SEDENTARY BEHAVIOUR IN HUMAN HEALTH AND DISEASE

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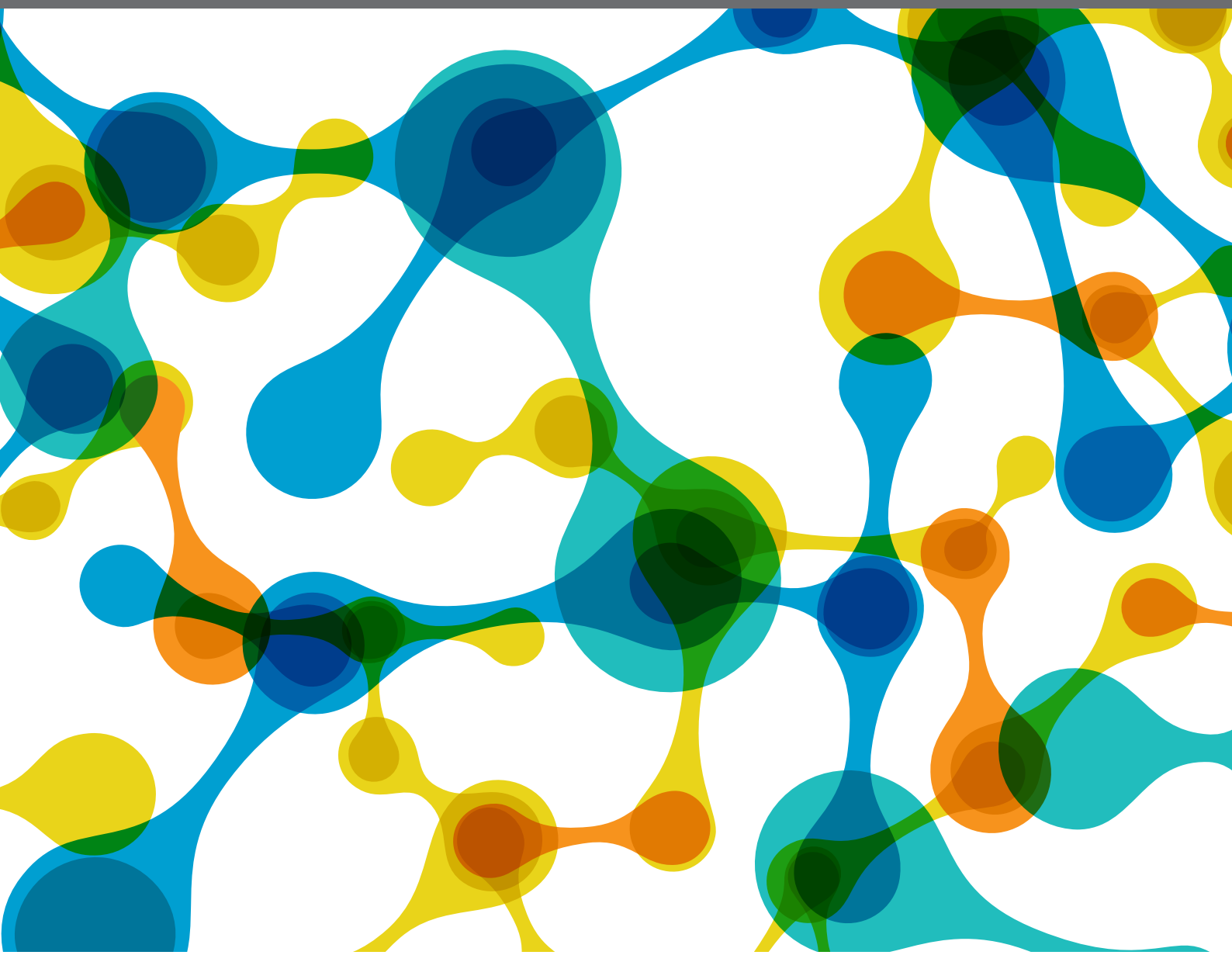

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SEDENTARY BEHAVIOUR IN HUMAN HEALTH AND DISEASE

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Sedentary behaviour – too much sitting as distinct from too little physical activity – is now recognised as an independent risk factor for several health outcomes and premature mortality. This is problematic as technological advancements in transportation, communications, workplaces, and domestic entertainment has created environments that encourage engagement in sedentary behaviour. Evidence from observational epidemiology shows that prolonged sitting is associated with increased risk of disease and adverse risk marker levels including type 2 diabetes, cardiovascular diseases, some cancers, obesity, glucose tolerance, and lipids. Importantly, the associations between prolonged sitting and these health markers are independent of time spent in moderate-to-vigorous physical activity.

Intriguingly, observational studies employing objective measures of sedentary time patterns using accelerometry have shown that adults who interrupt their sedentary time more frequently (breaks in sedentary time) have improved cardiometabolic profiles than those whose sedentary time is mostly uninterrupted. These beneficial associations are independent of total sedentary time and time spent in moderate-to-vigorous physical activity. In light of this evidence, experimental studies are now being conducted to identify novel mechanisms and potential causal relationships. It has been suggested that loss of muscular contractile stimulation induced through sitting impairs skeletal muscle metabolism of lipids and glucose and that the molecular processes through which these responses occur may be separate from the pathways activated when engaging in exercise.

This Research Topic aims to bring together contributions from researchers to advance the sedentary behaviour research agenda and strengthen the case for reducing and breaking up sitting time in primary prevention and disease management contexts.

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EDITORIAL
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Editorial: Sedentary Behavior in Human Health and Disease

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Keywords: sedentary behavior, sitting, physical activity, energy expenditure, breaks in sedentary time

Editorial on the Research Topic

Sedentary Behavior in Human Health and Disease

Sedentary behavior, defined as any waking behavior characterized by an energy expenditure ≤1.5 metabolic equivalents (METs) while in a sitting or reclining posture, has become a recognized independent risk factor for a wide array of health outcomes (Biswas et al., 2015). Technological advancements in modern society have created environments that encourage engagement in sedentary behavior, making this a public health concern. This Research Topic brings together contributions from researchers to advance the sedentary behavior research agenda and consider the case for reducing and breaking up sedentary time in primary prevention and disease management contexts.

The dangers of sedentary behavior may be particularly relevant to older adults who exhibit the highest amounts of sedentary time and are vulnerable to the adverse health effects of aging (Harvey et al., 2013). In this topic, Virtuoso et al. investigated whether self-reported sitting time could be used as a discriminator of frailty in hospitalized older adults (aged ≥ 60 years). Total daily sitting time was identified as a predictor of frailty with cut-points of >257 min/day and >330 min/day being predictive of the presence of frailty for males and females, respectively. In a slightly younger sample (40–75 years), van der Velde contributed a cross-sectional analysis of 1,932 adults from The Maastricht Study. Using an objective measure of sedentary behavior, total sedentary time were associated with a shorter 6 min walk test and lower relative elbow extension strength. There were favorable associations between the number of breaks in sedentary time per day and timed chair rise stand test performance. However, these associations were relatively weak, whereas associations between physical function measures with total and higher-intensity physical activity were stronger. These studies suggest that although sedentary time may increase the risk of frailty and reduce physical function, regular engagement in physical activity may be more important for improving and maintaining physical function in an older population.

In another cross-sectional study, Sardinha et al. contributed findings that total sedentary time and the number of breaks in sedentary time were associated with metabolic health in Type 2 diabetes, independent of moderate-to-vigorous physical activity and cardiorespiratory fitness. However, adjusting for cardiorespiratory fitness attenuated the association between total sedentary time and all but one glycaemic indicator, whereas the number of breaks in sedentary time had a favorable association with several glycaemic indicators independent of cardiorespiratory fitness. This suggests that high levels of cardiorespiratory fitness may neutralize the harmful effects of total sedentary time, but not *prolonged* sedentary time, in Type 2 diabetes.

To complement the growing experimental evidence that supports a causal relationship between sedentary behavior and metabolic health, Altenburg et al. contributed a pilot study that explored the effects of six consecutive days of increased prolonged sedentary time in free-living conditions in physically active young adult males. An increase in postprandial C-peptide was observed despite

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only a relatively small and insignificant increase in interrupted and uninterrupted sedentary time. No changes in glucose or triglycerides were observed, which may have been due to the relatively small increase in sedentary time during the experimental period. The authors provide important recommendations to overcome this limitation in future free-living research, such as objectively evaluating participants' normal baseline physical activity and sedentary time to ensure there is opportunity for them to substantially change their sedentary behavior during the experimental period. In a similar contribution, Duvivier et al. effectively changed participants' free-living sedentary behavior to permit a valid comparison between four consecutive days of (a) increased sedentary time and (b) substituting $\geq$7 h/day of sitting with light walking and standing in overweight adults. This resulted in a mean 13.5 and 7.6 h/day of sedentary time in these respective conditions. Favorable changes in insulin sensitivity, C-peptide, lipids and diastolic blood pressure were observed, which the authors suggest were similar in magnitude to responses observed when adhering to the 150 min/week physical activity guidelines. This highlights the potential importance of substituting sitting with light activities to reduce cardiometabolic disease risk in at-risk populations.

Based on growing evidence that reducing sedentary time may improve health, it is important to identify effective and feasible interventions for at-risk groups, such as office workers. Koepp et al. contributed an evaluation of an under-the-table-leg-movement apparatus. This apparatus was used during seated computer work at a desk and significantly increased energy expenditure by 18% compared to a standard office chair. However, this was not as high as the 107 and 155% increase in response to walking at 1 and 2 mph, respectively. Standing has also been recommended as an intervention to reduce sedentary time, although the benefits to metabolic health are inconsistent (Benatti and Ried-Larsen, 2015). Miles-Chan and Dulloo contributed a review of the large inter-individual variability in the energy cost of standing and identify that the energy cost of steady-state standing posture maintenance is considerably lower than the 1.5 METs threshold. However, regular postural transitioning (sitting to standing) appears to increase energy expenditure considerably more and may be most beneficial for overweight and obese individuals due to an increased postural transition energy cost. Naik et al. investigated electromyography muscle activities around the knee during sit-to-stand and returning task in females wearing shoes with different heel heights. Muscle imbalance around the knee during these tasks increased with increasing heel height, which may contribute to fatigue and knee problems, such as osteoarthritis. This should be considered when prescribing regular posture transitions as an intervention.

This research topic contributes to the mounting evidence highlighting the importance of avoiding high amounts of sedentary time, which may help in formulating public health guidelines. However, intervention development must take into account the population for which it is intended to ensure the strategies used are effective and do not predispose individuals to other health risks.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

REFERENCES

Benatti, F. B. and Ried-Larsen, M. (2015). The effects of breaking up prolonged sitting time: a review of experimental studies. *Med. Sci. Sports Exerc.* 47, 2053–2061. doi: 10.1249/MSS.0000000000000654

Biswas, A., Oh, P. I., Faulkner, G. E., Bajaj, R. R., Silver, M. A., Mitchell, M. S., et al., (2015). Sedentary time and its association with risk for disease incidence, mortality, and hospitalization in adults: a systematic review and meta-analysis. *Ann. Intern. Med.* 162, 123–132. doi: 10.7326/M14-1651

Harvey, J. A., Chastin, S. F. M., and Skelton, D. A. (2013). Prevalence of sedentary behavior in older adults: a systematic review. *Int. J. Environ. Res. Public Health* 10, 6645–6661. doi: 10.3390/ijerph10126645

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ORIGINAL RESEARCH
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Does Heel Height Cause Imbalance during Sit-to-Stand Task: Surface EMG Perspective

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The purpose of this study was to determine whether electromyography (EMG) muscle activities around the knee differ during sit-to-stand (STS) and returning task for females wearing shoes with different heel heights. Sixteen healthy young women (age = 25.2 ± 3.9 years, body mass index = 20.8 ± 2.7 kg/m^2) participated in this study. Electromyography signals were recorded from the two muscles, vastus medialis (VM) and vastus lateralis (VL) that involve in the extension of knee. The participants wore shoes with five different heights, including 4, 6, 8, 10, and 12 cm. Surface electromyography (sEMG) data were acquired during STS and stand-to-sit-returning (STSR) tasks. The data was filtered using a fourth order Butterworth (band pass) filter of 20–450 Hz frequency range. For each heel height, we extracted median frequency (MDF) and root mean square (RMS) features to measure sEMG activities between VM and VL muscles. The experimental results (based on MDF and RMS-values) indicated that there is imbalance between vasti muscles for more elevated heels. The results are also quantified with statistical measures. The study findings suggest that there would be an increased likelihood of knee imbalance and fatigue with regular usage of high heel shoes (HHS) in women.

Keywords: high heel shoes, imbalance, surface electromyography, sit-to-stand, vastus medialis, vastus lateralis

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INTRODUCTION

High heel shoes (HHS) are major sources for foot problems and chronic lower limb pain. They induce chronic muscle shortening with possible alterations in the muscle-tendon unit dynamic behavior and are associated with discomfort, fatigue and increased injury risk (Orizio et al., 2007; Cronin, 2014; Zöllner et al., 2015). Research shows that more than one-third of all women compromise health for looks and wears HHS on a daily basis (Cronin, 2014; Moore et al., 2015; Zöllner et al., 2015). HHS forces the foot into a plantarflexed position associated with shortening of the calf muscle–tendon unit (Cronin, 2014). Regularly wearing HHS alters neuromechanics of walking, compromise muscle efficiency, causes discomfort, and increase the risk of strain injuries (Cronin, 2014; Zöllner et al., 2015). Additionally, it has been proposed that HHS may contribute to the development and progression of knee osteoarthritis (OA) (Edwards et al., 2008; Kim et al., 2011). As wearing HHS in working environment is very common for women in today's modern society (Hsue and Su, 2009; Jung and Lee, 2014; Nam et al., 2014; Hapsari and Xiong, 2015), it is important to recognize the problems it causes.

Sit-to-stand (STS) and stand-to-sit-returning (STSR) tasks are some of the most frequently performed activities in daily life (Linder and Saltzman, 1998; Kim et al., 2011). These tasks are described as a motion of human body from a stable sitting-down position to a straight-up-standing position and vice versa (Kerr et al., 1994; Cronin, 2014). These tasks require higher muscle strength and coordination in balance system than other daily tasks, such as walking and stair climbing (Dall and Kerr, 2010; Hong et al., 2013), which demands an optimal neuromuscular coordination and posture adjustments (Dall and Kerr, 2010; Kim et al., 2011; Nam et al., 2014). It is recommended to perform the STS and STSR tasks frequently, as sedentary behavior such as prolonged sitting in office environment increases the health risks in both men and women (Neuhaus et al., 2014). Research shows that sedentary behaviors during prolonged sitting has been associated with cardiovascular disease and several musculoskeletal disorders (Wilmot et al., 2012; Costigan et al., 2013). Likewise, several tasks of daily living such as STS, STSR, walking, and stair climbing have been shown to be related to the ability to generate strength and power around the knee joint (Mizner and Snyder-Mackler, 2005; Brech et al., 2013).

Both STS and STSR tasks demand quadriceps muscle strength and postural control, so that balance is maintained during the postural transition and while standing upright (Carter et al., 2002; Brech et al., 2013). The quadriceps muscles such as vastus medialis (VM) and vastus lateralis (VL) are responsible for straightening (extending) knee joint and are the primary power source for daily activities like walking, running, squatting, and cycling. The VM and VL share the functional role of knee extension with the rectus femoris and vastus intermedius muscles (Hug et al., 2015). The functional importance of the VM is to dynamically stabilize the patella on the medial side and prevent lateral deviation and rotation of the patella caused by the lateral pull of the larger VL muscle (Grabiner et al., 1994; Christou, 2004). Several studies have used electromyography (EMG) to investigate VM and VL during motor activities, such as HHS walking, in musculoskeletal disorders, such as patellofemoral pain syndrome (PFPS) (Edwards et al., 2008; Jung and Lee, 2014) and also in knee OA (Simonsen et al., 2012; Nascimento et al., 2014; Tengman et al., 2015).

Muscle balance can be described as the respective equality between the antagonist and agonist muscles; this balance is essential for normal muscle movement and roles. It can be characterized by either front-to-back (agonist vs. antagonist) or side-to-side (right vs. left) differences in muscle length or strength. Muscle imbalance' occurs when opposing muscles provide different directions of tension due to tightness and/or weakness (Franettovich et al., 2011). The quadriceps (VM, VL, and rectus femoris) and hamstrings (semitendinosus, biceps femoris) of the knee joint perform opposite motions; an imbalance between the two could put undue stress on the joint (Page et al., 2010). Previously it has been shown that either a delay in EMG onset timing or a reduced EMG intensity in VM relative to VL may lead to a biomechanical imbalance in PFPS patients (Hug et al., 2015). In addition, it has been reported that HHS increases external adduction moment at the knee joint which may cause knee imbalance while wearing HHS in women (Lee et al., 2001; Kerrigan et al., 2005). However, to the best of our knowledge the imbalance using variety of HHS in women has not been investigated in the previous studies.

Surface Electromyography (sEMG) is widely used to measure muscle activation of isometric and dynamic actions of upper and lower limbs (Naik et al., 2016; Schmidt et al., 2016). The sEMG amplitude and frequency have been regarded as indicators of the localized muscular fatigue (Rainoldi et al., 2004; Cifrek et al., 2009). The amplitude [average rectified value and root mean square (RMS)] and spectral information [median frequency (MDF), mean frequency, peak frequency] of sEMG have also been exploited to estimate the level of muscle contraction and torque, respectively (Gerdle et al., 2000; Karlsson and Gerdle, 2001). The changes of sEMG characteristic values, such as decrease of the median power frequency (MDF) and increase of the root-mean-square (RMS), are very often used to estimate muscle fatigue and endurance limits. As stated previously, it has been shown that either a delay in EMG onset timing or a reduced EMG intensity in VM relative to VL may lead to a biomechanical imbalance. Hence, it is very reasonable to employ the above two parameters (RMS and MDF) to assess the imbalance in women wearing HHS.

Surface EMG has been widely used for assessing HHS muscle activation and other physiological problems such as lower limb joint moments, OA of the knee, patella tendon strain, and patellofemoral joint pressure in women (Edwards et al., 2008; Cronin, 2014). Hertel et al. (2005) reported that lateral and medial orthotics increased EMG activity in VM and decreased EMG activity in VL. Edwards et al. (2008) assessed the effect of shoe heel height on VM and VL muscles during STS task. However, their study did not find any changes in the relative EMG intensity of VM and VL as measured by the VM:VL ratio. This research study aims to examine whether EMG muscle activities around the quadriceps (VM and VL) muscles differ during STS and returning task with HHS that have heel heights ranging from 4 to 12 cm. As wearing HHS is a task with greater muscle demand than gait, it is possible that any effect of heel height on muscle activation patterns may be greater and more detectable than in gait. Considering the importance of these muscles in knee stability, and OA, it is necessary to investigate the effect of heel height on their activation. It is hypothesized that increasing heel height would elicit increased VM activity, relative to that of VL, to stabilize the patellofemoral joint in women wearing HHS.

MATERIALS AND METHODS

For the proposed research, an exploratory repeated measures study was conducted using data collected from young female participants. Materials and methods used for this study are explained in the next section.

Participants

Sixteen healthy young women (age $= 25.2 \pm 3.9$ years, body mass index $= 20.8 \pm 2.7$ kg/m^2) participated in this study. All participants were healthy, active women without prior histories of musculoskeletal disorders and injuries. An information sheet

was given and a consent form was signed before the experiment. The University Human Research Ethics Committee approved the study.

Instrumentation

The sEMG data were acquired at 2,048 samples/s using a MyoScan™ sEMG (SA9503M) silver-silver triode sensors; with three snap style receptacles representing two active (positive and negative) electrodes and one reference (ground) electrode (Thought Technology, Montreal, Quebec, Canada; input impedance ≥10 GΩ, CMRR >130 dB, bandwidth: 10–1,000 Hz and input/output gain: 500). The data was gathered via the Flexcomp Infiniti encoder system (Thought Technology, Montreal, Quebec, Canada) and was transmitted to a computer wirelessly through a Bluetooth device.

Procedures

The Stiletto type of shoes was used in this study, which means the surface of the heels that contact with the floor is no more than 1 cm^2. Participants self-identified the most suitable size for five different heights, including 4, 6, 8, 10, and 12 cm heels. The order of wearing different height of shoes was randomly assigned to avoid learning effect.

Surface EMG records muscle activities from the skin over the muscle belly. It offers a wealth of information concerning muscle activation patterns that makes it suitable for both research and clinical settings. EMG signals were recorded from the two muscles, VM and VL that involve in the extension of knee. Two sEMG electrodes (one for VM and one for VL) were placed on the dominant leg. Participants were asked which leg they would choose to kick a ball; and the chosen leg was identified as the dominant one (Mostamand et al., 2016). Also, from the previous studies it has been found that sEMG activity of quadriceps and calf muscles are significantly higher in the dominant leg as compared to non-dominant leg (Mostamand et al., 2016). The placement of electrodes was configured according to SENIAM guidelines (Hermens et al., 1999). Skin was cleaned by alcohol wipe before the placement.

An armless chair was used for STS and STSR experiments. During the experiment, the participants were asked to sit on the chair with their shanks 90° to the floor and their arms cross and rest on the chest. This is to avoid the assistance from arms when they stand. Trials were performed before real experiments started. Five-second sitting was recorded and participants stood up when hearing the signal word "stand." They remained standing for another 5 s and sat down after the signal word "sit." Three-time repetitions were recorded.

Data Processing and Analysis

Data analysis was performed on raw EMG data collected with sEMG electrodes using a custom MATLAB software program (The MathWorks Inc., Massachusetts, USA). In this research, sEMG data normalization was not needed since the participants acted as their own control and all procedures were performed in the same session, without the sEMG electrode positions being altered (Soderberg and Knutson, 2000; Edwards et al., 2008). Due to movement artifacts in the initial and final transient phases of the test, the signals generated during these periods (i.e., before 5% and after 95% of the total time of the test) were discarded. These (raw) sEMG signals from individual muscles were detrended and filtered with a fourth order Butterworth band pass filter with frequency range of 20–450 Hz to remove background instrumentation noise and its harmonics. Prior to data analysis the cut-off points for each sEMG burst and onset time of each muscle was computed, which is defined as the point at which the signal amplitude exceeded the mean amplitude plus 3 standard deviations (*SD*) during the 200 ms before the start of the STS task (Dehail et al., 2007; Kim et al., 2011). The same procedure was also adopted for STSR task. For each participant, the RMS and MDF were calculated for VM and VL in each sit to stand repetition by dividing the sEMG integral by the contraction time interval. RMS is one of the popular features used in the analysis of sEMG signals. It quantifies the degree of muscle activity and power of the signal of muscle voluntary contraction in EMG (Hapsari and Xiong, 2015). MDF is a parameter that is often used for muscle fatigue assessment, where EMG spectrum is divided into two regions with equal amplitude and the middle value is selected (Cifrek et al., 2009).

The ratio of the magnitudes of the VM and VL muscles (VM:VL ratio) using the RMS and MDF were computed for each of the heel heights for all participants. For each of these variables, pairwise repeated measures analysis of variance (ANOVA) was carried using the MATLAB and Statistics Toolbox Release 2012a (The MathWorks Inc., Massachusetts, USA) to determine statistically significant differences between the five heel heights (4, 6, 8, 10, and 12 cm). The statistical level of significance was fixed at an α level <0.05 (95% confidence intervals).

RESULTS

The RMS of the sEMG data for VM and VL are calculated and its mean and standard deviation (SD) are shown in **Table 1**. When pooled across all three recording sessions, there was a significant increase of muscle activities in VM:VL ratio ($p < 0.05$) for elevated heel heights. Also, for the pooled results significant decline (lower VM:VL ratio) in MDF were observed for elevated heel heights ($p < 0.05$). Similarly, the mean differences and 95% confidence intervals between the different HHS conditions (RMS and MDF) are presented in **Table 2**.

Pairwise ANOVA results (*p*-values) for RMS for all five heel heights (4, 6, 8, 10, and 12 cm) are shown in **Table 3**. From the results, it can be seen that RMS results are statistically significant ($p < 0.05$) for all heel heights, indicating significant differences in muscle activities for each heel height. The repeated measures ANOVA revealed that the difference between the conditions in the VM:VL ratio was statistically significant ($p < 0.05$) for all heel heights. At baseline, the onset of VL occurred before VM for all HHS. For elevated HHS (>6 cm), we observed greater change in sEMG onset timing difference for VM compared to VL ($p < 0.05$). The mean and standard deviation of onset time of VM and VL for different HHS are shown in **Figure 1**.

The box plots of VM:VL ratios corresponding to the MDF and RMS for STS and STSR are depicted in **Figures 2**, **3**,

TABLE 1 | Average RMS-values (mean ± *SD*) of VM and VL for different STS and STSR tasks.

Heel height (cm)	STS		STSR	
	VM (mv)	VL (mv)	VM (mv)	VL (mv)
4	92.6 ± 4.8	71.8 ± 4.2	61.2 ± 3.9	53.3 ± 3.7
6	124.2 ± 5.1	81.4 ± 4.3	74.3 ± 4.1	57.2 ± 3.1
8	141.2 ± 5.3	89.2 ± 4.3	93.5 ± 3.9	65.6 ± 3.4
10	176.8 ± 4.7	98.6 ± 4.8	123.6 ± 4.7	74.3 ± 3.6
12	182.5 ± 4.9	105.8 ± 4.6	134.1 ± 5.2	83.7 ± 4.9

Vm, Vastus medialis;, VL, Vastus lateralis; STS, Sit to stand; STSR, Sit to stand return; SD, Standard deviation.

TABLE 2 | The average results showing VM and VL ratio (VM:VL) of MDF and RMS values for different STS and STSR tasks.

Heel height (Mean ± *SD*)		4 cm	6 cm	8 cm	10 cm	12 cm
STS	MDF	1.36 ± 0.04	1.24 ± 0.03	1.08 ± 0.04	0.96 ± 0.04	0.86 ± 0.03
	RMS	1.13 ± 0.05	1.31 ± 0.03	1.47 ± 0.05	1.60 ± 0.04	1.77 ± 0.07
STSR	MDF	1.22 ± 0.03	1.10 ± 0.04	0.95 ± 0.03	0.84 ± 0.03	0.76 ± 0.03
	RMS	1.09 ± 0.06	1.25 ± 0.03	1.38 ± 0.04	1.50 ± 0.03	1.60 ± 0.03

Vm, Vastus medialis; VL, Vastus lateralis; STS, Sit to stand; STSR, Sit to stand return; SD, Standard deviation, MDF, Median frequency; RMS, Root mean square.

TABLE 3 | Mean (95% confidence interval) difference between conditions in average RMS-values of sEMG activity (μV) of VM and VL during sit to stand task.

Comparison	STS	
	VM	VL
4 vs. 6 cm	−31.691* (−38.667 to −24.715)	−9.627* (−16.921 to −2.333)
4 vs. 8 cm	−48.627* (−57.341 to −39.913)	−17.445* (−23.182 to −11.709)
4 vs. 10 cm	−84.182* (−92.520 to −75.843)	−26.809* (−34.211 to −19.407)
4 vs. 12 cm	−89.909* (−96.551 to −83.267)	−34.073* (−39.448 to −28.697)
6 vs. 8 cm	−16.936* (−23.992 to −9.880)	−7.818* (−14.403 to −1.234)
6 vs. 10 cm	−52.491* (−61.153 to −43.828)	−17.182* (−24.238 to −10.126)
6 vs. 12 cm	−58.218* (−65.842 to −50.595)	−24.445* (−32.285 to −16.606)
8 vs. 10 cm	−35.555* (−44.859 to −26.250)	−9.364* (−17.087 to −1.641)
8 vs. 12 cm	−41.282* (−48.614 to −33.950)	−16.627* (−20.926 to −12.328)
10 vs. 12 cm	−5.727 (−13.605 to 2.151)	−7.264* (−14.467 to −0.060)

*Significant heel height-associated differences are indicated by * $p < 0.05$.*

respectively. It is interesting to note that the box plots of STS and STSR parameters for all five-heel heights show significant separation, indicating the good discrimination ability of all five-heel heights for STS and STSR tasks. The results also indicate that each of the heel heights starting from 4 cm is responsible for knee imbalance in women wearing HHS. The higher VM:VL ratio for wearing heels that are >6 cm in height indicates carrying out a sit to stand task requires greater muscle activation in both VM and VL and also there could be further knee issues (imbalance) for women wearing HHS on a regular basis.

DISCUSSION

We examined whether wearing different HHS causes muscle imbalance on quadriceps muscles. Comparison of the results of our study with those previously published research is interesting. The results of the study based on MDF and RMS values confirming that wearing HHS that are higher than 6 cm results in significant amount of muscle imbalance as compared to HHS that are lesser than 6 cm height. These results are different from a previous study, where Edwards et al. (2008) found no statistically significant differences (VM:VL ratio) among different heel elevations. This might be due to the use of wedges instead of actual heels and also, they only used two heel heights (3 and 5 cm). Moreover, the wooden device (used in their study) that simulated the HHS had a broader base, while in this study the shoes had thin heels. In another study, Kim et al. (2011) reported significant differences among different heel heights in terms of muscle timings and activities. Similarly, Batista et al. (2013) compared the muscle activity between healthy women and PFPS patients when they performed the same task. They showed that wearing HHS significantly decreases the VM and the VL ratio in the patient group. On the other hand, Lee et al. (2001) study revealed that VL activity is not affected by wearing heels during gait. Also, Kerrigan et al. (2005) reported that high-heeled shoes increase the external adduction moment at the knee joint which implies an increased medial compartment load (Edwards et al., 2008). From the above, it is clear that there is clear relationship between our study and previous studies because, each of the above studies elicited the adverse effect of wearing HHS in women and like ours some even highlighted potential knee issues in both healthy and women with OA and PFPS. However, clear comparison of our study with the previous studies cannot be drawn because, each of the above studies was conducted on different experimental settings, for different population groups and moreover, they have been evaluated using different parameters.

Our experimental results show a significant increase in VM:VL ratio (RMS measure) when the subjects wore HHS >6 cm. One reason for this VM:VL imbalance could be an inconsistent increase of VM with late activation of VL. Hence, it seems that the high heel can beneficially increase the VM activation. We consider the reason behind the enhanced activity of the VM for elevated HHS is the fact that STS and STSR tasks make maintaining ankle joint positions difficult, so that more effort is required to maintain posture (Kang and Hyong, 2012; Hyong and Kang, 2013). This is in agreement with Hertel et al. (2005) who stated that lateral and medial orthotics increases EMG activity in VM and decreased EMG activity in VL.

According to Neptune et al. (2000), either a delay in EMG onset timing or a reduced EMG intensity in VM relative to VL may lead to a biomechanical imbalance. As stated by other researchers, this fact may be related to the increased external knee adduction moment due to the use of HHS (Simonsen et al., 2012; Batista et al., 2013). According to Batista et al. (2013), in order to avoid knee imbalance an increased activity of the VL muscle should be followed by a simultaneous increase of the VM. This is in agreement with the study of Foster et al. (2012),

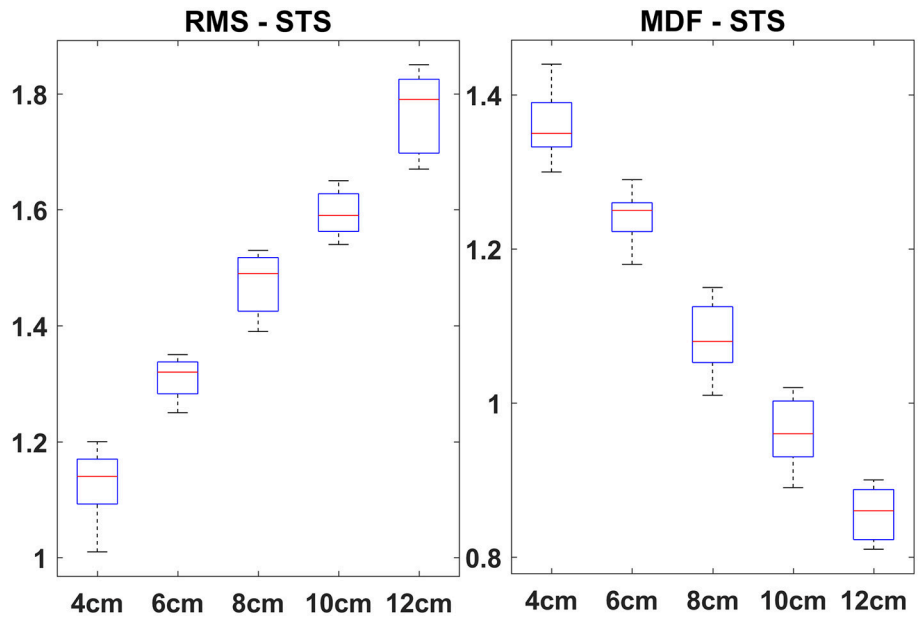

FIGURE 1 | Onset time (mean ± *SD*) of VM and VL for different HHS.

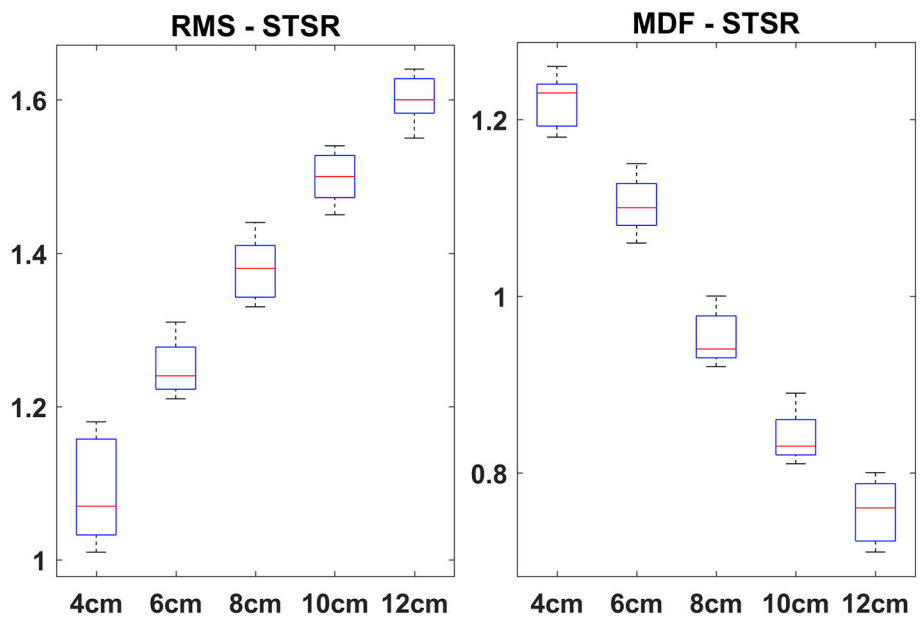

FIGURE 2 | Averaged VM:VL ratio (RMS—left side and MDF—right side) vs. heel heights across 16 subjects during STS. On each box, the red mark is the mean; the edges of the box are the 25th and the 75th percentiles.

who demonstrated that a 9.5 cm heel significantly increases the plantarflexion angles of the ankle and inversion of the foot. This condition may have required from the subjects some different strategies in order to keep the balance during the execution of STS and STSR task, and may have caused changes in the balance of forces not only in the sagittal plane, but also in the other planes.

A number of studies used MDF for assessing the effect of HHS in women. Gefen et al. (2002), compared the MDF of lower limb muscles from habitual and non-habitual HHS wearers following a fatiguing exercise. They reported a significant decrease of MDF for lower limb muscles in habitual wearers as compared to non-habitual wearers. They also argue that when the HHS are regularly used one of the lower limb muscles

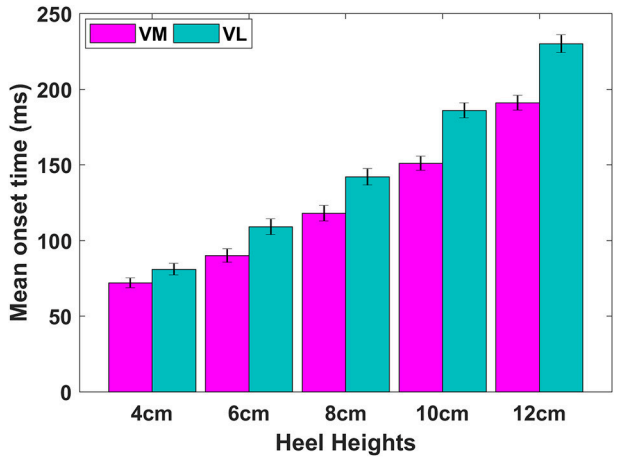

FIGURE 3 | Averaged VM:VL ratio (RMS—left side and MDF—right side) vs. heel heights across 16 subjects during STSR. On each box, the red mark is the mean; the edges of the box are the 25th and the 75th percentiles.

may act more intensively to produce the forces required to raise the foot from midstance to push-off leading to asymmetric muscle activity (Gefen et al., 2002). Millington et al. (1992) have reported that during STS task, the VM and rectus femoris become active before knee extension begins, whereas the gluteus maximus and medial hamstrings become active after the movement begins. According to muscle theory, in terms of muscle fibers, slow-twitch fibers (type I fibers) are thought to be represented by low-frequency band components and fast-twitch fibers (type II fibers) by high-frequency band components (Komi and Tesch, 1979; Moritani and Muro, 1987). This means, lower frequency values of VM and VL muscles for the elevated HHS indicate that type I fibers are more active when wearing HHS.

Over the past decades, technological advances, societal influences and environmental attributes have significantly influenced the way we socialize, work etc., resulting in substantial proportions of the day spent in sedentary pursuits, or sitting (Clemes et al., 2014). Sedentary behaviors during prolonged sitting has been associated with several musculoskeletal disorders (Costigan et al., 2013). Because of the adverse effect of sedentary tasks, including sitting, people need to perform to stand up more frequently. There is limited evidence on the association between sedentary behavior related to occupational sitting; prolonged sitting-time during leisure; and total sitting time (Chen et al., 2009; Wærsted et al., 2010). Furthermore, there are no available studies/literature suggesting the effect of sedentary behavior during wearing shoes or HHS in office/home settings. Also, it is unlikely that wearing HHS will make any impact on sedentary behavior in healthy women. However, more studies are warranted to research on the effect of HHS on either walking or STS after sedentary tasks such as prolonged sitting during office or watching movie in theater etc.

CONCLUSION

The purpose of the current study was to determine whether elevated heel heights causes' knee imbalance during STS tasks. Consistent with our hypothesis, RMS of VM:VL ratio was found to increase with heel height and similarly, MDF of VM:VL ratio decrease with heel height. Also, statistically significant changes were observed in the relative levels of muscle activity as measured by the VM:VL ratio for all heel heights. The study findings suggest that there would be an increased likelihood of fatigue or impending knee issues with regular usage of HHS in women. Moreover, decreased MDF and RMS ratios characterize muscle imbalance and indicate that women tend to get fatigue while wearing HHS of higher elevation due to imbalance between VM and VL muscles.

While findings for the present study are only examined on healthy younger women this need to be quantified with other age groups as well. Moreover, the finding of this research needs to be further validated with both kinetic and biomechanical analysis. Despite this, based on the results from the current study it is evident that there might be risk in wearing HHS during STS and STSR tasks.

ETHICS STATEMENT

The study was approved by the Human Research Ethics Committee of the University of Technology Sydney (UTS). Human subjects were given a consent form, which described the experimental procedure and any risks involved (which were minimal). After reading the form, human subjects were asked if they had any questions. Next, human subjects signed the consent form, and then the investigator signed the consent form. The consent forms were stored in a secure filling cabinet in the laboratory.

AUTHOR CONTRIBUTIONS

GN: performed all data analysis and wrote the manuscript. AA, MG, and HN: advised the analysis and edited the manuscript. GN and AA: conceptualized the experiment and edit the manuscript. HN: supervised the study, advised the analysis and edited the manuscript.

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REFERENCES

Batista, L. S. P., de Oliveira, V. M. A., de Souza, L. P. L., Pitangui, A. C. R., and de Araújo, R. C. (2013). Influence of high-heeled shoes on the quadriceps electromyographic activity in women with and without patellofemoral pain syndrome during the sit-to-stand task. *Fisioterapia e Pesquisa* 20, 2–10. doi: 10.1590/S1809-29502013000100002

Brech, G., Alonso, A., Luna, N., and Greve, J. (2013). Correlation of postural balance and knee muscle strength in the sit-to-stand test among women with and without postmenopausal osteoporosis. *Osteoporos. Int.* 24, 2007–2013. doi: 10.1007/s00198-013-2285-x

Carter, N. D., Khan, K. M., Mallinson, A., Janssen, P. A., Heinonen, A., Petit, M. A., et al. (2002). Knee extension strength is a significant determinant of static and dynamic balance as well as quality of life in older community-dwelling women with osteoporosis. *Gerontology* 48, 360–368. doi: 10.1159/000065504

Chen, S.-M., Liu, M.-F., Cook, J., Bass, S., and Lo, S. K. (2009). Sedentary lifestyle as a risk factor for low back pain: a systematic review. *Int. Arch. Occup. Environ. Health* 82, 797–806. doi: 10.1007/s00420-009-0410-0

Christou, E. A. (2004). Patellar taping increases vastus medialis oblique activity in the presence of patellofemoral pain. *J. Electromyogr. Kinesiol.* 14, 495–504. doi: 10.1016/j.jelekin.2003.10.007

Cifrek, M., Medved, V., Tonković, S., and Ostojić, S. (2009). Surface EMG based muscle fatigue evaluation in biomechanics. *Clin. Biomech.* 24, 327–340. doi: 10.1016/j.clinbiomech.2009.01.010

Clemes, S. A., O'connell, S. E., and Edwardson, C. L. (2014). Office workers' objectively measured sedentary behavior and physical activity during and outside working hours. *J. Occup. Environ. Med.* 56, 298–303. doi: 10.1097/JOM.0000000000000101

Costigan, S. A., Barnett, L., Plotnikoff, R. C., and Lubans, D. R. (2013). The health indicators associated with screen-based sedentary behavior among adolescent girls: a systematic review. *J. Adolesc. Health* 52, 382–392. doi: 10.1016/j.jadohealth.2012.07.018

Cronin, N. J. (2014). The effects of high heeled shoes on female gait: a review. *J. Electromyogr. Kinesiol.* 24, 258–263. doi: 10.1016/j.jelekin.2014.01.004

Dall, P. M., and Kerr, A. (2010). Frequency of the sit to stand task: an observational study of free-living adults. *Appl. Ergon.* 41, 58–61. doi: 10.1016/j.apergo.2009.04.005

Dehail, P., Bestaven, E., Muller, F., Mallet, A., Robert, B., Bourdel-Marchasson, I., et al. (2007). Kinematic and electromyographic analysis of rising from a chair during a "Sit-to-Walk" task in elderly subjects: role of strength. *Clin. Biomech.* 22, 1096–1103. doi: 10.1016/j.clinbiomech.2007.07.015

Edwards, L., Dixon, J., Kent, J. R., Hodgson, D., and Whittaker, V. J. (2008). Effect of shoe heel height on vastus medialis and vastus lateralis electromyographic activity during sit to stand. *J. Orthop. Surg. Res.* 3:2. doi: 10.1186/1749-799X-3-2

Foster, A., Blanchette, M. G., Chou, Y.-C., and Powers, C. M. (2012). The influence of heel height on frontal plane ankle biomechanics: implications for lateral ankle sprains. *Foot Ankle Int.* 33, 64–69. doi: 10.3113/FAI.2012.0064

Franettovich, M., Hides, J., Mendis, M., and Littleworth, H. (2011). Muscle imbalance among elite athletes. *Br. J. Sports Med.* 45, 348–349. doi: 10.1136/bjsm.2011.084038.109

Gefen, A., Megido-Ravid, M., Itzchak, Y., and Arcan, M. (2002). Analysis of muscular fatigue and foot stability during high-heeled gait. *Gait Posture* 15, 56–63. doi: 10.1016/S0966-6362(01)00180-1

Gerdle, B., Larsson, B., and Karlsson, S. (2000). Criterion validation of surface EMG variables as fatigue indicators using peak torque: a study of repetitive maximum isokinetic knee extensions. *J. Electromyogr. Kinesiol.* 10, 225–232. doi: 10.1016/S1050-6411(00)00011-0

Grabiner, M. D., Koh, T. J., and Draganich, L. F. (1994). Neuromechanics of the patellofemoral joint. *Med. Sci. Sports Exerc.* 26, 10–21. doi: 10.1249/00005768-199401000-00004

Hapsari, V. D., and Xiong, S. (2015). Effects of high heeled shoes wearing experience and heel height on human standing balance and functional mobility. *Ergonomics* 59, 249–64. doi: 10.1080/00140139.2015

Hermens, H. J., Freriks, B., Merletti, R., Stegeman, D., Blok, J., Rau, G., et al. (1999). *European Recommendations for Surface Electromyography*. Enschede: Roessingh Research and Development.

Hertel, J., Sloss, B. R., and Earl, J. E. (2005). Effect of foot orthotics on quadriceps and gluteus medius electromyographic activity during selected exercises. *Arch. Phys. Med. Rehabil.* 86, 26–30. doi: 10.1016/j.apmr.2004.03.029

Hong, W.-H., Lee, Y.-H., Lin, Y.-H., Tang, S. F., and Chen, H.-C. (2013). Effect of shoe heel height and total-contact insert on muscle loading and foot stability while walking. *Foot Ankle Int.* 34, 273–281. doi: 10.1177/1071100712465817

Hsue, B. J., and Su, F. C. (2009). Kinematics and kinetics of the lower extremities of young and elder women during stairs ascent while wearing low and high-heeled shoes. *J. Electromyogr. Kinesiol.* 19, 1071–1078. doi: 10.1016/j.jelekin.2008.09.005

Hug, F., Hodges, P. W., and Tucker, K. (2015). Muscle force cannot be directly inferred from muscle activation: illustrated by the proposed imbalance of force between the vastus medialis and vastus lateralis in people with patellofemoral pain. *J. Orthop. Sports Phys. Ther.* 45, 360–365. doi: 10.2519/jospt.2015.5905

Hyong, I. H., and Kang, J. H. (2013). Activities of the vastus lateralis and vastus medialis oblique muscles during squats on different surfaces. *J. Phys. Ther. Sci.* 25, 915–917. doi: 10.1589/jpts.25.915

Jung, J., and Lee, S.- Y. (2014). The effects of wearing high heels while pressing a car accelerator pedal on lower extremity muscle activation. *J. Phys. Ther. Sci.* 26:1715. doi: 10.1589/jpts.26.1715

Kang, J. H., and Hyong, I. H. (2012). Analysis of electromyographic activities of ankle muscles at different levels of instability of unstable surfaces. *J. Phys. Ther. Sci.* 24, 1333–1335. doi: 10.1589/jpts.24.1333

Karlsson, S., and Gerdle, B. (2001). Mean frequency and signal amplitude of the surface EMG of the quadriceps muscles increase with increasing torque—a study using the continuous wavelet transform. *J. Electromyogr. Kinesiol.* 11, 131–140. doi: 10.1016/S1050-6411(00)00046-8

Kerr, K., White, J., Barr, D., and Mollan, R. (1994). Standardization and definitions of the sit-stand-sit movement cycle. *Gait Posture* 2, 182–190. doi: 10.1016/0966-6362(94)90006-X

Kerrigan, D. C., Johansson, J. L., Bryant, M. G., Boxer, J. A., Della Croce, U., and Riley, P. O. (2005). Moderate-heeled shoes and knee joint torques relevant to the development and progression of knee osteoarthritis. *Arch. Phys. Med. Rehabil.* 86, 871–875. doi: 10.1016/j.apmr.2004.09.018

Kim, M. H., Chung, H. Y., Yoo, W. G., and Choi, B. R. (2011). EMG and kinematics analysis of the trunk and lower extremity during the sit-to-stand task while wearing shoes with different heel heights in healthy young women. *Hum. Mov. Sci.* 30, 596–605. doi: 10.1016/j.humov.2010.09.003

Komi, P. V., and Tesch, P. (1979). EMG frequency spectrum, muscle structure, and fatigue during dynamic contractions in man. *Eur. J. Appl. Physiol. Occup. Physiol.* 42, 41–50. doi: 10.1007/BF00421103

Lee, C.-M., Jeong, E.-H., and Freivalds, A. (2001). Biomechanical effects of wearing high-heeled shoes. *Int. J. Ind. Ergon.* 28, 321–326. doi: 10.1016/S0169-8141(01)00038-5

Linder, M., and Saltzman, C. L. (1998). A history of medical scientists on high heels. *Int. J. Health Serv.* 28, 201–225. doi: 10.2190/GA2M-FLA2-17FB-V5PE

Millington, P. J., Myklebust, B. M., and Shambes, G. M. (1992). Biomechanical analysis of the sit-to-stand motion in elderly persons. *Arch. Phys. Med. Rehabil.* 73, 609–617.

Mizner, R. L., and Snyder-Mackler, L. (2005). Altered loading during walking and sit-to-stand is affected by quadriceps weakness after total knee arthroplasty. *J. Orthop. Res.* 23, 1083–1090. doi: 10.1016/j.orthres.2005.01.021

Moore, J. X., Lambert, B., Jenkins, G. P., and McGwin, G. (2015). Epidemiology of high-heel shoe injuries in US women: 2002 to 2012. *J. Foot Ankle Surg.* 54, 615–619. doi: 10.1053/j.jfas.2015.04.008

Moritani, T., and Muro, M. (1987). Motor unit activity and surface electromyogram power spectrum during increasing force of contraction. *Eur. J. Appl. Physiol. Occup. Physiol.* 56, 260–265. doi: 10.1007/BF00690890

Mostamand, J., Bader, D. L., and Hudson, Z. (2016). Does leg predomination affect the measurement of vasti muscle activity during single leg squatting? A reliability study. *J. Bodyw. Mov. Ther.* 20, 728–733. doi: 10.1016/j.jbmt.2016.04.012

Naik, G. R., Al-Timemy, A. H., and Nguyen, H. T. (2016). Transradial amputee gesture classification using an optimal number of sEMG sensors: an approach using ICA clustering. *IEEE Trans. Neural Syst. Rehabil. Eng.* 24, 837–846. doi: 10.1109/TNSRE.2015.2478138

Nam, S. J., Kim, M. J., Yim, S. J., Oh, D. W., Park, H. J., and Kim, C. Y. (2014). Influence of walking speed on electromyographic activity of

the rectus abdominis and erector spinae during high-heeled walking. *J. Back Musculoskeletal Rehabil.* 27, 355–360. doi: 10.3233/BMR-140455

Nascimento, N. I. C., Saraiva, T. S., Cruz Jr, A. T. V., Silva Souza, G., and Callegari, B. (2014). Barefoot and high-heeled gait: changes in muscles activation patterns. *Health* 6:2190. doi: 10.4236/health.2014.616254

Neptune, R., Wright, I., and van den Bogert, A. J. (2000). The influence of orthotic devices and vastus medialis strength and timing on patellofemoral loads during running. *Clin. Biomech.* 15, 611–618. doi: 10.1016/S0268-0033(00)00028-0

Neuhaus, M., Healy, G. N., Dunstan, D. W., Owen, N., and Eakin, E. G. (2014). Workplace sitting and height-adjustable workstations: a randomized controlled trial. *Am. J. Prev. Med.* 46, 30–40. doi: 10.1016/j.amepre.2013.09.009

Orizio, C., Gobbo, M., Diemont, B., and Solomonow, M. (2007). Force dynamic response of tibialis anterior—ankle joint unit in humans. *J. Electromyogr. Kinesiol.* 17, 194–202. doi: 10.1016/j.jelekin.2006.01.012

Page, P., Frank, C., and Lardner, R. (2010). *Assessment and Treatment of Muscle Imbalance: The Janda Approach*. Baton Rouge, LA: Human Kinetics.

Rainoldi, A., Melchiorri, G., and Caruso, I. (2004). A method for positioning electrodes during surface EMG recordings in lower limb muscles. *J. Neurosci. Methods* 134, 37–43. doi: 10.1016/j.jneumeth.2003.10.014

Schmidt, A., Stief, F., Lenarz, K., Froemel, D., Lutz, F., Barker, J., et al. (2016). Unilateral hip osteoarthritis: its effects on preoperative lower limb muscle activation and intramuscular coordination patterns. *Gait Posture* 45, 187–192. doi: 10.1016/j.gaitpost.2016.01.028

Simonsen, E. B., Svendsen, M. B., Nørreslet, A., Baldvinsson, H. K., Heilskov-Hansen, T., Larsen, P. K., et al. (2012). Walking on high heels changes muscle activity and the dynamics of human walking significantly. *J. Appl. Biomech.* 28, 20–28. doi: 10.1123/jab.28.1.20

Soderberg, G. L., and Knutson, L. M. (2000). A guide for use and interpretation of kinesiologic electromyographic data. *Phys. Ther.* 80, 485–498.

Tengman, T., Coleman, S., Grävare Silbernagel, K., Karlsson, J., and Riad, J. (2015). Muscle fatigue after Achilles tendon rupture: a limited heel-rise test with electromyography reveals decreased endurance. *Eur. J. Physiother.* 17, 200–207. doi: 10.3109/21679169.2015.1078842

Wærsted, M., Hanvold, T. N., and Veiersted, K. B. (2010). Computer work and musculoskeletal disorders of the neck and upper extremity: a systematic review. *BMC Musculoskelet. Disord.* 11:79. doi: 10.1186/1471-2474-11-79

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

Zöllner, A. M., Pok, J. M., McWalter, E. J., Gold, G. E., and Kuhl, E. (2015). On high heels and short muscles: a multiscale model for sarcomere loss in the gastrocnemius muscle. *J. Theor. Biol.* 365, 301–310. doi: 10.1016/j.jtbi.2014.10.036

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frontiers in Physiology

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Time Spent Sitting Is Associated with Changes in Biomarkers of Frailty in Hospitalized Older Adults: A Cross Sectional Study

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Background: Sedentary behavior has gained prominence in the literature as a risk factor for health and mortality independent of physical activity level; however, little is known about the relationship of sedentary behavior with frailty in older adults. The aim of this study was to investigate if time spent sitting can be used as a discriminator of frailty in older hospitalized persons.

Methods: The study included 162 hospitalized inpatients aged ≥60 years. Blood samples were taken for analyzing leukocyte counts and serum concentrations of C-reactive protein (CRP). Participants also answered a questionnaire about time spent sitting. Frailty was determined from a combination of CRP concentration and leukocyte count. Receiver operating characteristic (ROC) curves were constructed to analyse the predictive power and cut-points for time spent sitting and the presence of frailty.

Results: The areas under the ROC curves indicated that time spent sitting was an independent indicator of frailty (area under curve >0.6). The cut-off points for time spent sitting as an indicator of frailty were >257 min/day for men and >330 min/day for women.

Conclusions: Time spent sitting is associated with biomarkers of frailty in persons aged ≥60 years, indicating a need for interventions aimed at reducing sedentary behavior in this age group.

Keywords: sedentary behavior, frailty, ROC curve, inflammation, older adults

INTRODUCTION

It has been estimated that the number of older adults worldwide will reach 1 billion in the next 10 years (United Nations Population Fund, 2016). Concomitant with this increase, there will be an expansion in the use of technology, including appliances, automobiles, smartphones, and Internet. This will contribute to higher prevalence of time spent in sedentary behavior across the different age groups (Owen et al., 2010). Nevertheless, older adults will likely continue to be the segment of the population with the highest rates of sedentary behavior (Matthews et al., 2008), as they usually present with comorbidities that limit their activities of daily life.

Long periods spent in sedentary behavior are reportedly associated with elevated inflammatory state (Healy et al., 2011; Allison et al., 2012; Gennuso et al., 2013; Hamer et al., 2013; León-Latre et al., 2014; Parsons et al., 2017). Inflammation contributes to disorders such as cardiovascular disease (Emerging Risk Factors Collaboration et al., 2010), diabetes (Marques-Vidal et al., 2012), cognitive decline (Metti et al., 2014), and frailty (Gale et al., 2013).

Frailty is an important geriatric syndrome characterized by substantial declines in the function of multiple organ systems, functional capacity and higher risk for mortality (Fried et al., 2009; Li et al., 2011; Abizanda et al., 2013). Because frailty is associated with adverse health outcomes, its early identification could facilitate interventions aimed at minimizing such problems (Tribess et al., 2012). One way to diagnose frailty is examining the combination of inflammatory biomarkers, such as the serum concentration of C-reactive protein (CRP) and leukocyte count (Li et al., 2011). Elevated inflammatory state contributes to the decrease of muscle mass, strength, power, and motor performance, aspects that play an important role in the pathogenesis of frailty (Chen et al., 2014).

Due to overlapping of primary and secondary effects of aging, older adults represent the age group most vulnerable to adverse health effects. This place older adults as a susceptible group to hospitalization, wherein diagnosis of the frailty is often difficulty because of associated clinical conditions. Although, it has been shown that sedentary behavior is associated with high values of inflammatory biomarkers, limited evidence is available on the association of time spent in sedentary behavior and such biomarkers. This study aimed to analyse time spent sitting as an indicator of the presence of frailty, indicated by increased levels of inflammatory biomarkers, in hospitalized older adults.

MATERIALS AND METHODS

Study Sample

This cross-sectional study is part of the research project titled "Prevalence and associated factors of frailty in older adults of a university hospital."

The study sample comprised 1,455 persons aged 60 years or more of both sexes who were inpatients of medical and surgical wards of a university hospital from April 2013 to March 2014. The required sample size was calculated by estimating the prevalence of frailty in older adults, which was identified as 30% (Khandelwal et al., 2012). After calculation of the 95% confidence interval and a tolerable error of 5%, the required sample was estimated to be 168 study subjects.

The inclusion criteria were as follows: (1) age over 60 years; (2) agreement to participate in the study by signing an informed consent form; (3) achieving the minimum score in the Mini-Mental State Examination (Folstein et al., 1975) according to level of education specified by the criteria of Bertolucci et al. (1994); and (4) ability to walk.

Exclusion criteria were as follows: absence of serious sequelae of stroke such as localized loss of strength, aphasia, or other speech disorders that would prevent study assessments; presence of Parkinson's disease with severe impairment of motor function, speech, ability to communicate, or emotional issues that would prevent study assessments; severe deficits of vision and/or hearing that would substantially hinder communication; participation in the study during previous hospitalization; and being in the terminal stage of an illness.

Instruments and Procedures for Data Collection

Participants were asked to respond to a structured questionnaire administered in the form of a face-to-face interview. The questionnaire assessed relevant economic and social factors, functional disability, and sedentary behavior. Subsequently, participants provided a blood sample for determination of inflammatory biomarkers of frailty, namely C-reactive protein (CRP) and leukocyte count. Data collection was carried out by 12 appropriately trained researchers in the health field.

Economic and Social Variables

A self-report instrument developed by our Research Group on Public Health was used to gather data on sex (male/female), age (60–69 years, 70−79 years, and 80 years or more) marital status (single, married or living with a partner, and widowed, separated, or divorced), living arrangements (living alone and with others), and monthly personal income (income less or greater than one minimum wage).

Functional Disability

Functional disability was assessed by self-reported ability to carry out basic activities of daily living (BADL) and instrumental activities of daily living (IADL). The Brazilian versions of the "Index of Independence in Activities of Daily Living" (Lino et al., 2008) and "Scale of Instrumental Activities of Daily Living" (Santos and Virtuoso-Júnior, 2008) were used, respectively.

Sedentary Behavior

Time spent sitting was assessed with the following questions: "How long, in total, did you spend sitting during a weekday prior to hospitalization?" and "How long, in total, did you spend sitting on a weekend day prior to hospitalization?" These questions are similar to those that evaluate time spent sitting in the International Physical Activity Questionnaire (Rosenberg et al., 2008), according to another study conducted in Brazil (Santos et al., 2017). The overall sitting time in minutes/day was determined by calculating the weighted mean of the time spent sitting on a weekday and on a weekend day with the following formula:

$$\text{Overall time spent sitting} = ((\text{time spent sitting on a weekday}^{*}5) + (\text{time spent sitting on a weekend day}^{*}2))/7. \quad (1)$$

Frailty

Frailty was assessed by serum concentrations of the inflammatory biomarkers C-reactive protein (CRP) and leukocyte count. Two blood samples were obtained: the first tube without anticoagulant, for the determination of serum CRP concentration, and the second with ethylenediamine tetraacetic acid for the overall leukocyte count.

Serum CRP concentrations were measured by an immunoturbidimetry method using a Cobas Integra 400-Plus (Roche Diagnostics, Basel, Switzerland), and the overall white blood cell count with XE2100-D equipment (Roche Diagnostics). The cut-off values for frailty were CRP>2.6 mg/dL (Puzianowska-Kuźnicka et al., 2016) and white cell count >9290 mm^3 (Bovill et al., 1996), which corresponded to the 4th quartile. If both values were abnormal according to these criteria, the participant was classified as frail, whereas if one or both were normal they were classified as non-frail.

Data Analysis

Data were double entered on a Microsoft Office 2007 Excel spreadsheet. Statistical analyses were performed with the Statistical Package for Social Sciences software (SPSS), version 20.0, and Medcalc, version 11.4.4. The c^2-test was used to compare social and economic variables and functional disability according to the presence of frailty. Cut-points for time spent sitting and their predictive power for presence of frailty were identified by receiver operating characteristic curves (ROC) as well as their sensitivity and specificity values. The larger the area under the ROC curve, the greater the power of cut-points for identifying presence of frailty. The lower limit of the area under of the ROC for accepting the cut-points as predictive of frailty was set at 0.60 (Schisterman et al., 2001) with a confidence interval (CI) of 95%. Cut-points for time spent sitting as a predictor of frailty were determined after calculating sensitivity and specificity values. The significance level was set at 5% ($p \leq 0.05$).

Ethical Considerations

This study was in accordance with the Helsinki declaration and ethical principles of the Resolution No. 466 of December 12, 2012 of the National Health Council from Brazil. The study protocol was approved by the Ethics Committee in Research with Human Beings of the Federal University of Triângulo Mineiro (Protocol number No. 2511/2012).

RESULTS

Of the 168 participants, 57.1% ($n = 96$) were male and 64.3% (n = 108) between 60 and 69 years old. Most participants were married or living with partners (61.3%; $n = 103$), not living alone (84.5%; $n = 142$), and had monthly incomes of less than one minimum wage (65.5%; $n = 110$). Regarding their functional capacity, 6.0% ($n = 10$) were scored as dependent for BADL, whereas 65.5% ($n = 110$) were scored as dependent for IADL (**Table 1**).

The prevalence of frailty was 15.5%, being more frequent in men and in those who were dependent for BADL (**Table 1**). The median time spent sitting was 231 min/day for men and 223 min/day for women. The time spent sitting was confirmed as a discriminator of frailty, with areas under the ROC > 0.60.

The areas under the ROC curve were 0.61 for men (CI: 0.51−0.71) and 0.62 for women (CI: 0.50−0.73). **Figure 1** shows the sensitivity and specificity values associated with the aforementioned areas under the ROC. The cut-points for time spent sitting as a predictor of frailty were >257 min/day and >330 min/day for men and women, respectively.

TABLE 1 | Distribution of social and economic variables, functional disability, and sedentary behavior in the sample of older inpatients.

Variable	Overall		Not frail		Frail		p*
	%	n	%	n	%	n	
GENDER							
Male	57.1	96	52.8	75	80.8	21	0.008
Female	42.9	72	47.2	67	19.2	5	
AGE GROUP							
60–69 years	64.3	108	66.9	95	50.0	13	0.147
70–79 years	30.4	51	28.9	41	38.5	10	
80 years or more	5.4	9	4.2	6	11.5	3	
MARITAL STATUS							
Single	3.0	5	3.5	5	0.0	0	0.487
Married or living with spouse or partner	61.3	103	59.9	85	69.2	18	
Widowed or separated, divorced	35.7	60	36.6	52	30.8	8	
LIVING ARRANGEMENT							
Alone	15.5	26	14.8	21	19.2	5	0.565
Accompanied	84.5	142	85.2	121	80.8	21	
INCOME							
>1 min wage	34.5	58	33.1	47	42.3	11	0.364
<1 min wage	65.5	110	66.9	95	57.7	15	
BASIC ACTIVITIES OF DAILY LIVING							
Independent	94.0	158	95.8	136	84.6	22	0.027
Dependent	6.0	10	4.2	6	15.4	4	
INSTRUMENTAL ACTIVITIES OF DAILY LIVING							
Independent	34.5	58	36.6	52	23.1	6	0.182
Dependent	65.5	110	63.4	90	76.9	20	
SEDENTARY BEHAVIOR							
<240 min/day	58.3	98	62.0	88	38.5	10	0.025
≥240 min/day	41.7	70	38.0	54	61.5	16	

χ2-test. One minimum salary/month = $260.00.

DISCUSSION

This study aimed to identify the predictive power of time spent sitting as a discriminator of frailty in hospitalized older adults. Previous studies have highlighted that prolonged sitting time is associated with greater vulnerability to adverse health outcomes in older persons; these include metabolic syndrome (Gardiner et al., 2011), reduced muscle strength (Hamer and Stamatakis, 2013), excessive body weight (Gómez-Cabello et al., 2012), and increased risk of mortality from all causes (Pavey et al., 2015; Lee, 2016). However, little is known concerning the association between sedentary behavior and frailty (Blodgett et al., 2015; da Silva Coqueiro et al., 2016).

In this study, a greater proportion of men were considered frail in comparison to women. One possible explanation would be a greater engagement in light intensity physical activities by older women compared to men. Sedentary behavior is usually replaced with light intensity physical activity (Buman et al., 2010). In this regard, women tend to perform more domestic activities

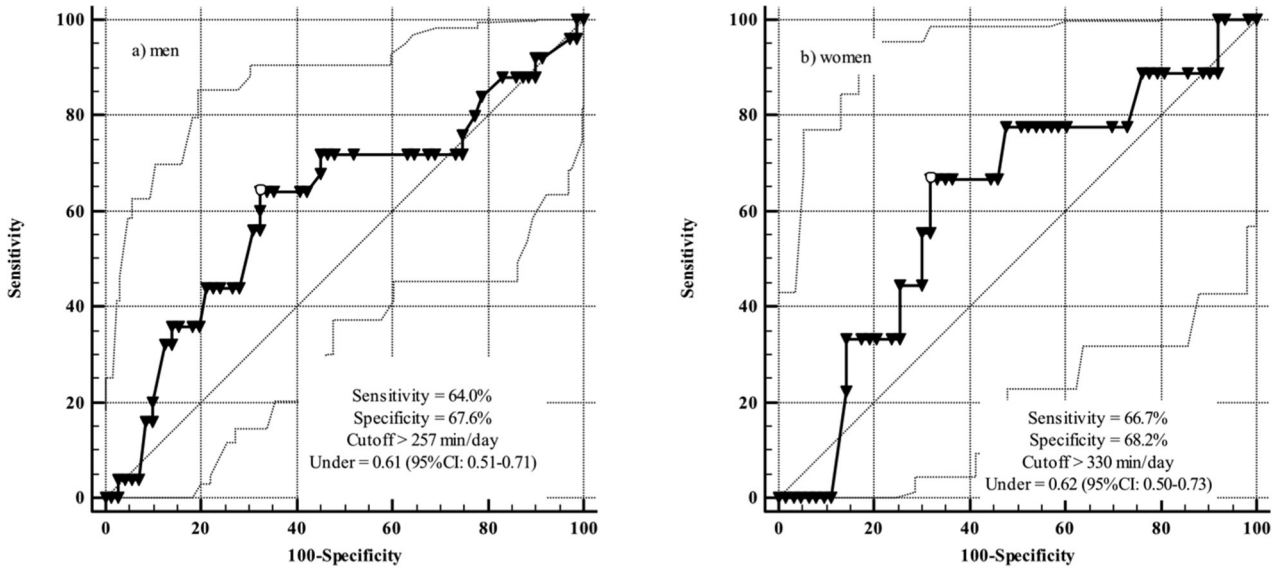

FIGURE 1 | Sitting time cut-points for discriminating frailty in older adults.

than men, thus presenting less exposure to sedentary behavior (Murphy et al., 2013).

This study determined frailty status based on simultaneous abnormalities in two inflammatory biomarkers, namely serum concentrations of CRP and leukocyte count (Li et al., 2011). These biomarkers have been associated with frailty and also with morbidity and mortality in older adults (Willems et al., 2010; Kim et al., 2013; Salazar et al., 2014). The association between time spent in sedentary behavior and increased CRP concentrations and leukocyte counts identified in large population studies (Healy et al., 2011; Pinto Pereira et al., 2012; León-Latre et al., 2014) has received considerable attention in recent years. A study with Americans found that sedentary behavior was associated with limited mobility and that participants with reduced mobility had higher CRP concentrations and leukocyte counts than those with unrestricted mobility (Loprinzi, 2013). A linear association of time spent sitting at work with CRP concentration and leukocyte count has been reported in a study with Spanish workers (León-Latre et al., 2014). It has also been shown that in older persons from UK, longer television viewing times were associated with higher CRP concentrations; this association remains significant even after controlling for confounding variables (Hamer et al., 2013). Similar findings have been reported in older American individuals (Gennuso et al., 2013).

The relationship between sedentary behavior and dysfunction of the immune system might be explained by the reduced muscle contraction, which can result in increased muscle glucose and decreased insulin sensitivity (Charansonney and Després, 2010; Charansonney, 2011). Sparing glucose is then metabolized by the liver into fat and stored in central adipocytes (Meneguci et al., 2015). Adipose tissue, in turn, releases a variety of synthesized proteins termed adipokines (Charansonney, 2011), among which, resistin is positively correlated with the immune and inflammatory system (Kunnari et al., 2006). Increases in resistin concentrations induce increases in leukocyte counts and concentrations of C-reactive protein (Kunnari et al., 2006).

Although, participants from this study were medical or surgical clinic inpatients of a university hospital, they were all able to walk independently. It is also important to emphasize that participants reported time spent sitting from the period prior to hospitalization, not during hospitalization. Hospitalization can increase the risk of deleterious health effects in older adults (Graf, 2006). Thus, appropriate intervention strategies for interrupting sedentary behavior are necessary in the hospitalization period. Interruption of prolonged periods of sedentary behavior can contribute to reduction of adverse health outcomes (Bailey and Locke, 2015; Júdice et al., 2015). A recent randomized study showed that 2 min of moderate walking for every 20 min spent sitting is associated with a reduction in postprandial blood glucose concentrations (Bailey and Locke, 2015). Interruptions in sedentary behavior have been associated with positive metabolic effects, such as smaller waist circumference and body mass index, as well as lower serum triglyceride and glucose concentrations (Healy et al., 2008). Breaks in sedentary behavior also protect against frailty and are associated with positive changes in concentrations of C-reactive protein (Healy et al., 2011). Additionally, a recent study showed that breaks in sedentary behavior are positively associated with components of physical fitness (Sardinha et al., 2015); and the latter has been directly related to frailty (Fried et al., 2001). In view of this, the cut-points presented in this study may be used to identify those individuals who need special attention for avoiding the frailty syndrome. In addition, the cut-points can be used in interventions as target values for guiding reductions in sedentary behavior in older adults.

The present study has limitations. Diagnosis of frailty was based on only two inflammatory biomarkers, which in hospitalized older adults may be altered for many different reasons, including acute infections. Thus, it is likely that some participants may have been misclassified by the criteria herein adopted for determining frailty status. Another limitation was the lack of an objective method to assess sedentary behavior. The use of an accelerometer would have resulted in more accurate measures of sedentary behavior, as older adults might present difficulties recalling their daily routine. The sampling procedure may also have introduced bias in selecting participants. Ideally, the stratification of participants by reasons for hospital admission as well as type of medications used would have minimized selection bias in this study. Finally, the cross-sectional design of the study is a limitation that precludes conclusions about cause and effect.

In conclusion, this study demonstrated that time spent sitting >257 min/day for men and >330 min/day for women are discriminators for the presence of frailty in older persons. While our results need to be interpreted with caution, they do support that sedentary behavior may be related to frailty in older adults, defined as elevated inflammatory biomarkers in this study. Future studies are needed to examine the relationship between sedentary behavior and frailty in hospitalized older adults. These studies should follow-up participants after hospital discharge in order to identify their susceptibility to adverse events and, at the same time, examine predictive validity of inflammatory biomarkers for frailty.

AUTHOR CONTRIBUTIONS

JV and LR initiated the article and wrote the first draft. ST, JM, EM, MP, FD, DD, and JS helped writing and provided input into subsequent revisions.

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REFERENCES

Abizanda, P., Romero, L., Sánchez-Jurado, P. M., Martínez-Reig, M., Gómez-Arnedo, L., and Alfonso, S. A. (2013). Frailty and mortality, disability and mobility loss in a Spanish cohort of older adults: the FRADEA study. *Maturitas* 74, 54–60. doi: 10.1016/j.maturitas.2012.09.018

Allison, M. A., Jensky, N. E., Marshall, S. J., Bertoni, A. G., and Cushman, M. (2012). Sedentary behavior and adiposity-associated inflammation: the multi-ethnic study of atherosclerosis. *Am. J. Prev. Med.* 42, 8–13. doi: 10.1016/j.amepre.2011.09.023

Bailey, D. P., and Locke, C. D. (2015). Breaking up prolonged sitting with light-intensity walking improves postprandial glycemia, but breaking up sitting with standing does not. *J. Sci. Med. Sports Med. Aust.* 18, 294–298. doi: 10.1016/j.jsams.2014.03.008

Bertolucci, P. H. F., Brucki, S. M. D., Campacci, S. R., and Juliano, Y. (1994). The mini-mental state examination in an outpatient population: influence of literacy. *Arq. Neuropsiquiatr.* 52, 1–7. doi: 10.1590/S0004-282X19940001 00001

Blodgett, J., Theou, O., Kirkland, S., Andreou, P., and Rockwood, K. (2015). The association between sedentary behaviour, moderate-vigorous physical activity and frailty in NHANES cohorts. *Maturitas* 80, 187–191. doi: 10.1016/j.maturitas.2014.11.010

Bovill, E. G., Bild, D. E., Heiss, G., Kuller, L. H., Lee, M. H., Rock, R., et al. (1996). White blood cell counts in persons aged 65 years or more from the cardiovascular health study. Correlations with baseline clinical and demographic characteristics. *Am. J. Epidemiol.* 143, 1107–1115. doi: 10.1093/oxfordjournals.aje.a008687

Buman, M. P., Hekler, E. B., Haskell, W. L., Pruitt, L., Conway, T. L., Cain, K. L., et al. (2010). Objective light-intensity physical activity associations with rated health in older adults. *Am. J. Epidemiol.* 172, 1155–1165. doi: 10.1093/aje/kwq249

Charansonney, O. L. (2011). Physical activity and aging: a life-long story. *Discov. Med.* 12, 177–185.

Charansonney, O. L., and Després, J.-P. (2010). Disease prevention–should we target obesity or sedentary lifestyle? *Nat. Rev. Cardiol.* 7, 468–472. doi: 10.1038/nrcardio.2010.68

Chen, X., Mao, G., and Leng, S. X. (2014). Frailty syndrome: an overview. *Clin. Interv. Aging* 9, 433–441. doi: 10.2147/CIA.S45300

da Silva Coqueiro, R., de Queiroz, B. M., Oliveira, D. S., das Merces, M. C., Oliveira Carneiro, J. A., Pereira, R., et al. (2016). Cross-sectional relationships between sedentary behavior and frailty in older adults. *J. Sports Med. Phys. Fitness* 57, 825–830. doi: 10.23736/S0022-4707.16.06289-7

Emerging Risk Factors Collaboration, Kaptoge, S., Di Angelantonio, E., Lowe, G., Pepys, M. B., Thompson, S. G., et al. (2010). C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet* 375, 132–140. doi: 10.1016/S0140-6736(09)61717-7

Folstein, M. F., Folstein, S. E., and McHugh, P. R. (1975). "Mini-mental state". a practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* 12, 189–198. doi: 10.1016/0022-3956(75)90026-6

Fried, L. P., Tangen, C. M., Walston, J., Newman, A. B., Hirsch, C., Gottdiener, J., et al. (2001). Frailty in older adults: evidence for a phenotype. *J. Gerontol. A Biol. Sci. Med. Sci.* 56, M146–M156. doi: 10.1093/gerona/56. 3.M146

Fried, L. P., Xue, Q.-L., Cappola, A. R., Ferrucci, L., Chaves, P., Varadhan, R., et al. (2009). Nonlinear multisystem physiological dysregulation associated with frailty in older women: implications for etiology and treatment. *J. Gerontol. A Biol. Sci. Med. Sci.* 64, 1049–1057. doi: 10.1093/gerona/glp076

Gale, C. R., Baylis, D., Cooper, C., and Sayer, A. A. (2013). Inflammatory markers and incident frailty in men and women: the english longitudinal study of ageing. *Age Dordr.* 35, 2493–2501. doi: 10.1007/s11357-013-9528-9

Gardiner, P. A., Healy, G. N., Eakin, E. G., Clark, B. K., Dunstan, D. W., Shaw, J. E., et al. (2011). Associations between television viewing time and overall sitting time with the metabolic syndrome in older men and women: the Australian diabetes obesity and lifestyle study. *J. Am. Geriatr. Soc.* 59, 788–796. doi: 10.1111/j.1532-5415.2011.03390.x

Gennuso, K. P., Gangnon, R. E., Matthews, C. E., Thraen-Borowski, K. M., and Colbert, L. H. (2013). Sedentary behavior, physical activity, and markers of health in older adults. *Med. Sci. Sports Exerc.* 45, 1493–1500. doi: 10.1249/MSS.0b013e318288a1e5

Gómez-Cabello, A., Pedrero-Chamizo, R., Olivares, P. R., Hernández-Perera, R., Rodríguez-Marroyo, J. A., Mata, E., et al. (2012). Sitting time increases the

overweight and obesity risk independently of walking time in elderly people from Spain. *Maturitas* 73, 337–343. doi: 10.1016/j.maturitas.2012.09.001

Graf, C. (2006). Functional decline in hospitalized older adults. *Am. J. Nurs.* 106, 58–67. doi: 10.1097/00000446-200601000-00032

Hamer, M., Poole, L., and Messerli-Bürgy, N. (2013). Television viewing, C-reactive protein, and depressive symptoms in older adults. *Brain Behav. Immun.* 33, 29–32. doi: 10.1016/j.bbi.2013.05.001

Hamer, M., and Stamatakis, E. (2013). Screen-based sedentary behavior, physical activity, and muscle strength in the English longitudinal study of ageing. *PLoS ONE* 8:e66222. doi: 10.1371/journal.pone.0066222

Healy, G. N., Dunstan, D. W., Salmon, J., Cerin, E., Shaw, J. E., Zimmet, P. Z., et al. (2008). Breaks in sedentary time: beneficial associations with metabolic risk. *Diabetes Care* 31, 661–666. doi: 10.2337/dc07-2046

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

Júdice, P. B., Silva, A. M., Santos, D. A., Baptista, F., and Sardinha, L. B. (2015). Associations of breaks in sedentary time with abdominal obesity in Portuguese older adults. *Age* 37:23. doi: 10.1007/s11357-015-9760-6

Khandelwal, D., Goel, A., Kumar, U., Gulati, V., Narang, R., and Dey, A. B. (2012). Frailty is associated with longer hospital stay and increased mortality in hospitalized older patients. *J. Nutr. Health Aging* 16, 732–735. doi: 10.1007/s12603-012-0369-5

Kim, K.-I., Lee, J., Heo, N. J., Kim, S., Chin, H. J., Na, K. Y., et al. (2013). Differential white blood cell count and all-cause mortality in the Korean elderly. *Exp. Gerontol.* 48, 103–108. doi: 10.1016/j.exger.2012.11.016

Kunnari, A., Ukkola, O., Päivänsalo, M., and Kesäniemi, Y. A. (2006). High plasma resistin level is associated with enhanced highly sensitive C-reactive protein and leukocytes. *J. Clin. Endocrinol. Metab.* 91, 2755–2760. doi: 10.1210/jc.2005-2115

Lee, P. H. (2016). Examining non-linear associations between accelerometer-measured physical activity, sedentary behavior, and all-cause mortality using segmented cox regression. *Front. Physiol.* 7:272. doi: 10.3389/fphys.2016.00272

León-Latre, M., Moreno-Franco, B., Andrés-Esteban, E. M., Ledesma, M., Laclaustra, M., Alcalde, V., et al. (2014). Sedentary lifestyle and its relation to cardiovascular risk factors, insulin resistance and inflammatory profile. *Rev. Esp. Cardiol.* 67, 449–455. doi: 10.1016/j.recesp.2013.10.017

Li, H., Manwani, B., and Leng, S. X. (2011). Frailty, inflammation, and immunity. *Aging Dis.* 2, 466–473.

Lino, V. T. S., Pereira, S. R. M., Camacho, L. A. B., Filho, R., Telles, S., and Buksman, S. (2008). Cross-cultural adaptation of the independence in activities of daily living index (Katz index). *Cad. Saúde Pública* 24, 103–112. doi: 10.1590/S0102-311X2008000100010

Loprinzi, P. D. (2013). Accelerometer-determined sedentary and physical activity behavior among U.S. older adults: considerations by comorbidity characteristics. *J. Aging Phys. Act.* 22, 432–440. doi: 10.1123/JAPA.2013-0019

Marques-Vidal, P., Schmid, R., Bochud, M., Bastardot, F., von Känel, R., Paccaud, F., et al. (2012). Adipocytokines, hepatic and inflammatory biomarkers and incidence of type 2 diabetes. The CoLaus study. *PLoS ONE* 7:e51768. doi: 10.1371/journal.pone.0051768

Matthews, C. E., Chen, K. Y., Freedson, P. S., Buchowski, M. S., Beech, B. M., Pate, R. R., et al. (2008). Amount of time spent in sedentary behaviors in the United States, 2003–2004. *Am. J. Epidemiol.* 167, 875–881. doi: 10.1093/aje/kwm390

Meneguci, J., Santos, D. A. T., Silva, R. B., Santos, R. G., Sasaki, J. E., Tribess, S., et al. (2015). Sedentary behavior: concept, physiological implications and the assessment procedures. *Motricidade* 11, 160–174. doi: 10.6063/motricidade.3178

Metti, A. L., Yaffe, K., Boudreau, R. M., Simonsick, E. M., Carnahan, R. M., Satterfield, S., et al. (2014). Trajectories of inflammatory markers and cognitive decline over 10 years. *Neurobiol. Aging* 35, 2785–2790. doi: 10.1016/j.neurobiolaging.2014.05.030

Murphy, M. H., Donnelly, P., Breslin, G., Shibli, S., and Nevill, A. M. (2013). Does doing housework 349 keep you healthy? The contribution of domestic physical activity to meeting current 350 recommendations for health. *BMC Public Health* 13:966. doi: 10.1186/1471-2458-13-966

Owen, N., Healy, G. N., Matthews, C. E., and Dunstan, D. W. (2010). Too much sitting: the population health science of sedentary behavior. *Exerc. Sport Sci. Rev.* 38, 105–113. doi: 10.1097/JES.0b013e3181e373a2

Parsons, T. J., Sartini, C., Welsh, P., Sattar, N., Ash, S., Lennon, L. T., et al. (2017). Physical activity, sedentary behavior, and inflammatory and hemostatic markers in men. *Med. Sci. Sports Exerc.* 49, 459–465. doi: 10.1249/MSS.0000000000001113

Pavey, T. G., Peeters, G. G., and Brown, W. J. (2015). Sitting-time and 9-year all-cause mortality in older women. *Br. J. Sports Med.* 49, 95–99. doi: 10.1136/bjsports-2012-091676

Pinto Pereira, S. M., Ki, M., and Power, C. (2012). Sedentary behaviour and biomarkers for cardiovascular disease and diabetes in mid-life: the role of television-viewing and sitting at work. *PLoS ONE* 7:e31132. doi: 10.1371/journal.pone.0031132

Puzianowska-Kuźnicka, M., Owczarz, M., Wieczorowska-Tobis, K., Nadrowski, P., Chudek, J., Slusarczyk, P., et al. (2016). Interleukin-6 and C-reactive protein, successful aging, and mortality: the PolSenior study. *Immun. Ageing* 13:21. doi: 10.1186/s12979-016-0076-x

United Nations Population Fund (2016). *Ageing in the Twenty-First Century | UNFPA - United Nations Population Fund.* Available online at: http://www.unfpa.org/publications/ageing-twenty-first-century (Accessed February 2, 2016).

Rosenberg, D. E., Bull, F. C., Marshall, A. L., Sallis, J. F., and Bauman, A. E. (2008). Assessment of sedentary behavior with the international physical activity questionnaire. *J. Phys. Act. Health* 5(Suppl. 1), S30–S44. doi: 10.1123/jpah.5.s1.s30

Salazar, J., Martínez, M. S., Chávez, M., Toledo, A., Añez, R., Torres, Y., et al. (2014). C-reactive protein: clinical and epidemiological perspectives. *Cardiol. Res. Pract.* 2014:e605810. doi: 10.1155/2014/605810

Santos, R. L., and Virtuoso-Júnior, J. S. (2008). Reliability of the Brazilian version of the scale of instrumental activities of daily living. *Rev. Bras. Promoç. Saúde* 2008, 290–296. doi: 10.5020/18061230.2008.p290

Santos, D. A., Virtuoso, J. S. Jr., Meneguci, J., Sasaki, J. E., and Tribess, S. (2017). Combined associations of physical activity and sedentary behavior with depressive symptoms in older adults. *Issues Ment. Health Nurs.* 38, 272–276. doi: 10.1080/01612840.2016.1263695

Sardinha, L. B., Santos, D. A., Silva, A. M., Baptista, F., and Owen, N. (2015). Breaking-up sedentary time is associated with physical function in older adults. *J. Gerontol. A Biol. Sci. Med. Sci.* 70, 119–124. doi: 10.1093/gerona/glu193

Schisterman, E. F., Faraggi, D., Reiser, B., and Trevisan, M. (2001). Statistical inference for the area under the receiver operating characteristic curve in the presence of random measurement error. *Am. J. Epidemiol.* 154, 174–179. doi: 10.1093/aje/154.2.174

Tribess, S., Virtuoso-Júnior, J. S., and Oliveira, R. J. (2012). Physical activity as a predictor of absence of frailty in the elderly. *Rev. Assoc. Méd. Bras.* 58, 341–347. doi: 10.1590/S0104-42302012000300015

Willems, J. M., Trompet, S., Blauw, G. J., Westendorp, R. G. J., and de Craen, A. J. M. (2010). White blood cell count and C-reactive protein are independent predictors of mortality in the oldest old. *J. Gerontol. A Biol. Sci. Med. Sci.* 65, 764–768. doi: 10.1093/gerona/glq004

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Posture Allocation Revisited: Breaking the Sedentary Threshold of Energy Expenditure for Obesity Management

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There is increasing recognition that low-intensity physical activities of daily life play an important role in achieving energy balance and that their societal erosion through substitution with sedentary (mostly sitting) behaviors, whether occupational or for leisure, impact importantly on the obesity epidemic. This has generated considerable interest for better monitoring, characterizing, and promoting countermeasures to sedentariness through a plethora of low-level physical activities (e.g., active workstations, standing desks, sitting breaks), amid the contention that altering posture allocation (lying, sitting, standing) can modify energy expenditure to impact upon body weight regulation and health. In addressing this contention, this paper first revisits the past and more recent literature on postural energetics, with particular emphasis on potential determinants of the large inter-individual variability in the energy cost of standing and the impact of posture on fat oxidation. It subsequently analyses the available data pertaining to various strategies by which posture allocations, coupled with light physical activity, may increase energy expenditure beyond the sedentary threshold, and their relevance as potential targets for obesity management.

Keywords: energy expenditure, posture, obesity, spontaneous physical activity, thermogenesis

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Evolutionary scientists still are not sure why our ancestors became bipedal, but along with the evolution of the major traits and behaviors that define humans (such as large brains, language, art, technology), walking upright - and the performance of a plethora of activities while maintaining standing posture—is a most fundamental human characteristic (Wayman, 2012). Yet, Modern Man (and Woman) is sedentary for much larger proportions of the day than ever before (Ng and Popkin, 2012). Indeed, a modern lifestyle involves a large variety of seated activities, whether they be occupational or for leisure. The rise in the prevalence of such activities has led to the notion of a major shift in posture allocation from standing in favor of sitting on a population basis. With this belief has come a myriad of correlative analyses showing a positive relationship between sitting time and cardiometabolic disease risk (Henson et al., 2016; Young et al., 2016; Tigbe et al., 2017). In addition, studies have now shown that obese individuals spend significantly more time sitting and less time standing than their lean counterparts (Levine et al., 2005; Johannsen et al., 2008). This therefore begs the question as to whether or not modifying posture allocation could sufficiently alter energy expenditure (EE) in order to impact body weight regulation over time; an idea that requires us to revisit the literature concerning postural energetics.

HISTORICAL INTEREST IN POSTURE ALLOCATION

Whilst, the interest in posture allocation as a potential target in obesity prevention has increased over recent years, interest in quantifying its energetic cost originated in an entirely different scientific and social context.

During the first half of the twentieth century there was considerable attention on improving guidelines of energy requirements at the individual and population level; with such information required to provide aid and assistance to developing and war-torn countries as well as to optimize military performance. A major hurdle in estimating energy requirements was the need to establish a database of the energy cost of common, standardized physical activities. The breakthrough came in the 1940s with the development of the Kofranyi-Michaelis or Max Planck respirometer (Passmore and Durnin, 1955). Despite being comparatively heavy compared to modern devices, this respirometer allowed researchers for the first time to measure EE by indirect calorimetry during a host of daily-life activities, in the field, and in very diverse populations (for example: Passmore et al., 1952; Passmore and Durnin, 1955).

It is noticeable from these early studies that considerable emphasis was put on variability in the energy cost of standardized low-level physical activities both between and within individuals—an important aspect of human energetics which has been largely overlooked in more recent studies. For example, the classic studies of Passmore et al. (1952) and Edholm et al. (1955) both reported large inter-individual variability in the energy cost for performing the same activity, with EE during standing compared to sitting increasing by anything from ∼0% to >30% in individuals from relatively homogenous study groups. In addition to this inter-individual variability, Miller (1982) reported intra-individual variability in the energy cost of sitting and standing in six individuals to range from 5 to 13% and 4 to 7%, respectively.

It was not until the demonstration by Zurlo et al. (1992) of an inverse correlation between spontaneous physical activity (SPA) and body weight gain in Pima Indians that research interest in low-level physical activities and sedentary behaviors in the context of obesity development really began; SPA being a term that encapsulates posture maintenance, fidgeting and other essentially subconscious low-level movement (Dulloo et al., 2012). However, the watershed moment occurred at the turn of this century, with the observation by Levine et al. (1999) of an increase in non-exercise activity thermogenesis (NEAT) in individuals showing a relative resistance to fat gain during overfeeding; NEAT being estimated by subtraction of basal and postprandial EE from total daily EE. Whilst posture allocation is just one component of SPA and NEAT (**Figure 1**), two subsequent studies (Levine et al., 2005; Johannsen et al., 2008), each involving 10 lean and 10 obese individuals, have provided evidence of a difference in posture allocation between these two population groups—therefore highlighting a new potential target for obesity treatment and prevention.

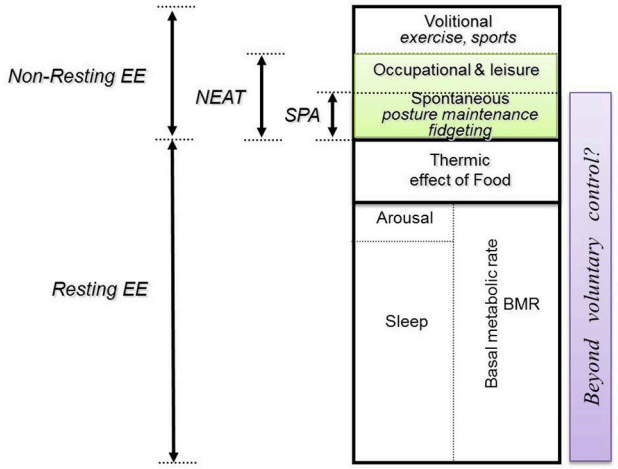

FIGURE 1 | Compartments of daily energy expenditure. Daily energy expenditure (EE) can be divided into resting and non-resting EE. Non-resting EE can further be divided into (i) volitional EE related to structured physical activities, such as sports and exercise, which are usually of moderate-to-vigorous intensity; and (ii) non-exercise activities (NEAT). These non-exercise activities include both those under voluntary (conscious) control associated with occupation and leisure, and those that are involuntary (subconscious) in nature. This sub-compartment of spontaneous physical activity (SPA) includes low-level physical activities such as postural maintenance and fidgeting. Adapted from Dulloo et al. (2012).

ENERGY COST OF POSTURE MAINTENANCE

As a result of these two observational studies, it has been suggested that if obese individuals were to match the posture allocation of lean individuals—i.e., by re-allocating 2–2.5 h of sitting time to standing per day—then daily EE would be increased by ∼300–350 kcal or ∼10–20% (Levine et al., 2005; Johannsen et al., 2008); potentially resulting in a weight loss of ∼15 kg over a year (Levine et al., 2005). These calculations are based on the following three key assumptions:

1. That standing is not a sedentary behavior, and as such its energetic cost is more than 1.5 times the energy cost of sitting at rest (i.e., >1.5 METs);
2. That the energy cost of standing is constant across the entire standing period regardless of duration; and,
3. That the energy cost of standing is the same or similar between individuals.

However, our analysis of the available literature reveals a number of challenges to these assumptions; these are elaborated below.

Energy Cost of Steady-State Standing Posture Maintenance

Since 1952, there have been just over 30 studies presenting measurements of both the energetic cost of standing and sitting, comprising of >60 experimental groups (**Table 1**; Passmore et al., 1952; Donald and Davidson, 1954; Edholm et al., 1955; Garry et al., 1955; Durnin and Passmore, 1967; Banerjee et al.,

TABLE 1 | Table of existing studies published from 1952 to 2017, specifying the values of energy expenditure during sitting vs. standing.

Study	Ethnicity (if stated)	*n* (gender)	Mean EE		ΔEE from sitting (%)	Length of measurement (if stated)
			Sitting	Standing		
Bandyopadhyay and Chattopadhyay, 1980	Indian	11♂	1.01 kcal/min	1.07 kcal/min	5.9	–
Banerjee et al., 1971	Asian	37♀	0.90 kcal/min	1.02 kcal/min	13.3	–
Banerjee and Saha, 1972	–	10♂	1.03 kcal/min	1.39 kcal/min	35	5 min
		10♀	0.68 kcal/min	0.89 kcal/min	30.9	∼10 min
Beers et al., 2008	–	12♂	80 kcal/h*	86 kcal/h*	7.5	Last 15 min of 20 min of activity
		12♀	55 kcal/h*	57 kcal/h*	3.6	Last 15 min of 20 min of activity
Bleiberg et al., 1980	African	27♀	1.29 kcal/min	1.35 kcal/min	4.7	∼10 min
Brun et al., 1981	African	29–33♂	1.38 kcal/min	1.44 kcal/min	4.3	∼10 min
Buckley et al., 2014	–	8♀, 2♂	1.49 kcal/min	2.32 kcal/min	56	210 mins!
Cole and Ogbe, 1987	African	7–10♂	5.55 kJ/min	11.51 kJ/min	107.4	10–15 min
Creasy et al., 2016	–	7♂, 11♀	1.31 kcal/min	1.43 kcal/min	9.2	15 min
de Guzman et al., 1978	Filipino	10♂	0.026 kcal/kg/min	0.029 kcal/kg/min	11.5	8–10 min
		10♀	0.026 kcal/kg/min	0.027 kcal/kg/min	3.8	
de Guzman et al., 1979	Filipino	25♀	0.022 kcal/kg/min	0.024 kcal/kg/min	9.1	10 min
		14♂	0.024 kcal/kg/min	0.024 kcal/kg/min	0	
de Guzman et al., 1984	Filipino	10♂	0.027 kcal/kg/min	0.027 kcal/kg/min	0	8–10 min
		10♀	0.027 kcal/kg/min	0.029 kcal/kg/min	7.4	
Donald and Davidson, 1954	–	13♂	0.25 L/min	0.27 L/min	8	–
Durnin and Passmore, 1967	–	♂	1.28 kcal/kg weight/h	1.61 kcal/kg weight/h	25.8	–
		♀	1.25 kcal/kg weight/h	1.49 kcal/kg weight/h	19.2	
Edholm et al., 1955	–	12♂	1.60 kcal/min	1.82 kcal/min	13.8	–
Edmundson and Edmundson, 1988	Indian	20–24♂	0.026 kcal/kg/min	0.03 kcal/kg/min	15.4	
Fountaine et al., 2016	–	10♂, 8♀	1.69 kcal/min	1.86 kcal/min	10	20 min
Garry et al., 1955	Scottish	10♂	1.6 kcal/min	1.8 kcal/min	12.5	
Gibbs et al., 2017	Mixed	9♂, 9♀	1.18 kcal/min	1.32 kcal/min	11.5	60 min
Geissler et al., 1981	Iranian	32♀/18♀	1.14 kcal/min	1.24 kcal/min	8.8	10–15 min
Geissler and Aldouri, 1985	European	15♂	1.42 kcal/min	1.59 kcal/min	12	10 min
	Asian	15♂	1.31 kcal/min	1.42 kcal/min	8.4	
	African	15♂	1.29 kcal/min	1.49 kcal/min	15.5	
Judice et al., 2016	–	25♂	1.14 kcal/min	1.23 kcal/min	∼8	10 min
		25♀	0.88 kcal/min	0.92 kcal/min	∼6.0	
Kanade et al., 2001	Indian	24♂	5.00 kJ/min	5.74 kJ/min	14.8	
		40♀	4.03 kJ/min	4.35 kJ/min	7.9	
Katzmarzyk et al., 1996	Siberia	30♂	6.67 kJ/min	7.09 kJ/min	6.3	3 min
		14♀	4.97 kJ/min	5.35 kJ/min	7.6	

(Continued)

TABLE 1 | Continued

Study	Ethnicity (if stated)	*n* (gender)	Mean EE		ΔEE from sitting (%)	Length of measurement (if stated)
			Sitting	Standing		
	Highland Ecuador	17♂	6.74 kJ/min	6.84 kJ/min	1.5	
		12♀	5.60 kJ/min	4.94 kJ/min	−11.8	
	Coastal Ecuador	5♂	7.42 kJ/min	7.00 kJ/min	−5.7	
		5♀	6.22 kJ/min	5.64 kJ/min	−9.3	
Lawrence et al., 1985	African	113♀	1.25 kcal/min	1.26 kcal/min	0.8	Last 5 mins of 8 min activity
Levine and Miller, 2007	–	14♀, 1♂	1.20 kcal/min	1.37 kcal/min	14.2	20 min
Levine et al., 2000	White	7♂, 17♀	5.6 kJ/min	6.1 kJ/min	8.9	20 min
Li and Yan, 1991	Chinese	319♂	0.839 kcal/m^2/min	0.886 kcal/m^2/min	5.6	Unknown
		287♀	0.818 kcal/m^2/min	0.846 kcal/m^2/min	3.4	
Malhotra et al., 1976	Indian	24♂	4.85 kJ/min	5.31 kJ/min	9.5	Unknown
McAlpine et al., 2007	–	11♂, 8♀	1.47 kcal/min	1.62 kcal/min	10.2	20 min
Norgan et al., 1974	New Guinean (Kaul)	40–41♂	1.23 kcal/min	1.32 kcal/min	7.3	Unknown
		41♀	1.08 kcal/min	1.19 kcal/min	10.2	
	New Guinean (Lufa)	32–34♂	1.36 kcal/min	1.47 kcal/min	8.1	
		29–30♀	1.21 kcal/min	1.29 kcal/min	6.6	
Passmore et al., 1952	–	5 ♂	1.82 kcal/min	2.02 kcal/min	10.9	6–15 min
Rao et al., 2008	Indian	4♀/3♀	3.66 kJ/min	4.10 kJ/min	12	6 min
Reiff et al., 2012	–	10♂, 10♀	1.02 kcal/min	1.36 kcal/min	33.3	last 30 min of 45 min activity
Speck and Schmitz, 2011	Indian	5♂, 8♀	1.26 kcal/min	1.287 kcal/min	2.1	6 min
Steeves et al., 2012	–	11♂, 12♀	86 kcal/h*	93 kcal/h*	8.1	5 min
Strickland and Ulijaszek, 1990	Gurkha	11♂	6.3 kJ/min	6.7 kJ/min	6.3	10 min
	British	11♂	5.9 kJ/min	6.8 kJ/min	15.3	
Sujatha et al., 2000	Indian	98♀	3.15 kJ/min	3.430 kJ/min	8.8	6 min
Viteri et al., 1971	Central American	18–19♂	1.21 kcal/min	1.28 kcal/min	5.8	Last 10 min of 15–20 min activity
Whybrow et al., 2013	–	7♂, 7♀	6.1 kJ/min	6.9 kJ/min	13.1	5 min

*Information obtained from original reference or compilations of Passmore and Durnin (1955) and Vaz et al. (2005). *Estimated from graphic.*

1971; Viteri et al., 1971; Banerjee and Saha, 1972; Norgan et al., 1974; Malhotra et al., 1976; de Guzman et al., 1978, 1979, 1984; Bandyopadhyay and Chattopadhyay, 1980; Bleiberg et al., 1980; Brun et al., 1981; Geissler et al., 1981; Geissler and Aldouri, 1985; Lawrence et al., 1985; Cole and Ogbe, 1987; Edmundson and Edmundson, 1988; Strickland and Ulijaszek, 1990; Li and Yan, 1991; Katzmarzyk et al., 1996; Levine et al., 2000; Sujatha et al., 2000; Kanade et al., 2001; Levine and Miller, 2007; McAlpine et al., 2007; Beers et al., 2008; Rao et al., 2008; Speck and Schmitz, 2011; Reiff et al., 2012; Steeves et al., 2012; Whybrow et al., 2013; Buckley et al., 2014; Creasy et al., 2016; Fountaine et al., 2016; Judice et al., 2016; Gibbs et al., 2017).

By comparing these values of standing relative to sitting (**Figure 2**), we can observe considerable variability amongst these studies, with the energy cost of standing ranging from a 10% decrease in EE during standing relative to sitting (measured in females of two subsistence-level populations in Ecuador; Katzmarzyk et al., 1996) to increases in EE of >30% above sitting values (with one study observing a mean increase of >100%; Cole and Ogbe, 1987); with an overall mean increase in EE during standing posture maintenance of 11.6%, and a median increase of 8.6%, above sitting EE. It is important to note that these studies differed considerably in terms of methodology, their level of standardization, presentation of results (i.e., integrated mean over entire standing period vs. average of last 5 min) and their definition of standing itself (i.e., with or without fidgeting, length of standing period), thus making direct comparison between these studies difficult. However, regardless of these inconsistencies, it appears that the true energy cost of steady-state standing posture maintenance is considerably lower than the commonly described sedentary threshold of 1.5 METs (Sedentary Behaviour Research Network, 2012).

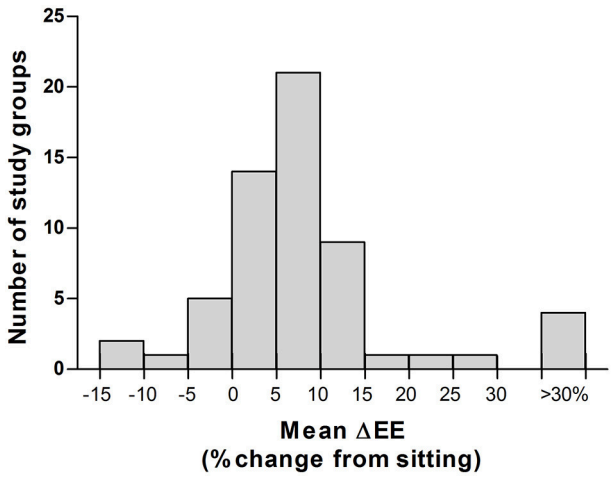

FIGURE 2 | Inter-study variability in the energy cost of standing vs. sitting. Histogram of all energy cost of standing vs sitting reported by all studies published 1952–2017 ($n = 32$ studies, 59 study groups). Mean ± SEM: 11.6 ± 2.1%; Median: 8.4%; Range −11.8% to +107.4%. Please refer to **Table 1** for further details of individual studies.

Time-Course of Energy Cost of Standing Posture Maintenance

Investigations of the energy cost of standing posture maintenance almost exclusively present the EE during standing (and therefore the calculation of its energy cost) as an integrated mean across the entire standing period, regardless of its duration. However, there seems to be little evidence to support the notion that EE is indeed constant during standing. In fact studies conducted in our laboratory using minute-by-minute EE monitoring have shown that the majority of individuals demonstrate an initial increase in EE (most likely due to the postural transition) and then rather quickly (within 5 min) decrease their EE back to sitting values (Miles-Chan et al., 2013, 2017; Monnard and Miles-Chan, 2017; **Figure 3**). The rise in EE during postural transitioning is expected given the large amount of muscular contraction required to move the body weight from, for example, a sitting to standing position; but it is perhaps inclusion of this transitional period of EE, rather than consideration of only the steady-state period of posture maintenance, that has led to some of the large discrepancies in calculated energy costs.

The exact mechanisms by which the majority of individuals are able to maintain a standing posture at the same energetic cost as sitting remain to be elucidated, although it appears somewhat analogous to the adaptation in energy cost observed during other physical activities. For example, a large volume of research now supports the notion that locomotion is quickly and precisely optimized in order to minimize its energetic cost. Such optimization may occur in response factors like pregnancy (Poppitt et al., 1993), load-carrying (Maloiy et al., 1986; Jones et al., 1987; Lloyd et al., 2010), or exogenous gait disturbance (Koller et al., 2015; Selinger et al., 2015), and is not unique to humans—with locomotive optimization demonstrated across a large number of species (Tucker, 1970; Alexander, 1989). Also, importantly when considering time-course of relatively short physical activities such as standing maintenance, recent studies involving the perturbation of human gait have shown that adaptations that minimize energetic cost of locomotion occur within minutes (Selinger et al., 2015); i.e., within the timescale over which standing is usually performed.

Variability in Energy Cost of Standing Posture Maintenance

As discussed earlier, there is considerable variability in the energy cost of posture maintenance in healthy individuals. Whilst a certain amount of variability may be accounted for by differences in standardization and methodology, large levels of within-study variability (i.e., amongst individuals measured under identical experimental conditions) strongly suggests a large degree of true biological variability. Indeed the inter-individual variability shown in the early studies of Edholm et al. (1955) and Passmore et al. (1952), is almost identical to that which we have recently observed in our laboratory using contemporary equipment (Miles-Chan et al., 2013, 2017; Monnard and Miles-Chan, 2017)—i.e., ranging from individuals who showed no increase in EE during steady-state standing relative to sitting ("energy savers") to those who showed sustained increases in EE

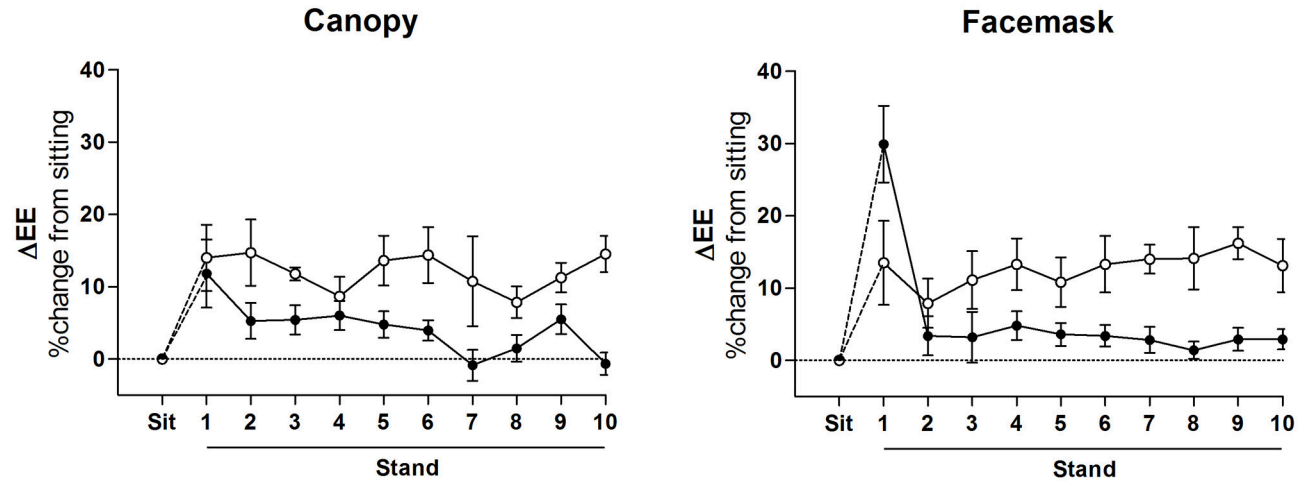

FIGURE 3 | Time course of energy cost of standing posture maintenance. Change in energy expenditure (EE) measured during 10 min of steady-state standing (i.e., after postural transition) as a percentage of resting, sitting EE. Mean ± SEM. **Left panel**: measurements obtained using posture-adapted ventilated canopy indirect calorimetry (Deltatrac II, Datex-Ohmeda, Instrumentarium Corp, Helsinki, Finland) (Miles-Chan et al., 2013); **Right panel**: measurements obtained using facemask indirect calorimetry (Cosmed Quark, Cosmed srl, Rome, Italy) (Miles-Chan et al., 2017); Closed circles: represent "Energy-savers," i.e., those who showed little or no change in EE (a rise in EE of <5%) during 10 min standing period relative to sitting, and also those who increased EE (a rise in EE of >5%) during first 5 min of the 10 min standing period relative to sitting but subsequently decreased EE (by >30% of the rise) during the second 5 min of this standing period; $n = 18$ (**left panel**) and 29 (**right panel**); *Open circles:* represent "Energy-spenders," i.e., those who increased EE (a rise in EE of >5%) during first 5 min of the 10 min standing period relative to sitting, and maintained an elevated EE throughout the entire 10 min standing period (drop in EE during second 5 min <30% of the rise in EE during first 5 of standing period); $n = 4$ (**left panel**) and 7 (**right panel**).

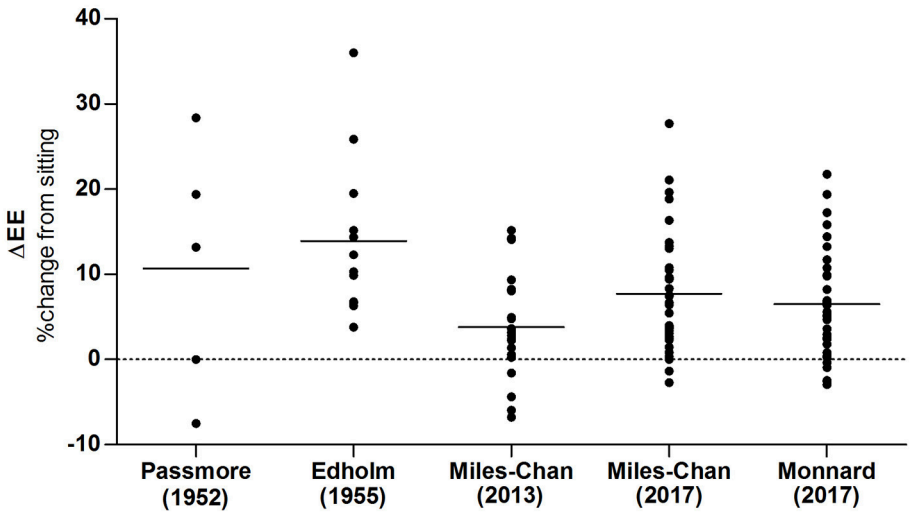

FIGURE 4 | Inter-individual (intra-study) variability in the energy cost of standing vs. sitting. Scatter plot of variability in the energy cost of standing vs. sitting between individual subjects in the studies of Edholm et al. (1955), Passmore et al. (1952), Miles-Chan et al. (2013), Miles-Chan et al. (2017), and Monnard and Miles-Chan (2017). Each point represents an individual study participant; horizontal lines indicate median and interquartile range.

of 25–35% ("energy spenders;" **Figure 4**). This is in sharp contrast to a relatively low intra-individual coefficient of variation in the energy cost of standing—reported by Miller to range from 4 to 7% (Miller, 1982), and the intra-individual coefficient of variation in EE during standing within our own laboratory to range from 0 to 7% (Miles-Chan et al., 2017). Nevertheless, using standardized experimental conditions, we have yet to observe any difference in terms of sex (Miles-Chan et al., 2013, 2017) or ethnic group (Monnard and Miles-Chan, 2017) between these two EE phenotypes. Furthermore, given that during standing posture maintenance individuals appear to differ in terms of the degree and pattern of weight-shifting behavior (i.e., the redistribution of body-weight from one foot to the other), we have recently investigated if an overt difference in terms of spontaneous weight-shifting behavior could be detected between these two EE phenotypes (Miles-Chan et al., 2017). However, no

such difference was apparent amongst the healthy young adults who participated in the study. It therefore remains unclear as to whether or not this apparent adaptive failure resides in a physiological difference between "energy spenders" and "energy savers," is related to psychological factors (for example, a strong preference for one posture over the other), or a combination of the two.

Moreover, given earlier demonstrations that the energy cost of physical activities such as walking may vary by 46% depending on energy intake (Apfelbaum et al., 1971), further investigations are warranted to assess the energy cost of standing posture maintenance in the postprandial phase, particularly given that the majority of the day is spent in the absorptive state. But perhaps most importantly, given the postulation that matching posture allocation of obese individuals to that of lean may significantly increase EE, it is of fundamental importance to comprehensively establish whether or not the energy cost of standing posture maintenance is altered in the obese state. Indeed, body geometry, and more specifically the distribution of adipose mass, has been shown to influence postural stability (Corbeil et al., 2001; Gilleard and Smith, 2007; Blaszczyk et al., 2009; Singh et al., 2009; Cruz-Gomez et al., 2011; Villarrasa-Sapina et al., 2016). With increased abdominal obesity shown to increase postural sway, and presumably increased muscle work being required to maintain balance, one might hypothesize that the energy cost of postural maintenance may be elevated in individuals with abdominal obesity or certain body morphologies, although this remains to be tested.

ENERGY COST VS. CARDIOVASCULAR RESPONSE

When considering the assessment of physical activity under free-living conditions, heart rate has traditionally been used as an objective, proxy measurement for EE. Indeed, while the recent advances in accelerometric devices are now allowing more accurate detection of body posture, commercially-available heart rate-based activity monitors are now widely used by the general public to monitor physical activity levels. However, it is important to note that although the relationship between these two variables is approximately linear during traditional, moderate-to-vigorous physical activity (Spurr et al., 1988), the same is not true of low-intensity physical activities (Ceesay et al., 1989). In order to maintain blood pressure during orthostasis, the autonomic nervous system works to increase both vasoconstriction in the extremities and heart rate. This increased heart rate persists across the standing period, and can occur in the absence of any obvious change in EE; as consistently observed in our recent studies where all individuals showed comparable increases in heart rate during steady-state standing (∼15 beats per minute), despite responses in terms of EE ranging from little or no change compared to sitting to an increase of ∼25% (Miles-Chan et al., 2013, 2017). Similarly, despite no detectable change in EE, we have also shown a significant difference in heart rate during sitting compared to supine ∼7 beat per minute (Miles-Chan et al., 2014). Further, dissociation between the heart rate and EE response to altered body posture can be demonstrated in our preliminary study in healthy young men, performed using a clinical tilting table. With the body weight supported entirely by the tilting table, and thereby minimizing any muscular work required for posture transition and maintenance, we were able to observe a "dose-response" relationship between tilt angle (from supine to 60°) and heart rate, but no change in EE (**Figure 5**). Studies reporting values of EE estimated from heart rate in situations where postural allocation is not controlled (i.e., free-living conditions) should therefore be interpreted with considerable caution.

BREAKING THE SEDENTARY THRESHOLD

The energy cost of steady-state posture maintenance is relatively small (<35% above sitting). Bodily movements, e.g., displacement of the body (i.e., at least one step to be taken), are needed to increase EE beyond 1.5 times resting metabolic rate (Miles-Chan et al., 2017)—the level of EE commonly defined as the cut-off between sedentary and physical activities (Sedentary Behaviour Research Network, 2012). But there are two aspects of posture allocation that could potentially be exploited to increase EE, as described below.

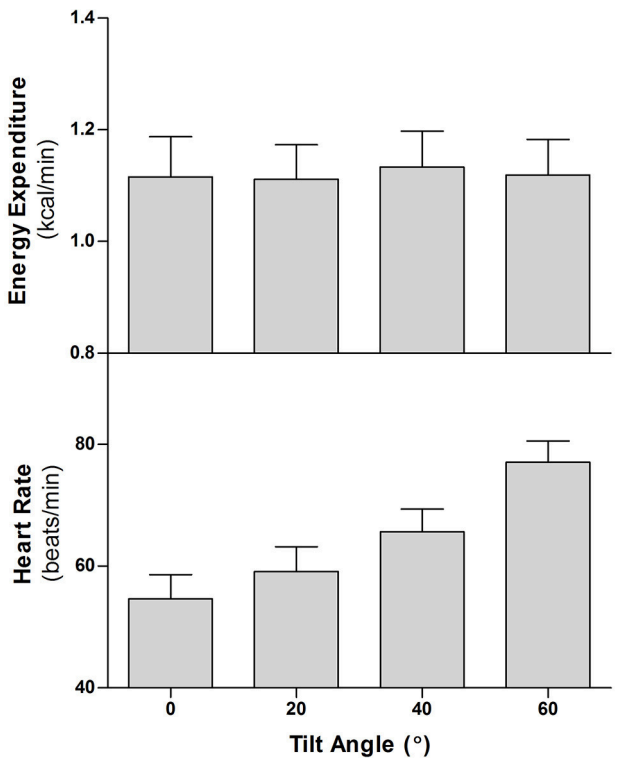

FIGURE 5 | Energy expenditure (EE) and heart rate (HR) of 6 healthy men during graded, incremental head-up tilting on a clinical table. After a baseline measurement period of 40–45 min in the supine position, the subjects were passively tilted in increasing increments of 20 degrees (i.e., supine, 20°, 40°, 60°), remaining at each head-up tilt angle for 16 min. The motorized tilt table achieved each 20° of tilt within 4–5 s. Data are presented as Mean of last 4 min at each tilt angle ± SEM.

Energy Cost of Muscle Activation (Isometric Contraction)

Maintaining posture, whether upright or seated, requires a certain degree of muscle tone and isometric contraction of stabilizing muscles. As the skeletal muscles involved in this stabilization and increased tonus are comprised of predominately oxidative fibers, increasing postural muscle activation could present not only an opportunity to increase EE, but also to increase the relative rate of fat oxidation. However, despite daily life activities consisting of a large amount of low-level isometric contraction, compared to dynamic exercise, its energy cost has been much less studied and quantified (Dulloo et al., 2017).

So how might isometric contraction be amplified in order to maximize EE during postural maintenance? Perhaps the simplest answer would be to alter posture allocation, so as to replace time spent in one posture with that of a potentially higher energetic cost (i.e., replace sitting time with standing time). However, this alone may not be sufficient to noticeably increase EE. Indeed, in addition to demonstrating that the majority of individuals (>75%) are able to maintain a standing posture at a similar level of EE to sitting (Miles-Chan et al., 2013, 2017), we have also shown that sitting in a comfortable chair, with the body weight well-supported, does not significantly increase EE above supine levels (<2% difference; Miles-Chan et al., 2014). In fact, based on these findings, replacing 2.5 h per day of lying or sitting by standing is in itself unlikely to increase daily EE by any more than 20 kcal (i.e., <1%); this is considerably less than the amount postulated by others (Levine et al., 2005; Johannsen et al., 2008). Similarly, Beers et al. (2008) have calculated that even sitting on a stability (exercise) ball—where the back is not supported—would still only result in an increase in sitting EE in the order of only around 0.07 kcal/min (∼7%). This marginal increase in EE, combined with studies showing increased levels of discomfort when sitting on such a ball compared to a traditional office chair (Gregory et al., 2006; McGill et al., 2006; Kingma and van Dieen, 2009), suggest that the use of such sitting balls does not present an effective obesity prevention/treatment strategy.

However, several other methods of enhancing muscle activation during postural maintenance have demonstrated the ability to appreciably increase EE. For example: (i) whole body vibration during standing has been shown to increase expenditure by ∼30% compared to standing without vibration (Fares et al., 2016); and (ii) Maffiuletti et al. (2012) have shown that standing in unstable shoes modestly increase EE (by ∼5% on average) in patients with obesity as compared to conventional shoes, with increases in postural sway and electromyographic activity of the leg and foot muscle also having been demonstrated when using such shoes (Landry et al., 2010). It is perhaps worth noting that some of the large discrepancy in energetic response to these two methods of enhancing muscle activation may lie in the timescale of the muscle contraction itself—with studies in isolated muscle suggesting that a series of brief contractions may be more energetically costly than a single muscle contraction of a longer duration (Chasiotis et al., 1987; Bergstrom and Hultman, 1988; Hogan et al., 1998); the former also resulting in a larger increase in glycolysis and greater fatigue (Spriet et al., 1988; Hogan et al., 1998).

Energy Cost of Postural Transitioning and Low-Level Physical Activities

Whilst, the energy cost of maintaining posture may be marginal, the energy cost of transitioning between postures (in particular, from sitting to standing) is receiving much attention as a potential interventional target. The reasoning for this interest is two-fold: Firstly, breaking sitting time has been shown to decrease metabolic risk independently of moderate-to-vigorous physical activity (Honda et al., 2016), with length of sitting bouts positively correlated with waist circumference and obesity prevalence (Healy et al., 2008; Gupta et al., 2016), and frequent interruptions to sitting time improving postprandial glucose metabolism (Bergouignan et al., 2016), triglyceride levels, waist circumference and BMI (Hamilton et al., 2008; Healy et al., 2008). Secondly, the energy cost of postural transitioning is much higher than that of postural maintenance—with a sit-to-stand transition increasing EE ∼35% above sitting metabolic rate (Judice et al., 2016), and showing a positive linear relationship with transition frequency (Hatamoto et al., 2016). Furthermore, the latter study (Hatamoto et al., 2016) demonstrated a four-fold increase in metabolic rate above resting during the performance of sit-to-stand transitions at a rate of 15 per minute, with the exercise still perceived as "light" by the participants. Importantly, while considerable inter-individual variability can be observed in the slope of this transition frequency vs. energy cost relationship, the cost is strongly correlated with body weight, thereby indicating that increasing postural transitioning may be of particular benefit to individuals who are overweight or obese (Hatamoto et al., 2016).

As mentioned earlier, in order to consistently increase EE beyond the sedentary threshold of 1.5 times resting metabolic rate (i.e., 1.5 METs), bodily movement is required. However, the physical activity need only be of a very low-level to achieve such an increase; with our own study finding that intermittent body displacement (stepping) increases EE to 1.5–1.6 METs (Miles-Chan et al., 2017). The low-level activities that comprise a large component of daily-life (e.g., domestic and household activities like carrying shopping, ironing, washing dishes, etc.) therefore present an ideal opportunity to elevate EE sufficiently to impact body weight management. The energetic cost of these activities was historically well-characterized in the context of estimating energy requirements (Passmore et al., 1952; Passmore and Durnin, 1955). Although, due to the myriad of technological advances made over recent decades, designed to make household activities quicker and easier, these early estimations are now largely redundant. There is hence a need to revisit such domestic activities in order to determine their contemporary energy cost. Recent investigations have shown that despite improved technologies, routine household activities easily reach energetic costs sufficient to be classified as low-intensity (>1.5 METs) to moderate-intensity (>3 METs; Gunn et al., 2002; Withers et al., 2006; Goh et al., 2016). To what extent the energy cost of these low-level physical activities of everyday life would differ if undertaken while standing compared to sitting (or vice versa) remains to be investigated. However, difficulties arise when comparing between population and study groups owing to a lack of standardized tests to assess the energy cost of low-level physical

activity. Furthermore, there is a need to explore human variability in this cost, which may have important implications for the efficacy of the use of low-level physical activity for body weight management. With the majority of daily-life activities consisting of both isometric and dynamic activity (Dulloo et al., 2017), we have recently developed and validated two such standardized methodologies; one involving an isometric leg press protocol of low-intensity (Sarafian et al., 2013), and the other a low-intensity cycle ergometer protocol (Fares et al., 2017). These standardized approaches are applicable to a vast range of population groups (i.e., healthy, elderly, or diseased populations) and pave the way for a more comprehensive examination of inter-individual variability in both our susceptibility to obesity and the efficacy of body weight maintenance strategies.

CONCLUDING REMARKS

Whilst altering posture represents a simple target for body weight management, the gains in EE achieved by changing postural allocation *per-se* are unlikely to be of significant importance. However, increases in postural transitioning, either alone, or in combination with low-level physical activities presents a much more efficacious method; with the relatively minor increases in EE easily accumulated over the course of our daily activities. Whether, breaking the sedentary threshold will lead to compensatory increases in energy intake (or not) remains to be investigated. However, it should be emphasized that not only are these types of movements both attainable and sustainable by the majority of the general population, but such modest increases in physical activity may lead to a better coupling of energy intake to energy expenditure, and hence facilitate the achievement of energy balance—as suggested by the J-shaped curves of Mayer et al. (1956) and more recently revisited by Blundell et al. (2015) and Hopkins and Blundell (2016). Therefore, with suggestions that an energy imbalance of 100–200 kcal/day (i.e., <10% of average daily energy expenditure) may be sufficient to address the obesity crisis at the broad population level (Butte and Ellis, 2003; Hill et al., 2003), the role of posture allocations coupled with inter-individual variability in our metabolic response to low-level physical activities deserve considerable research attention.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct, and intellectual contribution to the work, and approved it for publication.

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REFERENCES

Alexander, R. M. (1989). Optimization and gaits in the locomotion of vertebrates. *Physiol. Rev.* 69, 1199–1227.

Apfelbaum, M., Bostsarron, J., and Lacatis, D. (1971). Effect of caloric restriction and excessive caloric intake on energy expenditure. *Am. J. Clin. Nutr.* 24, 1405–1409.

Bandyopadhyay, B., and Chattopadhyay, H. (1980). Energy metabolism in male college students. *Indian J. Med. Res.* 71, 961–969.

Banerjee, B., Khew, K. S., and Saha, N. (1971). A comparative study of energy expenditure in some common daily activities of non-pregnant and pregnant Chinese, Malay and Indian women. *J. Obstet. Gynaecol. Br. Commonw.* 78, 113–116. doi: 10.1111/j.1471-0528.1971.tb00243.x

Banerjee, B., and Saha, N. (1972). Resting metabolic rate and energy cost of some common daily activities of trained and untrained tropical people: energy metabolism in training in Tropics. *J. Sports Med. Phys. Fitness* 12, 111–116.

Beers, E. A., Roemmich, J. N., Epstein, L. H., and Horvath, P. J. (2008). Increasing passive energy expenditure during clerical work. *Eur. J. Appl. Physiol.* 103, 353–360. doi: 10.1007/s00421-008-0713-y

Bergouignan, A., Latouche, C., Heywood, S., Grace, M. S., Reddy-Luthmoodoo, M., Natoli, A. K., et al. (2016). Frequent interruptions of sedentary time modulates contraction- and insulin-stimulated glucose uptake pathways in muscle: ancillary analysis from randomized clinical trials. *Sci. Rep.* 6:32044. doi: 10.1038/srep32044

Bergstrom, M., and Hultman, E. (1988). Energy cost and fatigue during intermittent electrical stimulation of human skeletal muscle. *J. Appl. Physiol.* 65, 1500–1505.

Blaszczyk, J. W., Cieslinska-Swider, J., Plewa, M., Zahorska-Markiewicz, B., and Markiewicz, A. (2009). Effects of excessive body weight on postural control. *J. Biomech.* 42, 1295–1300. doi: 10.1016/j.jbiomech.2009.03.006

Bleiberg, F. M., Brun, T. A., Goihman, S., and Gouba, E. (1980). Duration of activities and energy expenditure of female farmers in dry and rainy seasons in Upper-Volta. *Br. J. Nutr.* 43, 71–82. doi: 10.1079/BJN19800065

Blundell, J. E., Gibbons, C., Caudwell, P., Finlayson, G., and Hopkins, M. (2015). Appetite control and energy balance: impact of exercise. *Obes. Rev.* 16(Suppl. 1), 67–76. doi: 10.1111/obr.12257

Brun, T., Bleiberg, F., and Goihman, S. (1981). Energy expenditure of male farmers in dry and rainy seasons in Upper-Volta. *Br. J. Nutr.* 45, 67–75. doi: 10.1079/BJN19810077

Buckley, J. P., Mellor, D. D., Morris, M., and Joseph, F. (2014). Standing-based office work shows encouraging signs of attenuating post-prandial glycaemic excursion. *Occup. Environ. Med.* 71, 109–111. doi: 10.1136/oemed-2013-101823

Butte, N. F., and Ellis, K. J. (2003). Comment on "Obesity and the environment: where do we go from here?" *Science* 301, 598; author reply:598. doi: 10.1126/science.1085985

Ceesay, S. M., Prentice, A. M., Day, K. C., Murgatroyd, P. R., Goldberg, G. R., Scott, W., et al. (1989). The use of heart rate monitoring in the estimation of energy expenditure: a validation study using indirect whole-body calorimetry. *Br. J. Nutr.* 61, 175–186. doi: 10.1079/BJN19890107

Chasiotis, D., Bergstrom, M., and Hultman, E. (1987). ATP utilization and force during intermittent and continuous muscle contractions. *J. Appl. Physiol.* 63, 167–174.

Cole, A. H., and Ogbe, J. O. (1987). Energy intake, expenditure and pattern of daily activity of Nigerian male students. *Br. J. Nutr.* 58, 357–367. doi: 10.1079/BJN19870105

Corbeil, P., Simoneau, M., Rancourt, D., Tremblay, A., and Teasdale, N. (2001). Increased risk for falling associated with obesity: mathematical modeling of postural control. *IEEE Trans. Neural Syst. Rehabil. Eng.* 9, 126–136. doi: 10.1109/7333.928572

Creasy, S. A., Rogers, R. J., Byard, T. D., Kowalsky, R. J., and Jakicic, J. M. (2016). Energy expenditure during acute periods of sitting, standing, and walking. *J. Phys. Act. Health* 13, 573–578. doi: 10.1123/jpah.2015-0419

Cruz-Gomez, N. S., Plascencia, G., Villanueva-Padron, L. A., and Jauregui-Renaud, K. (2011). Influence of obesity and gender on the postural stability during upright stance. *Obes. Facts* 4, 212–217. doi: 10.1159/000329408

de Guzman, M. P. E., Cabrera, J. P., Basconcillo, R. O., Gaurano, A. L., Yuchingtat, G. P., Tan, R. M., et al. (1978). A study of the energy expenditure, dietary intake and pattern of daily activity among various occupational groups. Clerk-typist. *Philipp. J. Nutr.* 31, 147–156.

de Guzman, M. P. E., Cabrera, J. P., Yuchingtat, G. P., Abanto, Z. U., and Gaurano, A. L. (1984). A study of the energy expenditure, dietary intake and pattern of

daily activity among various occupational groups. Laguna Rice farmers. *Philipp. J. Nutr.* 37, 163–174.

de Guzman, M. P. E., Recto, R. C., Cabrera, J. P., Basconcillo, R. O., Gaurano, A. L., Yuchingtat, G. P., et al. (1979). A study of the energy expenditure, dietary intake and pattern of daily activity among various occupational groups. Textile Mill workers. *Philipp. J. Nutr.* 32, 134–148.

Donald, K. W., and Davidson, W. M. (1954). Oxygen uptake of booted and fin swimming divers. *J. Appl. Physiol.* 7, 31–37.

Dulloo, A. G., Jacquet, J., Montani, J. P., and Schutz, Y. (2012). Adaptive thermogenesis in human body weight regulation: more of a concept than a measurable entity? *Obes. Rev.* 13(Suppl. 2), 105–121. doi: 10.1111/j.1467-789X.2012.01041.x

Dulloo, A. G., Miles-Chan, J. L., Montani, J. P., and Schutz, Y. (2017). Isometric thermogenesis at rest and during movement: a neglected variable in energy expenditure and obesity predisposition. *Obes. Rev.* 18(Suppl. 1), 56–64. doi: 10.1111/obr.12505

Durnin, J. V. G. A., and Passmore, R. (1967). *Energy, Work and Leisure*. London: Heinemann.

Edholm, O. G., Fletcher, J. G., Widdowson, E. M., and McCance, R. A. (1955). The energy expenditure and food intake of individual men. *Br. J. Nutr.* 9, 286–300. doi: 10.1079/BJN19550040

Edmundson, W. C., and Edmundson, S. A. (1988). Food intake and work allocation of male and female farmers in an impoverished Indian village. *Br. J. Nutr.* 60, 433–439. doi: 10.1079/BJN19880115

Fares, E. J., Charriere, N., Montani, J. P., Schutz, Y., Dulloo, A. G., and Miles-Chan, J. L. (2016). Energy expenditure and substrate oxidation in response to side-alternating whole body vibration across three commonly-used vibration frequencies. *PLoS ONE* 11:e0151552. doi: 10.1371/journal.pone.0151552

Fares, E. J., Isacco, L., Monnard, C. R., Miles-Chan, J. L., Montani, J. P., Schutz, Y., et al. (2017). Reliability of low power cycling efficiency in energy expenditure phenotyping of inactive men and women. *Physiol. Rep.* 5:e13233. doi: 10.14814/phy2.13233

Fountaine, C. J., Johann, J., Skalko, C., and Liguori, G. A. (2016). Metabolic and energy cost of sitting, standing, and a novel sitting/stepping protocol in recreationally active college students. *Int. J. Exerc. Sci.* 9, 223–229.

Garry, R. C., Passmore, R., Warnock, G. M., and Durnin, J. V. (1955). Studies on expenditure of energy and consumption of food by miners and clerks, Fife, Scotland, 1952. *Spec. Rep. Ser. Med. Res. Counc.* 289, 1–70.

Geissler, C. A., and Aldouri, M. S. H. (1985). Racial differences in the energy cost of standardised activities. *Ann. Nutr. Metab.* 29, 40–47. doi: 10.1159/000176952

Geissler, C., Brun, T., Mirbagheri, I., Soheli, A., Naghibi, A., and Hedayat, H. (1981). The energy expenditure of female carpet weavers and rural women in Iran. *Am. J. Clin. Nutr.* 34, 2776–2783.

Gibbs, B. B., Kowalsky, R. J., Perdomo, S. J., Grier, M., and Jakicic, J. M. (2017). Energy expenditure of deskwork when sitting, standing or alternating positions. *Occup. Med.* 67, 121–127. doi: 10.1093/occmed/kqw115

Gilleard, W., and Smith, T. (2007). Effect of obesity on posture and hip joint moments during a standing task, and trunk forward flexion motion. *Int. J. Obes.* 31, 267–271. doi: 10.1038/sj.ijo.0803430

Goh, H. J., Govindharajulu, P., Camps, S. G., Tan, S. Y., and Henry, C. J. (2016). Gross and relative energy cost of domestic household activities in Asian men. *Eur. J. Clin. Nutr.* 70, 1414–1419. doi: 10.1038/ejcn.2016.134

Gregory, D. E., Dunk, N. M., and Callaghan, J. P. (2006). Stability ball versus office chair: comparison of muscle activation and lumbar spine posture during prolonged sitting. *Hum. Factors* 48, 142–153. doi: 10.1518/001872006776412243

Gunn, S. M., Brooks, A. G., Withers, R. T., Gore, C. J., Owen, N., Booth, M. L., et al. (2002). Determining energy expenditure during some household and garden tasks. *Med. Sci. Sports Exerc.* 34, 895–902. doi: 10.1097/00005768-200205000-00026

Gupta, N., Hallman, D. M., Mathiassen, S. E., Aadahl, M., Jorgensen, M. B., and Holtermann, A. (2016). Are temporal patterns of sitting associated with obesity among blue-collar workers? A cross sectional study using accelerometers. *BMC Public Health* 16:148. doi: 10.1186/s12889-016-2803-9

Hamilton, M. T., Healy, G. N., Dunstan, D. W., Zderic, T. W., and Owen, N. (2008). Too little exercise and too much sitting: inactivity physiology and the need for new recommendations on sedentary behavior. *Curr. Cardiovasc. Risk Rep.* 2, 292–298. doi: 10.1007/s12170-008-0054-8

Hatamoto, Y., Yamada, Y., Higaki, Y., and Tanaka, H. (2016). A novel approach for measuring energy expenditure of a single sit-to-stand movement. *Eur. J. Appl. Physiol.* 116, 997–1004. doi: 10.1007/s00421-016-3355-5

Healy, G. N., Dunstan, D. W., Salmon, J., Cerin, E., Shaw, J. E., Zimmet, P. Z., et al. (2008). Breaks in sedentary time: beneficial associations with metabolic risk. *Diabetes Care* 31, 661–666. doi: 10.2337/dc07-2046

Henson, J., Dunstan, D. W., Davies, M. J., and Yates, T. (2016). Sedentary behaviour as a new behavioural target in the prevention and treatment of type 2 diabetes. *Diabetes Metab. Res. Rev.* 32(Suppl. 1), 213–220. doi: 10.1002/dmrr.2759

Hill, J. O., Wyatt, H. R., Reed, G. W., and Peters, J. C. (2003). Obesity and the environment: where do we go from here? *Science* 299, 853–855. doi: 10.1126/science.1079857

Hogan, M. C., Ingham, E., and Kurdak, S. S. (1998). Contraction duration affects metabolic energy cost and fatigue in skeletal muscle. *Am. J. Physiol.* 274, E397–E402.

Honda, T., Chen, S., Yonemoto, K., Kishimoto, H., Chen, T., Narazaki, K., et al. (2016). Sedentary bout durations and metabolic syndrome among working adults: a prospective cohort study. *BMC Public Health* 16:888. doi: 10.1186/s12889-016-3570-3

Hopkins, M., and Blundell, J. E. (2016). Energy balance, body composition, sedentariness and appetite regulation: pathways to obesity. *Clin. Sci.* 130:1615. doi: 10.1042/CS20160006

Johannsen, D. L., Welk, G. J., Sharp, R. L., and Flakoll, P. J. (2008). Differences in daily energy expenditure in lean and obese women: the role of posture allocation. *Obesity* 16, 34–39. doi: 10.1038/oby.2007.15

Jones, C. D., Jarjou, M. S., Whitehead, R. G., and Jequier, E. (1987). Fatness and the energy cost of carrying loads in African women. *Lancet* 2, 1331–1332. doi: 10.1016/S0140-6736(87)91221-9

Judice, P. B., Hamilton, M. T., Sardinha, L. B., Zderic, T. W., and Silva, A. M. (2016). What is the metabolic and energy cost of sitting, standing and sit/stand transitions? *Eur. J. Appl. Physiol.* 116, 263–273. doi: 10.1007/s00421-015-3279-5

Kanade, A. N., Gokhale, M. K., and Rao, S. (2001). Energy costs of standard activities among Indian adults. *Eur. J. Clin. Nutr.* 55, 708–713. doi: 10.1038/sj.ejcn.1601211

Katzmarzyk, P. T., Leonard, W. R., Stephen, M. A., Berti, P. R., and Ross, A. G. (1996). Differences between observed and predicted energy costs at rest and during exercise in three subsistence-level populations. *Am. J. Phys. Anthropol.* 99, 537–545. doi: 10.1002/ajpa.1330990402

Kingma, I., and van Dieen, J. H. (2009). Static and dynamic postural loadings during computer work in females: sitting on an office chair versus sitting on an exercise ball. *Appl. Ergon.* 40, 199–205. doi: 10.1016/j.apergo.2008.04.004

Koller, J. R., Jacobs, D. A., Ferris, D. P., and Remy, C. D. (2015). Learning to walk with an adaptive gain proportional myoelectric controller for a robotic ankle exoskeleton. *J. Neuroeng. Rehabil.* 12:97. doi: 10.1186/s12984-015-0086-5

Landry, S. C., Nigg, B. M., and Tecante, K. E. (2010). Standing in an unstable shoe increases postural sway and muscle activity of selected smaller extrinsic foot muscles. *Gait Posture* 32, 215–219. doi: 10.1016/j.gaitpost.2010.04.018

Lawrence, M., Singh, J., Lawrence, F., and Whitehead, R. G. (1985). The energy cost of common daily activities in African women: increased expenditure in pregnancy? *Am. J. Clin. Nutr.* 42, 753–763.

Levine, J. A., and Miller, J. M. (2007). The energy expenditure of using a "walk-and-work" desk for office workers with obesity. *Br. J. Sports Med.* 41, 558–561. doi: 10.1136/bjsm.2006.032755

Levine, J. A., Eberhardt, N. L., and Jensen, M. D. (1999). Role of nonexercise activity thermogenesis in resistance to fat gain in humans. *Science* 283, 212–214. doi: 10.1126/science.283.5399.212

Levine, J. A., Lanningham-Foster, L. M., McCrady, S. K., Krizan, A. C., Olson, L. R., Kane, P. H., et al. (2005). Interindividual variation in posture allocation: possible role in human obesity. *Science* 307, 584–586. doi: 10.1126/science.1106561

Levine, J. A., Schleusner, S. J., and Jensen, M. D. (2000). Energy expenditure of nonexercise activity. *Am. J. Clin. Nutr.* 72, 1451–1454.

Li, J., and Yan, W. Y. (1991). The energy expenditure and nutritional status of college students. I. The energy cost and the total energy expenditure per day. *Biomed. Environ. Sci.* 4, 295–303.

Lloyd, R., Parr, B., Davies, S., and Cooke, C. (2010). No 'free ride' for African women: a comparison of head-loading versus back-loading among Xhosa women. *S. Afr. J. Sci.* 106:153. doi: 10.4102/sajs.v106i3/4.153

Maffiuletti, N. A., Malatesta, D., Agosti, F., and Sartorio, A. (2012). Unstable shoes increase energy expenditure of obese patients. *Am. J. Med.* 125, 513–516. doi: 10.1016/j.amjmed.2012.01.001

Malhotra, M. S., Chandra, U., and Sridharan, K. (1976). Dietary intake and energy requirement of Indian submariners in tropical waters. *Ergonomics* 19, 141–148. doi: 10.1080/00140137608931526

Maloiy, G. M., Heglund, N. C., Prager, L. M., Cavagna, G. A., and Taylor, C. R. (1986). Energetic cost of carrying loads: have African women discovered an economic way? *Nature* 319, 668–669. doi: 10.1038/319668a0

Mayer, J., Roy, P., and Mitra, K. P. (1956). Relation between caloric intake, body weight, and physical work: studies in an industrial male population in West Bengal. *Am. J. Clin. Nutr.* 4, 169–175.

McAlpine, D. A., Manohar, C. U., McCrady, S. K., Hensrud, D., and Levine, J. A. (2007). An office-place stepping device to promote workplace physical activity. *Br. J. Sports Med.* 41, 903–907. doi: 10.1136/bjsm.2006.034900

McGill, S. M., Kavcic, N. S., and Harvey, E. (2006). Sitting on a chair or an exercise ball: various perspectives to guide decision making. *Clin. Biomech.* 21, 353–360. doi: 10.1016/j.clinbiomech.2005.11.006

Miles-Chan, J. L., Fares, E. J., Berkachy, R., Jacquet, P., Isacco, L., Schutz, Y., et al. (2017). Standing economy: does the heterogeneity in the energy cost of posture maintenance reside in differential patterns of spontaneous weight-shifting? *Eur. J. Appl. Physiol.* 117, 795–807. doi: 10.1007/s00421-017-3563-7

Miles-Chan, J. L., Sarafian, D., Montani, J. P., Schutz, Y., and Dulloo, A. (2013). Heterogeneity in the energy cost of posture maintenance during standing relative to sitting: phenotyping according to magnitude and time-course. *PLoS ONE* 8:e65827. doi: 10.1371/journal.pone.0065827

Miles-Chan, J. L., Sarafian, D., Montani, J. P., Schutz, Y., and Dulloo, A. G. (2014). Sitting comfortably versus lying down: is there really a difference in energy expenditure? *Clin. Nutr.* 33, 175–178. doi: 10.1016/j.clnu.2013.11.009

Miller, D. S. (1982). Factors affecting energy expenditure. *Proc. Nutr. Soc.* 41, 193–202. doi: 10.1079/PNS19820030

Monnard, C. R., and Miles-Chan, J. L. (2017). Energy cost of standing in a multi-ethnic cohort: are energy-savers a minority or the majority? *PLoS ONE* 12:e0169478. doi: 10.1371/journal.pone.0169478

Ng, S. W., and Popkin, B. (2012). Time use and physical activity: a shift away from movement across the globe. *Obes. Rev.* 13, 659–680. doi: 10.1111/j.1467-789X.2011.00982.x

Norgan, N. G., Ferro-Luzzi, A., and Durnin, J. V. (1974). The energy and nutrient intake and the energy expenditure of 204 New Guinean adults. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 268, 309–348. doi: 10.1098/rstb.1974.0033

Passmore, R., and Durnin, J. V. (1955). Human energy expenditure. *Physiol. Rev.* 35, 801–840.

Passmore, R., Thomson, J. G., Warnock, G. M., Dixon, C. M., Kitchin, A. H., Smith, G., et al. (1952). A balance sheet of the estimation of energy intake and energy expenditure as measured by indirect calorimetry, using the Kofranyi-Michaelis calorimeter. *Br. J. Nutr.* 6, 253–264. doi: 10.1079/BJN19520028

Poppitt, S. D., Prentice, A. M., Jequier, E., Schutz, Y., and Whitehead, R. G. (1993). Evidence of energy sparing in Gambian women during pregnancy: a longitudinal study using whole-body calorimetry. *Am. J. Clin. Nutr.* 57, 353–364.

Rao, S., Gokhale, M., and Kanade, A. (2008). Energy costs of daily activities for women in rural India. *Public Health Nutr.* 11, 142–150. doi: 10.1017/S1368980007000055

Reiff, C., Marlatt, K., and Dengel, D. R. (2012). Difference in caloric expenditure in sitting versus standing desks. *J. Phys. Act. Health* 9, 1009–1011. doi: 10.1123/jpah.9.7.1009

Sarafian, D., Miles-Chan, J. L., Yepuri, G., Montani, J. P., Schutz, Y., and Dulloo, A. G. (2013). A standardized approach to study human variability in isometric thermogenesis during low-intensity physical activity. *Front. Physiol.* 4:155. doi: 10.3389/fphys.2013.00155

Sedentary Behaviour Research Network (2012). Letter to the editor: standardized use of the terms "sedentary" and "sedentary behaviours." *Appl. Physiol. Nutr. Metab.* 37, 540–542. doi: 10.1139/h2012-024

Selinger, J. C., O'Connor, S. M., Wong, J. D., and Donelan, J. M. (2015). Humans can continuously optimize energetic cost during walking. *Curr. Biol.* 25, 2452–2456. doi: 10.1016/j.cub.2015.08.016

Singh, D., Park, W., Levy, M. S., and Jung, E. S. (2009). The effects of obesity and standing time on postural sway during prolonged quiet standing. *Ergonomics* 52, 977–986. doi: 10.1080/00140130902777636

Speck, R. M., and Schmitz, K. H. (2011). Energy expenditure comparison: a pilot study of standing instead of sitting at work for obesity prevention. *Prev. Med.* 52, 283–284. doi: 10.1016/j.ypmed.2011.02.002

Spriet, L. L., Soderlund, K., and Hultman, E. (1988). Energy cost and metabolic regulation during intermittent and continuous tetanic contractions in human skeletal muscle. *Can. J. Physiol. Pharmacol.* 66, 134–139. doi: 10.1139/y88-024

Spurr, G. B., Prentice, A. M., Murgatroyd, P. R., Goldberg, G. R., Reina, J. C., and Christman, N. T. (1988). Energy expenditure from minute-by-minute heart-rate recording: comparison with indirect calorimetry. *Am. J. Clin. Nutr.* 48, 552–559.

Steeves, J. A., Thompson, D. L., and Bassett, D. R. Jr. (2012). Energy cost of stepping in place while watching television commercials. *Med. Sci. Sports Exerc.* 44, 330–335. doi: 10.1249/MSS.0b013e31822d797e

Strickland, S. S., and Ulijaszek, S. J. (1990). Energetic cost of standard activities in Gurkha and British soldiers. *Ann. Hum. Biol.* 17, 133–144. doi: 10.1080/03014469000000882

Sujatha, T., Shatrugna, V., Venkataramana, Y., and Begum, N. (2000). Energy expenditure on household, childcare and occupational activities of women from urban poor households. *Br. J. Nutr.* 83, 497–503. doi: 10.1017/S0007114500000635

Tigbe, W. W., Granat, M. H., Sattar, N., and Lean, M. E. (2017). Time spent in sedentary posture is associated with waist circumference and cardiovascular risk. *Int. J. Obes.* 41, 689–696. doi: 10.1038/ijo.2017.30

Tucker, V. A. (1970). Energetic cost of locomotion in animals. *Comp. Biochem. Physiol.* 34, 841–846. doi: 10.1016/0010-406X(70)91006-6

Vaz, M., Karaolis, N., Draper, A., and Shetty, P. (2005). A compilation of energy costs of physical activities. *Public Health Nutr.* 8, 1153–1183. doi: 10.1079/PHN2005802

Villarrasa-Sapina, I., Garcia-Masso, X., Serra-Ano, P., Garcia-Lucerga, C., Gonzalez, L. M., and Lurbe, E. (2016). Differences in intermittent postural control between normal-weight and obese children. *Gait Posture* 49, 1–6. doi: 10.1016/j.gaitpost.2016.06.012

Viteri, F. E., Torun, B., Galicia, J. C., and Herrera, E. (1971). Determining energy costs of agricultural activities by respirometer and energy balance techniques. *Am. J. Clin. Nutr.* 24, 1418–1430.

Wayman, E. (2012). *Becoming Human: The Evolution of Walking Upright.* Available online at: http://www.smithsonianmag.com/science-nature/becoming-human-the-evolution-of-walking-upright-13837658/

Whybrow, S., Ritz, P., Horgan, G. W., and Stubbs, R. J. (2013). An evaluation of the IDEEA activity monitor for estimating energy expenditure. *Br. J. Nutr.* 109, 173–183. doi: 10.1017/S0007114512000645

Withers, R. T., Brooks, A. G., Gunn, S. M., Plummer, J. L., Gore, C. J., and Cormack, J. (2006). Self-selected exercise intensity during household/garden activities and walking in 55 to 65-year-old females. *Eur. J. Appl. Physiol.* 97, 494–504. doi: 10.1007/s00421-006-0177-x

Young, D. R., Hivert, M. F., Alhassan, S., Camhi, S. M., Ferguson, J. F., Katzmarzyk, P. T., et al. (2016). Sedentary behavior and cardiovascular morbidity and mortality: a science advisory from the american heart association. *Circulation* 134, e262–e279. doi: 10.1161/CIR.0000000000000440

Zurlo, F., Ferraro, R. T., Fontvielle, A. M., Rising, R., Bogardus, C., and Ravussin, E. (1992). Spontaneous physical activity and obesity: cross-sectional and longitudinal studies in Pima Indians. *Am. J. Physiol.* 263, E296–E300.

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Benefits of Substituting Sitting with Standing and Walking in Free-Living Conditions for Cardiometabolic Risk Markers, Cognition and Mood in Overweight Adults

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Background: We investigated whether substituting sitting with standing and self-perceived light walking in free-living conditions would improve cardiometabolic risk factors, mood, and cognition in overweight/obese adults.

Methods: In a randomized, cross-over study, 24 (m/f: 13/11) sedentary overweight/obese participants (64 ± 7 years, BMI 29 ± 2 kg/m^2) followed two activity regimens of each 4 days in free-living conditions: "Sit": sitting 13.5 h/day, standing 1.4 h/day, self-perceived light-intensity walking 0.7 h/day; for "SitLess" these activities lasted 7.6, 4.0, and 4.3 h/day, respectively. Meals were standardized and physical activity was assessed by accelerometry (activPAL). Insulin sensitivity (expressed as Matsuda-index based on an oral glucose tolerance test), circulating lipids, blood pressure, mood (pleasantness and arousal), and cognition were assessed on the morning after the activity regimens. Quality of life and sleep were assessed on the last day of the activity regimens.

Results: We observed that AUC (0–190 min) for insulin decreased by 20% after SitLess vs. Sit [10,125 (656) vs. 12,633 (818); $p = 0.006$]. Insulin sensitivity improved by 16% after SitLess vs. Sit [Matsuda-index, mean (SEM): 6.45 (0.25) vs. 5.58 (0.25) respectively; $p = 0.007$]. Fasting triglycerides, non-HDL-cholesterol, and apolipoprotein B decreased by 32, 7, and 4% respectively, whereas HDL-cholesterol increased by 7% after SitLess vs. Sit (all $p < 0.01$). Diastolic blood pressure was lower after SitLess vs. Sit ($p < 0.05$). Pleasantness (as one marker of mood status) after the oral glucose tolerance test was higher after SitLess vs. Sit ($p < 0.05$). There was no significant difference between regimens for cognition, quality of life and sleep.

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Conclusions: Reducing sitting time in free-living conditions markedly improved insulin sensitivity, circulating lipids, and diastolic blood pressure. Substituting sitting with standing and self-perceived light walking is an effective strategy to improve cardiometabolic risk factors in overweight/obese subjects.

Keywords: exercise, insulin sensitivity, light-intensity physical activity, lipids, sedentary behavior, sitting, standing, walking

Clinical Trial Registration: http://www.clinicaltrials.gov, NCT02394249.

INTRODUCTION

Observational studies suggest that the majority of the Western population spends more than half of the waking day sedentary (Matthews et al., 2008; van der Berg et al., 2016b). Mounting evidence shows an association between a high sitting time and obesity (Levine et al., 2005; Chastin et al., 2015; de Rooij et al., 2016). In addition to the health risks associated with overweight and obesity (Hubert et al., 1983; Mokdad et al., 2003), a sedentary lifestyle has been associated with an increased risk of type 2 diabetes, metabolic syndrome, and premature mortality (Biswas et al., 2015; van der Berg et al., 2016b). This negative consequence of sitting seems to be independent of the time spent in moderate-to-vigorous physical activity (Biswas et al., 2015; van der Berg et al., 2016b). Hence, interventions reducing sitting time may improve cardiometabolic health in these individuals. Indeed, laboratory studies showed beneficial effects on circulating glucose and insulin in overweight and obese adults when sitting was interrupted every 20–30 min with light walking (Dunstan et al., 2012; Bailey and Locke, 2015; Henson et al., 2016). However, as recently pointed out by the American Heart Association, interventions in free-living conditions that reduce sitting time are very scarce (Young et al., 2016).

Apart from its cardiometabolic consequences, obesity has also been associated with an increased risk of mood disorders (McElroy et al., 2004) and reduced cognitive function (Smith et al., 2011). This increased risk may partly originate from obesity related insulin resistance in the brain (Lamport et al., 2009). Vice versa, improvements in insulin sensitivity have been linked to improvements in mood and cognition (Kim and Feldman, 2012; Heni et al., 2015). Several studies have shown that engaging in moderate-to-vigorous physical activity not only improves insulin sensitivity (Wojtaszewski et al., 2000), but also mood (Brown et al., 2009) and cognition (Smith et al., 2010). However, to which extent these beneficial effects also hold true for light-intensity physical activity is unclear.

In the present study, we investigated whether substituting sitting with standing and self-perceived light walking in free-living conditions improved insulin sensitivity and other cardiometabolic risk factors in sedentary overweight/obese individuals. Moreover, we explored whether reducing sitting time also improved mood and cognition.

Abbreviations: OGTT, oral glucose tolerance test.

METHODS

Participants

Adults aged 40–80 years with a BMI between 25 and 35 kg/m^2, were recruited through paper advertisements at Maastricht University and through online and newspaper advertisements outside Maastricht University. During screening, every individual performed a 1 day try-out of the SitLess regimen to ensure that the participant was able to carry out the SitLess regimen in free-living conditions. Physical activity was measured during 4 days (including one weekend day) in free-living conditions before the start of the study. Exclusion criteria were more than 2.5 h/week of moderate-to-vigorous physical activity based on self-report, diseases which interfered with physical activities, weight loss (>2 kg) in the last 3 months, alcohol abuse, experimental drug use, use of glucose lowering drugs, corticosteroids, or coumarins or fasting plasma glucose >6.9 mmol/l. Throughout the study, drug administration and usage remained unaltered. All participants provided written informed consent. The study was conducted at Maastricht University between February and September 2015. (www.clinicaltrials.gov, NCT02394249). This study was carried out in accordance with the recommendations of the Local Ethics Committee of the Maastricht University Medical Centre+ with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Local Ethics Committee of the Maastricht University Medical Centre+.

Study Design

The primary outcome was Area Under the Curve (AUC) for plasma insulin during an Oral Glucose Tolerance Test (OGTT). Based on an earlier study in healthy subjects with a similar design (Duvivier et al., 2013), the number of subjects required was calculated. Based on mean difference ± SD in AUC for insulin (1257.5 ± 2293.5 mU/l × min) between the two activity regimens and a two-sided alpha of 0.05, we calculated that 21 subjects would be needed to detect a difference of 1,500 mU/l × min between the SitLess and the Sit regimen with a power of 80% using a paired-samples *t*-test. To account for a 15% drop-out after randomization, 25 subjects were included.

The Activity Regimens

All participants were instructed to follow two activity regimens in free-living conditions, lasting 4 days each (Sit and SitLess). The study had a randomized cross-over design. Randomization was performed by a computer program with a block size of

two intervention orders; each pair of included persons received another regimen order. The study design is displayed in **Figure 1**. During Sit, participants were instructed to restrict walking and standing to ≤1 h/day each, spending the remainder of the waking day sitting. During SitLess, participants were instructed to substitute at least 7 h/day of sitting with ≥4 h of self-perceived light walking and ≥3 h of standing; and to interrupt sitting preferably every 30 min with standing/walking bouts. Subjects were instructed to walk at a self-perceived light-intensity. Adherence to these instructions was monitored by accelerometry (see below). There was a wash-out period of at least 10 days between the screening session and the first activity regimen, and between the two activity regimens. During the wash-out, participants were instructed to maintain their habitual pattern of daily life activities, not to perform more than 1 h/week of moderate-to-vigorous physical activity and to consume a maximum of 1 unit/day of alcohol.

Meal Standardization

During the activity regimens, subjects were instructed to adhere to their normal diet. During the first regimen, participants carefully recorded everything they ate, and drank of these consumptions in a diary. These records were returned to the participants who were instructed to consume the same diet during the second activity regimen. Alcohol was not permitted during the activity regimens. In order to achieve identical energy intake and meal composition in the 12 h before the final measurements, participants received identical pre-packaged meals for dinner on the last day of each activity regimen. The pre-packaged meals included a main meal (vegetables, potatoes, and chicken or pork, 409–437 kcal, 11.3–15.8 g fat, 45.0–51.8 g carbohydrates, 20.3–22.5 g protein) and a dessert (yogurt, 150 kcal, 3.8 g fat, 13.1 g carbohydrates, 2.9 g protein). The subjects were instructed to consume this meal at home before 22.00 and to refrain from food or drinks after this meal except for water.

Assessment of Physical Activity

Physical activity and posture allocation were measured 24 h/day using an activPAL3 activity monitor (PAL Technologies, Glasgow, Scotland). The monitor was attached waterproof to the skin on the anterior thigh using Tegaderm (3M, St. Paul, Minnesota, USA) at least 1 day before each activity regimen. This accelerometer accurately discriminates between time spent inactive (sitting or lying), standing, walking (Berendsen et al., 2014), and step number (Ryan et al., 2006). Since the activPAL program does not provide sleeping time automatically, sleeping time was determined with a validated algorithm (van der Berg et al., 2016a), which was implemented as a Matlab (Mathworks, Natick, MA) program. Diary data for self-reported physical activity were compared with the activPAL3 data to formulate tailor-made instructions on how to change daily activities after the first and third days of each activity regimen to guarantee optimal compliance to each activity regimen.

Oral Glucose Tolerance Test

After each activity regimen (day 5), the subjects came to the research center between 8:30 and 9:30 AM after an overnight fast and an OGTT was performed. After an acclimatization period of 10 min, blood pressure was measured three times with an Omron 705IT blood pressure monitor (Omron Healthcare Europe B.V., Hoofddorp, The Netherlands). An i.v. catheter was placed in an antecubital vein for blood sampling. At baseline, blood was sampled for analysis of glucose, insulin, C-peptide, triglycerides, free fatty acid (FFA) levels, total cholesterol, high-density-lipoprotein (HDL) and low-density-lipoprotein (LDL) cholesterol, non-HDL-cholesterol, apo A-I, and B100. After ingestion of 75 g of glucose in water (200 ml in total), blood samples were drawn for glucose, insulin and C-peptide levels at 15, 30, 45, 60, 90, 120, and 190 min. Blood samples were stored at −80°C until analysis after the end of the study. Insulin and C-peptide were measured using a Human Insulin Specific RIA kit (HI-14K, Millipore) and a Human C-peptide RIA kit (HCP-20K, Millipore) respectively. Radioactivity was count on a 2,470 Automatic Gamma Counter (Perkin Elmer). Plasma glucose, total cholesterol, HDL-cholesterol, triglycerides, free fatty acids, apo A-I, and apo B100 were spectrophotometrically analyzed on the ABX Pentra 400 (Horiba) and free glycerol on a Cobas Fara (Roche). Plasma samples were precipitated with 1/10 volume of sulfosalicylic acid, placed on ice for 25 min, and then centrifuged at maximal speed. Free glycerol was measured in the supernatant. LDL-cholesterol was calculated using the Friedewald formula (Friedewald et al., 1972). Non-HDL-cholesterol was calculated as total cholesterol minus HDL-cholesterol.

Mood and Cognition

Cognitive performance and mood were measured before and after the OGTT, based on the principle that by applying a challenge (in this case the glucose load), one might be better

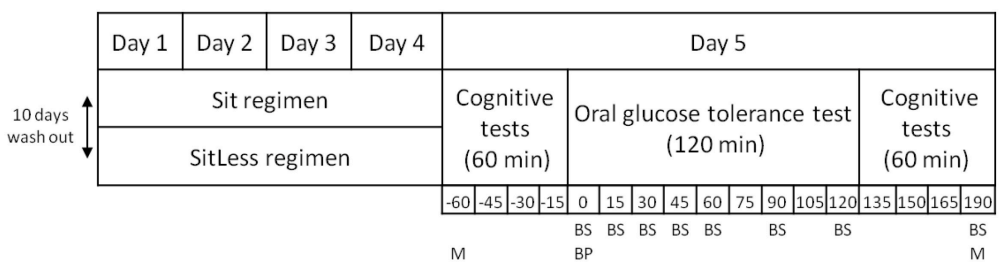

FIGURE 1 | Study design. BP, blood pressure; BS, blood sample; M, mood assessment.

able to measure the impact of interventions, such as physical activity (van Ommen et al., 2014). Mood was assessed with the Affect Grid test; which is a 19 × 19 single-item measure, assessing the self-reported degree of pleasantness and arousal of the participants (Russell et al., 1989). Verbal memory (immediate and delayed) was assessed with Rey's Verbal Learning Test (Van der Elst et al., 2005), executive function was assessed with the Trail Making Test (Bowie and Harvey, 2006; Oosterman et al., 2010), and attention with the Attention Network Test covering the dimensions alerting, orienting, and executive function (Fan et al., 2005). On day 4 of each activity regimen, quality of life was assessed with a 32-item questionnaire of Gill et al. (2013) and sleep quality was assessed with the 10-item Pittsburgh Sleep Quality Index (Buysse et al., 1989).

Data Processing and Statistical Analysis

The AUC over a period of 190 min after glucose ingestion was calculated for insulin and C-peptide using the trapezoidal rule approach (Brouns et al., 2005). For glucose, the positive incremental area under the curve (iAUC) was calculated as the AUC above the baseline level. Insulin sensitivity, expressed as the Matsuda index, was calculated based on glucose and insulin values during the first 120 min of the OGTT (Matsuda and DeFronzo, 1999).

All statistical calculations were performed using SAS (version 9.4, Cary, NC, USA) or IBM SPSS (version 21, Armonk, NY, USA). The differences in blood related outcome parameters and blood pressure between the activity regimens were analyzed using linear mixed model analyses including the activity regimen, order of the activity regimens and baseline characteristics as fixed factors. Since associations between sedentary behavior and cardiometabolic risk factors have previously been reported to be stronger in women (Owen et al., 2010), sex was added to the model as a co-variate. For the AUC and iAUC calculations, values at $t = 0$ were added as fixed factor to the model. For the mood scores (arousal and pleasantness), the linear mixed model included time as a categorical variable including its interaction with activity regimen, values at $t = 0$ and order of testing. The residual error structure was described with an ARH(1)-covariance matrix to handle variance heterogeneity at the time points. Similar analyses were performed for the cognitive parameters. For some subjects, part of the mood and cognition data was excluded from the statistical analysis due to technical errors during the mood and cognition tests. A log transformation was performed for glucose, insulin, C-peptide, and diastolic blood pressure. Numerical variables are presented as mean ± SD for baseline characteristics, mean ± standard error (SEM) for cardiometabolic risk factors and LSmeans (95% CI) for mood and cognition. *P*-values ≤ 0.05 were considered statistically significant.

RESULTS

Subjects

After screening 25 subjects (13 men, 12 women) were included. Before completing the protocol, one female participant withdrew because of cholangitis. The remaining 24 participants had a mean age of 64 ± 7 years and BMI of 29.4 ± 2.3 kg/m^2 (**Table 1**). Female participants had a significantly higher BMI and lower age and height than male participants. Five participants were using cholesterol lowering drugs (statins) and six participants were using blood pressure lowering drugs (3 angiotensin receptor blockers, 2 calcium channel blockers, 1 ACE-inhibitor, 1 beta blocker).

TABLE 1 | Subject characteristics.

Variables	Total	Men	Women
N	24	13	11
Age (years)*	64 ± 7	67 ± 2	59 ± 9
Height (m)*	1.72 ± 0.08	1.76 ± 0.07	1.68 ± 0.07
Weight (kg)	87.1 ± 9.7	88.3 ± 9.6	85.7 ± 10.1
BMI (kg/m^2)*	29.4 ± 2.3	28.5 ± 1.7	30.5 ± 2.5
Waist circumference (cm)†	104 ± 10	104 ± 8	103 ± 11
Systolic blood pressure (mmHg)	143 ± 17	148 ± 15	136 ± 18
Diastolic blood pressure (mmHg)	83 ± 9	83 ± 9	82 ± 8
Fasting glucose (mmol/l)	5.5 ± 0.6	5.5 ± 0.5	5.4 ± 0.7

*Data are presented as mean ± SD. *$p < 0.05$ for sex; †$n = 12$ for men; $n = 10$ for women.*

TABLE 2 | Cardiometabolic risk factors.

Variables	Sit	SitLess	*P*-value
Fasting glucose (mmol/l)	5.1 (0.1)	5.2 (0.1)	0.153
Glucose iAUC (mmol/l × min)	367 (40)	325 (36)	0.159
Fasting insulin (mU/l)	13.2 (1.0)	11.4 (0.9)	**0.003**
Insulin AUC (mU/l × min)	12,633 (818)	10,125 (656)	**0.006**
Fasting C-peptide (ng/ml)	1.75 (0.12)	1.53 (0.10)	**<0.001**
C-peptide AUC (ng/ml × min)	1,187 (42)	1,104 (39)	**0.032**
Apolipoprotein A-I (g/l)	1.45 (0.03)	1.46 (0.03)	0.366
Apolipoprotein B100 (g/l)	1.07 (0.04)	1.03 (0.03)	**0.007**
Free fatty acids (mmol/l)	0.59 (0.03)	0.69 (0.04)	**0.014**
Free glycerol (mmol/l)	0.14 (0.01)	0.16 (0.01)	0.062
Systolic BP (mmHg)	138 (4)	137 (3)	0.729
Diastolic BP (mmHg)	81 (1)	79 (1)	**0.043**
HR (beats/min)	64 (2)	62 (2)	0.170

Data are presented as mean (SEM). BP, blood pressure; HR, heart rate; iAUC, incremental AUC. Bold values indicate $p < 0.05$.

Insulin Sensitivity

After the activity regimens, there was no significant difference in the iAUC for glucose between Sit and SitLess (**Table 2**). AUC for insulin (**Table 2**; **Figure 2**) decreased by 20% after SitLess vs. Sit [mean (SEM): 10,125 (656) vs. 12,633 (818); $p = 0.006$]. As a result, insulin sensitivity (**Figure 3**) was 16% higher after SitLess vs. Sit [Matsuda-index: 6.45 (0.25) vs. 5.58 (0.25) respectively; $p < 0.001$]. The AUC for C-peptide was 7% lower ($p = 0.032$) after SitLess vs. Sit. In subgroup analyses the iAUC for glucose in women was lower after SitLess vs. Sit (−32%; $p = 0.006$), while no significant difference was observed in men (+14%; $p = 0.266$). No sex-differences were observed in Matsuda-index and AUC for insulin and C-peptide.

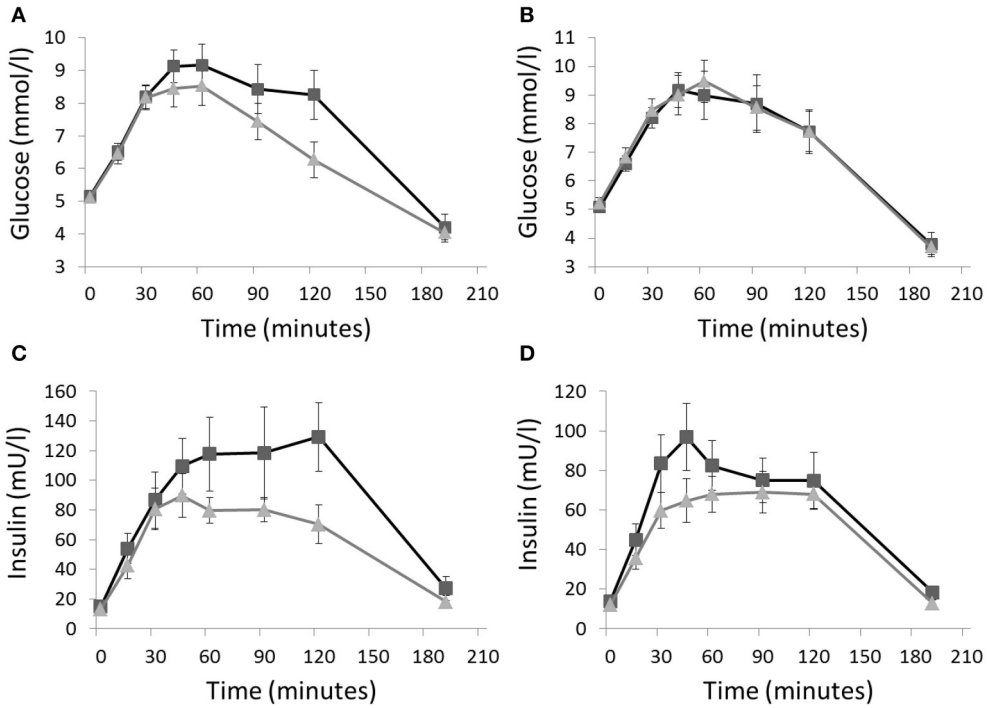

FIGURE 2 | Glucose and insulin responses to an oral glucose tolerance test on the morning after the Sit (■) and SitLess (▲) regimens for respectively women **(A,C)** and men **(B,D)**. iAUC for glucose in women was lower after SitLess vs. Sit ($p = 0.006$), but not in men ($p = 0.266$). AUC for insulin was significantly lower after SitLess vs. Sit in men and women ($p = 0.006$). Means and standard error bars are presented.

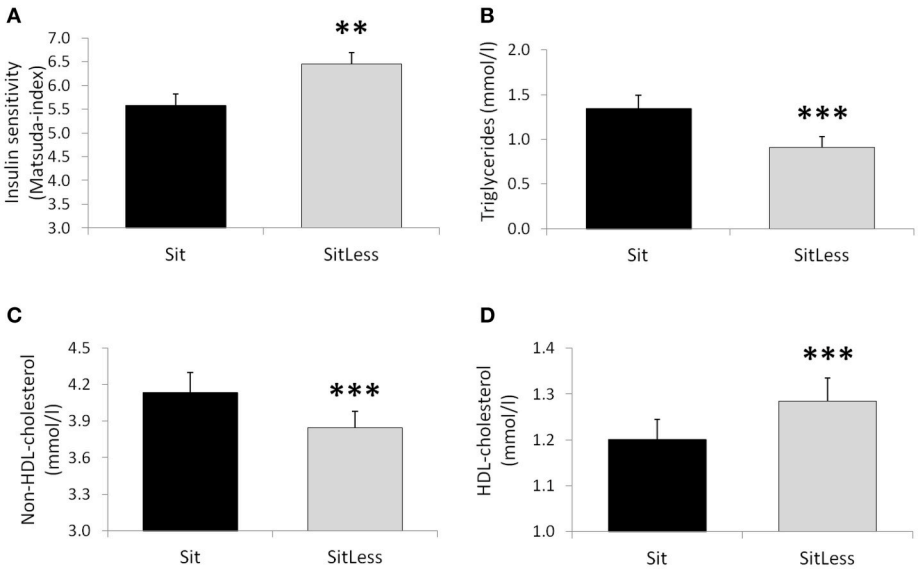

FIGURE 3 | Insulin sensitivity (Matsuda-index; **A**), triglycerides **(B)**, non-HDL-cholesterol **(C)** and HDL-cholesterol **(D)** on the morning after the activity regimens. Means and standard error bars are presented. $^{**}p < 0.01$; $^{***}p < 0.001$.

Circulating Lipids and Blood Pressure

After the activity regimens, triglycerides, total cholesterol, non-HDL-cholesterol, and apolipoprotein B were lower following SitLess vs. Sit by 32, 4, 7, and 4% respectively (all $p < 0.01$; **Table 2**; **Figure 3**). HDL-cholesterol was 7% higher ($p < 0.001$) and FFA levels were 17% higher ($p = 0.014$) after SitLess vs. Sit. Diastolic blood pressure was lower after SitLess vs. Sit ($p = 0.043$). Systolic blood pressure, heart rate, apolipoprotein A, and free

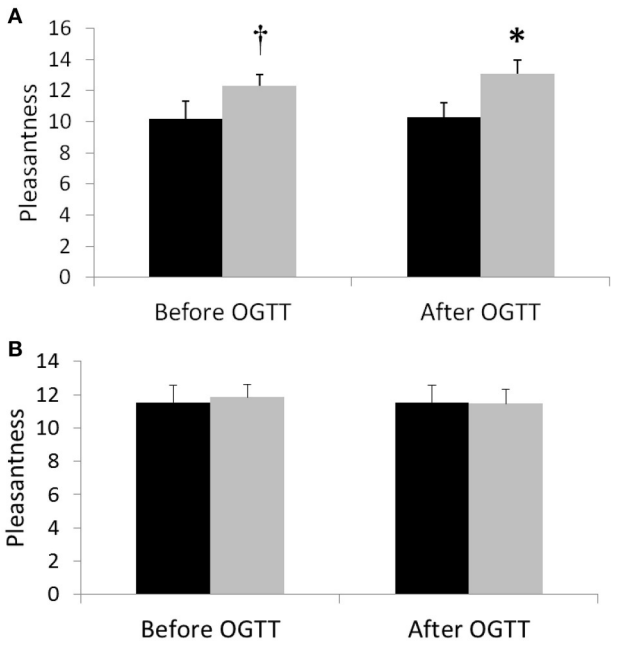

FIGURE 4 | Pleasantness 1 day after the SitLess (gray) and Sit (black) regimens in women **(A)** and men **(B)**. Pleasantness was measured before (−60 min) and 190 min after administering an oral glucose drink (OGTT). $^{*}p < 0.05$; $^{\dagger}p = 0.059$.

glycerol did not differ significantly between Sit and SitLess. In subgroup analyses, the magnitude of triglyceride attenuation was significantly greater in men (−38%; $p < 0.001$) than in women (−27%; $p < 0.001$) after SitLess vs. Sit. No sex-differences were observed in the other lipid variables, blood pressure, and heart rate.

Mood and Cognition

After the activity regimens, we performed measurements of mood and cognition both before the OGTT in the fasted state, as well as after an OGTT. Before the OGTT, pleasantness was not different between the activity regimens for the total group, although a non-significant improvement ($p = 0.059$) was observed in women after SitLess vs. Sit (estimated change 2.20, 95% CI: −0.08–4.48; $n = 10$; **Figure 4**). After the OGTT, pleasantness was significantly higher after SitLess vs. Sit (1.67; CI: 0.09–3.25; $n = 21$) in the total group; this could mainly be explained by a significant difference in pleasantness in the female subjects after SitLess vs. Sit (2.80; CI: 0.52–5.08; $n = 10$). There was no significant difference in the alerting, orienting and executive dimensions of attention between the activity regimens, neither before nor after the OGTT. Only in female subjects after the OGTT, alertness was significantly higher (−14.8 ms; CI: −29.1 to −0.5; $n = 11$) after SitLess vs. Sit. There were no significant differences in memory, executive function, quality of life, and sleep between the activity regimens.

TABLE 3 | Physical activity and diet.

Variables	Sit	SitLess	*P*-value
PHYSICAL ACTIVITY			
Sitting (h/day)	13.5 (0.2)	7.6 (0.3)	**<0.001**
Standing (h/day)	1.4 (0.1)	4.0 (0.2)	**<0.001**
Walking (h/day)	0.7 (0.1)	4.3 (0.1)	**<0.001**
Sleeping (h/day)	8.2 (0.2)	8.0 (0.2)	**0.027**
Steps/day (n)	3,228 (187)	24,626 (509)	**<0.001**
Sedentary bouts >30min (n/day)	8.5 (0.3)	3.9 (0.2)	**<0.001**
DIET			
Energy intake (kcal/day)	1,930 (77)	1,943 (94)	0.669
Carbohydrates (%)	47.3 (1.4)	47.9 (1.3)	0.422
Protein (%)	17.8 (0.7)	18.0 (0.8)	0.491
Fat (%)	34.8 (1.3)	34.1 (1.2)	0.205
Saturated fat (%)	13.3 (0.5)	13.3 (0.5)	0.723

Daily activities (activPAL data) and diet (diary data) during each activity regimen. Data are presented as mean (SEM). Bold values indicate p < 0.05.

Physical Activity and Diet

At baseline (before the start of the study), time spent sitting/lying was 18.4 ± 1.6 h/day, walking 1.8 ± 0.6 h/day and standing 3.8 ± 1.2 h/day. During the activity regimens, time spent sitting, walking, and standing in free-living conditions were successfully altered in accordance with the protocol (**Table 3**). During SitLess, time spent sitting (7.6 h/day), walking (4.0 h/day) and standing (4.3 h/day) were significantly different than during Sit (13.5 h/day sitting, 0.7 h/day walking, and 1.4 h/day standing). Sedentary bouts >30 min were significantly lower during SitLess (3.9 bouts) compared to Sit (8.5 bouts). Sleeping time was comparable between SitLess (8.0 h/day) and Sit (8.2 h/day). Energy intake did not differ significantly between the activity regimens, neither did the percentage macronutrients consumed (**Table 3**).

DISCUSSION

In the current study, we observed that substituting sitting with standing and self-perceived light walking improved insulin sensitivity, circulating lipids and diastolic blood pressure in overweight/obese subjects. Interestingly, while other studies reported positive effects on plasma glucose and insulin during interruptions in sitting time (Dunstan et al., 2012; Peddie et al., 2013; Blankenship et al., 2014), we observed improvements in insulin sensitivity 1 day after the SitLess intervention, suggesting that this beneficial effect persists into the next day. These results build on our previous findings in young healthy (Duvivier et al., 2013) and diabetic adults (Duvivier et al., 2017), strongly suggesting that light activities are a very effective measure to improve insulin sensitivity.

In addition to the effects on insulin sensitivity, we observed major improvements in circulating lipids after the SitLess regimen. Interestingly, the magnitude of the changes was comparable or larger than observed with exercise. Thus, exercise training has consistently been shown to increase HDL-cholesterol levels; a meta-analysis of RCT's reported an average 0.06

mmol/l increase when adhering to the exercise (∼150 min/week) guidelines (Kodama et al., 2007). In comparison, the SitLess regimen in our study resulted in an HDL-cholesterol increase of 0.08 mmol/l. To our knowledge, we are the first to show an increase in HDL-cholesterol after an acute sit less intervention. Hence, light activities such as standing and light walking seem to be effective in increasing HDL-cholesterol levels to a similar degree as exercise. In line with this result, we also observed a profound reduction in triglycerides (−32%) as well as a reduction in non-HDL-cholesterol, apolipoprotein B and diastolic blood pressure after the SitLess regimen, suggesting that reducing sitting time improves the cardiometabolic profile even further.

Our results may be especially important for sedentary overweight/obese subjects as these individuals are at high risk of developing cardiometabolic disease (Hubert et al., 1983; Mokdad et al., 2003). It was recently observed that each additional hour of sitting increased the odds for type 2 diabetes and metabolic syndrome by 22 respectively 39% (van der Berg et al., 2016b). Engaging in structured exercise as a countermeasure is a challenge for many individuals. Less than 5% of the population adheres to the exercise guidelines (Troiano et al., 2008) and physical activity has been reported to be even lower in people who are obese (Levine et al., 2005; de Rooij et al., 2016). Hence, reducing sedentary behavior might be a more feasible alternative. Strategies to reduce sitting time are generally considered less demanding than structured exercise programs and hence are more likely to have long term compliance (Martin et al., 2015). Our observations suggest that substituting sitting with light activities may have major cardiometabolic benefits and could potentially reverse the adverse cardiometabolic risk that is associated with sedentary behavior.

We observed sex-differences in glucose tolerance between the activity regimens. In comparison to the Sit regimen, SitLess lowered glucose iAUC levels significantly in female participants (−32%), but did not differ significantly in male participants (+14%). In contrary, the magnitude of triglyceride attenuation was significantly greater in men than in women after the SitLess regimen. These differences could not be explained by sex-differences in physical activity or diet during the activity regimens. The sex-differences for glucose are in line with a recent intervention study in obese adults with type 2 diabetes (Dempsey et al., 2016), in which postprandial glucose levels were also significantly lower in women (–58%) than in men (–26%) when sitting was interrupted with self-perceived light-intensity walking. It is possible that sex-differences in adipose and lean muscle mass can explain our observations; however, these variables were not measured in our study. Further studies should shed light on the underlying mechanisms explaining these possible sex-differences.

We observed that insulin sensitivity improved after the SitLess intervention, which is consistent with previous findings reporting an upregulation of the insulin signaling pathway after 3 days of interrupting sitting with light-intensity walking (Bergouignan et al., 2016). The decrease in triglyceride levels after the SitLess regimen could possibly be explained by enhanced lipoprotein lipase activity; thus, physical activity increases lipoprotein lipase mRNA and typically peaks ≥4 h after physical activity (Seip et al., 1997) and our results suggest that light-intensity activity may already be sufficient to elicit such effect. An inverse relationship is known to exist between the triglycerides and HDL-cholesterol levels. During exercise, the action of cholesterol esther transfer protein (CETP) produces triglyceride-rich HDL2 particles, resulting in an HDL-cholesterol increase (Zhang et al., 2013). Therefore, the reduction in triglycerides could have contributed to the increase in HDL-cholesterol following the SitLess regimen. We also observed, in line with previous exercise (Bilet et al., 2011) and light-intensity activity studies (Henson et al., 2016; Duvivier et al., 2017), that FFA levels were higher following the SitLess regimen. This increase in FFA levels was accompanied by a non-significant ($p = 0.06$) increase in free glycerol and may therefore result from elevation of adipose tissue lipolysis to fuel muscle for contractile activity (Jocken and Blaak, 2008).

In addition to cardiometabolic risk factors, we also explored the effects of reducing sitting time on mood and cognition. We observed significant improvements following the SitLess regimen in pleasantness after the OGTT in women. This result is in line with a recent study that observed sex-differences in mood response to exercise (McDowell et al., 2016). Also, alertness was somewhat higher after the OGTT in women following the SitLess regimen. Further research is necessary to assess the robustness of these sex-differences observed.

Strengths of our study include the cross-over randomized design in free-living conditions. Also, adherence to the activity regimens was according to the protocol which was measured 24 h/day by a validated activity monitor. Diet was standardized and energy intake and macronutrient percentage did not differ between the activity regimens. However, the study was not powered to detect differences in mood and cognition or to detect sex-differences. Hence, these findings should be considered exploratory and need replication. This study was a proof-of-concept study of short duration, and as a result the number of steps during the SitLess regimen (about 25,000 steps/day) was well above what is on average observed in a healthy population (about 6,000–13,000 steps/day; Tudor-Locke and Myers, 2001). Thus, the next logical step is to perform dose-response studies to inform about the optimal duration and pattern of time spent standing and light walking and its feasibility in real life circumstances. It also needs to be established whether the acute changes observed in this study persist on the longer-term.

CONCLUSION

In conclusion, our study suggests that substituting sitting with standing and self-perceived light walking is a very effective strategy to improve insulin sensitivity, circulating lipids, and diastolic blood pressure in sedentary overweight/obese subjects. Particularly for overweight/obese individuals, these results may be important as strategies to reduce sitting time are generally considered less demanding than structured exercise programs.

AUTHOR CONTRIBUTIONS

BD, HS, PS, HP, JA, Lv, and TG conceived and designed the experiments. BD and LV performed the experiments, enrolled patients, and performed the data collection. BD, EK, MH and PW performed the data analysis. BD, HS, PS, HP, JA, TG, NS, AK, and MH were involved data interpretation. BD wrote the first draft of the manuscript. All authors contributed to the writing of the manuscript and approved the final version of the manuscript.

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REFERENCES

Bailey, D. P., and Locke, C. D. (2015). Breaking up prolonged sitting with light-intensity walking improves postprandial glycemia, but breaking up sitting with standing does not. *J. Sci. Med. Sport.* 18, 294–298. doi: 10.1016/j.jsams.2014.03.008

Berendsen, B. A., Hendriks, M. R., Meijer, K., Plasqui, G., Schaper, N. C., and Savelberg, H. H. (2014). Which activity monitor to use? Validity, reproducibility and user friendliness of three activity monitors. *BMC Public Health* 14:749. doi: 10.1186/1471-2458-14-749

Bergouignan, A., Latouche, C., Heywood, S., Grace, M. S., Reddy-Luthmoodoo, M., Natoli, A. K., et al. (2016). Frequent interruptions of sedentary time modulates contraction- and insulin-stimulated glucose uptake pathways in muscle: ancillary analysis from randomized clinical trials. *Sci. Rep.* 6:32044. doi: 10.1038/srep32044

Bilet, L., van de Weijer, T., Hesselink, M. K., Glatz, J. F., Lamb, H. J., Wildberger, J., et al. (2011). Exercise-induced modulation of cardiac lipid content in healthy lean young men. *Basic Res. Cardiol.* 106, 307–315. doi: 10.1007/s00395-010-0144-x

Biswas, A., Oh, P. I., Faulkner, G. E., Bajaj, R. R., Silver, M. A., Mitchell, M. S., et al. (2015). Sedentary time and its association with risk for disease incidence, mortality, and hospitalization in adults: a systematic review and meta-analysis. *Ann. Intern. Med.* 162, 123–132. doi: 10.7326/M14-1651

Blankenship, J. M., Granados, K., and Braun, B. (2014). Effects of subtracting sitting versus adding exercise on glycemic control and variability in sedentary office workers. *Appl. Physiol. Nutr. Metab.* 39, 1286–1293. doi: 10.1139/apnm-2014-0157

Bowie, C. R., and Harvey, P. D. (2006). Administration and interpretation of the Trail Making Test. *Nat. Protoc.* 1, 2277–2281. doi: 10.1038/nprot.2006.390

Brouns, F., Bjorck, I., Frayn, K. N., Gibbs, A. L., Lang, V., Slama, G., et al. (2005). Glycaemic index methodology. *Nutr. Res. Rev.* 18, 145–171. doi: 10.1079/NRR2005100

Brown, A. K., Liu-Ambrose, T., Tate, R., and Lord, S. R. (2009). The effect of group-based exercise on cognitive performance and mood in seniors residing in intermediate care and self-care retirement facilities: a randomised controlled trial. *Br. J. Sports Med.* 43, 608–614. doi: 10.1136/bjsm.2008.049882

Buysse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R., and Kupfer, D. J. (1989). The pittsburgh sleep quality index: a new instrument for psychiatric practice and research. *Psychiatry Res.* 28, 193–213. doi: 10.1016/0165-1781(89)90047-4

Chastin, S. F., Palarea-Albaladejo, J., Dontje, M. L., and 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:e0139984. doi: 10.1371/journal.pone.0139984

Dempsey, P. C., Larsen, R. N., Sethi, P., Sacre, J. W., Straznicky, N. E., Cohen, N. D., et al. (2016). Benefits for Type 2 diabetes of interrupting prolonged sitting with brief bouts of light walking or simple resistance activities. *Diabetes Care* 39, 964–972. doi: 10.2337/dc15-2336

de Rooij, B. H., van der Berg, J. D., van der Kallen, C. J., Schram, M. T., Savelberg, H. H., Schaper, N. C., et al. (2016). Physical activity and sedentary behavior in metabolically healthy versus unhealthy obese and non-obese individuals - the maastricht study. *PLoS ONE* 11:e0154358. doi: 10.1371/journal.pone.0154358

Dunstan, D. W., Kingwell, B. A., Larsen, R., Healy, G. N., Cerin, E., Hamilton, M. T., et al. (2012). Breaking up prolonged sitting reduces postprandial glucose and insulin responses. *Diabetes Care* 35, 976–983. doi: 10.2337/dc11-1931

Duvivier, B. M., Schaper, N. C., Bremers, M. A., van Crombrugge, G., Menheere, P. P., Kars, M., et al. (2013). Minimal intensity physical activity (standing and walking) of longer duration improves insulin action and plasma lipids more than shorter periods of moderate to vigorous exercise (cycling) in sedentary subjects when energy expenditure is comparable. *PLoS ONE* 8:e55542. doi: 10.1371/journal.pone.0055542

Duvivier, B. M., Schaper, N. C., Hesselink, M. K., van Kan, L., Stienen, N., Winkens, B., et al. (2017). Breaking sitting with light activities vs structured exercise: a randomised crossover study demonstrating benefits for glycaemic control and insulin sensitivity in type 2 diabetes. *Diabetologia* 60, 490–498. doi: 10.1007/s00125-016-4161-7

Fan, J., McCandliss, B. D., Fossella, J., Flombaum, J. I., and Posner, M. I. (2005). The activation of attentional networks. *Neuroimage* 26, 471–479. doi: 10.1016/j.neuroimage.2005.02.004

Friedewald, W. T., Levy, R. I., and Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* 18, 499–502.

Gill, D. L., Hammond, C. C., Reifsteck, E. J., Jehu, C. M., Williams, R. A., Adams, M. M., et al. (2013). Physical activity and quality of life. *J. Prev. Med. Public Health* 46(Suppl. 1), S28–S34. doi: 10.3961/jpmph.2013.46.s.s28

Heni, M., Kullmann, S., Preissl, H., Fritsche, A., and Haring, H. U. (2015). Impaired insulin action in the human brain: causes and metabolic consequences. *Nat. Rev. Endocrinol.* 11, 701–711. doi: 10.1038/nrendo.2015.173

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

Hubert, H. B., Feinleib, M., McNamara, P. M., and Castelli, W. P. (1983). Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation* 67, 968–977. doi: 10.1161/01.CIR.67.5.968

Jocken, J. W., and Blaak, E. E. (2008). Catecholamine-induced lipolysis in adipose tissue and skeletal muscle in obesity. *Physiol. Behav.* 94, 219–230. doi: 10.1016/j.physbeh.2008.01.002

Kim, B., and Feldman, E. L. (2012). Insulin resistance in the nervous system. *Trends Endocrinol. Metab.* 23, 133–141. doi: 10.1016/j.tem.2011.12.004

Kodama, S., Tanaka, S., Saito, K., Shu, M., Sone, Y., Onitake, F., et al. (2007). Effect of aerobic exercise training on serum levels of high-density lipoprotein cholesterol: a meta-analysis. *Arch. Intern. Med.* 167, 999–1008. doi: 10.1001/archinte.167.10.999

Lamport, D. J., Lawton, C. L., Mansfield, M. W., and Dye, L. (2009). Impairments in glucose tolerance can have a negative impact on cognitive function: a systematic research review. *Neurosci. Biobehav. Rev.* 33, 394–413. doi: 10.1016/j.neubiorev.2008.10.008

Levine, J. A., Lanningham-Foster, L. M., McCrady, S. K., Krizan, A. C., Olson, L. R., Kane, P. H., et al. (2005). Interindividual variation in posture allocation: possible role in human obesity. *Science* 307, 584–586. doi: 10.1126/science.1106561

Martin, A., Fitzsimons, C., Jepson, R., Saunders, D. H., van der Ploeg, H. P., Teixeira, P. J., et al. (2015). Interventions with potential to reduce sedentary time in adults: systematic review and meta-analysis. *Br. J. Sports Med.* 49, 1056–1063. doi: 10.1136/bjsports-2014-094524

Matsuda, M., and DeFronzo, R. A. (1999). Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 22, 1462–1470. doi: 10.2337/diacare.22.9.1462

Matthews, C. E., Chen, K. Y., Freedson, P. S., Buchowski, M. S., Beech, B. M., Pate, R. R., et al. (2008). Amount of time spent in sedentary behaviors in the United States, 2003-2004. *Am. J. Epidemiol.* 167, 875–881. doi: 10.1093/aje/kwm390

McDowell, C. P., Campbell, M. J., and Herring, M. P. (2016). Sex-related differences in mood responses to acute aerobic exercise. *Med. Sci. Sports Exerc.* 48, 1798–1802. doi: 10.1249/MSS.0000000000000969

McElroy, S. L., Kotwal, R., Malhotra, S., Nelson, E. B., Keck, P. E., and Nemeroff, C. B. (2004). Are mood disorders and obesity related? A review for the mental health professional. *J. Clin. Psychiatry* 65, 634–651. doi: 10.4088/JCP.v65n0507

Mokdad, A. H., Ford, E. S., Bowman, B. A., Dietz, W. H., Vinicor, F., Bales, V. S., et al. (2003). Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA* 289, 76–79. doi: 10.1001/jama.289.1.76

Oosterman, J. M., Vogels, R. L., van Harten, B., Gouw, A. A., Poggesi, A., Scheltens, P., et al. (2010). Assessing mental flexibility: neuroanatomical and neuropsychological correlates of the Trail Making Test in elderly people. *Clin. Neuropsychol.* 24, 203–219. doi: 10.1080/13854040903482848

Owen, N., Healy, G. N., Matthews, C. E., and Dunstan, D. W. (2010). Too much sitting: the population health science of sedentary behavior. *Exerc. Sport Sci. Rev. Vol.* 38, 105–113. doi: 10.1097/JES.0b013e3181e373a2

Peddie, M. C., Bone, J. L., Rehrer, N. J., Skeaff, C. M., Gray, A. R., and Perry, T. L. (2013). Breaking prolonged sitting reduces postprandial glycemia in healthy, normal-weight adults: a randomized crossover trial. *Am. J. Clin. Nutr.* 98, 358–366. doi: 10.3945/ajcn.112.051763

Russell, J. A., Weis, A., and Mendelsohn, G. A. (1989). Affect grid: a single-item scale of pleasure and arousal. *J. Pers. Soc. Psychol.* 57, 493–502. doi: 10.1037/0022-3514.57.3.493

Ryan, C. G., Grant, P. M., Tigbe, W. W., and Granat, M. H. (2006). The validity and reliability of a novel activity monitor as a measure of walking. *Br. J. Sports Med.* 40, 779–784. doi: 10.1136/bjsm.2006.027276

Seip, R. L., Mair, K., Cole, T. G., and Semenkovich, C. F. (1997). Induction of human skeletal muscle lipoprotein lipase gene expression by short-term exercise is transient. *Am. J. Physiol.* 272(2 Pt 1), E255–E261.

Smith, E., Hay, P., Campbell, L., and Trollor, J. N. (2011). A review of the association between obesity and cognitive function across the lifespan: implications for novel approaches to prevention and treatment. *Obes. Rev.* 12, 740–755. doi: 10.1111/j.1467-789X.2011.00920.x

Smith, P. J., Blumenthal, J. A., Hoffman, B. M., Cooper, H., Strauman, T. A., Welsh-Bohmer, K., et al. (2010). Aerobic exercise and neurocognitive performance: a meta-analytic review of randomized controlled trials. *Psychosom. Med.* 72, 239–252. doi: 10.1097/PSY.0b013e3181d14633

Troiano, R. P., Berrigan, D., Dodd, K. W., Masse, L. C., Tilert, T., and McDowell, M. (2008). Physical activity in the United States measured by accelerometer. *Med. Sci. Sports Exerc.* 40, 181–188. doi: 10.1249/mss.0b013e31815a51b3

Tudor-Locke, C. E., and Myers, A. M. (2001). Methodological considerations for researchers and practitioners using pedometers to measure physical (ambulatory) activity. *Res. Q. Exerc. Sport* 72, 1–12. doi: 10.1080/02701367.2001.10608926

van der Berg, J. D., Stehouwer, C. D., Bosma, H., van der Velde, J. H., Willems, P. J., Savelberg, H. H., et al. (2016a). Associations of total amount and patterns of sedentary behaviour with type 2 diabetes and the metabolic syndrome: the Maastricht Study. *Diabetologia* 59, 709–718. doi: 10.1007/s00125-015-3861-8

van der Berg, J. D., Willems, P. J., van der Velde, J. H., Savelberg, H. H., Schaper, N. C., Schram, M. T., et al. (2016b). Identifying waking time in 24-h accelerometry data in adults using an automated algorithm. *J. Sports Sci.* 34, 1867–1873. doi: 10.1080/02640414.2016.1140908

Van der Elst, W., van Boxtel, M. P., van Breukelen, G. J., and Jolles, J. (2005). Rey's verbal learning test: normative data for 1855 healthy participants aged 24-81 years and the influence of age, sex, education, and mode of presentation. *J. Int. Neuropsychol. Soc.* 11, 290–302. doi: 10.1017/s1355617705050344

van Ommen, B., van der Greef, J., Ordovas, J. M., and Daniel, H. (2014). Phenotypic flexibility as key factor in the human nutrition and health relationship. *Genes Nutr.* 9:423. doi: 10.1007/s12263-014-0423-5

Wojtaszewski, J. F., Hansen, B. F., Gade, J., Kiens, B., Markuns, J. F., Goodyear, L. J., et al. (2000). Insulin signaling and insulin sensitivity after exercise in human skeletal muscle. *Diabetes* 49, 325–331. doi: 10.2337/diabetes.49.3.325

Young, D. R., Hivert, M. F., Alhassan, S., Camhi, S. M., Ferguson, J. Katzmarzyk, F., et al. (2016). Sedentary behavior and cardiovascular morbidity and mortality: a science advisory from the american heart association. *Circulation* 134, e262–279. doi: 10.1161/CIR.0000000000000440

Zhang, B., Kawachi, E., Miura, S., Uehara, Y., Matsunaga, A., Kuroki, M., et al. (2013). Therapeutic approaches to the regulation of metabolism of high-density lipoprotein. Novel HDL-directed pharmacological intervention and exercise. *Circ. J.* 77, 2651–2663. doi: 10.1253/circj.CJ-12-1584

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The other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
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An Under-the-Table Leg-Movement Apparatus and Changes in Energy Expenditure

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Introduction: Deskwork contributes substantially to sedentariness. Here, we evaluated an under-the-table apparatus that was designed to promote leg movement (fidgeting) while seated. Our hypothesis was that the under-the-table apparatus would increase energy expenditure.

Methods: We measured energy expenditure and heart rate in 26 people while they sat and worked using a standard chair, walked on a treadmill, and sat and worked using an under-the-desk apparatus that encouraged leg movement.

Results: Energy expenditure increased significantly while using the under-the-table apparatus when compared to the standard office chair (standard chair, 81 ± 18 kcal/h; under-the-table apparatus, 96 ± 23 kcal/h) ($P < 0.001$); representing an 18 ± 16% increase. The changes in energy expenditure were not as great as walking (1 mph, 168 ± 46 kcal/h, $P < 0.001$; 2 mph, 205 ± 51 kcal/, $P < 0.001$), representing 107 ± 37% and 155 ± 48% increases over baseline, respectively.

Conclusions: An under-the-table apparatus that promotes leg movement can increase energy expenditure by approximately 20%. Dynamic sitting is promoted by this apparatus and may be among a lexicon of options to help people move more while seated at work.

Keywords: energy expenditure, fidget, non-exercise activity thermogenesis, sedentary behavior, sitting disease

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INTRODUCTION

Sitting excessively, as occurs with any desk-bound job, is associated with increased rates of obesity, impaired cognition, and numerous other chronic diseases (Dunstan et al., 2011; Thyfault et al., 2014; Falck et al., 2016). The majority of adults' weekly waking hours are spent at work, which is invariable sedentary (McCrady and Levine, 2009). Hence, solutions to reverse work-time sitting and encourage daily movement (non-exercise activity thermogenesis [NEAT]) are necessary (Levine, 2010).

Excessive sitting can, in part, be attributed to the computer-based nature of modern work and to the standard office design, both of which encourage employees to remain seated throughout the workday (McCrady and Levine, 2009). Walking or standing while at work are 2 possible solutions for disrupting total workplace sitting time (Dempsey et al., 2016); however, these options are often not practical (Judice et al., 2015; Levin and Chisholm, 2016) because leaving a workstation or office can hinder workflow (Stengard et al., 2016). New methods are needed to help sedentary workers move more.

One approach to decreasing workplace sitting is to transform sitting into an active behavior, termed *dynamic sitting*. Laboratory studies have shown that people who fidget (move) while sitting increase energy expenditure by up to 10% more than those who do not (Levine et al., 2000). In one example of dynamic sitting, office chairs are replaced with large rubber balls (exercise stability ball) (Marks et al., 2012) so that a worker has to continuously fine-tune his or her balance and trunk musculature to maintain posture. Another dynamic sitting solution, such as with the apparatus we tested, is to encourage fidgeting and/or leg movements while seated (Pynt, 2015).

Walking, even slowly, doubles energy expenditure (Bouten et al., 1996; Westerterp et al., 1996); however, sitting, in general, is not exothermic (0–10% increase above basal metabolic rate) (Bouten et al., 1996; Westerterp et al., 1996). Here, we examine whether a commercial apparatus that promotes dynamic sitting can increase energy expenditure and heart rate above resting values. We compared these values to low-speed walking, which is known to improve overall health (Buckley et al., 2015). We hypothesized that the under-the-table dynamic-sitting apparatus we tested was associated with increased energy expenditure compared to sitting in a standard office chair. Because exercise is associated with increased heart rate, which in turn is linked to decreased morbidity and mortality (Chave et al., 1978; Pratley et al., 2000), we assessed the impact of the under-the-table dynamic-sitting apparatus on heart rate as well.

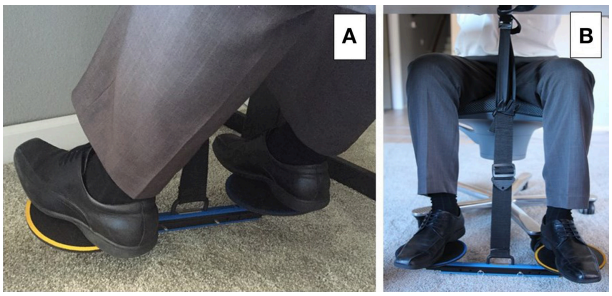

FIGURE 1 | Under-the-table leg-movement apparatus (A,B).

PARTICIPANTS AND METHODS

Participants

Participants provided informed written consent and the Mayo Clinic Institutional Review Board approved the protocol. Twenty-six participants (14 women and 12 men) were included with a mean (±SD) age of 23 ± 5 years and a body mass index (BMI) of 26 ± 5.5 kg/m^2.

Standard Office Chair

The criterion model chair (the "control chair") used is a standard office chair (Steelcase, Grand Rapids, MI).

Under-the-Table Leg-Movement Apparatus

The HOVR (Active Ideas LLC, Chicago, IL) is a pendulum attached to the underside of a desk or a portable stand. At the end of the pendulum are two discs mounted on an adjustable balanced beam (**Figure 1**).

Attachment to the Pre-existing Desk

At the top of the pendulum is a dense plastic clip. The clip is hung from a metal hook on the bottom side of the desk or 40-cm portable stand designed to fit under a standard office desk. The fastener mounted to the underside of the desk is securely attached with 4 screws. The pendulum may be moved up and down, forward and backward to achieve the user's desired position for both attachment options. In this study, the under-the-desk mount was used.

The pendulum is constructed from a 5-cm-wide nylon webbed strap and is adjustable from approximately 20 to 70 cm. At the bottom of the pendulum is another dense plastic clip, identical to one at the top. This clip is fastened to a metal pin (3.5-cm long and 0.5-cm in diameter). The metal pin is the fulcrum for the adjustable balanced beam and discs (**Figure 1**).

The balanced beam is constructed from dense plastic and rubber. It is adjustable to 37, 42, and 47 cm with 2 screws to accommodate users' varying sizes and preferences. At each end of the balance beam, identical 16-cm diameter metal discs are mounted with "ball and socket" metal hardware that allows for approximately 20° of motion in any direction relative to the position of the balance beam. The edge (circumference) and the top of the disc are covered by rubber to add a greater friction coefficient, which prevents the participant's feet from slipping. The discs also spin freely on the z-axis to allow leg movement without needing to readjust the feet (**Figure 1**).

Protocol

Prior to testing, participants were shown the equipment and the experimental protocol was explained. Body composition, height, weight, and blood pressure were all measured. Participants confirmed that they had not consumed any food or beverage aside from water in the 2 h preceding testing. Patients then rested, sitting comfortably at rest in a shaded quiet room for 30 min. They were not permitted to speak, eat, or use mobile devices during testing.

Participants were tested in thermal comfort (25.2 ± 0.7°C, 956.9 + 1.8 mBar barometric pressure, and 57.0 + 2.1% humidity).

Sitting energy expenditure and heart rate were measured for 20 min via indirect calorimetry. During this time, participants worked at a computer and sat on a standard office chair (Criterion; Steelcase, Grand Rapids, MI) in an effort to simulate their normal work activity. Data for the first 2 and final 2 min were excluded. Following this, subjects rested for 20 min while sitting (not working).

For the next timed interval, participants used the under-the-table dynamic-sitting apparatus while working at the computer. As with the previous segment, energy expenditure and heart rate were measured for 20 min, and data for the first 2 and final 2 min were excluded. Participants again rested in a sitting position for 20 min following testing.

Finally, participants were asked to walk on a calibrated treadmill (4Front; Woodway, Waukesha, WI) at 1 mph and 2 mph, each for 20 min. These speeds were thought to be comparable to the rates of people walking while at work (Ben-Ner et al., 2014). Energy expenditure and heart rate were measured throughout the walks, and data for the first 2 and final 2 min of each velocity were excluded.

The order of the sitting and walking phases were not randomized. This was to avoid the effect of high energy expenditure (as occurs after walking) on lower exertion measurements (e.g., fidgeting). This approach was used when measuring small changes in energy expenditure (Levine et al., 1999, 2000).

METHODS

Body Composition

Participants' body composition and weight were measured using a calibrated Seca Medical Body Composition Analyzer 514 (Seca, Hamburg, Germany) (Heymsfield et al., 2000) while they were wearing light clothing (athletic shorts and t-shirt); height (without shoes) was measured using a Seca 217 stadiometer (Seca, Hamburg, Germany).

Energy Expenditure

Energy expenditure was measured using indirect calorimetry (Metamax 3B; Cortex, Leipzig, Germany) (Levine et al., 2000). The calorimeter was calibrated using 5.0% CO_2 15.0% O_2 balance nitrogen (Praxair Inc., Danbury, CT) and ambient air according to the manufacturer's specifications. In addition, the calorimeter was volume calibrated before each participant using a 3 L syringe. The calorimeter was able to collect breath-by-breath CO_2 and O_2 production and consumption, respectively, and energy expenditure was calculated using standard formulae (Weir, 1949).

Heart Rate Monitoring

Participants were also fitted with a Polar Heart Rate Monitor H7 (Polar Inc., Lake Success, NY). Heart rate samples were synchronized and recorded for each breath.

Statistical Analysis

Analysis of data with repeated measures needs to consider the covariance structure due to correlations between repeated measures across time or different conditions on each participant. Failure to properly take care of this issue could result in biased estimates. The univariate analysis of variance (ANOVA) assumes equal variances or correlations across time or conditions on each participant, and this might not be true. In many cases, participant correlations tend to decrease with increasing lag time between measures. To overcome this limitation of univariate ANOVA, the general linear mixed model is used in this manuscript. This model allows for different correlations between measures.

For analysis, the PROC MIXED with REPEATED statement was used in SAS (SAS Institute Inc., Cary, NC). The model assumed no specific variance-covariance structure (unstructured) based on Akaike Information Criterion values and –2 log likelihood scores of 4 models (unstructured, compound symmetry, auto-regressive, and auto-regressive heterogeneous variance-covariance). The original data in wide format consisted of 26 participants (14 women, 12 men), and 2 outcomes (energy expenditure and heart rate) were measured under 5 different conditions for each participant. The data were transposed to a long format for the linear mixed model, and total available sample size for analysis was 130 person-conditions (26 individuals × 5 conditions).

RESULTS

Participants tolerated the protocol without complaint. Anthropometric and body composition data are shown in **Table 1**. Four additional participants were studied (3 women, 1 man), but their data are not included in the analysis because it was incomplete due to technical failures. Omitting these 4 subjects did not influence the principal conclusion because, in all 4 cases, energy expenditure increased using the under-the-table dynamic-sitting apparatus.

Twenty-three of the participants reported that their jobs were sedentary in nature, whereas the remaining 3 reported having employment that necessitated a degree of movement throughout

TABLE 1 | Demographic and body composition information for 26 study volunteers[a].

	Women				Men				Total			
	Mean	***SD***	**Min**	**Max**	**Mean**	***SD***	**Min**	**Max**	**Mean**	***SD***	**Min**	**Max**
Height (cm)	165.2	3.7	156.0	170.7	176.9	5.7	167.4	187.5	170.6	7.5	156.0	187.5
Weight (kg)	71.7	20.4	46.4	118.1	81.5	15.4	66.8	121.3	76.2	18.6	46.4	121.3
BMI (kg/m^2)	26.4	8.0	16.6	42.3	26.0	4.6	20.9	37.3	26.2	6.5	16.6	42.3
Age (years)	38.2	16.7	19.0	64.0	26.7	8.5	18.0	44.0	32.9	14.5	18.0	64.0
BP: Systolic	116.3	18.8	94.0	157.0	114.6	14.2	93.0	145.0	115.5	16.6	93.0	157.0
BP: Diastolic	76.2	11.4	63.0	104.0	75.8	10.9	56.0	98.0	76.0	11.0	56.0	104.0
Body fat (%)	38.1	19.3	16.0	94.5	20.0	9.4	9.0	38.0	29.8	17.8	9.0	94.5
No. of patients	14				12				26			

[a] *Body fat was measured using bioelectrical impedance (Falck et al., 2016).*
BMI, body mass index; BP, blood pressure; SD, standard deviation.

TABLE 2 | Energy expenditure and heart rate by sex.

Phase	Energy expenditure (kcal per h)			Heart rate (bpm)		
	Women	Men	Total	Women	Men	Whole group
	Mean ± *SD*	Mean ± *SD*	Mean ± *SD*	Mean ± *SD*	Mean ± *SD*	Mean ± *SD*
Sitting	69.7 ± 12.8	94.5 ± 14.2	81.2 ± 18.2	71.2 ± 8.6	75.7 ± 15.9	73.2 ± 12.4
Using apparatus	81.7 ± 17.9[a]	112.1 ± 116.1[a]	95.7 ± 22.8[a]	73.4 ± 9.0[a]	77.6 ± 17.6	75.3 ± 13.6[a]
Walking at 1 mph	150.8 ± 43.8[a,b]	187.0 ± 42.5[a,b]	167.5 ± 46.1[a,b]	87.2 ± 13.4[a,b]	94.0 ± 41.4[a,b]	90.3 ± 29.3[a,b]
Walking at 2 mph	186.8 ± 55.4[a,b,c]	225.9 ± 35.8[a,b,c]	204.9 ± 50.5[a,b,c]	88.7 ± 13.4[a,b]	101.3 ± 51.2[a,b]	94.5 ± 35.9[a,b,c]
Walking at 3 mph	261.4 ± 87.6[a,b,c,d]	294.3 ± 48.6[a,b,c,d]	276.6 ± 72.8[a,b,c,d]	106.1 ± 17.7[a,b,c,d]	115.7 ± 73.6[a,b,c,d]	110.6 ± 50.7[a,b,c,d]

[a] *Significantly different from "sitting" condition at the P-value 0.05 level.*
[b] *Significantly different from "surfing" condition at the P-value 0.05 level.*
[c] *Significantly different from "1 mph" condition at the P-value 0.05 level.*
[d] *Significantly different from "2 mph" condition at the P-value 0.05 level. "Apparatus" refers to the apparatus to promote leg movement. SD, standard deviation.*

the workday. Of the 23 participants, 7 self-reported as being sedentary, 12 as being moderately active, and 6 as exercising regularly.

Energy expenditure for the 2 seated conditions (standard chair and under-the-table dynamic-sitting apparatus) and slow walking (1 and 2 mph) are shown in **Tables 2**, **3**. Energy expenditure while sitting in a standard chair showed a positive correlation with body weight ($r = 0.55$, $P = 0.003$). The relationship was described by the following equation:

Sitting energy expenditure (kcal/hr) = 0.544 × weight (kg) + 39.7.

Energy expenditure increased considerably while using the under-the-table dynamic-sitting apparatus when compared to a standard office chair (**Tables 2, 3**). Energy expenditure increased in 25 of 26 participants, from a mean of 81 ± 18 kcal/hr to 96 ± 23 kcal/h ($P < 0.001$), representing a mean increase of 18.4 ± 16.2%. There was a strong association between energy expenditure while sitting on a standard chair and energy expenditure using the under-the-table leg-movement apparatus ($r^2 = 0.76$; $P < 0.001$). Heart rate did not increase substantially when using the under-the-table leg-movement apparatus compared to sitting on a standard office chair without the apparatus (73 ± 12 cf 75 ± 14 beats/min) (**Figure 2**).

Changes in energy expenditure for the under-the-table leg-movement apparatus vs. the standard office chair were not as great as for walking at a speed of 1 or 2 mph (**Tables 2, 3**). The changes in energy expenditure were 15 ± 11 kcal/hr for the under-the-table leg-movement apparatus, 86 ± 24 kcal/hr for walking at 1 mph, and 124 ± 39 kcal/hr for walking at 2 mph. Slow walking at 1 and 2 mph were associated with significant increases in heart rate (rest, 73 ± 12 bpm; 1 mph, 90 ± 36 bpm; [$P < 0.001$]; and 2 mph, 111 ± 51 bpm [$P < 0.001$]) when compared to sitting in a standard office chair.

The results show that there is a significant difference in the overall level of energy expenditure between men and women ($P = 0.04$). However, these differences disappear after body weight is accounted for (**Table 3**). There were no differences in the overall heart rate level between men and women. However, there is a considerable conditioning effect whereby heart rate increased with walking, as was expected.

TABLE 3 | Energy expenditure and heart rate by weight and sex.

	Energy Expenditure (kcal/h/kg)		
	Women	Men	Total
Phase	Mean ± *SD*	Mean ± *SD*	Mean ± *SD*
Sitting	1.0 ± 0.2	1.2 ± 0.2	1.1 ± 0.2
Using apparatus	1.2 ± 0.2[a]	1.4 ± 0.2[a]	1.3 ± 0.2[a]
1 mph	2.1 ± 0.3[a,b]	2.3 ± 0.3[a,b]	2.2 ± 0.3[a,b]
2 mph	2.6 ± 0.3[a,b,c]	2.8 ± 0.2[a,b,c]	2.7 ± 0.3[a,b,c]
3 mph	3.6 ± 0.3[a,b,c,d]	3.6 ± 0.3[a,b,c,d]	3.6 ± 0.3[a,b,c,d]

[a] *significantly different from "sitting" condition at the P-value 0.05 level.*
[b] *Significantly different from "surfing" condition at the P-value 0.05 level.*
[c] *Significantly different from "1 mph" condition at the P-value 0.05 level.*
[d] *Significantly different from "2 mph" condition at the P-value 0.05 level. "Apparatus" refers to the apparatus to promote leg movement. SD, standard deviation.*

DISCUSSION

Excessive sitting is linked with chronic disease, impaired cognition, and obesity (Dunstan et al., 2011; Thyfault et al., 2014; Falck et al., 2016). The majority of adults' weekly waking hours are spent at work, which is invariable sedentary (McCrady and Levine, 2009). Hence, solutions to considerably decrease work-time sitting and encourage daily movement are necessary. In this study, we found that when a person sat and used an under-the-table dynamic-sitting apparatus, energy expenditure increased by about 20%. Heart rate, however, did not increase substantially. The reason for this is that the movement promoted by the under-the-table apparatus is sufficient to increase energy expenditure through leg muscle activity, but not sufficiently intense enough to accelerate heart rate markedly (Levine et al., 2000). It is not surprising that energy expenditure increased significantly based on leg movements alone because gluteal-femoral muscular contractions contribute substantially to human energy expenditure (Westerterp et al., 1996; Westerterp and Bouten, 1997). What is important to note is that these types of movements may directly impact glycemic control and other

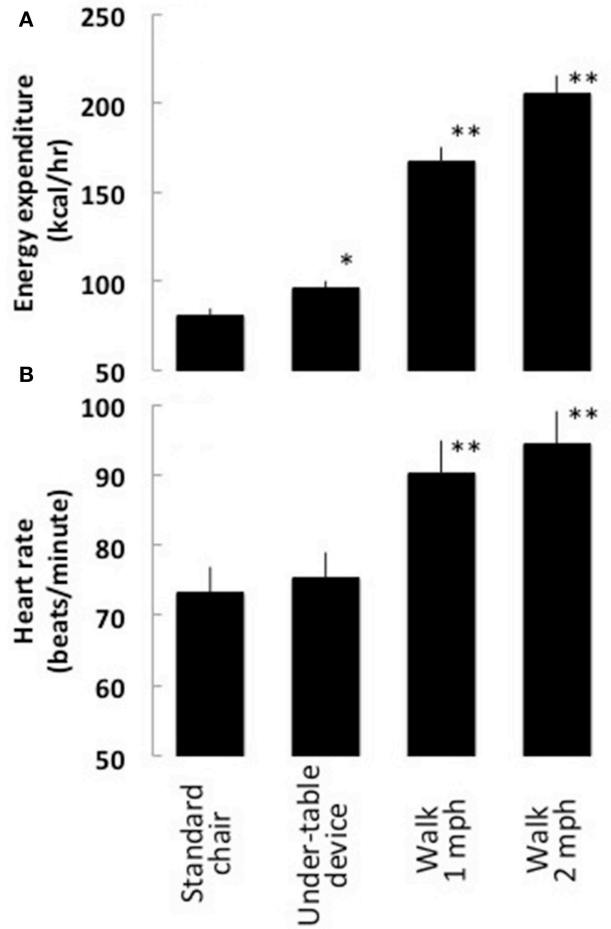

FIGURE 2 | Energy expenditure and heart rate.

health outcomes (Kadam and Chuan, 2016; Dempsey et al., 2017; Fanchamps et al., 2017; Larsen et al., 2017) although we did not measure these outcomes. The under-the-table dynamic-sitting apparatus we tested was exothermic but unlikely to contribute to aerobic fitness. Noting that heart rate did not increase with the use of the under-the-table dynamic-sitting apparatus it could be assumed that such a device doesn't contribute to physical fitness. It may not. However, it is possible that by using the under-the-table dynamic-sitting apparatus a person becomes more active throughout their day and daily physical activity increases. However, this was not tested here. Other studies show that office furniture, such as treadmill desks, can promote NEAT and daily activity (Koepp et al., 2013; Ben-Ner et al., 2014). These approaches, while expensive, have improved health care outcomes and workplace productivity (Koepp et al., 2013; Ben-Ner et al., 2014). Active work has the potential to improve overall health.

LIMITATIONS

Our study had several limitations. As this was a laboratory study conducted only to examine the effects of an apparatus on energy expenditure and heart rate, we did not examine whether the apparatus would impact productivity (positively or negatively), health outcomes, or standing time; these would be goals of future studies. There is solid evidence that breaking up sitting time can benefit glycemic variables (Dunstan et al., 2012). We did not examine whether the apparatus we studied could benefit blood glucose; this too would be a beneficial future study. Similarly, more time spent walking is known to improve overall health (Levine, 2007). It would be interesting to assess whether using a dynamic-sitting apparatus could help increase daily walking. In spite of these limitations, these experiments are encouraging. It would be worthwhile to examine dynamic-sitting interventions in real-world offices.

CONCLUSIONS

In conclusion, new approaches are needed to help decrease excessive sitting and the poor health linked with this prolonged lack of physical activity. Here, we have shown that an under-the-table dynamic-sitting apparatus can improve energy expenditure while a person sits. The applicability of such an apparatus in real-world offices remains to be seen.

AUTHOR CONTRIBUTIONS

GK was responsible for study design, IRB approvals, patient recruitment, data collection, data analysis, and manuscript preparation. JL was responsible for study design, IRB approvals, data analysis, and manuscript preparation. GM was responsible for data collection and data analysis.

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REFERENCES

Ben-Ner, A., Hamann, D. J., Koepp, G., Manohar, C. U., and Levine, J. (2014). Treadmill workstations: the effects of walking while working on physical activity and work performance. *PLoS ONE* 9:e88620. doi: 10.1371/journal.pone.0088620

Bouten, C. V., Verboeket-van de Venne, W. P., Westerterp, K. R., Verduin, M., and Janssen, J. D. (1996). Daily physical activity assessment: comparison between movement registration and doubly labeled water. *J. Appl. Physiol.* 81, 1019–1026.

Buckley, J. P., Hedge, A., Yates, T., Copeland, R. J., Loosemore, M., and Hamer, M. (2015). The sedentary office: an expert statement on the growing case for change towards better health and productivity. *Br. J. Sports Med.* 49, 1357–1362. doi: 10.1136/bjsports-2015-094618

Chave, S. P., Morris, J. N., Moss, S., and Semmence, A. M. (1978). Vigorous exercise in leisure time and the death rate: a study of male civil servants. *J. Epidemiol. Commun. Health* 32, 239–243. doi: 10.1136/jech.32.4.239

Dempsey, P. C., Grace, M. S., and Dunstan, D. W. (2017). Adding exercise or subtracting sitting time for glycaemic control: where do we stand? *Diabetologia* 60, 390–394. doi: 10.1007/s00125-016-4180-4

Dempsey, P. C., Larsen, R. N., Sethi, P., Sacre, J. W., Straznicky, N. E., Cohen, N. D., et al. (2016). Benefits for Type 2 Diabetes of Interrupting prolonged sitting with brief bouts of light walking or simple resistance activities. *Diabetes Care* 39, 964–972. doi: 10.2337/dc15-2336

Dunstan, D. W., Kingwell, B. A., Larsen, R., Healy, G. N., Cerin, E., Hamilton, M. T., et al. (2012). Breaking up prolonged sitting reduces postprandial glucose and insulin responses. *Diabetes Care* 35, 976–983. doi: 10.2337/dc11-1931

Dunstan, D. W., Thorp, A. A., and Healy, G. N. (2011). Prolonged sitting: is it a distinct coronary heart disease risk factor? *Curr. Opin. Cardiol.* 5, 412–419. doi: 10.1097/hco.0b013e3283496605

Falck, R. S., Davis, J. C., and Liu-Ambrose, T. (2016). What is the association between sedentary behaviour and cognitive function? A systematic review. *Br. J. Sports Med.* 51, 759–759. doi: 10.1136/bjsports-2015-095551

Fanchamps, M. H., van den Berg-Emons, H. J., Stam, H. J., and Bussmann, J. B. (2017). Sedentary behavior: different types of operationalization influence outcome measures. *Gait Posture* 54, 188–193. doi: 10.1016/j.gaitpost.2017.02.025

Heymsfield, S. B., Nunez, C., Testolin, C., and Gallagher, D. (2000). Anthropometry and methods of body composition measurement for research and field application in the elderly. *Eur. J. Clin. Nutr.* 54 (Suppl. 3), S26–S32. doi: 10.1038/sj.ejcn.1601022

Judice, P. B., Hamilton, M. T., Sardinha, L. B., and Silva, A. M. (2015). Randomized controlled pilot of an intervention to reduce and break-up overweight/obese adults' overall sitting-time. *Trials* 16:490. doi: 10.1186/s13063-015-1015-4

Kadam, P. D., and Chuan, H. H. (2016). Erratum to: rectocutaneous fistula with transmigration of the suture: a rare delayed complication of vault fixation with the sacrospinous ligament. *Int. Urogynecol. J.* 27:505. doi: 10.1007/s00192-016-2952-5

Koepp, G. A., Manohar, C. U., McCrady-Spitzer, S. K., Ben-Ner, A., Flint-Paulson, D., Runge, C. F., et al. (2013). Treadmill desks: a 1-year prospective trial. *Obesity* 2013, 705–711. doi: 10.1002/oby.20121

Larsen, R. N., Dempsey, P. C., Dillon, F., Grace, M., Kingwell, B. A., Owen, N., et al. (2017). Does the type of activity 'break' from prolonged sitting differentially impact on post-prandial blood glucose reductions? An exploratory analysis. *Appl. Physiol. Nutr. Metab.* doi: 10.1139/apnm-2016-0642. [Epub ahead of print].

Levin, C., and Chisholm, D. (2016). "Cost-effectiveness and affordability of interventions, policies, and platforms for the prevention and treatment of mental, neurological, and substance use disorders," in Vol. 4, *3rd Edn*., eds V. Patel, D. Chisholm, T. Dua, R. Laxminarayan, M. E. Medina-Mora (Washington, DC: International Bank for Reconstruction and Development/The World Bank), 219–236.

Levine, J. A. (2007). Exercise: a walk in the park? *Mayo Clin. Proc.* 82, 797–798. doi: 10.4065/82.7.797

Levine, J. A. (2010). Health-chair reform: your chair: comfortable but deadly. *Diabetes* 59, 2715–2716. doi: 10.2337/db10-1042

Levine, J. A., Baukol, P. A., and Pavlidis, Y. (1999). The energy expended chewing gum. *N. Engl. J. Med.* 341:2100. doi: 10.1056/nejm199912303412718

Levine, J. A., Schleusner, S. J., and Jensen, M. D. (2000). Energy expenditure of nonexercise activity. *Am. J. Clin. Nutr.* 72, 1451–1454.

Marks, C. R., Dupuie, L., and Patros, J. (2012). Stability ball sitting elevates peak arm ergometry oxygen consumption and heart rate. *Int. J. Exerc. Sci.* 5, 360–366.

McCrady, S. K., and Levine, J. A. (2009). Sedentariness at work: how much do we really sit? *Obesity* 17, 2103–2105. doi: 10.1038/oby.2009.117

Pratley, R. E., Hagberg, J. M., Dengel, D. R., Rogus, E. M., Muller, D. C., and Goldberg, A. P. (2000). Aerobic exercise training-induced reductions in abdominal fat and glucose-stimulated insulin responses in middle-aged and older men. *J. Am. Geriatr. Soc.* 48, 1055–1061. doi: 10.1111/j.1532-5415.2000.tb04780.x

Pynt, J. (2015). Rethinking design parameters in the search for optimal dynamic seating. *J. Bodyw. Mov. Ther.* 19, 291–303. doi: 10.1016/j.jbmt.2014.07.001

Stengard, J., Bernhard-Oettel, C., Berntson, E., Leineweber, C., and Aronsson, G. (2016). Stuck in a job: being "locked-in" or at risk of becoming locked-in at the workplace and well-being over time. *Work Stress* 30, 152–172. doi: 10.1080/02678373.2016.1163804

Thyfault, J. P., Du, M., Kraus, W. E., Levine, J. A., and Booth, F. W. (2014). Physiology of sedentary behavior and its relationship to health outcomes. *Med. Sci. Sports Exerc.* 47, 1301–1305. doi: 10.1249/MSS.0000000000000518

Weir, J. B. (1949). New methods for calculating metabolic rate with special reference to protein metabolism. *Nutrition* 6, 213–221. doi: 10.1113/jphysiol.1949.sp004363

Westerterp, K. R., and Bouten, C. V. (1997). Physical activity assessment: comparison between movement registration and doubly labeled water method. *Z. Ernahrungswiss* 36, 263–267. doi: 10.1007/BF01617795

Westerterp, K. R., Verboeket-van de Venne, W. P., Bouten, C. V., de Graaf, C., van het Hof, K. H., and Weststrate, J. A. (1996). Energy expenditure and physical activity in subjects consuming full-or reduced-fat products as part of their normal diet. *Br. J. Nutr.* 76, 785–795. doi: 10.1079/BJN19960086

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frontiers in Physiology

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Sedentary Patterns, Physical Activity, and Cardiorespiratory Fitness in Association to Glycemic Control in Type 2 Diabetes Patients

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Background: Sedentary behavior has been considered an independent risk factor for type-2 diabetes (T2D), with a negative impact on several physiological outcomes, whereas breaks in sedentary time (BST) have been proposed as a viable solution to mitigate some of these effects. However, little is known about the independent associations of sedentary pursuits, physical activity, and cardiorespiratory fitness (CRF) variables with glycemic control. We investigated the independent associations of total sedentary time, BST, moderate-to-vigorous physical activity (MVPA), and CRF with glycemic outcomes in patients with T2D.

Methods: Total sedentary time, BST, and MVPA were assessed in 66 participants (29 women) with T2D, using accelerometry. Glucose and insulin were measured during a mixed meal tolerance test, with the respective calculations of HOMA-IR and Matsuda index. Glycated hemoglobin (HbA1c) was also analyzed. CRF was measured in a maximal treadmill test with breath-by-breath gases analysis. Multiple regressions were used for data analysis.

Results: Regardless of CRF, total sedentary time was positively associated with HbA1c ($\beta = 0.25$, $p = 0.044$). Adjusting for MVPA, total sedentary time was related to fasting glucose ($\beta = 0.32$, $p = 0.037$). No associations between total sedentary time and the remaining glycemic outcomes, after adjusting for MVPA. BST had favorable associations with HOMA-IR ($\beta = -0.28$, $p = 0.047$) and fasting glucose ($\beta = -0.25$, p=0.046), when adjusted for MVPA, and with HOMA-IR ($\beta = -0.25$, $p = 0.036$), Matsuda index ($\beta = 0.26$, $p = 0.036$), and fasting glucose ($\beta = -0.22$, $p = 0.038$), following adjustment for CRF. When adjusting for total sedentary time, only CRF yielded favorable associations with HOMA-IR ($\beta = -0.29$, $p = 0.039$), fasting glucose ($\beta = -0.32$, $p = 0.012$), and glucose at 120-min ($\beta = -0.26$, $p = 0.035$), and no associations were found for MVPA with none of the metabolic outcomes.

Conclusion: The results from this study suggest that sedentary time and patterns are relevant for the glycemic control in patients with T2D. Still, MVPA and CRF counteracted most of the associations for total sedentary time but not for the BST. MVPA was not

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associated with metabolic outcomes, and CRF lost some of the associations with glycemic indicators when adjusted for total sedentary time. Future interventions aiming to control/improve T2D must consider reducing and breaking up sedentary time as a viable strategy to improve glycemic control.

Keywords: sedentary time, breaks in sedentary time, physical activity, cardiorespiratory fitness, glycemic control, type 2 diabetes

INTRODUCTION

Global age-standardized diabetes prevalence has increased from 4.3% in 1980 to 9.0% in 2014 in men, and from 5.0 to 7.9% in women, which together with the population growth and aging has led to a near quadrupling of the number of adults with diabetes worldwide (American Diabetes Association, 2016). Prospective studies (Pan et al., 1997; Tuomilehto et al., 2001; Knowler et al., 2002) have shown that moderate-to-vigorous physical activity (MVPA) is associated with a reduction in the risk of type 2 diabetes (T2D). More recently the Look Ahead Multicenter Study concluded that enhancements in MVPA significantly improved the management of cardiovascular diseases risk factors, and thereby reduced the use of medication and expenses associated with T2D treatments (Redmon et al., 2010; Moura et al., 2014). Similarly to the effects of structured exercise, a recent systematic review (Smith et al., 2016) showed that higher leisure time physical activity (PA) was also associated with lower incidence of T2D, and that additional benefits can be achieved if participants engage in considerably higher doses of PA than those suggested by public health recommendations (Smith et al., 2016).

Exercise-stimulated signal transduction can restore glucose metabolism in insulin-resistant muscle through both acute activation of glucose transport and by improving insulin sensitivity for up to 48 h after exercise (Sylow et al., 2016). Increasing PA in adults with T2D has resulted in partial or complete remission of T2D in 11.5% of participants within the first year of intervention and an additional 7% had partial or complete remission of T2D after 4 years of exercise intervention (Gregg et al., 2012). Transgenerational epigenetic research found that acute exercise also leads to transient changes in DNA methylation in adult skeletal muscle (Barres et al., 2012), that may improve glucose homeostasis.

Recently, sedentary behavior has been associated with hyperinsulinemia (Helmerhorst et al., 2009), and increased risk of T2D in the short (Rockette-Wagner et al., 2015) and long term (Hu et al., 2003; Helmerhorst et al., 2009; Grontved and Hu, 2011; Lahjibi et al., 2013), and has also been considered as an independent risk factor for T2D and premature mortality (Grontved and Hu, 2011; van der Ploeg et al., 2012). In a 4-year follow-up, T2D patients who increased sedentary behavior had the greatest increase in waist circumference, independently of MVPA (Lamb et al., 2016). In the short term, sedentary pursuits are related with hyperglycemia (Fritschi et al., 2015), also suggesting acute metabolic effects (Fritschi et al., 2015). Regularization of metabolic control can be achieved by introducing low-intensity physical activity (LIPA), and this can be tracked with an increased mRNA expression of mitochondrial and metabolic genes in skeletal muscle (Osler et al., 2015). However, for individuals presenting a greater imbalance in glycemia, the potential for clinical improvements after these LIPA protocols appears to be limited (Osler et al., 2015).

Preliminary findings indicate that time spent in sedentary behaviors can be reallocated into LIPA or MVPA, with differences in insulin sensitivity, but with greater results for MVPA (Yates et al., 2015b). Replacing sedentary time with LIPA was associated with a 3.0% lower fasting insulin values and a 3.1% lower insulin resistance, using the homeostatic model assessment (HOMA-IR) (Ekblom-Bak et al., 2016). Healy et al. (2011) have previously documented that breaking up sedentary time may be associated with favorable changes in the cardio-metabolic and inflammatory risk profile in adults. These findings have been recently extended to the T2D population, in which interrupting sedentary time by introducing short LIPA breaks may also have the same beneficial effects (Chastin et al., 2015; Duvivier et al., 2016; Dempsey et al., 2016c).

Mounting evidence suggests that breaking up prolonged sedentary time by light ambulation is an effective strategy for improving postprandial glucose regulation (Dunstan et al., 2012; Howard et al., 2013; Latouche et al., 2013; Larsen et al., 2014; Bailey and Locke, 2015; Dempsey et al., 2016a,b), and a recent meta-analysis revealed that breaks of at least light intensity in sedentary periods may have a positive effect on glycemia, independently of total sedentary time (Chastin et al., 2015). Dunstan et al. (2012) found that introducing light walking breaks every 20 min (2-min breaks) reduced 5 h glucose incremental area under the curve (iAUC) by 24% and 5 h insulin iAUC by 23%. From this same experiment, interrupting sedentary behavior reduced blood pressure (Larsen et al., 2014), attenuated the increase in plasma fibrinogen (Howard et al., 2013), and it also induced changes in the expression of skeletal muscle genes involved in cellular development, growth and proliferation, and lipid and CHO metabolism in non-diabetic adults (Latouche et al., 2013). Another study with a similar experimental approach also found that light walking reduced 5 h blood glucose iAUC by 15.9% compared to prolonged sitting in healthy individuals (Bailey and Locke, 2015), and that interrupting sedentary time by standing-up did not improve glucose tolerance (Bailey and Locke,

Abbreviations: BMI, Body mass index; BST, breaks in sedentary time; CRF, cardiorespiratory fitness; HbA1c, Glycated hemoglobin; HOMA-IR, homeostatic model assessment; iAUC, incremental area under the curve; LIPA, low-intensity physical activity; MVPA, moderate-to-vigorous physical activity; T2D, type-2 diabetes.

2015). Introducing light walking breaks reduced T2D patients' 7 h glucose, insulin, and C-peptide iAUC, compared with prolonged sitting (Dempsey et al., 2016b). Interestingly, 22 h hyperglycemia was also reduced and glycemic improvements persisted nocturnally, until the following morning (Dempsey et al., 2016a).

Experimental evidence is paramount to establish causal relationships, but the controlled conditions and sometimes unrealistic protocols makes it difficult for an ecological transfer to the real-life settings. Understanding if the associations between breaks in sedentary time (BST) and metabolic indicators remain while in free-living conditions is still unknown. Moreover, patients with lower fitness and high fasting glucose levels benefited more from replacing the same amount of sedentary time with LIPA and MVPA, compared with participants with normal to high cardiorespiratory fitness levels (CRF) (Ekblom-Bak et al., 2016). Additionally, CRF seems to be positively associated with glycemic control (Rohling et al., 2016), and may be an important mediator in the relationship between sedentary behavior and MVPA with metabolic outcomes (Rohling et al., 2016).

Notably, the acute experimental findings have mainly resulted from healthy and overweight participants, and the results seem to be less consistent for T2D patients, with one study showing no association between the number of BST with insulin levels or HOMA-IR (Cooper et al., 2012). Thus, the aim of this study was to cross-sectionally analyze the independent associations for total sedentary time, BST, and MVPA, with fasting glucose, glucose tolerance at 120 min, HOMA-IR and Matsuda index, and glycated hemoglobin (HbA1c), in free-living conditions, and examine if CRF may counteract these associations in T2D patients.

MATERIALS AND METHODS

Study Design and Participants

Sample recruitment was carried out by media, e-mails, or community events. For this cross-sectional study, a total of 96 participants were recruited but, given that 30 participants have not completed all the assessments, the results are based on the 66 participants (29 women) from which we have complete data (accelerometer, blood sample collection, and CRF assessment). In order to be included in this investigation, the participants had to be adults previously diagnosed with T2D in accordance with the ADA criteria (American Diabetes Association, 2016). This study was carried out in accordance with the recommendations of the Declaration of Helsinki for Human Studies (World Medical Association, 2008). The protocol was approved by the Ethics Committee of the Portuguese Diabetes Association (approval number: 07/17/2013). Written informed consent was obtained from all participants before entering the study and prior to any protocol-specific procedures.

Anthropometry and Body Composition

Participants were weighed to the nearest 0.01 kg while wearing minimal clothes and without shoes, on an electronic scale (Seca, Hamburg, Germany). Height was measured to the nearest 0.1 cm with a stadiometer (Seca, Hamburg, Germany) according to the standardized procedures described elsewhere (Lohman et al., 1988). Body mass index (BMI) was calculated as body mass (kg)/height^2 (m). BMI was further categorized into normal ($<$25 kg/m^2), overweight (25–24.9 kg/m^2), and obese ($\geq$30 kg/m^2).

Waist circumference measurement was taken with the participant in a standing position, over the naked skin, to the nearest 0.1 cm. The tape was applied horizontally just above the uppermost lateral border of the right ilium at the end of normal expiration (CDC, 2016). The mean of two measurements was considered. If the two measurements differed by more than 1 cm, a third measurement was necessary, and the two closest measurements were averaged. Dual energy X-ray absorptiometry (Hologic Explorer-W, fan-beam densitometer, software QDR for windows version 12.4, Waltham, USA) was used to estimate total body fat. A whole-body scan was performed and the attenuation of X-rays pulsed between 70 and 140 kV synchronously with the line frequency for each pixel of the scanned image was measured. The same laboratory technician positioned the subjects, performed the scans and executed the analyses according to the operator's manual using the standard analysis protocol (Santos et al., 2013). Based on ten participants, the coefficient of variation (CV) in our laboratory for fat mass was 1.7%.

Objective Measures of Sedentary Time and Physical Activity

Sedentary time and PA were assessed by accelerometry (ActiGraph, GT3X+ model, Fort Walton Beach, FL, USA). The accelerometer is a small device that measures the acceleration of normal human movements, ignoring high-frequency vibrations associated with mechanical equipment. All participants were asked to wear the accelerometer on the right hip, close to the iliac crest. The device activation, download, and processing were performed using the software Actilife (v.6.9.1) (ActiGraph, Fort Walton Beach, FL, USA). The devices were activated on the first day in the morning and data were recorded using the raw mode with a 100 Hz frequency, and posteriorly downloaded into 15-s epochs. Apart from accelerometer non-wear time (i.e., when it was removed during sleep and water activities), periods of at least 60 consecutive minutes of zero activity intensity counts were also considered as non-wear time. A valid day was defined as having 600 min (10 h) or more of monitor wear, and all participants with at least three valid days (including 1 weekend day) were included in the analyses. Each minute during which the accelerometer counts were below 100 cpm was defined as sedentary time. A break in sedentary time was defined as all interruptions (lasting at least 1-min) in sedentary time when the recorded counts value were $>$100 cpm. BST were divided by total sedentary time and the variable hourly breaks in sedentary time (BST/ST) was used in the analysis. Accelerometer counts $\geq$100 cpm were classified as PA with additional separation into light-intensity (LIPA: 100–2,019 cpm) and moderate-to-vigorous intensity (MVPA $\geq$ 2,020 cpm) (Troiano et al., 2008; Colley et al., 2010). There are no cutoffs for the sedentary time using the three-axial information from this new generation Actigraph GT3X+ accelerometer; therefore we used the previous cutoffs which are based on the vertical-axis only. Compliance with PA recommendations for public health

was assessed according to the WHO recommendations (Adults: 150 min/week of MVPA defined as ≥21.4 min/day).

Cardiorespiratory Fitness

Cardiorespiratory fitness was determined using a Bruce standard protocol (Bruce, 1971) on a motorized treadmill to exhaustion (model Q-65, Quinton, Cardiac Science Corp; Bothell, WA, USA). All graded exercise tests were monitored using a 12 lead electrocardiogram PC-based acquisition module (model Quark C12, Cosmed, Rome, Italy) and all data, including heart rate, were monitored and recorded using Cosmed software (Cosmed, Rome, Italy. Inspired and expired gases were continuously analyzed, breath-by-breath, through a portable gas analyzer (K4b2, Cosmed, Rome, Italy). Participants exercised until at least two of the following test termination criteria were reached: (1) participants volitional fatigue; (2) respiratory exchange ratio reached 1.1 or higher; (3) participants reached predicted maximal heart rate; (4) oxygen uptake did not increase in spite of increasing workload. Plateau in oxygen consumption with an increase in workload. The highest 20-s value for oxygen consumption (ml/kg/min) attained in the last minute was used in the analysis.

Laboratory Measurements

After the recruitment process, participants underwent biochemical assessments, including a mixed meal tolerance test and analysis of the HbA1c. Blood samples were collected from an indwelling catheter for the assessment of glucose, insulin, and HbA1c before ingesting the meal, and 30, and 120 min after the beginning of the meal consumption (2 bottles of Boost Complete Nutritional Drink), for glucose and insulin. Samples were drawn into chilled, heparinized tubes and centrifuged rapidly to avoid glycolysis. Plasma glucose was measured by photometry (auto analyzer Olympus AU640, Beckman Coulter). Plasma insulin was analyzed using electrochemiluminescence immunoassays (Liaison, Diasorin). HbA1c was analyzed by immunoassay (auto analyzer Hb9210 Premier A. Menarini diagnostics). Homeostasis model assessments of insulin resistance (HOMA-IR) and the Matsuda index were calculated (Matthews et al., 1985; Matsuda and DeFronzo, 1999) using their respective formulas.

Statistical Analysis

Data analyses were performed using IBM SPSS Statistics version 22.0 (SPSS Inc., an IBM Company, Chicago, Illinois, USA). Descriptive statistics including means ± SD were calculated for all outcome variables. Normality was tested using Q-Q plots. Comparisons between sexes were performed using independent sample *T*-test or the non-parametric Mann-Whitney-Wilcoxon approach.

Multiple regression analyses were performed to understand the associations between total sedentary time, breaks in sedentary time, MVPA (linear and dichotomized as compliance with PA guidelines), and CRF with metabolic variables (HOMA-IR, Matsuda index, HbA1c, fasting glucose, glucose at 120 min). Model adjustments included age, sex, time with diagnosed diabetes, and wear time of the accelerometer. To analyze the independent effects, additional adjustments were performed to for MVPA, CRF, or sedentary time (except when exposure).

During model development, normality and homoscedasticity of residuals were tested. If normality was rejected during model development, a logarithmic function of the dependent variable was used. If more than one variable was a predictor in the model, a variance inflation factor for each independent variable was calculated to evaluate multicollinearity, and values bellow 5 were considered not to have multicollinearity issues (Montgomery and Peck, 1982). For all tests statistical significance was set at $p < 0.05$.

RESULTS

Descriptive characteristics of the participants are presented in **Table 1**, for both sexes and for the overall sample.

Overall, 51.5% of the sample was categorized as obese, 31.8% as overweight, and 16.7% as normal weight. No differences were found for age, time of diagnosed diabetes, BMI, and waist circumference, between men and women. CRF ($p = 0.002$) was higher in men when compared to women, whereas percentage body fat ($p < 0.001$) was higher in women compared to men. Regarding metabolic and inflammatory variables, with the exception of glucose at 120 min ($p = 0.022$) where males presented higher values, there were no differences between both sexes. Compared to men, women spent a higher amount of time per day in light PA ($p = 0.005$). There were no differences between men and women for sedentary time, breaks in sedentary time per sedentary hour, and time spent engaging in MVPA.

In the multicollinearity diagnosis, we found no variation inflation factor above 5, which is the rule of thumb used in regression models to assess if the β is affected.

Associations for total sedentary time and breaks in sedentary time with metabolic variables are presented in **Table 2**.

Following adjustment for covariates, including age, sex, time of diabetes diagnosis, and wear time (**Table 2**, model 1), detrimental linear associations for total sedentary time with all glycemic outcomes were found. Conversely, except for glucose measured at 120 min, favorable associations were found for the breaks in sedentary time with all the metabolic variables.

Following an additional adjustment for time spent in MVPA (**Table 2**, model 2), total sedentary time yielded a detrimental association with fasting glucose ($\beta = 0.32$, $p = 0.037$), whereas, breaks in sedentary time remained favorably associated with HOMA-IR ($\beta = -0.28$, $p = 0.047$) and fasting glucose ($\beta = -0.25$, $p = 0.046$). The remaining metabolic outcomes were no longer associated with both total sedentary time and breaks in sedentary time after adjusting for MVPA.

In the last model (**Table 2**, model 3), adjusted for both the covariates of model 1 and CRF, total sedentary time remained detrimentally associated with HbA1c ($\beta = 0.25$, $p = 0.044$), while breaks in sedentary time had favorable associations with HOMA-IR ($\beta = -0.25$, $p = 0.036$), Matsuda index ($\beta = 0.26$, $p = 0.036$), and fasting glucose ($\beta = -0.22$, $p = 0.038$). Similarly to model 2, total sedentary time and breaks in sedentary time were no longer associated with all the remaining metabolic variables, after adjusting for CRF.

Table 3 reports the standardized coefficients for MVPA and CRF with metabolic outcomes. Overall, MVPA was negatively associated with glucose at 120 min ($\beta = -0.31$, $p = 0.006$) and with HbA1c ($\beta = -0.26$, $p = 0.026$), whereas, CRF had favorable

TABLE 1 | Participants' characteristics, according to sex and all sample.

	All Sample ($n = 66$)	Women ($n = 29$)	Men ($n = 37$)	*p*-Value
Age (years)	58.9 ± 8.2	58.7 ± 7.8	59.0 ± 8.6	0.914
Time of Diabetes Diagnosis (years)	7.2 ± 5.0	6.8 ± 4.9	7.6 ± 5.2	0.516
Height (cm)	164.7 ± 8.8	157.2 ± 5.9	170.8 ± 5.5	<0.001*
Weight (kg)	83.5 ± 15.6	77.6 ± 12.8	88.2 ± 16.2	<0.001*
Body Mass Index (kg/m^2)	30.8 ± 5.2	31.5 ± 5.2	30.2 ± 5.1	0.335
Waist Circumference (cm)	103.5 ± 12.5	102.0 ± 11.5	104.6 ± 13.4	0.425
Percentage Body Fat (%)	34.4 ± 7.1	40.7 ± 3.1	29.4 ± 5.2	<0.001*
Cardiorespiratory Fitness (VO_2, ml/kg/min)	25.8 ± 5.5	23.4 ± 3.5	27.6 ± 6.1	0.002
Fasting Glucose (mg/dl)	159.6 ± 56.4	153.5 ± 50.9	164.3 ± 60.7	0.486
Glucose 120 min (mg/dl)	272.2 ± 124.5	233.1 ± 91.9	303.7 ± 138.9	0.022*
Fasting Insulin (UI/l)	12.9 ± 8.3	13.8 ± 7.6	12.2 ± 8.9	0.451
HbA1c (%)	7.1 ± 1.3	7.1 ± 1.2	7.2 ± 1.4	0.919
HOMA-IR	5.2 ± 4.3	5.6 ± 4.3	4.9 ± 4.3	0.289
Matsuda Index	4.6 ± 6.5	4.3 ± 7.1	4.8 ± 6.3	0.482
Total Sedentary Time (min/day)	582.3 ± 79.8	575.8 ± 71.4	587.5 ± 86.7	0.573
Breaks in Sedentary Time per Sedentary Hour (number/h)	7.8 ± 3.8	8.6 ± 3.6	7.2 ± 3.9	0.055
Light Physical Activity (min/day)	214.9 ± 71.0	241.5 ± 66.5	193.8 ± 68.1	0.005*
Moderate-to-Vigorous Physical Activity (min/day)	33.7 ± 24.4	26.4 ± 16.9	39.5 ± 27.9	0.072

HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment; VO_2, oxygen consumption.
**Significant differences between sexes.*

TABLE 2 | Multiple regression analyses for total sedentary time and breaks in sedentary time with metabolic variables.

	Model 1[a]		Model 2[a,b]		Model 3[a,c]	
	β (CI 95%)	*p-value*	β (CI 95%)	*p-value*	β (CI 95%)	*p-value*
HOMA-IR						
Sedentary Time	0.30 (0.04;0.55)	0.023*	0.33 (−0.001;0.35)	0.051	0.25 (−0.01;0.49)	0.058
BST-ST	−0.28 (−0.51;−0.05)	0.020*	−0.28 (−0.54;−0.01)	0.046*	−0.25 (−0.47;−0.02)	0.036*
MATSUDA INDEX						
Sedentary Time	−0.33 (−0.58;−0.05)	0.020*	−0.32 (−0.64;0.02)	0.065	−0.26 (−0.52;0.01)	0.058
BST-ST	0.30 (0.05;0.53)	0.017*	0.27 (−0.03;0.53)	0.052	0.26 (0.02;0.48)	0.036*
HbA1c						
Sedentary Time	0.28 (0.04;0.54)	0.022*	0.18 (−0.12;0.48)	0.237	0.25 (0.01;0.49)	0.044*
BST-ST	−0.23 (−0.45;−0.01)	0.038*	−0.15 (−0.40;0.10)	0.240	−0.21 (−0.43;0.01)	0.059
FASTING GLUCOSE						
Sedentary Time	0.29 (0.05;0.52)	0.018*	0.32 (0.02;0.62)	0.037*	0.23 (−0.002;0.45)	0.052
BST-ST	−0.26 (−0.47;−0.04)	0.021*	−0.25 (−0.50;-0.004)	0.047*	−0.22 (−0.43;−0.01)	0.038*
GLUCOSE 120 MIN						
Sedentary Time	0.29 (0.05;0.52)	0.020*	0.14 (−0.16;0.43)	0.362	0.24 (−0.001;0.47)	0.051
BST-ST	−0.20 (−0.42;0.02)	0.075	−0.08 (−0.32;0.17)	0.530	−0.17 (−0.38; 0.05)	0.124

β, standardized beta coefficient; CI, confident interval; HOMA-IR, homeostatic model assessment; BST-ST, breaks in sedentary time per sedentary hour; HbA1c, glycated hemoglobin.
**Significant at $p < 0.05$.*
[a]Adjusted for age, sex, time with diagnosed diabetes, and wear time of the accelerometer.
[b]Adjusted for moderate-to-vigorous physical activity.
[c]Adjusted for cardiorespiratory fitness.

associations with HOMA-IR (β = −0.34, $p = 0.016$), Matsuda index (β = 0.32, $p = 0.025$), fasting glucose (β = −0.36, $p = 0.004$), and glucose at 120 min (β = −0.31, $p = 0.014$). When adjusting for total sedentary time, only CRF yielded favorable associations with HOMA-IR (β = −0.29, $p = 0.039$), fasting glucose (β = −0.32, $p = 0.012$), and glucose at 120 min (β = −0.26, $p = 0.035$), and no associations were found for the remaining metabolic outcomes.

Further analyses were conducted to analyze if complying with PA guidelines for MVPA was associated with the metabolic

TABLE 3 | Multiple regression analyses for moderate-to-vigorous physical activity and cardiorespiratory fitness with metabolic variables.

	Model 1[a]		Model 2[a,b]	
	β (CI 95%)	*p-value*	β (CI 95%)	*p-value*
HOMA-IR				
MVPA	−0.15 (−0.40;0.10)	0.241	0.04 (−0.27;0.35)	0.785
CRF	−0.34 (−0.67;−0.07)	0.016*	−0.29 (−0.62;-0.02)	0.039*
MATSUDA INDEX				
MVPA	0.18 (−0.08; 0.44)	0.162	0.01 (−0.31;0.32)	0.971
CRF	0.32 (0.05;0.66)	0.025*	0.26 (−0.03; 0.59)	0.075
HbA1c				
MVPA	−0.26 (−0.49;−0.03)	0.026*	−0.15 (−0.44; 0.13)	0.290
CRF	−0.22 (−0.53;0.04)	0.096	−0.17 (−0.47;0.10)	0.200
FASTING GLUCOSE				
MVPA	−0.14 (−0.37;0.10)	0.246	0.37 (−0.23;0.34)	0.716
CRF	−0.36 (−0.68;−0.14)	0.004*	−0.32 (−0.63;−0.08)	0.012*
GLUCOSE 120 MIN				
MVPA	−0.31 (−0.55;−0.10)	0.006*	−0.24 (−0.53;0.04)	0.088
CRF	−0.31 (−0.63;−0.07)	0.014*	−0.26 (−0.57;−0.02)	0.035*

β, *standardized beta coefficient; CI, confident interval; HOMA-IR, homeostatic model assessment; MVPA, moderate-to-vigorous physical activity; CRF, cardiorespiratory fitness; HbA1c, glycated hemoglobin.*

**Significant at $p < 0.05$.*

[a]*Adjusted for age, sex, time with diagnosed diabetes, and wear time of the accelerometer.*

[b]*Adjusted for total sedentary time.*

outcomes. In these analyses we verified that not performing at least 150 min of MVPA per week (average 24.1 min/day of MVPA) was associated with Matsuda index ($\beta = -0.36$, $p = 0.005$) and with HOMA-IR ($\beta = 0.32$, $p = 0.011$), but not with fasting glucose ($\beta = 0.08$, $p = 0.490$), HbA1c ($\beta = 0.167$, $p = 0.152$), nor glucose at 120 min ($\beta = 0.16$, $p = 0.174$). After additional adjustment for total sedentary time, only the association between meeting PA guidelines with Matsuda index remained significant ($\beta = -0.29$, $p = 0.043$).

DISCUSSION

The main findings from this study suggest that total time spent in sedentary activities and the patterns of accumulation are detrimental to the metabolic health of T2D patients. Still, MVPA seemed to offset the associations for both total sedentary time and BST with all metabolic outcomes, except for BST with fasting glucose and HOMA-IR. CRF only counteracted the associations for total sedentary time, whereas the associations for BST with most of the main outcomes remained unaltered. Our results suggest that future interventions aimed to control/improve T2D must consider BST as a viable strategy to improve glycemic control.

Both total MVPA and sedentary time have been consistently associated with HOMA-IR and Matsuda index, but after adjustment for each other, only associations with Matsuda index remained (Yates et al., 2015a). In the current investigation it was verified that, when considering MVPA as a continuous variable, only associations with HbA1c were verified. Interestingly, when considering compliance with PA guidelines as the independent variable, associations were observed for the Matsuda index and HOMA-IR. This finding suggests that there may be a threshold (i.e., 150 min of MVPA per week) to experience the metabolic benefits that are related to MVPA, and therefore more minutes of PA at these intensities do not necessarily relate to glycemic control. From the different variables used to assess glycemic control, both Matsuda index and HOMA-IR have been shown to correlate well with euglycemic-hyperinsulinemic clamp on cross-sectional level, making them suitable insulin resistance surrogates (Lorenzo et al., 2010). Observational evidence suggests that a 30-min difference in total sedentary time was inversely associated with a 4% difference in Matsuda index, whereas every 30 min in MVPA was positively associated with a 13% difference (Yates et al., 2015a). Reallocating 30 min of sedentary time into MVPA was associated with a 15% difference in HOMA-IR and an 18% difference in Matsuda index (Yates et al., 2015b). Our findings suggest that when considering these insulin resistance indexes, no associations remained when adjusting total sedentary time for MVPA and vice-versa.

Baseline MVPA has been documented as a predictor of fasting insulin at follow-up, with a borderline significance for HOMA-IR, regardless of total sedentary time (Ekelund et al., 2009). In contrast, each additional daily hour spent sedentary was cross-sectional associated with a 3% higher fasting insulin and HOMA-IR, but did not predict 5-year changes in metabolic parameters or incidence of metabolic disorders (Barone Gibbs et al., 2015). Experimental data has previously suggested that performing 45 min of MVPA following more than 10 h of sitting had beneficial effects on glucose metabolism in T2D patients (van Dijk et al., 2013), thus, some of the contradicting results may be explained by the specific window of time that both sedentary time and MVPA have in their ability to alter these specific metabolic indicators. Similar to the results observed for mortality, in a harmonized meta-analysis involving more than 1 million men and women (Ekelund et al., 2016), we found in our sample of T2D that adjusting for MVPA eliminated almost all the associations for total sedentary time with glycemic indicators, except fasting glucose. The results for the fasting glucose are in accordance with the findings from a previous systematic review (Brocklebank et al., 2015), and a longitudinal analysis that found higher baseline sedentary time to be associated with 3-year increases in fasting glucose, fasting insulin and HOMA-IR, regardless of MVPA (Lahjibi et al., 2013).

A new finding from the present investigation with T2D patients was that, the associations for BST with HOMA-IR and fasting glucose were not affected by the adjustment for MVPA. These findings further highlight the important role of breaking up sedentary time to improve cardiometabolic markers in the general population (Healy et al., 2011) and in T2D patients using an isotemporal substitution modeling approach (Healy et al., 2011; Falconer et al., 2015), and therefore to encourage adults with diagnosed T2D to adopt BST as a strategy for improving metabolic health. The underlying mechanisms explaining the associations between BST and glycemic control are still relatively unknown, but acute light exercise bouts may activate alternative molecular signals that can bypass defects

in insulin signaling in skeletal muscle, resulting in an insulin-independent increase in glucose uptake (Stanford and Goodyear, 2014) through several signal transduction pathways (Sylow et al., 2016), including the AMPK signaling network (Kjobsted et al., 2017), a function that remains intact in T2D patients (Kjobsted et al., 2016). It is important to highlight that the AMPK signaling is intensity-dependent (Birk and Wojtaszewski, 2006), however, it may also be stimulated by an increased energy expenditure resulting from skeletal muscle contractions. Breaking up sedentary time may have benefits that go beyond the physiological mechanisms, including certain energetic changes (i.e., increasing energy expenditure 35% above sitting, and 28% compared to standing while motionless) (Judice et al., 2016), that can justify why BST (frequent muscle contractions throughout the day) were favorably associated with glycemic outcomes in the present study.

Nonetheless, breaking up sedentary time was not independent of MVPA for some metabolic outcomes, particularly the Matsuda index, and HbA1c, which is in line with the results reported by some investigations (Cooper et al., 2012; van der Berg et al., 2016). For example, in a study with 528 adults with newly diagnosed T2D, no associations were found between BST and insulin levels or HOMA-IR (Cooper et al., 2012). Similar results were found in the Maastricht Study with 2497 participants, where an extra hour of sedentary time was associated with increased odds for T2D (22%), but the pattern of sedentary time accumulation was weakly associated with the incidence of metabolic impairment (van der Berg et al., 2016). With different results, Healy et al. (2011) found that, regardless of total sedentary time and MVPA, increased BST were beneficially associated with plasma glucose at 120 min. Additionally, an investigation based on 4935 adults found that total sedentary time was associated with higher insulin, and each additional 10 breaks/day were related to 0.57% lower glucose, and 4.19% lower insulin (Carson et al., 2014).

When considering experimental evidence (Dunstan et al., 2012; Howard et al., 2013; Latouche et al., 2013; Larsen et al., 2014; Bailey and Locke, 2015; Dempsey et al., 2016a,b), breaking up prolonged sedentary time with light ambulation is still an effective strategy for improving glucose regulation, which further clarifies the need to expand current diabetes-related PA guidelines, by introducing regular breaks in prolonged sedentary time (Dempsey et al., 2016c). Dunstan et al. (2012) found that breaking up sedentary time with LIPA bouts reduced 5 h glucose iAUC by 24% and 5 h insulin iAUC by 23%. When considering T2D patients, introducing light walking breaks reduced 7 h glucose, insulin, and C-peptide, compared with prolonged sitting (Dempsey et al., 2016b). The same authors verified that the glycemic improvements that arise from breaking up sedentary time persist until the next morning, indicating that there may be medium to long term benefits in T2D patients (Dempsey et al., 2016a). Even though these experimental findings are of great importance (because they allow establishing causal relationships between BST and metabolic outcomes) the laboratorial settings and protocols in which they are performed, do not mimic real-life conditions and limit their ecological transfer. On the other hand, the presented investigation collected free-living accelerometry data that may reflect a more realistic PA and sedentary pattern profile.

Breaking up sedentary time seems to reverse the effects of chronic inactivity on the expression of some specific genes and molecular processes (Latouche et al., 2013), but some of the contradicting findings for the independent associations of sedentary patterns with glycemic indicators may be explained by CRF, which is usually not accounted for most of the models. CRF is a reliable metric to assess the ability of the cardiovascular system to sustain prolonged physical work, and has been shown to be one the most powerful predictors of mortality and morbidity (Despres, 2016). Poor CRF is an independent risk factor for cardiovascular diseases and related mortality (Despres, 2016), and it appears to be a link between changes in CRF and glycemic control (Larose et al., 2011; Sui et al., 2012; Dickie et al., 2016). Alongside with these results, replacing 30 min of sedentary time with LIPA provided higher benefits in metabolic profile in participants with lower CRF when compared with those with normal to high CRF levels (Ekblom-Bak et al., 2016), suggesting that the associations between sedentary pursuits and metabolic outcomes may be moderated by CRF. There is a lack of studies that analyzed the associations for sedentary time and respective patterns with glycemic indicators while adjusting for CRF (Ekblom-Bak et al., 2016; Rohling et al., 2016). In the present study, after adjusting for CRF, it was observed that total sedentary time was only associated with HbA1c, whereas BST had favorable associations with HOMA-IR, Matsuda index, and fasting glucose. Thus, as previously shown (Ekblom-Bak et al., 2016; Rohling et al., 2016), CRF can neutralize most of the associations for total sedentary time with glycemic outcomes, and this may be explained by the association between total sedentary time and CRF itself (Krogh-Madsen et al., 2010).

A sedentary lifestyle is usually associated with poor levels of CRF (Lakka et al., 2003), but the fact that the associations for BST with HOMA-IR, Matsuda index, and fasting glucose remained independent of CRF, is another novel finding and suggests that BST may not be as influenced by CRF as total sedentary time. To the authors' knowledge, there is no evidence on the associations for BST with CRF in T2D patients, making it necessary to further investigate the plausible mechanisms that underlie these findings. CRF was not associated with all metabolic outcomes, after adjustment for total sedentary time, contradicting previous findings on the independent associations for CRF with metabolic outcomes (Larose et al., 2011; Dickie et al., 2016). Sedentary behavior accumulating pattern is a relatively new research topic and these contradicting findings in the literature reinforce the need for further experimental investigations that may help to uncover this subject.

Regardless of the amount of observational and experimental studies showing the deleterious effects of prolonged sedentary time and the benefits associated with breaking up sedentary time, few studies have focused on T2D patients and none controlled for their CRF levels. These were major strengths of the present study, and one must cautiously account for CRF when examining the associations of PA/sedentary variables with

metabolic outcomes in T2D patients, as this covariate may explain some of the variability found in previous investigations (Bouchard et al., 2015). Another important message is that the relative role of total sedentary time, BST, MVPA, and CRF may depend on the glycemic indicators that are being considered, and interpretation must be careful when considering different outcomes in patients with T2D. The present investigation is not without limitations, the inability to establish causality due to the cross-sectional nature of the data is by far the major problem. However, this study provides a basis for future interventional studies to confirm our findings in T2D patients.

CONCLUSIONS

The results from this study suggest that sedentary time and its patterns can be relevant for the glycemic control in patients with T2D. Current international recommendations include 150 min of moderate-intensity activity, or 75 min of vigorous-intensity activity, or some combination of moderate and vigorous activity with at least 2-days of resistance exercise. Thus, the present findings suggest that it will be equally important for T2D prevention and management programs to broaden the focus of public health message, and not only target MVPA, but also endorse people to reduce and interrupt sedentary time more often and improve CRF. Future interventions aiming to control/improve T2D must target reductions in sedentary behavior and increase the number of breaks in sedentary time as a viable strategy to improve glycemic control.

AUTHOR CONTRIBUTIONS

LBS contributed to the conception and design of the study. JM, DS, and PJ were responsible for data acquisition, analysis, and interpretation. LBS and PJ drafted the manuscript and DS and JM revised it critically for important intellectual content. All authors gave approval of the final version and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

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REFERENCES

American Diabetes Association, (2016). Standards of medical care in diabetes-2016 abridged for primary care providers. *Clin. Diabetes* 34, 3–21. doi: 10.2337/diaclin.34.1.3

Bailey, D. P., and Locke, C. D. (2015). Breaking up prolonged sitting with light-intensity walking improves postprandial glycemia, but breaking up sitting with standing does not. *J. Sci. Med. Sport* 18, 294–298. doi: 10.1016/j.jsams.2014.03.008

Barone Gibbs, B., Pettee Gabriel, K., Reis, J. P., Jakicic, J. M., Carnethon, M. R., and Sternfeld, B. (2015). Cross-sectional and longitudinal associations between objectively measured sedentary time and metabolic disease: the Coronary Artery Risk Development in Young Adults (CARDIA) study. *Diabetes Care* 38, 1835–1843. doi: 10.2337/dc15-0226

Barres, R., Yan, J., Egan, B., Treebak, J. T., Rasmussen, M., Fritz, T., et al. (2012). Acute exercise remodels promoter methylation in human skeletal muscle. *Cell Metab.* 15, 405–411. doi: 10.1016/j.cmet.2012.01.001

Birk, J. B., and Wojtaszewski, J. F. (2006). Predominant alpha2/beta2/gamma3 AMPK activation during exercise in human skeletal muscle. *J. Physiol.* 577(Pt 3), 1021–1032. doi: 10.1113/jphysiol.2006.120972

Bouchard, C., Blair, S. N., and Katzmarzyk, P. T. (2015). Less sitting, more physical activity, or higher fitness? *Mayo Clin. Proc.* 90, 1533–1540. doi: 10.1016/j.mayocp.2015.08.005

Brocklebank, L. A., Falconer, C. L., Page, A. S., Perry, R., and Cooper, A. R. (2015). Accelerometer-measured sedentary time and cardiometabolic biomarkers: a systematic review. *Prev. Med.* 76, 92–102. doi: 10.1016/j.ypmed.2015.04.013

Bruce, R. A. (1971). Exercise testing of patients with coronary heart disease. Principles and normal standards for evaluation. *Ann. Clin. Res.* 3, 323–332.

Carson, V., Wong, S. L., Winkler, E., Healy, G. N., Colley, R. C., and Tremblay, M. S. (2014). Patterns of sedentary time and cardiometabolic risk among Canadian adults. *Prev. Med.* 65, 23–27. doi: 10.1016/j.ypmed.2014.04.005

CDC (2016). *National Health and Nutrition Examination SURVEY (NHANES) - Anthropometry Procedures Manual.* Available online at: https://www.cdc.gov/nchs/nhanes/nhanes2015-2016/manuals15_16.htm.

Chastin, S. F., Egerton, T., Leask, C., and Stamatakis, E. (2015). Meta-analysis of the relationship between breaks in sedentary behavior and cardiometabolic health. *Obesity (Silver. Spring)* 23, 1800–1810. doi: 10.1002/oby.21180

Colley, R., Connor Gorber, S., and Tremblay, M. S. (2010). Quality control and data reduction procedures for accelerometry-derived measures of physical activity. *Health Rep.* 21, 63–69.

Cooper, A. R., Sebire, S., Montgomery, A. A., Peters, T. J., Sharp, D. J., Jackson, N., et al. (2012). Sedentary time, breaks in sedentary time and metabolic variables in people with newly diagnosed type 2 diabetes. *Diabetologia* 55, 589–599. doi: 10.1007/s00125-011-2408-x

Dempsey, P. C., Blankenship, J. M., Larsen, R. N., Sacre, J. W., Sethi, P., Straznicky, N. E., et al. (2016a). Interrupting prolonged sitting in type 2 diabetes: nocturnal persistence of improved glycaemic control. *Diabetologia* 60, 499–507. doi: 10.1007/s00125-016-4169-z

Dempsey, P. C., Larsen, R. N., Sethi, P., Sacre, J. W., Straznicky, N. E., Cohen, N. D., et al. (2016b). Benefits for Type 2 diabetes of interrupting prolonged sitting with brief bouts of light walking or simple resistance activities. *Diabetes Care* 39, 964–972. doi: 10.2337/dc15-2336

Dempsey, P. C., Owen, N., Yates, T. E., Kingwell, B. A., and Dunstan, D. W. (2016c). Sitting less and moving more: improved glycaemic control for type 2 diabetes prevention and management. *Curr. Diab. Rep.* 16, 114. doi: 10.1007/s11892-016-0797-4

Despres, J. P. (2016). Physical activity, sedentary behaviours, and cardiovascular health: when will cardiorespiratory fitness become a vital sign? *Can. J. Cardiol.* 32, 505–513. doi: 10.1016/j.cjca.2015.12.006

Dickie, K., Micklesfield, L. K., Chantler, S., Lambert, E. V., and Goedecke, J. H. (2016). Cardiorespiratory Fitness and light-intensity physical activity are independently associated with reduced cardiovascular disease risk in Urban Black South African Women: a cross-sectional study. *Metab. Syndr. Relat. Disord.* 14, 23–32. doi: 10.1089/met.2015.0064

Dunstan, D. W., Kingwell, B. A., Larsen, R., Healy, G. N., Cerin, E., Hamilton, M. T., et al. (2012). Breaking up prolonged sitting reduces postprandial glucose and insulin responses. *Diabetes Care* 35, 976–983. doi: 10.2337/dc11-1931

Duvivier, B. M., Schaper, N. C., Hesselink, M. K., van Kan, L., Stienen, N., Winkens, B., et al. (2016). Breaking sitting with light activities vs structured exercise: a randomised crossover study demonstrating benefits for glycaemic control and insulin sensitivity in type 2 diabetes. *Diabetologia* 60, 490–498. doi: 10.1007/s00125-016-4161-7

Ekblom-Bak, E., Ekblom, O., Bolam, K. A., Ekblom, B., Bergstrom, G., and Borjesson, M. (2016). SCAPIS pilot study: sitness, fitness and fatness - is sedentary time substitution by physical activity equally important for everyone's markers of glucose regulation? *J. Phys. Act. Health* 13, 697–703. doi: 10.1123/jpah.2015-0611

Ekelund, U., Brage, S., Griffin, S. J., Wareham, N. J., and ProActive, U. K. Research Group. (2009). Objectively measured moderate- and vigorous-intensity physical activity but not sedentary time predicts insulin resistance in high-risk individuals. *Diabetes Care* 32, 1081–1086. doi: 10.2337/dc08-1895

Ekelund, U., Steene-Johannessen, J., Brown, W. J., Fagerland, M. W., Owen, N., Powell, K. E., et al. (2016). Does physical activity attenuate, or even eliminate, the detrimental association of sitting time with mortality? A harmonised meta-analysis of data from more than 1 million men and women. *Lancet* 388, 1302–1310. doi: 10.1016/S0140-6736(16)30370-1

Falconer, C. L., Page, A. S., Andrews, R. C., and Cooper, A. R. (2015). The potential impact of displacing sedentary time in adults with type 2 diabetes. *Med. Sci. Sports Exerc.* 47, 2070–2075. doi: 10.1249/MSS.0000000000000651

Fritschi, C., Park, H., Richardson, A., Park, C., Collins, E. G., Mermelstein, R., et al. (2015). Association between daily time spent in sedentary behavior and duration of hyperglycemia in type 2 diabetes. *Biol. Res. Nurs.* 18, 160–166. doi: 10.1177/1099800415600065

Gregg, E. W., Chen, H., Wagenknecht, L. E., Clark, J. M., Delahanty, L. M., Bantle, J., et al. (2012). Association of an intensive lifestyle intervention with remission of type 2 diabetes. *JAMA* 308, 2489–2496. doi: 10.1001/jama.2012.67929

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

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

Helmerhorst, H. J. F., Wijndaele, K., Brage, S., Wareham, N. J., and Ekelund, U. (2009). Objectively measured sedentary time may predict insulin resistance independent of moderate- and vigorous-intensity physical activity. *Diabetes* 58, 1776–1779. doi: 10.2337/db08-1773

Howard, B. J., Fraser, S. F., Sethi, P., Cerin, E., Hamilton, M. T., Owen, N., et al. (2013). Impact on hemostatic parameters of interrupting sitting with intermittent activity. *Med. Sci. Sports Exerc.* 45, 1285–1291. doi: 10.1249/MSS.0b013e318285f57e

Hu, F. B., Li, T. Y., Colditz, G. A., Willett, W. C., and Manson, J. E. (2003). Television watching and other sedentary behaviors in relation to risk of obesity and type 2 diabetes mellitus in women. *JAMA* 289, 1785–1791. doi: 10.1001/jama.289.14.1785

Judice, P. B., Hamilton, M. T., Sardinha, L. B., Zderic, T. W., and Silva, A. M. (2016). What is the metabolic and energy cost of sitting, standing and sit/stand transitions? *Eur. J. Appl. Physiol.* 116, 263–273. doi: 10.1007/s00421-015-3279-5

Kjobsted, R., Munk-Hansen, N., Birk, J. B., Foretz, M., Viollet, B., Bjornholm, M., et al. (2017). Enhanced muscle insulin sensitivity after contraction/exercise is mediated by AMPK. *Diabetes* 66, 598–612. doi: 10.2337/db16-0530.

Kjobsted, R., Pedersen, A. J., Hingst, J. R., Sabaratnam, R., Birk, J. B., Kristensen, J. M., et al. (2016). Intact regulation of the ampk signaling network in response to exercise and insulin in skeletal muscle of male patients with type 2 diabetes: illumination of ampk activation in recovery from exercise. *Diabetes* 65, 1219–1230. doi: 10.2337/db15-1034

Knowler, W. C., Barrett-Connor, E., Fowler, S. E., Hamman, R. F., Lachin, J. M., Walker, E. A., et al. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N. Engl. J. Med.* 346, 393–403. doi: 10.1056/NEJMoa012512

Krogh-Madsen, R., Thyfault, J. P., Broholm, C., Mortensen, O. H., Olsen, R. H., Mounier, R., et al. (2010). A 2-wk reduction of ambulatory activity attenuates peripheral insulin sensitivity. *J. Appl. Physiol. (1985)* 108, 1034–1040. doi: 10.1152/japplphysiol.00977.2009

Lahjibi, E., Heude, B., Dekker, J. M., Hojlund, K., Laville, M., Nolan, J., et al. (2013). Impact of objectively measured sedentary behaviour on changes in insulin resistance and secretion over 3 years in the RISC study: interaction with weight gain. *Diabetes Metab.* 39, 217–225. doi: 10.1016/j.diabet.2012.12.006

Lakka, T. A., Laaksonen, D. E., Lakka, H. M., Mannikko, N., Niskanen, L. K., Rauramaa, R., et al. (2003). Sedentary lifestyle, poor cardiorespiratory fitness, and the metabolic syndrome. *Med. Sci. Sports Exerc.* 35, 1279–1286. doi: 10.1249/01.MSS.0000079076.74931.9A

Lamb, M. J., Westgate, K., Brage, S., Ekelund, U., Long, G. H., Griffin, S. J., et al. (2016). Prospective associations between sedentary time, physical activity, fitness and cardiometabolic risk factors in people with type 2 diabetes. *Diabetologia* 59, 110–120. doi: 10.1007/s00125-015-3756-8

Larose, J., Sigal, R. J., Khandwala, F., Prud'homme, D., Boule, N. G., Kenny, G. P., et al. (2011). Associations between physical fitness and HbA(1)(c) in type 2 diabetes mellitus. *Diabetologia* 54, 93–102. doi: 10.1007/s00125-010-1941-3

Larsen, R. N., Kingwell, B. A., Sethi, P., Cerin, E., Owen, N., and Dunstan, D. W. (2014). Breaking up prolonged sitting reduces resting blood pressure in overweight/obese adults. *Nutr. Metab. Cardiovasc. Dis.* 24, 976–982. doi: 10.1016/j.numecd.2014.04.011

Latouche, C., Jowett, J. B., Carey, A. L., Bertovic, D. A., Owen, N., Dunstan, D. W., et al. (2013). Effects of breaking up prolonged sitting on skeletal muscle gene expression. *J. Appl. Physiol. (1985)* 114, 453–460. doi: 10.1152/japplphysiol.00978.2012

Lohman, T. G., Roche, A. S., and Martorell, R. (1988). *Anthropometric Standardization Reference Manual.* Champaign, IL: Human Kinetics.

Lorenzo, C., Haffner, S. M., Stancakova, A., and Laakso, M. (2010). Relation of direct and surrogate measures of insulin resistance to cardiovascular risk factors in nondiabetic finnish offspring of type 2 diabetic individuals. *J. Clin. Endocrinol. Metab.* 95, 5082–5090. doi: 10.1210/jc.2010-1144

Matsuda, M., and DeFronzo, R. A. (1999). Insulin sensitivity indices obtained from oral glucose tolerance testing - Comparison with the euglycemic insulin clamp. *Diabetes Care* 22, 1462–1470. doi: 10.2337/diacare.22.9.1462

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., and Turner, R. C. (1985). Homeostasis model assessment - insulin resistance and beta-cell function from fasting plasma-glucose and insulin concentrations in man. *Diabetologia* 28, 412–419. doi: 10.1007/BF00280883

Montgomery, D. C., and Peck, E. C. (1982). *Introduction to Linear Regression Analysis*. New York, NY: John Wiley & Sons.

Moura, B. P., Amorim, P. R., Silva, B. P., Franceschini, S. C., Reis, J. S., and Marins, J. C. (2014). Effect of a short-term exercise program on glycemic control measured by fructosamine test in type 2 diabetes patients. *Diabetol. Metab. Syndr.* 6:16. doi: 10.1186/1758-5996-6-16

Osler, M. E., Fritz, T., Caidahl, K., Krook, A., Zierath, J. R., and Wallberg-Henriksson, H. (2015). Changes in gene expression in responders and nonresponders to a low-intensity walking intervention. *Diabetes Care* 38, 1154–1160. doi: 10.2337/dc14-2606

Pan, X. R., Li, G. W., Hu, Y. H., Wang, J. X., Yang, W. Y., An, Z. X., et al. (1997). Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing, IGT and Diabetes Study. *Diabetes Care* 20, 537–544. doi: 10.2337/diacare.20.4.537

Redmon, J. B., Bertoni, A. G., Connelly, S., Feeney, P. A., Glasser, S. P., Glick, H., et al. (2010). Effect of the look AHEAD study intervention on medication use and related cost to treat cardiovascular disease risk factors in individuals with type 2 diabetes. *Diabetes Care* 33, 1153–1158. doi: 10.2337/dc09-2090

Rockette-Wagner, B., Edelstein, S., Venditti, E. M., Reddy, D., Bray, G. A., Carrion-Petersen, M. L., et al. (2015). The impact of lifestyle intervention on sedentary time in individuals at high risk of diabetes. *Diabetologia* 58, 1198–1202. doi: 10.1007/s00125-015-3565-0

Rohling, M., Strom, A., Bonhof, G., Puttgen, S., Bodis, K., Mussig, K., et al. (2016). Differential patterns of impaired cardiorespiratory fitness and cardiac autonomic dysfunction in recently diagnosed type 1 and type 2 diabetes. *Diabetes Care* 40, 246–252. doi: 10.2337/dc16-1898

Santos, D. A., Gobbo, L. A., Matias, C. N., Petroski, E. L., Goncalves, E. M., Cyrino, E. S., et al. (2013). Body composition in taller individuals using DXA: a validation study for athletic and non-athletic populations. *J. Sports Sci.* 31, 405–413. doi: 10.1080/02640414.2012.734918

Smith, A. D., Crippa, A., Woodcock, J., and Brage, S. (2016). Physical activity and incident type 2 diabetes mellitus: a systematic review and dose-response meta-analysis of prospective cohort studies. *Diabetologia* 59, 2527–2545. doi: 10.1007/s00125-016-4079-0

Stanford, K. I., and Goodyear, L. J. (2014). Exercise and type 2 diabetes: molecular mechanisms regulating glucose uptake in skeletal muscle. *Adv. Physiol. Educ.* 38, 308–314. doi: 10.1152/advan.00080.2014

Sui, X., Jackson, A. S., Church, T. S., Lee, D. C., O'Connor, D. P., Liu, J., et al. (2012). Effects of cardiorespiratory fitness on aging: glucose trajectory in a cohort of healthy men. *Ann. Epidemiol.* 22, 617–622. doi: 10.1016/j.annepidem.2012.05.005

Sylow, L., Kleinert, M., Richter, E. A., and Jensen, T. E. (2016). Exercise-stimulated glucose uptake - regulation and implications for glycaemic control. *Nat. Rev. Endocrinol.* 13, 133–148. doi: 10.1038/nrendo.2016.162

Troiano, R. P., Berrigan, D., Dodd, K. W., Masse, L. C., Tilert, T., and McDowell, M. (2008). Physical activity in the United States measured by accelerometer. *Med. Sci. Sports Exerc.* 40, 181–188. doi: 10.1249/mss.0b013e31815a51b3

Tuomilehto, J., Lindstrom, J., Eriksson, J. G., Valle, T. T., Hamalainen, H., Ilanne-Parikka, P., et al. (2001). Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N. Engl. J. Med.* 344, 1343–1350. doi: 10.1056/NEJM200105033441801

van der Berg, J. D., Stehouwer, C. D., Bosma, H., van der Velde, J. H., Willems, P. J., Savelberg, H. H., et al. (2016). Associations of total amount and patterns of sedentary behaviour with type 2 diabetes and the metabolic syndrome: the Maastricht Study. *Diabetologia* 59, 709–718. doi: 10.1007/s00125-015-3861-8

van der Ploeg, H. P., Chey, T., Korda, R. J., Banks, E., and Bauman, A. (2012). Sitting time and all-cause mortality risk in 222 497 Australian adults. *Arch. Intern. Med.* 172, 494–500. doi: 10.1001/archinternmed.2011.2174

van Dijk, J. W., Venema, M., van Mechelen, W., Stehouwer, C. D., Hartgens, F., and van Loon, L. J. (2013). Effect of moderate-intensity exercise versus activities of daily living on 24-hour blood glucose homeostasis in male patients with type 2 diabetes. *Diabetes Care* 36, 3448–3453. doi: 10.2337/dc12-2620

World Medical Association (2008). Declaration of Helsinki - ethical principles for medical research involving human subjects. *JAMA* 54, 122–125. doi: 10.1001/jama.2013.281053

Yates, T., Davies, M. J., Henson, J., Edwardson, C., Webb, D., Bodicoat, D. H., et al. (2015a). Effect of the PPARG2 Pro12Ala polymorphism on associations of physical activity and sedentary time with markers of insulin sensitivity in those with an elevated risk of type 2 diabetes. *PLoS ONE* 10:e0124062. doi: 10.1371/journal.pone.0124062

Yates, T., Henson, J., Edwardson, C., Dunstan, D., Bodicoat, D. H., Khunti, K., et al. (2015b). Objectively measured sedentary time and associations with insulin sensitivity: importance of reallocating sedentary time to physical activity. *Prev. Med.* 76, 79–83. doi: 10.1016/j.ypmed.2015.04.005

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Sedentary Behavior Is Only Marginally Associated with Physical Function in Adults Aged 40–75 Years—the Maastricht Study

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Background: In an aging population, regular physical activity (PA) and exercise have been recognized as important factors in maintaining physical function and thereby preventing loss of independence and disability. However, (older) adults spent the majority of their day sedentary and therefore insight into the consequences of sedentary behavior on physical function, independent of PA, is warranted.

Objective: To examine the associations of objectively measured sedentary time (ST), patterns of sedentary behavior, overall PA, and higher intensity PA (HPA) with objective measures of physical function.

Methods: This is a cross-sectional study in 1,932 men and women (aged 40–75 years) participating in The Maastricht Study. The activPAL3 was used to assess daily sedentary behavior: ST (h), sedentary breaks (n), prolonged (≥30 min) sedentary bouts (n), and to assess time spent in (H)PA (h). Measures of physical function included: covered distance during a 6 min walk test [6MWD (meters)], timed chair rise stand test performance [$TCST_{time}$ (seconds)], grip strength (kg kg^{-1}), and elbow flexion and knee extension strength (Nm kg^{-1}). Linear regression analyses were used to examine associations between daily sedentary behavior and PA with physical function.

Results: Every additional hour ST was associated with shorter 6MWD [B = −2.69 m (95% CI = −4.69; −0.69)] and lower relative elbow extension strength (B = −0.01 Nm kg^{-1} (−0.02; 0.00). More sedentary breaks were associated with faster $TCST_{time}$: B = −0.55 s (−0.85; −0.26). Longer average sedentary bout duration was associated with slower $TCST_{time}$ [B = 0.17 s (0.09; 0.25)] and lower knee extension strength

[B = −0.01 Nm kg^{-1} (−0.02; 0.00)]. Every hour of PA and HPA were associated with greater 6MWD [B_{PA} = 15.88 m (9.87; 21.89), B_{HPA} = 40.72 m (30.18; 51.25)], faster $TCST_{time}$ [B_{PA} = −0.55 s (−1.03; −0.07), B_{HPA} = −2.25 s (−3.09; −1.41)], greater elbow flexion strength [B_{PA} = 0.03 Nm kg^{-1} (0.01; 0.07)], [B_{HPA} = 0.05 Nm kg^{-1} (0.01; 0.08)], and greater knee extension strength [B_{PA} = 0.04 Nm kg^{-1} (0.01; 0.07)], [B_{HPA} = 0.13 Nm kg^{-1} (0.06; 0.20)].

Conclusion: In adults aged 40–75 years, sedentary behavior appeared to be marginally associated with lower physical function, independent of HPA. This suggests that merely reducing sedentary behavior is insufficient to improve/maintain physical function. In contrast, engaging regularly in PA, in particular HPA, is important for physical function.

Keywords: accelerometry, muscle strength, sedentary lifestyle, pattern, physical fitness

INTRODUCTION

Physical function, or physical capability, can be defined as the degree to which a person can manage the physical tasks of daily living. This can be objectified by several performance tests such as strength, walking speed, and mobility. Deterioration in physical function has been associated with loss of independence, a reduced quality of life, disability, and mortality (Cooper et al., 2010, 2011). Limitations in physical functioning occur more often in later stages of life. For example, in the European Union 27% of the total population reported limitations in daily activities, for adults aged >65 years this was ∼40%, and for adults aged >75 years in excess of 60% (Statistical Office of the European Communities, 2015). In an aging population, such as in many European countries, the number of people at risk for functional limitations will increase further. Thus, identifying modifiable determinants that are important for improving or maintaining physical function is imperative. One of these determinants is physical activity (PA; Paterson and Warburton, 2010).

PA, particularly PA of higher intensity, often termed moderate to vigorous PA (MVPA), has been recognized as a major determinant for overall physical well-being (Warburton et al., 2010). Positive associations of MVPA with physical function (Paterson and Warburton, 2010; Bauman et al., 2016) and with leg strength (Volkers et al., 2012) have been reported. The importance of MVPA is nowadays well-recognized and PA guidelines worldwide advocate to spend at least 150 min per week in MVPA (Kahlmeier et al., 2015). Nonetheless, MVPA only comprises a small part of daily activities. Most of the day is generally spent in sedentary behavior in current Westernized societies (Owen et al., 2010). In recent years, there has been a growing interest in sedentary behavior as a determinant for adverse health outcomes.

Sedentary behavior refers to any waking behavior, characterized by an energy expenditure ≤1.5 metabolic equivalents (METs) while in a sitting or reclining position (Sedentary Behaviour Research Network, 2012). An increasing number of studies have associated a larger amount of sedentary time (ST) with unfavorable metabolic and cardiovascular risk markers, independent of MVPA (Wilmot et al., 2012; Brocklebank et al., 2015). However, whether or not a larger amount of ST is associated with lower physical function is less clear. Several population based studies have examined the association of ST and physical function (Santos et al., 2012; Cooper et al., 2015; Lee et al., 2015; Keevil et al., 2016; Reid et al., 2016; Rosenberg et al., 2016). Findings from these studies were inconsistent as some studies did report an association between larger amounts of ST and worse physical function (Santos et al., 2012; Cooper et al., 2015; Lee et al., 2015; Rosenberg et al., 2016), whereas other studies reported no such association (Keevil et al., 2016; Reid et al., 2016). Additionally, not only total ST, but also the pattern in which it is accumulated may be relevant for health. This pattern can be expressed by the number of interruptions in ST (sedentary breaks), by the average duration of uninterrupted periods of sitting or by the number of prolonged (e.g., ≥30 min) uninterrupted sedentary bouts. In studies with older adults (mean age >70 years), more sedentary breaks have been associated with a higher score on the senior fitness test and physical performance tests (Davis et al., 2014; Sardinha et al., 2015). Whether or not these patterns are associated with physical function at younger ages is uncertain.

As sedentary behavior appears to increase with age (Matthews et al., 2008; Evenson et al., 2012), an improved insight into the associations of ST (and the pattern in which this is accumulated) with physical function is warranted. If such associations exist, reducing sedentary behavior could be important in the prevention of functional limitations. Therefore, our objective was to examine the associations of objectively measured ST, patterns of sedentary behavior, overall PA, and higher intensity PA (HPA) with objective measures of physical function in an adult population aged 40–75 years.

METHODS

Population

We used data from The Maastricht Study, an observational prospective population-based cohort study. The rationale and methodology have been described previously (Schram

Abbreviations: PA, physical activity; HPA, higher intensity physical activity; ST, sedentary time; 6MWD, distance (meters) covered during the 6 min walk test; $TCST_{time}$, time (in seconds) needed for timed chair rise stand test.

et al., 2014). In brief, the study focuses on the etiology, pathophysiology, complications and comorbidities of type 2 diabetes mellitus (T2DM) and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known T2DM status, with an oversampling of individuals with T2DM, for reasons of efficiency. The present report includes cross-sectional data from a convenience sample of the first 3,451 participants, who completed the baseline survey between November 2010 and September 2013. Data were available for 1,932 participants, after excluding participants that did not receive an accelerometer due to logistics ($n = 673$), with invalid accelerometer readings ($n = 136$), with missing/unperformed physical function testing ($n = 629$) or with missing covariates ($n = 81$). The examinations of each participant were performed within a time window of 3 months. The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131088-105234-PG). All participants gave written informed consent in accordance with the Declaration of Helsinki.

Accelerometry: Sedentary Behavior, PA, and HPA

Daily activity levels were measured using the activPAL3™ PA monitor (PAL Technologies, Glasgow, UK). The activPAL3 is a small (53 × 35 × 7 mm), lightweight (15 g) triaxial accelerometer that records movement in the vertical, anteroposterior and mediolateral axes, and also determines posture (sitting or lying, standing, and stepping) based on acceleration information. The device was attached directly to the skin on the front of the right thigh with transparent 3M Tegaderm™ tape, after the device had been waterproofed using a nitrile sleeve. Participants were asked to wear the accelerometer for 8 consecutive days, without removing it at any time. To avoid inaccurately identifying non-wear time, participants were asked not to replace the device once removed. Data were uploaded using the activPAL software and processed using customized software written in MATLAB R2013b (MathWorks, Natick, MA, USA). Data from the first day were excluded from the analysis because participants performed physical function tests at the research center after the device was attached. In addition, data from the final wear day providing ≤14 waking hours of data were excluded from the analysis. Participants were included if they provided at least 1 valid day (≥10 h of waking data).

The total amount of ST was based on the sedentary posture (sitting or lying), and calculated as the mean time spent in a sedentary position during waking time per day. The method used to determine waking time has been described elsewhere (van der Berg et al., 2016). The total amount of standing time was based on the standing posture, and calculated as the mean time spent standing during waking time per day. The total amount of stepping was based on the stepping posture, and calculated as the mean time stepping during waking time per day. Stepping time (PA) was further classified into higher intensity physical activity (HPA; minutes with a step frequency >110 steps/min during waking time) and lower intensity physical activity (LPA; minutes with a step frequency ≤110 steps/min during waking time; Tudor-Locke et al., 2011).

The number of sedentary breaks during waking time was determined as each transition from a sitting or lying position to standing or stepping with a duration of at least 1 min, and the mean number of breaks per day was calculated. ST accumulated in a consecutive period ≥30 min was defined as a prolonged sedentary bout, and the mean number of prolonged sedentary bouts during waking time per day was calculated.

Physical Function

Physical function was assessed by four different tests: a fast paced 6 min walk test, the timed chair stand test (TCST), hand grip strength, and isometric strength tests of the knee extensors and elbow flexors.

Six Minute Walk Test

Participants were excluded from this test if they had experienced cardiovascular complications in the preceding 3 months, had severe hypertension (SBP ≥ 180 and/or DBP ≥ 110 mmHg), a resting heart rate of <40 of >110 beats min^{-1}, used a walker, or had other medical conditions which prevented them from walking independently. In a hallway, two cones were placed 20 meters apart around which the participants had to make turns. Participant were instructed to walk as many laps as possible in 6 min at a fast pace without running. Standardized encouragement was given every minute during the test. After 6 min, or when the participant was unwilling or unable to continue, the covered distance was measured. The covered distance (6MWD) in meters was used as measure for analyses.

Timed Chair Rise Stand Test

The timed chair rise stand test (TCST) was performed on a 46 cm high chair with a straight back and no arm-rests. The test started with the participant in a sitting position with his/her arms crossed over the chest. Participants were instructed to stand up to a full up-right position and to sit down again, as quickly as possible, without using their arms or hands to support. The time (in seconds) needed for 10 repetitions ($TCST_{time}$) was measured to the nearest of one decimal and was used for analyses.

Handgrip Strength

Handgrip strength was measured with the Jamar handheld dynamometer (SEHAN Corp., Korea-Biometrics Europe BV, Almere). During the test the participant was standing straight against the wall, with the upper arm along the trunk and the elbow in 90° flexion. Participants were instructed to squeeze as hard as possible in the dynamometer for 3–5 s, while given standard encouragement. The measurement was performed three times on each hand, alternating hands. Maximal strength (kg) from every trial was recorded. Maximum strength (in kg) out of all trials was normalized for body mass and was used for analyses.

Isometric Muscle Strength Test

Isometric muscle strength of the knee extensors and elbow flexors was assessed in a customized set-up with 2 dynamometers (Futek LSB302, FUTEK Advanced Sensor Technology Inc., Irvine, CA, USA) and recorded with the M-PAQ (Maastricht Instruments, Maastricht, the Netherlands). Measurements were performed on the right leg and arm. Participants were (partly) excluded from the test if they had undergone surgery on the right arm or leg in the preceding 3 months, or reported relevant injuries on the right arm or leg.

Participants were positioned up-right in the chair (hip angle 110°) with their knees flexed in a 90° angle and the upper leg fixated. A strap connected to the dynamometer (with the axis of the dynamometer corresponding to the knee-joint axis) was secured 2 cm above the lateral malleolus. Participants were instructed to extend their knee as powerful as possible for 5 s. Three trials were performed. For the measurement of elbow flexion strength, the participant remained up-right in the chair with the elbow flexed in a 90° angle. A strap connected to the dynamometer was secured 2 cm proximally from the wrist (with the axis of the dynamometer corresponding to the elbow-joint axis). Participants were instructed to flex their elbow as powerful as possible for 5 s. Three trials were performed. Participants were able to see the force generated on a monitor. During the trials participants were instructed to refrain from compensatory movements.

To calculate joint torques (Nm) for elbow and knee, the force applied on the dynamometers (N) was multiplied by the corresponding moment-arm (distance from the strap of the dynamometer to the rotation point of the knee joint and elbow joint, respectively). The joint torques were normalized for body mass (Nm/kg). The maximal normalized joint torques out of three trials for knee extension and elbow flexion were used in the analyses.

Covariates

Questionnaires were conducted to collect information on age (in years), sex, educational level, smoking behavior, alcohol consumption, cardiovascular disease history (CVD), self-reported physical functioning, and health status. Educational level was divided into low, middle, and high. Smoking behavior was divided into three categories: non-smoker, former smokers, and current smokers. Alcohol consumption was divided into three categories: non-consumers, low-consumers (for women $\leq$7 glasses alcohol per week; for men $\leq$14 glasses alcohol per week), and high-consumers (for women >7 glasses per week; for men >14 glasses alcohol per week). CVD was defined as a (self-reported) history of any of the following conditions: myocardial infarction, cerebrovascular infarction or hemorrhage, percutaneous artery angioplasty of, or vascular surgery on, the coronary, abdominal, peripheral, or carotid arteries. Self-reported physical functioning was based on the physical function score, ranging from 0 to 100, as obtained from the 36-Item Short Form Health Survey (SF-36). Health status was obtained from self-reported general health status on a 5-point scale ranging from "weak" to "excellent." BMI was calculated as: body mass (kg)/height (m)^2. For this, mass and height were measured to the nearest of 0.5 kg or 0.1 cm during physical examination. Type 2 diabetes was defined according to the World Health Organization 2006 criteria (World Health Organization, 2006), based on glucose levels in fasting state and directly after an oral glucose tolerance test. For details on this procedure see Schram et al. (2014).

Statistical Analyses

Descriptive statistics were presented for the included population and according to sex. Normally distributed variables were presented as mean (*SD*), skewed variables were presented as median [25–75%]. Percentages were provided for categorical variables.

Linear regression analyses were performed to assess the associations of ST, number of sedentary breaks, average sedentary bout duration, and number of prolonged sedentary bouts, total PA and HPA with the physical function measures. Associations were expressed as regression coefficients (B) with 95% confidence intervals. The associations in models 1 were adjusted for waking time, age, sex, education level, and type 2 diabetes (to account for oversampling in the study design). To assess if the associations were mutually independent, in models 2 HPA, was added to the models describing ST, ST was added in the models describing the associations of HPA (due to collinearity models of total PA were not adjusted for ST), and ST and HPA were both added in the models describing sedentary breaks, mean sedentary bout duration and number of prolonged sedentary bouts. Models 3 were additionally adjusted for several health-related factors: BMI, alcohol use, smoking status, CVD history, and health status. We chose to add these health-related factors in models 3 as some of these factors may cause overadjustment bias (in particular BMI and health status). For the ease of interpretation we chose to express the associations of ST, total PA and HPA per 1 h. In additional analyses, we have standardized these three exposure variables to allow a better comparison of strengths of the associations. Additionally, the analyses were repeated after excluding all participants with <4 valid days of activPAL data (n = 78) and after excluding participants who reported functional limitations, defined as having difficulty walking 500 m or climbing one flight of stairs as reported on the SF-36 (n = 328). All analyses were performed using IBM SPSS Statistics for Windows, Version 22.0. (Armonk, NY, USA: IBM Corp.).

RESULTS

Population Characteristics

Of the 1,932 participants, 51.4% were men. The mean (±*SD*) age was 59.7 (8.2) years and BMI was 26.8 (4.4; **Table 1**). In over 95% of participants 4 or more days with valid accelerometer data were obtained. During waking time 9.4 (1.6) h/day were spent in sedentary positions and 2.0 (0.7) h/day were spent in PA. The remainder of time was spent standing. Women spent less time in sedentary behavior and more time in HPA than men. Mean $TCST_{time}$ was similar between men and women. Mean 6MWD and strength measures were greater for men compared with women. When strength measures were adjusted for body

TABLE 1 | Descriptive characteristics of the study population (N = 1,932).

	Total population		Men (n = 993)		Women (n = 939)	
Age	59.7	(8.2)	60.8	(8.1)	58.6	(8.1)
Educational level (% high)	39.3		43.5		34.9	
Smoking status (% current)	12.5		13.5		11.5	
Alcohol consumption (% high)	26.3		23.7		29.2	
BMI	26.8	(4.4)	27.5	(4.0)	26.1	(4.6)
History of CVD (%)	15.7		18.8		12.4	
Type 2 diabetes mellitus (%)	26.0		36.0		15.5	
SF-36 physical function score	95	[85–100]	95	[85–100]	95	[80–100]
Valid days accelerometer data (n)	6.3	1.2	6.3	1.2	6.4	1.1
Waking time (h/day)	15.7	(0.9)	15.8	(0.9)	15.7	(0.9)
Sedentary time (h/day)	9.4	(1.6)	9.9	(1.5)	8.8	(1.6)
Total PA (h/day)	2.0	(0.7)	2.0	(0.7)	2.1	(0.6)
High intensity PA (min/day)	19.2	[9.6–32.0]	14.1	[6.9–26.5]	23.6	[14.5–35.7]
Sedentary breaks (N/day)	37.6	(8.5)	37.7	(9.0)	37.5	(8.0)
Average sedentary bout duration (min)	11.1	3.4	11.8	(3.7)	10.4	(2.9)
Sedentary bouts ≥30 min (N/day)	4.8	(1.5)	5.1	(1.6)	4.5	(1.4)
6 MWD (m)	585.1	(80.5)	594.1	(86.0)	575.5	(73.0)
Timed chair stand test (s)	23.8	(5.5)	23.8	(5.7)	23.7	(5.2)
Grip strength (kg)	35.7	(10.6)	43.6	(8.1)	27.4	(5.4)
Normalized grip strength (kg kg^{-1})	0.45	(0.12)	0.50	(0.11)	0.39	(0.09)
Elbow flexion strength (Nm)	59.2	(23.5)	73.2	(21.4)	44.2	(14.5)
Normalized elbow flexion (Nm kg^{-1})	0.75	(0.27)	0.86	(0.26)	0.64	(0.22)
Knee extension strength (Nm)	134.9	(44.8)	161.5	(39.8)	106.9	(30.4)
Normalized knee extension (Nm kg^{-1})	1.72	(0.48)	1.88	(0.46)	1.54	(0.45)

BMI, body mass index; CVD, cardiovascular disease; PA, physical activity; 6MWD, distance covered during six min walk test. Values expressed as mean (SD), median [25–75%], or percentages.

mass the differences between sexes were reduced, but relative measures of strength were still greater in men.

Sedentary Time and Patterns of Sedentary Behavior and Physical Function

Table 2 describes the associations of the sedentary behavior variables (sedentary time, sedentary breaks, average sedentary bout duration, and prolonged sedentary bouts) with measures of physical function. An additional hour of ST was associated with shorter 6MWD [B = −2.69 m (95% CI = −4.69; −0.69)] and lower elbow flexion strength [B = −0.01 Nm kg^{-1} (−0.02; 0.00)] independent of HPA and other potential confounders (model 3). Every 10 additional sedentary breaks per day were associated with better $TCST_{time}$ [B = −0.55 s (−0.85; −0.26)] in model 3, but not with the other measures of physical function. A longer average sedentary bout duration was associated with poorer performance on the $TCST_{time}$ [B = 0.17 s (0.09; 0.25)] and with lower relative knee extension strength [B = −0.01 Nm kg^{-1} (−0.02; 0.00)].

Physical Activity and Physical Function

Table 3 describes the associations of total PA and HPA with measures of physical function. Total PA was associated with all the different physical function outcome measures in models 1. After additional adjustment for BMI, alcohol use, smoking status, cardiovascular disease, and health status (models 3) an additional hour of total PA was statistically significant associated with longer 6MWD [B = 16.45 m (11.89; 21.02)], better $TCST_{time}$ [B = −0.67 s (−1.03; −0.30)], and greater elbow flexion strength [B = 0.03 Nm kg^{-1} (0.01; 0.07)] and knee extension strength [B = 0.04 Nm kg^{-1} (0.01; 0.07)]. Associations between HPA and physical function were observed independent of ST in models 2. In the fully adjusted models (models 3) an additional hour of HPA was associated with longer 6MWD [B = 40.72 m (30.18; 51.25)], $TCST_{time}$ [B = −2.25 s (−3.09; −1.41)], and greater relative elbow flexion strength [B = 0.05 Nm kg^{-1} (0.01; 0.08)] and knee extension strength [B = 0.13 Nm kg^{-1} (0.06; 0.20)].

Additional Analyses

To allow a better comparison of the strength of the associations of ST, total PA, and HPA with the physical function outcomes, differences in physical function outcomes were expressed per one standard deviation (*SD*) of ST, total PA, and HPA. Results are presented in Supplemental Table 1 and underline that associations of total PA and HPA with physical function were stronger than associations of ST with physical function.

All analyses were repeated after excluding participants with manifest functional limitations (n = 328). The association between ST and 6MWD was attenuated and no longer significant [B = −2.42 (−6.72; 1.86)]. Other results were similar as described above (data not tabulated). Additionally, results were similar

TABLE 2 | Associations of sedentary time and sedentary behavior pattern variables with distance during a six min walk test (6WMD), timed chair rise stand test performance (TCST time), grip strength, elbow flexion strength, and knee extension strength.

		Model 1		Model 2		Model 3	
		B	95% CI	B	95% CI	B	95% CI
Sedentary time (h/day)	6 MWT distance (m)	**−7.46**	**(−9.51; −5.40)**	**−4.39**	**(−6.49; −2.29)**	**−2.69**	**(−4.69; −0.69)**
	TCST time (s)*	**0.30**	**(0.15; 0.46)**	**0.16**	**(0.00; 0.32)**	0.11	(−0.05; 0.27)
	Grip strength (kg kg^{-1})	**−0.01**	**(−0.01; −0.01)**	**−0.01**	**(−0.01; 0.00)**	0.00	(−0.01; 0.00)
	Elbow flexion strength (Nm kg^{-1})	**−0.02**	**(−0.03; −0.01)**	**−0.02**	**(−0.02; −0.01)**	**−0.01**	**(−0.02; 0.00)**
	Knee extension strength (Nm kg^{-1})	**−0.03**	**(−0.04; −0.01)**	**−0.02**	**(−0.03; 0.00)**	−0.01	(−0.02; 0.01)
Sedentary breaks (10/day)	6 MWT distance (m)	**8.46**	**(4.53; 12.39)**	**5.32**	**(1.46; 9.18)**	2.57	(−1.13; 6.28)
	TCST time (s)*	**−0.71**	**(−1.00; −0.42)**	**−0.59**	**(−0.88; −0.30)**	**−0.55**	**(−0.85; −0.26)**
	Grip strength (kg kg^{-1})	**0.01**	**(0.01; 0.02)**	**0.01**	**(0.00; 0.01)**	0.00	(−0.01; 0.01)
	Elbow flexion strength (Nm kg^{-1})	**0.03**	**(0.01; 0.04)**	**0.02**	**(0.01; 0.03)**	0.01	(−0.01; 0.02)
	Knee extension strength (Nm kg^{-1})	**0.05**	**(0.02; 0.07)**	**0.04**	**(0.01; 0.06)**	0.02	(−0.01; 0.04)
Average sedentary bout duration (min)	6 MWT distance (m)	**−3.46**	**(−4.41; −2.52)**	**−1.81**	**(−2.89; −0.72)**	−0.68	(−1.74; 0.37)
	TCST time (s)*	**0.22**	**(0.15; 0.29)**	**0.18**	**(0.10; 0.26)**	**0.17**	**(0.09; 0.25)**
	Grip strength (kg kg^{-1})	**−0.01**	**(−0.01; 0.00)**	**−0.00**	**(−0.01; 0.00)**	0.00	(0.00; 0.00)
	Elbow flexion strength (Nm kg^{-1})	**−0.01**	**(−0.01; −0.01)**	**−0.01**	**(−0.01; 0.00)**	0.00	(−0.01; 0.00)
	Knee extension strength (Nm kg^{-1})	**−0.02**	**(−0.03; −0.01)**	**−0.02**	**(−0.02; −0.01)**	**−0.01**	**(−0.02; 0.00)**
≥30 min sedentary bout (n/day)	6 MWT distance (m)	**−7.75**	**(−9.83; −5.68)**	**−4.08**	**(−7.36; −0.79)**	−2.85	(−5.98; 0.28)
	TCST time (s)*	**0.34**	**(0.18; 0.49)**	0.23	(−0.24; 0.48)	0.21	(−0.04; 0.46)
	Grip strength (kg kg^{-1})	**−0.01**	**(−0.01; −0.01)**	**−0.01**	**(−0.01; 0.00)**	0.00	(−0.01; 0.00)
	Elbow flexion strength (Nm kg^{-1})	**−0.02**	**(−0.03; −0.01)**	−0.01	(−0.02; 0.00)	0.00	(−0.01; 0.01)
	Knee extension strength (Nm kg^{-1})	**−0.03**	**(−0.04; −0.02)**	**−0.02**	**(−0.04; 0.00)**	−0.01	(−0.03; 0.01)

*Results are presented as unstandardized regression coefficients (B) with 95% confidence interval (95% CI). *Positive coefficient indicates poorer performance. Associations were adjusted for the following covariates; Model 1: waking time, age, sex, type 2 diabetes, and education level. Model 2: model 1 + HPA. Model 3: model 2 +BMI, alcohol use, smoking status, cardiovascular disease, and health status. Bold fonts indicate statistical significance ($p < 0.05$).*

TABLE 3 | Associations of total physical activity (PA), and higher intensity physical activity (HPA) in hours per day with distance during a six min walk test (6WMD), timed chair rise stand test performance (TCST time), grip strength, elbow flexion strength, and knee extension strength.

		Model 1		Model 2		Model 3	
		B	95% CI	B	95% CI	B	95% CI
Total PA (h/day)	6 MWT distance (m)	**24.45**	**(19.74; 29.15)**			**16.45**	**(1189; 21.02)**
	TCST time (s)*	**−0.88**	**(−1.24; −0.52)**			**−0.67**	**(−1.03; −0.30)**
	Grip strength (kg kg^{-1})	**0.02**	**(0.01; 0.03)**			0.01	(0.00; 0.01)
	Elbow flexion strength (Nm kg^{-1})	**0.05**	**(0.04; 0.07)**			**0.03**	**(0.01; 0.04)**
	Knee extension strength (Nm kg^{-1})	**0.08**	**(0.05; 0.11)**			**0.04**	**(0.01; 0.07)**
HPA (h/day)	6 MWT distance (m)	**61.25**	**(50.73; 71.77)**	**54.51**	**(43.55; 65.48)**	**40.72**	**(30.18; 51.25)**
	TCST time (s)*	**−2.82**	**(−3.62; −2.03)**	**−2.58**	**(−3.42; −1.75)**	**−2.25**	**(−3.09; −1.41)**
	Grip strength (kg kg^{-1})	**0.04**	**(0.03; 0.06)**	**0.03**	**(0.02; 0.05)**	0.01	(0.00; 0.02)
	Elbow flexion strength (Nm kg^{-1})	**0.11**	**(0.08; 0.12)**	**0.09**	**(0.05; 0.13)**	**0.05**	**(0.01; 0.08)**
	Knee extension strength (Nm kg^{-1})	**0.22**	**(0.16; 0.29)**	**0.20**	**(0.13; 0.27)**	**0.13**	**(0.06; 0.20)**

*Results are presented as unstandardized regression coefficients (B) with 95% confidence interval (95% CI). *Negative coefficient indicates better performance.*
Associations were adjusted for the following covariates Model 1: waking time, age, sex, type 2 diabetes, and education level. Model 2:Models describing HPA were additionally adjusted for ST (due to collinearity models of total PA were not adjusted for ST). Model 3: model 2 +BMI, alcohol use, smoking status, cardiovascular disease, and health status. Bold fonts indicate statistical significance ($p < 0.05$).

after excluding participants with <4 valid days of accelerometer monitoring ($n = 78$).

DISCUSSION

This study examined the associations of objectivity measured ST, (patterns of) sedentary behavior, PA, and HPA with physical function in a large sample of adults aged 40–75 years. Our results showed that a larger amount of ST was associated with shorter 6MWD, and lower grip strength and elbow flexion strength. Additionally, more sedentary breaks were associated with faster $TCST_{time}$. Longer average sedentary bout duration was associated with slower $TCST_{time}$ and lower knee extension strength. However, the strength of these associations was relatively weak. PA and HPA were associated with greater 6MWD, faster TCSTtime, greater elbow flexion and knee extension strength. The associations of PA and HPA with physical function were stronger than the associations of sedentary behavior variables with physical function.

Sedentary Time

In our study, we observed a weak association between a large amount of ST and lower physical function. Several other epidemiological studies have examined objectively measured ST as a determinant of physical function expressed as gait speed or chair rise test (Santos et al., 2012; Davis et al., 2014; Cooper et al., 2015; Lee et al., 2015; Keevil et al., 2016; Reid et al., 2016; Rosenberg et al., 2016). Findings from these studies were inconsistent as in some studies an association was observed between larger amounts of ST and worse physical function (Santos et al., 2012; Davis et al., 2014; Cooper et al., 2015; Lee et al., 2015; Rosenberg et al., 2016), whereas in other studies no association was observed (Keevil et al., 2016; Reid et al., 2016). To our knowledge, three studies examined associations between objectively measured ST and knee extension strength (Willoughby and Copeland, 2015; Foong et al., 2016; Reid et al., 2016). In agreement with our results, these studies reported no association between ST and knee extension strength. Two other studies reported on associations between objectively measured ST and hand grip strength with different results. Cooper et al. (2015) did observe an association between ST and grip strength, while Keevil et al. (2016) did not. A difference between our study and the others was that we normalized measures of strength for body mass. Normalization for body mass allows better comparisons of strength measures between individuals of different body sizes (Jaric, 2002). We argued that an individual with greater body mass needs more strength to carry his/her own weight, thus a relative measure of strength would better reflect physical function. In addition, compared with absolute measures, normalized measures of hand grip strength and knee extension strength have been associated more strongly with functional limitations (Barbat-Artigas et al., 2013; Dong et al., 2016).

Patterns of Sedentary Behavior

In this study we observed some associations between the patterns of sedentary behavior with physical function. However, strength of these associations was rather weak. Few other studies have examined patterns of sedentary behavior and associations with physical function. In a small study ($n = 44$, mean age 70 ± 8 years), Genusso et al. reported that the number of sedentary breaks were positively and the number of prolonged sedentary bouts were negatively associated physical function (Gennuso et al., 2016). In addition, Sardinha et al. reported a positive association between sedentary breaks and physical function in a study with older adults (mean age 73 ± 6 years; Sardinha et al., 2015). In contrast, in the study by Reid et al., sedentary breaks and prolonged sedentary bouts were not associated with physical function (Reid et al., 2016). In our study, which was comparable to the study by Reid et al. in terms of age, we did however observe a small, beneficial association between the number of sedentary breaks and $TCST_{time}$.

Inconsistencies in outcomes between studies may have resulted from a difference in study populations. For example, the study by Reid et al. (2016), who reported no association between sedentary behavior and physical function, had the youngest study population (mean age 58 ± 10 years). The majority of the studies in which a negative association between large amounts of ST and physical function was reported comprised an older population, with mean age >65 years (Santos et al., 2012; Davis et al., 2014; Lee et al., 2015; Rosenberg et al., 2016). A younger population would generally be healthier and have a higher physical functioning. In our study, this was seen by a very high median [25–75%] SF-36 physical function score: 95 [85–100]. Consequently the measures of physical function may have a limited range due to a ceiling effect.

Physical Activity

Positive associations between PA, in particular HPA, and physical function are in line with the literature as summarized in reviews (Paterson and Warburton, 2010; Volkers et al., 2012). Both reviews incorporated longitudinal and/or intervention studies based on self-reported measures of PA. In addition, more recent studies that cross-sectionally examined associations between objectively measured PA and/or MVPA reported a positive association with physical function as well (Santos et al., 2012; Reid et al., 2016). As mentioned, the strength of the associations of sedentary behavior was small compared with the associations of PA and HPA. It is unlikely that the associations of sedentary behavior represent clinically meaningful differences. For example, in a population of COPD patients, ∼30 m was found to be the minimal clinically important difference in 6WMD (Polkey et al., 2013). In our study, each additional hour of ST was associated with 2.69 meters shorter 6MWD.

Future studies should examine the associations between objectively measured sedentary behavior and physical function in populations of different ages. Preferably these studies should have a longitudinal design to establish temporality. Importantly, future studies should provide answer to the important question: how much ST is too much? For instance in bed-rest studies, regarded as extreme conditions of ST, substantial muscle mass loss has been observed (Dirks et al., 2016). In our study [and others (Gennuso et al., 2016; Reid et al., 2016)], prolonged bouts of 30 min were used, but perhaps 30 min is not long enough to negatively affect physical function.

Strengths and Limitations

A strength of this study was the use of a posture based accelerometer. The activPAL3 has been found to measure ST and posture transitions (sedentary breaks) more accurately than accelerometers that determine ST based on acceleration data, which have been used in the majority of the studies (Kozey-Keadle et al., 2011; Berendsen et al., 2014). Therefore, estimations of ST were probably more accurate than those in studies using other types of accelerometers. Further, we used multiple objective measures of physical function that reflect upper and lower body function including several measures for muscle strength. However, this study is not without limitations. Importantly, due to the cross-sectional study design, caution is required with regard to causal inferences. It cannot be excluded that due to physical limitations, people engage less time in (H)PA and/or more in sedentary behaviors. However, in additional analyses we have demonstrated that after excluding individuals with mobility limitations the majority of the associations persisted. In addition, step frequency was used to determine HPA. This method may be less precise to determine intensity of PA compared with estimations based on acceleration data. However, we used a step frequency of >110 steps/min which has been reported to correspond to a MET score >3.0 (a commonly used as cut-off value for MVPA). Further, although the actviPAL3 may capture movement and intensity (based on step frequency), it does not provide context of activities. For example, the activPAL3 will classify (strength) training exercises as sedentary when performed in a sitting or lying position. Finally, our study population consisted of a highly functioning population aged 40–75 years. This was partly a result from the exclusion of participants that were unable to perform any of the physical function tests, introducing selection bias. In addition, The Maastricht Study population comprises adults of predominantly Caucasians from European descent. Therefore, generalizability of our results to other populations and ages may be limited. It is not unlikely that associations of sedentary behavior and PA with physical function are different in, for example, frail or institutionalized populations, which have other activity patterns and lower physical function.

CONCLUSION

In conclusion, in adults aged 40–75 years, sedentary behavior appeared to be marginally associated with lower physical function, independent of HPA. This suggests that merely reducing sedentary behavior is insufficient to improve/maintain physical function. On the other hand, engaging regularly in PA, and in particular HPA, is important for physical function.

AUTHOR CONTRIBUTIONS

JvV: Data analysis and writing the first draft of the manuscript. HS, AK, and NS: Study conception, interpretation of the results, and critically reviewed manuscript. JvB: Data acquisition, interpretation of the results, edited, and critically reviewed manuscript. SS, CvK, PD, MS, RH, PR, TvG, and CS: The Maastricht Study design and critically reviewed manuscript. All authors read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fphys.2017.00242/full#supplementary-material

REFERENCES

Barbat-Artigas, S., Rolland, Y., Cesari, M., Abellan Van Kan, G., Vellas, B., and Aubertin-Leheudre, M. (2013). Clinical relevance of different muscle strength indexes and functional impairment in women aged 75 years and older. *J. Gerontol. A Biol. Sci. Med. Sci.* 68, 811–819. doi: 10.1093/gerona/gls254

Bauman, A., Merom, D., Bull, F. C., Buchner, D. M., and Singh, M. A. F. (2016). Updating the evidence for physical activity: summative reviews of the epidemiological evidence, prevalence, and interventions to promote "Active Aging." *Gerontologist* 56, S268–S280. doi: 10.1093/geront/gnw031

Berendsen, B. A., Hendriks, M. R., Meijer, K., Plasqui, G., Schaper, N. C., and Savelberg, H. H. (2014). Which activity monitor to use? Validity, reproducibility and user friendliness of three activity monitors. *BMC Public Health* 14:749. doi: 10.1186/1471-2458-14-749

Brocklebank, L. A., Falconer, C. L., Page, A. S., Perry, R., and Cooper, A. R. (2015). Accelerometer-measured sedentary time and cardiometabolic biomarkers: a systematic review. *Prev. Med.* 76, 92–102. doi: 10.1016/j.ypmed.2015.04.013

Cooper, A. J., Simmons, R. K., Kuh, D., Brage, S., Cooper, R., Scientific, N., et al. (2015). Physical activity, sedentary time and physical capability in early old age: british birth cohort study. *PLoS ONE* 10:e0126465. doi: 10.1371/journal.pone.0126465

Cooper, R., Kuh, D., Cooper, C., Gale, C. R., Lawlor, D. A., Matthews, F., et al. (2011). Objective measures of physical capability and subsequent health: a systematic review. *Age Ageing* 40, 14–23. doi: 10.1093/ageing/afq117

Cooper, R., Kuh, D., Hardy, R., Mortality Review, G., Falcon, and Teams, H. A. S. (2010). Objectively measured physical capability levels and mortality: systematic review and meta-analysis. *BMJ* 341:c4467. doi: 10.1136/bmj.c4467

Davis, M. G., Fox, K. R., Stathi, A., Trayers, T., Thompson, J. L., and Cooper, A. R. (2014). Objectively measured sedentary time and its association with physical function in older adults. *J. Aging Phys. Act.* 22, 474–481. doi: 10.1123/japa.2013-0042

Dirks, M. L., Wall, B. T., Van De Valk, B., Holloway, T. M., Holloway, G. P., Chabowski, A., et al. (2016). One week of bed rest leads to substantial muscle atrophy and induces whole-body insulin resistance in the absence of skeletal muscle lipid accumulation. *Diabetes.* 65, 2862–2875. doi: 10.2337/db15-1661

Dong, R., Wang, X., Guo, Q., Wang, J., Zhang, W., Shen, S., et al. (2016). Clinical relevance of different handgrip strength indexes and mobility limitation in the elderly adults. *J. Gerontol. A. Biol. Sci. Med. Sci.* 71, 96–102. doi: 10.1093/gerona/glv168

Evenson, K. R., Buchner, D. M., and Morland, K. B. (2012). Objective measurement of physical activity and sedentary behavior among US adults aged 60 years or older. *Prev. Chronic Dis.* 9:110109. doi: 10.5888/pcd9.110109

Foong, Y. C., Chherawala, N., Aitken, D., Scott, D., Winzenberg, T., and Jones, G. (2016). Accelerometer-determined physical activity, muscle mass, and leg strength in community-dwelling older adults. *J. Cachexia Sarcopenia Muscle* 7, 275–283. doi: 10.1002/jcsm.12065

Gennuso, K. P., Thraen-Borowski, K. M., Gangnon, R. E., and Colbert, L. H. (2016). Patterns of sedentary behavior and physical function in older adults. *Aging Clin. Exp. Res.* 28, 943–950. doi: 10.1007/s40520-015-0386-4

Jaric, S. (2002). Muscle strength testing: use of normalisation for body size. *Sports Med.* 32, 615–631. doi: 10.2165/00007256-200232100-00002

Kahlmeier, S., Wijnhoven, T. M. A., Alpiger, P., Schweizer, C., Breda, J., and Martin, B. W. (2015). National physical activity recommendations: systematic overview and analysis of the situation in European countries. *BMC Public Health* 15:133. doi: 10.1186/s12889-015-1412-3

Keevil, V. L., Cooper, A. J., Wijndaele, K., Luben, R., Wareham, N. J., Brage, S., et al. (2016). Objective sedentary time, moderate-to-vigorous physical activity, and physical capability in a british cohort. *Med. Sci. Sports Exerc.* 48, 421–429. doi: 10.1249/MSS.0000000000000785

Kozey-Keadle, S., Libertine, A., Lyden, K., Staudenmayer, J., and Freedson, P. S. (2011). Validation of wearable monitors for assessing sedentary behavior. *Med. Sci. Sports Exerc.* 43, 1561–1567. doi: 10.1249/MSS.0b013e31820ce174

Lee, J., Chang, R. W., Ehrlich-Jones, L., Kwoh, C. K., Nevitt, M., Semanik, P. A., et al. (2015). Sedentary behavior and physical function: objective evidence from the Osteoarthritis initiative. *Arthritis Care Res. (Hoboken)* 67, 366–373. doi: 10.1002/acr.22432

Matthews, C. E., Chen, K. Y., Freedson, P. S., Buchowski, M. S., Beech, B. M., Pate, R. R., et al. (2008). Amount of time spent in sedentary behaviors in the United States, 2003–2004. *Am. J. Epidemiol.* 167, 875–881. doi: 10.1093/aje/kwm390

Owen, N., Healy, G. N., Matthews, C. E., and Dunstan, D. W. (2010). Too much sitting: the population health science of sedentary behavior. *Exerc. Sport Sci. Rev.* 38, 105–113. doi: 10.1097/JES.0b013e3181e373a2

Paterson, D. H., and Warburton, D. E. (2010). Physical activity and functional limitations in older adults: a systematic review related to Canada's Physical Activity Guidelines. *Int. J. Behav. Nutr. Phys. Act.* 7:38. doi: 10.1186/1479-5868-7-38

Polkey, M. I., Spruit, M. A., Edwards, L. D., Watkins, M. L., Pinto-Plata, V., Vestbo, J., et al. (2013). Six-minute-walk test in chronic obstructive pulmonary disease: minimal clinically important difference for death or hospitalization. *Am. J. Respir. Crit. Care Med.* 187, 382–386. doi: 10.1164/rccm.201209-1596OC

Reid, N., Daly, R. M., Winkler, E. A., Gardiner, P. A., Eakin, E. G., Owen, N., et al. (2016). Associations of monitor-assessed activity with performance-based physical function. *PLoS ONE* 11:e0153398. doi: 10.1371/journal.pone.0153398

Rosenberg, D. E., Bellettiere, J., Gardiner, P. A., Villarreal, V. N., Crist, K., and Kerr, J. (2016). Independent associations between sedentary behaviors and mental, cognitive, physical, and functional health among older adults in retirement communities. *J. Gerontol. A Biol. Sci. Med. Sci.* 71, 78–83. doi: 10.1093/gerona/glv103

Santos, D. A., Silva, A. M., Baptista, F., Santos, R., Vale, S., Mota, J., et al. (2012). Sedentary behavior and physical activity are independently related to functional fitness in older adults. *Exp. Gerontol.* 47, 908–912. doi: 10.1016/j.exger.2012.07.011

Sardinha, L. B., Santos, D. A., Silva, A. M., Baptista, F., and Owen, N. (2015). Breaking-up sedentary time is associated with physical function in older adults. *J. Gerontol. Series A Biol. Sci. Med. Sci.* 70, 119–124. doi: 10.1093/gerona/glu193

Schram, M. T., Sep, S. J., van der Kallen, C. J., Dagnelie, P. C., Koster, A., Schaper, N., et al. (2014). The Maastricht study: an extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. *Eur. J. Epidemiol.* 29, 439–451. doi: 10.1007/s10654-014-9889-0

Sedentary Behaviour Research Network (2012). Letter to the editor: standardized use of the terms "sedentary" and "sedentary behaviours." *Appl. Physiol. Nutr. Metab.* 37, 540–542. doi: 10.1139/h2012-02

Statistical Office of the European Communities (2015). *Functional and Activity Limitations Statistics.* Available online at: http://ec.europa.eu/eurostat/statistics-explained/index.php/Functional_and_activity_limitations_statistics Luxembourg, Eurostat (Accessed January 09, 2017).

Tudor-Locke, C., Craig, C. L., Brown, W. J., Clemes, S. A., De Cocker, K., Giles-Corti, B., et al. (2011). How many steps/day are enough? For adults. *Int. J. Behav. Nutr. Phys. Act.* 8:79. doi: 10.1186/1479-5868-8-79

van der Berg, J. D., Willems, P. J., van der Velde, J. H., Savelberg, H. H., Schaper, N. C., Schram, M. T., et al. (2016). Identifying waking time in 24-h accelerometry data in adults using an automated algorithm. *J. Sports Sci.* 34, 1867–1873. doi: 10.1080/02640414.2016.1140908

Volkers, K. M., De Kieviet, J. F., Wittingen, H. P., and Scherder, E. J. A. (2012). Lower limb muscle strength (LLMS): why sedentary life should never start? A review. *Arch. Gerontol. Geriatr.* 54, 399–414. doi: 10.1016/j.archger.2011.04.018

Warburton, D. E., Charlesworth, S., Ivey, A., Nettlefold, L., and Bredin, S. S. (2010). A systematic review of the evidence for Canada's physical activity guidelines for adults. *Int. J. Behav. Nutr. Phys. Act.* 7:1. doi: 10.1186/1479-5868-7-39

Willoughby, T., and Copeland, J. L. (2015). Sedentary time is not independently related to postural stability or leg strength in women 50-67 years old. *Appl. Physiol. Nutr. Metab.* 40, 1123–1128. doi: 10.1139/apnm-2015-0066

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

World Health Organization (2006). *Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia: Report of a WHO Consultation*. Geneva: World Health Organization.

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Effects of Multiple Sedentary Days on Metabolic Risk Factors in Free-Living Conditions: Lessons Learned and Future Recommendations

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Background: Recent experimental studies in adults have demonstrated that interruptions to prolonged sitting have beneficial effects on metabolic risk factors in adults, compared to prolonged sitting. We explored the hypothesis that multiple consecutive days of predominantly prolonged sedentary time may have an unfavorable effect on the postprandial response of C-peptide, glucose, and triglycerides in free-living healthy young men.

Methods: In this explorative pilot study, healthy young men (n = 7; 18–23 years) consumed standardized mixed meals at 1 and 5 h during two experimental laboratory-sitting days, with 6 days of predominantly prolonged sedentary time in between. Serum and plasma samples were obtained hourly from 0 to 8 h for measurement of glucose, C-peptide, and triglycerides. Participant's sedentary time was monitored using an accelerometer during the prolonged sedentary days as well as during 6 normal days prior to the first laboratory day. Differences in postprandial levels were assessed using generalized estimating equations analysis. Due to the explorative nature of this study and the small sample size, p-value was set at <0.10.

Results: Overall, when expressed as % of wear time, sedentary time was 5% higher during the 6 prolonged sedentary days, which was not significantly different compared to the 6 normal days (n = 4). Following 6 prolonged sedentary days, postprandial levels of C-peptide were significantly higher than at baseline (B = 0.11; 90%CI = [0.002; 0.22]; n = 7). Postprandial levels of glucose and triglycerides were not significantly different between the 2 laboratory days.

Conclusions: Due to the relatively high sedentary time at baseline, participants were unable to increase their sedentary time substantially. Nevertheless, postprandial C-peptide levels were slightly higher after 6 prolonged sedentary days than after 6 normal days.

Keywords: sedentary lifestyle, uninterrupted sitting, metabolic risk, postprandial, healthy adults

INTRODUCTION

Accumulating evidence from population-based studies indicates that prolonged sitting may have negative health effects in adults (Healy et al., 2008; Henson et al., 2013). Experimental studies on the acute effects of prolonged sitting in overweight/obese (Dunstan et al., 2012) and healthy (Peddie et al., 2013; Bailey and Locke, 2015) adults demonstrated that 1 day of uninterrupted sitting resulted in significant higher postprandial glucose and insulin levels, when compared to brief walking interruptions, i.e., 2 min interruptions every 20 min (Dunstan et al., 2012; Bailey and Locke, 2015) and 1 min 40 s interruptions every 30 min (Peddie et al., 2013), during prolonged sitting. However, brief standing interruptions, i.e., 2 min every 20 and 30 min, did not lower postprandial glucose and insulin levels in healthy adults (Miyashita et al., 2013; Bailey and Locke, 2015). Additionally, Altenburg et al. (2013) showed that 1 day of uninterrupted sitting resulted in significantly higher postprandial levels of C-peptide [i.e., reflecting endogenous insulin (Polonsky and Rubenstein, 1984; Van Cauter et al., 1992)] in healthy young men, when compared to hourly 8-min moderate-intensity physical activity interruptions to sitting.

Two recent studies compared the metabolic effects of sustained days of prolonged sitting in overweight/obese adults with sustained days of reduced (Thorp et al., 2014) and interrupted (Larsen et al., 2015) sitting in a laboratory setting. In a 3-day randomized crossover study Thorp et al. (2014) demonstrated that postprandial glucose level, but not insulin and triglyceride levels, was higher over the course of a day during prolonged sitting compared to a day of alternate standing and sitting in 30-min bouts. No temporal changes (day 1 vs. 5) were found in this study (Thorp et al., 2014). Similarly, in a 3-day randomized crossover study Larsen et al. (2015) found that sustained days of prolonged sitting resulted in higher postprandial glucose and insulin levels when compared to days with 2-min light activity interruptions every 20 min (i.e., treadmill walking), but no temporal changes (day 1 vs. 3) were found.

To date, only one study examined sustained days of prolonged sitting in free-living conditions (Lyden et al., 2015). In this study, young and healthy participants ($n = 10$; 4 males) were asked to increase their sitting time as much as possible for 7 consecutive days, limit their standing and walking and refrain from structured exercise and physical activity (Lyden et al., 2015). After 7 days of increased sitting time (i.e., sedentary time increased from 61 to 76% of wear time), glucose concentrations in response to a 2-h glucose tolerance test were similar, whereas insulin concentrations were significantly elevated. The current pilot study is the second study exploring the effects of 6 or more consecutive days of predominantly prolonged sitting in free-living conditions on postprandial glucose and lipid metabolism in healthy young adults. Confirmation of the findings from Lyden et al. (2015) is necessary to gain insight in the potential unfavorable health effects of consecutive days of prolonged sitting in free-living conditions. We hypothesized that 6 consecutive days of predominantly prolonged sitting may have an unfavorable effect on postprandial levels of C-peptide, glucose, and triglycerides in healthy young men.

MATERIALS AND METHODS

Participants

Seven males aged 18–23 years participated in this exploratory pilot study. Participants were recruited through distribution of flyers, announcements on University websites and Dutch recruitment websites. Participants were included if they (1) were normal weight (i.e., BMI<25), (2) were apparently healthy, (3) spent at least 30 min/day of moderate-to-vigorous physical activity (MVPA), (4) spent at least 20 min/day of vigorous physical activity on at least 2 days/week, (5) spent on average <2 h/day on prolonged sedentary time, (6) were Dutch or English speaking, and (7) signed a written informed consent. Exclusion criteria were major illness/injury or physical problems. Participants were screened using a health check questionnaire, including questions on medical history (e.g., heart, kidney, joint, muscle, asthmatic complaints; coagulation problems; chest pain) and usual pattern of sedentary behavior and physical activity was screened by dialogue. Participants were requested to refrain from any MVPA for at least 48 h prior to the experiment, and to avoid drinking alcohol and smoking for at least 24 h prior to the laboratory days. Finally, participants were requested to use passive transport (i.e., public transport or car) on the laboratory days.

Study Design and Procedures

This explorative pilot study involved 2 experimental laboratory-sitting days (i.e., pre- and post-test), with 6 consecutive increased prolonged sitting days in between (see **Figure 1**). As sitting time during the 6 consecutive sitting days was measured using accelerometers, these days were referred to as days of increased prolonged sedentary time (i.e., prolonged sedentary days). Accelerometer data of the prolonged sedentary days was used to check for the total (prolonged) sedentary time. Participants also wore an accelerometer during a normal week prior to the first laboratory day, to assess their "regular" daily activity. This study was approved by the Medical Ethics Committee of the VU University Medical Center in Amsterdam (No. 2011/171) and is in accordance with the Declaration of Helsinki.

On the evening before each laboratory-sitting day, participants consumed a standardized meal and snack. On the laboratory sitting days, participants visited the research unit after a 10-h fast. During the first visit, the informed consent and a health history were completed, and baseline anthropometrics were obtained ($t = 0$). Subsequently, an indwelling venous catheter was inserted in the antecubital vein of the left arm, to collect a baseline blood sample and to allow hourly blood sampling during the laboratory-sitting days. Participants then sat quietly for 1 h, in order to achieve a "steady state." After 1 h, participants consumed a standardized liquid high fat mixed meal, which they were requested to drink within 10 min. Then participants remained seated in a comfortable reclining chair for the next 7 h. They were allowed to use the computer

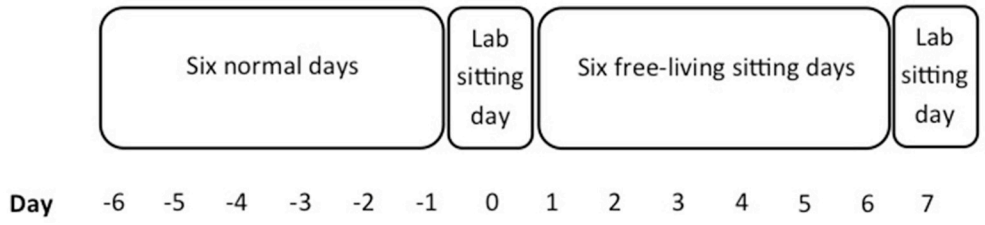

FIGURE 1 | Study design.

(e.g., watching movies, surfing on the Internet, or reading). Participants were instructed to minimize excessive movement but were allowed to visit the toilet. After 5 h of sitting ($t = 5$), participants consumed a standardized solid high fat mixed meal. Blood samples were collected hourly during each 8-h laboratory-sitting day (i.e., nine blood samples).

Prolonged Sedentary Days

During the 6 days of increased prolonged sedentary time participants were requested to increase their sedentary time as much as possible, directing them to remain seated for at least 8 h per day between 7 a.m. and 8 p.m. Participants were requested to spend four of these eight sedentary hours uninterrupted, except for visiting the toilet. During the remaining four sedentary hours, participants were allowed to interrupt their sedentary time once per hour, up to a maximal duration of 15 min, at a light- or moderate-intensity. Since total accumulated sedentary time had to be 8 h, participants had to lengthen (i.e., compensate) their sedentary time for each interruption. Participants were requested to refrain from vigorous physical activity during the 6 prolonged sedentary days.

Standardized Meals

Participants were studied in the postprandial state, as this is more likely to represent "normal daily life" than the fasted state. Importantly, the peaks in glucose and lipids induced by high-calorie (i.e., high carbohydrate and saturated fat content) meals are associated with biochemical inflammation, endothelial dysfunction and sympathetic hyperactivity (Eberly et al., 2003; Ceriello et al., 2004, 2006; O'Keefe and Bell, 2007). When repeated multiple times each day, these peaks in glucose and lipids increase the risk for atherosclerosis and CVD (O'Keefe and Bell, 2007). Maintenance of normal fasting and postprandial glucose levels depends on the ability to create an adequate insulin response to a meal. Participants were requested to consume a standardized meal dinner and an optional snack on the evening before each laboratory-sitting day. The dinner (three choices) consisted of 15 ± 5 g fat, 70 ± 10 g carbohydrates, and 25.8 ± 5.2 g proteins (in total 526.7 ± 40.4 kcal). The snack consisted of 0.6 g fat, 48.6 g carbohydrates, and 2 g proteins (in total 213 kcal). Participants were requested to consume the same meal (and snack) on the evening before each laboratory-sitting day.

The standardized liquid high fat mixed meal given after the first hour of "steady state" sitting (i.e., breakfast) consisted of 58.8 g fat, 92.0 g carbohydrates, and 15.6 g proteins (in total 843 kcal). The standardized solid high fat mixed meal consumed after 5 h of sitting (i.e., lunch) consisted of ∼ 77.1 g fat, 116.7 g carbohydrates, and 27.7 g proteins (in total 1190 kcal). The fat, carbohydrate, and protein content of the standardized meals were based on previous studies by our group in young adults, demonstrating postprandial increases in levels of HDL cholesterol, triglyceride, insulin, and glucose (Rotteveel et al., 2008; Altenburg et al., 2013).

Measurements

Height was measured with a Harpenden stadiometer with an accuracy of 0.1 cm, averaging three measurements. Weight was measured with a calibrated electronic scale (SECA 703) with an accuracy of 0.1 kg. Waist and hip circumference were measured with a flexible band with an accuracy of 0.5 cm. Body fat percentage was measured in a lying position using Bio-electrical Impedance Analysis (Maltron Body Composition Analyzer, BF-906) with an accuracy of 0.1%.

Plasma glucose levels were immediately assessed, within 10 s after collection, using the YSI2300 STAT Plus Analyzer (YSI, Yellow Springs, OH, USA) with an accuracy of 0.2 mmol/l. The second sample was centrifuged (10 min at a frequency of 3000 rpm) and subsequently stored at −80°C. From this sample, C-peptide and triglycerides were determined in heparin gel samples. All samples were analyzed in the same assay. Area under the curve (AUC) and incremental area under the curve (iAUC) were calculated for glucose, C-peptide, and triglyceride using the trapezoidal method, both for the 4- and 7-h postprandial period.

Participants wore an accelerometer (ActiTrainer, ActiLife v5.2.0) for 6 consecutive days, both during the 6 prolonged sedentary days and during 6 normal days before the start of the experiment. Epoch time was set at 15 s to capture the pattern of short duration interruptions to sedentary time. Participants were asked to wear the accelerometer at their right waist (using an elastic belt) during all waking hours, except for water-based activities. Periods of more than 60 min of consecutive zeros were considered as non-wear time and excluded from data analysis. A minimum of 8 h wearing time per day was required to include data in the analysis (Chinapaw et al., 2014).

A cut point of <100 counts per minute (cpm) was selected for overall sedentary time, between 100 and ≤1952 for light physical activity (LPA) and >1952 for MVPA (Freedson et al., 1998). A period of 10 or more consecutive minutes below 100 cpm was defined as a sedentary bout, and time spent

sedentary accumulated in sedentary bouts of ≥10 min was defined as prolonged sedentary time (Altenburg and Chinapaw, 2015). To adjust for differences in wear time, overall sedentary time, prolonged sedentary time, LPA, and MVPA time were additionally calculated as relative to wear time.

Statistics

Descriptive participant characteristics (median [min; max]) were calculated for baseline measures. The blood sample at the end of the first hour of each laboratory-sitting day was considered as steady state and used as baseline blood sample. Related Samples Wilcoxon Signed Rank Tests were used to test for baseline differences in blood levels between the first and the second laboratory day, and to test for differences in accelerometer-derived data (i.e., sedentary time, LPA time, and MVPA time expressed as percentage of wear time) during 6 normal days and 6 increased sedentary days. Generalized Estimating Equations (GEE; univariate) were used to assess the difference in blood levels between both laboratory-sitting days. This longitudinal analysis technique was used to correct for dependency of measures within each participant. All statistical procedures were performed using SPSS software (version 22.0.0). Due to the small sample size and explorative nature of our study we considered a *p*-value below 0.10 as statistically significant.

RESULTS

Table 1 shows baseline participant characteristics and metabolic risk factors at the start of each laboratory day. Steady state blood values for glucose, C-peptide and triglycerides were not different between the two laboratory-sitting days.

Table 1 presents overall and prolonged sedentary time, LPA time, and MVPA time during 6 normal days before the experiment and during the 6 prolonged sedentary days. Unfortunately, due to technical problems with the accelerometers, only four participants had valid data for both the 6 normal days as well as the 6 prolonged sedentary days.

TABLE 1 | Descriptive participant characteristics (mean ± *SD*; *n* = 7).

	Baseline anthropometrics		**Differences[a] (*p*-value)**
Age (years)	21.4 ± 2.3		
Height (cm)	183.2 ± 9.2		
Weight (kg)	72.9 ± 2.3		
BMI	21.8 ± 1.4		
Waist/hip	0.9 ± 0.1		
Body fat (%)	13.9 ± 5.2		
Blood measurements	**Steady state 1st laboratory day (pre-test)**	**Steady state 2nd laboratory day (post-test)**	
Glucose (mmol/l)	4.5 ± 0.3	4.3 ± 0.1	0.31
C-peptide (mmol/l)	0.35 ± 0.10	0.35 ± 0.08	0.61
Triglycerides (mmol/l)	0.86 ± 0.28	0.99 ± 0.19	0.18
Accelerometer-derived data	**Normal days[#] Median [min; max]**	**Increased sedentary days[#] Median [min; max]**	
Total wear time (min/day)	765 [668; 863]	882 [713; 922]	
Sedentary time (min/day)	557 [338; 591]	667 [638; 724]	
Prolonged sedentary time[b] (min/day)	220 [72; 342]	304 [162; 435]	
Number of sedentary bouts per day	12 [4; 15]	16 [11; 20]	
LPA time (min/day)	152 [88; 274]	131 [52; 239]	
MVPA time (min/day)	45 [13; 151]	39 [20; 53]	
Relative to total wear time (%)			
Overall sedentary time	75 [51; 85]	80 [70; 90]	0.14
Prolonged sedentary time	30 [8; 53]	34 [18; 62]	0.14
LPA time	20 [13; 32]	15 [7; 26]	0.07*
MVPA time	6 [2; 18]	4 [3; 6]	0.27

LPA, light physical activity; MVPA, moderate-to-vigorous physical activity.

[a] Differences in baseline blood levels and accelerometer-derived data (i.e. data relative to wear time) were tested using Wilcoxon Signed Rank Tests.

[b] Prolonged sedentary time was defined as the time spent sedentary accumulated in bouts of ≥10 min.

[#] Due to technical problems, valid accelerometer data were not available for one participant during the normal days, for one participant during the prolonged sedentary days and for one participant during both the normal days and the prolonged sedentary days. In total, valid accelerometer data for both the normal days and the prolonged sedentary days was available for four participants.

**Significantly higher during prolonged sedentary days when compared to normal days.*

Strikingly, during the 6 normal days participant's median sedentary time was quite high: 9.3 h/day sedentary, of which 3.7 h/day prolonged. During the prolonged sedentary days, participants spent 14.7 h/day sedentary, of which 5.1 h/day prolonged, thereby meeting the requests of interrupted and uninterrupted sedentary time. After adjusting for wear time, overall sedentary time, and prolonged sedentary time were slightly but not significantly higher during the prolonged sedentary days compared to the normal days. LPA was 5% lower during the 6 prolonged sedentary days compared to the normal days (i.e., 5% of wear time), whereas MVPA time was similar (**Table 1**).

Figure 2 demonstrates the levels of C-peptide, glucose, and triglycerides throughout 1 day of prolonged sedentary time before (closed circles) and after (open circles) 6 prolonged sedentary days. GEE analysis for the 7-h period, including the response to both standardized meals revealed a significant higher postprandial C-peptide levels during the second laboratory day following the 6 prolonged sedentary days compared to the first laboratory day (B = 0.11, 90% CI = [0.002; 0.22]; **Table 2**). Median C-peptide AUC and iAUC for the 7-h period were 7 and 16% larger after 6 prolonged sedentary days (**Table 3**). Glucose and triglycerides levels were not significantly different between the 2 laboratory days. Results for the first 4-h period (including the response to the first standardized meal only) were similar (**Table 2**).

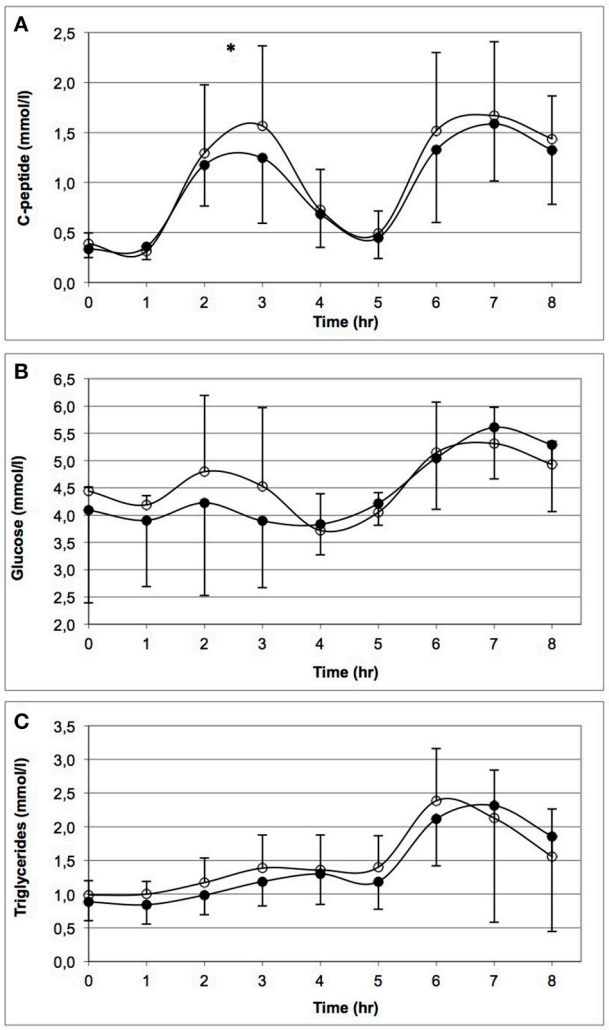

FIGURE 2 | Levels of C-peptide (A), glucose **(B)**, and triglycerides **(C)** throughout 1 day of prolonged sitting before (closed circles) and after (open circles) 6 prolonged sedentary days. *Indicates significant higher levels of C-peptide for the second laboratory-sitting day compared to the first day, i.e., following the 6 prolonged sedentary days. Note that the baseline measurements are slightly different between time points ($t = 0$ and 1) and laboratory days. Standardized high fat mixed meals were consumed at $t = 1$ and 5.

DISCUSSION

This pilot study explored the postprandial effects of multiple days of prolonged sedentary time in free-living conditions on metabolic risk factors in healthy young men. During the execution of this study, we encountered a number of important implications for future studies. Therefore, we first discuss the findings of our pilot study on the metabolic risk factors in healthy young men. Subsequently, we discuss the lessons we have learned from this study and recommendations for future studies.

Pilot Findings on Metabolic Risk Factors

Despite the relatively small increase in interrupted and uninterrupted sedentary time, we found higher postprandial levels of C-peptide following 6 prolonged sedentary days, when compared to baseline. The higher levels of postprandial C-peptide during several prolonged sedentary days is in contrast with previous studies in middle-aged, overweight/obese adults (Thorp et al., 2014; Larsen et al., 2015), but in line with a previous study in free-living, healthy, young adults (Lyden et al., 2015). Maintenance of normal fasting and postprandial glucose levels depend on the ability to create an adequate insulin response to a meal. The loss of local contractile stimulation in weight-bearing muscles may lead to reduced triglycerides uptake, through the suppression of skeletal muscle lipoprotein lipase (LPL) activity (Bey and Hamilton, 2003; Hamilton et al., 2004), as well as reduced glucose uptake. The contrasting findings regarding the potential unfavorable effects of consecutive prolonged sedentary on postprandial (endogenous) insulin may be explained by the number of prolonged sedentary days. Thorp et al. (2014) and Larsen et al. (2015) examined postprandial effects after 3 prolonged sedentary days, whereas Lyden et al. (2015) and this study examined postprandial effects after seven and 6 prolonged sedentary days, respectively. The potential adverse effects of prolonged sedentary time may only emerge when sustained for a number of consecutive days (e.g., six or more). Possibly, a healthy lifestyle may hold off unfavorable adverse effects of prolonged sedentary time, e.g., by sleeping adequately (Morselli et al., 2010). Future studies should examine this hypothesis. Another explanation may be that the participants in our study and the study of Lyden et al. (2015) were healthy and physically active

TABLE 2 | Difference (unstandardized regression coefficient (B) and 90% CI) in cardiometabolic risk factors between laboratory sitting day at baseline and following 6 prolonged sedentary days.

	B [90% CI]	
	7-h period	4-h period
Glucose (mmol/l)	0.08 [−0.07; 0.24]	0.10 [−0.10; 0.30]
C-peptide (mmol/l)	0.11 [0.002; 0.22]*	0.10 [0.01; 0.18]*
Triglycerides (mmol/l)	0.08 [−0.21; 0.36]	0.16 [−0.02; 0.35]

**Significantly higher postprandial C-peptide during the second laboratory-sitting day following 6 prolonged sedentary days compared to the first laboratory-sitting day. Note that a positive B indicates a higher blood level for the second laboratory-sitting day following 6 prolonged sedentary days compared to the first laboratory day.*

TABLE 3 | Postprandial plasma glucose, C-peptide, and triglyceride area under the curve (median [min; max]) before and after 6 prolonged sedentary days.

	Time (h)	1st laboratory day	2nd laboratory day
AUC			
Glucose	7	31.5 [21.9; 39.1]	31.4 [26.0; 39.0]
	4	16.4 [10.9; 21.6]	16.1 [14.4; 23.5]
C-peptide	7	7.1 [3.8; 11.4]	7.7 [4.0; 13.6]
	4	3.5 [1.9; 5.2]	3.9 [1.6; 6.8]
Triglyceride	7	9.9 [6.3; 15.8]	10.2 [7.5; 15.5]
	4	4.3 [2.8; 7.3]	4.5 [3.8; 7.6]
iAUC			
Glucose	7	2.3 [0.1; 4.7]	2.2 [0.1; 7.8]
	4	0.3 [0; 1.8]	0.2 [0; 5.1]
C-peptide	7	4.5 [2.0; 8.0]	4.9 [2.3; 10.3]
	4	1.5 [0.7; 2.9]	2.2 [0.6; 4.5]
Triglyceride	7	4.0 [2.0; 8.8]	3.8 [1.0; 8.3]
	4	1.2 [0.3; 1.6]	0.6 [0; 2.7]

AUC, area under the curve; iAUC, incremental area under the curve.

adults, while participants in the studies of Thorp et al. (2014) and Larsen et al. (2015) were overweight/obese and low active adults.

Our pilot study demonstrated that the postprandial level of C-peptide was 0.11 mmol/l higher after 6 days of increased sedentary time. The clinical importance of this finding needs further study, by prospectively examining the effects of prolonged sedentary time on metabolic risk factors and the incidence of type 2 diabetes and cardiovascular disease.

Although the design of the study of Lyden et al. (2015) is similar to the present study, the difference in postprandial response measurement (i.e., 2-h oral glucose tolerance test vs. 4- and 7-h meal response in our study, and insulin vs. C-peptide) hampers comparison between the two studies. Moreover, in the study of Lyden et al. (2015) participants spent more time sedentary during the increased free-living sedentary days (i.e., 15 vs. 5% in the present study). The 7 and 16% larger 7-h C-peptide AUC and iAUC, respectively, may have a substantial detrimental effect on cardiometabolic risk, especially when considering the small increase in sedentary time. A *post-hoc* sample size calculation based on the present findings revealed that 64 participants are needed to detect a 0.11 mmol higher postprandial C-peptide level after 6 prolonged sedentary days, using a significance level of 0.05 and a power of 80%.

In line with previous studies examining multiple prolonged sedentary days (Thorp et al., 2014; Larsen et al., 2015; Lyden et al., 2015) we found no significant difference in postprandial glucose levels. As proposed by Lyden et al. (2015), the lack of changes in postprandial glucose, as opposed to increases in postprandial insulin, may indicate the importance of insulin action in the development of cardiometabolic ill-health induced by prolonged sitting.

We found no significant difference in postprandial triglyceride levels following the 6 consecutive prolonged sedentary days. A postprandial increase in triglycerides has been related to decreased insulin sensitivity (Axelsen et al., 1999; Annuzzi et al., 2004; Madhu et al., 2008), which is not expected in young and healthy subjects. However, when analyzing only the first 4 h of the laboratory days, including the response of the first standardized meal only, the effect size for triglyceride levels doubled (**Table 2**). This might be caused by the difference in consistency of the standardized meals, i.e., the first meal was liquid, whereas the second meal was solid. Since liquid food is more rapidly emptied from the stomach than solid food (Read and Houghton, 1989), the first meal might have raised blood lipids to a higher extent than the second meal.

Lessons Learned and Recommendations

Our first recommendation is that future experimental studies should examine the potential adverse metabolic health effects of sedentary patterns that are more realistic in real life. Participants were slightly but not significantly more sedentary during the 6 prolonged sedentary days when compared to the normal days, both overall (i.e., 5% of wear time) and prolonged (i.e., 4% of wear time), and spent slightly less time on LPA (i.e., 5% of wear time). The relatively small increase in interrupted and uninterrupted sedentary time in our study may indicate that in free-living conditions it is difficult for young, physically active males to increase their sedentary time substantially. Thus, patterns of 6–8 h of prolonged sitting, as examined in previous experimental laboratory studies on the adverse health effects of prolonged sitting, are rare in young and healthy males. Additionally, we recommend future studies to monitor a full day including sleep, as adequate sleep may influence participants' metabolism (Morselli et al., 2010).

Secondly, we recommend future studies examining potential adverse effects of increased sedentary time to check participants' normal PA and sedentary behavior using objective measures, i.e., as a pre-study screening, to make sure the requested increase in prolonged sedentary time is indeed a substantial increase compared to normal weeks. Additionally, when participants know their normal prolonged sedentary time it may be more feasible for them to reach a certain sedentary time prescription. In the present study participants were screened by dialogue to check whether they spent <2 h on prolonged sedentary time

on a regular day. Baseline accelerometer data demonstrated that the healthy young men in our study spent on average 9.3 h/day sedentary of which 3.7 h/day prolonged, indicating that they underestimated their normal prolonged sedentary time during screening. As a consequence of the considerable amount of their baseline sedentary time, participants had limited opportunity to further increase their sedentary time substantially.

Another recommendation for future studies is to examine the longer-term health effects of both overall and prolonged sitting time in free-living conditions, thereby including measures that can differentiate between lying, sitting and standing (e.g., ActivPAL). Hip-worn accelerometers are widely used to measure sedentary time yet they cannot distinguish between various postures (i.e., lying, sitting, standing). The limitation that accelerometers are not accurate enough for assessing sedentary time may be another explanation for the relatively small increase in interrupted and uninterrupted sedentary time in our study.

Next, we recommend future intervention studies that targeting to increase LPA time may be a potential effective strategy when aiming to reduce sedentary time. Our study demonstrated that the small decrease in LPA time coincided the small increase in sedentary time may, indicating that participants substituted their LPA time with sedentary time.

Strengths and Limitations

Strengths of this study include the hourly blood collection and the focus on both glucose and lipid metabolism. The inclusion of healthy young men additionally strengthens our study, since the influence of confounding of disease processing (i.e., obesity, type 2 diabetes), and menstrual cycle was eliminated. The "real life" setting (i.e., imposing days of predominantly sitting) further strengthens our study. A limitation is the small sample size. Moreover, due to incomplete accelerometer data we cannot confirm that all participants actually increased their (uninterrupted) sedentary time. Finally, we did not standardize dietary intake during the 6 prolonged sedentary days. However, as participants consumed a standardized meal on the evening before each laboratory days, we expect this influence to be minimal. Future studies should examine this.

CONCLUSION

We conclude that multiple days of prolonged sedentary time may have an unfavorable effect on postprandial C-peptide levels, even in healthy young men. Acute metabolic effects of prolonged sedentary time may accumulate when sustained for multiple days, and therefore needs further study. Besides hypothesis testing experimental studies, we recommend future studies to examine metabolic effects of sedentary patterns that fit real life conditions.

AUTHOR CONTRIBUTIONS

TA conceived and designed the study, collected, analyzed and interpreted the data, and wrote the manuscript. JR, ES, and MC were involved in conceiving and designing the study, data interpretation, and drafting the manuscript. All authors read and approved the final manuscript.

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REFERENCES

Altenburg, T. M., and Chinapaw, M. J. (2015). Bouts and breaks in children's sedentary time: currently used operational definitions and recommendations for future research. *Prev. Med.* 77, 1–3. doi: 10.1016/j.ypmed.2015.04.019

Altenburg, T. M., Rotteveel, J., Dunstan, D. W., Salmon, J., and Chinapaw, M. J. (2013). The effect of interrupting prolonged sitting time with short, hourly, moderate-intensity cycling bouts on cardiometabolic risk factors in healthy, young adults. *J. Appl. Physiol.* 115, 1751–1756. doi: 10.1152/japplphysiol.00662.2013

Annuzzi, G., De Natale, C., Iovine, C., Patti, L., Di Marino, L., Coppola, S., et al. (2004). Insulin resistance is independently associated with postprandial alterations of triglyceride-rich lipoproteins in type 2 diabetes mellitus. *Arterioscler. Thromb. Vasc. Biol.* 24, 2397–2402. doi: 10.1161/01.ATV.0000146267.71816.30

Axelsen, M., Smith, U., Eriksson, J. W., Taskinen, M. R., and Jansson, P. A. (1999). Postprandial hypertriglyceridemia and insulin resistance in normoglycemic first-degree relatives of patients with type 2 diabetes. *Ann. Intern. Med.* 131, 27–31. doi: 10.7326/0003-4819-131-1-199907060-00006

Bailey, D. P., and Locke, C. D. (2015). Breaking up prolonged sitting with light-intensity walking improves postprandial glycemia, but breaking up sitting with standing does not. *J. Sci. Med. Sport* 18, 294–298. doi: 10.1016/j.jsams.2014.03.008

Bey, L., and Hamilton, M. T. (2003). Suppression of skeletal muscle lipoprotein lipase activity during physical inactivity: a molecular reason to maintain daily low-intensity activity. *J. Physiol.* 551, 673–682. doi: 10.1113/jphysiol.2003.045591

Ceriello, A., Davidson, J., Hanefeld, M., Leiter, L., Monnier, L., Owens, D., et al. (2006). Postprandial hyperglycaemia and cardiovascular complications of diabetes: an update. *Nutr. Metab. Cardiovasc. Dis.* 16, 453–456. doi: 10.1016/j.numecd.2006.05.006

Ceriello, A., Quagliaro, L., Piconi, L., Assaloni, R., Da Ros, R., Maier, A., et al. (2004). Effect of postprandial hypertriglyceridemia and hyperglycemia on circulating adhesion molecules and oxidative stress generation and the possible role of simvastatin treatment. *Diabetes* 53, 701–710. doi: 10.2337/diabetes.53.3.701

Chinapaw, M. J. M., de Niet, M., Verloigne, M., De Bourdeaudhuij, I., Brug, J., and Altenburg, T. M. (2014). From sitting time to sitting pattern: accelerometer data reduction decisions in youth. *PLoS One* 9:e111205. doi: 10.1371/journal.pone.0111205

Dunstan, D. W., Kingwell, B. A., Larsen, R., Healy, G. N., Cerin, E., Hamilton, M. T., et al. (2012). Breaking up prolonged sitting reduces postprandial glucose and insulin responses. *Diabetes Care* 35, 976–983. doi: 10.2337/dc11-1931

Eberly, L. E., Stamler, J., and Neaton, J. D. (2003). Relation of triglyceride levels, fasting and nonfasting, to fatal and nonfatal coronary heart disease. *Arch. Intern. Med.* 163, 1077–1083. doi: 10.1001/archinte.163.9.1077

Freedson, P. S., Melanson, E., and Sirard, J. (1998). Calibration of the Computer Science and Applications, Inc. accelerometer. *Med. Sci. Sports Exerc.* 30, 777–781. doi: 10.1097/00005768-199805000-00021

Hamilton, M. T., Hamilton, D. G., and Zderic, T. W. (2004). Exercise physiology versus inactivity physiology: an essential concept for understanding lipoprotein lipase regulation. *Exerc. Sport Sci. Rev.* 32, 161–166. doi: 10.1097/00003677-200410000-00007

Healy, G. N., Dunstan, D. W., Salmon, J., Cerin, E., Shaw, J. E., Zimmet, P. Z., et al. (2008). Breaks in sedentary time: beneficial associations with metabolic risk. *Diabetes Care* 31, 661–666. doi: 10.2337/dc07-2046

Henson, J., Yates, T., Biddle, S. J., Edwardson, C. L., Khunti, K., Wilmot, E. G., et al. (2013). Associations of objectively measured sedentary behaviour and physical activity with markers of cardiometabolic health. *Diabetologia* 56, 1012–1020. doi: 10.1007/s00125-013-2845-9

Larsen, R. N., Kingwell, B. A., Robinson, C., Hammond, L., Cerin, E., Shaw, J. E., et al. (2015). Breaking up of prolonged sitting over three days sustains, but does not enhance, lowering of postprandial plasma glucose and insulin in overweight and obese adults. *Clin. Sci.* 129, 117–127. doi: 10.1042/CS20140790

Lyden, K., Keadle, S. K., Staudenmayer, J., Braun, B., and Freedson, P. S. (2015). Discrete features of sedentary behavior impact cardiometabolic risk factors. *Med. Sci. Sports Exerc.* 47, 1079–1086. doi: 10.1249/MSS.0000000000000499

Madhu, S. V., Kant, S., Srivastava, S., Kant, R., Sharma, S. B., and Bhadoria, D. P. (2008). Postprandial lipaemia in patients with impaired fasting glucose, impaired glucose tolerance and diabetes mellitus. *Diabetes Res. Clin. Pract.* 80, 380–385. doi: 10.1016/j.diabres.2008.01.016

Miyashita, M., Park, J. H., Takahashi, M., Suzuki, K., Stensel, D., and Nakamura, Y. (2013). Postprandial lipaemia: effects of sitting, standing and walking in healthy normolipidaemic humans. *Int. J. Sports Med.* 34, 21–27. doi: 10.1055/s-0032-1321897

Morselli, L., Leproult, R., Balbo, M., and Spiegel, K. (2010). Role of sleep duration in the regultation of glucose metabolism and appetite. *Best Pract. Res. Clin. Endocrinol. Metab.* 24, 687–702. doi: 10.1016/j.beem.2010.07.005

O'Keefe, J. H., and Bell, D. S. (2007). Postprandial hyperglycemia/hyperlipidemia (postprandial dysmetabolism) is a cardiovascular risk factor. *Am. J. Cardiol.* 100, 899–904. doi: 10.1016/j.amjcard.2007.03.107

Peddie, M. C., Bone, J. L., Rehrer, N. J., Skeaff, C. M., Gray, A. R., and Perry, T. L. (2013). Breaking prolonged sitting reduces postprandial glycemia in healthy, normal-weight adults: a randomized crossover trial. *Am. J. Clin. Nutr.* 98, 358–366. doi: 10.3945/ajcn.112.051763

Polonsky, K. S., and Rubenstein, A. H. (1984). C-peptide as a measure of the secretion and hepatic extraction of insulin. Pitfalls and limitations. *Diabetes* 33, 486–494. doi: 10.2337/diab.33.5.486

Read, N. W., and Houghton, L. A. (1989). Physiology of gastric emptying and pathophysiology of gastroparesis. *Gastroenterol. Clin. North Am.* 18, 359–373.

Rotteveel, J., van Weissenbruch, M. M., Twisk, J. W., and Delemarre-Van de Waal, H. A. (2008). Abnormal lipid profile and hyperinsulinaemia after a mixed meal: additional cardiovascular risk factors in young adults born preterm. *Diabetologia* 51, 1269–1275. doi: 10.1007/s00125-008-1029-5

Thorp, A. A., Kingwell, B. A., Sethi, P., Hammond, L., Owen, N., and Dunstan, D. W. (2014). Alternating bouts of sitting and standing attenuate postprandial glucose responses. *Med. Sci. Sports Exerc.* 46, 2053–2061. doi: 10.1249/MSS.0000000000000337

Van Cauter, E., Mestrez, F., Sturis, J., and Polonsky, K. S. (1992). Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41, 368–377. doi: 10.2337/diabetes.41.3.368

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