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### **Title: Acute sedentary behavior and cardiovascular disease research: standardizing the methodological posture**

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**Running Head:** Standardizing Sitting Research - Posture

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**Abstract:**

Sedentary behavior has been identified as an independent predictor of future cardiovascular disease risk and all-cause mortality. To explain this association, a growing body of literature has sought to investigate the physiological underpinnings of this association with the goal of developing a biologically plausible model. In time, this biologically plausible model can be tested, and effective, translatable public health guidelines can be developed. However, to ensure that evidence across studies can be effectively synthesized, it is necessary to ensure their congruency and comparability. Whilst there are several key factors that should be considered and controlled across prolonged sitting studies, one pertinent issue is that of participant posture. There is currently a discourse within the literature regarding the posture that cardiovascular assessments are performed in and rest periods between posture transitions and subsequent measures. This perspectives piece makes the case for standardizing approaches across the research area and offers practical recommendations for future work.

### 1. Sitting and Cardiovascular Health

Increased exposure to sedentary behaviors, (waking behaviors in a seated, reclined, or lying posture with an energy expenditure  $\leq 1.5$  metabolic equivalents) (1), has been identified as an independent risk factor for cardiovascular disease (CVD) (2–4). As such, public health agencies around the world, including the World Health Organization, now include sedentary behavior guidelines in tandem with physical activity guidelines (5). However, current sedentary behavior guidelines are vague and non-specific in many countries, limited to encouraging people to reduce sedentary time (i.e., “Move more and sit less”) and replace it with light-intensity physical activity but without practical guidance on how to achieve these outcomes. In contrast, physical activity guidelines follow the FITT (Frequency, Intensity, Time, and Type) principle of prescription and are readily translatable to the public. As highlighted by recent calls to action, and to support policy development, there is a need to first develop a biologically plausible model explaining the link between sedentary behaviors, such as prolonged sitting, and CVD, against which detailed public health guidelines can be generated and tested (5–7).

Across the growing literature examining the physiological underpinnings of sitting-induced cardiovascular burden, several assessments of cardiovascular health and function have been utilized. These assessments include but are not limited to, flow-mediated dilation (FMD), central and peripheral blood pressure (BP), and arterial stiffness estimated by pulse wave velocity (PWV). Recent meta-analyses by our group have shown that acute bouts of prolonged sitting negatively impact both FMD (8) and peripheral BP (9). These findings and the work of others have led to the development of a working model whereby repeated acute exposures to prolonged sitting may contribute to increased cardiovascular burden via increases in central arterial stiffness. This augmented stiffness may, in turn, contribute to increased CVD risk (10). However, key methodological inconsistencies currently limit evaluation of the plausibility of such models by preventing the comparison of findings from randomized controlled trials, our principal unit of experimental evidence for guiding policy development. Whilst a great number of factors may confound the results of prolonged sitting research, including but not limited to, sitting duration, sleep quality and duration, time of day, hydration status, food consumption, and menstrual cycle phase, this article will focus on the pertinent issue of participant posture during the assessment of cardiovascular outcomes and related complexities during experimental research, before suggesting ways to move the field towards methodological consensus.

### 2. The Posture Problem

Acute sitting studies have been invaluable in progressing sedentary behavior research to its current state. However, a critical methodological issue within such studies is that of participant posture during assessments. There is a desire to complete cardiovascular measures with participants in a seated posture as this is the posture of interest, however, many cardiovascular assessments lack validity outside of a supine posture. One such example is FMD; in a recent meta-analysis by our group, it was observed that 59% of included prolonged sitting trials performed FMD assessments with participants in either a seated or semi-recumbent posture (8) despite current guidelines and underlying assumptions of the assessment stating that measures should be made in the supine posture (10, 11). Without the requisite evidence to suggest that measures made in alternative postures are valid or reliable, questions remain about the value of such assessments. Similarly, previous research indicates that whilst PWV measures performed in a seated posture may have acceptable reliability, they too lack validity compared to measures in a supine posture (12–16). The instinctive solution to this issue of posture is to perform measurements in the validated supine posture following the prolonged sitting period, however, this solution holds its own inherent complexities that must be addressed.

#### 2.1. Rest period duration following posture transition

Whilst pre-sitting measures may readily be performed with participants in a supine posture before moving them to the experimental seated position, post-sitting measures are complicated by the

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necessity for rest periods following the posture transition. Posture transitions following a sitting bout may create a transient hemodynamic response which could mask any effects of the sitting bout. To offset this, previous studies have employed post-posture transition rest periods ranging from 5 (17) to 20 (18, 19) minutes before FMD or PWV assessment, with many other studies failing to report the length of this rest period. Given that the purported stimulus for sitting-induced cardiovascular dysfunction is blood pooling in the lower limbs (10), the rest period employed may be of critical importance. It could be argued that longer rest periods of 20-minutes may mask the true effect of prolonged sitting as blood pooling dissipates. Conversely, shorter rest periods of 5-minutes may be capturing the hemodynamic effect of a posture transition. To address this issue and improve congruency between studies, we recommend researchers across the field adopt a standardized 10-minute rest period following posture transition in line with published recommendations for FMD and PWV assessments (11, 20).

A further consideration for posture transitions is the potential for participants to need to move between a test bed and chair. The desire to have participants sit in a more comfortable chair for any sitting bout is understandable as whilst folding test beds may allow participants to remain stationary and be passively transitioned between postures, typically these beds are less comfortable than a chair. Additionally, the cost of such test beds may be prohibitively expensive for some research labs. The authors believe that the ideal scenario is a folding test bed that reduces the need for participants actively transitioning between postures. However, when this is not possible, several steps should be taken to reduce participant exertion and thus muscle activation. It is necessary to reduce the distance between the test bed and chair as much as possible, ideally one or two steps. Further, the height of the test bed should be adjusted to prevent participants from needing to exert additional effort to climb onto the bed. Whilst the argument could be made that the minimal movement described in such a procedure may constitute a sitting interruption, the recommended 10-minute rest period should prevent any carry-over effect from such minimal movement.

Whilst the 10-minute rest describes the minimum time between posture transitions and subsequent measures, further consideration should be given to the maximum time between posture transitions and measures. It is currently not known how long sitting-induced changes in cardiovascular measures persist following a sitting bout and which factors may be of influence, and it would perhaps be inappropriate for authors to speculate. However, researchers should be mindful that the effects of a sitting bout are likely to be finite and transient and that the longer they wait following posture transition, and the greater number of consecutive vascular assessments included in the study protocol, the less representative those measures are likely to be of sitting-induced dysfunction. To address this methodological gap in the literature, we encourage researchers to investigate how long sitting-induced cardiovascular dysfunction is likely to persist following a bout of sitting.

### ***2.2. Timing of posture transitions and measurements relative to interruption strategies***

Studies implementing sitting interruption strategies are subject to further methodological complications beyond that of uninterrupted sitting studies. A pertinent issue is the timing of interruptions relative to the end of the sitting bout and subsequent cardiovascular measures. Previous work utilizing FMD and PWV has completed final measures anywhere from 10 (17, 19) to 60 (21) minutes after the final interruption. Similar to the issues posed by divergent post-posture transition rest periods, shorter periods of  $\leq 10$ -minutes between cessation of interruption strategy and final measures likely capture the hemodynamic effect of not only the postural transition but also the isolated activity bout, rather than the impact of prolonged sitting. Alternatively, longer periods of 60-minutes may allow the effects of any interruption strategies to dissipate prior to assessment. Additionally, the wide-ranging interruption strategies that have been employed in this research area, ranging from high-intensity stair climbing to passive standing, mean that standardizing a post-exercise washout period may be untenable. As there is unlikely to be a 'one-size-fits-all' approach to this complicated matter, researchers should consider the type, intensity, and duration of any interruption strategy utilized and employ what they deem to be an appropriate 'washout' period

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before post-sitting measures. Whilst not a perfect solution, the additional standardization of the time between post-sitting posture transitions and final measures noted above may absolve some of the issues.

### 3. Conclusion to Move the Field Forward

To improve the congruency of prolonged sitting research we argue that future work in this area should i) ensure that primary outcome measures are performed in a validated posture (i.e., supine), ii) adopt a standardized rest period of 10-minutes following a posture transition prior to any cardiovascular assessments, clearly stating this rest period within their methodological description, iii) make a concerted effort to reduce participant exertion as they transition between postures, and iv) carefully consider the 'washout' period between sitting interruption strategies and final cardiovascular measures. The proposed 10-minute rest period is in line with the minimum recommendations for both FMD and PWV assessments (11, 20), allowing a requisite level of hemodynamic stability, without leaving an extended rest period whereby the effect of the sitting bout may be lost. The standardization of approaches employed by researchers in this area will facilitate a more coherent synthesis of results in the future and we hope that by doing so we can progress towards better informed public health policy.

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### X. Author Contributions

CP generated the idea. CP and SH wrote the first draft of the manuscript. All authors edited subsequent drafts and approved the final version of this manuscript.

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