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Protocol

Effects of different intensities of sedentary behavior interruption on cardiovascular parameters and muscle activity in healthy adults: study protocol and rationale

Efectos de diferentes intensidades de interrupción del comportamiento sedentario sobre parámetros cardiovasculares y actividad muscular en adultos sanos: protocolo de estudio y justificación

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Abstract

Prolonged sedentary behavior is associated with acute impairments in cardiovascular, autonomic, and muscular function, even in physically active adults. Interruptions may mitigate these effects; however, evidence remains limited due to heterogeneous protocols and a lack of studies comparing different intensities and simultaneously assessing multiple physiological responses. **Objective:** To analyze the acute effects of interrupting SB with light- and moderate-intensity physical activity on cardiovascular parameters, autonomic function, muscle activity, and skin temperature in healthy adults. **Methods:** In this randomized, controlled, crossover clinical trial, 21 participants will complete three conditions. Blood pressure, heart rate, heart rate variability, muscle activity, and skin temperature will be assessed at standardized intervals throughout the sessions. Statistical analyses will be performed using linear mixed models with significance set at 5%. **Conclusion:** By integrating cardiovascular, automatic, muscular, and thermal outcomes, this study will provide comprehensive evidence on how interruption intensity modulates the acute physiological effects of SB. It is expected that moderate-intensity interruptions will promote more favorable responses than light-intensity activity and uninterrupted sitting, helping refine recommendations aimed at reducing sedentary time and improving cardiovascular and muscular health.

Keywords: sedentary behavior; cardiovascular parameters; muscle activity; autonomic function

Resumen

El comportamiento sedentario prolongado se asocia con deterioros agudos en las funciones cardiovascular, autonómica y muscular, incluso en adultos físicamente activos. Las interrupciones pueden mitigar estos efectos; sin embargo, la evidencia sigue siendo limitada debido a protocolos heterogéneos y a la falta de estudios que comparen diferentes intensidades y evalúen múltiples respuestas simultáneamente. **Objetivo:** Analizar los efectos agudos de interrumpir el comportamiento sedentario con actividades físicas de intensidad ligera y moderada sobre parámetros cardiovasculares, función autonómica, actividad muscular y temperatura de la piel en adultos. **Métodos:** En este ensayo clínico aleatorizado, controlado y cruzado, 21 participantes completarán tres condiciones experimentales. La presión arterial, la frecuencia cardíaca, la variabilidad de la frecuencia cardíaca, la actividad muscular y la temperatura cutánea serán evaluadas en intervalos estandarizados a lo largo de las sesiones. Los análisis estadísticos se realizarán mediante modelos lineales mixtos, con un nivel de significación del 5%. **Conclusión:** Este estudio proporcionará evidencia integral sobre cómo la intensidad de las interrupciones modula los efectos fisiológicos agudos del comportamiento sedentario, contribuyendo a mejorar recomendaciones para la salud.

Palabras clave: comportamiento sedentario; parámetros cardiovasculares; actividad muscular; función autonómica

Key points

- The study will evaluate the acute effects of interrupting sedentary behavior with light- and moderate-intensity physical activities on cardiovascular, muscular, and autonomic parameters in healthy adults.
- Moderate-intensity interruptions of sedentary behavior are expected to elicit greater improvements in blood pressure, heart rate, heart rate variability, and muscle activation compared with uninterrupted sitting and light-intensity breaks.
- The protocol will integrate cardiovascular, neuromuscular, and thermographic measures, providing a comprehensive assessment of physiological responses to sedentary behavior and active breaks under controlled laboratory conditions.

Introduction

Sedentary behavior (SB) is defined as any waking activity performed with an energy expenditure ≤ 1.5 metabolic equivalents, in a seated, reclining, or lying posture¹. Evidence consistently demonstrates an association between prolonged SB and the development of cardiovascular and metabolic diseases, as well as increased cardiovascular and all-cause mortality²⁻⁶. Even among physically active individuals, long uninterrupted periods of SB are associated with increased blood pressure (BP) and heart rate (HR), vascular dysfunction, and reduced muscle activity, thereby compromising cardiovascular health^{5,7-9}.

The mechanisms involved include reduced shear stress and blood flow in the lower limbs during prolonged SB, resulting in vascular dysfunction and impaired BP regulatory mechanisms^{10,11}. Additionally, decreased calf and thigh muscle activity reduces the action of the skeletal muscle pump, compromising venous return¹⁰ and being associated with pressor alterations¹². Evidence indicates that acute exposures of ≥ 1 hour of uninterrupted SB are sufficient to negatively impact cardiometabolic health, with vascular alterations occurring within up to 180 minutes and changes in muscle activity and elevations in BP observed within as little as 60 minutes^{9,13-16}.

Conversely, regular interruption of SB has demonstrated the potential to attenuate these adverse effects, promoting improvements in BP and vascular function^{14,17-20}. However, the strategies employed vary widely in terms of the type, duration, frequency, and intensity of the breaks²¹, and to date only light-intensity interruptions have been extensively studied, showing apparently limited effects, particularly on BP²². Studies comparing different intensities of SB interruption on cardiovascular parameters and muscle activity in healthy adults remain scarce.

Therefore, the aim of the present protocol is to present the rational and scientific basis for investigating the effects of interrupting sedentary behavior on cardiovascular parameters and muscle activity in healthy adults. In particular, there is limited evidence regarding the intensity of such interruptions, especially in relation to vascular function²³⁻²⁵, highlighting the need for studies to support the development of more effective intervention strategies²⁶.

Methods

The section will describe the methodological approach of this randomized clinical trial. The study will be conducted in a laboratory setting and consists of comparing a period of uninterrupted SB with periods of SB interrupted by physical activities performed at different intensities. The experimental conditions will be applied in a controlled manner, allowing the evaluation of the effects of interrupting SB on cardiovascular parameters and muscle activity in healthy adults.

The study was conducted in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines, following the extension for randomized crossover trials^{27,28}. The study will be carried out by the Physical Activity & Health Research Group and conducted at the facilities of the

Exercise Science, Health, and Performance Research Laboratory of the Federal University of Triângulo Mineiro (UFTM).

This study will be conducted in accordance with the Declaration of Helsinki and with Resolution No. 196/96 of the Brazilian National Health Council. The research protocol has been approved by the UFTM Research Ethics Committee (protocol number 8.040.795) and was prospectively registered in December 2025. The study will be conducted between January and February 2026.

Participants were undergraduate students regularly enrolled at the UFTM, Uberaba, MG, Brazil. A convenience sample was recruited through in-person invitations in classrooms, posters, and social media announcements. Inclusion criteria comprised healthy adults aged 18–59 years, without diagnosed chronic diseases, not using continuous medication or ergogenic aids, and without injuries or conditions that could prevent prolonged sitting or walking. Failure to complete any study stage, including screening, assessments, or experimental sessions, was considered an exclusion criterion.

Sample size calculation

Sample size estimation was based on BP outcomes reported by Paterson et al.¹⁷. Effect sizes of 0.26 for SBP and 0.19 for DBP were considered using an F test for repeated-measures ANOVA (within- and between-subject interaction), with $\alpha = 0.05$, power $(1-\beta) = 0.80$, three conditions, four measurements, a correlation among repeated measures of 0.5, and sphericity correction (ϵ) set to 1. Under these assumptions, the minimum required sample was 30 participants for SBP and 52 participants for DBP.

Because DBP resulted in the larger required sample size and therefore provided a more conservative and statistically robust estimate, the final sample size determination was based on DBP. In a crossover design, all participants complete all experimental conditions, reducing the required sample to 17 participants for DBP. To account for potential attrition and data variability, a 20% increase was applied, yielding a final target sample of 21 participants. This approach ensured adequate statistical power and enhanced sensitivity to detect intervention effects.

Study design

Participants completed three randomized experimental conditions in a crossover design, separated by at least 48 hours to minimize carryover effects. Condition order was determined by simple automated randomization prior to participant enrollment. The experimental conditions were: (i) Control condition, consisting of 3 hours of uninterrupted sitting; (ii) Light-intensity physical activity condition, consisting of 3 hours of sitting interrupted every 50 minutes by 5 minutes of treadmill walking at 3.2 km/h; and (iii) Moderate-intensity physical activity condition, consisting of 3 hours of sitting interrupted every 50 minutes by 5 minutes of treadmill walking at 6.0 km/h²⁹ (Figure 1).

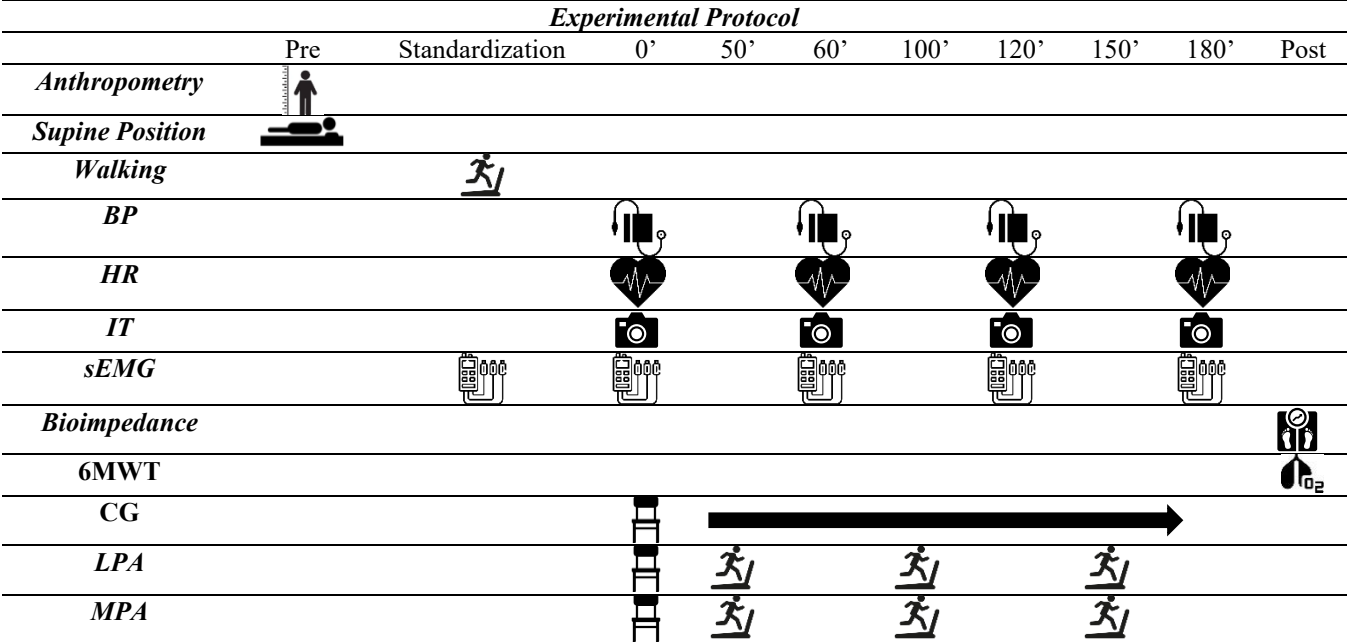
Procedures

During the initial contact, participants received detailed information about the study procedures. Eligible volunteers attended the laboratory for screening, sample characterization, anthropometric assessments, and completion of the experimental sessions. Written informed consent was obtained prior to participation.

Participants were instructed to avoid prolonged sun exposure for at least five days before testing, hot showers within two hours prior to the protocol, and the use of skin creams or lotions to ensure accurate thermal imaging. In addition, they were asked to refrain from moderate-to-vigorous physical activity, caffeine, and alcohol consumption for 24 hours before each experimental session, while maintaining their usual dietary habits and sleep patterns.

All laboratory visits will occur between 7:00 and 8:00 a.m. Anthropometric assessments, conducted during the first session, will include body mass, height, waist circumference, and hip circumference. Body mass and height were measured using a calibrated mechanical scale with an attached stadiometer. Body mass index and waist-to-hip ratio will be calculated using standard formulas³⁰.

Figure 1. Experimental Protocol.



BP = Blood Pressure; HR = Heart Rate; IT = Infrared Thermography; sEMG = Surface Electromyography; 6MWT = 6-Minute Walk Test; CG = Control Group; LPA = Light Physical Activity Group; MPA= Moderate Physical Activity Group.

Experimental protocol

Prior to all experimental sessions, all anatomical markings, researcher positioning, and equipment requiring thermal stabilization were prepared at least one hour before participant arrival.

At the beginning of each session, participants rested in the supine position on a stretcher for 10 minutes to achieve physiological stabilization, allow skin preparation, electrode placement for electromyography, and environmental acclimatization. After this period, participants completed a 5-minute treadmill walk at 3.2 km/h (Athletic Professional 6000T), during which muscle electrical activity was continuously recorded for standardization purposes.

Following the standardization procedure, participants remained seated for 3 hours in a standardized chair-and-desk setup, with reading and electronic device use permitted. From this point onward, measurements of BP, HR, heart rate variability (HRV), muscle electrical activity, and infrared thermography were collected every 60 minutes, with the first assessment performed immediately upon sitting. Cardiorespiratory fitness testing was conducted at the end of each experimental session.

During sedentary behavior interruptions, occurring every 50 minutes, perceived exertion during walking was assessed using the 0–10 Rating of Perceived Exertion scale^{31,32}. Perceived exertion was measured to ensure that walking interruptions remained at a light to moderate intensity, in line with study objectives and participant safety. If any session needed to be interrupted outside the predefined protocol, it was cancelled and rescheduled.

Participant characterization

Physical activity level and sedentary behavior were assessed using the short version of the International Physical Activity Questionnaire^{33,34}. The questionnaire evaluates moderate- and vigorous-intensity physical activities performed during a typical week across work, leisure, transportation, and household domains, as well as sedentary time. Only activities lasting at least 10 minutes were considered. Weekly physical activity time (min/week) was calculated as the sum of moderate activity time plus twice the time spent in vigorous activity. Sedentary behavior was estimated based on total sitting time (min/day), calculated as a weighted average of weekdays and weekend days³⁵.

Body composition was assessed using tetrapolar bioelectrical impedance analysis, a safe and non-invasive method, performed according to the manufacturer's guidelines. Measurements included fat-free mass, muscle mass, and body fat percentage, processed using proprietary software. Body composition assessment was conducted only after completion of the control session.

Autonomic function

Autonomic function was assessed through measurements of BP, HR, and HRV. SBP and DBP were measured hourly using an oscillometric device (Omron® HEM-780-E, Japan), in triplicate with 1-minute intervals, on the participant's left arm. Mean arterial pressure (MAP) was calculated from systolic and diastolic BP using the equation $MAP = 1/3 SBP + 2/3 DBP$ ³⁶⁻³⁸.

HR and HRV were recorded for 5 minutes every 60 minutes using a Polar® H10 heart rate monitor. R-R intervals were exported and analyzed using Kubios HRV Standard software (v.3.4.2), applying standard filtering procedures (Tarvainen et al., 2014; Plaza-Florido et al., 2020). Time-domain HRV indices included the root mean square of successive differences (RMSSD), the standard deviation of normal R-R intervals (SDNN), and the percentage of adjacent R-R intervals differing by more than 50 ms (pNN50). Frequency-domain indices included high-frequency power (HF: 0.15–0.40 Hz) and low-frequency power (LF: 0.04–0.15 Hz), calculated using the Fast Fourier Transform.

All HRV analyses followed the recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology³⁹.

Muscle Activity

Muscle activity was recorded for 5 minutes every hour using surface electromyography (sEMG) with a DELSYS® DSY-DS-T01-8 system. Data from four channels were simultaneously sampled at 2,000 Hz via TRIGNO® software and processed using custom routines in Matlab® and Octave⁴⁰ to extract mathematical features and identify potential patterns in muscle activation.

Electrode placement followed SENIAM recommendations⁴¹ and skin preparation procedures, targeting the Rectus Femoris (RF), Vastus Lateralis (VL), Vastus Medialis (VM), and Medial Gastrocnemius (GM) muscles. RF electrodes were placed at 50% of the distance between the anterior superior iliac spine and the superior patella; VL at 2/3 of the distance from ASIS to the lateral patella; VM at 80% of the distance from ASIS to the medial patellar ligament; and GM at the site of greatest muscle circumference.

sEMG signals were filtered, rectified, and normalized to the initial treadmill walking period. Quadriceps activity was calculated as the mean of normalized EMG signals from RF, VL, and VM over the analysis window, using the root mean square method to assess temporal changes in muscle activation^{12,42}.

Infrared Thermography

Skin temperature was assessed using infrared thermography (IT), a non-invasive and non-radiative method that indirectly evaluates physiological changes related to skin blood flow⁴³. A FLIR E76 thermal camera (Flir® Systems) was used, with the device stabilized for 60 minutes prior to data collection and a constant skin emissivity set at 0.98^{44,45}. Data collection followed the Thermographic Imaging in Sports and Exercise Medicine (TISEM) checklist⁴⁵. Regions of interest (ROIs) were defined for the anterior thigh and calf, and all measurements were analyzed bilaterally for each participant. Mean temperature of each ROI was calculated using FLIR Thermal Studio® software.

Cardiorespiratory capacity

Maximal cardiorespiratory capacity (CRF) was assessed using the 6-minute walk test (6MWT) at the end of each experimental session⁴⁶. Participants walked as far as possible along a 15-meter indoor course for 6 minutes, with heart rate, perceived exertion (Borg 0–10), and distance recorded^{31,32}. CRF was estimated from the average 6MWT distance across sessions using sex-specific equations accounting for age, BMI, height, weight, and HR⁴⁶.

Statistical analysis

Descriptive statistics were calculated for all variables and presented as mean $\pm$ SD. Participants who begin the experimental procedures and sign the informed consent form but subsequently withdraw from the study or fail to attend one or more scheduled sessions will be considered sample losses. As each experimental session is independently structured and generates a complete set of measurements, data from sessions that are fully completed will be retained and included in the analysis, while sessions that are not performed will be classified as missing.

Regarding missing data, no imputation procedures will be applied, as each session represents an independent and self-contained block of measures. Therefore, a per-protocol analytical approach will be adopted, using only the available data from completed sessions without substitution or estimation of missing values.

The variables — physical activity level and sedentary behavior, body composition, and respiratory capacity — will be used primarily for descriptive purposes to characterize the sample and, if necessary, as covariates in the analysis, in order to control for potential confounding effects, ensuring that the responses observed to sedentary behavior interruptions may or may not be influenced by these variables.

Data normality and homogeneity of variance were assessed using Shapiro-Wilk and Levene tests. Baseline comparisons between experimental conditions were performed using repeated-measures ANOVA or the Friedman test, depending on normality.

Mixed linear models were used to evaluate the effects of experimental conditions on blood pressure, heart rate, skin temperature, and muscle activity, considering Group (Control, Light, Moderate) and Time (baseline, 1, 2, and 3 hours) as factors. Post-hoc comparisons were adjusted with Bonferroni correction. Effect sizes were calculated using Hedge's *g* and interpreted as small (0.20–0.49), moderate (0.50–0.79), or large (≥ 0.80)⁴⁷. Significance was set at $p < 0.05$, and analyses were performed in SPSS 20.

Discussion

The randomized clinical trial described in this protocol will analyze the effects of interrupting prolonged sedentary behavior SB with light- and moderate-intensity physical activities on cardiovascular parameters, muscle activity, and autonomic function in healthy adults. Despite evidence supporting the benefits of breaking up SB, there is still limited understanding of how different interruption intensities influence acute physiological responses, specifically affect vascular function^{23–25}, representing a gap this study aims to address^{10,12,14,22}.

It is expected that regular breaks during prolonged periods of SB will attenuate adverse effects such as increases in BP, reductions in muscle activity, and impairments in vascular function^{12,14,17–20}. Previous studies suggest that interruptions, particularly at moderate intensity, can acutely improve BP, HR, and HRV, highlighting the importance of intervention intensity for cardiovascular and autonomic outcomes^{7,17,18,26}. Even over a short experimental period, regular SB interruptions are expected to elicit favorable acute cardiovascular and neuromuscular responses, supporting the use of active breaks as an effective strategy to mitigate the harmful effects of prolonged sitting^{14,17–19,22}.

During active breaks, HR is expected to increase, reflecting activation of the cardiovascular system and greater metabolic demand of the muscles involved⁷. This HR increase is likely to be accompanied by improvements in HRV, indicating enhanced autonomic modulation and a better balance between sympathetic and parasympathetic systems⁷. Additionally, quadriceps and calf muscle activity is expected to increase during the interruptions, whereas prolonged sitting tends to reduce muscle activation, demonstrating the direct effect of SB interruption on muscle recruitment^{12,16}.

In summary, this study aims to address gaps in the current literature regarding the intensity of sedentary behavior interruptions in healthy adults. By systematically examining different interruption intensities, it will provide evidence on their effects on cardiovascular responses and muscle activation. These findings are expected to inform more effective interventions for reducing sedentary time and promoting cardiovascular and muscular health, offering practical guidance for future recommendations.

The study will present important strengths. The interventions will be conducted in a laboratory setting, allowing rigorous control of external factors, such as temperature and physiological signal acquisition conditions, thereby ensuring standardization of the intensity and duration of the interruptions. Another relevant strength is the use of integrated measures, including surface electromyography, infrared thermography, BP, HR, and HRV, enabling a comprehensive assessment of the physiological responses to SB and active breaks.

One limitation of the present study is the use of infrared thermography as an indirect method to infer blood flow. Although it is a widely used and non-invasive technology, IT measures skin temperature, which is influenced by various environmental and physiological factors, and does not directly assess blood flow. Therefore, the interpretation of thermal results should be approached with caution.

Conclusion

This study aims to address an important research gap, as the integrated understanding of the immediate effects of different interruption intensities of sedentary behavior on multiple cardiovascular, autonomic, muscular, and thermal outcomes in adults remains limited. Although previous studies have examined isolated components—such as blood pressure or peripheral blood flow have simultaneously evaluated hemodynamic, autonomic, and muscular responses within the same controlled experimental protocol.

By examining these variables in combination, this study offers an important advancement by providing more comprehensive evidence on how different interruption intensities can acutely modulate the risks associated with prolonged sitting. Consequently, the expected findings have the potential to inform more precise recommendations regarding the characteristics of active breaks, contributing to the refinement of intervention strategies targeting cardiovascular health and sedentary behavior.

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Conflict of Interest

The authors declare no conflicts of interest.

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Declaration of generative AI and AI-assisted technologies in the writing process

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