Reallocating sitting time to standing or stepping through isotemporal analysis: associations with markers of chronic low-grade inflammation

Running title: Reallocating sitting time to standing or stepping and inflammation

Joseph Henson[a,b] (jjh18@le.ac.uk), Charlotte L. Edwardson[a,b] (ce95@le.ac.uk), Danielle H. Bodicoat[a,b,c] (dhm6@le.ac.uk), Kishan Bakrania[a,b,c] (kb318@le.ac.uk), Melanie J. Davies[a,b] (melanie.davies@uhl-tr.nhs.uk), Kamlesh Khunti[a,c] (kk22@le.ac.uk), Duncan C.S. Talbot[d] (Duncan.Talbot@unilever.com), Thomas Yates[a,b] (ty20@le.ac.uk)

[a] *Diabetes Research Centre, University of Leicester, UK*

[b] *NIHR Leicester Biomedical Research Centre, UK*

[c] *NIHR Collaborations for Leadership in Applied Health Research and Care (CLAHRC) East Midlands, UK and Diabetes Research Centre, University of Leicester, UK*

[d] *Unilever R&D, Colworth Science Park, Sharnbrook, Bedfordshire, UK.*

Corresponding Author:

Dr Joseph Henson, Email address:jjh18@le.ac.uk; Tel: +44 116 258 4389; Fax: +44 116 258 4053.

Main text word count =3939; Number of Tables = 3; Number of Supplementary Tables = 2; Number of pages = 28

Abstract

Aim: Although high levels of sitting time are adversely related to health, it is unclear whether moving from sitting to standing provides a sufficient stimulus to elicit benefits upon markers of chronic low-grade inflammation in a population at high risk of type 2 diabetes (T2DM).

Methods: 372 participants (age=66.8±7.5years; body mass index (BMI) =31.7±5.5kg/m^2; Male=61%) were included. Sitting, standing and stepping was determined using the activPAL3^{TM} device. Linear regression modelling employing an isotemporal substitution approach was used to quantify the association of theoretically substituting 60 minutes of sitting per day for standing or stepping on interleukin-6 (IL-6), C-reactive protein (CRP) and leptin.

Results: Reallocating 60 minutes of sitting time per day for standing was associated with a -4% (95% CI -7%, -1%) reduction in IL-6 (p=0.048). Reallocating 60 minutes of sitting time for light stepping was also associated with lower IL-6 levels (-28% (-46%, -4%; p=0.025)). Substituting sitting for moderate-to-vigorous (MVPA) stepping was associated with lower CRP (-41% (-75%, -8%; p=0.032)), leptin (-24% (-34%, -12%; p≤0.001)) and IL-6 (-16% (-28%, 10%; p=0.036).

Conclusion: Theoretically replacing 60 minutes of sitting per day with an equal amount of either standing or stepping yields beneficial associations upon markers of chronic-low grade inflammation.

Keywords: Sitting; standing; stepping; type 2 diabetes; inflammation.

Introduction

Low-grade inflammation has been proposed to be involved in the underlying pathogenesis of type 2 diabetes mellitus (T2DM) (Wang et al., 2013). This manifestation is thought to be a result of an ongoing acute-phase response, primarily characterised by alterations in acute-phase proteins, such as C-reactive protein (CRP) (Pradhan, Manson, Rifai, Buring, & Ridker, 2001). More specifically, mediators of inflammation which include the interleukin 6 (IL-6) family of cytokines have been proposed to affect glucose homeostasis and metabolism directly and indirectly by action on skeletal muscle cells (Kristiansen & Mandrup-Poulsen, 2005). Previous studies have established an inverse relationship between the amount of physical activity and proinflammatory cytokines in obesity, T2DM, and the metabolic syndrome (Hamer et al., 2012; Kasapis & Thompson, 2005). Therefore, the beneficial effects of physical activity may be partly mediated by changes in the adipokines profile.

Recent advances in physical activity research have suggested that sedentary behaviour, defined as any waking sitting activity with a low energy expenditure, is an independent risk factor for several health outcomes (Biswas et al., 2015; Edwardson et al., 2012; Thorp, Owen, Neuhaus, & Dunstan, 2011; Wilmot et al., 2012), including markers of inflammation (Healy, Matthews, Dunstan, Winkler, & Owen, 2011; Henson et al., 2013; Yates et al., 2012). However, not all studies have confirmed an association with inflammation after adjustment for key lifestyle confounders (adiposity and/or moderate-to-vigorous physical activity (MVPA))

(Allison, Jensky, Marshall, Bertoni, & Cushman, 2012; Falconer et al., 2014; Yates et al., 2012). These discrepancies may be partly explained by the population under investigation (high risk vs. general population), the potential interaction with physical activity, the measure of exposure (self-report vs. objective) or the statistical methods employed. Most previous investigations have examined each type of activity (sedentary, light, MVPA) without considering the time-dependent behaviours that are being displaced. Isotemporal substitution was developed as a methodology to study the time-substitution effects of one type of activity for another in a dataset consisting of continuous outcomes (Buman et al., 2014; Mekary, Willett, Hu, & Ding, 2009; Mekary et al., 2013).

Given that time is finite, the heterogenous effects of an activity undertaken at a certain time point will be largely driven by the other activities being displaced. Previous studies employing this method have found that reallocating time from sedentary time into physical activity is associated with improvements in insulin sensitivity (Yates et al., 2015), glucose (Healy, Winkler, Owen, Anuradha, & Dunstan, 2015), triglycerides (Buman et al., 2014), markers of adiposity (Falconer, Page, Andrews, & Cooper, 2015) and all-cause mortality risk (Matthews et al., 2015). However, only two studies have been able to isolate the effect of displacing sitting with standing using objective measurement (Edwardson et al., 2017; Healy, Winkler, Owen, Anuradha, & Dunstan, 2015), with both demonstrating beneficial associations with cardio-metabolic markers. This is important as habitual standing is behaviourally more ubiquitous than MVPA and may provide an appealing interventional target in the promotion of health. However, the associations of displacing sedentary time on markers of chronic low-grade inflammation have yet to

be explored. Therefore, the aim of this paper is to extend previous research by quantifying reallocation from sitting into standing and stepping (split into light and MVPA intensity) in a population at high risk of T2DM and to determine whether results were modified high/low overall sitting time, length of sitting bout (<30 minutes or ≥30 minutes), sex or glucose regulation.

Methods

Participants

Participants at increased risk of T2DM (aged 30-75) were originally recruited through 10 primary care practices in Leicestershire, UK, in 2010–2011. Individuals with an increased risk of impaired glucose regulation (IGR; any combination of impaired glucose tolerance (IGT) and/or impaired fasting glycaemia (IFG) or undiagnosed T2DM) were identified for recruitment using a modified version of the Leicester Risk Score (Gray et al., 2012). Those individuals scoring within the 90th percentile in each practice were invited to take part in the study. This approach has reasonable sensitivity and specificity for identifying participants with IGR (Gray et al., 2012). Individuals were unaware of their diabetes risk status before entering the study. We excluded those who had previously diagnosed T2DM, were currently taking steroids or were unable to take part in any walking. At baseline, individuals were randomised to usual care or the 3 hour Walking Away structured education programme with ongoing annual support (Yates, Davies et al., 2012). This paper reports 36-month data from 372 participants, collected 2013-2014 as this was the only time point at which participants wore the activPAL3^{TM} device. The trial details

and results have been published in detail elsewhere (Yates et al., 2016). There was no difference between groups in levels of physical activity, sedentary behaviour or markers of metabolic health after 36 months. Ethical approval was obtained from the Nottingham Research Ethics Committee, UK. Informed consent was obtained from all individual participants included in the study.

Sitting, standing and stepping quantification

Quantification of sitting, standing and stepping was determined using the activPAL3^{TM} device. This is a small, slim and highly accurate thigh worn monitor that uses accelerometer-derived information in order to determine body posture (i.e., sitting, upright and upright with stepping) (Grant, Ryan, Tigbe, & Granat, 2006; Kozey-Keadle, Libertine, Lyden, Staudenmayer, & Freedson, 2011; Lyden, Kozey Keadle, Staudenmayer, & Freedson, 2012). By using proprietary algorithms (Intelligent Activity Classification) it is able to provide the start and end time of each body posture (i.e., sitting, standing and stepping) as well as number of steps taken, step cadence, transitions between sitting and upright posture and metabolic equivalents (METs). In order to investigate the role of physical activity intensity on inflammation, stepping was further split into light (<3METs) and MVPA (≥3METs).

The activPAL3^{TM} was initialised using the manufacturer's software with default settings (i.e., 20Hz, 10s minimum sitting-upright period) and participants were asked to wear the device continuously 24 hours/day for 7 days. The device was waterproofed by wrapping in one nitrile sleeve and waterproof dressing. Participants wore the activPAL3^{TM} on the midline anterior aspect of the upper thigh and secured it using Hypafix dressing.

Participants completed a log of sleep, wake and any removal times during the seven days. ActivPAL data were downloaded using the manufacturer's software (activPAL Professional Research Edition, PAL Technologies, Glasgow, UK). A validated, automated algorithm in STATA (StataCorp LP) used the activPAL event files to isolate waking hours from 'sleeping' (time in bed), prolonged non-wear periods and invalid data (Winkler et al., 2016). Heatmaps of the included and excluded data were created and visually checked. The self-reported wake and sleep times were also referred to if any data was coded incorrectly. A valid day was defined as a day with <95% spent in any one behaviour (eg, standing or sitting), >500 steps and ≥10 hours of waking hours data. Participants were required to have at least four valid days of data to be included in the analysis. For isotemporal analysis, data are expressed in units of 60 $min \cdot d^{-1}$.

Demographic, anthropometric and medication status

Body mass (Tanita TBE 611, Tanita, West Drayton, UK), height and waist circumference (midpoint between the lower costal margin and iliac crest) were measured to the nearest 0.1 kg and 0.5 cm, respectively. Information on current smoking status, medication and ethnicity was obtained following an interview administered protocol with a health care professional.

Biomarker measurements

Biomarkers were selected a priori. CRP was analysed using a high sensitivity (Minimum Interpretation Limit = 0.1 mg/L) HORIBA ABX clinical chemistry analyser. IL-6 was analysed using quantikine high-sensitivity enzyme-linked immonosorbent assays (R&D systems). Leptin was analysed using AlphaLISA no wash fluorescence immunoassay kits (Perkin Elmer). All ELISA and fluorescence immunoassays were conducted in replicate on the same sample and the average value obtained. If the intra-assay coefficient of variation exceeded 10% for leptin, the assay was repeated using the same technique. Similarly, the IL-6 assay was repeated if the concentration was >2 pg/ml and the coefficient of variation >20% or the concentration was <2 pg/ml and the coefficient of variation >25%.

Statistical Analysis

Forced-entry linear regression modelling employing an isotemporal substitution approach was used to quantify the association of substituting 60 minutes of sitting for standing, light and MVPA stepping on markers of chronic low-grade inflammation. We chose 60 minute blocks as previous experimental and epidemiological work has demonstrated that breaking sitting time with 60 minutes of either standing or walking across the day significantly reduces the postprandial response in several markers of cardio-metabolic health in high risk of T2DM individuals (Henson et al., 2016; Yates et al., 2015).

In order to investigate the association between sitting time and markers of chronic low grade inflammation, isotemporal substitution requires that average waking wear time, standing activity and time in stepping activity (both light and MVPA) are simultaneously entered into a linear regression model; the resulting regression coefficient for standing activity and stepping activity represent the association of substituting a given unit of sitting time into each category. These models have been described in detail elsewhere (Buman et al., 2014; Mekary et al., 2009; Mekary et al., 2013).

Model 1 was adjusted for measured potential confounding variables defined as treatment group (intervention/control), age (continuous), sex (male/female), ethnicity (White European/South Asian/other), smoking status (current smoker/former smoker/never smoked), medication status (beta-blocker (non-steroidal anti-inflammatory drugs (NSAIDs), angiotensin-converting-enzyme (ACE) inhibitors and statin use; all yes/no). As obesity plays a central role in the expression of inflammatory markers (Wisse, 2004), results were further adjusted for waist circumference (Model 2).

The derived indexes of inflammatory markers displayed non-normal distributions, therefore all dependent variables were log-transformed with resulting regression coefficients back transformed; displayed coefficients consequently represent the value by which the dependent variable is multiplied by for a given unit of time in standing or stepping activity.

As the association between sitting time and markers of cardio-metabolic health may be influenced by the patterns in which the total volume is accumulated, significant observations were followed up with interaction terms to assess associations between bout length of sitting time (sitting time accumulated in bouts <30 minutes or ≥30 minutes) and markers of inflammation. We also tested the interaction for high/low sitting time (based on the median split) and whether results differed based upon sex and IGR status. All interaction analyses were adjusted for the same covariates as the main analysis.

Assumptions of linearity for each model were verified and multicollinearity was checked using the variance inflation factor (VIF). VIF values in all models were less than 5 indicating that multicollinearity was low. *P* values of <0.05 were considered significant for main effects and $p<0.1$ for interactions. IBM SPSS Statistics v22.0 (Chicago, IL, USA) was used to conduct all statistical analyses.

Sensitivity analyses were also conducted to investigate whether results were affected if a different measure of adiposity (BMI) was used as a covariate in model 2. In order to be consistent with previous studies (Best et al., 2005; Lee et al., 2009), a sensitivity analysis also investigated whether results were affected by removing participants with a CRP level >10mg/L, as this may be indicative of acute inflammation (Ridker, 2003).

A single activity model (not an isotemporal model) was also run in order to assess each activity component separately (e.g., sitting, standing and walking), without taking into account the other activity types.

Results

Of the 648 participants that attended at 36 months, 459 participants (70.8%) had valid activPAL3^{TM} data. Of these, 372 (57.4%) had complete adipokine and activPAL3^{TM} data and were subsequently included in these analyses. Although those individuals with missing data (n=276) were more likely to be male (68% vs 32%), this was not statistically significant (p=0.082). There were no significant differences in age (66.8±7.5 vs. 65.8±8.0, p=0.287), body mass index (BMI) (31.7±5.5 vs. 31.4±5.5, p=0.258), glycaemic status (IGR; 32% vs. 31%, p=0.694), ethnicity (WE; 89.5% vs. 89.4%, p=0.794), medication use (beta blockers (16.9% vs. 17.1%, p=0.938; ACE inhibitors (18.8% vs. 17.5%, p=0.544); NSAIDs (4.8% vs. 5.3%, p=0.826; statins (32% vs. 31.9%, p=0.711) or smoking status (current smokers; 5.6% vs. 7.9%, p=0.115) between those included in the analyses compared with those excluded.

Table 1 displays the anthropometric, demographic and activity characteristics of those included. Participants wore the activPAL3^{TM} for (mean ± standard deviation) 15.6±1.3 hours per day and spent 9.4±2.0 hours sitting, 4.5±1.5 hours standing, 0.7±0.2 hours in light stepping and 1.0±0.4 hours in MVPA stepping per day, which equated to 3861±1664 steps per day. 55% of total sitting time was accrued in bouts of 30 minutes or longer.

Table 2 presents the results of the isotemporal substitution model (β coefficient and 95% CIs) of reallocating time from sitting to more active behaviours.

Reallocating 60 minutes of sitting to standing, light or MVPA stepping

Following adjustment for various confounders, reallocating 60 minutes of sitting time for standing was associated with -5% (-9%, -1%) lower IL-6 levels; p=0.013; -8% (-14%, 2%) lower CRP levels; p=0.110 and -3% (-7%, 1%) lower leptin levels; p=0.206. Even after adjusting for waist circumference the results for IL-6 remained significant (-4% (-7%, -1%; p=0.048)).

In the fully adjusted model, reallocating 60 minutes of sitting time for light stepping was associated with lower IL-6 (-28% (-46%, -4%; p=0.025)) only. Conversely theoretically substituting 60 minutes of sitting for MVPA stepping was associated with lower CRP (-41% (-75%, -8%; p=0.032)), leptin (-24% (-34%, -12%; p≤0.001)) and IL-6 (-16% (-28%, -10%; p=0.036)) values (Table 2).

In the single partition model all measured behaviours (sitting, standing, light and MVPA walking) were associated with all markers of inflammation after adjustment for the covariates listed above. Generally, the strongest associations were seen for sitting (direct) and MVPA stepping (inverse). After further adjustment for waist circumference, all results remained significant with the exception of replacing 60 minutes of standing on CRP and leptin (Table S1).

Interaction analyses

There was no interaction by sex, IGR status or length of sitting bout (<30 minutes or ≥30 minutes) for any measure (p > 0.1 for all). However, the interaction

for reallocating high /low sitting time to MVPA stepping was significant for IL-6 (p=0.017) and CRP (p=0.091). Results were only significant in those with high sitting time (>9.3h per day). Reallocating 60 minutes of sitting for MVPA stepping was associated with 42% lower CRP (-74%, -1%, p=0.048) and 26% lower IL-6 (-45%, -1%, p=<0.001) (Table 3). No interactions were found reallocating 60 minutes of sitting time for standing or light stepping (p values displayed in Table S2).

Results reported above were unaffected if BMI, rather than waist circumference was used in Model 2. From the 372 participants with CRP values, 39 (10.5%) had levels >10mg/L. After exclusion of these participants, the standardised beta-coefficients were largely unchanged across all markers of chronic low-grade inflammation (Table S3). However, of particular note, the significant associations between standing and light stepping with IL-6 were attenuated.

Discussion

This epidemiological study provides novel evidence on the replacement effects of sitting, standing and stepping on markers of chronic low-grade inflammation using statistical modelling. Our results indicate that in people at high risk of T2DM, replacing 60 minutes of sitting with equal amounts of either standing or stepping may yield beneficial associations upon markers of chronic-low grade inflammation; even after controlling for various confounding variables, including waist circumference. Overall, the greatest effects were shown when moving from sitting to MVPA stepping with those displaying the highest overall sitting time likely to accrue the greatest benefit.

This study extends previous research in high risk of T2DM individuals which demonstrated that theoretically reallocating short or prolonged sitting time with standing or stepping may positively influence 2-hour glucose, fasting and 2-hour insulin and insulin sensitivity (improvements ranging from 4-16%) (Edwardson et al., 2017). More specifically, results of this study show that reallocating 60 minutes of sitting to standing resulted in a 4% reduction in IL-6. Transitioning from sitting to light stepping resulted in a 28% reduction in IL-6. The results for MVPA stepping were more consistent, with significant improvements in CRP (-41%), IL-6 (-16%) and leptin (-24%). These findings are compatible with both epidemiological and early experimental work, which demonstrate that reducing sitting time by approximately 60 minutes per day is likely to be around the minimum needed to gain clinical benefit, with greater reductions (and intensity) resulting in greater health gain (Dunstan et al., 2012; Grontved & Hu, 2011; Henson et al., 2016; Matthews et al., 2015; Yates et al., 2015). Therefore, along with messages related to accumulating at least 150 min/week of MVPA, individuals should be further encouraged to simply sit less and move more. This has been demonstrated in a recent epidemiological study which showed that physical activity of any intensity influences markers of metabolic health but the higher the intensity, the greater the potential improvement for a given physical activity duration (Jelleyman et al., 2017). For example, individuals in this study would need to carry out 3.5 times more standing than walking in order to get the same hypothetical health benefit for IL-6.

The beneficial association between IL-6 and standing is a novel finding and may suggest a link with low-grade inflammation. These findings are broadly consistent with previous research which has demonstrated cross-sectional

associations between objectively measured (Henson et al., 2013) and self-reported (Yates et al., 2012) sedentary behaviour and IL-6, independent of MVPA. Furthermore, an increase in ambulatory activity, is known to have a strong inverse correlation with sedentary behaviour (Healy et al., 2011), and has been shown to be associated with reduced IL-6 in those with IGT, independent of obesity (Yates et al., 2010). IL-6 is a multifunctional proinflammatory cytokine produced by immune and non-immune cells (mainly adipose tissue and skeletal muscle) which acts upon a wide range of tissues through the modulation of cell growth and differentiation (Pedersen, 2012). Although primarily considered a proinflammatory hormone, it is known that the isoform released by skeletal muscle also has anti-inflammatory effects (Pedersen & Febbraio, 2012). Given its pleiotropic nature, IL-6 is one of the few genuine myokines that are produced by and/or act upon skeletal muscle. In response to muscle contractions, satellite cells are activated, proliferated, differentiated and fused to form new myofibres (Munoz-Canoves, Scheele, Pedersen, & Serrano, 2013). Subsequently, standing and stepping (both light and MVPA) may trigger and control the distinct actions of satellite cells through the myogenic process, increasing in an exponential fashion proportional to the length of activity and amount of muscle mass engaged, hence the more pronounced results for light (-28%) and MVPA stepping (-16%) vs. standing (-4%). Nevertheless, this speculative hypothesis of standing and stepping eliciting changes in IL-6 has not been previously tested in an experimental context as the large majority of studies have focused upon the release of IL-6 following bouts of MVPA.

Despite many of the associations persisting after exclusion of participants with a CRP-level >10mg/L, the associations between standing and light stepping

with IL-6 were attenuated. However, it could be argued that removal of such participants may exclude many with chronic inflammation who are at the highest risk for poor health outcomes. This is important as previous studies have demonstrated that >5% of the population exhibit such values at a given time-point (Alley et al., 2006; Visser, Bouter, McQuillan, Wener, & Harris, 1999). This number may be further exacerbated by individuals who exhibit characteristics such as obesity, smoking and physical inactivity, all known risk factors for chronic inflammation. Furthermore, it has been suggested that using CRP>10mg/L to distinguish between acute and chronic inflammation may lack sensitivity (Ishii et al., 2012). Nevertheless, caution needs to be applied when interpreting the results due to potential bias through acute inflammation.

Although CRP was beneficially associated with replacing 60 minutes of sitting for MVPA stepping, the association between moving from sitting to a standing posture or engaging in light stepping was found to be non-significant. This is largely unsurprising as CRP is considered a simple downstream mediator of the acute phase response and is primarily derived via IL-6 dependent hepatic biosynthesis, usually within 6 hours of the stimulus (Pradhan et al., 2001). Therefore, transitioning from sitting to MVPA stepping may be a reflection of upstream changes in the inflammatory response that are detected by downstream CRP levels, even if CRP itself has no direct activity. Leptin is also considered a proinflammatory adipocytokine that belongs structurally to the IL-6 family of cytokines (Harle & Straub, 2006). It is known that leptin is capable of directly regulating glucose and fatty acid metabolism in skeletal muscle and fat tissue (Wolsk, Mygind, Grondahl, Pedersen, & van Hall, 2012). Importantly, these tissues play fundamental roles in

whole-body glucose and lipid homeostasis, and are therefore implicated in the development of obesity, insulin resistance and T2DM (Karpe, Dickmann, & Frayn, 2011).

The subsequent reduction in leptin observed when moving from sitting to MVPA stepping may be associated with the elevated production of non-esterified fatty acids [NEFA] during activity, which has previously been shown to be associated with leptin levels (Ceddia, 2005). This is consistent with experimental evidence demonstrating that walking attenuates the postprandial suppression in plasma NEFA concentrations in high risk of T2DM individuals (Henson et al., 2016). In contrast, standing may not yield a sufficient stimulus in order to significantly alter lipid metabolism.

Importantly, the results for IL-6, CRP and leptin persisted after adjustment for waist circumference. Obesity-mediated cytokine production is an important and possible central mechanism for systemic elevations of these biomarkers (Wisse, 2004). However, these results suggest a possible role in the expression of inflammatory markers which is autonomously mediated.

This study has multiple strengths; most notably it provides novel evidence in a high risk primary care population. Our unique cohort of individuals are broadly representative of those referred onto diabetes prevention programmes, therefore having direct relevance for future diabetes prevention. The objective measure of time spent sitting, standing and stepping is also a strength. The activPAL3^{TM} has been shown to have almost perfect correlation with direct observation for sitting, sitting to

upright transitions and for detecting reductions in sitting (Kozey-Keadle et al., 2011; Kozey-Keadle, Libertine, Staudenmayer, & Freedson, 2012; Lyden et al., 2012). That said, the ActivPAL may not represent the full range of activities undertaken, since it does not include upper body activities. A further limitation of our work was that the exposures were only carried out at one time-point (up to 7 days), which precludes the drawing of causal inferences and may not be an accurate reflection of habitual activity. Furthermore, it is also possible that unmeasured lifestyle or demographic variables were confounding the observed relationships (e.g. alcohol intake). However, we adjusted for key medications and behaviours [smoking] known to affect inflammation and metabolic health. The cross-sectional design also means that the isotemporal substitution approach used in this study is not based on actual replacements of one activity for another; it should instead be viewed as a population level modelling study. More consistent regression estimates may have been derived from a compositional data analysis approach; where the relative distribution between all behaviours in the composition of the day is considered (Carson, Tremblay, Chaput, & Chastin, 2016; Chastin, Palarea-Albaladejo, Dontje, & Skelton, 2015; Dumuid et al., 2017).

In conclusion, findings from this study provide further encouraging evidence that simply substituting sitting for standing throughout the day may improve markers of health involved in the underlying pathophysiology of T2DM. That said, stronger and more consistent associations were observed for stepping (in particular MVPA), thus highlighting the continued importance of more intense physical activity. The results may also be particularly pertinent for those individuals with high overall sitting time. Given the limitations inherent in the design, our findings need

investigation in the general population. These results should also act as a stimulus for tightly controlled experimental studies elucidating potential mechanisms mediating the effect of low stimulus activities, such as standing in order to influence future physical activity and sedentary behaviour interventions and public health initiatives aimed at disease prevention.

Acknowledgements

The Walking Away trial was funded by The National Institute for Health Research [NIHR] Collaboration for Leadership in Applied Health Research and Care for Leicestershire, Northamptonshire and Rutland [NIHR CLAHRC – LNR] and East Midlands [NIHR CLAHRC EM]. The research was further supported by the University of Leicester Clinical Trials Unit and the NIHR Leicester Biomedical Research Centre which is a partnership between University Hospitals of Leicester NHS Trust, Loughborough University and the University of Leicester. The views expressed are those of the author[s] and not necessarily those of the NHS, the NIHR or the Department of Health. Analysis of IL-6, CRP and Leptin levels was funded by Unilever R&D, UK.

Clinical Trials Registration: ClinicalTrials.gov NCT: NCT00941954. The authors would also like to thank Charlotte Jelleyman and Matthew McCarthy for their help with analysing the inflammatory markers and the participants for taking the time to take part.

Conflict of Interest

The authors declare no conflict of interest

References

Alley, D. E., Seeman, T. E., Ki Kim, J., Karlamangla, A., Hu, P., & Crimmins, E. M. (2006). Socioeconomic status and C-reactive protein levels in the US population: NHANES IV. *Brain, Behavior, and Immunity, 20*(5), 498-504. doi:10.1016/j.bbi.2005.10.003

Allison, M. A., Jensky, N. E., Marshall, S. J., Bertoni, A. G., & Cushman, M. (2012). Sedentary behavior and adiposity-associated inflammation: The multi-ethnic study of atherosclerosis. *American Journal of Preventive Medicine, 42*(1), 8-13. doi:10.1016/j.amepre.2011.09.023; 10.1016/j.amepre.2011.09.023

Best, L. G., Zhang, Y., Lee, E. T., Yeh, J. L., Cowan, L., Palmieri, V., . . . Howard, B. V. (2005). C-reactive protein as a predictor of cardiovascular risk in a population with a high prevalence of diabetes: The strong heart study. *Circulation, 112*(9), 1289-1295. doi:10.1161/CIRCULATIONAHA.104.489260

Biswas, A., Oh, P. I., Faulkner, G. E., Bajaj, R. R., Silver, M. A., Mitchell, M. S., & Alter, D. A. (2015). Sedentary time and its association with risk for disease incidence, mortality, and hospitalization in adults: A systematic review and meta-analysis. *Annals of Internal Medicine, 162*(2), 123-132. doi:10.7326/M14-1651 [doi]

Buman, M. P., Winkler, E. A., Kurka, J. M., Hekler, E. B., Baldwin, C. M., Owen, N., . . . Gardiner, P. A. (2014). Reallocating time to sleep, sedentary behaviors,

or active behaviors: Associations with cardiovascular disease risk biomarkers, NHANES 2005-2006. *American Journal of Epidemiology, 179*(3), 323-334. doi:10.1093/aje/kwt292 [doi]

Carson, V., Tremblay, M. S., Chaput, J. P., & Chastin, S. F. (2016). Associations between sleep duration, sedentary time, physical activity, and health indicators among canadian children and youth using compositional analyses. *Applied Physiology, Nutrition, and Metabolism = Physiologie Appliquee, Nutrition Et Metabolisme, 41*(6 Suppl 3), S294-302. doi:10.1139/apnm-2016-0026 [doi]

Ceddia, R. B. (2005). Direct metabolic regulation in skeletal muscle and fat tissue by leptin: Implications for glucose and fatty acids homeostasis. *International Journal of Obesity (2005), 29*(10), 1175-1183. doi:0803025 [pii]

Chastin, S. F., Palarea-Albaladejo, J., Dontje, M. L., & Skelton, D. A. (2015). Combined effects of time spent in physical activity, sedentary behaviors and sleep on obesity and cardio-metabolic health markers: A novel compositional data analysis approach. *PloS One, 10*(10), e0139984. doi:10.1371/journal.pone.0139984 [doi]

Dumuid, D., Stanford, T. E., Martin-Fernandez, J. A., Pedisic, Z., Maher, C. A., Lewis, L. K., . . . Olds, T. (2017). Compositional data analysis for physical activity, sedentary time and sleep research. *Statistical Methods in Medical Research,* , 962280217710835. doi:10.1177/0962280217710835 [doi]

Dunstan, D. W., Kingwell, B. A., Larsen, R., Healy, G. N., Cerin, E., Hamilton, M. T., . . . Owen, N. (2012). Breaking up prolonged sitting reduces postprandial glucose and insulin responses. *Diabetes Care,* doi:10.2337/dc11-1931

Edwardson, C. L., Gorely, T., Davies, M. J., Gray, L. J., Khunti, K., Wilmot, E. G., . . . Biddle, S. J. (2012). Association of sedentary behaviour with metabolic syndrome: A meta-analysis. *PloS One, 7*(4), e34916. doi:10.1371/journal.pone.0034916

Edwardson, C. L., Henson, J., Bodicoat, D. H., Bakrania, K., Khunti, K., Davies, M. J., & Yates, T. (2017). Associations of reallocating sitting time into standing or stepping with glucose, insulin and insulin sensitivity: A cross-sectional analysis of adults at risk of type 2 diabetes. *BMJ Open, 7*(1), e014267-2016-014267. doi:10.1136/bmjopen-2016-014267 [doi]

Falconer, C. L., Cooper, A. R., Walhin, J. P., Thompson, D., Page, A. S., Peters, T. J., . . . Andrews, R. C. (2014). Sedentary time and markers of inflammation in people with newly diagnosed type 2 diabetes. *Nutrition, Metabolism, and Cardiovascular Diseases : NMCD, 24*(9), 956-962. doi:10.1016/j.numecd.2014.03.009 [doi]

Falconer, C. L., Page, A. S., Andrews, R. C., & Cooper, A. R. (2015). The potential impact of displacing sedentary time in adults with type 2 diabetes. *Medicine and Science in Sports and Exercise, 47*(10), 2070-2075. doi:10.1249/MSS.0000000000000651 [doi]

Gray, L. J., Davies, M. J., Hiles, S., Taub, N. A., Webb, D. R., Srinivasan, B. T., & Khunti, K. (2012). Detection of impaired glucose regulation and/or type 2 diabetes mellitus, using primary care electronic data, in a multiethnic UK community setting. *Diabetologia, 55*(4), 959-966. doi:10.1007/s00125-011-2432-x [doi]

Grontved, A., & Hu, F. B. (2011). Television viewing and risk of type 2 diabetes, cardiovascular disease, and all-cause mortality: A meta-analysis. *Jama, 305*(23), 2448-2455. doi:10.1001/jama.2011.812 [doi]

Hamer, M., Sabia, S., Batty, G. D., Shipley, M. J., Tabak, A. G., Singh-Manoux, A., & Kivimaki, M. (2012). Physical activity and inflammatory markers over 10 years: Follow-up in men and women from the whitehall II cohort study. *Circulation, 126*(8), 928-933. doi:10.1161/CIRCULATIONAHA.112.103879 [doi]

Harle, P., & Straub, R. H. (2006). Leptin is a link between adipose tissue and inflammation. *Annals of the New York Academy of Sciences, 1069*, 454-462. doi:1069/1/454 [pii]

Healy, G. N., Matthews, C. E., Dunstan, D. W., Winkler, E. A., & Owen, N. (2011). Sedentary time and cardio-metabolic biomarkers in US adults: NHANES 2003-06. *European Heart Journal, 32*(5), 590-597. doi:10.1093/eurheartj/ehq451 [doi]

Healy, G. N., Winkler, E. A., Owen, N., Anuradha, S., & Dunstan, D. W. (2015). Replacing sitting time with standing or stepping: Associations with cardio-metabolic risk biomarkers. *European Heart Journal,* doi:ehv308 [pii]

Henson, J., Davies, M. J., Bodicoat, D. H., Edwardson, C. L., Gill, J. M., Stensel, D. J., . . . Yates, T. (2016). Breaking up prolonged sitting with standing or walking attenuates the postprandial metabolic response in postmenopausal women: A randomized acute study. *Diabetes Care, 39*(1), 130-138. doi:10.2337/dc15-1240 [doi]

Henson, J., Yates, T., Edwardson, C. L., Khunti, K., Talbot, D., Gray, L. J., . . . Davies, M. J. (2013). Sedentary time and markers of chronic low-grade inflammation in a high risk population. *PloS One, 8*(10), e78350. doi:10.1371/journal.pone.0078350 [doi]

Ishii, S., Karlamangla, A. S., Bote, M., Irwin, M. R., Jacobs, D. R.,Jr, Cho, H. J., & Seeman, T. E. (2012). Gender, obesity and repeated elevation of C-reactive protein: Data from the CARDIA cohort. *PloS One, 7*(4), e36062. doi:10.1371/journal.pone.0036062; 10.1371/journal.pone.0036062

Jelleyman, C., Edwardson, C. L., Henson, J., Gray, L. J., Rowlands, A. V., Khunti, K., . . . Yates, T. (2017). Associations of physical activity intensities with markers of insulin sensitivity. *Medicine and Science in Sports and Exercise,* doi:10.1249/MSS.0000000000001381 [doi]

Karpe, F., Dickmann, J. R., & Frayn, K. N. (2011). Fatty acids, obesity, and insulin resistance: Time for a reevaluation. *Diabetes, 60*(10), 2441-2449. doi:10.2337/db11-0425 [doi]

Kasapis, C., & Thompson, P. D. (2005). The effects of physical activity on serum C-reactive protein and inflammatory markers: A systematic review. *Journal of the American College of Cardiology, 45*(10), 1563-1569. doi:S0735-1097(05)00470-5 [pii]

Kozey-Keadle, S., Libertine, A., Lyden, K., Staudenmayer, J., & Freedson, P. S. (2011). Validation of wearable monitors for assessing sedentary behavior. *Medicine and Science in Sports and Exercise, 43*(8), 1561-1567. doi:10.1249/MSS.0b013e31820ce174 [doi]

Kozey-Keadle, S., Libertine, A., Staudenmayer, J., & Freedson, P. (2012). The feasibility of reducing and measuring sedentary time among overweight, non-exercising office workers. *Journal of Obesity, 2012*, 282303. doi:10.1155/2012/282303 [doi]

Kristiansen, O. P., & Mandrup-Poulsen, T. (2005). Interleukin-6 and diabetes: The good, the bad, or the indifferent? *Diabetes, 54 Suppl 2*, S114-24. doi:54/suppl_2/S114 [pii]

Lee, C. C., Adler, A. I., Sandhu, M. S., Sharp, S. J., Forouhi, N. G., Erqou, S., . . . Wareham, N. J. (2009). Association of C-reactive protein with type 2 diabetes: Prospective analysis and meta-analysis. *Diabetologia, 52*(6), 1040-1047. doi:10.1007/s00125-009-1338-3; 10.1007/s00125-009-1338-3

Lyden, K., Kozey Keadle, S. L., Staudenmayer, J. W., & Freedson, P. S. (2012). Validity of two wearable monitors to estimate breaks from sedentary time. *Medicine and Science in Sports and Exercise, 44*(11), 2243-2252. doi:10.1249/MSS.0b013e318260c477 [doi]

Matthews, C. E., Moore, S. C., Sampson, J., Blair, A., Xiao, Q., Keadle, S. K., . . . Park, Y. (2015). Mortality benefits for replacing sitting time with different physical activities. *Medicine and Science in Sports and Exercise, 47*(9), 1833-1840. doi:10.1249/MSS.0000000000000621 [doi]

Mekary, R. A., Lucas, M., Pan, A., Okereke, O. I., Willett, W. C., Hu, F. B., & Ding, E. L. (2013). Isotemporal substitution analysis for physical activity, television watching, and risk of depression. *American Journal of Epidemiology, 178*(3), 474-483. doi:10.1093/aje/kws590 [doi]

Mekary, R. A., Willett, W. C., Hu, F. B., & Ding, E. L. (2009). Isotemporal substitution paradigm for physical activity epidemiology and weight change. *American Journal of Epidemiology, 170*(4), 519-527. doi:10.1093/aje/kwp163 [doi]

Munoz-Canoves, P., Scheele, C., Pedersen, B. K., & Serrano, A. L. (2013). Interleukin-6 myokine signaling in skeletal muscle: A double-edged sword? *The FEBS Journal, 280*(17), 4131-4148. doi:10.1111/febs.12338 [doi]

Pedersen, B. K. (2012). Muscular interleukin-6 and its role as an energy sensor. *Medicine and Science in Sports and Exercise, 44*(3), 392-396. doi:10.1249/MSS.0b013e31822f94ac; 10.1249/MSS.0b013e31822f94ac

Pedersen, B. K., & Febbraio, M. A. (2012). Muscles, exercise and obesity: Skeletal muscle as a secretory organ. *Nature Reviews.Endocrinology, 8*(8), 457-465. doi:10.1038/nrendo.2012.49; 10.1038/nrendo.2012.49

Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E., & Ridker, P. M. (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Jama, 286*(3), 327-334. doi:joc10096 [pii]

Ridker, P. M. (2003). Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation, 107*(3), 363-369.

Thorp, A. A., Owen, N., Neuhaus, M., & Dunstan, D. W. (2011). Sedentary behaviors and subsequent health outcomes in adults: A systematic review of longitudinal studies, 19962011. *American Journal of Preventive Medicine, 41*(2), 207-215.

Visser, M., Bouter, L. M., McQuillan, G. M., Wener, M. H., & Harris, T. B. (1999). Elevated C-reactive protein levels in overweight and obese adults. *JAMA : The Journal of the American Medical Association, 282*(22), 2131-2135.

Wang, X., Bao, W., Liu, J., Ouyang, Y. Y., Wang, D., Rong, S., . . . Liu, L. G. (2013). Inflammatory markers and risk of type 2 diabetes: A systematic review and meta-analysis. *Diabetes Care, 36*(1), 166-175. doi:10.2337/dc12-0702 [doi]

Wilmot, E. G., Edwardson, C. L., Achana, F. A., Davies, M. J., Gorely, T., Gray, L. J., . . . Biddle, S. J. (2012). Sedentary time in adults and the association with diabetes, cardiovascular disease and death: Systematic review and meta-analysis. *Diabetologia, 55*(11), 2895-2905. doi:10.1007/s00125-012-2677-z; 10.1007/s00125-012-2677-z

Winkler, E. A., Bodicoat, D. H., Healy, G. N., Bakrania, K., Yates, T., Owen, N., . . . Edwardson, C. L. (2016). Identifying adults' valid waking wear time by automated estimation in activPAL data collected with a 24 h wear protocol. *Physiological Measurement, 37*(10), 1653-1668. doi:10.1088/0967-3334/37/10/1653 [doi]

Wisse, B. E. (2004). The inflammatory syndrome: The role of adipose tissue cytokines in metabolic disorders linked to obesity. *Journal of the American Society of Nephrology : JASN, 15*(11), 2792-2800. doi:15/11/2792 [pii]

Wolsk, E., Mygind, H., Grondahl, T. S., Pedersen, B. K., & van Hall, G. (2012). Human skeletal muscle releases leptin in vivo. *Cytokine, 60*(3), 667-673. doi:10.1016/j.cyto.2012.08.021 [doi]

Yates, T., Edwardson, C. L., Henson, J., Gray, L. J., Ashra, N. B., Troughton, J., . . . Davies, M. J. (2016). Walking away from type 2 diabetes: A cluster randomised controlled trial
 .

Yates, T., Davies, M. J., Gorely, T., Talbot, D., Bull, F., Sattar, N., & Khunti, K. (2010). The effect of increased ambulatory activity on markers of chronic low-grade inflammation: Evidence from the PREPARE programme randomized controlled trial. *Diabetic Medicine : A Journal of the British Diabetic Association, 27*(11), 1256-1263. doi:10.1111/j.1464-5491.2010.03091.x; 10.1111/j.1464-5491.2010.03091.x

Yates, T., Davies, M. J., Henson, J., Troughton, J., Edwardson, C., Gray, L. J., & Khunti, K. (2012). Walking away from type 2 diabetes: Trial protocol of a cluster randomised controlled trial evaluating a structured education programme in those at high risk of developing type 2 diabetes. *BMC Family Practice, 13*, 46-2296-13-46. doi:10.1186/1471-2296-13-46 [doi]

Yates, T., Henson, J., Edwardson, C., Dunstan, D., Bodicoat, D. H., Khunti, K., & Davies, M. J. (2015). Objectively measured sedentary time and associations with insulin sensitivity: Importance of reallocating sedentary time to physical activity. *Preventive Medicine, 76*, 79-83. doi:10.1016/j.ypmed.2015.04.005 [doi]

Yates, T., Khunti, K., Wilmot, E. G., Brady, E., Webb, D., Srinivasan, B., . . . Davies, M. J. (2012). Self-reported sitting time and markers of inflammation, insulin resistance, and adiposity. *American Journal of Preventive Medicine, 42*(1), 1-7. doi:10.1016/j.amepre.2011.09.022 [doi]

Table 1. Characteristics of included participants

Variable	
N	372
Age (years)	66.8 ± 7.5
Male	227 (61.0)
Current smokers	21 (5.6)
Family History of Diabetes (1st degree)	135 (36.3)
Body Mass Index (BMI) (kg/m^2)	31.7 ± 5.5
Waist circumference (cm)	104.3 ± 13.0
Weight (kg)	90.2 ± 17.0
High sensitivity C-reactive protein (CRP) (mg/L)	1.9 (0.6-4.9)
Interleukin-6 (IL-6) (pg/mL)	2.0 (1.5-3.0)
Leptin (ng/mL)	12.0 (6.8-22.4)
Blood pressure medication β-blockers Angiotensin-converting enzyme (ACE) inhibitors	 63 (16.9) 70 (18.8)
Non-steroidal anti-inflammatory (NSAIDs) medication	18 (4.8)
Lipid lowering medication Fibrates Statins	 2 (0.5) 119 (32.0)
Impaired Glucose Regulation (IGR)	119 (32.0)
Ethnicity White European South Asian Other	 333 (89.5) 28 (7.5) 11 (3.0)
activPAL3^{TM} variables (time in hours per day)	
Number of valid days activPAL3^{TM} worn	6 ± 1
Waking wear-time	15.6 ± 1.3
Total sitting time	9.4 ± 2.0
Sitting time accumulated in long bouts (≥30 minutes)	5.2 ± 2.0
Sitting time accumulated in short bouts (<30 minutes)	4.2 ± 1.2
Standing time	4.5 ± 1.5
Stepping time	1.7 ± 0.6
Light stepping time	0.7 ± 0.2
MVPA stepping time	1.0 ± 0.4
Average steps per day	3861 ± 1664

Data presented as mean ± standard deviation, median (interquartile range) or number (column percent)

Table 2. Associations of substituting 60 minutes of sitting for standing or stepping with markers of chronic low grade inflammation using isotemporal substitution

Model 1						
	Sitting to standing	*P* value	**Sitting to light stepping**	*P* value	**Sitting to MVPA stepping**	*P* value
CRP	0.92 (0.84, 1.02)	0.110	0.79 (0.55, 1.14)	0.209	0.47 (0.21, 0.86)	**0.018**
IL-6	0.95 (0.91, 0.99)	**0.013**	0.69 (0.52, 0.93)	**0.015**	0.80 (0.69, 0.94)	**0.008**
Leptin	0.97 (0.93, 1.01)	0.206	0.76 (0.56, 1.03)	0.078	0.69 (0.59, 0.81)	**<0.001**
Model 2						
	Sitting to standing	*P* value	**Sitting to light stepping**	*P* value	**Sitting to MVPA stepping**	*P* value
CRP	0.94 (0.86, 1.04)	0.372	0.90 (0.63, 1.29)	0.575	0.59 (0.25, 0.92)	**0.032**
IL-6	0.96 (0.93, 0.99)	**0.048**	0.72 (0.54, 0.96)	**0.025**	0.84 (0.72, 0.90)	**0.036**
Leptin	1.00 (0.96, 1.04)	0.942	0.82 (0.63, 1.07)	0.139	0.76 (0.66, 0.88)	**<0.001**
Model 1 was adjusted for age, sex, smoking status, ethnicity, beta blockers, angiotensin-converting-enzyme inhibitors, lipid lowering, non-steroidal anti-inflammatory drugs and wear time						
Model 2 was additionally adjusted for waist circumference						

Coefficients represent the factor by which the measure of inflammatory markers are multiplied by (95% confidence interval) for a 60 minute difference in the substituted behaviour

Table 3. Associations of substituting 60 minutes of sitting for stepping with markers of chronic low grade inflammation in those with high (>=9.3h per day) and low (<9.3h per day) sitting time

Model 1				
	HIGH (n=186)		**LOW (n=186)**	
	Sitting to MVPA stepping	*P* value	**Sitting to MVPA stepping**	*P* value
CRP	0.58 (0.26, 0.99)	**0.048**	0.93 (0.68, 1.30)	0.651
IL-6	0.74 (0.55, 0.99)	**0.046**	0.95 (0.87, 1.26)	0.603
Model 1 was adjusted for age, sex, smoking status, ethnicity, beta blockers, angiotensin-converting-enzyme inhibitors, lipid lowering, non-steroidal anti-inflammatory drugs, waist circumference and wear time				

Supplementary Table 1. Associations of substituting 60 minutes of sitting for standing, light or MVPA stepping with markers of chronic low grade inflammation using a single activity model (each activity considered separately)

Model 1								
	Sitting	*P* value	**Standing**	*P* value	**Light Stepping**	*P* value	**MVPA Stepping**	*P* value
CRP	0.234 (0.055)	**<0.001**	-0.165 (0.052)	**0.002**	-0.203 (0.053)	**<0.001**	-0.235 (0.051)	**<0.001**
IL-6	0.303 (0.057)	**<0.001**	-0.228 (0.053)	**<0.001**	-0.296 (0.052)	**<0.001**	-0.268 (0.054)	**<0.001**
Leptin	0.242 (0.042)	**<0.001**	-0.154 (0.039)	**<0.001**	-0.226 (0.038)	**<0.001**	-0.274 (0.038)	**<0.001**
Model 2								
	Sitting	*P* value	**Standing**	*P* value	**Light Stepping**	*P* value	**MVPA Stepping**	*P* value
CRP	0.142 (0.056)	**0.012**	-0.092 (0.051)	0.073	-0.122 (0.053)	**0.021**	-0.161 (0.053)	**<0.002**
IL-6	0.232 (0.058)	**<0.001**	-0.169 (0.054)	**0.002**	-0.241 (0.053)	**<0.001**	-0.191 (0.055)	**<0.001**
Leptin	0.131 (0.039)	**0.001**	-0.065 (0.036)	0.076	-0.137 (0.037)	**<0.001**	-0.183 (0.036)	**<0.001**
Model 1 was adjusted for age, sex, smoking status, ethnicity, beta blockers, angiotensin-converting-enzyme inhibitors, lipid lowering, non-steroidal anti-inflammatory drugs and wear time								
Model 2 was additionally adjusted for waist circumference								

Results presented as the standardised beta coefficient (β) (standard error)

Supplementary Table 2. Interaction values for reallocating sitting time to standing, light or MVPA stepping in those with high (>=9.3h per day) and low (<9.3h per day) sitting time.

Model 1			
	Sitting to standing	**Sitting to light stepping**	**Sitting to MVPA stepping**
CRP	0.179	0.112	**0.091**
IL-6	0.300	0.102	**0.017**
Leptin	0.121	0.851	0.898
Model 1 was adjusted for age, sex, smoking status, ethnicity, beta blockers, angiotensin-converting-enzyme inhibitors, lipid lowering, non-steroidal anti-inflammatory drugs, waist circumference, wear time, sitting group (high/low) and interaction term			

Supplementary Table 3. Associations of substituting 60 minutes of sitting for standing, light or MVPA stepping with markers of chronic low grade inflammation using isotemporal substitution (participants with CRP >10mg/L removed)

Model 1						
	Sitting to standing	*P* value	**Sitting to light stepping**	*P* value	**Sitting to MVPA stepping**	*P* value
CRP	0.95 (0.87, 1.04)	0.275	0.85 (0.63, 1.14)	0.279	0.86 (0.69, 0.95)	**0.012**
IL-6	0.98 (0.95, 1.02)	0.160	0.72 (0.54, 0.96)	**0.029**	0.82 (0.70, 0.95)	**0.011**
Leptin	0.98 (0.94, 1.04)	0.457	0.74 (0.54, 1.01)	0.060	0.66 (0.55, 0.78)	**<0.001**
Model 2						
	Sitting to standing	*P* value	**Sitting to light stepping**	*P* value	**Sitting to MVPA stepping**	*P* value
CRP	0.98 (0.90, 1.07)	0.685	0.89 (0.66, 1.19)	0.452	0.89 (0.72, 0.99)	**0.047**
IL-6	0.99 (0.96, 1.03)	0.230	0.85 (0.56, 1.00)	0.054	0.85 (0.73, 0.99)	**0.048**
Leptin	0.99 (0.95, 1.03)	0.684	0.81 (0.60, 1.08)	0.160	0.73 (0.63, 0.86)	**<0.001**
Model 1 was adjusted for age, sex, smoking status, ethnicity, beta blockers, angiotensin-converting-enzyme inhibitors, lipid lowering, non-steroidal anti-inflammatory drugs and wear time						
Model 2 was additionally adjusted for waist circumference						

Coefficients represent the factor by which the measure of inflammatory markers are multiplied by (95% confidence interval) for a 60 minute difference in the substituted behaviour.