Universidade de Lisboa
Faculdade de Motricidade Humana

Metabolic and Energetic Cost of Intermittent Sitting and Standing in Adults

Dissertação elaborada com vista à obtenção do Grau de Mestre em
Exercício e Saúde

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Abbreviations

AEE – Activity energy expenditure

ALST - Appendicular lean soft tissue

BMI – Body mass index

BSB – Bout of sedentary behavior

BST – Beak in sedentary time

CRP - C-reactive protein

CV – Coefficient of variation

CVD – Cardiovascular diseases

DIT – Diet-induced thermogenesis

DLTII® **-** Deltatrac II®

DLW – Doubly labeled water

DXA – Dual energy x-ray absorptiometry

EE – Energy expenditure

EEE – Exercise energy expenditure

FM – Fat mass

FFM – Fat-free mass

HDL – High density lipoprotein

HR – Heart rate

iAUC - Positive incremental area under the curve

IC – Indirect calorimetry

LIPA – Light-intensity physical activity

LST – Lean soft tissue

MedU® - MedGraphics CPX Ultima

MET – Metabolic equivalent

MetS – Metabolic syndrome

MVPA – Moderate-to-vigorous physical activity

NEAT – Non-exercise activity thermogenesis

PA – Physical activity

REE – Resting energy expenditure

RER - Respiratory exchange ratio

RMR – Resting metabolic rate

RQ – Respiratory quotient

SB - Sedentary behavior

SBRN - Sedentary Behavior Research Network

SIT – Uninterrupted motionless sitting condition

SIT/STAND – Break motionless sitting with brief bouts of standing condition

SIT_STAND – Alternating between motionless sitting and standing condition

STAND – Uninterrupted motionless standing condition

TDEE – Total daily energy expenditure

T2DM – Type-2 diabetes mellitus

WC – Waist circumference

WHO – World Health Organization

VCO_2 – Carbon dioxide production

VO_2 – Oxygen consumption

Abstract

Breaking-up prolonged sitting with alternating bouts of sitting and standing may increase non-exercise energy expenditure and consequently influence the total daily energy expenditure.

Purpose: The main purpose of this work was to analyze the metabolic and energetic cost of alternating between specific postures (sitting and standing), in healthy adults.

Methods: A randomized crossover trial was conducted among 48 adults (25 males, aged 34.8 ± 14.0 years) who were randomly assigned to four sequential experimental conditions, of which three were included in our analysis: 1) uninterrupted motionless sitting (SIT); 2) uninterrupted motionless standing (STAND); and 3) alternating between motionless sitting and motionless standing (SIT_STAND). This last condition was further divided in two distinct sub-conditions, SS_SIT (sitting after standing) and SS_STAND (standing after sitting). Before the intervention, with the participant in 8 hours fasting condition, anthropometric measures were collected, followed by a body composition analysis trough dual-energy x-ray absorptiometry. Indirect calorimetry was used to assess both resting energy expenditure (REE) and the energy expenditure resulting from the assigned conditions. Repeated measures ANOVA was performed to examine the differences between all conditions (CI 0.95%).

Results: In women, oxygen consumption levels **(**VO_2) (ml·kg^{-1}·min^{-1}) and energy expenditure (EE) (kcal·min^{-1}) for SIT (2.86 ± 0.07 ml·kg^{-1}·min^{-1}; 0.88 ± 0.03 kcal·min^{-1}), STAND (3.03 ± 0.08 ml·kg^{-1}·min^{-1}; 0.94 ± 0.03 kcal·min^{-1}), SS_SIT (3.18 ± 0.08 ml·kg^{-1}·min^{-1}; 0.98 ± 0.02 kcal·min^{-1}) and SS_STAND (3.59 ± 0.13 ml·kg^{-1}·min^{-1}; 1.11 ± 0.04 kcal·min^{-1}) were significantly different, considering the randomly assigned order (p-value < 0.001). In men, VO_2 (ml·kg^{-1}·min^{-1}) and EE (kcal·min^{-1}) also differed from SIT (2.96 ± 0.13 ml·kg^{-1}·min^{-1}; 1.14 ± 0.05 kcal·min^{-1}), STAND (3.18 ± 0.14 ml·kg^{-1}·min^{-1}; 1.23 ± 0.05 kcal·min^{-1}), SS_SIT (3.34 ± 0.16 ml·kg^{-1}·min^{-1}; 1.28 ± 0.06 kcal·min^{-1}) and SS_STAND (3.68 ± 0.19 ml·kg^{-1}·min^{-1}; 1.43 ± 0.08 kcal·min^{-1}). Although interaction effect of the assigned order was considered, in men, no significant differences were found between STAND and SS_SIT (p-value ≥ 0.05). For both sexes, heart rate (HR) only differed significantly between SIT and the other conditions (~13 bpm) (p-value < 0.001), while no significant changes were found in respiratory quotient (RQ) (p-value ≥ 0.05). After further adjustment for age, no significant differences between conditions were found for all metabolic and energetic variables, in both sexes (p-value ≥ 0.05).

Conclusions: In a sample of adults, the metabolic and energetic cost of one specific posture was influenced by the posture executed immediately before, regarding an intermittent condition (alternating between sitting and standing). These findings suggest a potential cumulative effect resulting from breaking sitting with short bouts of standing. In this sense, global health messages encouraging individuals to avoid extended periods in sedentary behavior (SB), should informed about the potential metabolic and energetic benefit of interrupting this behavior as many times as possible.

Key-words: adults, bouts, breaks, energy expenditure, indirect calorimetry, sedentary behavior, sitting, standing

Resumo

Interromper longos períodos na postura sentada, alternando entre períodos de tempo na postura sentada e em pé, poderá contribuir para o aumento do dispêndio energético (DE) associado a atividades espontâneas, que por sua vez irá influenciar o DE total.

Objetivo: Numa amostra de indivíduos adultos saudáveis, determinar o contributo metabólico e energético resultante da alternância entre posturas (estar sentado e em pé).

Métodos: O estudo envolveu a participação de 48 indivíduos com uma média de idades de 34.8 ± 14.0 anos (25 homens), aos quais que foi aleatoriamente atribuída uma sequência com quatro condições experimentais, das quais três foram tratadas neste estudo: 1) postura sentada imóvel ao longo de 10 minutos (SIT); 2) postura em pé imóvel ao longo de 10 minutos (STAND); e 3) alternar entre a postura sentada imóvel e em pé imóvel a cada minuto, ao longo de 10 minutos (SIT_STAND). Esta última condição foi posteriormente dividida em duas subcondições distintas (SS_SIT – estar sentado depois de ter estado em pé; SS_STAND – estar em pé depois de ter estado sentado). Anteriormente à intervenção, e com o participante em jejum (8 horas), foram recolhidas medidas antropométricas, e de seguida, realizada uma avaliação de composição corporal por densitometria radiológica de dupla energia. Posteriormente, um método de calorimetria indireta foi utilizado para determinar o DE em repouso, assim como o DE relativo a cada condição experimental. As diferenças entre condições foram determinadas com recurso ao teste estatístico Anova com medidas repetidas (IC 95%).

Resultados: Nas mulheres, as condições SIT (2.86 ± 0.07 $ml \cdot kg^{-1} \cdot min^{-1}$; 0.88 ± 0.03 $kcal \cdot min^{-1}$), STAND (3.03 ± 0.08 $ml \cdot kg^{-1} \cdot min^{-1}$; 0.94 ± 0.03 $kcal \cdot min^{-1}$), SS_SIT (3.18 ± 0.08 $ml \cdot kg^{-1} \cdot min^{-1}$; 0.98 ± 0.02 $kcal \cdot min^{-1}$) e SS_STAND (3.59 ± 0.13 $ml \cdot kg^{-1} \cdot min^{-1}$; 1.11 ± 0.04 $kcal \cdot min^{-1}$) apresentaram diferenças significativas face às variáveis consumo de oxigénio (VO_2) (ml·kg^{-1}·min^{-1}) e dispêndio energético (DE) (kcal·min^{-1}), ajustando para a ordem das condições atribuída aleatoriamente (p-value < 0.001). Nos homens, as condições SIT (2.96 ± 0.13 ml·kg^{-1}·min^{-1}; 1.14 ± 0.05 kcal·min^{-1}), STAND (3.18 ± 0.14 ml·kg^{-1}·min^{-1}; 1.23 ± 0.05 kcal·min^{-1}), SS_SIT (3.34 ± 0.16 ml·kg^{-1}·min^{-1}; 1.28 ± 0.06 kcal·min^{-1}) e SS_STAND (3.68 ± 0.19 ml·kg^{-1}·min^{-1}; 1.43 ± 0.08 kcal·min^{-1}) apresentaram diferenças significativas face às variáveis VO_2 (ml·kg^{-1}·min^{-1}) e DE (kcal·min^{-1}). No entanto, apesar de ter sido considerada a ordem das condições atribuída aleatoriamente, não foram encontradas diferenças significativas entre as condições STAND e SS_SIT (p-value ≥ 0.05). Em ambos os sexos, a variável frequência cardíaca apenas variou significativamente entre a condição SIT e todas as outras (~13 bpm) (p-value < 0.001), enquanto que nenhuma diferença significativa para a variável quociente respiratório foi detetada (p-value ≥ 0.05). Após o ajustamento dos resultados para a variável idade, não foram encontradas diferenças significativas entre as condições nos indicadores metabólicas e energéticas considerados, em ambos os géneros (p-value $\geq$ 0.05).

Conclusões: Tendo por base uma condição intermitente (alternância entre estar sentado e de pé), verificou-se que custo metabólico e energético associado a uma determinada postura é influenciado pela postura em que o individuo se encontrava imediatamente antes. Os resultados sugerem que a interrupção de períodos contínuos na postura sentada com breves períodos na postura de pé, poderá resultar num potencial efeito cumulativo ao longo do tempo. Nesse sentido, as recomendações gerais para a redução do comportamento sedentário deverão ter em conta a sua implicação metabólica e energética associada à frequente interrupção deste comportamento.

Palavras-chave: adultos, calorimetria indireta, comportamento sedentário, dispêndio energético, interrupções, períodos de tempo, postura em pé, postura sentada

Introduction

Industrial innovation has contributed to the development of technologies that limit human intervention in several contexts. Particularly in high-income countries, the accessibility to sitting based occupations, such as labour and recreational-saving devices, concurred to decrease daily physical demands. As consequence, the prolonged exposure to sedentary behaviors (SB) is argued to be a major contributor to the numerous diseases (D. Dunstan, Healy, Sugiyama, & Owen, 2010; Healy, Matthews, Dunstan, Winkler, & Owen, 2011; Patterson et al., 2018; Wilmot et al., 2012).

In the last years, the scientific community intensified their research in the field of sedentariness, particularly, by analyzing the relationship between SB, energy expenditure (EE) and health. A growing body of observational evidence has shown that, regardless the level of physical activity (PA), the exposure to prolonged SB, and consequently low daily EE, is associated with the development of numerous deleterious health outcomes, such as all-cause mortality (Patterson et al., 2018), cardiovascular and metabolic events (Hamilton, Hamilton, & Zderic, 2007; Wilmot et al., 2012), cancer (Kerr, Anderson, & Lippman, 2017) and physical and cognitive function impairment (Gianoudis, Bailey, & Daly, 2015). In this sense, investigators expected that an increase in EE, resulting from breaking up sedentary time may have an inverse relationship with those conditions (Dohrn, Kwak, Oja, Sjostrom, & Hagstromer, 2018; D. W. Dunstan et al., 2012; Healy, Winkler, Owen, Anuradha, & Dunstan, 2015)

Although most of the present knowledge in this area is derived from observational data, there has been an emerging increase of experimental studies. Recent experimental evidence is derived from interventions that aimed to determine the health impact of replacing sitting by standing or other active pursuits (D. W. Dunstan et al., 2012; Healy, Winkler, Owen, Anuradha, & Dunstan, 2015). However, given the diversified number of samples and study designs reported, it is necessary to carefully investigate how the metabolic and energetic impact may vary depending on the condition's specificities (condition type, frequency, duration, intensity). As such, in order to clarify the current evidential burden about this topic and promote efficient alternatives to break prolonged sedentary time, further investment in the development of experimental trials is warranted.

SB is a reality common to all ages, especially in modern and developed societies. In Portugal, according to a national sample from 2008, female and male adolescents (10-19 years) spend 61.1% and 57.7% of daily accelerometer wear time in SB, respectively, while breaking up this behavior approximately 87 times per day (Baptista et al., 2012; Santos et al., 2018)**.** In adulthood (20-64 years), 56.5% and 60.2% of total wear time is spent in sedentary pursuits, for females and males, respectively (Baptista et al., 2012). Moreover, females have around 90 breaks of SB per day, while males only reach 86 (Santos et al., 2018). In older adults (≥ 65 years), the number of daily sedentary breaks decreases to 78 in females and 70.5 in males, while the % of total wear time spent in SB increases to 63.8% and 65.2%, in females and males, respectively (Baptista et al., 2012; Santos et al., 2018). Considering that total accelerometer wear time is approximately 14 hours per day for all age groups, it is expected that Portuguese adolescents and adults spend at least 8 hours in sedentary time per day, while older adults exceed 9 hours in the same behavior (Baptista et al., 2012)

Given the scenario, there is a clear need to develop sustainable behavioral strategies that decrease daily time spent in sedentary pursuits, particularly in critical environments where individuals are highly exposed to this type of activity, such as, schools, workplaces, day centers and nursing homes. Based on this, breaking-up prolonged sitting with standing time or other active pursuits emerges as one of the most effective solutions to reduce total sedentary time. Thus, in addition to the need of raising awareness of the positive effects that this may have on health, it is essential to invest in public health-related recommendations supporting frequent interruptions of SB.

Due to this, our contribution to the scientific community will be on the potential metabolic and energetic impact of alternating between short periods of time of two differentiated postures (sitting and standing).

The present thesis includes an introduction of our work, followed by a review of the available literature that will describe sedentariness-related definitions, epidemiological data and implications on health, through observational data. The topic related to the interruptions in SB will be further explored through the description of the current definitions, epidemiological data and the available observational and experimental evidence. Then, we will explore the issue of EE, regarding their components and related assessment methodologies. Moreover, the impact that several interventions have on EE will be further described. After that, we will present the methodology section, where the recruitment process, study design, intervention conditions, assessment instruments and protocols are discussed. Further on, all results will be outlined, and a discussion related to our major findings will be presented. Finally, the strengths and limitations of our work will be referred, as well as the main conclusions and future implications.

Literature Review

1. Sedentary Behavior (SB)

1.1 Definitions

SB is defined as any waking behavior with an EE below 1.5 metabolic equivalents (METs), while in a sitting, reclining or lying posture (Tremblay et al., 2017). Generally, this behavior is related to several low-intensity activities that are accumulated throughout the day and do not increase EE substantially above the rest (Thivel et al., 2018).

In the last century, the increase in the occurrence of SB reflected the process of modernization and technological automation that our societies have been experiencing. Occupational activities, such as TV-viewing, computer and mobile phone using, working and commuting for work while in a sitting position are the most common examples of SB nowadays (Thivel et al., 2018). Therefore, the amount of time spent in these pursuits represent a major concern to the scientific community, particularly due to its effects on health-related outcomes.

In 2008, Hamilton et al. (Hamilton, Healy, Dunstan, Zderic, & Owen, 2008) proposed a new paradigm shift (e.g. "physical inactivity paradigm") based on the premise that sitting too much and physically inactivity are distinct concepts that affect health through different specific mechanisms. As such, considering that the environmental and technological evolution foster sedentariness and physical inactivity in multiple ways, there is a clear need to further explore the previous conceptual approach by understanding the independent health impact of both dimensions (SB and PA).

Following the descriptions of SB and physical activity, van der Ploeg et al. (van der Ploeg & Hillsdon, 2017) strengthened that both concepts are based on different constructs and are not the opposite of each other. In this regard, the authors showed that although individuals are considered active when they reach PA recommendations, that does not prevent them from also devoting a significant part of the day in sedentary activities (van der Ploeg & Hillsdon, 2017). For example, an adult that meets the weekly PA recommendations, but is seated for most of his daily work time (e.g. call center assistant), is expected to be considered as both active and sedentary. In another perspective, an adult that stands up during his 8 hours work (e.g. supermarket cashier) but fails to accumulate more than 150 minutes/week of moderate-to-vigorous PA (MVPA), is classified as non-sedentary, but also as inactive or insufficiently active.

Nonetheless, while PA recommendations are globally recognized for each age group, there is insufficient evidence regarding SB public guidelines (Ku, Steptoe, Liao, Hsueh, & Chen, 2018). In fact, although some cut-off points have been suggested during the last years, such as Australian National Preventive Health Agency guidelines (2014), the construct on which they were based are inconsistent (Ku et al., 2018). Therefore, the most common recommendations of SB include minimizing the amount of time spent in SB and breaking up prolonged period of SB, as often as possible (John P Buckley et al., 2015; Ku et al., 2018). Thus, the adoption of these behaviors has been increasing not only due to the technological modernization process, but also due to the lack of consistent recommendations, in particular for adults and older adults.

1.2 Epidemiology of Sedentary

At present, the adoption of a sedentary lifestyle is a health issue that is cross-sectional to most of the high-income countries. Given the detrimental impact of this behavior on health, scientific community focused much of their work on monitoring sedentary time and targeting populations at risk. In a representative sample of 20 worldwide countries (49 493 participants;18 – 65 years), Bauman et al. (Bauman et al., 2011) noticed that adults subjectively (questionnaire) reported to spend approximately

300 minutes/day in SB (5 to 6 sitting hours per day). Moreover, a report including subjective data of 66 countries stated that 41.5% of the adult world population spent more than 4 hours per day sitting (Hallal et al., 2012). In addition, this surveillance system showed a wide variation between all World Health Organization (WHO) regions, with Europe having the greatest percentage of adults spending more than 4 hours per day sitting (64.1%) (Hallal et al., 2012).

In Europe, Bennie et al. (Bennie et al., 2013) examined the prevalence of sitting time of 32 countries and found that adults self-reported between 5 to 6 hours of daily sitting. In line with these findings, another study (Loyen, van der Ploeg, Bauman, Brug, & Lakerveld, 2016) reported a wide variation of sitting time - 2.5 hours/day up to 10 hours/day - across studies and countries, being the adults from north-western European countries (e.g. Denmark and Netherlands) more sedentary, compared to south-eastern Europe countries (e.g. Portugal and Spain). Thus, since these findings were based on the application of subjective methods to assess SB, such as questionnaires or diaries, results in a wide range of values which may lead to an underestimation of total SB time, especially when comparing with objective measurements (e.g. accelerometry) (Hills, Mokhtar, & Byrne, 2014). To overcome many of these issues and therefore, provide more accurate and comparable estimates of sedentary time across countries, a recent paper including 4 European countries (Loyen et al., 2017) indicated that, on average, an European citizen accumulates 8 to 9 hours of SB throughout the day. Additionally, it was found that 80% of adults spent at least 7.5 hours in SB per day, and 20% of them were sedentary for more than 10 hours per day (Loyen et al., 2017). According to the authors, the largest difference between self-reported and objectively measured sedentary time was found in Portugal, with the Portuguese population self-reporting 5 hours less sedentary time (180 minutes/day) than the objective assessments of 8 hours per day (Loyen et al., 2017).

In 2012, a cross-sectional study using data from 2008 assessed the prevalence of sedentary and PA time in the Portuguese population through objective monitoring (accelerometer) (Baptista et al., 2012). In this paper, the authors stated that the prevalence of Portuguese adults accumulating more than 7.5 and 10 hours of sedentary time per day was 66.7% and 12.1%, respectively (Baptista et al., 2012). Moreover, the overall average of SB time of the Portuguese population ranged between 8.3 to 8.8 hours per day [adolescents (10-17 years): 8.3 hours/day; adults (18-64 years): 8.3 hours/day; and older adults (≥ 65 years): 8.8 hours/day], representing more than one third of the day in this behavior (Baptista et al., 2012). In general, while adolescents were those who spent less time on SB in male groups, the less sedentary female group corresponded to adults (Baptista et al., 2012). For both genders, the age group that spent the most time in SB were older adults (Baptista et al., 2012). Using the same sample from 2008, Santos et al. (Santos et al., 2018) sought to complement the existent information, noting that for females age groups – adolescents, adults and older adults – sedentary time represented 61.1%, 56.5% and 63.8% of daily accelerometer wear time, respectively. In male age groups, adolescents, adults and older adults spent, respectively, 57.7%, 60.2% and 65.2% of total wear time in SB (Santos et al., 2018).

1.3 Observational Studies and Health-related outcomes

The associations between SB and health-related outcomes indicators have been extensively studied. SB has been directly and indirectly implicated in the development of numerous negative outcomes, particularly those related to non-communicable diseases and that are responsible for the increase in all-cause mortality (Ekelund et al., 2016). Conditions, such as, cardiovascular diseases (CVD) (Patterson et al., 2018), metabolic conditions [e.g. obesity, type-2 diabetes mellitus (T2DM), metabolic syndrome (MetS)] (Hamilton, Hamilton, & Zderic, 2007; Wilmot et al., 2012), cancer (Kerr, Anderson, & Lippman, 2017) and physical function impairment (e.g. frailty) (Gianoudis, Bailey, & Daly, 2015) are all adversely correlated with SB.

Mortality. According to Wilmot et al. (Wilmot et al., 2012), greater levels of SB represented an increased risk of 49% for all-cause mortality, 90% for cardiovascular mortality, 147% for cardiovascular events and 112% for T2DM, independently of amount of PA accumulated (Wilmot et al., 2012). In 2016, Ekelund et al. (Ekelund et al., 2016) reported that in inactive individuals (≤ 2.5MET-h/week) mortality pooled risk for sitting > 8 hours/day was 27% comparing with those sitting < 4 hours/day. Moreover, the authors documented that inactive individuals (≤ 2.5 MET-hour/week) watching > 5 hours/day of TV had an increased risk of 44% for all-cause mortality compared with the reference group (TV-viewing < 1 hour/day) (Ekelund et al., 2016). In line with these findings, a recent prospective study (Larsson & Wolk, 2018) found that the risk of all-cause mortality was 72% higher in those in the highest category of SB leisure-time (< 6 hours/day), comparing with those in the lowest category (< 1 hour/day).

Cardiovascular events. Regarding CVD, a recent study identified two independent thresholds – 6 hours of sitting and 4 hours of TV-viewing, above which the risk of CVD events increase (Patterson et al., 2018). Moreover, Grontved et al. (Grontved & Hu, 2011) found that prolonged TV-viewing (≥ 2 hours) was associated with an 15% increased risk of fatal or nonfatal CVD. In line with these findings, the EPIC Norfolk Study (Wijndaele et al., 2011) reported that each additional hour of TV-viewing per day increased the risk for any CVD event, nonfatal CVD and coronary heart disease by 6%, 6% and 8%, respectively. While using a different approach a research group led by Ekelund et al. (Ekelund et al., 2018) found that individuals performing < 2.5 MET-hour/week and sitting > 8 hours/day had a 32% increased risk for CVD mortality, compared to those sitting less than 4 hour/day and in the same PA level.

Type 2 Diabetes Mellitus (T2DM). In 2011, Grontved and Hu (Grontved & Hu, 2011) reported that subjective measurements of SB, such as TV viewing, were associated with a 20% higher risk of developing T2DM when accumulated for more than for 2 hours per day. Furthermore, a representative meta-analysis (Wilmot et al., 2012) showed that negative associations between SB time and T2DM were stronger, representing an increased risk of 112%. In line with these findings, Larsen et al. (Larsen et al., 2015) stated that each hour of sitting was associated with 4% increased odds of T2DM. However, although there is now a reasonably consistent base of epidemiologic evidence reporting deleterious associations between SB and T2DM (Larsen et al., 2015), recently, Patterson et al. (Patterson et al., 2018) reported that PA appeared to attenuate the effect size of SB on the development of T2DM.

Cardiometabolic biomarkers. A growing body of evidence has shown detrimental associations between total SB and several cardiometabolic biomarkers, such as high density lipoprotein (HDL), C-reactive protein (CRP), triglycerides, insulin and 2-hour plasma glucose (Healy et al., 2011; Henson et al., 2013). However, a recent study using data from 2008 Health Survey for England suggested that the magnitude of the association between SB time and metabolic biomarkers depend on the balance of time between SB, light-intensity PA (LIPA) and MVPA (McGregor, Palarea-Albaladejo, Dall, Stamatakis, & Chastin, 2019).

Metabolic Syndrome (MetS). In 2012, a meta-analysis including 10 cross-sectional studies documented that, independently of PA, a greater time spent in SB was associated with a 73% increased risk for MetS (Edwardson et al., 2012). Numerous prospective studies reported the presence of a negative association between SB and MetS in adolescents

(Salonen et al., 2015), adults (Gennuso, Gangnon, Thraen-Borowski, & Colbert, 2015; Honda et al., 2016; Saleh & Janssen, 2014) and older adults (Bankoski et al 2011). In adults, Gennuso et al. (Gennuso et al., 2015) found that, compared to low levels of SB (< 6.7 hours/day), the risk of developing MetS in those with higher levels of SB (> 9.5 hours/day) increased 58%. Moreover, the authors stated that for each 1 hour increase in daily SB time was associated with 9% increased odds of developing MetS (Gennuso et al., 2015).

Obesity and Body Composition. Hamilton et al. (Hamilton et al., 2007) examined the role of SB (e.g. sitting) on several health conditions and suggested that higher levels of SB have been linked to increased rates of overweight and obesity. In a population-based longitudinal study, Helajarvi et al. (Helajarvi et al., 2014) found that individuals with moderate (1 - 3 hours) and high levels (≥ 3 hours) of daily TV-viewing significatively increased BMI and waist circumference (WC), comparing to those with low levels in the same behavior. Moreover, the authors reported that during the 10-years follow-up, high levels of TV-viewing time had approximately two-fold increased risk of developing obesity compared to the group with constantly low TV-viewing time (Helajarvi et al., 2014). Regarding to central obesity, Júdice et al. (Judice, Silva, & Sardinha, 2015) reported that independently of total SB time, prolonged periods in SB of at least 1 hour are associated with 48% increased risk of developing abdominal obesity. In line with these findings, data from the English longitudinal study of ageing indicated that, compared to individuals with less than 2 hours of daily TV-viewing, those spending more than 6 hours per day in the same behavior have an increased risk of 48% of developing centrally obesity (Smith, Fisher, & Hamer, 2015).

Most of the above-mentioned evidence suggested that total SB time and prolonged periods in this behavior represent a trigger for the development of several negative health outcomes. However, this complex analysis should be explored with caution because there is recent evidence showing small or inexistent associations between SB and other health pursuits (Campbell et al., 2018; Evenson, Wen, & Herring, 2016; Pulsford, Stamatakis, Britton, Brunner, & Hillsdon, 2015).

2. Interruption of Sedentary Behavior

2.1 Definitions

In 2017, the Sedentary Behavior Research Network (SBRN) sought to clarify the definitions of SB and SB-related terms through the development of an innovative conceptual model (Tremblay et al., 2017). In this paper, the authors reported that in addition to the total volume of SB, sedentary patterns, seen as the way in which SB is accumulated throughout the day, may also be important (Tremblay et al., 2017). As such, while bout in sedentary behavior (BSB) was defined as any period of uninterrupted SB time, break in sedentary time (BST) represents a non-sedentary time in between two BSB (Tremblay et al., 2017). Moreover, an individual is considered *breaker* if sedentary time is accumulated with frequent interruptions and limited bouts, or *prolonger* if sedentary time results from the exposure to prolonged continuous bouts (D. Dunstan et al., 2010).

One of the most commonly used alternatives to interrupt prolonged sedentary time refers to the standing posture. In fact, standing without ambulation is already considered a relevant stationary behavior that can be further characterized as *active*, if the standing posture involves an EE > 2.0 METs, or *passive*, if the standing posture requires an EE ≤2.0 METs (Tremblay et al., 2017). Although SB patterns has seen an exponentially growth over the last years, there is still limited evidence regarding their description, particularly in national representative samples.

2.2 Epidemiology

In 2018, Santos et al. (Santos et al., 2018) described for the first time the patterns of SB across lifespan in a representative national sample of Portuguese adolescents, adults and older adults. In this paper, the authors observed that, in adolescents (10-19 years), the number of short BSB (1 to < 5 minutes) gradually decreased throughout the adolescence [10 - 14 years (~62 BSB/day), 15 - 19 years (53 BSB/day), in females; 10 - 14 years (~62 BSB/day), 15 - 19 years (~53 BSB/day) in males)], which may have contributed to a substantial increase in the time spent in prolonged BSB (≥ 30 minutes) [10 - 14 years (~3 BSB/day), 15-19 years (~4 BSB/day), in females; 10 - 14 years (~2 BSB/day), 15 - 19 years (~3 BSB/day) in males)] (Santos et al., 2018).

In adults (20 - 64 years), the number of short BSB (1 to < 5 minutes) increased during the first half of adulthood (20 - 49 years), beginning to decline thereafter [35 - 49 years (~67 BSB/day), 50 - 64 years (~63 BSB/day), in females; 35-49 years (~59 BSB/day), 50 - 64 years (~56 BSB/day), in males)] (Santos et al., 2018). In addition, the authors reported that, in females, the number of prolonged BSB (≥ 30 minutes) substantially decreased during the first half of adulthood (20 - 49 years), beginning to increase immediately after that moment [35 - 49 years (~2 BSB/day), 50 - 64 years (~3 BSB/day)] (Santos et al., 2018). However, in males, no differences were found throughout the adulthood, as the number of prolonged BSB remained relatively constant [20 - 64 years (~3 BSB/day)] (Santos et al., 2018).

In older adults (≥ 65 years), the authors found that number of short bouts (1 to < 5 minutes) dropped dramatically with age in both sexes [65 - 69 years (~59 BSB/day), ≥ 85 years (~38 BSB/day), in females; 65 - 69 years (~50 BSB/day), ≥ 85 years (~40 BSB/day), in males)] (Santos et al., 2018). Conversely, the number of prolonged BSB (≥ 30 minutes) gradually increased throughout the older adulthood [65 - 69 years (~3

BSB/day), ≥ 85 years (~5 BSB/day), in females; 65 - 69 years (~4 BSB/day), ≥ 85 years (~6 BSB/day), in males)], suggesting that over 85 years, approximately half of the daily SB time (48%) was accumulated in prolonged BSB (Santos et al., 2018).

Regarding daily BST, the authors found that, in females, the number of daily BST remains relatively high during the adolescence (87 BST/day) and adulthood (91 BST/day), however, gradually decreasing during the older adulthood [65 years (83 BST/day); ≥ 85 years (65 BST/day)] (Santos et al., 2018). In a similar perspective, for males the number of daily BST was higher during the adolescence (87 BST/day) and adulthood (86 BST/day), compared to older adulthood [65 years (73 BST/day); ≥ 85 years (63 BST/day)], where it considerably decreased (Santos et al., 2018).

For instance, Chen et al. (Chen et al., 2018) noted that the number of BST for each sedentary hour significantly decreased over the time, from 10 times in adults to 7.3 in older adults, representing a decline of 16 daily BST (70 to 54) in the transition to older adulthood. Moreover, the authors reported that adults over 40 years accumulated more than one-third of the daily sedentary time in uninterrupted SB (≥ 30 minutes) (Chen et al., 2018). In a different approach, a cross-sectional study highlighted the periods in which adolescents are particularly exposed to prolonged SB (e.g. school classes) and reported SB is interrupted, approximately, 50 times per day (3.15 BST/hour) (Arundell, Salmon, Koorts, Contardo Ayala, & Timperio, 2019). Based on these previous-mentioned findings, there is a clear need to target adolescence and the transition from adulthood to older adulthood as critical periods to intervene. As such, encouraging potential at-risk populations to break sedentary time more often and designing effective interventions to reduce total SB across all age groups may be urgent strategies to minimize the negative health impact of SB.

2.3 Observational Studies and Health-related outcomes

During the last decade, a large body of observational evidence emerged, concerning about the manner in which SB is accumulated. Besides the research of detrimental effects of total SB, recent evidence suggested that SB patterns may have singular implications on health-related outcomes. In 2016, Matthews et al. (Matthews et al., 2016) applied isotemporal substitution models to estimate the potential impact of replacing 1 hour/day of SB with LIPA and MVPA. Findings from this study suggested that for LIPA and MVPA mortality rates decreased 20% and 40%, respectively, in low-active individuals (Matthews et al., 2016). In a similar analysis, was found that over 5 years of follow up, individuals replacing 30 minutes/day of SB with LIPA decreased mortality risk by 20%, while substituting 30 minutes/day of SB with MVPA had a greater reduction of 51% (Fishman et al., 2016).

More recently, a national cohort study with a 15-year follow-up documented a risk reduction of 11% for all-cause mortality, 14% for cancer mortality and 24% for CVD mortality, when replacing 30 minutes of SB with LIPA, per day (Dohrn et al., 2018). Although no significant reductions in all-cause and cancer mortality were found when 10 and 30 min/day of SB were replaced with MVPA, for CVD mortality this substitution resulted in a significant decreased risk of 38% (10 minutes/day) and 77% (30 minutes/day) (Dohrn et al., 2018). As such, considering recent findings suggesting that interrupting SB with standing may have a limited impact on several cardiometabolic variables, some authors suggested that breaking prolonged sitting with LIPA or MVPA may be a more powerful alternative to motionless standing (Amirfaiz & Shahril, 2018; Chastin, Egerton, Leask, & Stamatakis, 2015; McGregor et al., 2019).

In another perspective, data derived from the Australian Diabetes, Obesity, and Lifestyle Study suggested, for the first time, that independently of total SB time, the total number of BST was positively associated with body mass index (BMI), WC, triglycerides and 2-h plasma glucose (Healy et al., 2015). Moreover, Júdice et al. (Judice et al., 2015) showed that the odds of abdominal obesity were positively associated with the continuous time spent in SB, in older adults. As such, while for each additional BSB of 10 < minutes < 20 abdominal obesity only increased 7%, for each 1-hour BSB increment, the pooled risk of becoming obese was 48% (Judice et al., 2015). In line with these findings, Carson et al. (Carson et al., 2014) reported that, independently of total SB time and MVPA, each additional 10 BST/day were significatively associated with 0.83 cm lower WC, 0.32 mm Hg lower systolic blood pressure (SBP), 0.01 mmol/L higher HDL-cholesterol, 4% lower triglycerides, 0.6% lower glucose and 4% lower insulin.

Regarding other health-related outcomes, as far as older adults are concerned, Sardinha et al. (Sardinha et al., 2015) showed that the total number of BST was significatively associated with an enhanced physical function, independently of potential confounders (e.g. SB and PA). Regarding lower extremity function, each additional BST in sedentary time per hour represented an increase of 58% in overall lower extremity function (Davis et al., 2014). These findings are in agreement with the Maastricht Study, which stated that, in adults and older adults (40 - 75 years), every 10 additional BST per day were positively associated with an improved physical function, especially in the lower extremity of the body (van der Velde et al., 2017).

During the last years, the growth of observational evidence suggested that patterns of SB, such as BSB, may have a negative health impact, independently of total SB and PA time. However, variables such as, the independent nature of SB, the type of SB behind the identified associations and the potential protective role of PA are still questioned. In this sense, considering that there are multiple types of study designs, the comparison between studies and the establishment of supportive conclusions are even more challenging.

2.4 Experimental Studies and Health-related outcomes

Besides the growing interest in observational evidence, during the last decade several experimental studies such as, randomized controlled trials (RCT's), emerged to describe the impact of replacing SB with other active pursuits. In this sense, as far as SB patterns are concerned, scientific community has been designing effective alternatives to this behavior, especially in environments where individuals are highly exposed. For instance, an innovative experimental crossover trial suggested that interrupting SB time with 2 minutes bouts of light- and moderate-intensity walking every 20 minutes, significatively lowered glycemic and insulinemic responses in 19 nondiabetic overweight/obese adults (45 - 65 years) (D. W. Dunstan et al., 2012). After adjustment for several confounders, the authors reported that breaking up prolonged sitting with light-intensity walking resulted in a beneficial decrease of 23% for insulin positive incremental area under the curve (iAUC) and 24% for plasma glucose iAUC, compared to uninterrupted sitting (D. W. Dunstan et al., 2012). Moreover, significant reductions of 30% insulinemic iAUC and 24% plasmatic glucose iAUC were also reported when sitting time was interrupted with brief moderate-intensity bouts of walking (D. W. Dunstan et al., 2012).

In other perspective, using randomized crossover design, Duvivier et al. (Duvivier et al., 2013) aimed to determine variation of insulin sensitivity and circulating lipids across three different free living conditions (4 days each): 1) sitting regime (14 hours/day sitting); 2) exercise regime (replacing 1 hour/day of sitting for vigorous-intensity cycling; 13 hours/day sitting); 3) minimal intensity PA regime (replacing 6 hour/day of sitting for 4 hours/day of leisure walking and 2 hours/day of standing; 8 hours/day sitting). The results of this study suggested that compared to sitting regime, minimal intensity PA regime significantly lowered the circulating levels of triglycerides (22%), non – HDL cholesterol (10%) and apo B concentration (8%) (Duvivier et al., 2013). Curiously, as the authors did not found significant improvements for the exercise regime comparing to the sitting one, they suggested that, if participants spend most of their day in sitting time, practicing 1 hour of structured PA per day may not prevent the negative impact on metabolic health outcomes (Duvivier et al., 2013).

In a similar sample, Thorp et al. (Thorp et al., 2014) showed that alternating between a sitting and standing posture every 30 minutes resulted in a significant decrease of 11% in mean glucose iAUC, compared to an uninterrupted sitting condition. However, although interchanging between sitting and standing only occasioned a modest effect on glucose responses, with no significant differences observed for serum insulin and plasma triglycerides (Thorp et al., 2014). Moreover, a recent experimental study including 14 inactive healthy adult males reported that breaking up sitting with 15 minutes bouts of non-ambulatory standing every 30 minutes lowered by 27% the cumulative postprandial glucose response, compared to time spent in continuous sitting (Benatti et al., 2017). Additionally, although interrupting sitting with standing resulted in a modest decrease of the postprandial insulin and C-peptide response, no statistical significance was reached (Benatti et al., 2017).

In 2015, Bailey et al. (Bailey & Locke, 2015) implemented a randomized crossover trial to explore the effects of breaking up sitting on a range of cardiometabolic risk markers in non-obese adults. In this study, participants took part in a three 5-hour trial conditions randomly ordered: 1) uninterrupted sitting; 2) sitting with 2 minutes bouts of standing (every 20 minutes); and 3) sitting with 2-minutes bouts of light-intensity walking (every 20 minutes) (Bailey & Locke, 2015). The results of this study suggested that sitting with 2-minutes bouts of light-intensity walking significantly lowered the postprandial glucose AUC (16%), compared to continuous sitting + sitting with 2 minutes bouts of standing (Bailey & Locke, 2015). However, it was found that interrupting sitting with short bouts of standing had no meaningful effect on cardiometabolic health (Bailey & Locke, 2015). Using a similar protocol, Pulsford et al. (Pulsford, Blackwell, Hillsdon, & Kos, 2017) reinforced these previous findings by observing that plasmatic insulin and glucose demands only significantly reduced when prolonged sitting was interrupted with 2-minutes bouts of light-intensity activity, every 20 minutes.

Given the results, interrupting prolonged sitting with brief bouts of LIPA, but not standing, may be a better option to significantly reduce the cardiometabolic risk of SB in adults (Bailey & Locke, 2015; MacEwen, Saunders, MacDonald, & Burr, 2017; Pulsford et al., 2017). However, there is a need to further explore the cardiometabolic impact of interrupting SB with longer bouts of standing or with activities that require a minimum threshold of EE (Bailey & Locke, 2015).

3. Energy Expenditure (EE)

3.1 Definition

EE, usually referred as total daily energy expenditure (TDEE), can be divided into resting energy expenditure (REE), diet-induced thermogenesis (DIT) and activity energy expenditure (AEE) (E Ravussin, Burnand, Schutz, & Jéquier, 1982) (Figure 1). REE, also defined as resting metabolic rate (RMR), corresponds to the minimal rate of EE compatible with life, representing approximately 60 - 70% of TDEE (E. Ravussin & Bogardus, 1992; E Ravussin et al., 1982). RMR magnitude is strongly dependent on fat-free mass (FFM), that accounts for at least 70% of its variance, however there are other significant contributors, such as fat mass (FM), gender and age (Weyer, Snitker, Rising, Bogardus, & Ravussin, 1999). In addition, to estimate REE, it is essential to measure individuals in standard conditions of resting, fasting, immobility, thermo-neutrality and mental relaxation (Levine, 2005).

Regarding DIT, this component represents the metabolic response to food consumption, that is, the required energy to process, absorb and store different types of nutrients (Tappy, 1996). Although DIT accounts for a relatively small portion of TDEE (10%), slight differences in the amount and type of nutrients consumed over time can result in significant changes in energy balance (de Jonge & Bray, 1997). Moreover, this response also depends on the size and body composition (e.g. FM and FFM) of the individual, as well as nutritional state (de Jonge & Bray, 1997). However, independently of food consumption, some conditions including aging, PA, obesity and insulin resistance, seem to considerably affect DIT (Weyer et al., 1999).

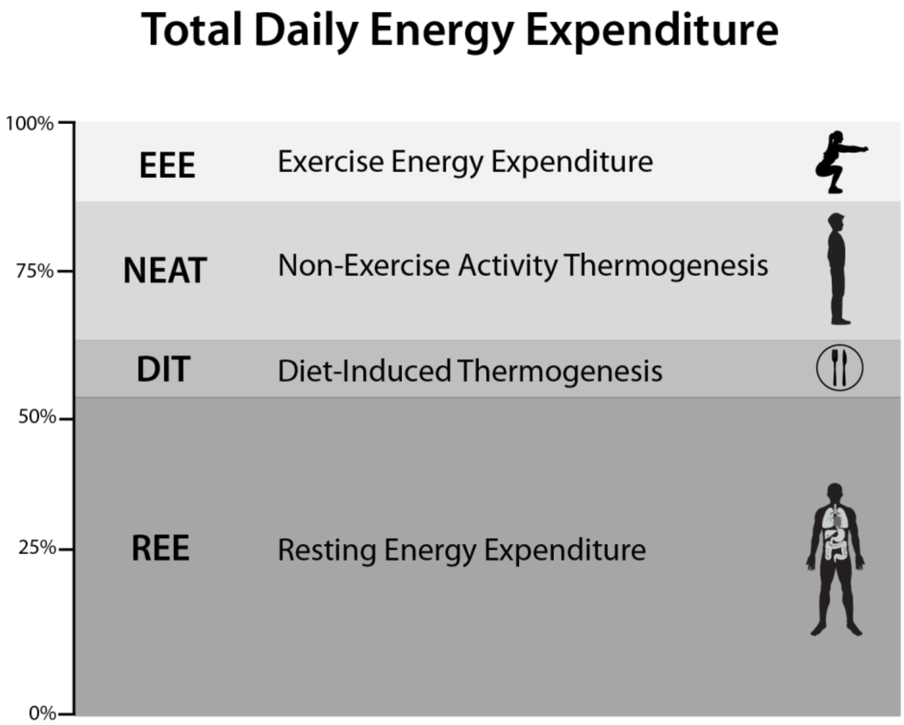

Figure 1 – Total Daily Energy Expenditure (TDEE) compartments

The energetic cost of PA (AEE), considered as the most variable component of TDEE, includes energy consumed from muscular work during spontaneous or structured activities of daily-living (Levine, 2004). In this sense, while there is a limited AEE in very sedentary individuals (15% of TDEE), in highly active individuals AEE accounts for more than 50% of TDEE (Levine, 2004). In addition to the contribution of AEE in TDEE, it is important to further describe each of the two sub-components, namely the exercise energy expenditure (EEE) and non-exercise activity thermogenesis (NEAT) (Levine, 2004). While NEAT corresponds to the energy expended in trivial daily activities, such as fidgeting, posture maintenance and non-specific ambulatory behavior, the magnitude of EEE is determined by the energetic cost of planned and structured activities (Garland et al., 2011; Levine, 2004). Particularly in EEE, generally known as the minor portion of AEE, the amount of energy spent may vary according to the intensity level and the energetic adaptation of the activities performed (Westerterp, 2016). However, both NEAT and EEE are influenced by several conditions, such as age, gender, genetic component, individuals physiological and biochemical pathways and their response to environmental requirements (Garland et al., 2011).

3.2 Methodologies to assess EE

Due the growing interest of scientific community in quantifying EE in laboratory and field settings, several subjective and objective methodologies are being used to characterize TDEE, as well as their sub-components (e.g. AEE).

3.2.1 Subjective Methods

Regarding AEE assessment, a range of subjective approaches including direct observation, questionnaires, interviews and diaries, are commonly used to assess different dimensions of an individual’s PA (Ceesay et al., 1989). Moreover, in large population-based studies, free-living activity is typically assessed through subjective methods, because they represent an inexpensive, easy applicable, non-invasive and valid technique to use (Hills et al., 2014). Although PA questionnaires are useful to determine the specific variables of PA, such as the type and the context of practice (Lam & Ravussin, 2016), most of them consistently underestimate the energetic cost of PA (AEE) (Shephard, 2003). Rather than determining EE through questionnaires, some studies used a Compendium of PA that characterizes each daily task or activity into domains and intensities (MET's) (Ainsworth et al., 2000). Although this approach enables the estimation of energy cost of several activities, it may not be applicable to all individuals, due to the fact that the energetic cost defined for each activity relies on group averages with specific characteristics (Byrne, Hills, Hunter, Weinsier, & Schutz, 2005). Therefore, based on the above-mentioned limitations, it is widely recognized that the validity of data derived from subjective methods seems to be questionable, especially when compared to objective approaches (Hills et al., 2014).

3.2.2 Objective Methods

The most popular objective methodologies used to characterize PA and indirectly estimate AEE are motion sensors (e.g. accelerometer, pedometer and inclinometer), heart rate (HR) monitoring devices (e.g. cardiofrequencimeter) and combined methods (e.g. accelerometer combined with cardiofrequencimeter) (Hills et al., 2014). Due to their practical, non-invasive, valid and relatively inexpensive character, these methods are typically used to quantify PA intensity and volume, in both free-living and laboratory settings (Hills et al., 2014).

Accelerometers have gained a substantial reputation within the scientific field of PA, due to their capacity to accurately measure different intensities of movement through the accelerations of the body (Freedson, Melanson, & Sirard, 1998). As such, to overcome some limitations related to other objective methods, such as inclinometers and pedometers, several large-scale studies have used accelerometers to quantify PA level, as well as minimal movement activities, such as SB and low levels of PA (Van

Cauwenberghe, Gubbels, De Bourdeaudhuij, & Cardon, 2011). Despite a favorable association between accelerometry and EE estimation for a wide range of activities, some limitations have been reported when using this method in specific activities, such as water-based activities and non-ambulatory exercises (e.g. cycling) (Bouten, Sauren, Verduin, & Janssen, 1997). Additionally, accelerometers are also unable to directly assess the internal stress load that an individual has when performing a specific task (Brage et al., 2004).

As none of the above-mentioned methods allow the assessment of all domains of PA in free-living settings, the use of methods combining accelerometry with HR monitoring is widely recommended (Hills et al., 2014). Based on the assumption that accelerometry confirms whether the raise in HR is due to PA or not (Hills et al., 2014), it is possible to precisely quantify PA and estimate EE through this combined methodology, independently of the limitations that each approach has (Brage et al., 2004).

3.2.3 Gold Standard Methods

Although the previous referred approaches represent practical tools to determine specific subcomponents of TDEE, they are not the reference methods to assess TDEE. In this sense, reference methodologies, also designed as “gold standard”, are indispensable to distinguish the various components of TDEE, that is REE, DIT and AEE (Levine, 2005). Generally, this set of criterion techniques focus on the estimation of O_2 consumption and CO_2 production [e.g. doubly labeled water (DLW) and indirect calorimetry (IC)], as well as quantification of heat production [e.g. direct calorimetry (DC)] (Levine, 2005).

Doubly Labeled Water (DLW). DLW is acknowledged as the criterion or “gold standard” to assess TDEE in a free-living context (Schoeller, 1988). This non-invasive technique consists in an isotope-ratio mass spectrometry analysis. The individual ingests two stable isotopes, deuterium (H_2) and oxygen-18 (O_{18}), via drinking water (Coward, 1988; Speakman, 1998) . Over 7 to 14 days of assessment, the daily collection of urine samples will allow the tracking of the elimination rate of these isotopes (Coward, 1988). Posteriorly, by measuring the difference between the elimination rates of H_2 and O_{18} is possible to determine CO_2 rate production and, therefore, estimate the average TDEE (Coward, 1988). Despite being the "gold standard" method to assess TDEE, DLW has some practical limitations. This technique does not provide information regarding the intensity and nature of daily activity and the time-course of EE is not possible to determine (Ainslie, Reilly, & Westerterp, 2003). Moreover, the elevated cost of the isotopes and correspondent equipment to perform isotope-ratio mass spectrometry limits the availability of this technique to specific clinical settings (Lam & Ravussin, 2016).

Direct Calorimetry (DC). Direct calorimetry assessment is based on the assumption that the energy expended during physiological processes is dissipated, in this regard, it is possible to determine TDEE by directly assessing heat production (Weir, 1949). This technique consists of an isotermic metabolic chamber with a ventilated hood system surrounded by a shell space that is maintained at the same temperature as the inside of the chamber (Jequier, 1986). Thus, by measuring the differences in the air temperature and humidity between the inside and the outside of the chamber, heat production is determined (Jequier, 1986). Although these metabolic chambers are effective to assess EE over prolonged periods of time (from 24-h to a large number of days), they are not able to detect acute variations in EE (Lam & Ravussin, 2016). In addition, one of the main limitations of this technique is that, as individuals are confined to a small chamber, it does not provide an accurate estimate of free-living activities (Carson et al., 2014).

Indirect Calorimetry (IC). Rather than measuring heat production or loss directly, IC determines EE through the real-time measurement of the amount of O_2 consumed and

CO_2 produced (Levine, 2005). This non-invasive and highly accurate method typically measures the flowing levels of O_2 and CO_2 to 1) determine the respiratory exchange ratio (RER), defined as the ratio between the amount of CO_2 production and O_2 utilization by metabolism and 2) identify the energetic substrates that are being predominantly metabolized (Levine, 2005). In this sense, by measuring the oxidation rate of macronutrients, it is possible to calculate heat production and consequently determine sub-components of TDEE (e.g. REE) (William D McArdle, Katch, & Katch, 1991).

Generally, IC consists of a gas collector in which the exhaled gas is captured using an adaptable mouthpiece, facial mask or a canopy connected to a gas analyzer (calorimeter) (Levine, 2005). Through a unidirectional valve, the calorimeter quantifies the volume of O_2 inspired and CO_2 expired minute by minute, typically over a minimum period of 30 minutes (Hills et al., 2014; Levine, 2005). After that, EE is estimated through the calculation of heat output from substrate oxidation, using the abbreviated Weir's equation (Weir, 1949).

$$\textbf{EE (kcal)} = \textbf{3.9} \times \textbf{O}_2 \textbf{ consumed [L]} + \textbf{1.11} \times \textbf{CO}_2 \textbf{ produced [L]}$$

The respiratory quotient (RQ), defined as the volume of CO_2 released over the O_2 consumed during respiration, also informs about the carbohydrate and fat oxidation. In general, only carbohydrates are consumed when RQ is 1, conversely, a RQ of 0.7 represents a complete fat oxidation (William D McArdle et al., 1991). Moreover, when RQ range within 0.7 and 1, it indicates that both subtracts are being utilized simultaneously (William D McArdle et al., 1991). However, significant deviations from this range (RQ < 0.7) may indicate relevant physiological changes, such as excessive production of ketone bodies (William D McArdle et al., 1991). Thus, the balance of the macronutrient utilization represents, from a clinical point of view, a precise metabolic predictor for some specific metabolic diseases (e.g. obesity, DMT2 and liver cirrhosis) (William D McArdle et al., 1991).

3.3 Indirect Calorimetry Assessment

Regarding the assessment of NEAT and EEE, instead of the measurements being performed at rest, they are typically performed during the practice of a determined activity. Therefore, to assess the EE of defined tasks such as sitting, standing, walking or MVPA, most of researchers assume that for each liter of O_2 consumed, approximately 5 kcal are spent by the body (William D McArdle et al., 1991).

Presently, to overcome some constrains including locomotion limitation and restricted protocols, there has been an increasing interest in quantifying EE in free-living settings through the use of innovative portable ventilated hood systems (Macfarlane, 2017). However, given the high number of spontaneous activities that an individual can perform throughout the day, there is an increased difficulty to quantify this component, particularly in free-living settings. One possible explanation to this fact is that NEAT represents the most significant contribution to inter- and intrapersonal variability in EE, independently of total body mass (Levine, 2004). Therefore, to precisely estimate the energetic cost of non-exercise activities under controlled conditions, most of the present IC assessments are carried out in laboratory settings (Levine, 2004).

Particularly in NEAT, there has been a growing interest in estimating EE particularly in elementary activities including fidgeting, sitting, standing or other active pursuits (Levine, 2004). The daily energy expended in these behaviors accounts for a large NEAT variance and substantially affects the daily energy balance. Based on the assumption that the variation of the energy balance informs about the predisposition to develop specific health-related outcomes, global recommendations suggesting the ideal amount of daily time spent in each of these behaviors should be ensured.

3.4 Experimental Studies – Assessing NEAT and EEE

During the last years, the role of a SB on health outcomes has been outlined by several studies, where it becomes clear that replacing common sitting with low-intensity activities, should be advocated, regardless of the time spent in MVPA. In this regard, several strategies can be used to replace prolonged sitting by activities with higher physiological impact, such as standing, stepping or walking, leading to increased daily values of EE and potentially contributing to small, but frequent long-term changes. (Carter, Jones, & Gladwell, 2015; McAlpine, Manohar, McCrady, Hensrud, & Levine, 2007; Miles-Chan, Sarafian, Montani, Schutz, & Dulloo, 2013; Saeidifard et al., 2018)

In 2007, McAlpine et al. (McAlpine et al., 2007) assessed and compared the EE of motionless lying, sitting and standing, treadmill walking and stepping, in 19 obese and non-obese adults. According to the results, walking (5.63 ± 1.57 $kcal \cdot min^{-1}$) and stepping (6.27 ± 1.93 $kcal \cdot min^{-1}$) were significantly associated with a 5- to 6-fold increase in EE above uninterrupted sitting (1.47 ± 0.35 $kcal \cdot min^{-1}$) and standing (1.62 ± 0.43 $kcal \cdot min^{-1}$) (McAlpine et al., 2007). Considering that stepping presented similar increases in EE as walking, the authors argued that replacing two daily hours of sitting by self-regulated stepping may represent a weight loss of 20 kg/year, if other components of the energy balance are considered (McAlpine et al., 2007). More recently, Carter et al. (Carter et al., 2015) suggested that using calisthenics to break up prolonged SB is a more time efficient strategy than standing or walking. In fact, the authors reported that compared to prolonged sitting (30 minutes), there was an additional 6.5% (3 kcal), 24.9% (10 kcal) and 37.8% (16 kcal) EE by breaking up this behavior with 2 minutes of standing, walking and calisthenics, respectively (Carter et al., 2015).

Although interrupting sitting with active bouts of walking, stepping or cycling, can significantly increase daily EE in the short-term, there is no evidence on their effect on sitting over a long-term period. Moreover, MacEwen et al. (MacEwen, MacDonald, & Burr, 2015) suggested that exercises with greater EE are typically related to larger decreases in productivity and motor ability while working. Therefore, to overcome some of these issues, it is suggested that the replacement of prolonged periods of sitting with continuous bouts of standing represents a more logistic and feasible burden to the person in their workplace (MacEwen et al., 2015).

In this perspective, Reiff et al. (Reiff, Marlatt, & Dengel, 2012) showed a significant increase of 33% in the energy cost in adults that where standing for 45 minutes at a standing desk (1.36 ± 0.20 $kcal \cdot min^{-1}$) compared with those who remained seated (1.02 ± 0.22 $kcal \cdot min^{-1}$). In line with these findings, Buckley et al. (J. P. Buckley, Mellor, Morris, & Joseph, 2014) argued that replacing sitting with standing-based work can further influence EE. According to the authors, the energetic cost of 15 minutes standing-based work (2.32 ± 0.83 $kcal \cdot min^{-1}$) represented an increase of 0.83 $kcal \cdot min^{-1}$ compared to sitting work (1.49 ± 0.66 $kcal \cdot min^{-1}$) (J. P. Buckley et al., 2014), which is in line with previous findings suggesting an approximately 0.8 $kcal \cdot min^{-1}$ difference between both behaviors (Levine, Schleusner, & Jensen, 2000).

However, Seaidifard et al. (Saeidifard et al., 2018) noted that the difference between sitting and standing was approximately 30% higher in adults that underwent an intervention using sit-stand desks to work (0.18 ± 0.11 $kcal \cdot min^{-1}$) compared to those who remained motionless in both sitting and standing postures (0.14 ± 0.03 $kcal \cdot min^{-1}$). A likely explanation for this fact is that, although the interventions were conducted under controlled conditions, in participant using a sit-stand workstation, the amount of movement fidget was not assessed (Saeidifard et al., 2018). As such, the difference between these subgroups (0.04 $kcal \cdot min^{-1}$) may be largely attributed to the occurrence of fidgeting-like movements at low work intensities that quantitatively lead to substantial increases in EE (Levine et al., 2000; Mansoubi et al., 2015).

Due to the difficulty to individually quantify fidgeting-like movements and determine the magnitude of its impact on EE, several scientific groups have opted to investigate the substitution of sitting with standing under more restricted laboratory conditions. For example, Miles-Chan et al. (Miles-Chan et al., 2013), based on a sample of 22 young adults, compared the magnitude of change in EE that occurs over 10 minutes of steady state standing versus sitting and conclude that the energetic cost of the standing condition was 5% higher (1.02 ± 0.04 $kcal \cdot min^{-1}$), compared to the mean sitting EE (0.97 ± 0.04 $kcal \cdot min^{-1}$). In line with these findings, an innovative randomized controlled trial including 50 adults suggested that mean EE of continuous sitting (10 minutes) (1.14 ± 0.18 $kcal \cdot min^{-1}$) also differed from continuous standing (10 minutes) (1.23 ± 0.19 $kcal \cdot min^{-1}$) (Judice, Hamilton, Sardinha, Zderic, & Silva, 2016). In addition, this research group found that a complete transition from sitting to standing (and return to sitting) represented an increase in EE of 0.32 $kcal \cdot min^{-1}$, above sitting (Judice et al., 2016). With a similar protocol, Popp et al. (Popp, Bridges, & Jesch, 2018) strengthened these previous findings, suggesting that a 15 minutes standing condition increased mean EE by 9% and 7% compared to continuous lying (15 minutes) and sitting (15 minutes), respectively. However, no changes were found in mean EE when comparing lying and sitting (Popp et al., 2018).

In order to systematize the available evidence, a recent systematic review summarized the information related to studies assessing the magnitude of change in EE between sitting and standing (Saeidifard et al., 2018). For instance, this paper stated that from 46 studies included, 44 reported a positive mean EE difference between sitting and standing, while only two reported no significant differences between both behaviors

(Saeidifard et al., 2018). Although the mean difference in EE between sitting and standing was approximately twice as high in males as in females, and twice as high in randomized trials as in observational studies, all participants modestly increased mean EE by 0.15 kcal·min^{-1} when replaced sitting (1.29 ± 0.24 kcal·min^{-1}) with standing (1.47 ± 0.33 kcal·min^{-1}) (Saeidifard et al., 2018).

Although this may be an efficient strategy to increase daily EE, there have been numerous studies, both laboratory and field interventions, suggesting that occupations involving extended periods of standing may result in the development of negative health outcomes. In 2007, Anderson et al. conducted a 1-year study in 5600 workers and found that those spending more than 30 minutes/hour in uninterrupted standing resulted in two-fold increased risk of developing low back and extremity pain (Andersen, Haahr, & Frost, 2007). In another perspective, recent findings suggested that the development of adverse conditions, such as lower back discomfort, limbs swelling and attention loss, were particularly high in adults spending more than 2 continuous hours of standing (Baker, Coenen, Howie, Williamson, & Straker, 2018; Fewster, Gallagher, Howarth, & Callaghan, 2017; Gallagher & Callaghan, 2015).

Moreover, Waters and Dick (Waters & Dick, 2015) pooled out the existing literature examining the potential health risks resulting from the exposure to prolonged standing and found that, considering a variable number of periods of uninterrupted standing (> 30 minutes/day to > 4 hours/day), studies consistently reported increased levels of low back pain, physical fatigue, muscle pain, tiredness and leg swelling. Based on these findings, this research group suggested that health problems may be minimized if body posture is modified along the day (Waters & Dick, 2015). So, instead of a vague recommendation for replacing prolonged sitting with uninterrupted static standing, individuals should be encouraged to reduce their sitting time with intermittent standing

(Agarwal, Steinmaus, & Harris-Adamson, 2018; Gallagher, Campbell, & Callaghan, 2014; Waters & Dick, 2015).

In a recent experimental study, Thorp et al. (Thorp et al., 2016) determined whether alternating bouts of sitting and standing at work influenced daily workplace EE, in both overweight and sedentary adults. The 23 included participants undertake two 5-day experimental conditions: 1) continuous sitting work for 8 hours (SIT-condition) and 2) alternating between sitting and standing every 30-minutes for 8 hours (STAND-SIT condition) (Thorp et al., 2016). Thus, in the fourth day of each condition, acute EE was measured during the first 30 minutes using an open-circuit IC (Thorp et al., 2016). The results showed that standing to work (1.3 ± 0.1 kcal·min^{-1}) significantly increased EE compared to sitting (1.1 ± 0.01 kcal·min^{-1}) (Thorp et al., 2016). According to the authors, if results were extrapolated to 8 hours of daily work, replacing 4 hours of sitting with standing could represent a slight increase of 29% (48 kcal) in mean EE (Thorp et al., 2016). Moreover, this study reported that standing to work resulted in a significant increase in all respiration values, with the exception of RER, which was consistent between conditions (Thorp et al., 2016). Although this study yielded interesting results, there is a need to further investigate whether intermittent standing may influence daily EE in a wider range of populations and be influenced by the time spent in each behavior.

4. Relevance of the study

The research field of SB has become increasingly relevant over the last decades given its impact on health status. While some experimental studies focused on associating prolonged sedentary time to specific health parameters over a medium and long-term, others concerned about the development of effective strategies that simultaneously decrease SB and increase EE. In this regard, replacing sitting with standing, walking, stepping or other activities with high EE are suggested (Carter et al., 2015; Healy et al., 2015; McAlpine et al., 2007). Thus, even activities as minimal as standing, rather than continuous sitting, have been shown to promote substantial increases in TDEE. Therefore, interrupting SB with frequent and short bouts of standing could be a simple and effective way to decrease total sedentary time and contribute to increase TDEE (Mailey, Rosenkranz, Casey, & Swank, 2016).

In this perspective, there has been an increasing interest in examining the metabolic and energetic response of intermittent transitions between sitting and standing postures. As previously mentioned, Júdice et al. (Judice et al., 2016) examined the metabolic and energetic effect of breaking-up prolonged sitting with brief standing BST (sit-to-stand and immediate stand-to-sit transitions). The authors reported a substantial EE increase, compared to continuous sitting, which was mainly justified by the direct effect that the complete transition had on the following sitting moments (Judice et al., 2016). Although these findings were interesting, given the minimal time that individuals spent in a standing posture during the BST (transition), it was not possible to determine the isolate EE of this posture (Judice et al., 2016).

In another perspective, Miles-Chan et al. (Miles-Chan et al., 2013) examined variation in EE over a standing posture, as soon as the individual shifted from sit-to-stand posture. According to their findings, it was suggested that after taking a transition from sit-to-stand, the EE increased significantly during the first moments of standing, but not thereafter (Miles-Chan et al., 2013). In this regard, although this study firstly quantified the EE of standing after sitting, only one period of standing was considered (Miles-Chan et al., 2013). Therefore, it would have been interesting to promote more intermittent periods of standing to understand how EE would change over time.

In this sense, Thorp et al. (Thorp et al., 2016) measured the energetic cost of adopting intermittent 30-minutes bouts of standing across the workday, in a specific population of middle-aged adults with overweight or obesity and at relatively high risk of developing chronic diseases. Although this intervention presented suggestive conclusions, based on previous findings, the difference in EE between postures would be even higher if shorter intermittent periods of sitting and standing were considered (Miles-Chan et al., 2013). In addition, although there were performed several 30-minutes bouts of intermittent sitting and standing, the authors limited their IC analysis to the first 30-minutes bout of each posture (Thorp et al., 2016). Thus, instead of continuously measuring the impact of alternate between sitting and standing, the authors extrapolated their initial findings for all the remaining sitting and standing periods (Thorp et al., 2016).

In this sense, given the fact that some studies sought to describe the EE change based on an intermittent condition of sitting and standing postures, there is a considerable heterogeneity in the current samples and study designs utilized. Therefore, to the best of our knowledge, at the present date no study using IC has yet determined whether alternating short and continuous bouts of sitting with standing can affect the EE of one specific posture (e.g. sitting or standing), particularly in a healthy population sample.

4.1 Thesis purpose

In order to clarify some of these issues, the main purpose of our study is to determine if the energetic cost of one specific posture (e.g. sitting or standing), was influenced by the posture previously executed, using an intermittent protocol with short bouts of sitting and standing. Additionally, we also aimed to compare the differences of these findings with those related to continuous sitting and standing. In this regard, we hypothesized that the mean EE accumulated during intermittent sitting and standing postures would be greater that the mean EE measured in the conditions of continuous sitting and standing, respectively.

Methodologies

1. Sample Recruitment

All participants were recruited trough media advertisement and attendance to university classes at Faculdade de Motricidade Humana – Universidade de Lisboa. Interested individuals had asses to a detailed explanation of the study, that included: the main purposes of the study, a detailed description of the intervention procedures, schedule availability to perform the intervention and specific requirements to take part in the intervention.

In order to integrate this intervention, all the interested participant should be healthy adults, both men or women, aged between 18 and 65 years-old. Individuals taking regular medication with metabolic effect, with cardio-metabolic or pulmonary disease, with locomotion limitations, in a pregnancy condition or engaged to any weight loss program were excluded. After validating which participants were in accordance with the inclusion criteria, a written informed consent was obtained from all participants. The present study was approved by Ethics Committee of the Faculdade de Motricidade Humana (approval number: 14/2013) and conducted according with the 2013 Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects and the 1997 Convention on Human Rights and Biomedicine.

Prior to our intervention, an initial power and sample size were calculated (G*Power software, version 3.1.9.2) according to a pilot study (n = 15) using IC, where the obtained effect size was approximately 0.39 for the differences between sitting and standing, while using repeated measures ANOVA, a power of 0.80 and a significance of 0.05. Based on the expecting drop rate of 10%, an overall sample size of 50 participants was suggested. In line with the proposed sample, of the 50 participants recruited, 48 (23 women, 25 men) successfully completed all assessments.

2. Study Design

The randomized crossover study took place at Exercise and Health Laboratory (EHLAB) of Faculdade de Motricidade Humana – Universidade de Lisboa between November of 2014 and February of 2015. The 1-day trial consisted in a set of laboratory measurements (anthropometric, body composition and metabolic and energetic assessments) performed between 7 and 10 a.m. of each experimental day according to the participant's availability. The participant was instructed to attend the study on a complete fasting condition, instructed to avoid consuming stimulants (e.g. caffeine) and practicing planned MVPA, within 48 hours prior to their visit.

Regarding the set metabolic and energetic measurements, beyond the determination of REE, all participants underwent a sequence of four randomly ordered experimental conditions with 10 minutes length each: uninterrupted motionless sitting, uninterrupted motionless standing, breaking motionless sitting with brief bouts of standing and alternating between motionless sitting and motionless standing (Figure 2).

Considering that, from the above-mentioned conditions, breaking motionless sitting with brief bouts of standing has been previously studied (Judice et al., 2016), the focus of the present study was to determine and compare the EE across the other three experimental conditions (motionless sitting, motionless standing and alternating between postures), and also, determine metabolic and energetic contributions of sitting after standing and standing after sitting actions (alternating between postures).

Therefore, while the main outcome of this study is related to the variation of the EE ($kcal \cdot min^{-1}$) between these conditions, metabolic and ventilatory parameters, resulting from the intervention, are considered as secondary outcomes.

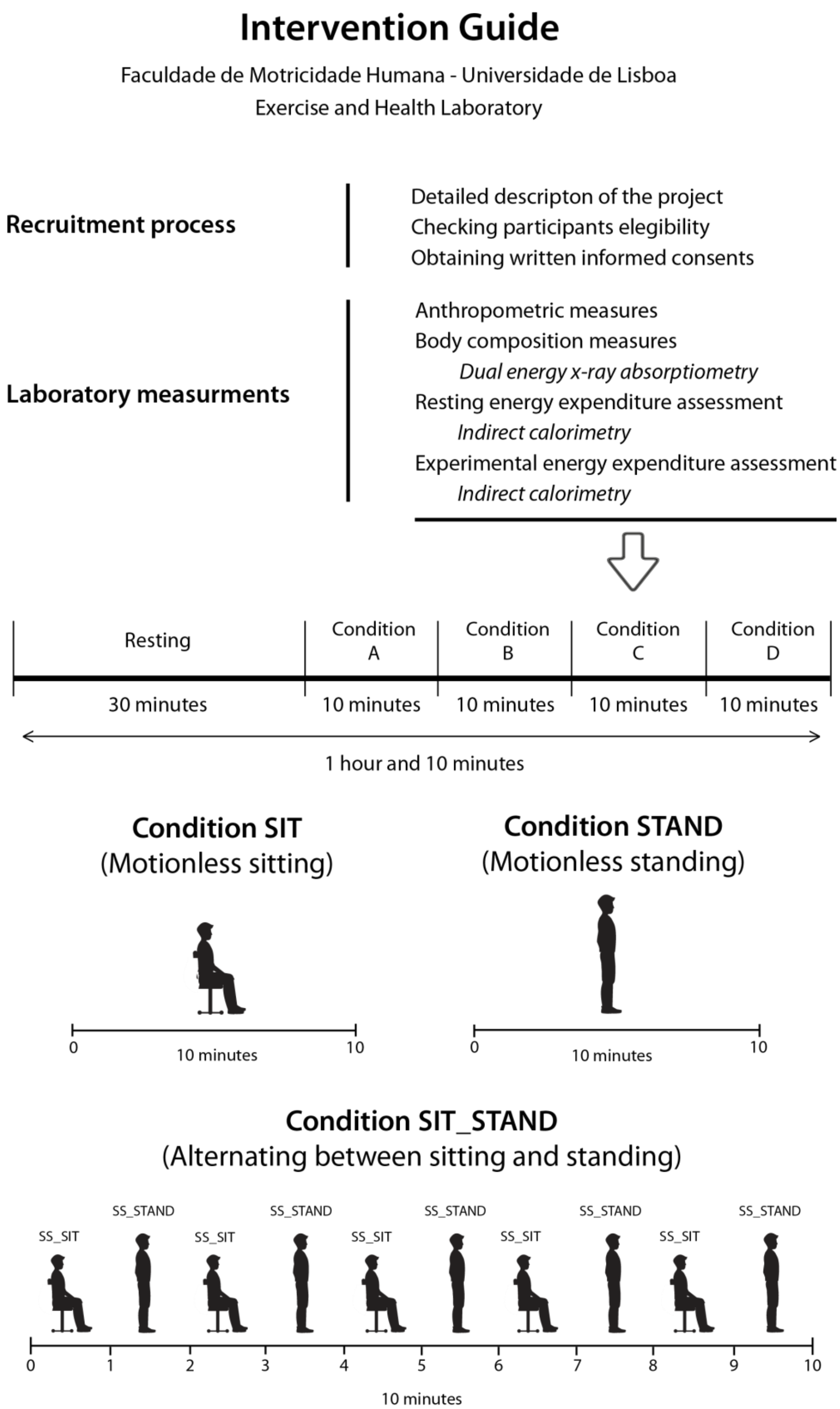

Figure 2 – Intervention Guide

2.1 Intervention Protocol

To assure similar baseline conditions, three days before the intervention, all participants were verbally instructed (via telephone call) to have minimum of 8 hours fast prior to their visit, not engage any structured MVPA in the last 24 hours and avoid consuming caffeine or other stimulants in the last 48 hours.

On the assessment day, after being confirmed the eligibility criteria of each participant for the study, an automated computer-generated randomization scheme (Excel, 2013) was used to determine the order in which each participant would perform the four experimental conditions. Thus, to determine the intervention sequence for each participant, the four experimental conditions were categorized as 1 – uninterrupted motionless sitting; 2 - uninterrupted motionless standing; 3 – breaking motionless sitting with brief bouts of standing (not included in our analysis); and 4 - alternating between motionless sitting and motionless standing. A total of 24 possible combinations per participant were generated.

After assigning an intervention sequence to the participant, the course of the intervention was remembered and the participant underwent a set of sequential laboratory assessments that involved anthropometric measures, imaging analysis of body composition through dual energy x-ray absorptiometry (DXA) and measure of REE using IC. Continuously after REE, each participant performed the four sequential conditions randomly ordered.

Prior and over the course of the intervention (in the last minute of each condition) the participant was remembered of their sequence. If the attributed sequence or following condition was forgotten, the participant was instructed to indicate it with a right-hand signal. During all the intervention, the research technician continuously supervised the participant to ensure an appropriate course of the assessments. Thus, a maximum of two participants were assessed each day.

2.2 Experimental Conditions

In the present trial, the designed intervention consisted in performing a set of four experimental conditions with 10 minutes length, randomly ordered and sequentially executed without any interruptions in between. The performance of all experimental conditions accounted for a minimum time of 40 minutes (4 x 10 minutes condition).

Regarding the procedures for each experimental condition explored in this study, they are followed described as: 1) uninterrupted motionless sitting (**SIT**), the participants were asked to remain in motionless upright sitting with hands on thighs during 10 minutes; 2) uninterrupted motionless standing (**STAND**), the participants were instructed to stand up motionless with arms resting alongside the body throughout 10 minutes; and 3) alternating between sitting with standing (**SIT_STAND**), the participants were instructed to continuously alternate between 1 minute of motionless upright sitting with hands on thighs (**SS_SIT**) with 1 minute of motionless standing with arms resting alongside the body (**SS_STAND**) over 10 minutes. In SIT_STAND condition, participants completed a total of 10 minutes of which 5 minutes were spent in SS_SIT and the other 5 minutes were spent in SS_STAND.

All instructions related to the experimental conditions, including selected sequence, continuous time tracking, correct body posture and other relevant details, were precisely reminded before the initiation of REE assessment.

3. Baseline assessments

3.1 Anthropometry

Participants were weighed barefoot to the nearest 0.1 kg wearing minimal clothes and height was measured to the nearest 0.1 cm on a digital scale with an integrated stadiometer (SECA-769 Hamburg, Germany), according to a standardized protocol (Lohman, Roche, & Martorell, 1988). BMI was calculated dividing weight (kg) by square of height (m).

3.2 Body Composition

Absolute (kg) and percentage values (%) of FM and FFM were estimated by DXA (Hologic Explorer-W, fan-beam densitometer software QDR for windows version 13.3, Waltham, Massachusetts, USA). This equipment measures the attenuation of X-rays pulsed between 70 and 140 kV synchronously with the line frequency for each pixel of the scanned image. According to the protocol for DXA described by the manufacturer, a step phantom with six fields of acrylic and aluminum of varying thickness and known absorptive properties was scanned to serve as an external standard for the analysis of different tissue components. Following the operator manual, the same experienced technician positioned the participants, performed the whole-body scan and executed the analysis, using a standard analysis protocol. Total lean soft tissue (LST) and appendicular lean soft tissue (ALST) were also calculated trough DXA. Based on test-retest using ten participants, the coefficients of variation (CV) in our laboratory for FM, FFM, LST and ALST were respectively, 1.7%, 0.8%, 0.8% and 1.2%.

3.3 Energy Expenditure Measures

3.3.1 Resting Energy Expenditure (REE) assessment

REE was measured in the morning, between 7:00 and 10:00 a.m., with the participants in a minimum of 8 hours fast. All measurements took place in a quiet laboratory room with an environmental temperature of approximately 22ºC and humidity between 40-50%. The MedGraphics CPX Ultima (MedGraphics Corporation, Breezeex Software) (MedU®) indirect calorimeter was used to measure breath-by-breath O_2 consumption (VO_2) and CO_2 production (VCO_2). Before testing, the O_2 and CO_2

analyzers were calibrated using a known gas concentration (16.7% O_2 and 5.7% CO_2). The flow and volume were measured using a pneumotachograph calibrated with a 3 L-syringe (Hans Rudolph, inc.TM).

All participants were instructed about all the following procedures and asked to relax, breathe normally, not to sleep or talk during the assessment. After connecting a pulse oximeter to the participant, to monitor HR minute-by-minute, the same technician conducted all measurements. Total rest duration last 60 minutes, with the participant lied and covered with a blanket. After the first 30 minutes, the calorimeter extension was attached to an adjusted facial mask and breath-by-breath VO_2 and VCO_2 were measured for another 30 minutes period. According to MedU® operator's manual, outputs of VO_2, VCO_2, RER and ventilation were collected and averaged over 1-minute interval for data analysis. The first and the last 5 minutes of data collection were discarded and the lowest mean of 5 minutes steady state, between the 5 and the 25 minutes of REE assessment with RER between 0.7 and 1.0, was used to determine REE. Steady state was defined as a 5 minutes period with ≤ 10 % CV for VO_2 and VCO_2 (Compher, Frankenfield, Keim, & Roth-Yousey, 2006). The mean VO_2 and VCO_2 of 5 minutes steady state were used in Weir's equation (Weir, 1949) and the period with the lowest EE was considered for analysis. The automatic gas calibration was performed between participants' evaluation. Based on test–retest using seven participants, the CV in our laboratory for REE was 4.0%.

3.3.2 Experimental Conditions Assessment

The same equipment that measured REE was used to determine the metabolic and energetic cost of the three 10-minutes experimental conditions. In SIT and STAND conditions the initial 5 minutes measured allowed the participant to reach a VO_2 steady state, however, to avoid a potential overestimation resulting from the condition previously performed, they were excluded from further analysis**.** Therefore, only the last 5 minutes of each condition were used to determine RER and mean VO_2. In SIT_STAND condition, the participant alternated between 1 minute in SS_SIT and 1 minute in SS_STAND, performing a total of 5 minutes in each posture. In this condition the first 4 minutes were used to reach a VO_2 steady state, being then rejected to avoid potential overestimation. Therefore, only the remaining 6 minutes of the SIT_STAND condition, 3 minutes SS_SIT and 3 minutes SS_STAND, were considered to determine RER and mean VO_2.

VO_2 was presented in millimeters of oxygen consumption per body mass per minute ($ml·kg^{-1}·min^{-1}$) and in millimeters of oxygen consumption per FFM per minute ($ml·kg^{-1}_{FFM}\ ·min^{-1}$). EE, presented in kilocalories per minute ($kcal·min^{-1}$), was determined with the use of the specific caloric equivalent (5 kcal) for a liter of O_2 consumed, considering the RER of each test and assuming non-protein metabolic mixture. This option was based on the assumption that approximately 4.82 kcal are released when a mixture of carbohydrates, lipids and proteins are oxidized (for 1 liter of O_2) and that the caloric value for O_2 remains stable (2% - 4% of variation), even in large variation in this metabolic mixture (William D. McArdle, 1981). Mean oxygen consumption per condition ($ml\ min^{-1}$) was divided by oxygen consumption during REE assessment ($ml\ min^{-1}$) to estimate relative METs. Absolute METs were estimated by dividing VO_2 ($ml·kg^{-1}·min^{-1}$) by 3.5 $ml·kg^{-1}·min^{-1}$. Additionally, the percentages above resting and sitting were calculated for VO_2 variables.

4. Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 25.0, 2019 (SPSS Inc., New York, NY) for windows. Descriptive statistics, including means and standard deviations, were calculated for demographic, body composition, metabolic and energetic parameters.

Normality was confirmed using Kolmogorov–Smirnov test. A repeated measure ANCOVA with post hoc analysis (Bonferroni) was used to compare the differences between all experimental conditions (SIT, STAND, SS_SIT, SS_STAND), considering age as a covariate and the randomly assigned order as a between-subject effect. Mauchly's statistical test was used to test the assumption of sphericity. If the test was non-significant (p-value ≥ 0.05) the F-statistic ratios suggested by SPSS would be considered. If the test was significant (p-value < 0.05), no homogeneity of variances was assumed, and adjustment with Greenhouse and Geisser's test ($\varepsilon < 0.75$) or Huynh-Feldt's test ($\varepsilon \geq 0.75$) was considered. Statistical significance was set at p-value < 0.05.

Results

Forty-eight healthy males (N = 25) and female (N = 23) participants with a mean age of 32.5 ± 11.4 years and 37.4 ± 16.1 years respectively, completed the study. Mean BMI was 25.6 ± 3.19 $kg \cdot m^{-2}$ for males (48% overweight) and 24.6 ± 5.1 $kg \cdot m^{-2}$ for females (30% overweight). There were no interactions for sex among the changes in metabolic and energetic variables and HR between all conditions ($p \geq 0.05$), However, as some differences in body composition profiles were found, means, standard deviation (SD), maximum and minimum values of participants characteristics are presented separately by sex in Table 1.

Table 1 – Baseline demographic, body composition, metabolic and energetic characteristics of the participants.

	Males (N = 25)		Females (N = 23)	
	Mean ± SD	Min - Max	Mean ± SD	Min - Max
Age (years)	32.5 ± 11.4	20 – 64	37.4 ± 16.1	20 - 64
Weight (kg)	79.1 ± 11.6	65.7 – 108.3	63.2 ± 12.1	47.6 – 97.2
Height (cm)	175.7 ± 5.0	166.7 – 184.5	160.6 ± 7.1	148.3 – 171.5
BMI ($kg \cdot m^{-2}$)	25.6 ± 3.19	21.1 – 32.2	24.6 ± 5.1	19.1 – 41.0
FM (kg)	16.5 ± 7.37	7.59 – 35.19	21.3 ± 8.52	11.76 – 42.19
FM (%)	20.7 ± 7.09	11.4 – 33.1	33.5 ± 8.19	20.9 – 48.3
FFM (kg)	61.5 ± 7.41	45.81 – 74.63	40.8 ± 5.93	29.50 – 52.73
ALST (kg)	28.39 ± 3.93	20.55 – 35.04	17.48 ± 3.15	11.83 – 26.40
REE ($ml \cdot kg^{-1} \cdot min^{-1}$)	2.73 ± 0.52	2.03 – 4.24	2.62 ± 0.33	2.04 – 3.13
REE ($kcal \cdot day^{-1}$)	1476 ± 246	902 - 1820	1173 ± 166	966 - 1580
RQ	0.91 ± 0.08	0.70 – 1.06	0.89 ± 0.07	0.74 – 0.99
HR	56.8 ± 9.5	42.8 – 85.0	63.5 ± 9.1	51.0 – 84.0

ALST, appendicular lean soft tissue; BMI, body mass index; FM, fat mass; FFM, fat-free mass; HR, heart rate; Max, maximum; Min, minimum; REE, resting energy expenditure; RQ, respiratory quotient; SD, standard deviation

Mean values for VO_2 ($ml \cdot kg^{-1}_{FFM} \cdot min^{-1}$, $ml \cdot kg^{-1} \cdot min^{-1}$, % above REE, % above SIT) (Figure 3), EE ($kcal \cdot min^{-1}$, Absolute MET’s, Relative MET’s) (Figure 4), RQ, HR (Figure 5) and ANOVA differences for all conditions are presented in Table 2.

In women, VO_2, EE and MET values, significant differences were found between all experimental conditions (p-value < 0.001). However, for % above SIT no significant differences were detected between STAND and SS_SIT (p-value ≥ 0.05). In men, significant differences between all conditions (p-value < 0.001), except between STAND and SS_SIT (p-value ≥ 0.05) were found in all VO_2, EE and MET parameters.

For HR, significant changes were only found between SIT and the other conditions (p-value < 0.001), in both men and women. Non-significant changes in HR were detected between STAND, SS_SIT and SS_STAND (p-value ≥ 0.05). Moreover, across both sexes, RQ did not differed significantly between all conditions (p-value ≥ 0.05). For metabolic and energetic variables, the differences between conditions did not changed after considering the interaction effect of the randomly assigned order.

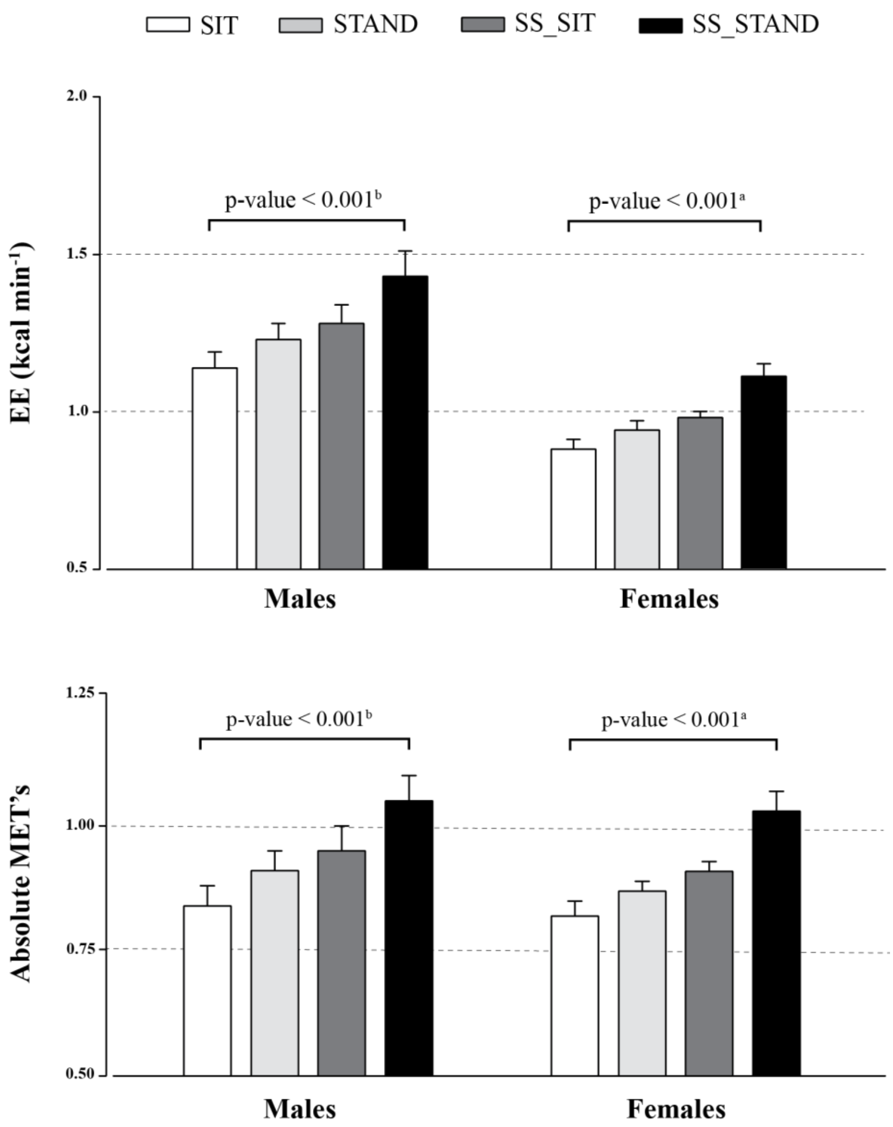

Figure 3 – Energy expenditure (EE) (kcal·min^{-1}) and Absolute MET's for SIT, STAND and both sub-components of the intermittent condition, only considering the randomly order of conditions as a between subject effect. *Bars* represent the mean and standard deviations values in both men and women. [a] Significant differences between all conditions (p-value < 0.001); [b] Significant differences between all experimental conditions (p-value < 0.001), except between STAND and SS_SIT (p-value ≥ 0.05)

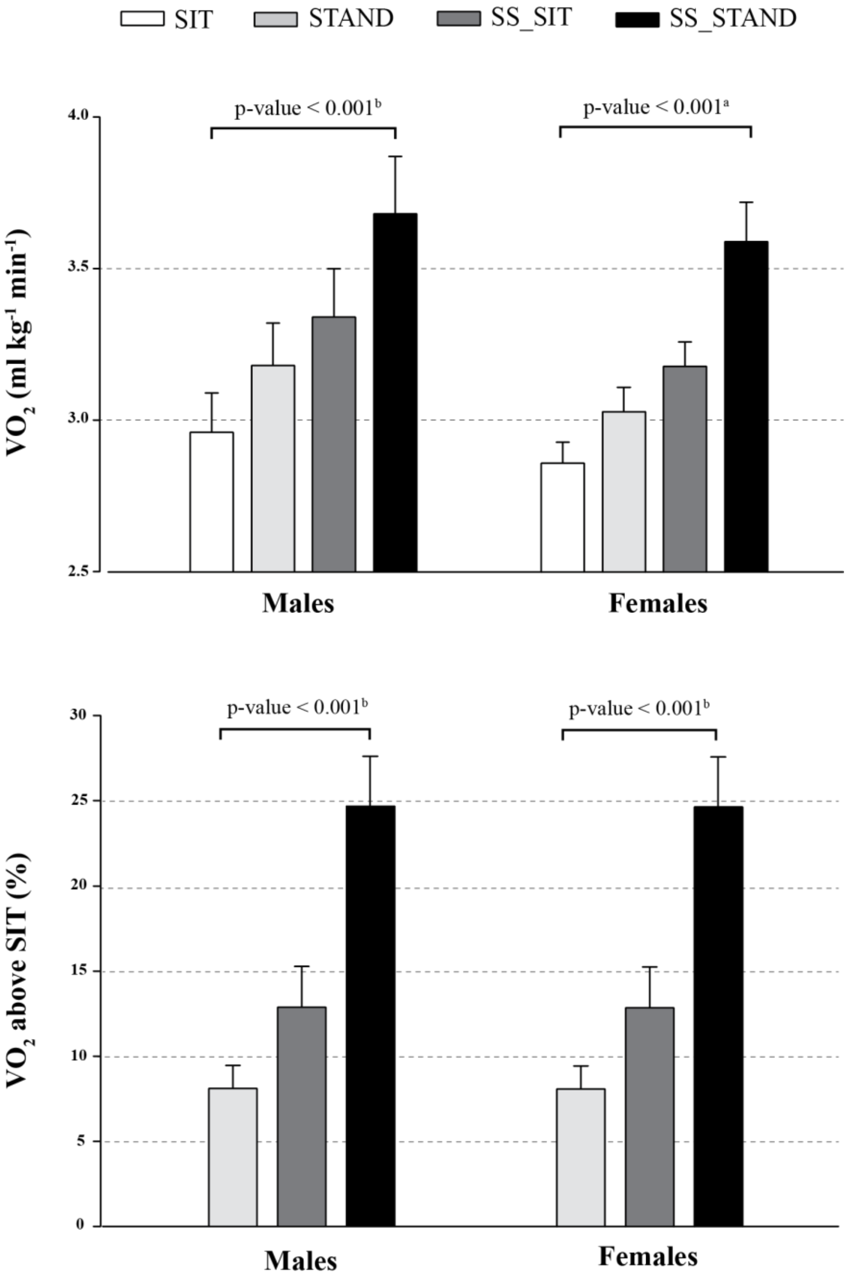

Figure 4 – VO2 ml·kg−1·min−1 and VO^2 above SIT (%), for SIT, STAND and both sub-components of the intermittent condition, only considering the randomly order of conditions as a between subject effect. *Bars* represent the mean and standard deviations values in both men and women. [a] Significant differences between all conditions (p-value < 0.001); [b] Significant differences between all experimental conditions (p-value < 0.001), except between STAND and SS_SIT (p-value ≥ 0.05).

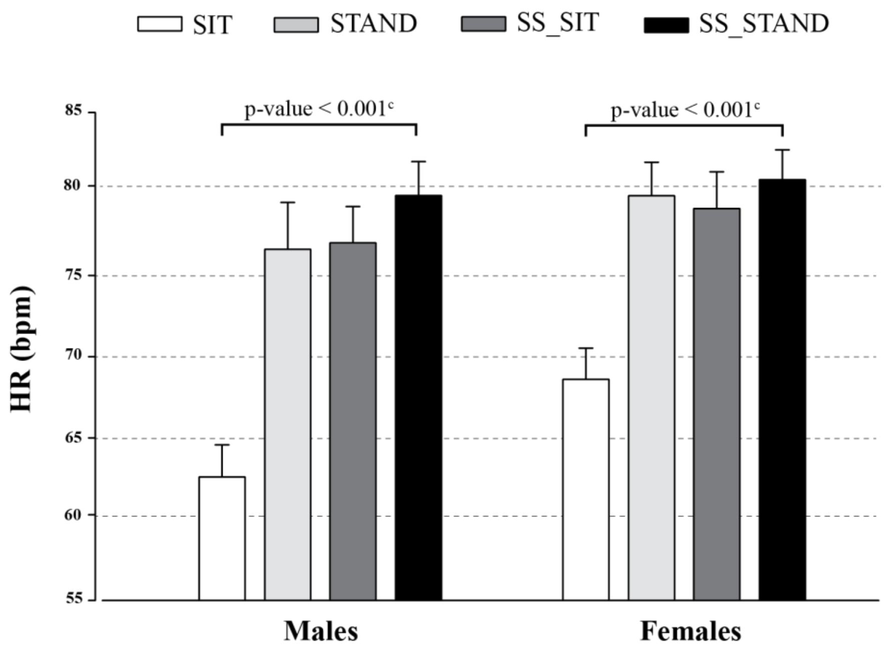

Figure 5 – Heart rate (HR) (bpm) for SIT, STAND and both sub-components of the intermittent condition, only considering the randomly order of conditions as a between subject effect. Bars represent the mean and standard deviations values in both men and women. [c] Significant differences only between SIT and the other experimental conditions (p-value < 0.001).

After further adjustment for age, most of the ANCOVA differences changed for the main variables (Table 3). All metabolic and energetic variables, except to HR, became non-significant for all conditions (p-value ≥ 0.05), in women. In men, although there was a similar trend for main variables (p-value ≥ 0.05), for VO_2 and Absolute MET's, the differences remained identical to those without adjustment (p-value < 0.001). HR differences have not changed significantly after adjustment for age, for both men and women. All the differences persisted after considering the interaction effect of the randomly assigned order.

Table 2 - Differences in metabolic and energetic parameters for all experimental conditions (SIT, STAND, SS_SIT and SS_STAND), in both men and women. 95% confidence interval.

	SIT	**STAND**	**SS_SIT**	**SS_STAND**	**p-value**
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Males (n = 25)					
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	2.96 ± 0.13	3.18 ± 0.14	3.34 ± 0.16	3.68 ± 0.19	< 0.001[b]
VO_2 ($ml \cdot kg^{-1}_{FFM} \cdot min^{-1}$)	3.78 ± 1.17	4.08 ± 0.19	4.26 ± 0.20	4.71 ± 0.25	< 0.001[b]
VO_2 % above REE	9.01 ± 1.51	17.62 ± 1.82	23.08 ± 2.86	35.68 ± 4.52	< 0.001[b]
VO_2 % above SIT		8.10 ± 1.35	12.88 ± 2.39	24.68 ± 3.92	< 0.001[b]
EE ($kcal \cdot min^{-1}$)	1.14 ± 0.05	1.23 ± 0.05	1.28 ± 0.06	1.43 ± 0.08	< 0.001[b]
EE (Absolute MET's)	0.84 ± 0.04	0.91 ± 0.04	0.95 ± 0.05	1.05 ± 0.05	< 0.001[b]
EE (Relative MET's)	1.09 ± 0.02	1.18 ± 0.02	1.23 ± 0.03	1.36 ± 0.05	< 0.001[b]
RQ	0.95 ± 0.02	0.96 ± 0.02	0.97 ± 0.02	0.94 ± 0.02	0.106
HR	62.7 ± 2.12	76.7 ± 3.29	77.1 ± 2.46	77.0 ± 2.34	< 0.001[c]
Females (n = 23)					
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	2.86 ± 0.07	3.03 ± 0.08	3.18 ± 0.08	3.59 ± 0.13	< 0.001[a]
VO_2 ($ml \cdot kg^{-1}_{FFM} \cdot min^{-1}$)	4.37 ± 0.13	4.62 ± 1.13	4.87 ± 0.15	5.49 ± 0.22	< 0.001[a]
VO_2 % above REE	7.95 ± 1.80	14.08 ± 1.92	20.25 ± 2.53	35.80 ± 4.05	< 0.001[a]
VO_2 % above SIT		5.79 ± 1.02	11.46 ± 1.41	24.89 ± 2.63	< 0.001[b]
EE ($kcal \cdot min^{-1}$)	0.88 ± 0.03	0.94 ± 0.03	0.98 ± 0.02	1.11 ± 0.04	< 0.001[a]
EE (Absolute MET's)	0.82 ± 0.02	0.87 ± 0.02	0.91 ± 0.02	1.03 ± 0.04	< 0.001[a]
EE (Relative MET's)	1.09 ± 0.02	1.15 ± 0.02	1.21 ± 0.03	1.36 ± 0.04	< 0.001[a]
RQ	0.99 ± 0.02	1.00 ± 0.03	0.97 ± 0.02	1.02 ± 0.11	0.900
HR	68.6 ± 1.86	80.5 ± 2.32	79.4 ± 2.27	81.4 ± 2.08	< 0.001[c]

EE, energy expenditure; FFM, fat-free mass; HR, heart rate; REE, resting energy expenditure; RQ, respiratory quotient; SD, standard deviation; Kcal, kilocalories; VO_2, oxygen consumption

[a] Significant differences between all conditions (p-value < 0.001).
[b] Significant differences between all conditions (p-value < 0.001), except between STAND and SS_SIT (p-value ≥ 0.05).
[c] Significant differences only between SIT and the other three conditions (p-value < 0.001).

Table 3 – Differences in metabolic and energetic parameters for all experimental conditions (SIT, STAND, SS_SIT and SS_STAND), with adjustment for age, in both men and women. 95% confidence interval.

	SIT	STAND	SS_SIT	SS_STAND	p-value
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Males (n = 25)					
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	2.95 ± 0.13	3.18 ± 0.14	3.33 ± 0.15	3.67 ± 0.18	0.018[b]
VO_2 ($ml \cdot kg^{-1}{}_{FFM} \cdot min^{-1}$)	3.78 ± 0.17	4.08 ± 0.19	4.26 ± 0.21	4.71 ± 0.26	0.070
VO_2 % above REE	9.01 ± 1.55	17.63 ± 1.86	23.06 ± 2.91	35.68 ± 4.64	0.071
VO_2 % above SIT		8.11 ± 1.35	12.87 ± 2.43	24.69 ± 4.02	0.129
EE ($kcal \cdot min^{-1}$)	1.14 ± 0.05	1.23 ± 0.06	1.28 ± 0.06	1.43 ± 0.08	0.105
EE (Absolute MET's)	0.84 ± 0.04	0.91 ± 0.04	0.95 ± 0.04	1.05 ± 0.05	0.018[b]
EE (Relative MET's)	1.09 ± 0.02	1.18 ± 0.02	1.23 ± 0.03	1.36 ± 0.05	0.104
RQ	0.95 ± 0.02	0.96 ± 0.02	0.97 ± 0.02	0.94 ± 0.02	0.522
HR	62.6 ± 1.96	76.6 ± 2.88	77.0 ± 2.21	79.9 ± 2.09	< 0.001[c]
Females (n = 23)					
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	2.88 ± 0.07	3.03 ± 0.09	3.18 ± 0.09	3.60 ± 0.14	0.090
VO_2 ($ml \cdot kg^{-1}{}_{FFM} \cdot min^{-1}$)	4.41 ± 0.10	4.66 ± 0.11	4.92 ± 0.11	5.55 ± 0.18	0.228
VO_2 % above REE	8.17 ± 1.80	14.19 ± 1.97	25.58 ± 2.51	36.50 ± 3.86	0.079
VO_2 % above SIT		5.69 ± 1.03	11.54 ± 1.45	25.31 ± 2.54	0.417
EE ($kcal \cdot min^{-1}$)	0.89 ± 0.02	0.94 ± 0.03	0.99 ± 0.02	1.11 ± 0.04	0.094
EE (Absolute MET's)	0.82 ± 0.02	0.87 ± 0.03	0.91 ± 0.03	1.03 ± 0.04	0.090
EE (Relative MET's)	1.09 ± 0.02	1.15 ± 0.02	1.21 ± 0.03	1.37 ± 0.04	0.071
RQ	0.98 ± 0.02	1.00 ± 0.03	0.97 ± 0.02	1.03 ± 0.12	0.425
HR	68.6 ± 1.93	79.9 ± 2.06	79.1 ± 2.26	80.9 ± 1.82	< 0.001[c]

EE, energy expenditure; FFM, fat-free mass, HR, heart rate; REE, resting energy expenditure; RQ, respiratory quotient; SD, standard deviation; Kcal, kilocalories; VO_2, oxygen consumption

[b] Significant differences between all conditions (p-value < 0.001), except between STAND and SS_SIT (p-value ≥ 0.05).
[c] Significant differences only between SIT and the other three conditions (p-value < 0.001).
Adjustment for covariate: age

Discussion

Although most of the current evidence is derived from experimental studies that aimed to replace SB with standing or other active pursuits (e.g. MVPA), to the best of our knowledge, no study to date has yet determined whether the energetic cost of one specific posture (e.g. sitting or standing), may be influenced by the posture previously executed. In addition, as interrupting SB with frequent and short bouts of standing may slightly increase daily EE (Miles-Chan et al., 2013), we seek to understand in more detail the metabolic and energetic response of 4 experimental conditions, including two standard conditions (SIT and STAND) and two sub-conditions (SS_SIT and SS_STAND), derived from an intermittent condition (SIT_STAND).

In the present study there was a similar distribution on participants sex and age, however, there were some differences regarding metabolic, energetic and body composition variables. Although there is a clear indication that, for both men and women, the metabolic and energetic cost of STAND is considerably higher than SIT, the same is not true when comparing SIT and STAND with the two sub-components of the intermittent condition (SS_SIT and SS_STAND). In fact, we found that women had significant differences between all experimental conditions for almost all variables analyzed (p-value < 0.001). However, in men, although there was a similar trend that in women, no significant differences were found between two conditions (STAND and SS_SIT) for a large set of variables (p-value ≥ 0.05).

Although these findings are quite suggestive about a positive metabolic and energetic influence of interrupting sitting with short standing bouts, throughout the discussion we will analyze in greater detail the magnitude of this relationship, as well as identify which are the variables most sensitive to this intervention. Furthermore, we will compare our findings with the existing evidence and present potential explanatory mechanisms that may justify these findings.

In this perspective, the present results suggest that the energetic differences between continuous motionless sitting (SIT) (1.14 ± 0.05 kcal·min^{-1} for men; 0.88 ± 0.03 kcal·min^{-1} for women) and motionless standing (STAND) (1.23 ± 0.05 kcal·min^{-1} for men; 0.94 ± 0.03 kcal·min^{-1} for women) are significant (p-value < 0.001), but relatively modest when compared to findings derived from other experimental trials that analyzed this difference (Saeidifard et al., 2018). Considering the mean differences between sitting and standing, above REE levels (1476 for men; 1173 for women), an increase of 22 kcal (men) and 19 kcal (women) per day would be expected, if the individual replaced at least half of his sitting working time (8 hours) with standing. Correspondingly, for a 5-day working week an additional 110 kcal (men) and 95 kcal (women) would be expended.

Although energetically different, our results seem to be comparable to previous findings that reported small energetic changes (Miles-Chan et al., 2013; Monnard & Miles-Chan, 2017; Pulsford et al., 2017). Thus, one possible explanation for the large difference between studies may be attributed to the lack of control over the individual's spontaneous movement, in particular fidgeting-like movement. In fact, the energetic cost of such movements, can considerably increase 20% to 40% over resting levels (Ferro-Luzzi, Scaccini, Taffese, Aberra, & Demeke, 1990). Therefore, the contamination of the exposure to these movements could have influenced the accuracy of the determined difference between both sitting and standing postures (Saeidifard et al., 2018).

We further explored the intermittent condition where the individual was asked to alternate between motionless sitting and motionless standing (each minute over 10 minutes), dividing it into two distinct moments, sitting after standing (SS_SIT condition) and standing after sitting (SS_STAND condition). Interestingly, our results suggested that, in men, for SS_SIT condition (1.28 ± 0.06 kcal·min^{-1}) EE levels were significantly higher (~12%) than continuous sitting condition (p-value < 0.001). However, although the EE was modestly higher in SS_SIT (~4%), compared to the continuous standing condition, no significant differences were found (p-value ≥ 0.05). In women, EE levels were ~11% and ~4% higher in SS_SIT condition (0.98 ± 0.02 kcal·min^{-1}; p-value < 0.001) than in continuous sitting and standing conditions, respectively. These findings suggest that, for both men and women, the nature of sitting (sitting continuously vs. sitting after standing) seems to significantly influence the magnitude of EE.

Curiously, this trend was also verified for the standing posture, were we found that, in men, the EE levels were significantly higher in the SS_STAND condition (1.43 ± 0.08 kcal·min^{-1}, p-value < 0.001) compared with continuous sitting (~25%), continuous standing (~16%) and sitting immediately after standing (~12%). With similar differences, we found that, in women, the EE related to SS_STAND condition (1.11 ± 0.04 kcal·min^{-1}, p-value < 0.001) was ~26%, ~18% and ~13% higher than continuous sitting, continuous standing and sitting immediately after standing, respectively. However, after further adjustment of these results for age (covariate), no significant changes were detected between all experimental conditions (p-value > 0.05). This fact may be partly explained by a non-proportional distribution of participant's age verified in a secondary analysis of our study (data not shown).

Nevertheless, these results represent a novel finding, since they indicate that the energetic cost of a specific posture (sitting or standing) can be further influenced by a set of variables, including the posture previously conducted. In this sense, if we replace, every half hour, at least 5 minutes of sitting with standing, this change would represent an additional 23 kcal (men) and 18 kcal (women) expended for 8 working hours, compared to continuous sitting. Consequently, at the end of a 5-day working week this increase would reach 116 kcal (men) and 92 kcal (women), which in turn, may yield an increase of nearly 510 kcal (men) and 405 (women) per month (22 working days). Interestingly, these findings suggest that the energetic benefit of alternating sitting and standing was slightly lower than Thorp et al. (Thorp et al., 2016) previously stated. These authors extrapolated their findings, reporting that alternating between 30 minutes bouts of sitting and standing would translate an increase of 40 kcal per work day (Thorp et al., 2016). However, considering that the IC analysis in this study (Thorp et al., 2016) was performed while individuals were working, it is expected that these findings may be slightly overestimated, due the potential contamination of unexpected factors (e.g. fidgeting-like movements and brain function while working). In this perspective, we believe that the magnitude of our results was similar to the findings previously reported (Thorp et al., 2016), with the particularity that, for the same daily working time (8 hours), in our analysis individuals spend three times less time in a standing posture (80 minutes vs. 240 minutes).

Theoretically, both sub-conditions derived from our intermittent condition (SIT_STAND) are characterized by having two distinct phases, where the former is related the moment of transition between postures, and the later concerns to the moment where the individual remains motionless in the desired posture, until it is time to make another transition. This observation led us to speculate about a distinct contribution that both phases may have throughout the corresponding sub-condition. Regarding the first moment, transition between postures, Júdice et al. (Judice et al., 2016) aimed to investigate this issue, by determining the metabolic and energetic cost of a single sit-to-stand and immediate stand-to sit transition. By examining the experimental condition not included in our analysis, this study reported that the EE related to this action was approximately 0.32 kcal, which represented an EE 35% and 28% above continuous sitting and standing, respectively (Judice et al., 2016).

In this sense, a likely explanation that justifies this significant increase in EE may be related to the singular muscular implications that this action (transition between postures) may have. Thus, based on the assumption that the EE of a specific activity is determined by the volume of contracting muscle mass (Hamilton et al., 2007; Tikkanen et al., 2013) and that the most of total-body muscle mass (74%) is located in the extremities, particularly in the lower limbs (Kim, Wang, Heymsfield, Baumgartner, & Gallagher, 2002; Shih, Wang, Heo, Wang, & Heymsfield, 2000), we expect that the substantial increase in EE over the three conditions may be largely attributed to the muscle mass activated during each condition.

In this perspective, Tikkanen et al. (Tikkanen et al., 2013) found that the muscle mass activity (quadriceps and hamstrings muscles) during standing was approximately 2.5 times greater than during sitting. Moreover, shifting from sitting to standing posture, can also have a specific muscular activation that is particularly high during the seat-off action (Roebroeck, Doorenbosch, Harlaar, Jacobs, & Lankhorst, 1994). This increase may be broadly explained by the concentrically contraction of quadriceps, that reach at least 50-80% of the required activity during maximal contractions, and also, the moderate co-contraction of hamstrings, which in turn, contribute to sustain the hip extension (Roebroeck et al., 1994).

Relatively to the later phase of both sub-conditions, where the individual was asked to remain motionless, it is expected that the energetic response during this moment is mainly explained by the acute increase in EE during the transition phase. In 2013, Miles-Chan et al. (Miles-Chan et al., 2013) aimed to determine the energetic response immediately after taking half of a complete transition (sit-to-stand transition). The authors reported that over the course of 10 minutes in continuous standing (after a sit-to-stand transition), a significant increase in EE (7.7%) was found within the first 5 minutes, compared to the previous continuous sitting condition (Miles-Chan et al., 2013). However, although there was a modest increase in EE (3.8%) in the second 5 minutes of continuous standing, compared to continuous sitting, these differences were not significant (Miles-Chan et al., 2013). In fact, this progressive decrease in EE over time may be explained by the mechanism of excess post-exercise oxygen consumption (EPOC), which suggests that, after performing a specific activity, the levels of oxygen consumption does not return to resting levels immediately, remaining relatively high during some period of time (depending on the type, intensity and duration of the activity) (Borsheim & Bahr, 2003).

If we transfer this logic to our analysis, it is expected that the isolate transition from sit-to-stand or from stand-to-sit may have a direct influence on the EE accumulated over the remaining time on the corresponding posture. Moreover, considering that, in the intermittent condition, the alternation between postures occurred at the end of each minute, it is possible that the recovery time to return to EE baseline levels, before performing another transition, was insufficient. Although this effect was not quantified in our analysis, it may indicate that the shorter the time between postural transitions (sitting and standing), the greater the potential cumulative effect of EE over time.

Furthermore, this variation in EE was not accompanied by significant changes in RQ and HR. For instance, we found that HR related to continuous sitting (63 bpm in men; 69 in women) differed significantly from continuous standing (77 bpm in men; 80 bpm in women), sitting after standing (77 bpm in men; 79 bpm in women) and standing after sitting (80 bpm in men; 81 bpm in women) (p-value < 0.001). Neither the randomly assigned order (interaction within-subjects) or the age (covariate) influenced the results (p-value ≥ 0.05). These findings are in line with previous studies that reported similar differences in HR between continuous sitting (64 to 70 bpm) and continuous standing (78 to 83 bpm) (Carter et al., 2015; Miles-Chan et al., 2013).

However, although there was a slight difference in HR between continuous standing condition and both sub-conditions of the intermittent condition, no significant changes were detected (p-value ≥ 0.05). One possible explanation to this fact is that the intensity stimulus may have been insufficient to activate the sympathoadrenal system (Borsheim & Bahr, 2003). Theoretically, after initiating a PA bout, the sympathoadrenal activity increases their activity by releasing catecholamines that influence several physiological parameters, such as heart chronotropy (HR) and inotropy (Borsheim & Bahr, 2003). However, if the external load of the activity is reduced, both the activation of this system and the subsequent EPOC effect will be limited (Borsheim & Bahr, 2003).

In a similar perspective, although there were some variations in RQ between continuous sitting (0.95 in men; 0.99 in women), continuous standing (0.96 in men; 1.00 women), sitting after standing (0.97 in men; 0.97 in women) and standing after sitting (0.94 in men; 1.02 in women), no significant differences were found between all conditions (p-value ≥ 0.05). These unexpected findings differed from the results of a previous study (Miles-Chan et al., 2013), that reported a modest but significant decrease in RQ immediately after moving from sitting (0.83) to standing (0.81), which may have represented a shift in favor of fat oxidation.

It would be expected that for a low-intensity activity, such as standing, RQ would range between 0.80 and 0.88 suggesting a dominant fat oxidation (William D. McArdle, 1981). However, the RQ measured our study was constantly higher, corresponding to high intensity activity (0.9 to 1.0), where the primary fuel is carbohydrates (William D. McArdle, 1981). Considering that we ensured similar baseline conditions for all participants (avoid PA in the last 24h and fasting at least 8h before the assessments), these findings are quite intriguing. In addition, it is unlikely that these differences were due an increased individual stress response, mainly because no significant raises in continuously measured HR were detected throughout our intervention, and besides that, we soften the potential stress effect by asking all the individuals to feel comfortable and, if necessary, to feel free to interrupt the intervention at any time.

In this sense, we believe that these divergent results may be due to the different types of IC devices used in the aforementioned studies (William D. McArdle, 1981; Miles-Chan et al., 2013). In fact, in our study we used MedU® to measure the metabolic and energetic cost of REE and across all experimental condition. However, some recent studies that compared the validity and reliability of different gas analysis systems, reported significant differences in both metabolic and energetic parameters, compared to the Deltatrac II® (DLTII®) known as the IC reference device. In 2009, Cooper et al. (Cooper et al., 2009) found that the metabolic and energetic response measured by MedU® device was significantly different (overestimation) from that measured by the reference device (DLTII®). Moreover, the authors reported that the reliability assessment for EE showed that MedU® had a significantly higher CV (10.9%), compared to DLTII® (3.0%) (Cooper et al., 2009). In line with these findings, Black et al. (Black, Grocott, & Singer, 2015) reported that, although the systematic error between MedU® and DLTII® for measurements of the metabolic activity was acceptable, the margins of agreement were wide. Therefore, given the less precision of MedU®, the authors suggested that for either research and clinical purposes, DLTII® should preferably be used (Black et al., 2015).

It is also important to note that all EE assessments, using an open-circuit indirect calorimeter (MedU®), were performed in a small laboratory room. Therefore, we speculate that over the assessment time, the proportion of air gases may have changed, contributing to slightly overestimate our findings.

It is important to acknowledge that our findings highlighted the significant contribution that alternating between short bouts of sitting and standing has on several energetic variables. However, given the newness that this intervention represents for the scientific community, some doubts remain about its medium to long-term metabolic impact. It is widely accepted that different exercises will have distinct effects on cellular and molecular regulatory mechanisms in different human body systems, especially in skeletal muscles and cardiovascular systems (Hamilton et al., 2007). Thus, in inactive or sedentary people, the deterioration process of these systems appears to be particularly accelerated, leading to the development of a set of unfavorable health conditions.

In this sense, much scientific interest has been emerging about the metabolic effect of interrupting prolonged SB time with short bouts of activity (Bey et al., 2003; D. W. Dunstan et al., 2012; Duvivier et al., 2013; Henson et al., 2013; Latouche et al., 2013; Peddie et al., 2013). Thus, while some authors examined the regulation in gene expression induced by breaking up prolonged sitting periods with brief bouts of PA (Bey et al., 2003; Hamilton, Hamilton, & Zderic, 2004; Latouche et al., 2013; Levine, 2004), others concerned about the overall effect of PA on specific metabolic markers of heath (D. W. Dunstan et al., 2012; Duvivier et al., 2013; Henson et al., 2013; Peddie et al., 2013).

However, due to some limitations related to with the integration of these PA bouts in specific daily-living environments (e.g. workplace) (MacEwen et al., 2015), scientific research became interested in the potential health effect that interrupting prolonged periods of sitting with intermittent light-intensity activity, such as standing, may have. As previously mentioned, Thorp et al. (Thorp et al., 2014) suggested that alternating between sitting and standing every 30 minutes could beneficially attenuate the effect of glucose responses. These findings were further supported by two other studies (Benatti et al., 2017; J. P. Buckley et al., 2014) that reported significant decreases in the cumulative postprandial glucose response when prolonged sitting was briefly interrupted with standing bouts. Conversely, some studies suggested that interrupting prolonged sitting with standing may be insufficient stimulus to enhance the cardiometabolic health, therefore, as alternative, brief bouts of LIPA should be implemented (Bailey & Locke, 2015; Pulsford et al., 2017).

Based on the potential effect that these interventions may have on several health outcomes, some recent recommendations have been established. In 2015, Buckley et al. (John P Buckley et al., 2015) proposed a set of recommendations which was supported by the existing evidence related to the reduction of SB during work. Based on their findings, the derived guidance suggested that workers, in which their occupation is predominantly sedentary, should accumulate at least 2 hours/day of standing and LIPA during working hours (John P Buckley et al., 2015). According to this guidance, a recent study (Ku et al., 2018) suggested that interrupting prolonged periods of SB as often as possible provides an opportunity to minimize the amount of time spent in this behavior. On the basis of our findings, that suggest an increased energetic response resulting from altering postures, the previous recommendations might best be if short bouts of sitting and standing are frequently interrupted.

1. Methodological Strengths and Limitations

1.1 Strengths

One of the main strengths of this experimental trial is related to the novelty that this study represents for the scientific community. In fact, to the best of our knowledge, no studies have previously determined whether the metabolic and energetic cost of one specific posture (e.g. sitting or standing) was influenced by the posture previously executed. Therefore, the present study adds relevant findings to the field by helping to elucidate the metabolic and energetic impact of alternative strategies, such as alternating between short bouts of sitting and standing.

Due to the high intra and inter-variability in the energetic response derived from fidgeting-like movements (Levine, 2004), all individuals were instructed to remain as motionless as possible during the intervention, only interrupting this behavior when needed. Regarding the current evidence, until to the present this approach has been quite underexplored, however, it may represent an opportunity to better understand more about the specific metabolic and energetic cost of each posture and how it changes in an intermittent condition. Thus, to avoid possible contamination of unexpected factors in our analysis (e.g. fidgeting-like movements or DIT), all measurements were conducted under restricted and controlled laboratory environment. However, if we extrapolate our findings for free-living settings, we expect that the metabolic and energetic cost of these conditions would be substantially higher.

Moreover, to accurately measure the energetic cost of each condition, besides randomizing the order in which the participants would perform the experimental conditions, we assumed that for each 10 minutes of EE assessment (SIT and STAND conditions), the first 5 minutes should be discarded, while the remaining 5 minutes having a CV < 10% should be considered (Compher et al., 2006). Finally, given the great contribution that body composition has on energy balance, we used valid objective method (DXA) to examine several body composition parameters.

1.2 Limitations

It is also important to address the current limitations of this study. Firstly, although we initially calculated the overall sample size needed (n = 50), we decided to ignore this assumption, by examining our findings separately by sex. However, considering the sample included in our study (25 men and 23 women), in a further analysis we calculated the current statistical power for the main variables and considering different conditions, which ranged constantly between 0.90 and 1.00.

Of the remaining sample, while men presented a balanced distribution according to their BMI (50% normal and 50% overweight/obese), the majority of women was considered as normal, regarding the weight category. In addition, age seemed to significantly influence the differences in metabolic and energetic variables between conditions, this fact may be in part explained by a non-proportional distribution of participant's age (data not shown).

Another limitation of this work is that our results can be only generalized to a adult population deprived of diagnosed diseases. Moreover, although performing fidgeting-like movements while seated or standing are considered significant contributors to increase TDEE, due to the restricted postures of our experimental conditions, the generalization of our findings may be limited. Furthermore, the fasting condition may also represent a potential limitation of generalization due to the difficulty to isolate thermic effect of feeding in and to the fact that individuals are not typically fasting throughout their day.

All individuals included in our analysis performed a 30 minutes REE assessment followed by a sequence of four experimental conditions with 10 minutes length, which implied the use of a mask attached to the face, continuously during 70 min. Therefore, it should be noted that this prolonged period of assessment could possibly have generated discomfort and stress, which in turn, resulted in an increased metabolic and energetic response, particularly at the end of our intervention. Based on this assumption, we consider that the 10 minutes duration of each condition can be considered itself as a limitation.

Finally, all inclusion criteria of the study were only verbally instructed between the researcher and the participant during the recruitment process. Considering that no questionnaires were applied to ascertain the individuals meeting the established criteria, this may have led to the inclusion of individuals with unwanted conditions. Moreover, despite of the recommendation to avoid MVPA practice in the 24 hours prior to their visit, individuals were not monitored through objective devices, such as pedometers, accelerometers or combined methods. However, on the day of assessment we confirmed whether the individual has complied or not with the recommendations.

Conclusions

In a sample of adults, the metabolic and energetic cost of one specific posture was substantially influenced by the posture executed immediately before, regarding an intermittent condition (alternating between sitting and standing). Although these findings are not large on a percentage basis, they suggest that there may be a potential cumulative effect resulting from breaking sitting with short bouts of standing.

In addition, we also conclude that the metabolic and energetic requirements of either SS_SIT and SS_STAND, of the intermittent condition, were slightly higher than in continuous sitting and standing conditions. In this sense, global health messages encouraging individuals to avoid extended periods in SB, should informed about modest, but relevant metabolic and energetic impact of interrupting this behavior as many times as possible.

Future Work

Based on our speculation about a possibility of a cumulative effect resulting from an intermittent condition of sitting and standing, the findings of this study have provided a window of opportunity for future studies to explore this issue. Although we found that alternating sitting with short bouts of standing (every minute) can modestly increase the metabolic and energetic response, compared to continuous sitting and standing, it is yet not known whether longer periods of alternation between postures (e.g. every 5 or 10 minutes) will have an identical metabolic and energetic impact.

Future studies should be encouraged to include larger samples integrating individuals of all age groups, with a balanced distribution by sex and age. In addition, future research on the effectiveness of this approach in different population groups in required (e.g. active vs. inactive individuals; healthy vs. unhealthy individuals).

Finally, given the growing evidence reporting a negative causal link between prolonged time in SB and health outcomes, may be a worth direction to develop sustained health recommendations encouraging people to frequently breaking-up this behavior with more active pursuits, especially in places where the susceptibility to this behavior is increased (e.g. workplaces).

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Appendix A – Informed consent

CONSELHO DE ÉTICA DA FACULDADE DE MOTRICIDADE HUMANA

CONSENTIMENTO INFORMADO LIVRE E ESCLARECIDO PARA INVESTIGAÇÃO CIENTÍFICA COM SERES HUMANOS

Título do projeto ou estudo: Qual é o dispêndio energético associado a estar sentado, de pé e às transições entre estes comportamentos? Um estudo randomizado e controlado

Pessoa responsável pelo projeto: Pedro B Júdice (doutorando) e Dra. Analiza M Silva (orientadora)

Instituição de acolhimento: CIPER, Exercise and Health Laboratory, Faculdade de Motricidade Humana, Universidade de Lisboa

Este documento, designado **Consentimento, Informado, Livre e Esclarecido**, contém informação importante em relação ao estudo para o qual foi abordado/a, bem como o que esperar se decidir participar no mesmo. Leia atentamente toda a informação aqui contida. Deve sentir-se inteiramente livre para colocar qualquer questão, assim como para discutir com terceiros (amigos, familiares) a decisão da sua participação neste estudo.

Informação geral
Está a ser convidado (a) a participar num projeto de investigação que pretende analisar e quantificar o dispêndio energético associado aos comportamentos “estar sentado”, “estar de pé”, e em duas formas de transição específicas entre estes dois tipos de comportamento, em pessoas adultas de ambos os sexos, aparentemente saudáveis com perfis de composição corporal diferenciados. A nossa hipótese é que, em homens e mulheres, a simples substituição do CS (que tem vindo a ser apresentado como um fator de risco para diversas doenças) por tempo passado “em pé” (estratégia levada a cabo em diversas intervenções) poderá não aumentar substancialmente o DE, mas ao invés, o maior contributo para o aumento do DE estar associado às transições entre estes dois estados de comportamento. A seleção para a participação baseia-se nos critérios de elegibilidade do estudo (idade acima de 18 anos, sem nenhuma doença diagnosticada ou limitação motora que possa limitar a locomoção e funcionalidade dos membros e não estar envolvido num programa de perda de peso. No caso de cumprir com todos os critérios acima mencionados, o participante compromete-se através deste documento, a continuar a sua participação no estudo.

Qual a duração esperada da minha participação?
Este estudo tem a duração total de aproximadamente 2 horas.
Quais os procedimentos do estudo em que vou participar?
A avaliação da composição corporal e dispêndio energético serão efetuadas durante uma manhã tendo o participante que estar em jejum até ao final das 2 horas de avaliação (sem comer e sem beber). Estas avaliações serão efetuadas através das seguintes técnicas: • Consumo de oxigénio em repouso: Este teste servirá para conhecer qual a energia necessária para manter todas as funções vitais da pessoa, quando em estado de repouso. O participante terá que permanecer deitado durante 45 minutos durante os quais lhe será colocada uma máscara ligada a um analisador de gases. • Consumo de oxigénio nas diferentes condições: Cada participante terá de completar 4 períodos (condições) de 10 minutos sequenciais com uma máscara ligado ao analisador de gases (como descrito em cima). A primeira condição é permanecer sentado numa cadeira, com as mãos colocadas em cima das coxas durante 10 minutos, mantendo o mínimo movimento possível. A segunda condição é idêntica à primeira, mas desta vez o participante está na posição ereta (de pé). A terceira condição será uma condição mista, em que o participante transita entre a condição 1 e a condição 2 a cada minuto. Por fim, na quarta condição, o participante estará sentado continuamente, levantando-se e voltando a sentar-se numa única ação, a cada minuto. A ordem das 4 condições será distribuída de forma aleatória. • Densitometria radiológica de dupla energia (DXA): realização de um scanner de corpo inteiro, com utilização de raio X (baixo nível de radiação e curto tempo de exposição; radiação equivalente a uma viagem de avião transcontinental) com a duração de 7 minutos que permite conhecer a composição do nosso peso, isto é, a massa gorda, a massa magra e o conteúdo mineral ósseo da pessoa. A avaliação das técnicas acima descritas será efetuada por técnicos especializados em cada um dos parâmetros avaliados.
A minha participação é voluntária?
A sua participação é voluntária e pode recusar-se a participar. Caso decida participar neste estudo é importante ter conhecimento que pode desistir a qualquer momento, sem qualquer tipo de consequência para si. No caso de decidir abandonar o estudo, a sua relação com a Faculdade de Motricidade Humana (FMH) não será afetada. Se for o caso, o seu estatuto enquanto estudante ou funcionário da FMH será mantido e não sofrerá nenhuma consequência da sua não-participação ou desistência.
Quais os possíveis benefícios da minha participação?
Terminadas as avaliações e os respetivos procedimentos analíticos, serão disponibilizados relatórios individuais com a descrição numérica e significado de cada uma das componentes da composição corporal avaliadas, nomeadamente a densidade óssea (fator importante por exemplo na prevenção de quedas), percentagem de gordura (fortemente associada a parâmetros de saúde). Ficará ainda a conhecer, quanto é que o seu corpo gasta (dispêndio energético) em repouso e nos vários comportamentos em estudo (sentado, de pé, etc).
Quais os possíveis riscos da minha participação?

É reconhecido que todas as técnicas utilizadas não causam qualquer dor ou desconforto e estão assegurados todos os princípios de defesa da saúde humana. O programa não possui riscos associados. É preciso ressaltar que as despesas eventuais, por exemplo, transporte até o local da atividade, serão de responsabilidade dos participantes.
Quem assume a responsabilidade, no caso de um evento negativo?
Investigador principal e orientador.
Há cobertura por uma companhia de seguros?
Tendo em conta a natureza do estudo, não encontrámos necessidade de realizar qualquer tipo de seguro para os participantes.
Quem deve ser contactado em caso de urgência?
Pedro B Júdice.
Como é assegurada a confidencialidade dos dados?
A informação obtida neste estudo será utilizada apenas pela equipa de investigação, sendo garantido o anonimato dos participantes e a confidencialidade dos dados.
O que acontecerá aos dados quando a investigação terminar?
Os dados serão guardados numa base de dados SPSS e excel, no servidor da Faculdade de Motricidade Humana afeto ao Laboratório de Exercício e Saúde. Os documentos em suporte de papel serão destruídos após a construção da matriz de tratamento dos dados.
Como irão os resultados do estudo ser divulgados e com que finalidades?
Os dados serão tratados na sua globalidade de forma anónima e utilizados para divulgar por meio de apresentações orais após a conclusão do estudo, e à comunidade científica, por meio de artigos.
Em caso de dúvidas quem devo contactar?
Para qualquer questão relacionada com a sua participação neste estudo, por favor, contactar: Pedro Júdice, email: ; tele.: 918301189

Assinatura do Consentimento Informado, Livre e Esclarecido

Li (ou alguém leu para mim) o presente documento e estou consciente do que esperar quanto à minha participação no estudo (Qual é o dispêndio energético associado a estar sentado, de pé e às transições entre estes comportamentos? Um estudo randomizado e controlado). Tive a oportunidade de colocar todas as questões e as respostas esclareceram todas as minhas dúvidas. Assim, aceito voluntariamente participar neste estudo. Foi-me dada uma cópia deste documento.

____

Nome do participante **Assinatura do participante**

____

Data

____

Nome do representante legal do participante
(se aplicável)

____

Grau de relação com o participante

Investigador/Equipa de Investigação

Os aspetos mais importantes deste estudo foram explicados ao participante ou ao seu representante, antes de solicitar a sua assinatura. Uma cópia deste documento ser-lhe-á fornecida.

____

Nome da pessoa que obtém o consentimento **Assinatura da pessoa que obtém o consentimento**

____

Data

Appendix B – Ethics Council – Study approval

Ethics Council

MEMBERS
Pedro Teixeira (President) Paulo
Armada (Vice-president) Analiza
Silva
Ana Rodrigues
Augusto Gil Pascoal
Margarida Matos
Paula Marta Bruno
Celeste Simões (supl.)
Hermínio Barreto (supl.)

To:

Dr. Pedro Júdice
Faculdade de Motricidade Humana

Date: March 30, 2015

Research Project: *What is the energy expenditure associated with sitting, standing, and transitions between these behaviors?*

CEFMH Status: Approved
CEFMH Approval Number: 8/2015

This Council has reviewed the project indicated above. We declare that this project is in accordance with Portuguese and international guidelines for scientific research involving human beings, including the 2013 Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects, and the 1997 Convention on Human Rights and Biomedicine (the "Oviedo Convention").

The President of the Ethics Council

Pedro J. Teixeira, Ph.D.

Ethics Council of the Faculty of Human Kinetics, University of Lisbon Conselho de Ética da Faculdade de Motricidade Humana, Universidade de Lisboa Faculdade de Motricidade Humana
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