



The University of Rome "Foro Italico"
The German Sport University, Cologne
The University of Southern Denmark, Odense
The Norwegian School of Sport Sciences, Oslo
The University of Vienna

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**“A comparison between two different
conditions of breaking up sedentary
behaviour on glucose metabolism during
prolonged sitting - a randomized,
exploratory, crossover study”**

Candidate
Edvard Hamnvik Sagelv

Supervisor
Ulf Ekelund

Co - Supervisor
Trine Stensrud

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Abstract

Introduction: Sedentary behaviour is suggested a risk factor for various health outcomes, independent of the amount of subcomponents of physical activity. The majority of evidence is obtained from observational studies, making it difficult to rule out if associations are explained by confounding and/or reverse causation. Experimental studies have until now been limited by examining the effect of breaking up sedentary behaviour by bouts of exercise. There is currently paucity of data comparing frequent breaks of sedentary time with one single long bout of exercise in an iso-caloric design. **Methods:** 12 healthy adults were recruited to visit the Norwegian School of Sport Sciences to undergo three different conditions in randomized order; 1) six hours of sitting; 2) six hours of sitting, with breaks of running on a treadmill for five minutes at 70 % of maximal oxygen uptake (VO_{2max}) each hour. In total for 30 minutes; 3) continuous running on a treadmill for 30 minutes at 70 % of VO_{2max} , followed by five hours and 30 minutes of sitting. For each condition, participants arrived after an overnight fast for an initial fasting blood sample followed by a standardized meal approximately 15 minutes after arrival consisting 646 kilocalories. Blood samples were collected and blood pressure measured every hour. Maximal aerobic capacity was assessed using indirect calorimetry and the running speed equivalent to 70 % of VO_{2max} was determined by extrapolation following a submaximal steady state exercise test prior to the experiment. Each condition was separated by a 6-day wash-out period. **Results:** Six out of 12 participants completed all conditions. Analyses were performed as both per protocol (PP) and intention to treat (ITT). Regardless of analyses being PP or ITT, there were no differences in insulin, glucose and triglyceride total and incremental area under curve, and one-hour postprandial insulin and glucose concentration, across conditions. **Conclusion:** There is no effect of either breaking up sedentary behaviour each hour or performing 30 minutes of vigorous physical activity in one bout compared with prolonged sitting for six hours on glucose and lipid metabolism.

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Abbreviations

ACC	Accelerometer
AUC	Total area under curve
iAUC	Incremental area under curve
BMI	Body mass index
BP	Blood pressure
BS	Blood sample
BSB condition	Breaking sedentary behaviour condition
Continuous VPA condition	Continuous vigorous physical activity condition
CRF	Cardiorespiratory fitness
CVD	Cardiovascular disease
EE	Energy expenditure
GLUT4	Glucose transporter type 4
h	Hour
HDL-C	High density lipoprotein cholesterol
HR	Hazard ratio
HR_{max}	Heart rate maximum
HR + ACC	Combined heart rate and accelerometer
Kcal	Kilocalories
Kg	Kilograms
LDL-C	Low density lipoprotein cholesterol
LPA	Light physical activity
LPL	Lipoprotein lipase
MET	Metabolic equivalent of task
mmHg	Millimeter hydrargyrum
mmol • L⁻¹	Milomole per liter blood
min	Minute
MetS	Metabolic syndrome
MPA	Moderate physical activity
MVPA	Moderate and vigorous physical activity
NDH	Norwegian directorate of health
NNR	Nordic nutrition recommendations
OR	Odds ratio

PA	Physical activity
PAEE	Physical activity energy expenditure
pmol • L⁻¹	Picomole per liter blood
RER	Respiratory exchange ratio
RR	Relative risk
SB	Sedentary behaviour
SB condition	Sedentary behaviour condition
SD	Standard deviation
TG	Triglyceride
T2DM	Type 2 diabetes mellitus
VLDL	Very low density lipoprotein
VO₂	Oxygen uptake
VO_{2max}	Maximal Oxygen uptake
VPA	Vigorous physical activity
WC	Waist circumference
WHO	World health organization
95 % confidence interval	95 % CI

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1.0 Introduction

In the last decades sedentary behaviour (SB) has emerged as a risk factor for various health outcomes, and some have suggested this behavioural risk is independent of the amount of moderate and vigorous physical activity (MVPA) (de Rezende, Lopes, Rey-López, Matsudo, & Luiz, 2014). In fact, the extent of evidence have led health authorities to recommend breaks and reduction in sedentary time (Biddle et al., 2010; NDH, 2014; Tremblay, LeBlanc, Janssen, et al., 2011). However, the majority of evidence is obtained by using subjective forms of assessments and when objective forms of assessments are used, associations of SB and health outcomes independent of MVPA are less clear (Saunders, Chaput, & Tremblay, 2014). Furthermore, at date there is no evidence for any causal relationship between SB and health risk independent of MVPA. Thus, recommendations in regard of SB seem premature (Ekelund, 2012).

Recently, the first review of experimental studies was conducted, where the effects of breaking up prolonged sitting were investigated. Conclusions were that type, frequency and intensity of breaks needed to reverse the effects of SB seems to depend on subjects' characteristics, in particular physical activity (PA) level. In other words, this suggest that the association between SB and health risk is not independent of MVPA. Further, there were undoubtedly more studies needed to identify the most feasible and effective PA protocol to counteract the detrimental effects of prolonged SB (Benatti & Ried-Larsen, 2015). To extend the evidence, an exploratory, randomized, three-treatment, crossover study was carried out. The objective was to examine if breaking up sedentary behaviour with short five minutes' bouts or one continuous 30-minute bout of vigorous intensity activity differentially affects metabolic risk markers compared with a control condition of prolonged sitting.

2.0 Research on sedentary behaviour

2.1 Definitions

For years, sedentary were by many perceived as the least active on a continuous scale. Today, this perception is outdated. Sedentary behaviour is defined as: *“Any waking behaviour characterized by an energy expenditure ≤ 1.5 METs while in a sitting or reclining posture.”*. This is distinct different from physical inactivity, which is: *“performing insufficient amounts of moderate to vigorous physical activity (i.e., not meeting specified physical activity guidelines)”* (Sedentary Behaviour Research Network, 2012). Physical activity is defined as: *Any bodily movement by skeletal muscles resulting in an increase in energy expenditure”*. Exercise is defined as: *“a subset of physical activity that is planned, structured, and repetitive and has as a final or an intermediate objective the improvement or maintenance of physical fitness”*. Finally, physical fitness is defined as: *“a set of attributes that are either health- or skill-related”* (Caspersen, Powell, & Christenson, 1985).

2.2 Physical activity recommendations

Physical activity (PA) can be performed in light, moderate and vigorous intensity, which is equivalent to 1.5-2.9, 3.0-5.9 and >6.0 Metabolic Equivalent of Tasks (MET)-values, respectively (WHO, 2010). MET is a way of expressing physiological energy expenditure (EE) from physical activities and is expressed in multiples of resting metabolic rate. One MET is equivalent to an oxygen uptake (VO_2) of $3.5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, derived from one individual at seventy kilograms (kg) which is suggested to be the reference point for the general population (Jette, Sidney, & Blumchen, 1990). For further reading, see Ainsworth et al. (2011a). The current PA recommendations from the World Health Organization (WHO) is 30 minutes for five days or a total of 150 minutes a week of moderate intensity-, or 20 minutes for three days or a total of 75 min a week of vigorous intensity, or a combination of these two (WHO, 2010).

2.3 Sedentary behaviour independent of physical activity

There are three primarily suggestions for why SB is a health risk factor independent of MVPA.

2.3.1 Displacement theory

The displacement theory suggests that by sitting, SB is chosen over PA. This will lead to lower level of PA, which again leads to lower EE. One meta-analysis investigated the association between SB and PA in children and adolescents. They found a small, significant, negative association between SB and PA in children and adolescents. However, this association is weak and based on 254 studies, of which 212 was cross-sectional and mainly self- or proxy-reports of PA (179 studies) or SB (209 studies). Thus, there is little support for the displacements hypothesis, suggesting that PA is not displaced by SB (Pearson, Braithwaite, Biddle, van Sluijs, & Atkin, 2014).

2.3.2 Reducing sedentary behaviour will increase total physical activity

By definition, reducing SB will increase total PA which will increase EE. Replacing LPA with SB is reported to result in increased cardio-metabolic risk (Buman et al., 2014). However, MVPA results in lower cardio-metabolic risk (Buman et al., 2014), and it is therefore unlikely that SB is a health risk independent of MVPA. Moreover, standing quietly (1.3 METs) does not necessarily result in an increase in EE compared with reading/writing while sitting (1.5 METs) (Ainsworth et al., 2011b). Furthermore, homeostasis will most likely ensure energy balance in SB/LPA ratio over time by consuming more/less nutrients (Hall et al., 2012).

2.3.3 Residual confounding

In both cross-sectional samples and prospective cohorts, there is always a possibility for residual confounding. This may be unmeasured or/and poorly measured confounders. For example, sitting time and in particular screen time may be associated with uncontrolled consumption of high energy dense food (Ekelund, 2012).

2.4 Assessment of sedentary behaviour

Both sedentary behaviours and PA are complex behaviours requiring accurate and precise assessment methods when used in research aimed at studying health associations.

2.4.1 Subjective assessments

Subjective assessment methods are usually self-reported and rely on the respondents' memory and recall. Strengths of self-reports include low-cost, easy to assess except long quantitative questionnaires, and it can explore all dimensions and domains (Sallis & Saelens, 2000). The most used method is questionnaires. Reliability of questionnaires are usually fair. Validity on the other hand, is variable ($r = 0.19 - 0.80$) (Atkin et al., 2012). In children, both reliability and validity is reported to variate (test-retest: $r = 0.13 - 0.98$, majority <0.50 ; validity: $r = 0.19 - 0.88$, majority <0.50). (Bryant, Lucove, Evenson, & Marshall, 2007; Lubans et al., 2011). In recall questionnaires it seems to be easier to remember weekdays compared with weekends, suggested to be because of more variability in behaviour patterns during weekends (Atkin et al., 2012). A widely used recall questionnaire, International Physical Activity Questionnaire (IPAQ) short version, was added a question of sitting time where the validity of this question is reported to be fair ($r = 0.5$) (Atkin et al., 2012). Proxy reports, especially parent-proxy reports, are widely used for assessments of children. Similar to questionnaires, reliability is fair ($r = 0.60-0.80$) while validity is variable ($r = 0.08-0.84$) (Atkin et al., 2012).

A particular concern in subjective assessment is information bias. In research, bias is deviation of results or inferences from the truth, and information bias is incorrect determination of exposure, outcome or both (Grimes & Schulz, 2002). This can be in form of recall bias and may be the greatest concern in self-report assessments (Hassan, 2006). Information is based on respondents' memory, and participants may be unaware of the amount of sitting and PA. Usually, recall bias is a concern in retrospective case-control studies where it is suggested that individuals with poor health/disease may recall factors that effects their condition more accurately compared with healthy controls (Schulz & Grimes, 2002). However, if disease onset resulted in more awareness of exposure measure, this phenomenon may be a concern in prospective cohorts as well (Hassan, 2006). For example, stress may impose relaxation which involves sitting. While stress explained the association between poor health and SB at follow-up, the individual was sitting for an equal amount unconsciously before the onset of the stressor and thereby reported less sitting time at baseline.

Diaries and ecological momentary assessment are used to some extent. Both these methods provide the opportunity to constantly assess behaviour as they occur and thereby avoiding recall bias (Atkin et al., 2012).

Information bias can also be caused by social desirability. For example, wanted behaviours is over-reported (e.g. PA), or/and unwanted behaviours is under-reported (e.g. sitting) (Sternfeld & Goldman-Rosas, 2012). Further, it may also be because of different understanding of words and cultural norms in a multicultural world (Atkin et al., 2012).

In summary, subjective assessment rely on respondents' memory, which is threatening the internal validity (Grimes & Schulz, 2002). Since the majority of validity studies are reporting $<r = 0.5$ (Atkin et al., 2012), one positive validity study may be coincidental rather than the true validity.

2.4.2 Objective assessments

To avoid some of the limitations of subjective assessments, use of objective assessments of SB is increasing (Atkin et al., 2012).

2.4.2.1 Direct observation

Direct observation can be both subjective and objective. It is objective because the one observing has an objective approach towards the research sample. It is subjective because it relies on the observant subjective memory/perception. Direct observation is usually carried out using a coding scheme, like the Physical Activity Compendium, which offer EE from sedentary to vigorous activities (Ainsworth et al., 2011b). A major limitation is that the observer can only observe one or a few subjects at the same time. Another limitation may be the Hawthorne effect, which will be explained below (see 2.4.2.6 hawthorne effect).

2.4.2.2 Inclinometers

Inclinometers measure the subjects' posture. A common device is ActivPAL, and this is placed on the anterior part of the thigh. It measures subjects' leg accelerations and distinguish between lying, sitting, standing and walking/stepping (Atkin et al., 2012). Reliability is reported to be good ($r = 0.97$), and the mean percentage between direct observation and ActivPAL was 0.3-, 1.4- and 2 % for sitting, standing and walking, respectively (Grant, Ryan, Tigbe, & Granat, 2006). Although it distinguishes walking from sitting and standing, it may have problems detecting MVPA. Thus in regard of identifying the potential association between SB and health outcome independent of MVPA, ActivPAL may not be appropriate.

2.4.2.3 Accelerometers

Accelerometers (ACC) measures frequency and amplitude of acceleration and is usually placed on the subjects' hip or lower back (Atkin et al., 2012). ACC values are expressed in counts per minute where SB is determined with low acceleration counts, usually a threshold below 100 counts per minute (Matthews et al., 2008; Trost, Loprinzi, Moore, & Pfeiffer, 2011). In contrast to ActivPAL, ACC do not distinguish between lying/sitting and standing. Further, there is no consensus regarding how data should be extracted in terms of epoch lengths. For comparison between studies, shorter epochs is suggested, as shorter epochs can be summed together to longer ones (Atkin et al., 2012). Strengths of both ACC and inclinometers includes is easy to use, information of activity pattern, data storage for longer periods (7-14 days) and not dependent on respondents' recall memory. It also shows great correlation with EE in walking but not cycling (Hansen et al., 2014).

In a recent validity study, the newest ACC of Actigraph (GT3X+ (Pensacola, FL, USA)) overestimated SB by $135 \text{ min} \cdot \text{day}^{-1}$ and underestimated breaks in SB by $78 \text{ times} \cdot \text{day}^{-1}$ compared with ActivPAL (Judice, Santos, Hamilton, Sardinha, & Silva, 2015). Another limitation is that ACC only measure acceleration where it is placed, thus hip or lower back placed ACC may exclude upper body activity while seated which exceed 1.5 METs, such as resistance type exercises (6.0 METs) (Ainsworth et al., 2011b). This may also be the explanation for poor correlation with EE in cycling as cycling does not create big movements of the hip or lower back (Hansen et al., 2014). Placement of the ACC on the wrist is suggested to be a feasible option as compliance rates is suggested to increase compared with hip worn ACC (Hildebrand, van Hees, Hansen, & Ekelund, 2014). It seems the accuracy in PA and SB assessments in wrist worn ACC is depended on ACC brand, where the common used Actigraph GTX3+ seem suitable for wrist assessments (Hildebrand et al., 2014; Rosenberger et al., 2013). In both children and adults, higher outputs are reported when wrist placements are compared with hip placement (Hildebrand et al., 2014). Thus comparison of results in studies using either hip or wrist worn ACC may not be appropriate. Nevertheless, wrist worn ACC seem accurate in estimating EE (K. Y. Chen et al., 2003; Ekblom, Nyberg, Bak, Ekelund, & Marcus, 2012; Hildebrand et al., 2014), and at least the wrist worn Actigraph GTX3+ seem feasible for assessment of both SB and PA (Hildebrand et al., 2014).

2.4.2.4 Heart rate

Heart rate (HR) has been widely used to assess physiological intensity in both activities and exercise. In epidemiology, the Flex-HR is been used for assessment of SB (Atkin et al., 2012). The Flex-HR is an individual threshold, which determines if the individual is resting or exercising. HR below or above the threshold value determines which regression formula to be used to calculate free-living behaviours to EE (Spurr et al., 1988). In assessment of SB, the flex-HR show high specificity, but low sensitivity (Atkin et al., 2012). One concern may be whether heart rate or EE alone can assess SB, as both methods measure physiological components and not posture.

2.4.2.5 Combined methods of heart rate and accelerometer

Combined methods of HR and ACC (HR+ACC) is suggested to reduce the limitations of both assessments alone (Atkin et al., 2012). Six activities (lying, sitting, slow and brisk walking, running and hopscotch) were assessed using combined HR+ACC in children. Both ACC and HR+ACC accurately predicted physical activity energy expenditure (PAEE) in the six activities and conclusion were that although systematic errors were present, combined HR+ACC may be more accurate than ACC alone for assessment of PAEE (Corder et al., 2007). The utility and validity of assessment of SB using combined HR+ACC are not fully explored (Atkin et al., 2012). One model, Actiheart, underestimated SB by 156 min • day⁻¹ and breaks in SB by 235 times • day⁻¹ (Judice et al., 2015). Combined heart rate and movement sensing is highly valid for assessing PA and PAEE but may underestimate SB. This, in addition to the limitation of individual calibration of heart rate, makes combined HR+ACC less feasible compared with ACC in epidemiology research.

2.4.2.6 Hawthorne effect

The Hawthorne effect is the effect of individuals giving more effort while being observed (Smith, 1968). This is called reactivity in assessment of PA and it is suggested that both direct observation and activity trackers may be exposed to reactivity (Dössegger et al., 2014). Observing participants without their consent is unethical and participants should be explained the purpose of the study orally and in writing (written informed consent). As a consequence, making participants aware of the purpose (measuring reactivity) may increase the individual's activity level during assessments compared with their normal daily PA level. Some have suggested to omit the first day of measurement, since PA is reported to decrease after the first

day of measurement (Dössegger et al., 2014). Further, it is suggested that reactivity is more prominent in children and adolescents compared with adults because of their competitive and curious nature (Dössegger et al., 2014). When reactivity is investigated, assessments in children suggest three percent more counts per minute on the first day of measurements compared with the mean of the remaining valid days (Mattocks et al., 2008), and 3-7 % higher the first day compared with the remaining days (Dössegger et al., 2014). The increase of four minutes more walking or running each day (10 %) in PA interventions for children is suggested to have little clinical relevance (Metcalf, Henley, & Wilkin, 2012), thus making the evidence for omitting the first day of measurement weak. To the author of this thesis' knowledge, reactivity have not been explored in regard of assessments of SB.

2.4.3 Conclusion; sedentary behaviour assessments

In conclusion, inclinometers, especially ActivPAL, seem most accurate in SB assessment. However, ACC measure in addition MVPA, allowing to assess SB as a health risk independent of MVPA. One solution in the future may be combined ACC and inclinometers.

2.4.4 Additional limitations in sedentary behaviour assessments

In addition to the limitations information bias and residual confounding, observational studies in general cannot rule out reverse causation (Ekelund, 2012) and selection bias (Grimes & Schulz, 2002). Reverse causation is the unknown direction of causality; does SB cause poor health or *vice versa*. Selection bias is related to the comparability between groups (Grimes & Schulz, 2002); does those who suffer from poor health differ in any other way of importance except higher amounts of SB.

2.5 Prevalence of sedentary behaviour

It seems to be a discrepancy in subjective and objective assessments of SB. For example, children report to spend 1.4-3.7 hour per day ($\text{h} \cdot \text{day}^{-1}$) watching television, while ACC measurements reveal that children spend 6-8 $\text{h} \cdot \text{day}^{-1}$ sitting or lying (Pate, Mitchell, Byun, & Dowda, 2011). This exemplifies why screen time is a poor estimate of total SB. This discrepancy may also be caused by information bias. Nevertheless, because of this, prevalence data of SB will be presented with studies using objective assessments. Cut-off

counts for SB vary from <100-1100 counts per minute (Pate et al., 2011). All studies included in the following summary used <100 counts per minute as threshold for SB.

2.5.1 Children/adolescents

Across countries, SB in children and adolescents seem to increase with increasing age (Pate et al., 2011). American 6-11-, 12-15- and 16-19 years-olds spent 6.1-, 7.5-, and 8 h • day⁻¹ in sedentary behaviours, respectively (Matthews et al., 2008). British 10 year-olds spent 7.6 h • day⁻¹ in sedentary behaviours (Steele et al., 2010). In Denmark, Portugal, Estonia and Norway, 9-year-olds spent 4.0-5.7 h • day⁻¹ sedentary, while 15 year olds spent 5.5-7. h • day⁻¹ (Nilsson et al 2009). However, a recent Norwegian surveillance survey (UngKan2) reported higher amounts for Norwegian children; 6-, 9- and 15-years-olds spent 6.4, 7.7 and 9.4 h • day⁻¹ in sedentary behaviours, respectively (Kolle, Stokke, Hansen, & Andersen, 2012). This indicate that the prevalence of SB in children is increasing in Norway.

2.5.2 Adults

Adults tend to spend between 7.5-9.4 h • day⁻¹ in sedentary behaviours. In Sweden, adults between 18-64 years spent 7.7 h • day⁻¹ in sedentary behaviours (Hagströmer, Oja, & Sjöström, 2007). In the United States, adults between 20-59 years spent 7.5 h • day⁻¹ in sedentary behaviours (Matthews et al., 2008). Chinese adults between 40-59 years spent 8.4 h • day⁻¹ in sedentary behaviours (Peters et al., 2010). Portuguese adults between 18-64 years spent 9.4 h • day⁻¹ in sedentary behaviours (Baptista et al., 2012). A Norwegian surveillance survey (Kan2) reported that adults between 20-64 years spent 9.1 h • day⁻¹ in sedentary behaviours (Hansen et al., 2015).

2.5.3 Elderly

In Sweden, elderly between 65-79 years spent 7.5 h • day⁻¹ in sedentary behaviours (Hagströmer et al., 2007). In the United States, elderly between 60-85 years spent 8.9 h • day⁻¹ in sedentary behaviours (Matthews et al., 2008). Chinese elderly between 65-74 years spent 9 h • day⁻¹ in sedentary behaviours (Peters et al., 2010). Portuguese elderly over 65 years (upper age limit not specified) spent 10.1 h • day⁻¹ in sedentary behaviours (Baptista et al., 2012). Norwegian elderly spent 9.3 h • day⁻¹ in sedentary behaviours (Hansen et al., 2015).

3.0 Sedentary behaviour and health outcomes

The following section will summarise the observed associations between SB and health outcomes throughout the life-course. More specifically, the pertinent question is whether these associations are independent of MVPA. This first part includes observational studies, and experimental studies are presented below (see 4.0 sedentary behaviour and health outcomes: experimental studies). Associations between SB and psychosocial and cognitive development have been investigated (de Rezende, Lopes, et al., 2014), however, psychosocial parameters are excluded in this thesis and the emphasis is associations between SB and physiological parameters. The majority of available evidence is based on subjective assessments of SB. Results from studies using objectively measured SB will be highlighted when available.

3.1 Sedentary behaviour and cardio-metabolic risk

Cardio-metabolic risk factors are predictors for metabolic syndrome (MetS), type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). Included cardio-metabolic risk factors in this summary are blood lipids (HDL-C, low density lipoprotein cholesterol (LDL-C), TG, total cholesterol and HDL-LDL-cholesterol ratio), impaired glucose metabolism (glucose and insulin) and high predictive CVD risk factors (Intima-media thickness and low ankle-brachial index). Blood pressure (BP) and overweight/obesity will be presented separately below.

3.1.1 Children/adolescents

Subjective assessments of SB reports moderate rated quality evidence for an association between SB and total cholesterol (Tremblay, LeBlanc, Kho, et al., 2011), however, these results are inconsistent (Chinapaw, Proper, Brug, Van Mechelen, & Singh, 2011). In a narrative review, 15 out of 18 studies reported no association between objectively assessed SB and cardio-metabolic risk after adjustment for MVPA (Saunders et al., 2014). Finally, a systematic review and meta-analysis of objectively measured SB and cardio-metabolic risk reported that eight out of 28 studies included reported at least one association between SB and cardio-metabolic risk. The pooled effect size of SB and glucose and insulin were significant ($r = 0.07$, 95 % CI: 0.01 to 0.13), while the pooled effect size of SB and HDL-C, which included five studies, was not significant. For both effect sizes, MVPA was a significant moderator that removed the association (Cliff et al., 2016).

3.1.2 Adults

According to an overview of systematic reviews there is insufficient evidence for an association between SB and cardio-metabolic risk in adults (de Rezende, Lopes, et al., 2014). In addition, a prospective cohort study with six year follow-up reported no association between self-reported SB and both visceral fat and other cardio-metabolic risk factors adjusted for MVPA (Saunders, Tremblay, et al., 2013).

In one cross-sectional study measuring SB using both self-report and ACC, objectively measured SB were only associated with total cholesterol, whereas self-reported SB were associated with additional cardio-metabolic risk factors (Stamatakis, Hamer, Tilling, & Lawlor, 2012). Moreover, another cross-sectional study reported that there was no significant greater risk for clustered cardio-metabolic risk in the objectively measured sedentary but active group, compared with the active and non-sedentary group. Both inactive groups which were sedentary and non-sedentary, had higher risk for clustered cardio-metabolic risk (Maddison et al., 2015).

In a prospective cohort study of subjects with a family history of T2DM, SB and LPA measured with ACC were not associated with insulin resistance. MVPA on the other hand, was a significant predictor of insulin resistance independent of SB and LPA (Ekelund, Brage, Griffin, & Wareham, 2009). In contrast, another prospective cohort study suggested that SB was positively associated with insulin resistance independent of MVPA assessed by minute-by-minute heart rate recording according to the Flex-HR method (Helmerhorst, Wijndaele, Brage, Wareham, & Ekelund, 2009).

Further, high estimated CRF (peak oxygen uptake: men: >43.3 -, women: >35.2 ml \cdot kg $^{-1}$ \cdot min $^{-1}$) resulted in a substantial lower risk for clustered cardio-metabolic risk compared with low CRF (peak oxygen uptake: men: <35.7 -, women: <28.4 ml \cdot kg $^{-1}$ \cdot min $^{-1}$) when both groups reported high sedentary time (>7 h \cdot day $^{-1}$) and meeting PA guidelines (high CRF: men: OR: 1.0, 95 % CI: 0.5-1.8; women: OR: 1.2, 95 % CI: 0.5-3.3; low CRF: men: OR: 24.2, 95 % CI: 12.7-46.3; women: OR: 36.0, 95 % CI: 15.3-84.6) (Nauman, Stensvold, Coombes, & Wisløff, 2015). Furthermore, one prospective cohort study with 31-year follow-up reported that with adjustment for MVPA, self-reported SB was associated with TG, TG-HDL-C ratio, body mass index (BMI), waist circumference (WC) and adiposity, but when

adjustment included CRF and MVPA, SB was only associated with TG-HDL-C ratio (Shuval et al., 2014).

Finally, there is cross-sectional associations between ACC measured SB and carotid Intima-media thickness, but not carotid injury, independent of MVPA (García-Hermoso et al., 2015) and low ankle-brachial index independent of VPA (Kulinski et al., 2015). Intima-media thickness is used to identify atherosclerotic disease, which is a predictor for CVD (Lorenz, Markus, Bots, Rosvall, & Sitzer, 2007). Carotid artery is the oxygen providing artery to the brain and neck. Low ankle-brachial index is the ratio between systolic BP of the ankle and the arm, and low ankle-brachial index (<0.9) is a high risk factor for peripheral arterial disease, which is atherosclerosis in the peripheral arteries (Kulinski et al., 2015)

3.1.3 Elderly

A recent review identified six cross-sectional studies, where four studies were rated as very low quality, and two were rated as moderate quality (de Rezende, Rey-López, Matsudo, & Luiz, 2014). Some reported screen time to be associated with low density lipoprotein cholesterol (LDL-C) and total cholesterol ratio (Gao, Nelson, & Tucker, 2007), however, this observation is not consistent (Gennuso, Gangnon, Matthews, Thraen-Borowski, & Colbert, 2013). Similar, screen time is reported to be associated with fasting glucose (Gennuso et al., 2013) but observations are inconsistent (Gao et al., 2007). Screen time is reported to be associated with C-reactive protein (Gennuso et al., 2013), and there is no association between screen time and TG (de Rezende, Rey-López, et al., 2014).

Finally, there is no cross-sectional associations between ACC measured SB and low ankle-brachial index independent of MVPA. Moreover, VPA showed stronger inverse association for low ankle-brachial index compared with MPA, and longer bouts (>10 minutes) of MVPA did not result in greater inverse association with low ankle-brachial index compared with shorter bouts. This suggest that higher intensity PA is more beneficial for low ankle-brachial index and that shorter bout lengths (<10 minutes) is equally protective for low ankle-brachial index compared with longer bouts (Parsons et al., 2016).

3.1.4 Brief conclusion

In children and adults, it appears that the detrimental associations between SB and health

outcomes are restricted to studies examining self-reported leisure time SB. Based on the conflicting results from self-reported SB and no association when SB is assessed with ACC in adults and elderly, there seem to be no high quality evidence for an association between SB and cardio-metabolic risk independent of MVPA in all age groups.

3.2 Sedentary behaviour and blood pressure

One recent systematic review and meta-analysis investigated the association between SB and BP. 31 studies of good quality were included of which 28 were included in the meta-analysis. 10 studies used ACC and the remaining used self-report assessments. 14 studies included children and adolescents, and 17 included adults and elderly. There were no difference for pooled estimate between adults and children, and therefore, the age groups were combined (P. H. Lee & Wong, 2015).

There were no association between SB and systolic and diastolic BP when SB were assessed with ACC. Results from the meta-analysis revealed that for each hour increase in self-reported SB, there was an increase of 0.06 (95 % CI: 0.01-0.11) and 0.25 (95 % CI: 0.10-0.29) mmHg in systolic- and diastolic BP, respectively. For each hour increase in SB, the risk for hypertension increased with 2 % (odds ratio (OR): 1.02, 95 % CI 1.003–1.03) (P. H. Lee & Wong, 2015). One study found that participants with worse health outcomes were less compliant to wear an ACC (P. H Lee, Macfarlane, & Lam, 2013). It was hypothesized that the lack of an observed association between ACC measured SB and BP was due to less compliance in wearing an ACC in individuals with high BP compared with those with normal BP (P. H. Lee & Wong, 2015).

3.3 Sedentary behaviour and overweight/obesity

Obesity is a predictor for several health outcomes, including MetS, T2DM and CVD (Alberti et al., 2009).

3.3.1 Children/adolescents

Some have reported moderate evidence for an association between television viewing and overweight in later childhood (Te Velde et al., 2012; Tremblay, LeBlanc, Kho, et al., 2011), however, this observation is inconsistent (Chinapaw et al., 2011). When objective

assessments of SB were applied, SB appeared not to predict later gains in adiposity (Collings et al., 2015).

3.3.2 Adults

According to the overview of systematic reviews, 14 reviews have investigated the association between SB and overweight/obesity (de Rezende, Lopes, et al., 2014). There is limited evidence of a longitudinal association between SB and weight gain or risk of obesity in adults (Proper, Singh, Van Mechelen, & Chinapaw, 2011; Van Uffelen et al., 2010). One prospective cohort study used heart rate monitors to investigate if obesity predicts SB, or *vice versa*. Body weight, WC, BMI and fat mass seemed to predict SB, but SB did not predict gains in obesity (Ekelund, Brage, Besson, Sharp, & Wareham, 2008). However, the authors mention that PA and SB are measured much less precise compared with body weight and BMI, thus it is not surprising that body weight predicts SB and PA level and not *vice versa*. Since it is not clear if SB predicts obesity in other populations who differ in age, baseline obesity and PA level, bidirectional associations cannot be ruled out (Ekelund et al., 2008).

3.3.3 Elderly

Six cross-sectional studies of low and moderate rated quality all found an association between self-reported SB and BMI (de Rezende, Rey-López, et al., 2014). Further, there is consistent evidence for an association between objectively assessed SB and WC independent of MVPA (Gennuso et al., 2013; Stamatakis, Davis, Stathi, & Hamer, 2012).

3.3.4 Brief conclusion

Longitudinal data suggest limited evidence for an association between SB and overweight and obesity in children/adolescents and adults. In elderly, there is cross-sectional associations between objectively measured SB and WC. However, there seem to be no available longitudinal evidence for an association between SB and overweight and obesity in this age group.

3.4 Sedentary behaviour and metabolic syndrome

Metabolic syndrome (MetS) is associated with increased risk for T2DM and CVD. The diagnostic criteria from the International Diabetes Federation are following; WC: >94

centimeters in men, > 80 centimeters in women (European populations, with ethnicity specific values for other groups), in addition to two of these four outcomes: Fasting glucose: >100 mg • dL⁻¹, 5,6 mmol • L⁻¹ or previously diagnosed T2DM, BP: >130 diastolic/85 systolic, triglycerides level: >150 mg • dL⁻¹ and HDL-C: <40 mg • dL⁻¹ (Alberti, Zimmet, & Shaw, 2006).

3.4.1 Children/adolescents

Independent of subjectively measured MVPA and using 1 h • day⁻¹ as the referent value, 2 h-, 3 h-, 4 h- and 5 h • day⁻¹ of screen time increased the OR for the MetS with 1.21 (95 % CI: 0.54–2.73), 2.12 (95 % CI: 0.99–4.74), 1.73 (95 % CI: 0.72–4.17) and 3.07 (95 % CI: 1.48–6.34), respectively (Mark & Janssen, 2008). However, when adiposity was excluded from the analysis, self-reported screen time was not associated with clustered metabolic risk adjusted for objectively measured MVPA. It is therefore suggested that the association between screen time and the MetS may be mediated by adiposity (Ekelund et al., 2006).

3.4.2 Adults

A meta-analysis including 10 cross-sectional studies (N = 21 393), were nine used subjective- and one used objective assessments of SB, reported that higher amounts of sedentary time was associated with an increased risk (OR: 1.73 95 % CI: 1.55–1.94) for having the MetS independent of MVPA (Edwardson et al., 2012).

3.4.3 Elderly

The systematic review of SB and health outcomes in elderly identified three cross-sectional studies who assessed the association between SB and the MetS. All studies in elderly, except the study by Gao et al. (2007) that was rated moderate, were rated very low in quality (de Rezende, Rey-López, et al., 2014). Watching television for more than 7 h • day⁻¹ increased the risk for the MetS compared with 1 h • day⁻¹ (OR: 2.2, 95 % CI: 1.1–4.2), and by each hour of sitting the risk for the MetS increased with 19 % (95 % CI: 1.1–1.3) (Gao et al., 2007). Similarly, sitting for three hours increased the odds for the MetS (men: OR 1.57, CI 95 %: 1.02 – 2.41; women: OR 1.56, CI 95 %: 1.09 – 2.24) compared with less sitting (<1.14 h • day⁻¹) (Gardiner et al., 2011). Finally, when the highest quartile was compared with the lowest quartile, percentage of ACC measured sedentary time of total wear time (OR: 1.61, 95 % CI:

0.97-2.67) and lower intensity during breaks in sedentary time (OR: 1.59, 95 % CI: 0.96-2.62) increased the risk for the MetS independent of MVPA. However, duration of sedentary time, average bout length of sedentary time and number of breaks did not show an increased risk for the MetS independent of MVPA (Bankoski et al., 2011).

3.4.4 Brief conclusion

There seem to be no cross-sectional associations between screen time and the MetS independent of MVPA in children/adolescents. For adults and elderly, SB is associated with an increased risk for the MetS independent of MVPA. There seem to be few studies investigating the association between objective assessments of SB and the MetS and there is no available longitudinal data for the association between SB and the MetS.

3.5 Sedentary behaviour and type 2 diabetes mellitus

In 2012, 1.5 million deaths were caused by diabetes and in 2030, diabetes will be the 7th leading cause for global mortality. Besides mortality, other complications caused by diabetes include heart attack, stroke, blindness, kidney failure and lower limb amputation. Although type 1 diabetes mellitus is included in these estimates, the majority of diagnosed diabetes is the non-insulin dependent type 2 diabetes mellitus (T2DM) (WHO, 2016). The onset of T2DM in children and adolescent is rapidly increasing (L. Chen, Magliano, & Zimmet, 2012), however, there seem to be no studies investigating a potential association between SB and T2DM in children. Surprisingly, in the systematic review of SB and health outcomes in elderly, T2DM is not mentioned (de Rezende, Rey-López, et al., 2014).

3.5.1 Adults

In the overview of systematic reviews, five reviews reported a significant association between self-reported SB and T2DM independent of MVPA (de Rezende, Lopes, et al., 2014). A meta-analysis including four prospective studies reported that for each two-hour increase in television viewing, there was a 20 % increased risk for T2DM (relative risk (RR): 1.20, 95 % CI: 1.14-1.27) (Grøntved & Hu, 2011). Similar, another meta-analysis including five prospective and five cross-sectional studies reported that, compared with less self-reported sedentary time, higher amounts of sedentary time increased the risk for T2DM more than twofold (RR: 2.12, 95 % CI: 1.61, 2.78) (Wilmot et al., 2012). More recently, a meta-analysis

including one cross-sectional and four prospective studies, reported that higher amounts of self-reported sedentary time was associated with an increased hazard ratio for T2DM independent of MVPA (HR: 1.91, 95 % CI: 1.62–2.22) (Biswas et al., 2015).

3.5.2 Brief conclusion

The available evidence suggest that TV viewing may be associated with an increased risk for T2DM independent of MVPA but more evidence is needed. In particular studies investigating an association between objective assessments of SB and T2DM are warranted.

3.6 Sedentary behaviour and cardiovascular disease

Cardiovascular disease (CVD) is the number one cause for global mortality. In 2012, 17.5 (37 %) million deaths were caused by CVD (Organization, 2014). The available evidence for an association between SB and CVD are restricted to self-reported SB. As expected, there seem to be no study investigating this association in children, since cardiovascular diseases is usually caused by multiple risk factors (e.g. hypertension, tobacco use, inactivity, unhealthy diet, impaired glucose tolerance, high lipid level, overweight/obesity) that evolves over time, thus CVD is usually not observed until adulthood (Mendis, Puska, & Norrving, 2011). Surprisingly, there seem to be no studies of SB and CVD in elderly (de Rezende, Rey-López, et al., 2014).

3.6.1 Adults

Compared with the general population, people with CVD spend between 0.5-1 h • day⁻¹ more in sedentary behaviours. In addition, compared with those without CVD, those with CVD reported higher amounts of television watching (Evenson, Butler, & Rosamond, 2013)

The overview of systematic reviews identified five reviews that investigated an association between self-reported SB and CVD (de Rezende, Lopes, et al., 2014). In meta-analyses, each two-hour increase in television viewing increased the risk for CVD with 15 % (RR: 1.15, 95 % CI: 1.06-1.23) (Grøntved & Hu, 2011) and 17 % (hazard ratio (HR): 1.17, 95 % CI: 1.13-1.20) (Ford & Caspersen, 2012). For total sitting time including two studies, each two-hour increase resulted in a 5 % increased risk for CVD (HR: 1.05, 95 % CI: 1.01–1.09) (Ford & Caspersen, 2012). When higher amounts of self-reported sedentary time was compared with

less sedentary time the increased risk for CVD was 147 % (RR 2.47; 95 % CI 1.44-4.24) (Wilmot et al., 2012).

More recently, a meta-analysis including three studies reported that higher amounts of self-reported sedentary time was associated with an increased hazard ratio (HR: 1.14, 95 % CI: 1.00–1.30), however, the confidence interval suggests a possibility for no risk for CVD independent of MVPA (Biswas et al., 2015). Moreover, in a recent cross-sectional study assessing the association between ACC measured SB and a predicted 10-year risk for first atherosclerotic cardiovascular disease event, the groups who were inactive, regardless of being categorised as sedentary or non-sedentary, had higher risks for CVD during 10 years follow up compared with the active and non-sedentary group. The sedentary and active group had no significant higher risk score for CVD compared with the active, non-sedentary group (Loprinzi & Davis, 2015).

3.6.2 Brief conclusion

There is inconsistent longitudinal evidence for an association of self-reported SB and CVD independent of MVPA. Future longitudinal studies employing objectively assessed SB in association with CVD are warranted.

3.7 Sedentary behaviour and mortality

In 2008, inactivity caused 5.3 millions of all 57 million (9 %) deaths worldwide (I. M. Lee et al., 2012). Further, sitting causes 430.000 deaths worldwide each year. However, in this estimate it is assumed that inactivity and sitting are independent risk factors for mortality, which is not yet confirmed (de Rezende et al., 2016). As expected, there is no studies investigating an association of SB and mortality in children, as the potential mortality caused by sitting probably is caused by one or several non-communicable diseases, which usually evolves throughout adulthood (Organization, 2014). Some of the following studies included both adults and elderly in their meta-analyses and therefore this summary of evidence between SB and mortality includes both adults and elderly.

3.7.1 Adults and elderly

The overview of systematic reviews identified seven reviews investigating SB and mortality

in adults and all studies report consistent findings (de Rezende, Lopes, et al., 2014). For example, each two-hour increase in television viewing resulted in a 13 % increased risk for mortality (RR: 1.13 (95 % CI, 1.07-1.18) (Grøntved & Hu, 2011).

Further, one meta-analysis including six prospective studies reported that the risk for mortality increased with 2 % (HR: 1.02, 95 %: 1.01-1.03) for each one-hour increase in screen time independent of MVPA. Without adjustment for MVPA, this risk was doubled (HR: 1.04, 95 % CI: 1.02-1.05). If placing subject in groups of <3 h-, 3-7 h- and >7 h • day⁻¹ of sitting, the hazard ratios were 1.00 (95 % CI: 0.98-1.03), 1.02 (95 % CI: 0.99-1.05) and 1.05 (95 % CI: 1.02-1.08), respectively (Chau et al., 2013). Furthermore, a meta-analysis of 14 studies reported that higher amounts of self-reported sedentary time was associated with a 22 % increased risk for mortality independent of MVPA (HR: 1.22, 95 % CI: 1.08–1.38). However, the hazards ratios varied depending on the amount of MVPA. In fact, the modifying effect of PA suggest no risk for mortality independent of MVPA (high MVPA, high SB; HR: 1.16, 95 %: 0.86-1.59). In comparison, high amounts of SB but low amounts of MVPA increased this risk (HR: 1.46, 95 % CI: 1.22–1.75) (Biswas et al., 2015).

Recently, using an isotemporal substitution model, it was demonstrated that replacing self-reported SB with standing, walking and MVPA decreased the hazard ratio for mortality with 0.95 (95 % CI: 0.94–0.96), 0.86 (95 % CI: 0.81–0.90) and 0.88 (95 % CI: 0.85–0.90), respectively (Stamatakis et al., 2015). An isotemporal substitution model allows one behaviour to be replaced with another where outcome data have continuous variables (Mekary, Willett, Hu, & Ding, 2009). Yet another similar study presenting results from an isotemporal substitution model demonstrated that replacing ACC measured SB with LPA reduced the risk of mortality with 14 % (HR: 0.86, 95 % CI: 0.83-0.90), and replacing SB with MVPA reduced this risk with 50 % (HR: 0.50 95 % CI: 0.31-0.80). If LPA was replaced with MVPA, the risk of mortality was reduced with 42 % (HR: 0.58, 95 % CI: 0.36-0.93) (Schmid, Ricci, Baumeister, & Leitzmann, 2016).

3.7.2 Brief conclusion

Based on the available evidence, it seems the association of SB and mortality may not be independent of MVPA.

3.8 Sedentary behaviour and cardiorespiratory fitness

Exercise physiology research has demonstrated that MVPA, in particular aerobic exercise, leads to improvements in CRF, and VO_{2max} is a major marker of CVD (Myers et al., 2015). However, there are few studies examining an association between SB and CRF.

In children, one systematic review including one high- and two low quality rated prospective studies reported moderate evidence for a longitudinal association between self-reported SB and CRF in children (Chinapaw et al., 2011). For example, the study rated high in quality was a birth cohort study, where screen time during childhood and adolescence remained a significant predictor of estimated CRF from heart rate in an all-out ergometer test at age 26, independent of MVPA at age 15 (Hancox, Milne, & Poulton, 2004). However, the biological plausibility is questionable and this may therefore be explained by confounding factors. In adults, 6-7 h • day⁻¹ of self-reported sedentary time was associated with negative effects on CRF equal to the positive effects of one hour of MPA (Kulinski et al., 2014). Unfortunately, there seem to be no available evidence using objective assessments of SB with CRF as outcome.

3.9 Sedentary behaviour and other health outcomes

In the overview of systematic reviews, SB was associated with some cancers, however, studies were few and were not adjusted for confounders such as BMI and MVPA. Further, there is insufficient evidence for any relationship between SB and bone mineral density in children and insufficient evidence for any association between SB and low back-, neck-, shoulder, hand- and arm pain in both children and adults (de Rezende, Lopes, et al., 2014). Finally, in elderly (>60 years), cross-sectional analysis reveal an increased risk for disability in activities of daily living for each one-hour increase of ACC measured SB independent of MVPA (OR: 1.46, 95 % CI: 1.07, 1.98) (Dunlop et al., 2015). However, since there is no association between SB and pain (de Rezende, Lopes, et al., 2014), a plausible explanation for the association between SB and disability in activities of daily living may be reverse causation but more studies are needed.

3.10 Observational studies: overall conclusion

Compared with objective assessments of SB (Saunders et al., 2014), subjective assessments suggest greater magnitude of associations with various health outcomes (Biswas et al., 2015; Ford & Caspersen, 2012; Grøntved & Hu, 2011). This may be explained by information bias. However, as those with poor health were less compliant in wearing an ACC compared with those with good health (P. H Lee et al., 2013), selection bias may also be present in objective assessments of SB. Overall, observational studies reported either no association or inconsistent evidence for an association between SB and health outcome independent of MVPA. And after all, causality remain unidentified.

4.0 Sedentary behaviour and health outcomes: experimental studies

4.1 Exercise physiology versus inactivity physiology

In 2004, it was proposed that the physiology during inactivity were different compared with the physiology during exercise (Hamilton, Hamilton, & Zderic, 2004).

“A goal of the inactivity physiology field is to recommend safe, sustainable, and science-based behaviours to the public. Behaviours at the lower intensities, if proven effective, hold great promise because if the perceived exertion is low enough, it might provide a more achievable solution for the large percentage of the population that is currently unfit, obese, aging, and too sedentary, yet desperately needs to be active” (Hamilton, Hamilton, & Zderic, 2014).

Four different concept within inactivity physiology were highlighted where research was warranted (Hamilton, Hamilton, & Zderic, 2007). The first concept is whether more sitting and less LPA would push people towards the high risk zone where people are least fit. For unfit individuals ($<40 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ in $\text{VO}_{2\text{max}}$), the lowest intensity threshold for improvements in CRF is observed at 30 % of VO_2 reserve, while improvements for fit individuals ($>40 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ in $\text{VO}_{2\text{max}}$) are observed above 45 % of VO_2 reserve (Garber et al., 2011). For unfit individuals, 30 % of VO_2 reserve is equal to a higher percentage of $\text{VO}_{2\text{max}}$ compared with fit individuals performing the same 30 % of VO_2 reserve (Swain & Franklin, 2002). This indicates that the determining factor for fitness improvements is performing sufficient amounts of percentage of $\text{VO}_{2\text{max}}$, which is LPA (<3 METs), MPA (3-6 METs) or VPA (>6 METs) (Ainsworth et al., 2011b) depending on fitness level. Thus, performing LPA (3 METs = $10.6 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ (Ainsworth et al., 2011b)) may in fact result in increases in CRF in unfit individuals, however, this increase is due to percentage of $\text{VO}_{2\text{max}}$ and not SB/LPA ratio *per se*.

The second concept is if SB and inactivity are two different behaviours distinct from each other. Today, this concept is clear and they are two distinct different behaviours (Sedentary Behaviour Research Network, 2012).

The third concept, and most central to a potential causal link between SB and health risk, is if some cellular or molecular processes are different in inactivity physiology and exercise physiology, which is supported by some observational studies (Hamilton et al., 2004). Based on studies of rodents (Bey & Hamilton, 2003), it is suggested that there is a potent regulatory process in lipoprotein lipase (LPL) that are only effected by SB and LPA. LPL is a key enzyme in lipid metabolism, and especially TG uptake into cells (Hamilton et al., 2004). In rodents, LPL activity was significantly decreased following 12 hours of inactivity but not following ambulatory activity. LPL activity was then reversed following four hours of ambulatory activity in the inactivity group (Bey & Hamilton, 2003). Further, LPL activity is reported to only increase in the glycolytic fast-twitch fibres and not in slow-twitch fibres following two weeks of intensive exercise (Hamilton, Etienne, McClure, Pavey, & Holloway, 1998; Seip, Mair, Cole, & Semenkovich, 1997). As a consequence, it is suggested that LPA should be performed daily in order to increase LPL-activity in slow-twitch fibres (Hamilton et al., 2007).

A follow-up study of Bey and Hamilton (2003) reported that one gene, LLP1, is down-regulated in both rats (N = 89) and humans (N = 3) following 12 hours of inactivity and did not return to normal levels following 4 • 30 minutes of treadmill exercise in rats, but did return to normal levels following 12 hour of standing in humans. LPP1 is proposed to have an antagonistic role in platelet aggregation and inflammation. Hence, LPP1 may play a role in the development of deep venous thrombosis, which is caused in part by no contractile activity of leg muscles (Zderic & Hamilton, 2012). Deep venous thrombosis is one condition that was suggested to be caused by prolonged sitting and not just lack of PA, and it is plausible that breaking SB may prevent blood to clot in the periphery vessels (Hamilton et al., 2007). However, it seems only those with additional risk factors is exposed to an increased risk for deep venous thrombosis following prolonged sitting during long-haul flights (Schwarz et al., 2003), indicating that sitting alone may not cause deep venous thrombosis.

Based on the LPL activity hypothesis, one randomized crossover study investigated the postprandial effect on LPL activity in humans undergoing three conditions; 1) sitting for seven hours and 45 minutes, 2) standing six times for 45 minutes during seven hours and 45 minutes, 3) 30 minutes walking at self-selected speed. Blood samples were collected in both fasting and postprandial state in two consecutive days where day two consisted six hours of sitting in all three conditions (Miyashita et al., 2013). In short, measuring LPL enzyme

activity in the rats were done by killing the rats (Bey & Hamilton, 2003). In the human study, measurements were performed by blood sampling. LPL protein mass, apolipoprotein C-II (activator of LPL) and apolipoprotein C-III (inhibitor of LPL) were not affected following the three conditions. However, a lower TG incremental area under curve (iAUC) were observed following 30 minutes of walking compared with the two other conditions. Thus, LPL activity may not be the dominating mechanism in lowering postprandial TG concentration in humans (Miyashita et al., 2013)

Finally, the fourth concept proposed is that increased rates of CVD, T2DM, MetS and obesity are observed in cohorts of people after adjustment for age, and this is not just caused by lack of exercise (Hamilton et al., 2004). However, plausible explanations may be other known risk factors, such as unbalanced energy intake/poor diet and smoking (Organization, 2014).

Even though the proposed hypothesis of down-regulated LPL activity maybe falsified in a human study (Miyashita et al., 2013), the study was not mentioned in Hamilton and colleague's latest review (Hamilton et al., 2014). To sum up, at date, there is no evidence to confirm that inactivity physiology is distinct different from exercise physiology.

4.2 Reduction and breaking up sedentary behaviour

Recently, the first systematic review on the effect of breaking up prolonged sitting time was published. There seem to be no randomized controlled trials examining the effect of breaking up prolonged sitting, and the majority of studies included were randomized crossover studies (Benatti & Ried-Larsen, 2015).

4.2.1 Children/adolescents

In two meta-analyses, the estimated intervention effect of reducing SB in obese children and adolescents were $-0.25 \text{ m} \cdot \text{kg}^{-2}$ (95 %: -0.40 - $-0.09 \text{ m} \cdot \text{kg}^{-2}$) (van Grieken, Ezendam, Paulis, van der Wouden, & Raat, 2012) and $-0.89 \text{ m} \cdot \text{kg}^{-2}$ (95 % CI: -1.67 ; $-0.11 \text{ m} \cdot \text{kg}^{-2}$) in BMI (Tremblay, LeBlanc, Kho, et al., 2011). $-0.25 \text{ m} \cdot \text{kg}^{-2}$ and $-0.89 \text{ m} \cdot \text{kg}^{-2}$ in BMI is equal to a weight loss of one and three kg, respectively, which is clinical relevant. However, some intervention durations seem short to reduce weight. Interventions were between one week and four years (van Grieken et al., 2012) and 8-12 weeks (Tremblay, LeBlanc, Kho, et al., 2011).

Moreover, only 13 out of 44 studies (van Grieken et al., 2012) and one out of four studies (Tremblay, LeBlanc, Kho, et al., 2011) reported significant decreases in BMI. Finally, growth in terms of height is a potential confounding factor for decreases in BMI in children and adolescents.

In 10-14-year-old children, eight hours of sitting, eight hours of sitting with breaks of two-min walking every 20 minutes and two 20-min bouts of continuous MPA in addition to breaks of two-min walking resulted in no significant differences in insulin, glucose, or TG iAUC (Saunders, Chaput, et al., 2013). In 14-year-old children, three hours of sitting compared with three times 45 minutes LPA resulted in no differences for insulin, triglycerides and HDL-C area under curve (AUC) (Sisson et al., 2013).

4.2.2 Adults

A systematic review reported moderate quality rated evidence for deleterious changes in insulin sensitivity, glucose tolerance and plasma triglyceride levels following acute prolonged sitting and lying. There was very low quality rated evidence for deleterious changes in fasting insulin, fasting glucose, HDL-C, and LDL-C. (Saunders, Larouche, Colley, & Tremblay, 2012).

In inactive, overweight and obese adults, changing from sitting to standing every 30 minutes for eight hours compared with continuous sitting resulted in a lower glucose iAUC (Thorp et al., 2014). In contrast, in non-obese adults, two-min bouts of standing every 20 minutes during five hours of sitting compared with prolonged sitting did not result in a lower postprandial glucose AUC, but two-min bouts of walking did (Bailey & Locke, 2015). However, in the interchanging standing and sitting condition, ambulatory activities were allowed when standing (Thorp et al., 2014). Thus, the ambulatory activities while standing may be equal to LPA. These results may corroborate the findings from Bailey and Locke (2015).

Further, in another study in inactive, overweight and obese individuals, postprandial glucose iAUC were 24- and 29 % lower following two-min bouts of LPA and two-min bouts of MPA every 20 minutes, respectively, compared with seven hours of sitting. In addition, insulin iAUC were 23 % lower following both activity conditions compared with sitting. All

conditions started with a two hour steady state sitting prior to meal ingestion and activities (Dunstan et al., 2012). However, these results seem to be transient, as three consecutive days with the same protocol resulted in no additional effect in glucose and insulin iAUC (Larsen et al., 2015). The same protocol resulted in lower systolic and diastolic blood pressure for both two-min bouts of LPA and MPA every 20 minutes in overweight and obese adults (Larsen et al., 2014).

Furthermore, in a sub-sample of the participants in the study by Dunstan et al. (2012), an observed increase in haematocrit, haemoglobin, red blood cells and plasma volume were attenuated in both activity conditions compared with sitting, while an observed increase in plasma fibrinogen were only attenuated in the two-min bouts of LPA (Howard et al., 2013). As proposed above (Hamilton et al., 2007), breaking up prolonged sitting may decrease the risk of deep venous thrombosis, which is supported by these results (Howard et al., 2013). This further suggest that subjects at risk should break their prolonged sitting in order to decrease the risk of deep venous thrombosis.

In apparently healthy young adults, eight minutes cycling bouts compared with eight hours of sitting resulted in a lower C-peptide level although all other metabolic risk factors were unaffected by the exercise. Differences between the abovementioned studies and this study may be explained by different blood sampling method and healthier subjects (Altenburg, Rotteveel, Dunstan, Salmon, & Chinapaw, 2013).

Some studies have included one condition of continuous activity in addition to one condition of small bouts of activity in comparison with prolonged sitting. In healthy young men, 30 minutes self-selected walking speed ($6.8 \text{ km} \cdot \text{h}^{-1} / 41 \% \text{ of } \text{VO}_{2\text{max}}$) accumulated in ten short bouts and one continuous bout resulted in equally lower postprandial TG, glucose, insulin and BP AUC compared with prolonged sitting when assessments were done the following day during seven hours of prolonged sitting (Miyashita, Burns, & Stensel, 2008). In contrast, one hour of running at 65 % of $\text{VO}_{2\text{max}}$ resulted in a lower postprandial TG, glucose and insulin incremental area under curve (iAUC) compared with small bouts of self-selected walking speed (approximately 25 % of $\text{VO}_{2\text{max}}$) and prolonged sitting in healthy young men when iAUC assessments were done the following day during six hours of prolonged sitting. PAEE were matched in both activity conditions (Kim, Park, Trombold, & Coyle, 2014).

In one study including healthy, normal weight adults, iAUC assessment were done the same days as activity conditions. Postprandial glucose and insulin iAUC were lower following 18 100 seconds bouts at 45 % of VO_{2max} compared with both 30 minutes continuous running at 60 % of VO_{2max} followed by eight hours and 30 minutes sitting and nine hours prolonged sitting. TG iAUC however, were significantly lower following continuous running compared with sitting, but not breaks with small bouts of 100 seconds. There were no differences in TG iAUC in prolonged sitting compared with small bouts of 100 seconds. PAEE were matched in both activity conditions (Peddie et al., 2013).

In T2DM subjects, 45 minutes of continuous VPA (6 METs) compared with prolonged sitting resulted in a significant lower glucose 24 hour iAUC and this was almost lower when compared with three 15-min bouts of MPA (3 METs) ($p = 0.06$). Insulin iAUC were lower following continuous VPA compared with both prolonged sitting and three bouts of MPA during 11 hours (Van Dijk et al., 2013). The discrepancy in glucose and insulin iAUC between T2DM subjects and healthy adults may be explained by adenosine monophosphate activated protein kinase, which is more prominent in T2DM compared with healthy individuals (Benatti & Ried-Larsen, 2015). Adenosine monophosphate activated protein kinase is an inhibitor of fat and glucose absorption in muscles (Sriwijitkamol et al., 2007). An additional suggested explanation for this discrepancy was higher intensity in the study by Van Dijk et al. (2013) (6 METs) compared with the study by Peddie et al. (2013) (60 % of VO_{2max}) (Benatti & Ried-Larsen, 2015). However, due to a possibly lower CRF in T2DM patients, 6 METs may be equal or even higher than 60 % of VO_{2max} .

In obese adults, two conditions with one continuous bout of activity with 50- and 65 % of VO_{2max} , respectively, after seven hours of prolonged sitting resulted in no significant different glucose and insulin AUC the following day (Newsom, Everett, Hinko, & Horowitz, 2013). Finally, in healthy young men, seven hours and 45 minutes of sitting compared with standing six times for 45 minutes and continuous running for 30 minutes at 60 % of age-predicted heart rate maximum (HR_{max}) resulted in no difference in oxidative stress markers in both the same and the following day (Takahashi, Miyashita, Park, Sakamoto, & Suzuki, 2015).

4.2.3 Free-living

A free-living study conducted for four consecutive days suggested that a large amount of LPA was more beneficial compared with one hour of exercise in relation to influence on TG, non HDL-C (total cholesterol-HDL-C) and post-prandial insulin (Duvivier et al., 2013). However, limitations include methods for assessing PAEE (ActivPAL for small bouts of LPA and heart rate for exercise) and not controlling for caloric intake (Benatti & Ried-Larsen, 2015).

In another free-living study, frequent long breaks for <20 min was compared with 30 minutes continuous MPA at lunch time with matched PAEE and one frequent short break condition in addition to long breaks of <20 min (higher PAEE) in overweight and obese adult office workers. There were no differences following any conditions in glucose and insulin iAUC following two hours of sitting in the afternoon. Secondary outcomes included nocturnal glucose and glycaemic variability. An elevated nocturnal glucose concentration was observed following continuous MPA during lunch time compared with both frequent breaks conditions. Glycaemic variability was lower following both frequent breaks conditions compared with continuous MPA at lunch time, which may indicate that individuals at risk (e.g. MS and T2DM) should conduct more frequent breaks of activity instead of one single bout to avoid hyperglycaemia (Blankenship, Granados, & Braun, 2014).

4.2.4 Gene expression

In a subsample of the participants in the study by Dunstan et al. (2012), seventy-five genes were identified to be expressed differently between conditions. Genes were assessed from muscle biopsies of the m. vastus lateralis. 10 genes involved in glucose metabolism were identified to be differently expressed between conditions. One gene (CCL13), involved in glucose osmosis into cells, were down-regulated with breaks of LPA, but up-regulated with breaks of MPA. PDK4, which inhibits pyruvate dehydrogenase complex and increases glucose metabolism, were up-regulated in both activity conditions, but were more up-regulated following bouts of MPA. All other genes were only up-regulated following activity bouts of MPA (Latouche et al., 2013). Muscle oxidative phosphorylation and mitochondrial function has been shown to be down-regulated during bed-rest (Ringholm et al., 2011), and this seem not to be up-regulated by two-min bouts of LPA and MPA (Latouche et al., 2013). One gene, USP6, which is observed to be up-regulated following 48 hours of immobilisation (Urso, Scrimgeour, Chen, Thompson, & Clarkson, 2006), were down-regulated with bouts of

MPA. USP6 plays a role in protein degradation and thereby is of relevance to muscle atrophy. Thus, this down-regulation of USP6 following bouts of MPA may theoretically reduce breakdown of protein and thereby avoid the observed muscle atrophy following prolonged sitting (Latouche et al., 2013).

4.2.5 Sit-to-stand workstations

One recent review including nine studies concluded that it is unclear if implementing sit-to-stand workstations can reverse the detrimental health outcomes caused by sitting because EE is only slightly increased. Sitting at work was reduced with 30 minutes per workday, however, there were no difference in work performance, musculoskeletal symptoms or sick leave (Shrestha et al., 2016). This may be due to compensation, because a compensation effect was observed in ACC assessment of SB by sitting more in non-working hours when implementing sit-to-stand workstations at work (Mansoubi, Pearson, Biddle, & Clemes, 2015).

4.3. Experimental studies: overall conclusion

There is limited evidence to suggest inactivity physiology is distinct different from exercise physiology in humans. Prolonged sitting results in increased cardio-metabolic risk, however, this is reversible with breaks of PA and PA of higher intensity results in more positive health outcomes. In children, there is low quality evidence to suggest reducing SB will result in decreased BMI. There is inconsistent evidence to suggest a specific activity bout length in breaks when sitting for prolonged time, and this seems to be dependent on subjects' characteristics such as weight and fitness level. Sit-to-stand workstations do not result in better health outcomes although sitting time is reduced in adults.

5.0 Objective and hypothesis

The objective of this study is to examine if breaking up sedentary behaviour with short five minutes' bouts or one continuous 30-minute bout of vigorous intensity activity differentially affects metabolic risk markers compared with a control condition of prolonged sitting. The primary outcome is postprandial insulin total area under curve (AUC) and incremental area under curve (iAUC). I hypothesise that *“30 minutes of continuous VPA /exercise at 70 % of VO_{2max} is associated with an equal postprandial insulin response compared with breaking up sedentary time every 60 minute keeping exercise energy expenditure and intensity constant”*.

Secondary outcomes include glucose, triglycerides, high density lipoprotein cholesterol (HDL-C) and systolic and diastolic blood pressure (BP) AUC and iAUC. The five outcomes measurements are, except insulin, the International Diabetes Federation's diagnostic criteria for the MetS (Alberti et al., 2006). WC was excluded because a change in WC seemed unrealistic with the present study design.

6.0 Methods

6.1 Design and subjects

This study is an exploratory study using a randomized crossover design. The experiment was carried out between October and December 2015. The three conditions were randomized using Research Randomizer (Urbaniak & Plous, 2013) to control for any confounding factors. All testing procedures were pilot tested prior to the start of the study.

Participants were recruited through posters at the University of Oslo, Campus Blindern, and through e-mail to the study coordinators who forwarded the mail to their students at four other educational institutions in the Oslo Area; Norwegian Academy of Music, Bjørknes College, Norwegian University of Life Sciences and Oslo National Academy of the Arts. The aim was to recruit participants who could represent the fitness level of the general population. Students from the Norwegian School of Sport Sciences were excluded as they are more likely to have a higher fitness level and be more active than the general population.

6.2 Inclusion and exclusion criteria

Inclusion criteria were age between 18 and 40 and BMI classification as normal- and overweight (18-30). Exclusion criteria were BMI classification as obese (>30) or underweight (<18), Norwegian School of Sport Sciences' students, disease that is known to influence the outcome measurements, such as MetS, T2DM, CVD and hypertension. And medication that is known to influence these outcome measurements.

6.3 Ethics

This study was done according to the Declaration of Helsinki (WMA, 2013). Prior to testing, the study design was explained verbally and in writing and all participants provided written informed consent. Participants were free, at any time, to withdraw from the study without giving any reason and without any form for consequences (Appendix 1).

The Regional committees for medical and health research ethics (REK) consider the study did not fall under the Law of Medical and Health Research and thus exempt from approval (Appendix 2). The Data Protection Official for Research (NSD) in Norway, approved the

storage of personal data (Appendix 3).

6.4 Protocol

Participants visited the laboratory at the Norwegian School of Sport Sciences for a pre-test and three additional visits with at least a 6-day washout period between each visit. The following experimental conditions were performed in random order:

1. Sedentary behaviour condition (SB)
Six hours of sitting.
2. Breaking sedentary behaviour condition (breaking SB)
Six hours of sitting, with breaks of running on a treadmill for five minutes at 70 % of VO_{2max} each hour. In total six times.
3. Continuous vigorous physical activity condition (continuous VPA)
Running on a treadmill for 30 minutes at 70 % of VO_{2max} , followed by five hours and 30 minutes of sitting.

Condition two (breaking SB) and three (continuous VPA) both included running for 30 minutes at 70 % of VO_{2max} with an equal duration of rest ensuring the two different conditions are iso-caloric. The process of the experiment is shown in figure 4.1.



Figure 4.1: showing the process of the experiment

The participants arrived in the laboratory following at least 12 hours in a fasted state between eight and nine am. Prior to each visit, the participants were instructed to avoid alcoholic beverages and refrain from exercise 24 hours prior to testing and refrain from tobacco/nicotine and caffeine on the assessment days. If for any reason these instructions were not met, the participants were instructed to undertake exactly the same routine each time before each condition. For example, if they had exercised the day before at 6 pm, they had to do the same exercise at the same hour prior to their next visit. All except one reported to have successfully avoided, alcohol, exercise, tobacco/nicotine and caffeine at every visit.

The experimental protocol is summarised in Figure 4.2. In short, seven blood samples (BS) and BP were collected at each occasion, one at baseline in a fasted state and thereafter one sample every hour. All BS were collected prior to running for 5 minutes in BSB condition. The blood pressure measurements followed immediately after the blood sampling. Following the fasting blood sample, participants received a standardized meal in form of a shake. The shake contained 646 kilocalories, of which 19 % (31 grams (g)) is protein, 51 % (82 g) is carbohydrates and 30 % (22 g) is fat. Except sugar (18 % (29 g)) and saturated fat (15 % (11 g)), this is within the recommended nutrition guidelines from Nordic Nutrition Recommendations of 2012 (NNR, 2012).

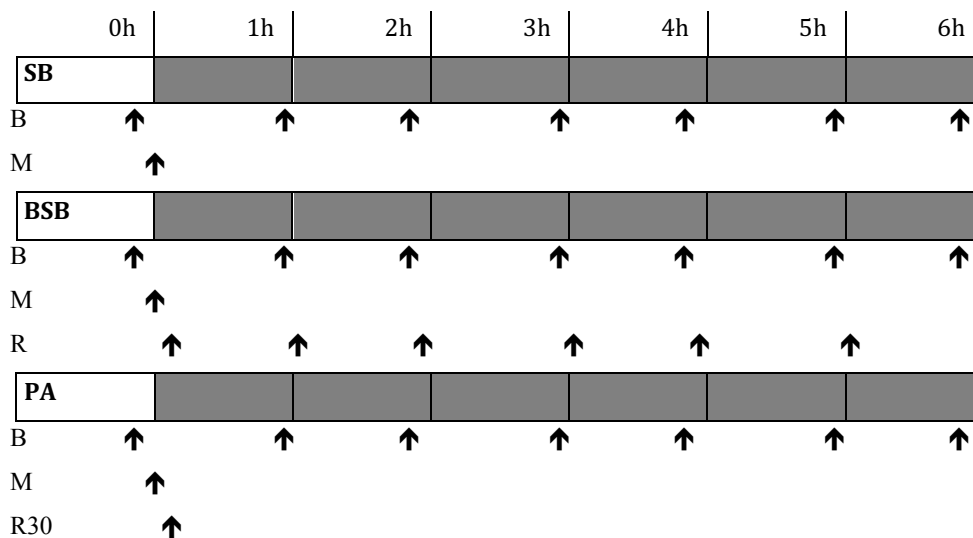


Figure 4.2: showing the protocol. SB= Sedentary behaviour condition, BSB= Breaking sedentary behaviour condition, PA= Physical activity condition, B= Blood Samples and blood pressure, M= Meal, R= Run 5 minutes, R30= Run 30 minutes continuously

6.5 Assessments

6.5.1 Blood samples

Blood samples were collected using a 1.3 x 32 mm peripheral catheter (BD Venflon™ Pro Safety Shielded IV Catheter, United States) connected to a 3-way stopcock (BD Connecta™,

United States) inserted in the median cubital vein. A medical laboratory technologist or a doctor inserted the catheter. Five ml of Saline water (9 mg/ml Natriumchlorid B. Braun Melsungen AG, Germany) was used to rinse the stopcock and catheter followed by drawing 2.5 ml of blood using a 5 ml syringe (B. Braun Melsungen AG, Germany) which was discarded before the actual blood sampling. The stopcock and catheter were thereafter rinsed which was repeated every 15 minutes during the test to avoid blood coagulation.

Blood were transferred into 8 ml serum tubes (BD Vacutainer® SST™ Serum Separation Tubes, United States) for measurements of glucose, triglyceride and HDL-C and five ml serum tubes (Vacuette® Z Serum Sep Clot Activator tubes, Austria) were used for insulin measurements. The tubes were then inverted eight times and the blood was allowed to clot for 30-120 minutes. The samples were further centrifuged at 3400 revolutions per minute for 10 minutes using a centrifuge machine (Heraeus Instruments, Megafuge 1.0R, United Kingdom) at 4 °C.

The samples for glucose, triglyceride and HDL-C in the 8 ml tubes were stored in a fridge overnight at 4 °C and thereafter transported to a certified Medical Laboratory for analysis. Analyses of glucose, triglyceride and HDL-C were performed using an automatic analysing machine (ADVIA 2400 Chemistry System, Siemens Medical Solutions Diagnostics, Japan). Insulin samples were further transferred into 75x13 mm tubes suitable for freezing, and were frozen at -80 °C. They were later transported to the Hormonal Laboratory at the Oslo University Hospital for analysis in one batch at the end of the project. Analyses of insulin were performed using an automatic analyzing machine (Modular E170, Roche Diagnostics, Switzerland).

6.5.2 Blood pressure

Systolic and diastolic blood pressure (BP) were measured in a sitting position using an automatic sphygmomanometer (Welch Allyn Spot Vital Signs LXi, United States). BP was measured after blood sampling in the opposite arm in all participants. BP was measured three times separated by one minute. The mean of the first two recordings were used as BP. If the difference between these recordings was greater than five mmHg, the mean of all three recordings were used. This procedure is recommended by the Subcommittee of Professional

and Public Education of the American Heart Association Council on High Blood Pressure Research (Pickering et al., 2005).

6.5.3 Weight, height and body mass index

Prior to pre-testing, participants stood on an automatic weighting scale (Seca 876, Seca GmbH & Co. KG, Germany) and in front of a stable stadiometer (Seca 217, Seca GmbH & Co. KG, Germany) for weight and height measurements, respectively. Body mass index (BMI) was then calculated using the following formula: $\text{kg} \cdot \text{m}^{-2}$.

6.5.4 Pre-test

Prior to start, all participants underwent a submaximal graded steady state exercise test followed by a test to maximal exhaustion while running on a motorized treadmill (Woodway pps55 sport, Woodway GmbH, weil an Rhein, Germany) where VO_2 was assessed using indirect calorimetry. Participants were fitted with a nose-clip and a two-way mouthpiece (Hans Rudolph Instr., Germany) connected to a mixing chamber, and VO_2 was recorded each 30 seconds in an integrated O_2 and CO_2 analyzer (Oxycon-Pro®, Jaeger Instr., Germany), which uses paramagnetic principle for analyses of O_2 and infrared absorption for CO_2 . Before measurements, sensors were automatically calibrated for O_2 and CO_2 using a gas tank with known concentrations of O_2 and CO_2 (approximately 14.93% and 5.99%, respectively) as well as the O_2 and CO_2 concentration of the room (approximately 20.90 % and 0.04 %, respectively). Volume was manually calibrated using a three-liters volume pump (Calibration Syringe, series 5530, Hans Ruolph Instr; MO, Germany). The inclination of the treadmill was set to 5.3 % during all tests.

6.5.4.1 Submaximal graded steady state exercise test

The submaximal graded steady state exercise test is showed in Figure 4.3. The test lasted in total for 16 minutes, with four one-kilometer per hour ($\text{km} \cdot \text{h}^{-1}$) increasing workloads. The starting workload was either 5- or 6 $\text{km} \cdot \text{h}^{-1}$, determined by a subjective assessment of the participants. Protocol is similar to the one conducted in the study by Carlsen et al. (2001) with one adjustment; no pause to collect fingertip blood samples were needed in this trial, and as three minutes is sufficient to reach a steady state plateau VO_2 (Ward & Palange, 2007), five minutes workloads was reduced to four minutes. Recordings of VO_2 were performed the last

two minutes and the mean of the two highest consecutive 30-seconds recordings were used as steady state.

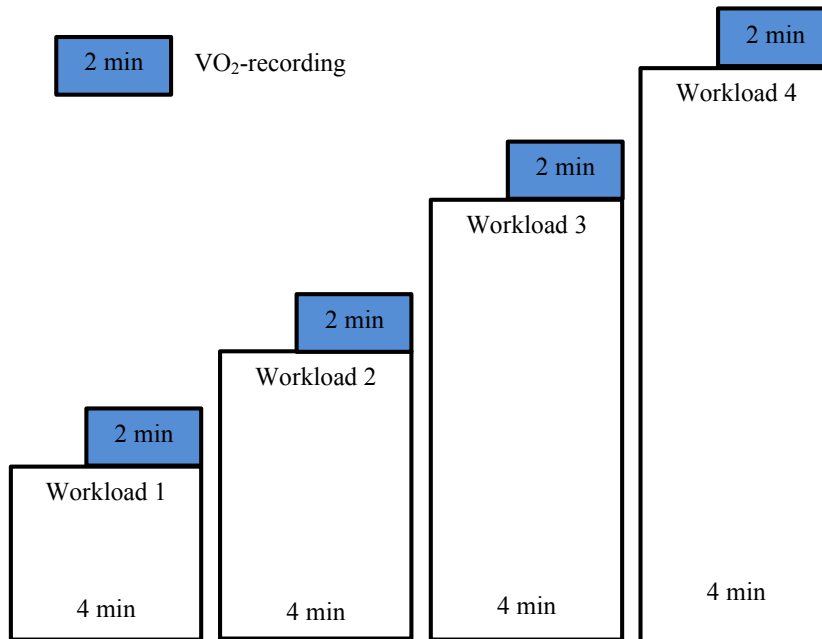


Figure 4.3: showing a graded steady state exercise test. Each workload increased with $1 \text{ km} \cdot \text{h}^{-1}$. VO_2 -recording = oxygen uptake recording.

An example of a relationship between treadmill speed and VO_2 is shown in Figure 4.4. By using the regression formula (y) obtained following the submaximal graded exercise test and $\text{VO}_{2\text{max}}$ -value following the maximal test (protocol explained below; 6.7.4.2, maximal aerobic capacity), 70 % of $\text{VO}_{2\text{max}}$ was calculated by extrapolation for each participant.

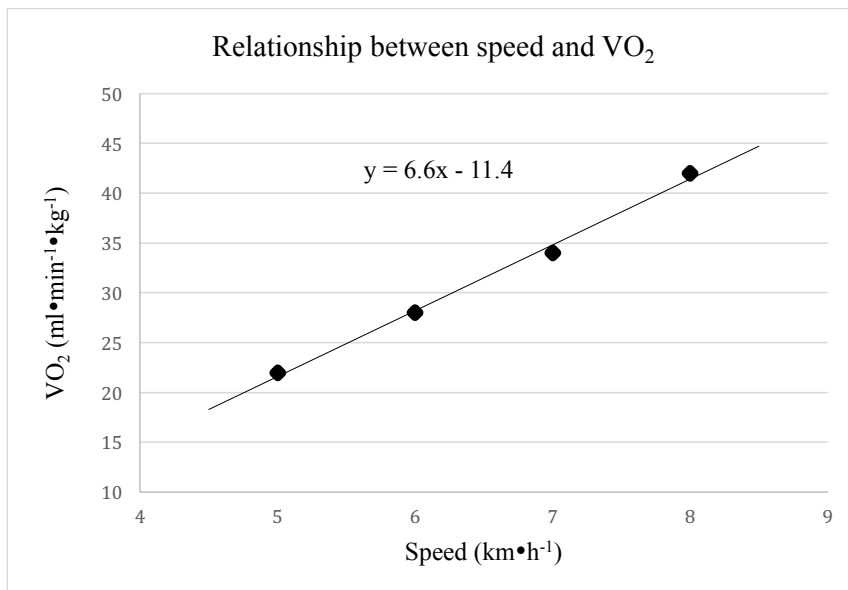


Figure 4.4: example of a scatter plot of speed and oxygen uptake (VO_2) following a submaximal graded steady state exercise test. $Km \cdot h^{-1}$ = kilometres per hour.

6.5.4.2 Maximal aerobic capacity

Immediately following the steady state test participants walked on the treadmill at four $km \cdot h^{-1}$ while they received instructions about the maximal exercise test. The maximal exercise test started at eight $km \cdot h^{-1}$ or nine $km \cdot h^{-1}$ depending on the respiratory exchange ratio (RER) during the final stage at the steady state test at 5.3 % degrees. The speed of the treadmill was increased with one $km \cdot h^{-1}$ each minute until exhaustion. At each workload, after 30 seconds the participant was asked if he/she could cope with an increase of one $km \cdot h^{-1}$. The participant communicated with a thumb up for yes and a thumb down for no. If no, the participant where asked if he/she could cope with an 0.5 $km \cdot h^{-1}$ increase. If no again the participant maintained the workload for at least one minute.

The following criteria were used to determine whether participants reached their maximal oxygen uptake (VO_{2max}); 1) A plateau in oxygen uptake despite increase in workload; 2) a RER-value of at least 1.05 (Åstrand, 2003). Due to the participants' inexperience with the test and ability to push themselves, a plateau despite increase in workload was only obtained for

three participants. VO_{2max} was defined as the mean of the two highest consecutive 30-seconds recordings, and expressed as $ml \cdot min^{-1} \cdot kg^{-1}$.

6.6 Outcome measurements

6.6.1 Total area under curve

AUC ground represents the entire area underneath the curve (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003) and will in this thesis be expressed as total area under curve (AUC). This method is the summation of trapezoids, where one trapezoid represents the area underneath the line between two measure points in a time series (Pruessner et al., 2003). The following mathematical formula was used to calculate AUC; $(a^1 + a^2) \cdot time^{-1} \cdot 2^{-1}$. a^1 is measure value one, a^2 is measure value two and time is time difference between two measurements. The calculated trapezoids were then summed for each participant, to provide the total area under curve (AUC).

6.6.2 Incremental area under curve

Incremental area under curve (iAUC) represents how much the AUC increments from baseline in form of triangles, and this is performed using a method where all values below baseline values for the time series are ignored (Wolever, 2004). iAUC was calculated using three different mathematical formulas depending on the start- and end measure value being above, subsiding below or rising above the baseline value for the time series.

The first formula was used when both values were either equal or above the baseline value in the time series. This formula is; $(a^1 + a^2) \cdot \frac{1}{2} time^{-1} - (b \cdot time)$. a^1 is measure value one, a^2 is measure value two, b is baseline value, $\frac{1}{2} time$ is half the time difference between measurements and time is time difference between measurements.

The second formula was used when the start value was above and the end value was subsiding below the baseline value. This formula is; $((a^1 - b) \cdot ((a^1 - b) \cdot (a^1 - a^2)^{-1} \cdot time) \cdot 2^{-1})$. a^1 is measure value one, a^2 is measure value two, b is baseline value and time is time difference between measurements.

The third formula was used when the start value was below and the end value was rising above the baseline value. This formula is; $((a^2 - b) \cdot ((a^2 - b) \cdot (a^2 - a^1)^{-1} \cdot \text{time}) \cdot 2^{-1})$. a^1 is measure value one, a^2 is measure value two, b is baseline value and time is time difference between measurements. Total iAUC for each participant was then calculated by summing each triangle for the entire time series.

6.7 Statistical analyses

Statistical Package for the Social Sciences for Mac, version 22 (International Business Machines Corporation, United States) was used to perform statistical analyses. Microsoft Excel 2016 for Mac (Microsoft Cooperation, United States) was used to calculate AUC and iAUC, and create figures. In all analyses, alpha value was set to 5 % ($\alpha = p = <0.05$). All analyses were performed as intention to treat (ITT) and per protocol (PP). In ITT analysis, the values of those who completed one condition, were carried forward for the other conditions. For the participant who completed two conditions, a mean of these values was carried forward. In PP analyses, only those who completed all conditions were included (Laake, Olsen, & Benestad, 2008). Due to the exploratory nature of this study, only results from PP analyses are shown in the results, if not otherwise is stated. Univariate analysis of variance (two-way ANOVA) was performed to assess sex differences in descriptive characteristics and between-condition effect for AUC, iAUC and one-hour postprandial insulin and glucose concentration.

7.0 Results

7.1 Participants

12 healthy adults, men (N = 3) and women (N = 9), volunteered to participate. Prior to pre-test, one of the participants withdrew due to milk allergy. All, except one, was classified as normal weight ($<25 \text{ kg} \cdot \text{m}^{-2}$) by BMI. Six participants completed all three conditions, one completed two conditions and four completed one of the three conditions. Reported reasons for dropout were illness, tiredness after visit and lack of time. Descriptive characteristics of the participants who completed all conditions are shown in total and in men and women separately in table 5.1. Of those who completed all conditions, all were classified as normal weight by BMI.

Table 5.1. *descriptive characteristics of participants. Data are Mean \pm standard deviation (SD). BMI = body mass index, kg = kilograms, $\text{VO}_{2\text{max}}$ = maximal oxygen uptake.*

Variable	Total (N = 6)	Men (N = 2)	Women (N = 4)
Age (years)	21.5 \pm 2.7	23.5 \pm 4.9	20.5 \pm 1.7
Height (centimeters)	172.4 \pm 11.6	187.0 \pm 2.8	165.1 \pm 2.7
Weight (kg)	65.0 \pm 10.5	76.8 \pm 4.3	59.1 \pm 6.3
BMI ($\text{kg} \cdot \text{m}^{-2}$)	21.6 \pm 1.7	21.8 \pm 1.9	21.6 \pm 1.9
$\text{VO}_{2\text{max}}$ ($\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$)	49.4 \pm 7.3	56.4 \pm 3.2	46 \pm 6.1

7.1.1 Descriptive differences in sex

There were no statistical significant differences in descriptive characteristics between men and women in terms of age ($p = 0.24$), BMI ($p = 0.92$) or $\text{VO}_{2\text{max}}$ adjusted for body weight ($p = 0.54$). To check for consistency, sex differences were also tested in those participants who completed one or two conditions (ITT-analysis). There was a tendency for a statistically significantly age difference ($p = 0.06$), but no statistically significantly differences in BMI ($p = 0.33$) and $\text{VO}_{2\text{max}}$, adjusted for body weight ($p = 0.12$).

7.2 Insulin

Results for insulin AUC and iAUC are shown in table 5.2.

Table 5.2: results of insulin AUC and iAUC. Data are mean (95 % confidence interval (95 % CI)) and p-value for between-condition effect. $\text{pmol} \cdot \text{L}^{-1}$ = Picomole per liter blood, AUC = total area under curve, iAUC = incremental area under curve.

Insulin	Sedentary Behaviour	Breaking Sedentary Behaviour	Continuous Physical Activity	p-value
AUC ($\text{pmol} \cdot \text{L}^{-1} \cdot 360$ min)				0.74
Mean (95 % CI)	1221 (818.4- 1566.2)	1091 (671.8- 1419.7)	985,7 (639.6- 1387.5)	
iAUC ($\text{pmol} \cdot \text{L}^{-1} \cdot 360$ min)				0.71
Mean (95 % CI)	60704.8 (36352.4- 79412.1)	53375.7 (28787.8- 71847.5)	44827.3 (24853- 67912.7)	

There were no statistical significant differences in insulin AUC and iAUC across conditions. Both AUC and iAUC were lower following continuous VPA condition compared with both Breaking SB and SB condition. Mean insulin concentration for each measure point during six hours are shown in Figure 5.1.

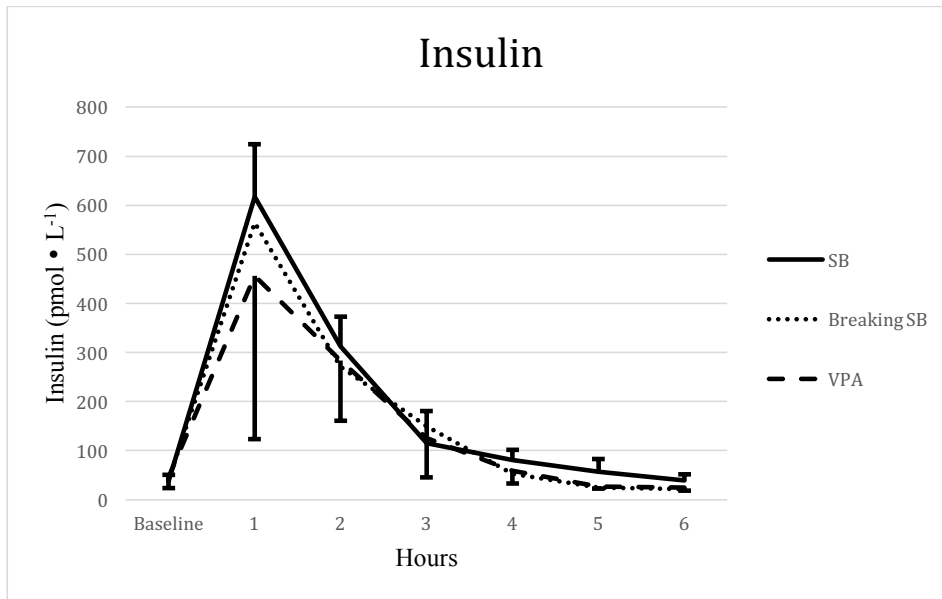


Figure 5.1: showing mean insulin concentration during six hours. Values are mean and error bars are SD. SB = sedentary behaviour condition, Breaking SB = Breaking sedentary behaviour condition, VPA = continuous vigorous physical activity condition.

Mean insulin concentration rises between baseline and one hour. Mean one-hour insulin concentration was lower following VPA condition ($456 \text{ pmol} \cdot \text{L}^{-1}$, SD: $303 \text{ pmol} \cdot \text{L}^{-1}$) compared with both Breaking SB ($564 \text{ pmol} \cdot \text{L}^{-1}$, SD: $142 \text{ pmol} \cdot \text{L}^{-1}$) and SB condition ($617 \text{ pmol} \cdot \text{L}^{-1}$, SD: $235 \text{ pmol} \cdot \text{L}^{-1}$). However, there were no significant differences across conditions for one-hour insulin concentration ($p = 0.63$). Mean insulin concentration during six hours were $180 \text{ pmol} \cdot \text{L}^{-1}$ (SD: $199 \text{ pmol} \cdot \text{L}^{-1}$), $160 \text{ pmol} \cdot \text{L}^{-1}$ (SD: $185 \text{ pmol} \cdot \text{L}^{-1}$) and $146 \text{ pmol} \cdot \text{L}^{-1}$ (SD: $152 \text{ pmol} \cdot \text{L}^{-1}$) for SB-, breaking SB- and continuous VPA condition, respectively.

7.3 Glucose

Results for glucose AUC and iAUC are shown in table 5.3.

Table 5.3: results of glucose AUC and iAUC. Data are mean (95 % CI) and p-value for between-condition effect. $\text{mmol} \cdot \text{L}^{-1}$ = Milimole per litre blood, AUC = total area under curve, iAUC = incremental area under curve.

Glucose	Sedentary Behaviour	Breaking Sedentary Behaviour	Continuous Physical Activity	p-value
AUC ($\text{mmol} \cdot \text{L}^{-1} \cdot$ 360 min)				0.44
Mean (95 % CI)	27 (25.9- 28.1)	25.9 (25- 27.3)	26.8 (25.8- 28.1)	
iAUC ($\text{mmol} \cdot \text{L}^{-1} \cdot$ 360 min)				0.36
Mean (95 % CI)	105 (27.6- 150)	48.4 (-88.6- 113.5)	105.8 (49.3- 171.6)	

There were no statistical significant differences in AUC and iAUC across conditions. Both AUC and iAUC were lower following breaking SB condition compared with both SB- and continuous VPA condition. Mean glucose concentration for each measure point during six hours are shown in figure 5.2.



Figure 5.2: showing mean glucose concentration during six hours. Values are mean and error bars are SD. SB = sedentary behaviour condition, Breaking SB = Breaking sedentary behaviour condition, VPA = continuous vigorous physical activity condition.

Mean glucose concentration rises between baseline and one hour. Mean one-hour glucose concentration was lower following breaking SB condition ($5.1 \text{ mmol} \cdot \text{L}^{-1}$, SD: $0.6 \text{ mmol} \cdot \text{L}^{-1}$) compared with both SB condition ($6.1 \text{ mmol} \cdot \text{L}^{-1}$, SD: $0.9 \text{ mmol} \cdot \text{L}^{-1}$) and continuous VPA condition ($6.5 \text{ mmol} \cdot \text{L}^{-1}$, SD: $1.3 \text{ mmol} \cdot \text{L}^{-1}$). However, there were no statistical significant differences between conditions for one-hour glucose concentration ($p = 0.20$). At three to six hours, mean glucose concentration seems to “level off” and return to baseline value in all conditions; $4\text{-}4.5 \text{ mmol} \cdot \text{L}^{-1}$ (SD: $0.05\text{-}0.4 \text{ mmol} \cdot \text{L}^{-1}$). Mean glucose concentration during six hours were $4.5 \text{ mmol} \cdot \text{L}^{-1}$ (SD: $0.7 \text{ mmol} \cdot \text{L}^{-1}$), $4.3 \text{ mmol} \cdot \text{L}^{-1}$ (SD: $0.4 \text{ mmol} \cdot \text{L}^{-1}$) and $4.5 \text{ mmol} \cdot \text{L}^{-1}$ (SD: $0.9 \text{ mmol} \cdot \text{L}^{-1}$) for SB-, breaking SB- and continuous VPA condition, respectively.

7.4 Triglycerides

Results for triglyceride AUC and iAUC are shown in table 5.4.

Table 5.4: results of triglyceride AUC and iAUC Data are mean (95 % CI) and p-value for between-condition effect. $\text{mmol} \cdot \text{L}^{-1}$ = Milimole per litre blood, AUC = total area under curve, iAUC = incremental area under curve.

Triglyceride	Sedentary Behaviour	Breaking Sedentary Behaviour	Continuous Physical Activity	p-value
AUC ($\text{mmol} \cdot \text{L}^{-1} \cdot 360$ min)				0.60
Mean (95 % CI)	4.64 (2.85- 6.42)	5.02 (3.44- 7.02)	5.63 (4.03- 7.61)	
iAUC ($\text{mmol} \cdot \text{L}^{-1} \cdot 360$ min)				0.53
Mean (95 % CI)	41 (10.4- 72.1)	60.63 (30- 91.5)	38.22 (9.4- 71.1)	

There were no statistical significant differences in TG AUC and iAUC across conditions. AUC was lower following SB condition compared with both breaking SB and continuous VPA condition. iAUC were lower following continuous VPA condition compared with both SB condition and breaking SB. Mean TG concentration at each measure point during six hours are shown in Figure 5.3.

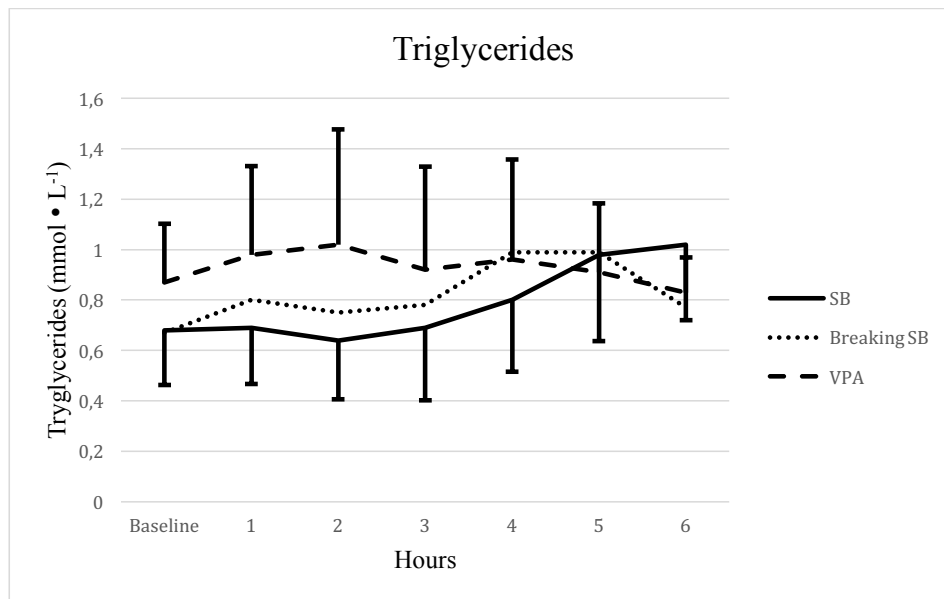


Figure 5.3: showing mean triglyceride concentration during six hours. Values are mean and error bars are SD. SB = sedentary behaviour condition, Breaking SB = Breaking sedentary behaviour condition, VPA = continuous vigorous physical activity condition.

Mean baseline value was different in continuous VPA ($0.87 \text{ mmol} \cdot \text{L}^{-1}$, SD: $0.23 \text{ mmol} \cdot \text{L}^{-1}$) condition compared with both Breaking SB ($0.67 \text{ mmol} \cdot \text{L}^{-1}$, SD: $0.19 \text{ mmol} \cdot \text{L}^{-1}$) and SB condition ($0.68 \text{ mmol} \cdot \text{L}^{-1}$, SD: $0.23 \text{ mmol} \cdot \text{L}^{-1}$), however, this difference was not statistical significant ($p = 0.24$). In ITT-analysis, this difference was attenuated ($p = 0.7$); continuous VPA: $0.90 \text{ mmol} \cdot \text{L}^{-1}$ (SD: $0.27 \text{ mmol} \cdot \text{L}^{-1}$), breaking SB: $0.80 \text{ mmol} \cdot \text{L}^{-1}$ (SD: $0.29 \text{ mmol} \cdot \text{L}^{-1}$) and SB: $0.81 \text{ mmol} \cdot \text{L}^{-1}$ (SD: $0.30 \text{ mmol} \cdot \text{L}^{-1}$). During six hours, mean baseline value and six-hour value were more approximated following continuous VPA condition (0.87 - $0.83 \text{ mmol} \cdot \text{L}^{-1}$) compared with breaking SB condition (0.67 - $0.77 \text{ mmol} \cdot \text{L}^{-1}$) and SB condition (0.68 - $1.02 \text{ mmol} \cdot \text{L}^{-1}$) Mean TG concentration during six hours were $0.78 \text{ mmol} \cdot \text{L}^{-1}$ (SD: $0.14 \text{ mmol} \cdot \text{L}^{-1}$), $0.82 \text{ mmol} \cdot \text{L}^{-1}$ (SD: $0.11 \text{ mmol} \cdot \text{L}^{-1}$) and $0.83 \text{ mmol} \cdot \text{L}^{-1}$ (SD: $0.06 \text{ mmol} \cdot \text{L}^{-1}$) for SB, breaking SB and continuous VPA condition, respectively.

7.5 High density lipoprotein cholesterol and blood pressure

HDL-C seems to not be affected of either acute sitting or PA. BP may yield interesting results given the right sample size, however, results from BP in this study seems to be random. As a consequence, results from HDL-C and both diastolic and systolic BP will not be presented in this thesis.

8.0 Discussion

The objective of this study was to examine if breaking up sedentary behaviour with short five minutes' bouts or one continuous 30-minute bout of vigorous intensity activity differentially affects metabolic risk markers compared with a control condition of prolonged sitting. The primary outcome was postprandial insulin total area under curve (AUC) and incremental area under curve (iAUC). There were no statistical significant differences in insulin, glucose and triglyceride AUC and iAUC, and no statistical significant differences in one-hour postprandial insulin and glucose concentration, across conditions.

8.1 Insulin

Mean insulin AUC, iAUC and one-hour postprandial insulin concentration were lower, although not statistically significantly lower, following one continuous bout of activity compared with short five minutes' bouts every hour. This is in contrast to previous observations in adults, where insulin iAUC were significantly lower following 18 activity bouts of 100 seconds (total 30 min) compared with one bout of continuous running and control condition of nine hours sitting (Peddie et al., 2013). The authors proposed that the non-significant effect on insulin sensitivity by one single 30 minutes' bout may be due to insufficient intensity. In iso-caloric experiments, higher intensity exercise appears to be associated with greater improvements in insulin sensitivity (Dube, Allison, Rousson, Goodpaster, & Amati, 2012).

In opposite to observation in adults, one previous study in children did not observe any differences in insulin iAUC between prolonged sitting, breaks in prolonged sitting and two 20-min bouts of PA in addition to breaks in prolonged sitting. The authors speculated that the results may be explained by their sample of healthy and fit children and that the results may have been similar to those in adults in more unfit children (Saunders, Chaput, et al., 2013). This is supported by observational studies, where unfit children seem to benefit more from participation in MVPA compared with fit children in relation to metabolic risk (Franks, Ekelund, Brage, Wong, & Wareham, 2004).

In studies assessing iAUC the following day, there were no differences across conditions when six 45 minutes bouts of standing were compared with walking at 60 % of HR_{max}

(Miyashita et al., 2013), and when 30 minutes of running at 65 % of VO_{2max} was compared with intermittent walking at 25 % of VO_{2max} in a iso-caloric design (Kim et al., 2014). As both studies primary objective was postprandial lipidemia, no explanation was provided in regard of postprandial insulin response. It is likely that intensity may have been insufficient to provide an increased insulin sensitivity. Alternatively, the assessment of iAUC the following day may also explain the lack of effect on insulin sensitivity.

In free-living, there is no differences between fasting and postprandial insulin assessments following breaks in sitting or continuous MPA. The authors proposed this was because breaks in SB were imposed where it was appropriate, which consisted of a combination of standing and walking, instead of planned breaks at specific times. Interestingly, these results in addition to results from studies over multiple days indicate that effects from breaks in SB are transient (Larsen et al., 2015; Thorp et al., 2014), suggesting that breaks should be continued throughout the postprandial period in order to produce beneficial effects (Blankenship et al., 2014).

8.2 Glucose

There were no significant differences in glucose AUC and iAUC across conditions. This is in contrast to previous observations in adults, suggesting a lower postprandial glucose response following 18 small bouts of 100 seconds compared with one single 30 minute' bout (Peddie et al., 2013). However, in this present study, the mean iAUC were $48 \text{ mmol} \cdot \text{L}^{-1} \cdot 360 \text{ min}$ following the breaking SB condition compared with $105 \text{ mmol} \cdot \text{L}^{-1} \cdot 360 \text{ min}$ and $105.8 \text{ mmol} \cdot \text{L}^{-1} \cdot 360 \text{ min}$ in the SB- and VPA condition, respectively. An increased glucose iAUC following continuous VPA may be explained by an increased hepatic glucose secretion (Howlett, Febbraio, & Hargreaves, 1999). On the other hand, mechanisms for a lower iAUC following breaks in SB are not clear. Suggested explanations include an increased permeability of glucose in muscle cells and an increased glucose transporter type 4 (GLUT4) due to muscle contraction (Holloszy, 2005), and subsequently a reduced blood glucose concentration following the short break while the short exercise duration in the breaking SB condition may not stimulate increased hepatic glucose secretion (Peddie et al., 2013).

In T2DM subjects, both activities of daily living at an intensity equal to MPA and continuous VPA improved postprandial glucose response compared with prolonged sitting. At the same

time, there was a tendency ($p = 0.06$) for glucose iAUC to be lower following continuous VPA compared with activities of daily living, which is likely to be caused by higher intensity during VPA compared with MPA (Van Dijk et al., 2013). However, observations from the present study although not significant, and those from the study by Peddie et al. (2013), suggest that if EE is iso-caloric in activity conditions, there seem to be an extra benefit from breaking SB compared with continuous PA. However, during a 24-hour protocol, glucose iAUC were significantly lower following continuous VPA compared with both activities of daily living and prolonged SB condition. This suggest that in order to maintain glycaemic control beyond the postprandial phase, higher intensity may be more beneficial (Van Dijk et al., 2013).

In children, there were no significant differences in glucose iAUC between prolonged SB, breaks in prolonged SB and two 20-min bouts of PA (Saunders, Chaput, et al., 2013). However, there was a tendency ($p = 0.09$) towards lower glucose iAUC following breaks in prolonged SB compared with prolonged SB and the two 20 minutes' bouts of PA condition. As discussed above, the lack of effect of breaks and the two 20 minutes' bouts of PA conditions compared with prolonged SB may be explained by the fit and healthy sample of children (Saunders, Chaput, et al., 2013).

The results suggested a transient, but not significant, lower one-hour postprandial glucose concentration following breaking SB condition compared with both SB and continuous VPA condition (figure 5.2). This is in agreement with one previous study using a similar protocol (Peddie et al., 2013). Moreover, this was also observed in obese/overweight individuals when sitting was compared with bouts of LPA (Bailey & Locke, 2015; Dunstan et al., 2012; Larsen et al., 2015) but in contrast to observations in healthy adults doing eight minutes bouts of VPA (Altenburg et al., 2013). Similar to the lower glucose iAUC, the observed lower one-hour postprandial glucose concentration may be explained by an increase in GLUT4 activity due to muscle contraction (Holloszy, 2005), and subsequently a reduced blood glucose concentration following the short break while the short exercise duration in breaking SB condition may not stimulate increased hepatic glucose secretion (Peddie et al., 2013). In addition, differences in fitness levels may explain different results for one-hour postprandial glucose, as fit children and adults seem unaffected by shorter bouts of activity (Altenburg et al., 2013; Saunders, Chaput, et al., 2013).

The results suggested that glucose and insulin concentration are not directly proportional in the continuous VPA condition, where insulin concentration is lowest while glucose concentration is highest (figure 5.1 and 5.2). This observation is in contrast to others (Peddie et al., 2013; Saunders, Chaput, et al., 2013). An increased hepatic glucose secretion likely explained the high glucose concentration in all studies. However, the intensity and overall PAEE was higher in this study compared with previous studies, which may explain a higher increase in insulin sensitivity following this study compared with other studies.

When glucose iAUC assessments were carried out the following day, iAUC was lower following continuous running at 65 % of VO_{2max} compared with intermittent walking at 25 % of VO_{2max} when PAEE were equal, but both activity conditions were significantly different from prolonged SB. The authors suggested this was caused by higher glycogen synthesis the next day following the continuous running protocol (Kim et al., 2014). Others compared six 45 minutes bouts of standing with 30 minutes walking at 60 % of HR_{max} , and glucose iAUC were significantly lower following continuous walking compared with prolonged SB suggesting intensity and/or overall EE is important for glucose iAUC (Miyashita et al., 2013).

Although not directly comparable, data from free-living studies suggest a greater glucose AUC following breaks in SB compared with continuous MPA in the postprandial state when activities are done in the pre-prandial state. However, there were no differences between conditions when assessed with continuous glucose monitoring (Blankenship et al., 2014). Further, nocturnal glucose concentrations were higher following continuous MPA compared with breaks in SB, suggesting greater benefits from breaking SB beyond the postprandial state if PAEE is equal in both conditions (Blankenship et al., 2014).

8.3 Triglyceride

Mean baseline TG concentration were higher, although not significantly higher, during the continuous VPA condition compared with breaking SB- and SB condition. The results should therefore be interpreted cautiously. Nevertheless, there were no significant differences in TG AUC and iAUC across conditions. In one previous study using a similar protocol as in the present study, TG iAUC were significantly lower following continuous VPA condition compared with breaking SB condition, but not SB condition (Peddie et al., 2013). The authors suggested the increased TG response in breaking SB condition was caused by the decrease in

insulin resulting in a lowered LPL activity in adipose tissue, whereas the lower TG response following continuous VPA was due to an up-regulation of LPL activity in muscles (Peddie et al., 2013). However, PAEE exceeding more than 1100 kcals is reported to up-regulate LPL activity in muscles (Peddie, Rehrer, & Perry, 2012), and 30 minutes of 60 % VO_{2max} is unlikely to exceed 1100 kcals in PAEE. Lower TG and VLDL concentrations were observed following PAEE of 800 kcals despite no increase in LPL activity (Peddie et al., 2012), which suggest another explanation for the observed lower TG iAUC following continuous VPA.

Data in children do not suggest a difference between different conditions possibly explained by high fitness levels in this age group (Saunders, Chaput, et al., 2013). When iAUC assessment were carried out the following day, significant lower TG iAUC were observed following continuous running at 65 % of VO_{2max} compared with multiple bouts of activity at 25 % of VO_{2max} (Kim et al., 2014), and following 30 minutes continuous walking at 60 % of HR_{max} compared with six bouts of 45 minutes standing (Miyashita et al., 2013). However, when 30 minutes of continuous MPA were compared with bouts of three min of MPA, TG iAUC was not different across conditions (Miyashita et al., 2008). The lower TG iAUC assessed the following day in the study by Miyashita et al. (2013) may be caused by a higher PAEE in continuous VPA compared with breaks in SB condition.

Except for the study by Peddie et al. (2013), insulin, which is suggested to inhibit LPL activity in muscles and enhance activity in adiposity (Peddie et al., 2012), was not different across conditions in studies where also TG was assessed (Blankenship et al., 2014; Kim et al., 2014; Miyashita et al., 2013). This difference may be explained by different timing of assessments. However, this also suggest that LPL activity may not be the primary mechanism in lowering TG concentration (Peddie et al., 2012).

Despite mean baseline TG concentration being different across conditions in this present study, the decrease in TG during six hours in continuous VPA condition may be caused by intramyocellular replenishment, together with decreased VLDL production. As triglyceride levels are changing throughout the day and LPL activity is reported highest 18 hours following exercise, which is returned to baseline after 42 hours, exercise may be needed in an almost daily or at least every second day in order to avoid acute deleterious TG levels. The duration, intensity and timing during the day when exercise is most beneficial is unknown (Peddie et al., 2012).

8.4 Breaking sedentary behaviour; a health protector?

There is currently controversy regarding MVPA's ability to reverse the deleterious outcomes caused by SB (Benatti & Ried-Larsen, 2015). However, evidence suggesting breaks and/or reduction in SB to be more beneficial for health outcomes compared with a single bout of longer MVPA is inconsistent.

First, the proposed inactivity physiology theory (Hamilton et al., 2004) are not supported in human studies (Miyashita et al., 2013). Further, the suggested mechanism for inactivity physiology, which is local contractile activity causing up-regulation of LPL activity leading to increased TG uptake into cells (Hamilton et al., 2004), seem to have stronger influence in rodents compared with humans (Fielding & Frayn, 1998; Peddie et al., 2012).

Furthermore, there is consistent evidence for prescribing interventions including exercise together with diet to decrease cardio-metabolic risk (Pedersen & Saltin, 2015). Based on glycated haemoglobin (HbA_{1c}) and its relationship to average glucose concentration (Nathan et al., 2008), continuous VPA seem more effective in glycaemic control compared with breaks with bouts of MPA (Van Dijk et al., 2013). This relationship seem to be mediated by PAEE rather than type of PA (e.g. multiple bouts versus single bout) (Benatti & Ried-Larsen, 2015; Dube et al., 2012; Peddie et al., 2012) suggesting overall activity may be most important for glycaemic control.

It is acknowledged that breaks in SB is reported to be beneficial to lower postprandial glycaemia in overweight and obese (Bailey & Locke, 2015; Dunstan et al., 2012), which is supported by one study in inactive, normal weight adults (Peddie et al., 2013). However, it is in contrast to observations in healthy adults where activity level is not specified (Altenburg et al., 2013), and the experimental studies including overweight and obese only compared breaks in sedentary time with a complete sedentary condition (Bailey & Locke, 2015; Dunstan et al., 2012). Prescribing interventions aimed to reduce cardio-metabolic risk by breaking up sedentary time rather than prescribing continuous bouts of activity does not currently seem prudent. More studies comparing breaks with different durations and intensities of activity in relation to cardio-metabolic risk factors are needed.

Finally, it is acknowledged that breaks in SB maybe seen as an alternative or supplement to

MVPA and to create a feasible option for those who refuse or are not able to meet PA guidelines (Hamilton et al., 2004; Peddie et al., 2013). However, recommendations for those who are not able to meet PA guidelines are already in place, where as much MVPA as possible is recommended (NDH, 2014; WHO, 2010). Despite this, prescribing breaks in SB as a supplement to MVPA in regard of controlling cardio-metabolic risk is still suggested (Peddie et al., 2013). Alternatively, based on the available evidence, a simple communicable message to be public could be “*sit less and move more*”.

8.5 Strengths and limitations

8.5.1 Strengths

There are several strengths of the present study. First, the randomized cross-over design with strict supervision of experimental conditions allowed for within subject comparison in random order, and to control for confounding factors. In addition, the 6-day washout period most likely controlled for any carryover effects of conditions.

Second, maximal aerobic capacity was measured by indirect calorimetry during an exercise test to exhaustion. In combination with steady state EE measurements, this allowed for accurate extrapolation of activity intensity, and thereby an iso-caloric protocol for the continuous and break conditions. Third, data on CRF level, which can explain discrepancies between studies since it is proposed a mediator for the association between SB and health (Kulinski et al., 2014), allows for comparison with future studies where CRF assessments are included.

Third, a standardized meal was provided following fasting BS in all conditions. In addition, except pre-test, a 12-hour fast was imposed prior to each visit to avoid any carryover-effect of dietary intake the day before.

In general, mainly men have been included in similar studies (Benatti & Ried-Larsen, 2015), and previous studies have highlighted the importance of including females in studies where breaking up prolonged sitting are the treatment (Thorp et al., 2014). In this study the majority of participants were females. However, the small sample size did not allow for stratification by sex. Future studies with larger sample sizes are needed to examine potential differences

between men and women.

8.5.2 Limitations

Although there are strengths in this study, there are several limitations as well.

8.5.2.1 Calculation of Power

The small sample size may have hindered the possibility to detect any between-conditions differences. Due to the explorative design of the study, sample size calculation was not pre-specified. However, given a larger sample size, it seems possible that results from the present study would be significant across conditions for both insulin and glucose AUC, iAUC and mean postprandial one-hour blood concentration.

Previous studies adopting almost similar designs and protocols used different methods for calculating sample size. For example, power calculations by Saunders, Chaput, et al. (2013) were based on the study by Dunstan et al. (2012) despite large differences in protocols and participants (overweight adults versus healthy children).

Nevertheless, post hoc power calculations from the present study can be used to inform future research. The difference in insulin iAUC between SB and continuous VPA condition was 26 %, and the difference between breaking SB and SB condition was 12 %. Assuming the difference between continuous VPA condition and SB condition (26 %) to be clinical relevant, $15000 \text{ pmol} \cdot \text{L}^{-1} \cdot 360 \text{ min}$ difference with assumed SD to be $\approx 23000 \text{ pmol} \cdot \text{L}^{-1} \cdot 360 \text{ min}$, 80 % power and an alpha level of 5 % will require a sample of 19 participants. However, assuming the difference between continuous VPA condition and breaking SB condition (12 %) to be clinical relevant, $\approx 8500 \text{ pmol} \cdot \text{L}^{-1} \cdot 360 \text{ min}$ difference with assumed SD to be $\approx 19000 \text{ pmol} \cdot \text{L}^{-1} \cdot 360 \text{ min}$, 80 % power and an alpha level of 5 % will require a sample of 40 participants. Power calculations for glucose requires 22 participants if assuming a clinical relevant difference to be $\approx 60 \text{ mmol} \cdot \text{L}^{-1} \cdot 360 \text{ min}$ (54 %) with assumed SD to be $\approx 100 \text{ mmol} \cdot \text{L}^{-1} \cdot 360 \text{ min}$, and choosing 80 % power and an alpha level of 5 %. Calculation of required participants for TG assessments is not recommended because of huge differences in SD between SB condition ($\approx 16 \text{ mmol} \cdot \text{L}^{-1} \cdot 360 \text{ min}$) and continuous VPA condition ($\approx 37 \text{ mmol} \cdot \text{L}^{-1} \cdot 360 \text{ min}$), in addition to different mean baseline TG concentrations between

conditions. One solution may be to emphasize sample size calculation for insulin and glucose and include TG assessments, and then calculate sample size from those findings.

Taking into account a 50 % dropout rate as in the present study at least 60 participants are needed in a future study given the most conservative power calculation. These calculations were performed according to the instructions in the book by Laake et al. (2008).

8.5.2.2 Drop-outs

50 % (six out of 12) of the recruited participants withdrew from the project before completing all conditions. One withdraw before pre-test due to milk allergy. Five participants completed pre-test and withdrew after completing one or two conditions. Other reported reasons for dropout were illness, tiredness after visit and lack of time. Illness, as it can happen to everyone without further notice, is hard to control. Rescheduling was tried but were unfortunately not possible because of a tight schedule for the participant and availability in the laboratory.

Fatigue with the protocol may contribute to the high dropout rate. The protocol including a liquid meal followed by six hours, only allowing consumption of water may have been too long for some of the participants. Participants' motivation was expected to drop if sitting for more than six hours, and six hours were chosen as an effect was observed five hours postprandial in studies using a similar protocol (Dunstan et al., 2012). In addition, due to the time limitation of a master thesis, longer recruitment time to find highly motivated participants willing to participate in a fairly extensive study seemed unfeasible.

Lack of time may have been due to an overestimation of time available for participation. Further, other reasons for the high drop-out than those reported cannot be ruled out. Some participants may have envisaged the project to be different in terms of protocol. Nevertheless, a dropout rate of 50 % is high, and needs to be considered in future research of similar type.

8.5.2.3 Other limitations

Continuous glucose monitoring throughout the experiment would likely provide a more accurate measure of glucose fluctuations induced by the different experimental conditions.

However, AUC calculation is a well-established method in examining treatments effect (Wolever, 2004) and is in fact a strength.

Even though a standardized meal was provided at all conditions following baseline fasting blood samples, this meal was not individualized to the participants resting EE or body weight and composition. However, the meal contained 646 kcals, which was supposed to provide energy containing sugar amounts well above recommended amounts to provide large postprandial responses in glucose and insulin. In addition, a liquid meal is unlikely to exactly mirror the solid food usually consumed (Saunders, Chaput, et al., 2013).

It is also possible that nutrients ingested prior to each visit may have had impact in the BS in this study, as dietary intake was not controlled for in the days prior to each visit as done in previous studies (Kim et al., 2014; Miyashita et al., 2013). In addition, reliance in participants to report absence of nicotine, alcohol, caffeine, exercise and food ingestion for 12 hours is necessary, however, it may not have been adhered. Nevertheless, randomization should control for these confounding factors.

The duration of conditions is arguably short, especially since TG seemed to further decrease and exceeding the six hours of assessment in this study. On the other hand, glucose and insulin seemed to “level off” after three hours, which suggest that longer protocols may not be needed when examining variables related to glucose metabolism. In addition, multiple meals are unlikely to provide other outcomes than one meal when observing an acute postprandial response in insulin and glucose. For more long-term effect of activity in a postprandial phase, protocols similar to Kim et al. (2014), Miyashita et al. (2013) and Blankenship et al. (2014) seem appropriate. Given the time limitation, a short acute postprandial response was a feasible objective for this study.

Current recommendations for physical activity and health recommend bouts of at least 10 minutes of MVPA for providing health benefits (NHR, 2012). However, recent studies indicate that bouts of less than 10 minutes are beneficial (Benatti & Ried-Larsen, 2015). Thus, the protocol in this study was appropriate for examining the proposed research question.

The fitness levels of the participants were higher compared with the general population (Edvardsen, Hansen, Holme, Dyrstad, & Anderssen, 2013). Thus, the results of this study may

not be generalizable beyond young, healthy participants.

It should also be noted that this was a laboratory based study, and the results are therefore less transferable into real life settings, such as an office or at home. However, the objective was to investigate the acute metabolic effects of prolonged sitting and different breaks in SB, and the experimental conditions allowed for close adherence to the design and protocol. Thus based on design and hypothesis, transferring the results to real life settings are not perceived as a limitation.

8.6 Future research

There is evidence for a dose-response relationship of exercise and insulin sensitivity and by now, no threshold for upper exercise volume is identified (Dube et al., 2012). An interesting objective for future research would be to investigate the postprandial effect of breaking up sitting with bouts of higher intensities than what was imposed in the present study, for example 80-90 % of VO_{2max} . As it may be hard to sustain such intensities for continuous 30 minutes, the single continuous bout can be carried out in an interval training session followed by prolonged sitting.

8.6.1 Long term effect

This study investigated acute effect of breaking up prolonged sitting, thus there is no way to infer any long-term effect of this or previous studies of similar design. To date, the long-term effect of daily prolonged sitting has not been investigated. A randomized controlled trial with randomization to three groups would be ideal; 1) control group engaging in high volumes of SB; 2) same as 1, but breaking this imposed SB throughout the day; 3) same as 1, but doing 30 minutes of continuous VPA for three days a week. Outcomes can include CRF, insulin sensitivity, platinum standard for research in MetS (Alberti et al., 2006), and physiological activities related to lipid metabolism in muscles obtained by muscle biopsies.

Ideally, duration of interventions should be several months to one year. However, there are ethical issues to consider. SB is suggested to be harmful and it is therefore doubtful if such projects are ethically tenable. Alternatively, since breaks in SB is reported to be protective of the deleterious effects of prolonged sitting (Benatti & Ried-Larsen, 2015), a randomization into two groups, number 2 and 3 in the above proposed study, may be appropriate.

An additional issue to solve is how to control participants to break their SB each hour with different activities (walk for five minutes in stairs, do calisthenics etc.). Direct observation is not feasible. ACC/inclinometers can be worn for one or two weeks as a proxy for the entire month, or be replaced with an ACC/inclinometer with fresh batteries for the next one-two weeks. The participants can be reminded to adhere to the imposed breaks in sitting in form of a text-message or call. Performing 30 minutes of VPA in the evening together with a trained supervisor is easier compared with other issues at hand.

Finally, finding participants can be done by screening for sedentary behaviour and select those most sedentary. However, free-living long term studies cannot be controlled similar to a lab study, thus, this proposed design is challenging. Nevertheless, results may yield many answers for unsolved questions regarding sedentary behaviours' independent impact on health.

8.7 Conclusion

In conclusion, there is no effect of either breaking up sedentary behaviour each hour or performing 30 minutes of vigorous physical activity in one bout compared with prolonged sitting for six hours on glucose and lipid metabolism.

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Appendix 1

Inquiry of participation in a research project [Forespørsel om deltakelse i forskningsprosjekt]

Forespørsel om deltakelse i forskningsprosjektet



“A comparison between two different conditions of breaking up sedentary behaviour on glucose metabolism during prolonged sitting - a randomized, exploratory, crossover study”

Kontaktinformasjon:

- Mastergradsstudent Edvard Hamnvik Sagelv - tlfnr: 97612520. E-post: edvardhs@student.nih.no
- Hovedveileder Professor Ulf Ekelund: +4723262239 / +4740629589. E-post: ulf.ekelund@nih.no
- Biveileder Førsteamanuensis Trine Stensrud: +4723262346 / +4741223979. E-post: trine.stensrud@nih.no

Bakgrunn og formål

Dette er et spørsmål til deg om å delta i en forskningsstudie for å undersøke hvor akutt helseskadelig det er å sitte stille, og om man kan reversere/kompensere for stillesittingen ved å gjøre fysisk aktivitet. Til nå anbefales det å redusere/bryte opp stillesitting med å gjøre noen aktiviteter. Dette blir gjort uten å helt forstå sammenhengen mellom stillesitting og helseskadene som medfølger. Derfor ønsker vi å undersøke om man kan sitte stille hele dagen så lenge man kompenserer dette med å trene.

Hypotesen som skal testes er: *“thirty minutes of continuous VPA /exercise at 70 % of VO_{2max} is associated with an equal postprandial insulin response compared with breaking up sedentary time every sixty minute keeping exercise energy expenditure and intensity constant”*
Oversatt til norsk: *“tretti minutter med kontinuerlig hard fysisk aktivitet / trening ved 70% av VO_{2max} er assosiert med en lik postprandial insulinrespons sammenlignet med å bryte opp stillesittende tid hver sekstiende minutt energiforbruk og intensitet holdes konstant”*

Denne studien er i forbindelse med en masteroppgave ved Norges Idrettshøgskole.

Denne studien er mest relevant for de som har en stillesittende jobb/studiehverdag, og derfor

er du valgt ut til å delta. Du ble trukket ut/vervet via mail til din institusjon som viderefremildte mailen til deg eller ved plakat hengt opp ved Universitetet i Oslo, campus Blindern.

Hva innebærer deltakelse i studien?

Å være deltaker krever 4 besøk ved Norges Idrettshøgskole:

Det vil bli notert alder, høyde og vekt. Med høyde og vekt blir det målt kroppsmasseindeks (KMI).

1. Først et besøk for å teste maksimalt oksygenopptak (VO_{2maks}). Denne testen skal gjøres for å finne din individuelle 70 % av VO_{2maks} for å stadfeste intensiteten som aktiviteten skal gjennomføres ved. Du kan beregne 1 time til dette besøket, men det kan fort ta litt kortere tid.
2. Deretter 3 besøk på 6 timer hvor alle deltakerne skal gjennom alle tre intervensjonene.
 - a. Den ene gangen skal du sitte i 6 timer
 - b. Den andre gangen skal du sitte i 6 timer, men hver time skal du jogge/gå i oppoverbakke i 5 minutter ved en intensitet som tilsvarer din 70 % av VO_{2maks} . Det vil til sammen bli 30 minutter med hard fysisk aktivitet/trening
 - c. Den tredje gangen skal du jogge/gå i oppoverbakke i 30 minutter ved en intensitet som tilsvarer din 70 % av VO_{2maks} , og deretter sitte i 5 og en halv time.

I løpet av disse 3 besøkene er det fullt mulig å ha med datamaskin og dermed jobbe akkurat som ved skolen/kontoret.

- Både menn og kvinner kan delta.

Ved de 3 besøkene hvor man skal være her i 6 timer:

- Må man sitte hele tiden. Det er lov å gå på toalettet når det er nødvendig
- Du må møte opp om morgenen uten å ha spist frokost. Du får frokost av meg en etter første blodprøve er tatt. Det er en shake med mye kalorier, så man skal ikke føle seg for sulten gjennom disse 6 timene. Sult er individuelt, så jeg kan ikke garantere at du ikke er sulten mot slutten av disse 6 timene.
 - o Tech Nutrition sponser shakene som du skal drikke ved disse tre besøkene. Hvis du er allergisk mot laktose kan det være en praktisk utfordring som ikke lar seg løse siden shaken inneholder laktose fra melkeproteiner som er i pulveret til shaken. Shaken blandes med helmelk og har smak av sjokolade eller jordbær, ettersom hva du ønsker.

- Neste måltid kan du spise når forsøket er ferdig. Det vil si 5 og en halv time etter du drakk shaken. Da kan du spise det du ønsker. Vi har ikke mat til deg da, så det kan være lurt å ha med en nistepakke. Det er og en kantine ved NIH som serverer en varmrett om dagen og har brød og salatbar. Grunnen til å bare drikke denne shaken det er for å passe på at ikke noe du spiser/drikker påvirker verdiene i blodet og blodtrykket.
- Du skal ikke trene dagen før. Hvis du må trene, gi beskjed om morgenen at du trente dagen før.
- Du skal ikke drikke alkohol dagen før
- Hvis du røyker eller snuser, kan du ikke gjøre det i løpet av disse 6 timene. Du skal helst ikke røyke/snuse om morgenen før du kommer til laben. Hvis du MÅ gjøre dette, må du gjøre det til samme tid og like lenge før alle 3 besøkene. Grunnen til det er at nikotinet i røyk/snus/nikotinplaster påvirker blodtrykket.
- Det blir tatt blodtrykk og blodprøver hver time (7 ganger for hvert besøk). Siden det skal tappes blod hver time vil du få en kateter, og dermed bare et stikk i løpet av dagen, også må dette kateteret sitte i gjennom disse 6 timene.
 - o Blodprøvene som registreres er glukose (blodsukker), insulin (hormon som hjelper kroppen å bruke blodsukker), triglyseridnivå (frie fettsyrer i blodet) og HDL-kolesterol (det gode kolesterolet)
 - o Hvis du sier ja til å delta i studien, gir du også ditt samtykke til at prøver sendes til Fürst Laboratorium for analyse, og til hormonlaboratoriet ved Oslo universitetssykehus for analyse av insulinprøvene.

Hvis du ønsker å delta må du svare på et veldig kort spørreskjema som kalles "International physical activity questionnaire, short version". Dette har du allerede fylt ut. Dette skjemaet er kortversjonen av et større spørreskjema og stiller 7 spørsmål om fysisk aktivitet som du har gjort de siste 7 dagene. Grunnen til det er for å kunne vite noe om ditt aktivitetsnivå selv om dette ikke er den beste metoden for å kartlegge ditt aktivitetsnivå. I tillegg er det et 2 spørsmål til om ditt aktivitetsnivå.

Mulige fordeler og ulemper

Fordelene med denne studien:

- Er at du får være med på å undersøke en helserisiko som er forbundet med ditt yrke/hverdag.

- Du vil og få en test for maksimalt oksygenopptak. Dette er et fint mål for din kardiovaskulære helse. Det vil si hvor sterkt ditt hjerte er. Maksimalt oksygenopptak sier noe om din evne til å takle alt av gjøremål - Alt fra å vaske huset til å løpe et maraton. Denne testen er tung, men kort.
 - o Denne testen kan oppleves hard. Men den tunge delen varer mellom 4-7 minutter. Resten er oppvarming og vårt forsøk på å finne din fart ved 70 % av VO₂maks.
 - o Du får svar umiddelbart fra testen, der Edvard vil forklare hva de forskjellige tallene betyr.
- Du får et innblikk i eksperimentell studiedesign. Du vil få muligheten til å lære om hvordan en type forskning er gjort i praksis.
- Du vil få en mulighet til å jobbe effektivt med jobb/skolerelatert arbeid siden du må sitte i 6 timer i strekk.
- Siden vi skal være sammen i 6 timer ved 3 anledninger vil du få muligheten til å snakke med meg om alt innen helse, trening, ernæring og idrett – som er det feltet jeg studerer. Hvis du ikke ønsker noen snakk og heller jobbe med jobb/skolerelatert arbeid vil det selvfølgelig bli akseptert.

Ulempene/risiko ved denne studien:

- Det kan være en risiko for å falle av tredemøllen når du jogger/går på tredemøllen. Du vil få god opplæring i å hoppe av tredemøllen på en forsvarlig måte. Edvard står alltid ved siden av og er klar til trykke på en stopknapp hvis uhellet skulle skje.
 - o Man kan slå seg ved å falle av, og i verste fall brette noe, men denne risikoen er liten.
- Blodprøvetaking kan for noen være ubehagelig. Men det gjør ikke nevneverdig vondt. Det blir gjort på en forsvarlig måte hvor autorisert personell vil sette kateteret, og dermed utgjør dette ingen risiko.
 - o Edvard får god opplæring i å tappe blod i prøveglassene fra kateteret.

Hva skjer med informasjonen om deg?

Alle personopplysninger vil bli behandlet konfidensielt. Det vil si at det ikke vil være mulig å gjenkjenne deg ved hjelp av opplysningene som publiseres i innlevering av masteroppgaven og eventuelt en vitenskapelig artikkel.

Prøvene tatt av deg og informasjonen som registreres om deg skal kun brukes slik som

beskrevet i hensikten med studien. Under hele forsøket vil opplysningene oppbevares
avidentifisert. En kodenøkkel knytter deg til dine opplysninger og prøver gjennom en
navneliste. Navnelisten vil jeg lagre med en passord-beskyttet fil. Det vil bli lagret en fil på
Edvard sin bærbare datamaskin og en backup-fil på Edvard sin server på skolen sitt nettverk.
Begge vil være passord-beskyttet

Prøvene vil bli sendt til laboratorium for analyse. Deretter vil de bli ødelagt. Jeg får tilbake
blodverdiene av laboratoriet. Alle dine opplysninger, alder, vekt, høyde, blodtrykk,
blodverdier (glukose, HDL-kolesterol, triglyseridnivå og insulin) er kun gjenkjennerbar med
navnelisten..

Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Eksperimentet skal etter planen avsluttes før 18.desember. Da skal alle deltakerne vært
igjennom alle besøkene ved NIH og alle blodprøvene blir sendt til laboratorium for analyse. I
januar vil Edvard begynne å sammenligne og analysere svarene fra blodprøvene.
Masteroppgaven vil bli levert inn 30. Mai 2016, det er også forventet prosjektslutt. Ditt navn
vil bli lagret frem til da i den kodebeskyttede navnelisten. Hvis det er ønskelig, kan du få
tilbake dine blodprøveverdien når de er kommet tilbake fra laboratorium.

Frivillig deltakelse

Det er frivillig å delta i studien, og du kan når som helst trekke ditt samtykke uten å oppgi
noen grunn. Dersom du trekker deg, vil du ikke oppleve noen form for etterspill eller straff.
Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Dersom du
senere ønsker å trekke ditt samtykke eller har spørsmål til studien, kan du en av de ansvarlige
personene for studien. Kontaktinfo finnes på første side.

Studien er meldt til Personvernombudet for forskning, Norsk samfunnsvitenskapelig
datatjeneste AS.

Samtykke til deltakelse i studien

Jeg har mottatt informasjon om studien, både skriftlig og muntlig, og er villig til å delta

(Signert av prosjektdeltaker, dato)

Appendix 2

Copy of the e-mail received from The Regional committees for medical and health research ethics (REK), which confirm the project did not fall under the Law of Medical and Health Research.

From: post@helseforskning.etikkom.no [<mailto:post@helseforskning.etikkom.no>]
Sent: 7. september 2015 13:53
To: Ulf Ekelund <ulf.ekelund@nih.no>
Subject: Sv: REK sør-øst 2015/1542 Kan fysisk aktivitet kompensere for stillesittende adferd

Hei,

Jeg viser til din forespørsel om framleggingsvurdering for prosjektet «Kan fysisk aktivitet kompensere for stillesittende adferd» (vår ref. 2015/1542), sendt inn den 21.08.2015.

Redegjørelse for prosjektet

Prosjektet skal undersøke om hard fysisk aktivitet i 30 minutter kan kompensere for stillesittende adferd i 6 timer. Deltakerne er voksne kvinner og menn over 18 år som ikke gjør mer enn 30 minutter fysisk aktivitet daglig. Deltakerne skal komme på laboratorium ved Norges Idrettshøgskole 4 ganger. Først skal de testes for maksimalt oksygenopptak. Deretter skal de komme på 3 besøk på 6 timer hver gang. Det skal måles blodtrykk og tas prøver av deltakerne 7 ganger.

Utfallsmålene er de kriteriene på metabolsk syndrom som kan måles med blodprøver. I blodprøvene skal det måles: «Non fasting glucose, triglycerides level, HDL-colesterol, Insulin, Atherogenic dyslipidaemia, Insulin resistance with HOMA-IR(glucose and insulin), Pro-inflammatory state (detailed LDL-colesterol), Hormonal factors related to the Pituitary-adrenal axis.»

Vurdering

Helseforskningsloven gjelder for medisinsk og helsefaglig forskning, det vil si virksomhet som utføres med vitenskapelig metodikk for å skaffe til veie ny kunnskap om helse og sykdom, jf. helseforskningsloven § 2, jf. § 4. Slik prosjektet er beskrevet i skjema for vurdering av framleggingsplikt og vedlagte prosjektbeskrivelse, virker det ikke som om prosjektet har som formål å fremskaffe ny kunnskap om helse og sykdom. Prosjektet ser derfor ut til å falle utenfor helseforskningslovens virkeområde. Prosjekter som faller utenfor helseforskningslovens virkeområde krever ikke forhåndsgodkjenning av REK.

Så lenge formålet med prosjektet ikke er å fremskaffe ny kunnskap om helse og sykdom, kreves det heller ikke godkjenning fra REK for å ta en blodprøve fra deltakerne.

Jeg gjør oppmerksom på at denne konklusjonen er å anse som veiledende jfr. forvaltningsloven § 11. Dersom du likevel ønsker å søke REK vil søknaden bli behandlet i komitémøte, og det vil bli fattet et enkeltvedtak etter forvaltningsloven.

Med vennlig hilsen

Jakob Elster
seniorrådgiver

post@helseforskning.etikkom.no

T: 22845530

Regional komité for medisinsk og helsefaglig forskningsetikk REK sør-øst-Norge (REK sør-øst) <http://helseforskning.etikkom.no>

Appendix 3

Comment on notice of storage of personal data [Tilbakemelding på melding om behandling av personopplysninger]

Norsk samfunnsvitenskapelig datatjeneste AS
NORWEGIAN SOCIAL SCIENCE DATA SERVICES



Harald Hårfagres gate 29
N-5007 Bergen
Norway
Tel: +47-55 58 21 17
Fax: +47-55 58 96 50
nsd@nsd.uib.no
www.nsd.uib.no
Org.nr. 985 321 884

Ulf Ekelund
Seksjon for idrettsmedisinske fag Norges idrettshøgskole
Postboks 4014 Ullevål Stadion
0806 OSLO

Vår dato: 22.10.2015

Vår ref: 44657 / 3 / LB

Deres dato:

Deres ref:

TILBAKEMELDING PÅ MELDING OM BEHANDLING AV PERSONOPPLYSNINGER

Vi viser til melding om behandling av personopplysninger, mottatt 13.09.2015. Meldingen gjelder prosjektet:

44657	<i>A comparison of breaking sedentary behaviour and physical activity during a 6 hour sitting condition – A randomized crossover study</i>
<i>Behandlingsansvarlig</i>	<i>Norges idrettshøgskole, ved institusjonens øverste leder</i>
<i>Daglig ansvarlig</i>	<i>Ulf Ekelund</i>
<i>Student</i>	<i>Edvard Hamnvik Sagelv</i>

Personvernombudet har vurdert prosjektet, og finner at behandlingen av personopplysninger vil være regulert av § 7-27 i personopplysningsforskriften. Personvernombudet tilrår at prosjektet gjennomføres.

Personvernombudets tilråding forutsetter at prosjektet gjennomføres i tråd med opplysningene gitt i meldeskjemaet, korrespondanse med ombudet, ombudets kommentarer samt personopplysningsloven og helseregisterloven med forskrifter. Behandlingen av personopplysninger kan settes i gang.

Det gjøres oppmerksom på at det skal gis ny melding dersom behandlingen endres i forhold til de opplysninger som ligger til grunn for personvernombudets vurdering. Endringsmeldinger gis via et eget skjema, <http://www.nsd.uib.no/personvern/meldeplikt/skjema.html>. Det skal også gis melding etter tre år dersom prosjektet fortsatt pågår. Meldinger skal skje skriftlig til ombudet.

Personvernombudet har lagt ut opplysninger om prosjektet i en offentlig database, <http://pvo.nsd.no/prosjekt>.

Personvernombudet vil ved prosjektets avslutning, 30.05.2016, rette en henvendelse angående status for behandling av personopplysninger.

Vennlig hilsen

Katrine Utaaker Segadal

Lene Christine M. Brandt

Kontaktperson: Lene Christine M. Brandt tlf: 55 58 89 26

Dokumentet er elektronisk produsert og godkjent ved NSDs rutiner for elektronisk godkjenning.

Avdelingskontoret / District Offices

OSLO NSD Universitetet i Oslo, Postboks 1055 Blindern, 0316 Oslo. Tel: +47-22 85 52 11. nsd@uio.no
TRONDHEIM NSD Norges teknisk-naturvitenskapelige universitet, 7491 Trondheim. Tel: +47-73 59 19 07. kyrra.svana@svt.ntnu.no
TROMSØ NSD SVF, Universitetet i Tromsø, 9037 Tromsø. Tel: +47-77 64 43 36. nsdmaa@svt.uo.no

Vedlegg: Prosjektvurdering
Kopi: Edvard Hamnvik Sagelv edvardhs@student.nih.no

Personvernombudet for forskning



Prosjektvurdering - Kommentar

Prosjektnr: 44657

Formålet med studien er å studere hvor helseskadelig det er å sitte stille, og om man kan reversere/kompensere for stillesittingen ved å gjøre fysisk aktivitet.

Utvalget består av friske personer som har en stillesittende jobb/hverdag. De rekrutteres ved at de henvender seg direkte til student etter å ha mottatt epost fra student eller å ha sett oppslag/plakater om prosjektet ved studieinstitusjon. Når det sendes ut eposter til ulike bedrifter anbefaler vi at kontaktperson ved bedriften videreformidler eposten til sine ansatte. Alternativt legger vi til grunn at bedriftene har anledning til å utlevere kontaktfinformasjon dersom rekrutteringen foregår slik.

Utvalget informeres skriftlig om prosjektet og samtykker til deltakelse. Informasjonsskrivet er godt utformet, såfremt følgende endres/tilføyes:

- Det må legges til en setning om at det er forventet at prosjektet avsluttes innen 30.05.2016, og at alle de innsamlede opplysningene anonymiseres senest da.
- Setningen "Under hele forsøket vil ditt navn være anonymisert" omskrives slik at det i stedet står "Under hele forsøket vil opplysningene oppbevares avidentifisert". Dette da det benyttes koblingsnøkkel/navneliste.
- Vi anbefaler at kontaktopplysninger til veileder tilføyes første del av skrevet.
- I selve samtykkeerklæringen ber vi om at delen om stedfortredende samtykke tas bort, da vi ikke kan se at det er relevant i dette prosjektet (da det ikke skal inkluderes myndige personer med redusert eller manglende samtykkekompetanse).

Det behandles sensitive personopplysninger om helseforhold, jf. personopplysningsloven § 2 nr. 8 c).

Personvernombudet legger til grunn at forsker etterfølger Norges idrettshøgskole sine interne rutiner for datasikkerhet. Gitt at biologisk materiale sendes til eksterne laboratorier forutsetter ombudet at det foreligger en databehandleravtale mellom laboratoriene og Norges idrettshøgskole for den behandling av data som finner sted, jf. personopplysningsloven § 15. For råd om hva databehandleravtalen bør inneholde, se Datatilsynets veileder på denne siden: <<http://www.datatilsynet.no/verktoy-skjema/Skjema-maler/Databehandleravtale---mal/>>

Forventet prosjektslutt er 30.05.2016. Ifølge prosjektmeldingen skal innsamlede opplysninger da anonymiseres. Anonymisering innebærer å bearbeide datamaterialet slik at ingen enkeltpersoner kan gjenkjennes. Det gjøres ved å:

- slette direkte personopplysninger (som navn/koblingsnøkkel)
- slette/omskrive indirekte personopplysninger (identifiserende sammenstilling av bakgrunnsopplysninger som f.eks. bosted/arbeidssted, alder og kjønn)
- slette alle biologiske prøver (dette gjøres fortløpende, innen 2 måneder ved laboratoriene, jf. telefonsamtale med Edvard Hamnvik Sagelv 21.10.2015).

Det er opplyst i meldingen at prosjektet er vurdert til å falle utenfor helseforskningslovens virkeområde. Ombudet ber om at REK-vedtaket ettersendes oss (til personvernombudet@nsd.uib.no).

