shown no effect of the menstrual cycle on other reactivity markers, including BP [60]. We decided to test during all phases of the menstrual cycle to increase ecological validity of our findings. Due to logistics, we were not able to collect saliva samples immediately post-stress, and our participants were asked to provide answers to the PASAT by pointing to a number on a sheet rather than verbally. This was due to collection of respiratory measures (data not reported) and is in line with our other previously published work [4]. While this may have further enhanced the stressful nature of the task, participants' self-reported perceptions of stress would indicate that the stressful nature of the task is consistent with other literature [61]. Due to the acute nature of this study the implications of our findings cannot be extrapolated to longer-term CVD outcomes. Finally, our sample were young and healthy (but highly sedentary) and therefore our results may not generalise to the general population, including to those with heightened CVD risk.

Conclusion

Our study provides the first evidence to show that manipulations to sedentary behaviour (frequently breaking-up prolonged sitting with body-weighted resistance activity) can impact psychobiological responses to acute psychological stress, evidenced by lowered SBP during CP recovery, increased HR throughout the stress protocol, elevated IL-6

concentrations during PASAT recovery, and increased total leukocyte and cortisol levels during the CP (relative to when psychobiological responses to stress were measured after a prolonged bout of uninterrupted sitting). Future research should explore at risk populations, investigate other intervention strategies (e.g., breaking up prolonged sitting with light walking every 20 min), and examine the longer-term implications for CVD outcomes.

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Table 1. Participant characteristics.

	Mean (SD) / N (%)
Age (years)	24.2 (0.6)
Sex (male)	9 (53)
Ethnicity (white)	12 (71)
A non-manual occupation category for the head of the household	14 (82)
Body mass index (kg/m^2)	25.2 (1.2)
Body fat percentage (%)	26.8 (2.4)
Average daily hours of habitual sedentary behaviour (activPAL)	10.7 (0.3)
Average daily minutes of habitual light physical activity (ActiGraph)	191.8 (12.3)
Average daily minutes of habitual moderate-to-vigorous physical activity (ActiGraph)	81.0 (7.9)

Table 2. Pre-intervention physiological data across the SIT and BREAK conditions.

	SIT condition	BREAK condition	Wald χ^2	p	Cramer's V (effect size)
Resting systolic blood pressure (SBP; mmHg)	110.1 [105.0, 115.4]	110.1 [105.0, 115.5]	0.01	.985	.02
Resting diastolic blood pressure (DBP; mmHg)	71.8 [67.6, 76.3]	69.7 [65.4, 74.2]	1.75	.185	.23
Resting heart rate (HR; bpm)	63.9 [60.0, 68.2]	66.3 [61.2, 71.9]	2.16	.142	.26
Resting interleukin-6 concentration (IL-6; pg/ml)	1.28 [0.94, 1.75]	1.49 [1.07, 2.08]	0.38	.536	.11
Resting total leukocyte count (10^9/l)	5.98 [5.32, 6.71]	6.18 [5.50, 6.94]	0.73	.393	.15
Resting neutrophil count (10^9/l)	3.06 [2.55, 3.68]	3.35 [2.79, 4.04]	1.86	.173	.25
Resting lymphocyte count (10^9/l)	2.09 [1.81, 2.41]	1.91 [1.69, 2.15]	1.64	.200	.23
Resting monocyte count (10^9/l)	0.52 [0.46, 0.59]	0.56 [0.46, 0.68]	1.42	.234	.22

Note. Data are mean [95% confidence intervals], presented statistics reflect main effect of condition with unadjusted p values.
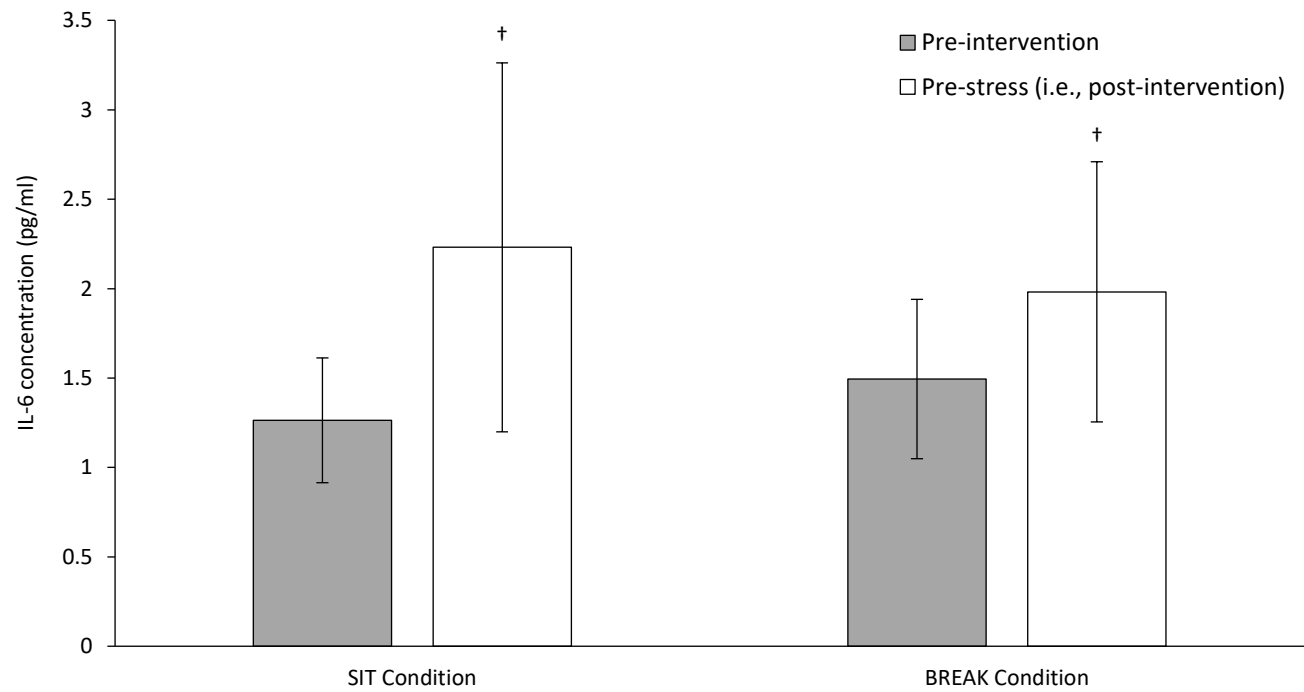
Figure 1. Generalized estimating equation time-by-condition interactions for interleukin-6 (IL-6), in terms of change from pre-intervention to post-intervention, with adjustment for intervention order as a covariate. Data provided as estimated marginal means with error bars that represent 95% confidence intervals. † indicates significant difference from pre-intervention as a main effect of time (p<.05). SIT condition=uninterrupted sitting condition, BREAK condition=interrupted sitting condition.
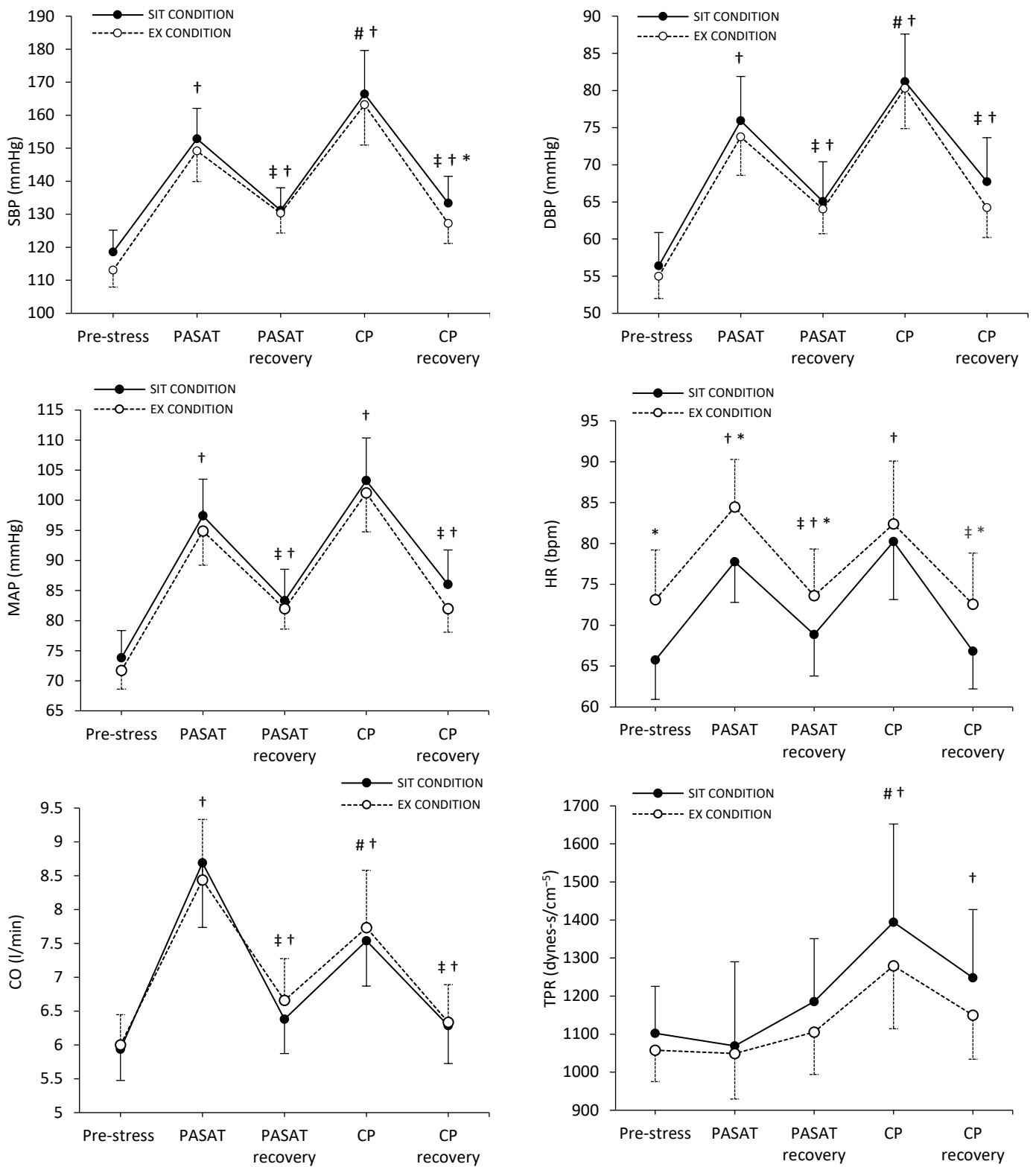
Figure 2. Time-by-condition interaction effects for systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (HR), cardiac output (CO), and total peripheral resistance (TPR), with adjustment for stress task and intervention order. PASAT = Paced Auditory Serial Addition Test, CP = Cold Pressor. Solid line = SIT condition, dashed line = BREAK condition. Data provided as estimated marginal means; error bars represent 95% confidence intervals. * Indicates significant difference across condition (Holm-Bonferroni adjusted $p < .05$), † indicates significantly different from pre-stress (unadjusted $p < .05$), ‡ indicates significant change from stress task to associated recovery period (unadjusted $p < .05$), # indicates significant difference when comparing the PASAT and CP (unadjusted $p < .05$).
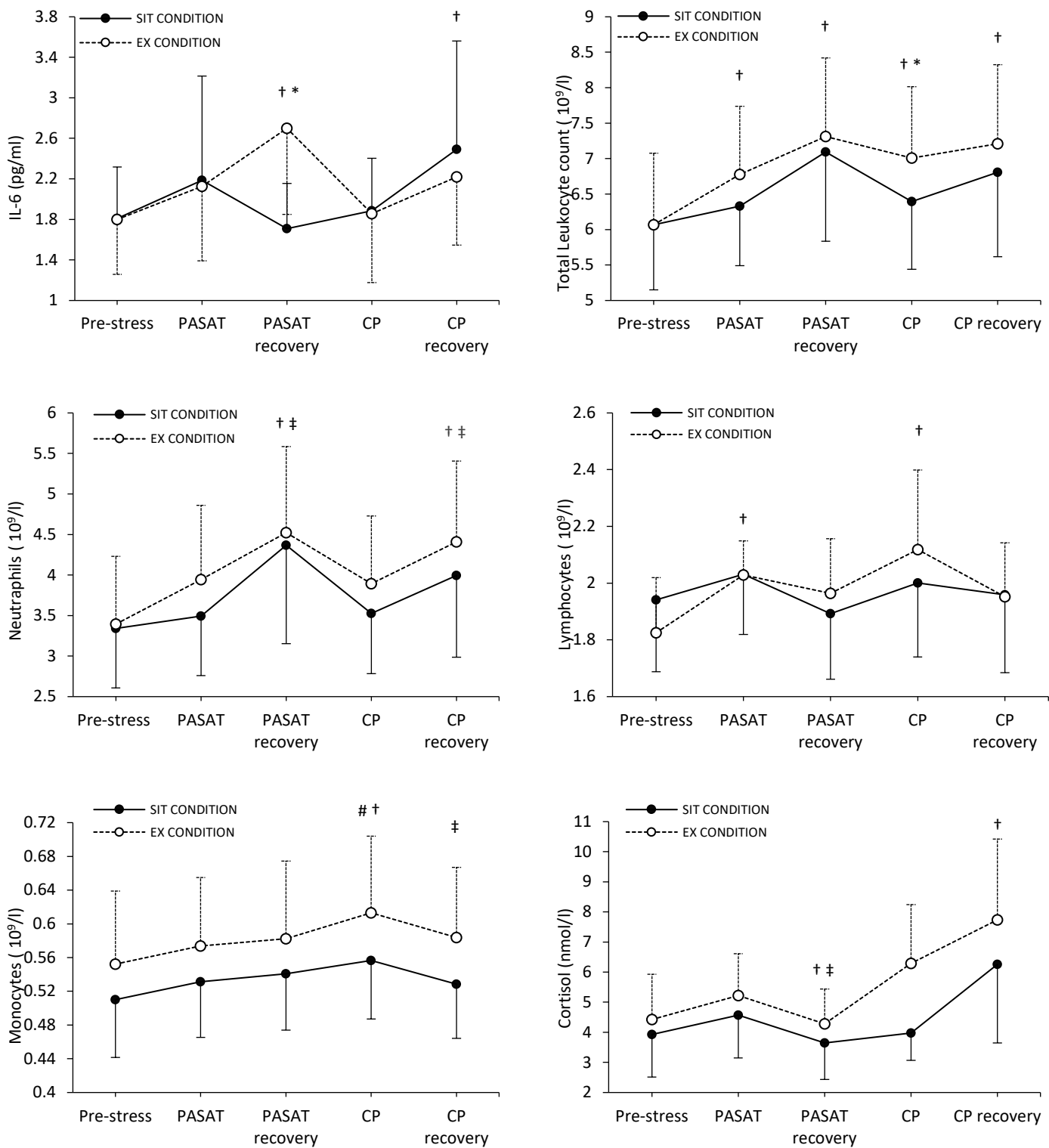
Figure 3. Time-by-condition interaction effects for interleukin-6 concentration, total leukocyte count, neutrophil count, lymphocyte count, monocyte count, and salivary cortisol concentration, with adjustment for stress task and intervention order. PASAT = Paced Auditory Serial Addition Test, CP = Cold Pressor. Solid line = SIT condition, dashed line = BREAK condition. Data provided as estimated marginal means; error bars represent 95% confidence intervals. * Indicates significant difference across condition (Holm-Bonferroni adjusted $p < .05$), † indicates significantly different from pre-stress (unadjusted $p < .05$), ‡ indicates significant change from stress task to associated recovery period (unadjusted $p < .05$), # indicates significant difference when comparing the PASAT and CP (unadjusted $p < .05$).
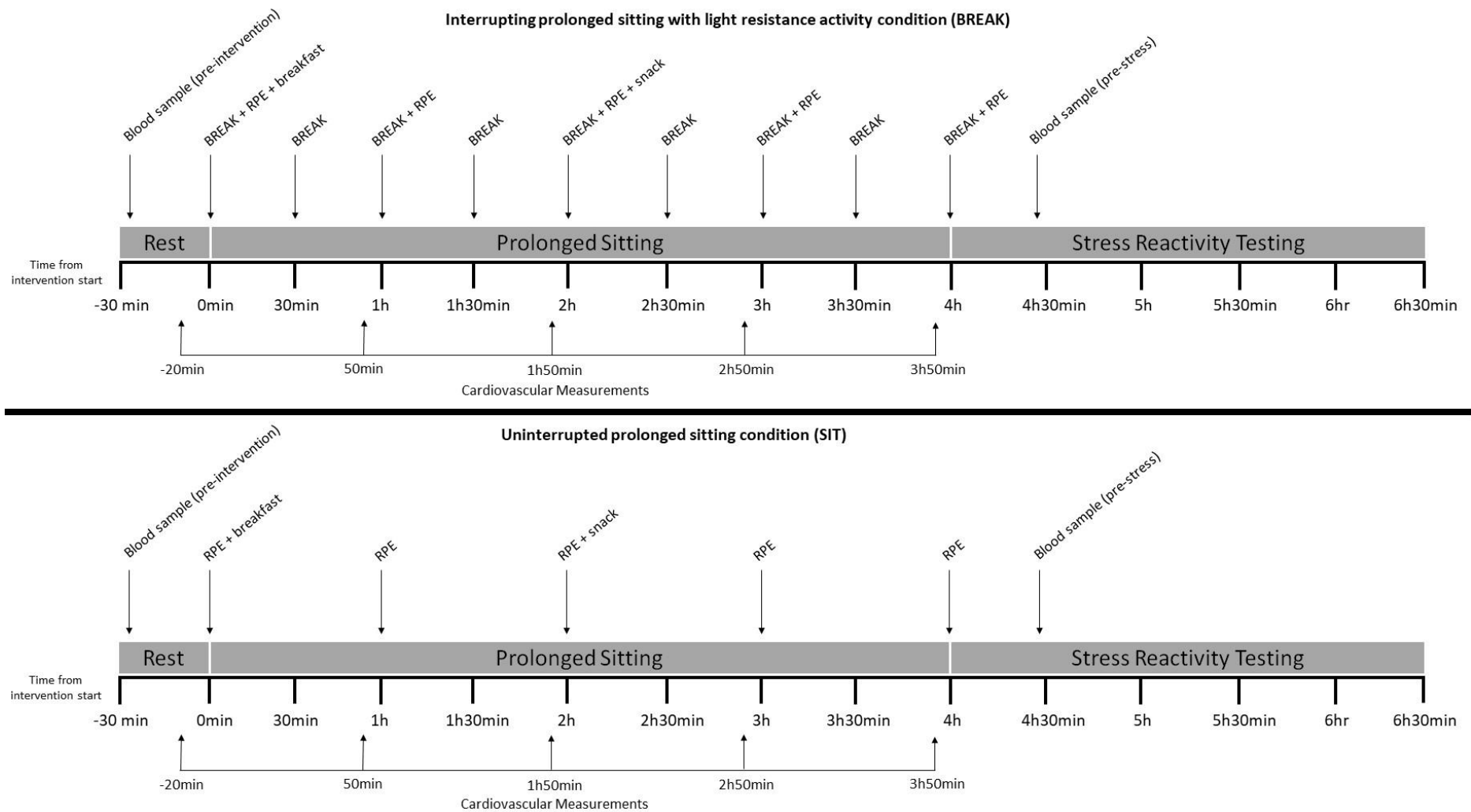
Supplementary Figure 1. Schematic depicting the intervention protocol. BREAK=4min bout of resistance activity, RPE= Rating of Perceived Exertion (BORG scale).
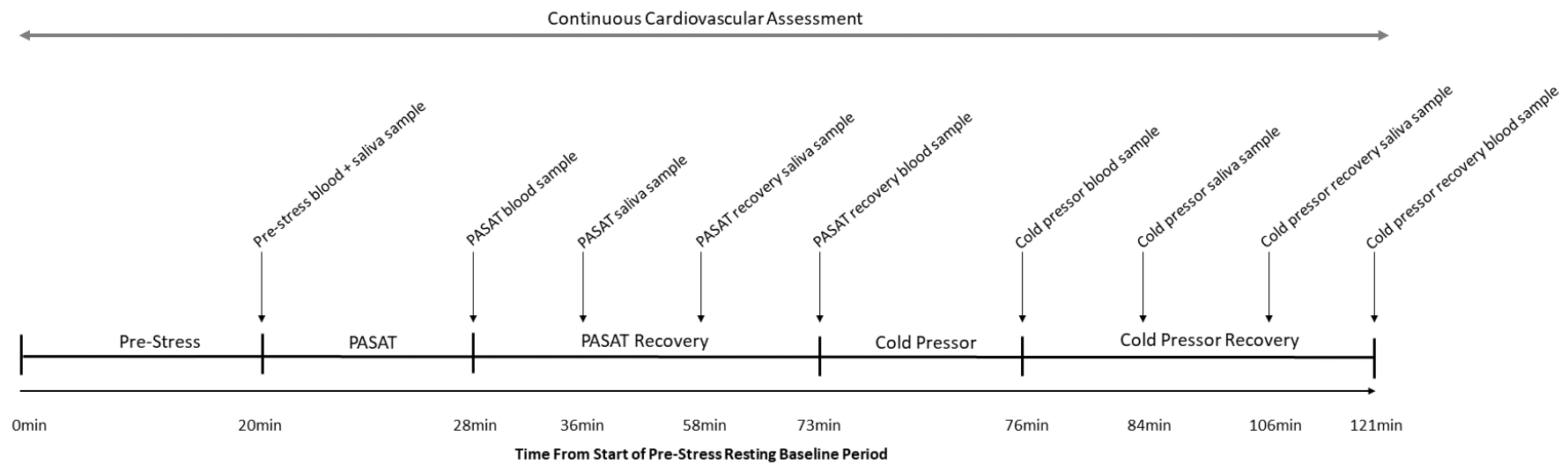
Supplementary Figure 2. Schematic depicting the stress protocol. The 8-min Paced Auditory Serial Addition Test (PASAT) and 3-min Cold Pressor (CP) task were presented in a randomised order across participants, but stress task order remained consistent for each participant during both experimental sessions. The PASAT first protocol is demonstrated above as an example.

Supplementary Table 1. Resting cardiovascular activity during the SIT and BREAK intervention windows.

	-20 min		50 min		1hr 50 min		2hr 50 min		3hr 50 min	
	SIT	BREAK	SIT	BREAK	SIT	BREAK	SIT	BREAK	SIT	BREAK
SBP (mmHg)	110.1 [105.2, 115.4]	110.1 [105.1, 115.4]	111.9 [107.6, 116.4]*	112.3 [106.5, 118.5]†	110.9 [105.3, 116.9]	112.2 [106.5, 118.3]	109.9 [104.1, 115.9]	111.3 [106.3, 116.5]	107.6 [103.1, 112.2]*	108.5 [103.2, 114.0]†
DBP (mmHg)	71.8 [67.7, 76.3]	69.7 [65.5, 74.2]	68.8 [65.3, 72.5]	69.6 [65.1, 74.5]	71.0 [67.3, 74.8]	69.5 [65.5, 73.8]	69.8 [65.9, 73.8]	69.8 [66.1, 73.8]	70.1 [65.7, 74.6]	71.5 [67.3, 76.0]
HR (bpm)	63.9 [59.9, 67.9]	67.3 [62.0, 72.6]	68.4 [63.2, 73.6]*	74.8 [67.8, 81.8]†	72.0 [66.1, 78.0]*	77.5 [71.3, 83.8]†	67.9 [62.2, 73.5]*	75.0 [68.9, 81.1]†	66.0 [60.5, 71.5]*	71.3 [65.9, 76.7]†

Note. SIT= uninterrupted sitting condition, BREAK= interrupted sitting condition, SBP=systolic blood pressure, DBP=diastolic blood pressure, HR=heart rate. Data reflects time-by-condition estimated marginal means [95% confidence intervals] with statistical adjustment for order of intervention allocation. *indicates significantly different from pre-intervention (i.e., -20 min) in the SIT condition ($p<.05$), †indicates significantly different from pre-intervention (i.e., -20min) in the BREAK condition ($p<.05$). Time reflects from start of intervention.