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ABSTRACT

The purpose of this thesis was to investigate whether, over a 6-month period, a 1-hour reduction in daily sedentary behaviour (SB) without adding intentional exercise training would improve insulin sensitivity in adults with metabolic syndrome.

The cross-sectional screening phase included 144 participants (42 men) with a mean age of 57 (SD 7) years and a mean BMI of 32 (SD 4) kg/m^2. Sixty-four of the participants (27 men) were found to be sedentary and inactive with metabolic syndrome and were randomized into intervention and control groups. The 6-month individualized behavioural intervention aimed at reducing daily SB for 1h compared to screening. SB and physical activity were measured with hip-worn accelerometers throughout the intervention. Whole-body insulin sensitivity by hyperinsulinemic euglycemic clamp, skeletal muscle insulin sensitivity by positron emission tomography, body composition by air displacement plethysmography and fasting blood samples were analysed before and after the intervention. During screening, insulin resistance was assessed with the surrogate marker HOMA-IR.

During the screening, all physical activity, regardless of intensity, associated with lower insulin resistance and a favourable plasma lipid profile, whereas a greater proportion of SB associated with higher insulin resistance. However, these associations partly depended on the duration of the accelerometer data collection. During the intervention, SB decreased in the intervention group by 40 min/day and moderate-to-vigorous physical activity increased by 20 min/day, with no change in the control group. After 6 months, fasting plasma insulin decreased in the intervention group compared to control, but insulin sensitivity in the whole body or thigh muscles did not change in either group. The changes in body mass or adiposity did not differ between groups. Among all participants, the changes in SB and body mass correlated inversely with the change in insulin sensitivity; the insulin sensitivity modestly increased among the participants that reduced SB for at least ~ 27 min /day.

The intervention resulted in a slight decrease in fasting insulin, but had no effect on insulin sensitivity. However, as the change in insulin sensitivity associated with the changes in SB, successfully reducing SB and increasing moderate-intensity activity may be beneficial in improving whole body insulin sensitivity.

KEYWORDS: Sedentary behaviour, Physical activity, Accelerometry, Behaviour change, Insulin sensitivity, Metabolic health, Metabolic syndrome

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
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TANJA SJÖROS: Paikallaanolon vähentämisen vaikutukset koko kehon ja luurankolihasten insuliiniherkkyyteen
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TIIVISTELMÄ

Tämän väitöstutkimuksen tarkoitus oli selvittää paikallaanolon vähentämisen vaikutusta koko kehon ja luurankolihasten insuliiniherkkyyteen 6 kuukauden tutkimusjakson aikana aikuisilla, joilla on metabolinen oireyhtymä.

Sisäänottovaiheessa tutkimukseen osallistui 144 henkilöä (42 miestä), ikä 57 (SD7) vuotta ja BMI 32 (SD 4) kg/m^2. Heistä 64 henkilöä (joista miehiä 27), joilla todettiin metabolinen oireyhtymä, satunnaistettiin koe- ja kontrolliryhmään. Intervention tavoitteena oli 6 kuukauden ajan vähentää paikallaanoloa 1h päivässä sisäänottovaiheeseen verrattuna. Paikallaanoloa ja liikkumista mitattiin lantiolla pidettävillä kiihtyvyysmittareilla koko tutkimuksen ajan. Koko kehon insuliiniherkkyyttä mitattiin hyperinsulineemisella euglykeemisella clamp –tutkimuksella ja lihasten insuliiniherkkyyttä positronemissiotomografialla. Lisäksi analysoitiin paastoverinäytteitä ja kehonkoostumusta.

Sisäänottovaiheessa liikkuminen oli yhteydessä alhaisempaan insuliiniresistenssiin ja paikallaanolo suurempaan insuliiniresistenssiin ja huonompaan veren lipidiprofiiliin. Nämä yhteydet riippuivat kuitenkin liikemittarimittauksen kestosta. Intervention aikana paikallaanolo väheni koeryhmässä 40 min/vrk ja reipas liikkuminen lisääntyi 20 min/vrk, muutos kontrolliryhmässä ei ollut merkitsevä. Tutkimusjakson jälkeen paastoinsuliini laski koeryhmässä kontrolliryhmään verrattuna, mutta insuliiniherkkyydessä ei tapahtunut muutosta. Kehonkoostumuksen muutoksissa ryhmien välillä ei ollut eroa. Paikallaanolon muutos korreloi negatiivisesti kehon insuliiniherkkyyden kanssa ja insuliiniherkkyys parani hieman niillä tutkittavilla, jotka onnistuivat vähentämään paikallaanoloa vähintään ~ 27 min päivässä.

Interventio laski hieman paastoinsuliinia, mutta insuliiniherkkyyteen sillä ei ollut vaikutusta. Paikallaanolon vähentäminen oli kuitenkin yhteydessä parempaan insuliiniherkkyyteen, joten paikallaanolon vähentäminen reippaan liikkumisen avulla saattaa hieman kohentaa insuliiniherkkyyttä.

AVAINSANAT: Paikallaanolo, fyysinen aktiivisuus, liikemittari, insuliiniherkkyys, insuliiniresistenssi, aineenvaihdunnallinen terveys, metabolinen oireyhtymä

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Abbreviations

3D-OSEM	Three-dimensional ordered subsets expectation-maximization method
^{18}F	Fluorine-18 radioisotope
APE	Angle for postural estimation
ATP	Adenosine triphosphate
AUC	Area under the curve
BMI	Body mass index
CT	Computer tomography
CVD	Cardiovascular disease
FDG	^{18}F-fluorodeoxyglucose
FFA	Free fatty acid
FUR	Fractional tracer uptake rate
g	Gravity unit
GU	Glucose uptake
HbA1c	Glycated haemoglobin
HDL	High-density lipoprotein
HEC	Hyperinsulinemic euglycemic clamp
HOMA-B	Homeostatic model assessment of beta-cell function
HOMA-IR	Homeostatic model assessment of insulin resistance
IDF	International Diabetes Federation
IQR	Interquartile range
LPA	Light physical activity
LV	Left ventricle
MAD	Mean amplitude deviation
MET	Metabolic equivalent
MetS	Metabolic Syndrome
MRI	Magnetic resonance imaging
MVPA	Moderate to vigorous physical activity
OGTT	Oral glucose tolerance test
PA	Physical activity
PET	Positron emission tomography
Q1	First quartile

Q3	Third quartile
QF	Quadriceps femoris muscle
RF	Rectus femoris muscle
ROI	Region of interest
SB	Sedentary behaviour
SBP	Systolic blood pressure
SBRN	Sedentary Behavior Research Network
SD	Standard deviation
SMD	Standardized mean difference
T2D	Type 2 diabetes
TAC	Time-activity curve
WHO	World Health Organisation

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Sjöros T, Vähä-Ypyä H, Laine S, Garthwaite T, Lahesmaa M, Laurila S M, Latva-Rasku A, Savolainen A, Miikkulainen A, Löyttyniemi E, Sievänen H, Kalliokoski K K, Knuuti J, Vasankari T, Heinonen I H A. Both sedentary time and physical activity are associated with cardiometabolic health in overweight adults in a 1 month accelerometer measurement. *Scientific Reports*, 2020; 10: 20578.

II Sjöros T, Vähä-Ypyä H, Laine S, Garthwaite T, Löyttyniemi E, Sievänen H, Kalliokoski K K, Knuuti J, Vasankari T, Heinonen I H A. Influence of the duration and timing of data collection on accelerometer-measured physical activity, sedentary time and associated insulin resistance. *International Journal of Environmental Research and Public Health* 2021; 18(9): 4950.

III Sjöros T, Laine S, Garthwaite T, Vähä-Ypyä H, Löyttyniemi E, Koivumäki, M, Houttu N, Laitinen K, Kalliokoski K K, Sievänen H, Vasankari T, Knuuti J, Heinonen I H A. The effect of reducing sedentary time on whole-body insulin sensitivity in adults with metabolic syndrome – A 6-month randomised controlled trial. *Manuscript*.

IV Sjöros T, Laine S, Garthwaite T, Vähä-Ypyä H, Koivumäki M, Eskola O, Löyttyniemi E, Houttu N, Laitinen K, Kalliokoski K K, Sievänen H, Vasankari T, Knuuti J, Heinonen I H A. The Effects of a 6-Month Intervention Aimed to Reduce Sedentary Time on Skeletal Muscle Insulin Sensitivity – A Randomized Controlled Trial. *Manuscript*.

1 Introduction

Physical activity (PA) has been identified as a key component in the prevention of many non-communicable diseases, including metabolic diseases such as type 2 diabetes (T2D) (Lee et al., 2012). The prevalence of T2D is globally increasing together with the prevalence of physical inactivity (Guariguata et al., 2014; Guthold et al., 2018; Zheng et al., 2018). Therefore, insufficient PA is a global health concern. PA is defined as any bodily movement produced by the skeletal muscles, which raises energy expenditure above the resting level (Bull et al., 2020). However, the evidence and knowledge about the benefits of PA is mainly based on the effects of exercise training performed at least at a moderate intensity. To prevent illness, it is recommended for all adults to do at least 150–300 min of moderate-intensity aerobic PA or at least 75–150 min of vigorous-intensity aerobic PA every week (Bull et al., 2020). Additionally, muscle strengthening activities at a moderate or greater intensity are recommended at least twice per week (Bull et al., 2020).

On the other hand, sedentary behaviour (SB) has been associated with an increased risk for cardiometabolic diseases and mortality (Ekelund et al., 2019, 2020; Powell et al., 2018). SB is defined as a sitting, lying or reclining posture combined with a low energy expenditure (Tremblay et al., 2017). The current global guidelines on PA for health promotion encourage people to limit the time spent being sedentary (Bull et al., 2020).

The field of SB research is rapidly developing. The early observations about the deleterious effects of SB were based on self-assessed information, such as questionnaire-based estimates. During the recent years, device-based assessments of SB and PA with, for example, accelerometers have become commonly used methods in SB research. However, the current knowledge about the detrimental effects of SB is mainly based on epidemiological and cross-sectional evidence, and therefore, no specific limits for a recommended maximal amount of SB can be given (Stamatakis et al., 2019). Since the narrative review of Stamatakis and colleagues was published, the amount of cross-sectional and surveillance-based evidence has continued to increase although only a few intervention studies have been published. There is only limited evidence about how reducing SB time can improve health (Hadgraft et al., 2021).

Therefore, there is an urgent need for experimental evidence about the health benefits of reducing SB. Both mechanistic studies about the possible intrinsic consequences of SB and real-life intervention studies about the effectiveness of reducing SB are lacking (Dempsey et al., 2016). Moreover, the effects of reducing SB on insulin sensitivity measured by the gold standard method of the hyperinsulinemic euglycemic clamp (HEC) are unknown. This thesis was designed to cover a part of this gap in the current knowledge. The purpose of this PhD study was to investigate whether reducing daily SB during a 6-month period would improve insulin sensitivity, which is a key component in the gradual development of T2D.

2 Review of the Literature

2.1 Sedentary behaviour and physical inactivity

SB is defined as a sitting, lying, or reclining posture with the relative energy consumption being less than or equal to 1.5 metabolic equivalents (MET)s (Tremblay et al., 2017). The MET is a reference value for the basal resting metabolic rate, in other words one MET equals to the amount of oxygen consumed while sitting quietly, i.e. 3.5 ml per kilogram per hour on average. On the other hand, physical inactivity is defined as not meeting the current PA recommendations (Tremblay et al., 2017). However, these definitions are rather new, a consensus statement by the Sedentary Behavior Research Network (SBRN) only being published in 2017 and consequently in earlier literature the terminology may be mixed; in previous research, the word sedentary was often used in the same meaning as physical inactivity.

The whole field of research considering SB has evolved during the 21st century. First, it was discovered that a great proportion of the world's population is insufficiently physically active to stay healthy and then later, when research methodology developed, it was possible to separate SB from physical inactivity using device-based measurements.

The current guidelines on PA and SB recommended by the World Health Organisation (WHO) are that all adults should undertake 150–300 minutes of moderate-intensity PA or 75–150 minutes of vigorous PA per week, or some equivalent combination of these two (Bull et al., 2020). In addition, regular muscle-strengthening activity and reducing SB is recommended. The latter is a novel addition to the previous guidelines, but no definite amounts of maximal recommended SB are given. Currently, there is insufficient evidence to specify any quantitative thresholds for SB (Bull et al., 2020). Therefore, it is currently impossible to report accurately if a person fully meets the recommendations. To be precise, in this thesis, being physically inactive refers to not meeting the recommended amount of moderate to vigorous PA (MVPA).

2.1.1 The epidemic of sedentary behaviour and physical inactivity

The worldwide prevalence of physical inactivity is increasing alarmingly. In 2016, based on questionnaire-derived data from large population-based cohorts, the global prevalence of insufficient PA was 27.5% and as much as 42.3% in western high-income countries (Guthold et al., 2018). Even if PA during free time has tended to increase in high-income countries, overall PA has decreased (Hallal et al., 2012). Measured by accelerometers, in a population-based sample of Swedish adults, 48% failed to accumulate MVPA for at least 30 min/day, which is a little more than the current recommendation of 150 min/week (Hagströmer et al., 2007). In a slightly larger Finnish study sample, the participants spent an average of 59% of the day being sedentary (Husu et al., 2016). However, one must bear in mind that results obtained by different study methodologies are not directly comparable, as explained later in the Chapter 2.1.2.

In the industrialised countries, increasingly less PA is required at work and deliberate exercise during free time has not been able to compensate for this reduction. Making work and transport less strenuous has been one of the greatest advantages of industrialisation, and although it has considerably increased the quality of life it seems that simultaneously it has also induced a vast number of non-communicable symptoms and diseases.

2.1.2 Measuring sedentary behaviour and physical activity with accelerometry

The associations between physical inactivity and adverse health consequences were first discovered due to the shorter life expectancy and higher disease incidence in people with physically inactive occupations (Morris et al., 1953). Subsequently, the phenomenon has been investigated further based on self-assessment tools such as questionnaires. During the last decades, advances in technology have introduced small wearable devices, such as pedometers, accelerometers, and inclinometers into the field of PA and SB research. Compared to accelerometry, questionnaires are considered to underestimate the total amount of SB (Prince et al., 2020), but also accelerometry results vary according to the analysis methods used. Therefore, the results of different methods cannot directly be compared with each other.

Accelerometry has become the most commonly used quantitative method for assessing PA and SB in clinical studies. However, despite the advantages, the current device-based methods are unable to separate different PA and SB domains, such as screen time, work, exercise, household activities etc. Therefore, self-assessment tools continue to be valuable means of providing complementary information to

device-based measurements. Moreover, there are several issues to consider when evaluating the validity, reliability, and accuracy of the accelerometer measurement.

2.1.2.1 Device placement

Accelerometers can be placed on several locations on the body. The most commonly used locations in clinical studies are the thigh, hip, and wrist. To separate SB from PA, the thigh has been proposed as the optimal device placement (Janssen & Cliff, 2015). However, the attachment of the device on the thigh often requires usage of adhesives that may cause hypersensitization and discomfort, and thus it can limit the duration of the measurement. Devises worn on the hip or wrist are more agreeable to the bearer and lengthy data collection periods become more feasible. However, the agreement between wrist- and hip- measured outcomes is poor (Kamada et al., 2016; Kerr et al., 2017). Compared to the wrist, hip-measured estimates of SB and PA are considered more accurate (Rosenberger et al., 2013). Device placement close to the centre of the mass of the body produces more accurate estimates of body motion, as well as posture.

To be able to quantify standing time separately from sitting, reclining or lying postures, the method should be able to distinguish between postures. The best precision in estimating body posture can be achieved by accelerometers placed on the thigh combined with inclinometry (Janssen & Cliff, 2015). However, it is also possible to reliably estimate body posture with a single tri-axial accelerometer placed on the hip with validated algorithms (Sievänen & Kujala, 2017; Vähä-Ypyä et al., 2018). With the angle for posture estimation (APE) method, the body posture can be determined from the incident accelerometer orientation in relation to the reference vector (i.e. gravity) (**Figure 1**). It is based on the assumptions that the earth's gravity vector is constant and the body posture during walking is upright. The APE method relies on the detection of a walking pattern. Therefore, there is no need to control for the exact orientation of the accelerometer on the hip, only a sufficiently firm fixation with a belt or a clip is required. This is an advantage compared to the more traditional methods that may require the usage of several devices simultaneously (e.g. on the thigh and trunk).

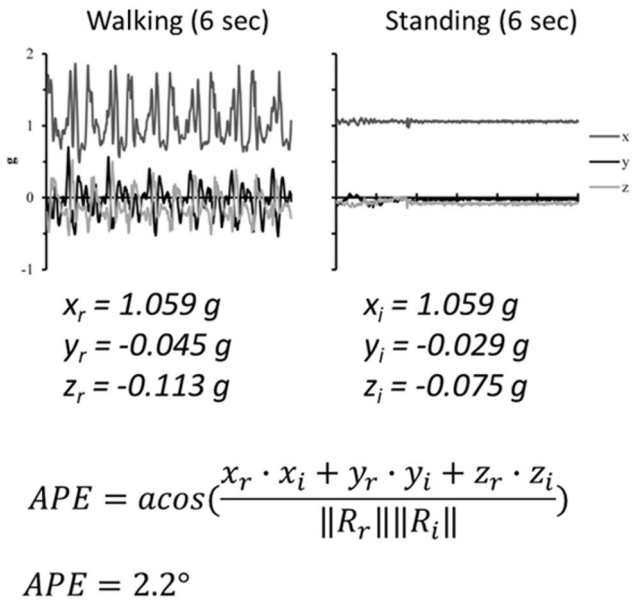

Figure 1. Angle for posture estimation (APE) analysis of a 6-s sample of tri-axial raw acceleration data (x-, y-, and z-axes). The left panel expresses walking and the right panel standing still. Illustration by Henri Vähä-Ypyä, modified from Vähä-Ypyä et al. 2018.

2.1.2.2 Data quantification

There are several possibilities for how to quantify the collected acceleration data. Currently there is no agreement about which method would be the most appropriate in each situation. Generally, the different quantification methods can provide information about SB and PA either as estimates of time spent in different PA categories during the day (calculated by setting certain cut-points for the acceleration magnitude) or as the magnitude of the acceleration (Migueles et al., 2022).

Reducing the acceleration data to a single estimate of the acceleration during the day (e.g. mean acceleration or step count) gives a simple estimate about the overall movement, and statistical interpretations are easy to make. However, presented as estimates of time in different PA categories, the figures are more informative and practical from the perspective of health messaging, but they pose more challenges for the statistical testing and interpretation (Migueles et al., 2022). Furthermore, the agreement between different quantification methods may be poor and one must be cautious when comparing the results of different studies with each other. For example, the commonly used acceleration quantification methods are activity counts and an Euclidean norm minus one (Sievänen & Kujala, 2017). The results and interpretation can be essentially different depending on the quantification method and the cut-points that were used to separate the different PA categories (Migueles,

Cadenas-Sanchez, et al., 2019; Migueles, Delisle Nyström, et al., 2019). However, if the device can store raw tri-axial acceleration data with a sufficient sampling rate, measurement range and resolution, it can be reliably analysed with a single algorithm irrespective of the device that was used in the data collection (Vähä-Ypyä, Vasankari, Husu, Suni, et al., 2015).

Traditionally the commercially available accelerometers have quantified the acceleration as activity counts, which is a proprietary algorithm and thus concealed. Another disadvantage in this method is, that a "count" is an artificial measure, and difficult to interpret. With the method of mean amplitude deviation (MAD), the raw acceleration data can be converted to METs and presented as hours per day in specific MET-categories (Sievänen & Kujala, 2017; Vähä-Ypyä, Vasankari, Husu, Mänttäri, et al., 2015). The MAD describes the average distance of the acceleration data points around the mean (**Figure 2**).

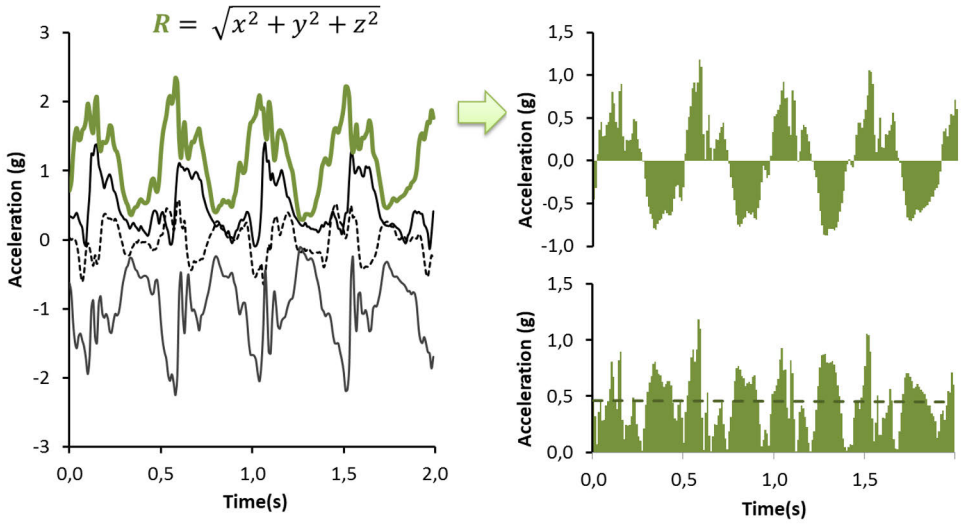

Figure 2. The mean amplitude deviation (MAD) describes the average distance of the resultant of the tri-axial acceleration signal around the mean. The MAD value (here 0.456 *g*) is the mean of the rectified dynamic absolute *g* values within the analysis epoch independent of the static (gravity vector) acceleration component. Illustration by Henri Vähä-Ypyä. Based on Vähä-Ypyä, Vasankari, Husu, Suni, et al., 2015 and Vähä-Ypyä, Vasankari, Husu, Mänttäri, et al., 2015.

2.1.2.3 Duration of data collection

The most commonly used accelerometry data collection period in clinical studies is one week. Often both weekend and weekdays are required for reliable data acquisition. In large population-based cohorts, this is probably a sufficient duration to detect the amount of SB and PA associated with the investigated outcomes.

However, in small study samples and intervention studies this might not be so. Nevertheless, the duration of the accelerometer data collection has rarely been an addressed issue (Bergman, 2018). Tested against 21 days of accelerometry, seven to ten days of monitoring was needed to reliably estimate overall PA (Aadland & Ylvisåker, 2015). Adjusted for the daily wear time of the accelerometer, six to eight days were needed for reliable estimates of SB (Aadland & Ylvisåker, 2015). However, whether or not 21 days is a sufficient duration to represent individuals' habitual PA and SB behaviours in the long term remains unresolved.

Accelerometer wear time (hours/day) may seriously impact the accelerometer-measured estimates of daily SB and PA (Aadland & Ylvisåker, 2015). Data collection for 10 h/day is considered sufficient for reliable estimates of both SB and PA. However, further adjustment by adding the daily accelerometer wear time as a covariate in statistical modelling or calculating outcomes as proportions of wear time can further augment the reliability and accuracy of the outcomes (Aadland & Ylvisåker, 2015; Bankoski et al., 2011).

2.1.3 Health consequences of insufficient physical activity

Physical inactivity has been identified as a risk factor for many non-communicable diseases and premature death. Based on questionnaire-derived data obtained by the WHO, physical inactivity is globally estimated to cause 6% of coronary artery disease, 7% of T2D, 10% of breast and colon cancer, and 9% of premature all-cause mortality (Lee et al., 2012). Additionally, PA is known to reduce the incidence of hypertension, metabolic syndrome (MetS), stroke, and depression and, on the other hand, improve cardiorespiratory fitness, body composition, and cognitive function (Bull et al., 2020). An estimated 15% of premature deaths could be averted worldwide if everyone was sufficiently physically active (Strain, Brage, et al., 2020). Estimated by devise-based measurements, a longer time spent in PA is associated with reduced all-cause mortality, and more strenuous PA is associated with even lower mortality rates than lower-intensity PA (Strain, Wijndaele, et al., 2020).

However, heritable factors are strong determinants of both physical fitness and longevity. Therefore, intervention studies are needed to estimate the health consequences of SB and physical inactivity, or reducing SB, in the general population as well as various clinical conditions.

2.1.4 Health consequences of sedentary behaviour

Currently there is no definite evidence that the health consequences of SB would be distinct from the effects of deficient PA, even if some suggestions have been made that SB would also have independent effects (Biswas et al., 2015). In practical

perspective this is, however, rather trivial. Almost any effort in reducing SB will inevitably result in increasing PA, unless replaced by sleep. However, these two behaviours, SB and PA can coexist; the same person can be highly physically active and also spend lengthy hours being sedentary. In contrast, another person might be physically inactive by not fulfilling the current PA recommendations but neither be sedentary. **Figure 3** represents two suppositional activity profiles during one day.

Figure 3. Two suppositional activity profiles during one day presented as metabolic equivalents (METs). A construction engineer (blue line) is lightly physically active at work and during free time, but does not exercise. An office employee (orange line) is sedentary at work and exercises at a vigorous level during free time. In the evening, both of them are sedentary. Mean MET during the day is 2.3 for both profiles.

Therefore, to be able to make impactful public health recommendations, it would be relevant to know which is more effective, reducing SB or increasing PA. Or rather, what is the combined amount of PA (duration and/or intensity) and SB that would promote health in the best possible way. The current recommendations emphasize that "every move counts", indicating that there is no lower threshold for the health benefits of being more active (Bull et al., 2020). The following chapters focus mainly on the evidence from population-based prospective and cross-sectional studies utilising devise-measured estimates of SB and PA.

2.1.4.1 Sedentary behaviour and mortality

SB has been associated with premature all-cause and cardiovascular as well as cancer mortality. Longer SB time is associated with higher all-cause mortality among inactive middle-aged and older adults (Ekelund et al., 2020). However, within individuals that accumulate 30–40 minutes of MVPA per day, SB is not associated with mortality (Ekelund et al., 2020). Earlier, based on questionnaire-based data, a dose-response association was discovered between sitting time and cardiovascular disease (CVD) mortality (9–32% higher risk) as well as sitting time and cancer mortality (6–21% higher risk) among people in the lowest quartile of PA, but not among the more physically active people (Ekelund et al., 2019). It was concluded, that a sufficient amount of MVPA can reduce, or even eliminate the SB-induced risk of premature death. However, opposite conclusions have also been made, namely that sitting and television viewing are associated with greater all-cause and CVD mortality risk, independent of PA (Patterson et al., 2018). Sitting time above 6–8 hours/day and television viewing for more than 3–4 hours/day have been associated with a higher mortality risk. However, in addition to the equivocality of self-estimated outcomes, in surveillance-based studies there is a risk of bias induced by reverse causation (Strain et al., 2019). This bias was controlled for by removing the deaths during the first two years from the analyses, with the result that the hazard ratios for all-cause and CVD mortality decreased although they remained significant, suggesting that analyses with short follow-up times show stronger associations (Strain et al., 2019).

2.1.4.2 Sedentary behaviour and metabolic health risks

SB has been associated with various metabolic health risks. Pooled from 46 cross-sectional studies, an increase in device-measured SB indicated detrimental associations with fasting plasma glucose, insulin, triglycerides, and high density lipoprotein (HDL) -cholesterol as well as waist circumference (Powell et al., 2018). The size of the reported association effect depended on the device and quantification method used in estimating SB. However, a meta-analysis of 23 prospective studies with mainly questionnaire-based estimates of SB showed no associations between baseline SB or a change in SB during follow-up and weight gain or body mass index (BMI) after five years (Campbell et al., 2018). Nevertheless, comparing high and low amounts of SB at baseline, with a mean difference of 8 h/day between groups, there was a significant risk of becoming overweight (odds ratio 1.33) in the high-SB group at a 5-year follow-up (Campbell et al., 2018). Furthermore, self-reported SB or television viewing has been strongly associated (hazard ratio 1.91) with the incidence of type 2 diabetes (Biswas et al., 2015).

Bedrest-studies

So-called bedrest-models have been utilised to estimate the effects of the absence of gravity during space travel. These studies have demonstrated a rapid loss of muscle mass and changes in metabolism upon reducing daily PA to the minimum by means of total bedrest. In some of the bedrest-protocols, exercise training has been used to counteract the negative effects of being extremely sedentary. Based on these findings, even a large volume of exercise cannot fully counteract the detrimental metabolic adaptations during bedrest (Roux et al., 2022). Therefore, it has been concluded that SB has some independent effects on human metabolism, irrespective of the amount of exercise. Resistance and aerobic exercise protect muscle mass and cardiorespiratory fitness against changes during excessive SB. However, even high amounts of exercise have not been able to fully prevent bedrest-interventions from inducing insulin resistance and glucose intolerance (Roux et al., 2022). This finding suggests that light PA (LPA) and daily non-exercise PA are important in maintaining healthy glucose metabolism.

2.1.5 The effects of reducing sedentary time

Thus far there is little evidence about the long-term effects of reducing SB time. The majority of publications until now are cross-sectional studies or short-term crossover interventions. A meta-analysis has been published consisting of interventions lasting for at least seven days (Hadgraft et al., 2021). This report was comprised of 56 studies, and included both randomised controlled trials and non-controlled interventions, with a total of 5217 participants (52% men). Fifty percent of the interventions lasted for up to three months, 47% took place at workplaces and 61% included environmental modifications (such as sit-stand desks) in the intervention. Some of the studies also included added MVPA or structured exercise and dietary components in the intervention. The pooled effects showed very small beneficial changes in fasting plasma insulin (≈ −1.4 pmol), HDL-cholesterol (≈ +0.04 mmol), body mass (≈ −0.6 kg), waist circumference (≈ −0.7 cm), body fat percentage (≈ −0.3 %), and systolic blood pressure (SBP) (≈ −1.1 mmHg) (Hadgraft et al., 2021).

Another review and meta-analysis consisting of 18 individual studies evaluated the effectiveness of behavioural lifestyle interventions targeting SB (including aims to increase MVPA or dietary components in some of the studies) in different clinical populations, i.e. people with overweight, T2D, CVD, or musculoskeletal or neurological diseases (Nieste et al., 2021). The pooled effects showed small significant decreases in HbA1c, body fat percentage, and waist circumference in favour of the intervention groups (Nieste et al., 2021). However, the effects on body weight, BMI, blood pressure, plasma lipids, or glucose were not significant (Nieste

et al., 2021). The effects on fasting insulin or insulin resistance were not tested in this meta-analysis.

2.1.5.1 The short-term effects of reducing sedentary time

In the short term, interrupting sitting with short activity breaks attenuate the rise of postprandial glucose and insulin. This has been investigated in crossover settings, where typically 5–8 h of sitting has been compared with frequently interrupted sitting (1–5 min activity or standing breaks repeated every 15–60 minutes). In a meta-analysis of 20 studies, the standardised mean difference (SMD) between the conditions in postprandial glucose was -0.36 and in insulin -0.37 in favour of interruptions (Saunders et al., 2018). In a meta-analysis of 37 studies, PA breaks attenuated postprandial plasma glucose, insulin and triglycerides (SMD -0.54, -0.56 and -0.26, respectively) (Loh et al., 2020). Interestingly, BMI was associated with the postprandial responses in plasma glucose and insulin, suggesting that the beneficial effects were greater in people with a higher BMI (Loh et al., 2020).

2.1.5.2 The long-term effects of reducing sedentary time

This chapter summarises findings about metabolic health indicators from randomised controlled trials aimed at reducing SB without adding exercise training where the intervention has lasted for at least three months. It seems that in interventions aimed at reducing SB there is potential to improve insulin sensitivity and body composition, but the evidence about the long-term effects are still contradictory at best.

After a 12-week intervention of reduced SB time, participants with an increased risk of cardiovascular diseases (n = 57) showed no significant changes in body fat, BMI, fasting plasma lipids, insulin, or glucose, or in insulin and glucose during an oral glucose tolerance test (OGTT), compared to the sedentary control group (Kozey Keadle et al., 2014). However, when combined with an incremental structured exercise intervention with added MVPA, the intervention led to a decrease in the insulin area under the curve (AUC) during OGTT; this was not observed with exercise alone. However, this was a pilot study, and the number of participants in each four arms of the study was only 8–16 participants. The study collected accelerometer data repeatedly before the intervention and during weeks 3, 6, 9, and 12 with an ActivPAL monitor, but the methodology is incompletely reported. The results report that the intervention was successful in reducing SB by five percentage points compared to the baseline and increasing daily steps by 3000 (Kozey Keadle et al., 2014).

Among office-workers with young children (n = 135), individual counselling to reduce SB led to a decrease in SB during free time after 3 months, even if the change in total SB was not significant (Pesola et al., 2017). The SB and PA data was collected with waist-worn accelerometers over a seven-day period at baseline and at 3, 6, 9, and 12 months. No significant changes in SB or PA were detected in the 6 to 12-month period. However, the fasting plasma glucose decreased at 3 months and the HOMA-B -index, that is a surrogate measure for pancreatic beta-cell function, increased at 3 and 6 months, these changes were more pronounced in the intervention group and the differences were significant compared to the control group. Interestingly, thigh lean mass measured by dual-energy x-ray absorptiometry decreased in the control group leading to a significant time * group interaction at 12 months (Pesola et al., 2017). This may indicate that even a small decrease in the leisure SB may protect against inactivity-induced muscle loss. Among the same participants (n = 48), there was an interesting short-term effect in the thigh muscle activity (m. quadriceps femoris, m. biceps femoris, m. semitendinosus) measured by surface-electromyography. Muscle inactivity time decreased by 33 (SD 72) minutes/day and light activity time increased by 21 (SD 53) minutes/day in the intervention group compared to the control group during the first two weeks of the intervention (Pesola et al., 2014).

Among young adults with an increased risk for T2D (n = 187), a group-based education intervention that aimed to reduce SB and was supported by a self-monitoring tool was unsuccessful in inducing changes at 3 and 12 months in SB and PA when measured with waist-worn accelerometers for 10 days (Biddle et al., 2015). Nevertheless, compared to the intervention group, fasting glucose increased and insulin tended to increase in the control group at 3 months. However, this difference between the groups was mitigated at 12 months (Biddle et al., 2015).

A motivational counselling intervention to reduce SB among self-reportedly sedentary adults led to more pronounced decreases in fasting insulin and HOMA-IR in the intervention group (n = 93) compared to the control group (n = 73) after 6 months (Aadahl et al., 2014). Additionally, waist circumference decreased in the intervention group by about 1.2 (SD 4) cm, and the change was significantly different from the control group. Regarding the accelerometer-measured SB that was collected during 7 days at baseline and at 6 months, the change during the intervention was non-significant compared to the control group. However, it should be noticed that the analyses were not adjusted for the wear time of the accelerometer, which tended to decrease in the control group compared to the intervention group in the post-intervention measurements. As discussed earlier in Chapter 2.1.2.3, the accelerometer wear time may significantly modify the daily mean estimates of SB and PA. Measured standing time increased in the intervention group by about 13

minutes/day, and the change was significant compared to the control group, in which the standing time decreased by a similar amount.

Several reports on workplace interventions aimed to reduce occupational SB have been published. According to a Cochrane review, they may be effective in reducing occupational sitting, especially if sit-stand desks are provided (Shrestha et al., 2018). A multicomponent workplace intervention that successfully reduced accelerometer-measured sedentary time, by mainly replacing it with standing time, mildly decreased fasting plasma glucose after 12 months (Healy et al., 2017). The intervention was carried out among office-workers (n = 231) with a mean age of 46 years and a mean BMI of 28.6 kg/m^2 (Healy et al., 2017). However, no differences between the intervention and control groups were detected in waist circumference, weight, blood lipids, insulin, or in other metabolic health markers. A similar setting (n = 317) resulted in a small but significant increase in bioimpedance-measured fat free mass leading to a decreased fat percentage in the intervention group in three months, however, no differences were observed in fat mass or waist circumference (Danquah et al., 2017). In these studies, estimates of occupational and total SB and PA were measured with accelerometers during 5-7 days at baseline and at the end of the intervention.

The effects of reducing SB on whole-body or skeletal muscle insulin sensitivity remain unknown.

2.2 The metabolic syndrome (MetS)

MetS refers to the clustering of metabolic risk factors potentially leading to cardiovascular diseases. The prevalence of MetS is increasing globally due to the disadvantages related to economic development and the consequent phenomenon called the 'overweight epidemic' (Afshin et al., 2017). While global data on the prevalence of MetS is lacking, it is estimated that as much as a quarter of the world's population may be affected by this problem (Saklayen, 2018). MetS is related to insufficient PA, a positive energy balance, and an unhealthy diet, and possibly also to sleep disturbances and inflammation (Nilsson et al., 2019). The criteria for MetS vary a little, but according to the consensus statement of the International Diabetes Federation (IDF), the National Heart, Lung, and Blood Institute, the American Heart Association, the World Heart Federation, the International Atherosclerosis Society and the International Association for the Study of Obesity, three of the following criteria need to be met to determine MetS:

- Elevated waist circumference with population-specific definitions (for Europeans ≥ 94 cm in men, ≥ 80 cm in women according to IDF)
- Elevated plasma triglycerides (≥ 1.7 mmol/l)

- Reduced HDL-Cholesterol (< 1.0 mmol/l) in men, < 1.3 mmol/l in women)
- Hypertension (SBP ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg)
- Elevated fasting glucose (≥ 5.6 mmol/l)(Alberti et al., 2009).

In addition to these deviant triglyceride, cholesterol, and glucose levels, drug treatment for the corresponding condition is an alternate indicator (Alberti et al., 2009).

The severity of the metabolic syndrome can be described with the MetS Score, that is the sum of z-scores for the following outcomes: waist circumference, mean blood pressure, fasting plasma glucose, insulin and HDL/triglyceride-ratio (Viitasalo et al., 2014).

2.3 Insulin resistance

One of the early manifestations of the progression of T2D is the slowly developing insulin resistance in different organs. This is a state, where the beta cells in the pancreas are still secreting insulin, but which gradually fails to stimulate glucose uptake (GU) in other tissues. This has been suggested to be the main pathophysiological cause of MetS (McCracken et al., 2018).

2.3.1 Normal glucose metabolism and insulin action

Under normal conditions blood glucose level is strictly regulated. Normally, arterial plasma glucose values fluctuate between maximum of 9 mmol/l after a meal and minimum of 3 mmol/l after strenuous PA, the average being approximately 5.5 mmol/l (Alsahli et al., 2017). Even a small decrease in plasma glucose to 3.8 mmol/l will increase glucagon release and suppress insulin secretion from the pancreas and activate the hypothalamic–pituitary–adrenal axis; this will trigger the release of hormones which will increase the plasma glucose concentration (Alsahli et al., 2017). Normoglycemia is especially important for brain functions, since plasma glucose is the necessary energy source for the brain.

After a meal, in the postprandial state, elevated plasma glucose level increases insulin secretion which induces GU in skeletal muscles, the heart, and adipose tissue by the glucose transporter pathway (Alsahli et al., 2017). Another important activator of the glucose transporter pathway is muscle contraction that is independent of insulin. After being taken up by the cell, glucose is either stored as glycogen (mainly in the skeletal muscles and liver) or glycolyzed. The glycolysis takes place non-

oxidatively in the cytosol or oxidatively in the mitochondria in the citric acid cycle to produce adenosine triphosphate (ATP) to provide energy for the cell.

Insulin regulates plasma glucose both directly and indirectly by affecting e.g. the amount of free fatty acids (FFA) that are the primary energy source for the majority of tissues at rest (Alsahli et al., 2017). Insulin induces suppression of glucose release from the liver and kidney, suppression of FFA release, and translocation of glucose transporters to muscle and adipose tissue cell membrane causing increased GU. In the postabsorptive state (as after a normal overnight fasting), an insulin concentration of 5–10 mU/l will suppress the glucose and FFA release by about 30–50% (Alsahli et al., 2017). Postprandially, plasma insulin increases to 40–50 mU/l and FFA and glucose release are further suppressed (Alsahli et al., 2017). The postprandial insulin levels can induce a maximal FFA suppression effect. However, full suppression of glucose production and stimulation of tissue GU require even higher plasma insulin levels. In healthy individuals under insulin infusion with euglycemia, only a small increment to the physiological plasma insulin (~ 60 mU/l) can almost totally suppress glucose production, but the maximal GU stimulation occurs with markedly higher insulin doses (200-700 mU/l) (Rizza et al., 1981). This indicates that endogenous glucose production is more sensitive to insulin compared to tissue glucose utilisation (DeFronzo et al., 1983; Rizza et al., 1981).

2.3.2 Impaired glucose metabolism and insulin resistance

The diagnosis of T2D is based on the understanding that when the fasting plasma glucose, glycated haemoglobin (HbA1c), or glucose intolerance in the OGTT exceed certain threshold values, the state is called T2D (Table 1). HbA1c reflects mean plasma glucose concentration during the previous 2–3 months. One abnormal fasting or 2h plasma glucose value cannot be utilised alone in the diagnosis of T2D, therefore, at least two samples from distinct time points are needed for a reliable diagnosis.

Table 1. The diagnostic criteria for impaired fasting glucose, impaired glucose tolerance, and type 2 diabetes according to the WHO.

	Fasting plasma glucose, mmol/l	2h glucose in OGTT, mmol/l	HbA1c, mmol/mol
Impaired fasting glucose	6.1–6.9	< 7.8	na
Impaired glucose tolerance	< 7.0	7.8–11.0	na
Type 2 diabetes	≥ 7.0	≥ 11.1	≥ 48

OGTT, oral glucose tolerance test; HbA1c, glycated haemoglobin; na, not applicable.

Insulin resistance as a protective mechanism

Insulin resistance can be seen as a protective mechanism against plasma hyperinsulinemia-induced glucolipotoxicity in the tissues (Nolan & Prentki, 2019). According to a novel concept presented by Nolan and Prentki, insulin hypersecretion is the driving force behind the harmful cascade of events leading to insulin resistance. Insulin sensitivity is a highly adaptive mechanism that can regulate nutrient partitioning between different tissues (Nolan & Prentki, 2019). For example, short-term overfeeding can induce a rapid decrease in insulin sensitivity (Wang et al., 2001). High amounts of glucose in the cell with occasionally concurrent high FFA availability will cause cell injury. High glucose availability will inhibit FFA oxidation and high FFA availability will inhibit glucose oxidation. Consequently, FFA metabolism will shift to esterification and other processes that lead to intracellular steatosis and accumulation of complex lipids. Additionally, excessive nutrient availability also overburdens the electron transport chain leading to mitochondrial dysfunction and reactive oxygen species production (Nolan & Prentki, 2019). Oxidative stress and inflammation are closely linked to MetS and CVD risk (Nilsson et al., 2019). Insulin resistance is a protective mechanism against this harmful flux of events, nevertheless it remains an important biomarker of poor metabolic health.

2.3.3 Measuring insulin sensitivity

There are several options regarding how to quantify insulin resistance or insulin sensitivity of the whole body and different organs.

2.3.3.1 Homeostasis model of insulin resistance (HOMA-IR)

The homeostatic model assessment of insulin resistance (HOMA-IR) is often used as a surrogate measure for insulin resistance because it is an inexpensive and rapid method. It requires only one blood sample acquired during a fasting state and an analysis of plasma insulin and glucose concentrations. It is calculated simply by the equation *insulin x glucose / 22.5* (Matthews et al., 1985). It is well correlated to the gold standard method of HEC.

2.3.3.2 Hyperinsulinemic euglycemic clamp (HEC)

HEC is considered the gold standard for measuring whole-body insulin sensitivity in humans. During HEC the plasma insulin concentration is elevated by infusion to a supraphysiological level to suppress endogenous glucose production by the liver and insulin production by the pancreas. Simultaneously, glucose is infused to maintain a

steady plasma glucose level. By applying a steady and constant insulin infusion combined with on-demand adjusted glucose infusion it is possible to calculate how much glucose is needed to maintain a normal plasma glucose level with a certain pre-determined insulin infusion rate (**Figure 4**). Indirectly it shows how much glucose is taken up from plasma to tissues stimulated by insulin. It is critically important that the study participant stays relaxed in a resting state during the whole HEC procedure to avoid muscle contraction-stimulated insulin-independent GU by the skeletal muscles. The method was originally described by DeFronzo and colleagues (DeFronzo et al., 1979).

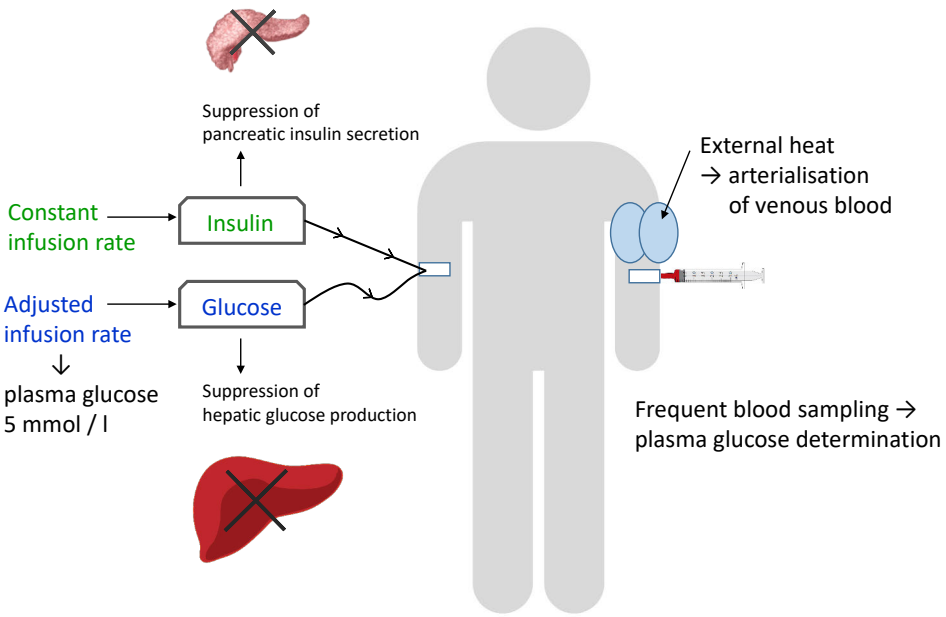

Figure 4. The execution of a hyperinsulinemic euglycemic clamp study.

Despite this method being the gold standard, HEC is a rather demanding and laborious and thus also a relatively costly method. Therefore, it is not used in clinical practise to diagnose insulin resistance or T2D. Nevertheless, some cut-off values for normal and impaired insulin sensitivity by HEC have been presented (Tam et al., 2012). However, the HEC protocols are variable and single cut-offs cannot be applied to all different protocols.

2.3.3.3 Tissue-specific insulin sensitivity by PET

To measure tissue-specific insulin sensitivity HEC can be combined with positron emission tomography (PET). With simultaneous HEC and PET imaging with the tracer ^{18}F-fluorodeoxyglucose (FDG) it is possible to detect site-specific insulin-stimulated GU (**Figure 5**). Other methods such as microdialysis can also be used.

Figure 5. The research technician injecting the FDG-radiotracer into the antecubital vein of the study participant who is lying supine inside the PET/CT-scanner with simultaneous HEC infusion. Photograph by Tanja Sjöros.

PET imaging is a non-invasive and accurate method to study human physiology (Baghaei et al., 2012). The method is based on the half-life of short-lived positron emitting radioisotopes, such as fluorine-18 (^{18}F). The half-life of ^{18}F is approximately 110 minutes (Mettler & Guiberteau, 2012). This allows a fairly flexible time frame but the imaging must take place within hours of tracer production.

After being released from the nucleus of the radioisotope the positron annihilates with an electron in the target tissue and two gamma-rays are created (Baghaei et al., 2012). The reconstruction of the PET image is based on the generalization that the gamma-rays are emitted in exactly opposite directions and thus the origin and the site of annihilation can be detected in a process called annihilation coincidence detection (Cherry et al., 2012). The spatial resolution of the reconstructed image depends on the distance between the tracer molecule where the positron is released and site of annihilation (Cherry et al., 2012). The maximal energy of the ^{18}F isotope is 635 keV, thus the distance remains short, about 0.2 mm, and therefore the resolution is accurate enough to detect differences in the tracer uptake in tissues proximate to each other (Baghaei et al., 2012; Cherry et al., 2012). Appropriate anatomical precision can be achieved by conjoining the PET image with computer tomography (CT) or magnetic resonance imaging (MRI) (Baghaei et al., 2012). One obvious limitation in PET studies is that both the radiotracer and CT induce small doses of ionising radiation to the study participants. Therefore, the number of repeated PET investigations with healthy volunteers is limited.

FDG is a glucose analogue that follows normal glucose pathway when injected into the bloodstream (Baghaei et al., 2012). Unlike glucose, FDG is not metabolized after the initial phosphorylation, but becomes trapped in the cells, in which it has been taken up. The GU rate of the tissues of interest can be quantified using dynamic or static PET imaging with repeated blood sampling to determine the input function.

The dynamic GU rate can be calculated from the PET images with graphical analysis with the Patlak plot, where the tracer transport rate is determined by integrating plasma and tissue time-activity curves (TACs) (Patlak et al., 1983; Patlak & Blasberg, 1985). The slope of the Patlak plot's linear phase is the net uptake rate (**Figure 6**). To calculate the GU this is further multiplied with the plasma glucose concentration determined from repeated venous blood samples during the scanning and divided by the lumped constant 1.2 to adjust the differences between the glucose and FDG transfer and phosphorylation rates (Peltoniemi et al., 2000).

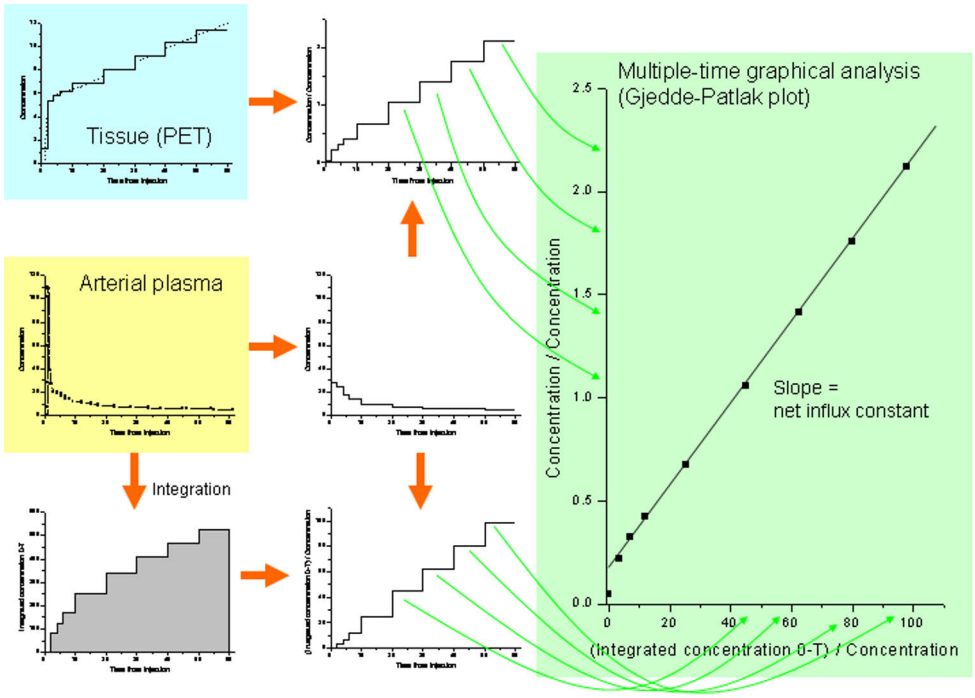

Figure 6. Calculating the Patlak plot from tissue activity curves in the region of interest in the PET image and plasma samples. The Patlak plot becomes linear when the radiopharmaceutical concentrations in the reversible compartments and in the plasma are in equilibrium. The slope of the linear phase of the plot is the net uptake rate constant K_i. © Turku PET Centre, University of Turku. http://www.turkupetcentre.net/petanalysis/model_mtga.html#patlak

During later scanning, i.e. minutes/hours after the tracer injection, GU can be determined with the fractional uptake rate (FUR) analysis (Rutland et al., 2000; Thie, 1995). Compared to the Patlak plot, only one static time point is used to determine tissue tracer concentration, but the dynamic plasma TAC is required (**Figure 7**). FUR overestimates the tracer transport rate compared to the Patlak plot, but this bias decreases towards the later calculation times, and becomes less than 5% after 60 min of scanning (Oikonen, 2007).

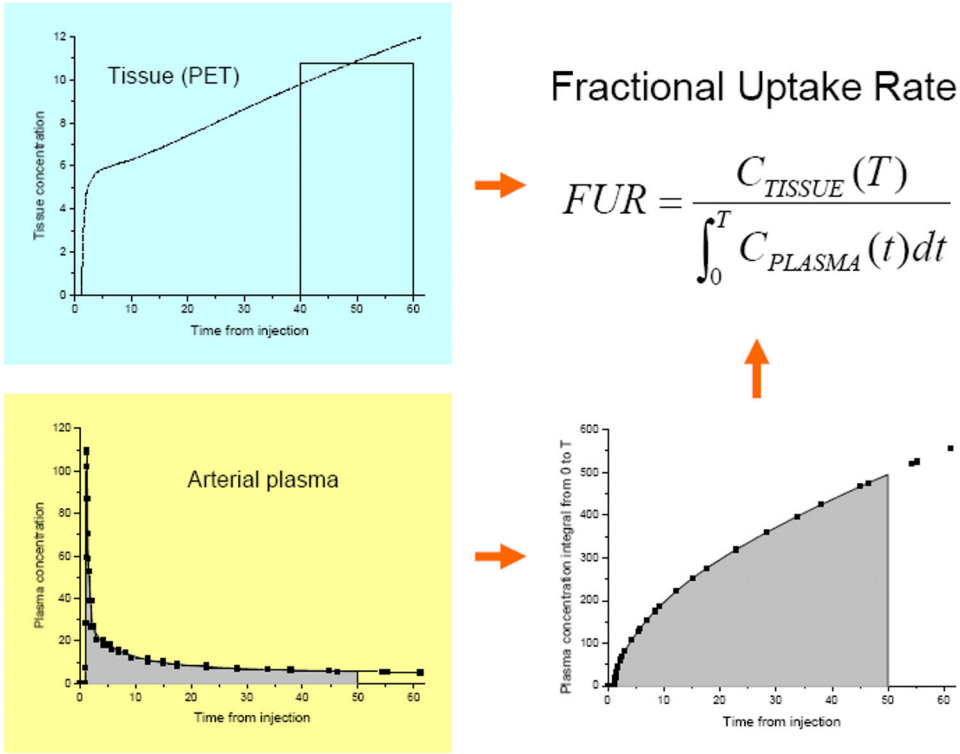

Figure 7. For the calculation of the fractional uptake rate (FUR), the plasma tissue activity curve is integrated from the injection time to the mid-point of the PET time range. The mean tissue concentration is then divided by the plasma integral to obtain the FUR value. © Turku PET centre, University of Turku. http://www.turkupetcentre.net/petanalysis/model_fur.html

3 Aims

The main objectives of this thesis were

1. To evaluate the associations of accelerometer-measured SB and PA with metabolic health indicators and the components of MetS in adults with an increased risk for metabolic diseases (Study I).
2. To investigate whether the measured SB and PA vary during four weeks of accelerometer data collection and if the duration of data collection has an effect on the observed association with insulin resistance (Study II).
3. To evaluate whether an intervention that was aimed to reduce daily SB for 1h had an impact on whole-body insulin sensitivity after six months in sedentary inactive adults with MetS (Study III).
4. To evaluate whether an intervention that was aimed to reduce daily SB for 1h had an impact on thigh muscle insulin sensitivity after six months in sedentary inactive adults with MetS (Study IV).

4 Materials and Methods

4.1 The course of events in the study

This study was conducted at the Turku PET Centre in two phases: the screening phase and intervention phase between April 2017 and March 2020. The study was conducted according to good clinical practice and the ethical principles of the Declaration of Helsinki. All study participants gave their informed consent before taking part in the study. The Ethics Committee of the Hospital District of Southwest Finland approved the study (16/1810/2017). The study is registered at ClinicalTrials.gov (NCT03101228, 05/04/2017).

4.1.1 Cross-sectional screening phase

The participants in this study were recruited through newspaper advertisements and bulletin leaflets from the area covered by the Hospital District of Southwest Finland. The recruitment lasted for 24 months from April 2017 to April 2019. Seven advertisements in local newspapers were published during the years 2017–2019 and the recruitment notice was displayed on the Turku University webpage during the whole recruitment period.

Criteria for entering the screening phase were as follows:

- Age 40–65 years
- BMI 25–40 kg/m^2
- According to self-reports, the participants should not meet the current recommendations for PA and additionally, in their own view, they should be sitting for a major proportion of the day.

The exclusion criteria were as follows:

- History of a cardiac event
- Insulin- or medically treated diabetes
- Abundant use of alcohol (according to national guidelines)
- Use of narcotics

- Smoking tobacco or consuming snuff tobacco
- Diagnosed depression or bipolar disorder
- Inability to understand written Finnish
- Previous participation in a PET-study or considerable exposure to ionising radiation
- Any chronic disease or condition that could create a hazard to the participant safety or endanger the study procedures.

The eligible volunteers were interviewed, and during the interview their BMI, waist circumference, and blood pressure were measured. Additionally, during the interview the participants received an accelerometer (UKK AM30, UKK Institute, Tampere, Finland) that was attached to a flexible belt, which they were instructed to wear on the right hip for four consecutive weeks, starting the following morning. They were instructed to wear the accelerometer during all waking hours, except for activities where the devise would be exposed to water, because the UKK AM30 is not waterproof. Moreover, they were advised to maintain their habitual activities and ways of life during the measurement. Because the storage capacity and the battery durability of the UKK AM30 sensor is limited, the participants were asked to revisit the PET Centre after two weeks to receive a new sensor with fresh batteries; after this exchange the accelerometer measurement was continued as before for two more weeks. During the four-week accelerometer measurement, at a time that was the most suitable for the participants, they were instructed to visit the nearest or most conveniently located Turku University Hospital Laboratory unit for fasting venous blood samples to be taken.

4.1.2 Randomised controlled study

In addition to the screening criteria, the inclusion criteria for entering the intervention phase were as follows:

- Fulfilling the criteria of MetS according to the consensus statement of the IDF and other organisations (Alberti et al., 2009) with diagnosed diabetes excluded
- An SB time of at least 10 h/day or 60% of the accelerometer wear time in the 4-week screening measurement.

If previous exposure to radiation or certain medical diagnoses could not be confirmed before the screening, they were verified at this point.

The participants were allocated into the intervention and control groups by random permuted blocks with a 1:1 allocation ratio and a block size of 44. The

randomisation was performed separately for men and women. A biostatistician, who took no part in the examinations, did the randomisation in advance and the envelopes containing the randomisation information for each participant were sealed. The envelope with the allocation result for each participant was opened after all the baseline measurements had been taken. Furthermore, the participants were randomised into either the HEC + FDG-PET examination (n = 44) or HEC only (additional 20 participants) with a 14:30 allocation ratio. The results of this randomisation were accessible to the researcher and participants in order to allow the scheduling of the measurements. The targeted duration of the intervention was 6 months and the minimum duration was 5 months, after which all baseline measurements were repeated in a convenient order.

4.1.2.1 Intervention

The participants in the intervention group received a one-hour tailored personal counselling session with a physiotherapist (the author), where they were instructed to reduce their daily sedentary time by one hour compared to the baseline. The means to achieve this goal were planned individually according to each participant's preferences and supported by an interactive mobile application (ExSed, UKK Institute, Tampere, Finland) connected to an accelerometer (ExSed Movesense, Suunto, Vantaa, Finland) worn on the hip during the whole intervention. A pre-designed form was utilised as the outline of the counselling session.

The mobile application provided a visual summary of the collected data as time spent in SB, standing, LPA, and MVPA as well as the numbers of steps and breaks in sedentary time during each day to allow self-monitoring by the participants (**Figure 8**). In the application, the target levels of SB and different PA categories (standing, LPA, MVPA) were set according to the baseline measurements during screening by reducing one hour of the SB measured during the screening phase and adding this to standing, LPA, and MVPA; it was divided equally or according to participant's preferences. A maximum of 1/3 (20 min) was added to the MVPA. Adding exercise was not recommended, but the participants were encouraged to increase the amount of PA in their daily activities at work and free time and to reduce the amount of sitting time. Introducing more daily walking was accepted since most of the participants considered this to be the easiest and most convenient method to replace sitting with PA. They were contacted by telephone approximately once a month and they visited the research centre at the midpoint of the intervention to receive support in achieving the goals of the intervention and to assure the functioning of the accelerometer and mobile application.

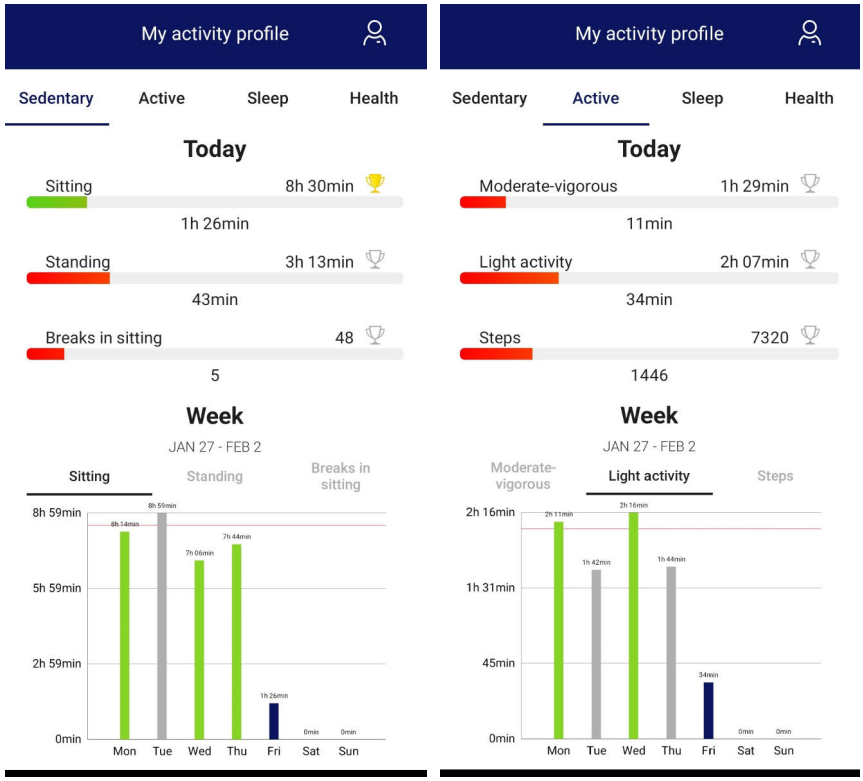

Figure 8. Two examples of the ExSed application display (UKK Institute, Tampere, Finland).

4.1.2.2 Control

The participants in the control group were asked to continue their normal daily routines and they received the same accelerometer with a mobile application as the intervention group. The target levels of PA and SB were set according to the accelerometry results measured during the screening phase. They were contacted by telephone approximately once a month and they visited the research centre at the midpoint of the intervention to ensure the functioning of the accelerometer. After the post-intervention measurements, the participants in the control group had the option of taking part in a similar counselling session to that initially received by the participants in the intervention group.

4.2 Accelerometry

The accelerometer data was collected in collaboration with the UKK Institute, Tampere, Finland. SB and PA were measured with hip-worn tri-axial accelerometers (during the screening phase UKK AM30, UKK Institute, Tampere, Finland; and

during the intervention phase Movesense, Suunto, Vantaa, Finland). Both accelerometers use digital tri-axial acceleration sensors. The sensor of the UKK AM30 accelerometer (ADXL345; Analog Devices, Norwood MA, USA) stores the acceleration signal with 100 Hz sampling frequency, ± 16 gravity unit (*g*) measurement range, and 4 m*g* measurement resolution; and the sensor of the Movesense (LSM6DS3; STMicroelectronics, Geneva, Switzerland) with 52 Hz frequency, ± 8 *g* range and 4 m*g* resolution.

The Movesense device was coupled with the ExSed application installed in the mobile phone of the participant. In cases where the participants did not have a smartphone, the study project lent a suitable phone.

After the screening measurement, the collected acceleration data were transferred to hard disk for further analysis. The acceleration data collected during the intervention was automatically stored in a secure cloud server and later downloaded for analysis. A researcher at the UKK Institute performed the analysis with a Microsoft Excel 2010 Visual Basic for Applications program (Microsoft Corporation, Santa Rosa, CA, USA).

A period was classified as non-wear time, if the acceleration of each three-measurement axis remained within 187.5 m*g* range for at least for 30 min time. A wear time of 10–19 h/day was considered valid. Daily measurement time exceeding 19 h indicates that a participant has likely slept with the accelerometer, and therefore measurement hours exceeding 19 h/day were subtracted from the SB time. Additionally, proportions of different activity intensities (i. e. SB; standing, LPA, MVPA) per day were calculated, and presented as a percentage of the wear time. The mean duration of daily SB and PA categories was calculated individually for weeks 1, 2, 3, and 4 in Study II. Moreover, the cumulative means of SB and the different categories of PA during weeks 1 to 4 were calculated.

4.2.1 Mean amplitude deviation (MAD) method

The collected raw acceleration data was analysed in six-second epochs using the validated MAD method (Vähä-Ypyä, Vasankari, Husu, Mänttäri, et al., 2015). The epoch-wise MAD values were converted to METs (3.5 ml/kg/min of oxygen consumption) (Vähä-Ypyä, Vasankari, Husu, Mänttäri, et al., 2015). LPA was defined as 1.5–3.0 MET (MAD value 22.5–91.5 m*g*), MVPA as > 3.0 MET (MAD > 91.5 m*g*) and vigorous PA as > 6.0 MET (MAD > 414.5 m*g*). However, vigorous PA was added to moderate-intensity PA and presented as MVPA, because only a few participants accumulated any VPA and therefore the data could not be reliably analysed. Additionally, in Study I the epoch-wise MET values were smoothed with 1-min exponential moving average and the daily peak 1-min exponential moving

average of epoch-wise MET values (METpeak) was determined. The daily mean MET value (METmean) was calculated from the epoch-wise MET values.

4.2.2 Angle for posture estimation (APE) method

The body posture was determined with the APE method only for the epochs, which had a MAD value lower than 22.5 mg (Vähä-Ypyä et al., 2018). The classification of body posture was based on two assumptions; the earth's gravity vector is constant and the body's posture while walking is upright. During walking, the accelerometer orientation in terms of the gravity vector was defined as the reference vector. The posture was determined from the incident accelerometer orientation in relation to the reference vector. Epochs having APE values less than 11.6° were classified as standing and epochs having an APE value of at least 11.6° as SB. In standardised conditions, SB can be separated from standing with 95% accuracy (Vähä-Ypyä et al., 2018). In free-living conditions, the agreement between the posture classification from simultaneous thigh-worn and hip-worn data has been about 90% (Vähä-Ypyä et al., 2018).

4.2.3 Steps

The step detection algorithm splits the measured acceleration into vertical and horizontal components. The vertical component is band-pass filtered (1–4 Hz) and positive values are integrated. When the integral value exceeds the specified limit, the step is detected. The step algorithm requires about 3 km/h walking speed to detect every step (Vähä-Ypyä et al., 2018).

4.2.4 Breaks in sedentary time

The daily number of breaks in sedentary time represents the number of SB periods during which the 1-min exponential moving average of the estimated MET value was < 1.5 and which ended in a clear vertical acceleration and subsequent movement (≥ 1.5 MET) or a standing position according to APE (Vähä-Ypyä et al., 2018).

4.3 Blood sample analyses

Blood samples collected after at least 10h of fasting were analysed at the Turku University Hospital laboratory with standard procedures. Plasma insulin was determined by electrochemiluminescence immunoassay (Cobas 8000 e801, Roche Diagnostics GmbH, Mannheim, Germany). Plasma glucose was determined by the enzymatic reference method with hexokinase GLUC3; and plasma triglycerides and

total, LDL and HDL cholesterol by enzymatic colorimetric tests (Cobas 8000 c702, Roche Diagnostics GmbH, Mannheim, Germany). HbA1c was determined by turbidimetric inhibition immunoassay (Cobas 6000 c501, Roche Diagnostics GmbH, Mannheim, Germany).

4.4 Measurements of insulin resistance

4.4.1 HOMA-IR

The HOMA-IR index was calculated from fasting venous plasma samples with the formula

insulin x glucose / 22.5

which was used as a surrogate measure of insulin resistance during Study I.

4.4.2 Hyperinsulinemic Euglycemic Clamp

HEC in Studies III and IV was modified from method originally described by DeFronzo and colleagues (DeFronzo et al., 1979). It was performed after at least 10 hours of fasting. The participants arrived at the research facility in the morning and were instructed to refrain from strenuous PA and alcohol during the previous day. They were instructed to limit all PA to the minimum during the same morning and they stayed rested on a bed for approximately 1h before the initiation of the HEC and during the whole procedure. The antecubital veins of both arms were cannulated. One for insulin and glucose infusions and the other for blood sampling during the study. The arm used for the blood sampling was proximally heated with hot water bottles for the arterialization of venous blood. The primed-constant insulin (Actrapid, 100 U/ml, Novo Nordisk, Bagsvaerd, Denmark) infusion rate was 160 mU/min/m^2 of the participant's body surface area during the first 4 min. From 4 to 7 min, the infusion rate was reduced to 80 mU/m^2/min, and from 7 min to the end of the clamp, it was kept constant at 40 mU/m^2/min. If the plasma glucose of the participant was within normal range, an exogenous 20% glucose infusion was started 4 min after the initiation of the insulin infusion, with a rate of ml/h per participant's body mass (kg) x 0.5, e.g. for a person weighing 80 kg, the rate was 40 ml/h ≈ 8 g of glucose per hour. At 10 min, the glucose infusion was doubled, and after that further adjusted according to the blood glucose concentration to keep it as close as possible to the level of 5 mmol/l. Arterialized venous blood samples were collected before the HEC and every 5 min during the first 30 min of the HEC and later, when a steady state was achieved, every 10 minutes to determine the glucose concentration for

adjusting the glucose infusion rate. The whole-body insulin-stimulated GU rate was calculated from the measured steady-state glucose values and glucose infusion rate, starting from 20 min after the start of the HEC. The outcome, M-value represents whole-body GU as µmol / kg body mass /min.

4.4.3 PET

To quantify tissue-specific insulin-stimulated GU in Study IV, HEC was combined with FDG-PET imaging that was conducted with a PET/CT scanner (GE D690 PET/CT, GE Healthcare, Milwaukee, US) and started during the steady state, 75 (SD 12) min after the initiation of HEC.

FDG-PET imaging was performed as previously described (Eskelinen et al., 2015; Sjöros et al., 2018). The tracer was injected into the antecubital vein and imaging of the thoracic region with the heart and left ventricle (LV) in the imaging area was started simultaneously and continued for 40 min. This was followed by the scanning of the abdominal region. The femoral region with the quadriceps femoris (QF) and hamstring muscle groups was scanned starting at 57 (SD 13) minutes after the tracer injection in 3 x 300 s time frames and continued for 15 minutes. Blood samples for plasma radioactivity determination (1480 Wizard 3; Wallac, Turku, Finland) and calculation of input function were collected at approximately 50 and 70 min after the tracer injection. The activity in the LV chamber during the first 40 min of PET imaging was used to determine the input function for that period. The FDG-PET imaging of the femoral region was successfully completed for 43 participants before the intervention and for 37 participants after the intervention.

All PET image data were corrected for dead time, decay, and photon attenuation and reconstructed using three-dimensional ordered subsets expectation-maximization (3D-OSEM) reconstruction method. The images were analysed with Carimas software (version 2.9, Turku PET Centre, Turku, Finland). Regions of interest (ROIs) were drawn manually into the fused PET/CT images. Femoral region ROIs encompassed the four heads of QF (rectus femoris (RF), vastus lateralis, vastus medialis, vastus intermedius) and the hamstring muscle group (semitendinosus, semimembranosus, and biceps femoris) in five cross-sectional mid-thigh planes (5 x 3.3 mm). Using these ROIs, tissue TACs were extracted from the PET data.

FUR was calculated using graphical analysis utilising tissue and plasma TACs (Rutland et al., 2000; Thie, 1995). The GU as µmol/100 cm^3 tissue/min was calculated by multiplying the FUR rate by the mean plasma glucose concentration from the repeated samples during the PET imaging (Analox GM7, Analox Instruments, London, UK). This was further divided by the lumped constant value of 1.2 to resolve the differences in transport and phosphorylation of FDG and glucose (Peltoniemi et al., 2000). Analyses of plasma radioactivity and plasma glucose

concentrations during PET scanning were performed at the Turku PET Centre clinical laboratory.

4.5 Other measurements

Resting blood pressure (SBP and diastolic blood pressure) in mmHg was measured with a digital blood pressure monitor (Apteq AE701f, Rossmax International LtD, Taipei, Taiwan) in a seated position after at least 5 minutes of sitting. The mean of 2–3 measurements was used as the outcome measure.

Body mass was measured in the screening phase (publications I-II) by scales (Seca 797, Vogel & Halke, Hamburg, Germany) and before and after the intervention (publications III-IV) by air displacement plethysmography (Bod Pod) (Cosmed USA, Concord, California, USA) in light clothing. Body height was measured without footwear with a wall-mounted stadiometer. BMI was calculated with the formula body mass (kg) / (body height $(m)^2$).

Body fat percentage and fat free mass were measured by air displacement plethysmography (Cosmed USA, Concord, California USA) after at least 4 hours of fasting in Studies III and IV. Waist circumference in 0.1 cm was measured with a flexible measuring tape midline between the iliac crest and the lowest rib, repeated twice or until the same measure was obtained twice; i. e. the mode of the repeated measures was used as the outcome measure. Because of great interrater variability, one researcher did all the waist circumference measurements.

The MetS score was calculated as a sum of z-scores of the following outcomes: waist circumference, the mean of systolic and diastolic blood pressures, fasting plasma glucose, fasting plasma insulin and fasting HDL/triglyceride-ratio, as previously described (Viitasalo et al., 2014).

The participants were instructed not to alter their diet during the intervention and this was controlled with structured food diaries before and at the end of the intervention. The participants were instructed to fill in all consumed food and drink (plain water excluded) during four consecutive days with one weekend day included. Detailed instructions of how to complete the diaries were given beforehand and the diaries were checked with a portion picture booklet by a trained researcher during a study visit to assure reliable reporting. The mean daily intake of energy was calculated with computerised software (AivoDiet 2.2.0.1, Aivo, Turku) utilising the Finnish Food Composition Database (*Fineli. Finnish Food Composition Database.*, 2019).

4.6 Statistical methods

4.6.1 Cross-sectional analyses

In the screening phase (Study I), there was a significant difference in the fasting insulin levels between the men and women. Therefore, sex was included as a variable in all the analyses. The associations were examined by linear models with one categorical (sex) and three continuous variables (age, the outcome (metabolic marker) and SB/PA measure) for BMI, waist circumference, glucose, HbA1c, insulin, and HOMA-IR. For lipid-related outcomes, the possible use of cholesterol-lowering medication (statins) was added to the model; and for blood pressure-related outcomes, the possible presence of medical treatment of hypertension was added to the model. Additionally, further analyses with a fourth continuous variable BMI were conducted to adjust for confounding overweight.

The differences in the accelerometer results during weeks 1–4 (Study II) were analysed by a mixed model for repeated measures with two categorical variables: time as a within-subject factor and sex as a between-subjects factor, and the interaction term (time*sex). Each participant had one period of accelerometer data collection that was conducted either during the winter months (November–March) or the summer months (April–October). The differences between the accelerometer results measured during winter and summer were analysed by a two-way ANOVA with the categorical variable sex included in the model. The associations between HOMA-IR and different durations of accelerometer measurements were tested by linear models with one categorical variable (sex) and three continuous variables (age, BMI, and accelerometer outcome) in the model, and therefore the association between continuous factors and the response is described as a slope.

4.6.2 Sample size

The sample size for the intervention phase was determined according to the following power calculations: Based on the earlier finding that insulin sensitivity measured by HEC was increased by 2.4 μmol/kg/min after two weeks of moderate intensity exercise (Eskelinen et al., 2015), we estimated that a reduced SB intervention would increase insulin sensitivity in the intervention group by 1.9 (SD 1.8) μmol/kg/min, representing a 6% change from the baseline. It was estimated that in the control group insulin sensitivity would increase by 0.2 μmol/kg/min. To detect a statistically significant change during the intervention and when compared to the control group, we calculated that 24 subjects were needed in both groups ($\alpha = 0.05$, $1 - \beta = 0.9$). To allow for possible drop-outs and technical problems in the measurements, 64 subjects were recruited.

4.6.3 Longitudinal analyses

In the intervention phase (Studies III and IV) the changes over time and across groups were tested by linear mixed models for repeated measurements with three categorical variables [time (within-factor), group, sex] and the interaction term (time*group). Pairwise comparisons were adjusted with a Tukey-Kramer adjustment for multiple comparisons. When evaluating the changes in measured SB and PA, mean daily accelerometer wear time was included as a covariate in the model. The associations between changes in the measured outcomes during the intervention were tested with the Pearson correlation coefficient.

4.6.4 Data handling

Logarithmic (log10) or square root transformations were performed when necessary to achieve normal distribution assumption of the residuals. The normal distributions of the residuals were examined visually, and sensitivity analyses were performed when needed by a leave-one-out method to assure the robustness of the findings. If not otherwise stated, data are expressed as means (SD) or model-based means with 95% confidence interval, when applicable. In the case of a skewed distribution, the median (IQR or Q1, Q3) is presented. The level of statistical significance was set at 5% (two-tailed). The correlation analyses were carried out with IBM SPSS Statistics 27.0 (IBM Corp., Armonk, NY, USA). All the other analyses were carried out with the SAS 9.4 and JMP pro 13.1 and 15 for Windows (SAS Institute Inc., Cary, NC, USA).

4.6.5 Additional analyses

Additional analyses were performed by dividing the participants into two groups according to the changes in measured SB as a proportion of the daily wear time of the accelerometer. The participants that reduced their daily SB by at least three percentage points compared to the baseline (that equals ~ 27 min reduction in SB with 15 h wear time) were defined as “more active” (n = 30) and the participants that either increased their SB or reduced it less than three percentage points compared to the baseline were defined as “continuously sedentary” (n = 26). This cut-point was chosen because it created a relatively even number of participants in each group and the assumption of normal distribution of the residuals in the statistical model was fulfilled. The participants with missing accelerometer data during the intervention (n = 8) were allocated according to the original randomisation, resulting in 34 participants (with 26 from the intervention and 8 from the control group) in the “more active” and 30 (with 7 from the intervention and 23 from the control group) in the “continuously sedentary” group. Similar statistical analyses with linear mixed

models for repeated measurements, as was done to compare the intervention and control groups, were performed between the ‘more active’ and ‘continuously sedentary’.

5 Results

5.1 Studies I and II, screening phase

In total, 263 people volunteered, of these volunteers 102 women and 42 men were found eligible for Study I and completed the 4-week accelerometer measurements in the screening phase. The mean accelerometer wear time was 14.37 (SD 1.04) h/day and the mean duration of the measurement was 25 (SD 4) days. The majority of the participants (99%) wore the accelerometer for more than 12 h/day on average. There were differences in SB time, SB percentage, and standing time between men and women, but not in daily steps or PA (Table 2). The participants spent 67.0 (SD 8.3)% of the accelerometer wear time in sedentary activities, and took on average 5265 (SD 2113) steps per day. Blood samples were successfully collected from 102 women and 40 men.

Table 2. Characteristics of the study participants by sex. If not otherwise stated, the results are reported as mean (SD).

	MEN	**WOMEN**
n (% of total)	42 (29)	102 (71)
Age, years	58.0 (6.0)	56.4 (6.7)
Height, cm	178.8 (7.1)	165.2 (6.1)***
Body mass, kg	101.8 (14.7)	86.7 (13.4)***
BMI, kg/m^2	31.8 (3.6)	31.7 (4.2)
Waist circumference, cm	116.3 (11.0)	106.7 (10.4)***
SBP, mmHg	149 (19)	147 (20)
DBP, mmHg	91 (11)	90 (12)
HR, bpm	70 (11)	71 (11)
BPL medication, n (%)	23 (55)	34 (33)*
CL medication (statins), n (%)	8 (19)	11 (11)

Table 2. continued.

	MEN	WOMEN
f-Glucose, mmol/l	5.9 (0.7)	5.8 (0.9)
f-Insulin, mU/l	16.0 (10.4)	11.8 (7.3)*
HOMA-IR	4.2 (3.0)*	3.2 (2.4)*
Triglycerides, mmol/l	1.6 (0.9)	1.4 (0.8)
Cholesterol, mmol/l	5.0 (0.7)	5.4 (0.9)**
HDL-cholesterol, mmol/l	1.28 (0.32)	1.66 (0.43)***
LDL-cholesterol, mmol/l	3.4 (0.7)	3.4 (0.8)
HbA1c, mmol/mol	37.5 (5.3)	36.9 (6.1)
Accelerometry, days	24 (5)	26 (4)
Wear time, h/day	14.27 (1.14)	14.41 (1.00)
Sedentary time, h/day	10.13 (1.24)	9.42 (1.31)**
Standing, h/day	1.44 (0.44)	2.18 (0.76)***
LPA, h/day	1.67 (0.61)	1.83 (0.45)
MVPA, h/day	1.03 (0.43)	0.98 (0.36)
PA, h/day	2.70 (0.92)	2.81 (0.70)
Sedentary proportion,%/day	71.0 (7.3)	65.4 (8.1)***
Standing proportion,%/day	10.1 (2.9)	15.0 (5.0)***
LPA proportion,%/day	11.7 (3.9)	12.8 (3.1)
MVPA proportion,%/day	7.3 (2.9)	6.8 (2.5)
PA proportion,%/day	19.0 (5.8)	19.6 (4.8)
Breaks in sedentary time/day	26 (7)	30 (8)***
Daily steps	5408 (2288)	5206 (2046)
MET peak/day	4.9 (0.6)	4.8 (0.7)
MET mean/day	1.3 (0.1)	1.3 (0.1)

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; BPL blood pressure lowering; CL, cholesterol lowering; LPA, light physical activity; MVPA, moderate to vigorous physical activity; PA, physical activity (LPA and MVPA together); MET, the metabolic equivalent. Sex difference in t-test (or Fisher's exact test, when applicable): Women vs. men, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Modified from Original publication I.

5.1.1 Associations between metabolic markers and PA, Study I

BMI associated negatively with MVPA and especially with daily steps, breaks in sedentary time, and MET peak (the mean of the daily MET peak values), when adjusted for age and sex (Table 3). BMI associated positively with SB percentage (of total accelerometer wear time) but not with total SB time. Waist circumference associated strongly with breaks in sedentary time, daily steps, MET peak, SB percentage, and standing time (p = <0.0001, 0.0002, 0.0002, 0.0011 and 0.0014, respectively), whereas with LPA there was no association.

Table 3. Associations between BMI and accelerometer measures, analysed with linear models in which age and sex are included in the model.

	df	F ratio	T ratio	p	r^2
Sedentary%	1	7.20	2.68	0.0082	0.05
Sedentary time	1	2.27	1.51	0.13	0.02
Standing%	1	5.91	-2.43	0.016	0.05
Standing time	1	6.73	-2.59	0.011	0.05
LPA%	1	0.93	-0.97	0.34	0.01
LPA time	1	1.61	-1.27	0.21	0.02
MVPA%	1	6.92	-2.63	0.0095	0.05
MVPA time	1	8.08	-2.84	0.0051	0.06
PA%	1	3.79	-1.95	0.054	0.03
PA time	1	4.88	-2.21	0.029	0.04
Steps / day	1	13.81	-3.72	0.0003	0.10
Breaks in sedentary time	1	16.24	-4.03	<.0001	0.11
MET mean / day	1	9.08	-3.01	0.0031	0.07
MET peak / day	1	12.63	-3.55	0.0005	0.09

LPA, light physical activity; MVPA, moderate to vigorous physical activity; PA, physical activity (LPA and MVPA together); MET, the metabolic equivalent. From Original publication I.

Fasting insulin and HOMA-IR associated negatively with PA, daily steps, breaks in sedentary time, and daily MET peak and mean values, when adjusted for age and sex (Table 4). Greater SB percentage associated with higher fasting insulin and HOMA-IR. The strongest associations with fasting insulin and HOMA-IR were found to be the daily steps and the MET mean (mean of MET mean values of each day).

Table 4. Associations between fasting insulin (log10) and accelerometer measures, analysed with linear models in which age and sex are included in the model.

	df	F ratio	T ratio	p	r^2
Sedentary%	1	11.15	3.34	0.0011	0.12
Sedentary time	1	3.25	1.80	0.073	0.07
Standing%	1	4.24	-2.06	0.041	0.08
Standing time	1	5.05	-2.25	0.026	0.09
LPA%	1	5.10	-2.26	0.026	0.09
LPA time	1	6.13	-2.48	0.015	0.09
MVPA%	1	12.16	-3.49	0.0007	0.13
MVPA time	1	14.03	-3.75	0.0003	0.14
PA%	1	10.56	-3.25	0.0015	0.12
PA time	1	12.05	-3.47	0.0007	0.13
Steps / day	1	19.41	-4.41	<.0001	0.17
Breaks in sedentary time	1	12.70	-3.56	0.0005	0.13
MET mean / day	1	15.43	-3.93	0.0001	0.15
MET peak / day	1	13.53	-3.68	0.0003	0.14

LPA, light physical activity; MVPA, moderate to vigorous physical activity; PA, physical activity (LPA and MVPA together); MET, the metabolic equivalent. From Original publication I.

Fasting glucose associated positively with the SB percentage and negatively with standing time, MVPA, daily steps, breaks in sedentary time, and MET mean and peak values, when adjusted for age and sex (Table 5).

However, in the sensitivity analyses after excluding one participant with an extreme glucose value, only the associations of glucose with standing, MVPA, and daily steps remained significant (p = 0.031, 0.028 and 0.0089, respectively).

Table 5. Associations between fasting plasma glucose and accelerometer measures, analysed with linear models in which age and sex are included in the model.

	df	F ratio	T ratio	p	r^2
Sedentary%	1	5.82	2.41	0.017	0.04
Sedentary time	1	1.87	1.37	0.17	0.01
Standing%	1	4.38	-2.09	0.038	0.03
Standing time	1	5.37	-2.32	0.022	0.04
LPA%	1	0.92	-0.96	0.34	0.01
LPA time	1	1.41	-1.19	0.24	0.01
MVPA%	1	5.79	-2.41	0.018	0.04
MVPA time	1	7.45	-2.73	0.0072	0.05
PA%	1	3.33	-1.82	0.070	0.02
PA time	1	4.41	-2.10	0.038	0.03
Steps / day	1	9.54	-3.09	0.0024	0.07
Breaks in sedentary time	1	4.98	-2.23	0.027	0.04
MET mean / day	1	5.54	-2.35	0.020	0.04
MET peak / day	1	4.54	-2.13	0.035	0.03

LPA, light physical activity; MVPA, moderate to vigorous physical activity; PA, physical activity (LPA and MVPA together); MET, the metabolic equivalent. From Original publication I.

HbA1c associated negatively with breaks in sedentary time when adjusted for age and sex ($p = 0.031$), but not with any other accelerometer measures. In the sensitivity analyses, after excluding one participant with an extreme HbA1c value, this association became non-significant ($p = 0.089$).

Fasting plasma triglycerides associated positively with SB percentage and negatively with PA, when adjusted for age, sex, and usage of cholesterol lowering medication (Table 6). The associations of triglycerides with total PA and LPA were stronger than associations between triglycerides and MVPA.

Table 6. Associations between fasting plasma triglycerides (log10) and accelerometer measures, analysed with linear models in which sex, age, and cholesterol lowering medication are included in the model.

	df	F ratio	T ratio	p	r^2
Sedentary%	1	11.05	3.32	0.0011	0.09
Sedentary time	1	2.30	1.52	0.13	0.04
Standing%	1	4.69	-2.16	0.032	0.05
Standing time	1	5.48	-2.34	0.021	0.06
LPA%	1	7.83	-2.80	0.0059	0.07
LPA time	1	9.20	-3.03	0.0029	0.08
MVPA%	1	6.11	-2.47	0.015	0.06
MVPA time	1	7.23	-2.69	0.0080	0.07
PA%	1	9.73	-3.12	0.0022	0.09
PA time	1	11.17	-3.34	0.0011	0.09
Steps / day	1	7.34	-2.71	0.0076	0.07
Breaks in sedentary time	1	7.25	-2.69	0.0080	0.07
MET mean / day	1	8.96	-2.99	0.0033	0.08
MET peak / day	1	3.98	-2.00	0.048	0.05

LPA, light physical activity; MVPA, moderate to vigorous physical activity; PA, physical activity (LPA and MVPA together); MET, the metabolic equivalent. From Original publication I.

HDL-cholesterol associated negatively with SB time and SB percentage, when adjusted for age, sex and usage of cholesterol lowering medication (Table 7). A longer duration of LPA, MVPA, and total PA were associated with higher HDL. No associations were found between accelerometer measures and total or LDL-cholesterol when adjusted for age, sex, and usage of cholesterol lowering medication (data not shown).

Table 7. Associations between plasma HDL-Cholesterol and accelerometer measures, analysed with linear models in which sex, age, and cholesterol lowering medication are included in the model.

	df	F ratio	T ratio	p	r^2
Sedentary%	1	9.60	-3.10	0.0024	0.25
Sedentary time	1	5.92	-2.43	0.016	0.23
Standing%	1	2.10	1.45	0.15	0.21
Standing time	1	2.65	1.63	0.11	0.21
LPA%	1	9.93	3.15	0.0020	0.25
LPA time	1	10.53	3.24	0.0015	0.25
MVPA%	1	7.12	2.67	0.0085	0.23
MVPA time	1	8.03	2.83	0.0053	0.24
PA%	1	11.98	3.46	0.0007	0.26
PA time	1	12.67	3.56	0.0005	0.26
Steps / day	1	9.05	3.01	0.0031	0.24
Breaks in sedentary time	1	5.56	2.36	0.020	0.23
MET mean / day	1	11.76	3.43	0.0008	0.26
MET peak / day	1	4.19	2.05	0.043	0.22

LPA, light physical activity; MVPA, moderate to vigorous physical activity; PA, physical activity (LPA and MVPA together); MET, the metabolic equivalent. From Original publication I.

There were no associations between accelerometer measures and blood pressure, even when adjusted for age, sex, and usage of antihypertensive medication (data not shown). Lower resting heart rate associated significantly with more breaks in sedentary time ($p = 0.018$).

All the above-mentioned associations are summarised below in Table 8.

Table 8. Associations between accelerometer measures and metabolic outcomes derived from clinical investigation and fasting blood samples measured by linear models in which age and sex were included in the analyses. For lipid-related outcomes, the possible use of cholesterol-lowering medication (statins) was included the model; and for blood pressure-related outcomes, the possible presence of medical treatment of hypertension was included the model. The significant positive and negative associations are indicated by upward ↑ and downward ↓ arrows, respectively.

	BMI	Waist	Insulin	HOMA-IR	Glucose	HbA1c	Triglyceride	HDL	LDL	Cholesterol	RHR	SBP	DBP
Sedentary%	↑	↑	↑	↑	↑	ns	↑	↓	ns	ns	ns	ns	ns
Sedentary time	ns	↑	ns	ns	ns	ns	ns	↓	ns	ns	ns	ns	ns
Standing%	↓	↓	↓	↓	↓	ns	↓	ns	ns	ns	ns	ns	ns
Standing time	↓	↓	↓	↓	↓	ns	↓	ns	ns	ns	ns	ns	ns
LPA%	ns	ns	↓	↓	ns	ns	↓	↑	ns	ns	ns	ns	ns
LPA time	ns	ns	↓	↓	ns	ns	↓	↑	ns	ns	ns	ns	ns
MVPA%	↓	↓	↓	↓	↓	ns	↓	↑	ns	ns	ns	ns	ns
MVPA time	↓	↓	↓	↓	↓	ns	↓	↑	ns	ns	ns	ns	ns
PA%	ns	↓	↓	↓	ns	ns	↓	↑	ns	ns	ns	ns	ns
PA time	↓	↓	↓	↓	↓	ns	↓	↑	ns	ns	ns	ns	ns
Steps / day	↓	↓	↓	↓	↓	ns	↓	↑	ns	ns	ns	ns	ns
Breaks in sedentary time	↓	↓	↓	↓	↓	↓	↓	↑	ns	ns	↓	ns	ns
MET mean / day	↓	↓	↓	↓	↓	ns	↓	↑	ns	ns	ns	ns	ns
MET peak / day	↓	↓	↓	↓	↓	ns	↓	↑	ns	ns	ns	ns	ns

BMI, body mass index; HOMA-IR, homeostasis model for insulin resistance; HbA1c, glycated haemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein; RHR, resting heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; ns, non-significant; LPA, light physical activity; MVPA, moderate to vigorous physical activity; PA, physical activity (LPA and MVPA together); MET, the metabolic equivalent. From Original publication I.

5.1.1.1 Associations adjusted with BMI

To account for confounding overweight, the models were further adjusted by adding BMI as a variable in the analyses. All the associations between breaks in sedentary time and metabolic markers became non-significant, when BMI was added to the model. However, fasting insulin and HOMA-IR associated persistently negatively with PA, daily steps and MET mean and peak values; and positively with SB percentage, when adjusted for age, sex, and BMI. Associations of plasma

triglycerides and HDL-cholesterol with SB percentage, PA, steps and MET mean values also remained significant, when adjusted for age, sex, BMI, and cholesterol lowering medication. However, all the associations of plasma glucose, HbA1c and resting heart rate with accelerometer measures became non-significant when BMI was added to the model. All the BMI-adjusted associations are summarised in Table 9.

Table 9. Associations between accelerometer measures and metabolic outcomes derived from clinical investigation and fasting blood samples measured by linear models in which age, sex, and BMI were included in the analyses. For lipid-related outcomes, the possible use of cholesterol-lowering medication (statins) was included in the model; and for blood pressure-related outcomes, the possible presence of medical treatment of hypertension was included in the model. The significant positive and negative associations are indicated by upward ↑ and downward ↓ arrows, respectively.

	Insulin	HOMA-IR	Glucose	HbA1c	Triglyceride	HDL	LDL	Cholesterol	RHR	SBP	DBP
Sedentary%	↑	↑	ns	ns	↑	↓	ns	ns	ns	ns	ns
Sedentary time	ns	ns	ns	ns	ns	↓	ns	ns	ns	ns	ns
Standing%	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Standing time	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
LPA%	↓	↓	ns	ns	↓	↑	ns	ns	ns	ns	ns
LPA time	↓	↓	ns	ns	↓	↑	ns	ns	ns	ns	ns
MVPA%	↓	↓	ns	ns	ns	ns	ns	ns	ns	ns	ns
MVPA time	↓	↓	ns	ns	ns	↑	ns	ns	ns	ns	ns
PA%	↓	↓	ns	ns	↓	↑	ns	ns	ns	ns	ns
PA time	↓	↓	ns	ns	↓	↑	ns	ns	ns	ns	ns
Steps / day	↓	↓	ns	ns	ns	↑	ns	ns	ns	ns	ns
Breaks in sedentary time	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
MET mean / day	↓	↓	ns	ns	↓	↑	ns	ns	ns	ns	ns
MET peak / day	↓	↓	ns	ns	ns	ns	ns	ns	ns	ns	ns

HOMA-IR, homeostasis model for insulin resistance; HbA1c, glycated haemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein; RHR, resting heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; ns, non-significant; LPA, light physical activity; MVPA, moderate to vigorous physical activity; PA, physical activity (LPA and MVPA together); MET, the metabolic equivalent. From Original publication I.

5.1.1.2 Associations with one-week accelerometry results

Since the majority of the previously published studies have reported results of accelerometer data collected during a one-week period, the 4-week results in the Study I were repeated with only the first week's accelerometer data (4–7 valid days). A number of results were different from the original findings calculated from the 4 weeks' accelerometer data. The significant associations between fasting insulin and standing percentage as well as LPA time and percentage became non-significant. Similarly, the associations between the following became non-significant when analysed with only the first week's accelerometer data: HOMA-IR and LPA time and percentage, triglycerides and standing time and percentage, fasting glucose and total PA and MET peak, HbA1c and daily steps, BMI and total PA time and percentage as well as waist circumference and SB time and total PA time and percentage (data not shown).

5.1.2 Accelerometry duration, Study II

Of the 144 eligible participants, 102 women and 41 men completed the accelerometer measurements with at least two valid weeks of data collection. Among this group, the mean accelerometer wear time was 14.38 (SD 1.04) h/day, and the mean duration of the data collection was 25 (SD 3) days. The duration varied from 10 to 28 days and 91% of the participants had valid data collected during all four weeks.

Measured SB time decreased during the measurement period. Measured mean SB time was 9.88, 9.82, 9.73, and 9.57 hours during the weeks 1–4, respectively (**Figure 9**). The fourth week differed significantly from the weeks 1 and 2 ($p = 0.0033$ and 0.021, respectively).

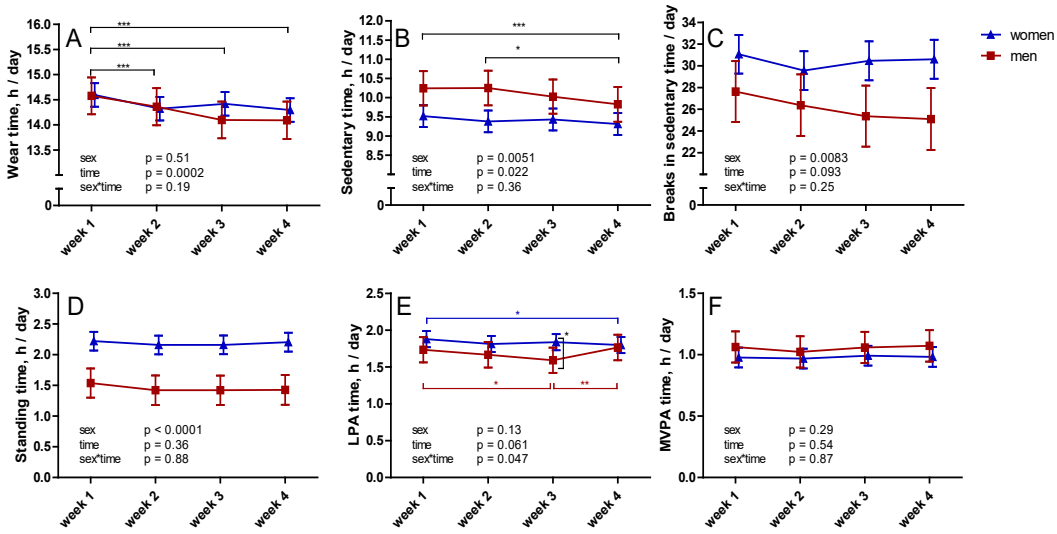

Figure 9. Changes in wear time of the accelerometer (A) and accelerometer-measured sedentary (B) and physical activity time (C–F) during four weeks of data collection. The mean results with a 95% confidence interval for women are represented by blue triangles and men by red squares. LPA, light physical activity; MVPA, moderate-to-vigorous physical activity; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. From Original publication II.

Men had significantly more SB time compared to women, with the 4-week average being 10.09 h/day for men and 9.41 h/day for women. During week 3, men had significantly less LPA compared to women ($p = 0.018$). For men, the mean daily LPA during week 3 (1.59 h) was significantly less than during weeks 1 (1.73 h, $p = 0.023$) and 4 (1.76 h, $p = 0.0066$). For women, the mean daily LPA during week 4 (1.80 h) was significantly lower than during week 1 (1.88 h, $p = 0.045$).

Standing time, MVPA, or breaks in sedentary time did not change significantly during measurement weeks 1–4. Accelerometer wear time decreased after the first week of measurement (**Figure 9**). The average wear time during the weeks 1–4 was 14.59, 14.34, 14.26, and 14.19 hours/day, respectively ($p = 0.0090$, 0.0004, and <0.0001 for week 1 vs weeks 2–4, respectively).

SB, standing, or MVPA percentages, estimated as a percentage of accelerometer wear time, did not change during the four weeks of accelerometry (**Figure 10**). However, there was a significant sex*time interaction in the LPA percentage. During week 3, men had a lower LPA percentage (11.3%) compared to women (12.8%, $p = 0.035$). Moreover, for men, the percentage of LPA during week 4 (12.6%) was significantly higher than during weeks 3 and 2 ($p = 0.0027$ and 0.022, respectively).

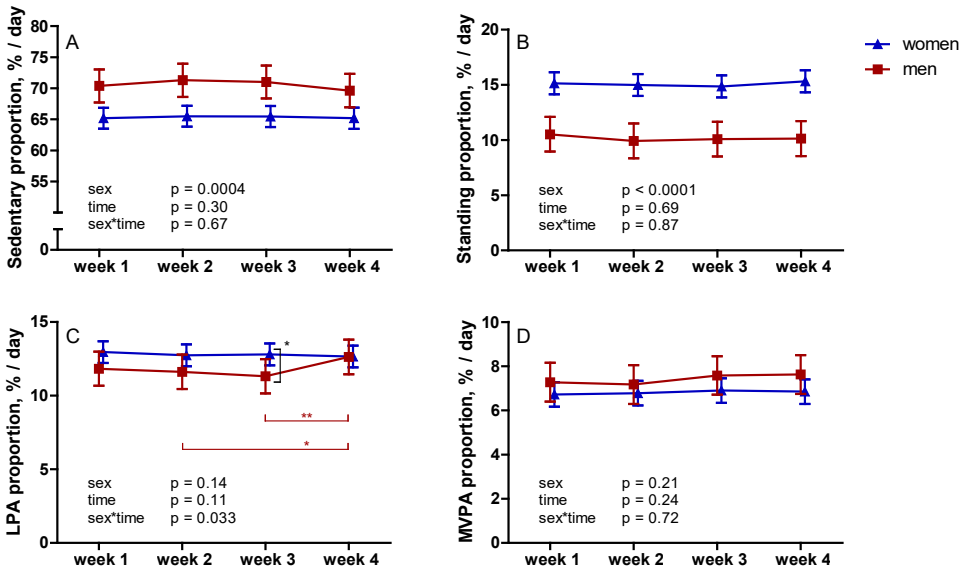

Figure 10. Changes in accelerometer-measured sedentary (A) and physical activity percentages (B–-D) during four weeks of data collection. The mean results with a 95% confidence interval for women are represented by blue triangles and men by red squares. LPA, light physical activity; MVPA, moderate-to-vigorous physical activity; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. From Original publication II.

The accelerometer data was collected during the winter months (November–March) for 69 participants and during the summer months (April–October) for 74 participants. More breaks in sedentary time were measured during the summer (mean 30 breaks/day) compared to the winter (mean 27 breaks/day). Women had significantly more standing time during the summer compared to the winter ($p < 0.0001$). Women had more standing time than men had, both during the winter and summer ($p = 0.014$ and < 0.0001, respectively). Both men and women had more MVPA during the summer compared to the winter (**Figure 11**).

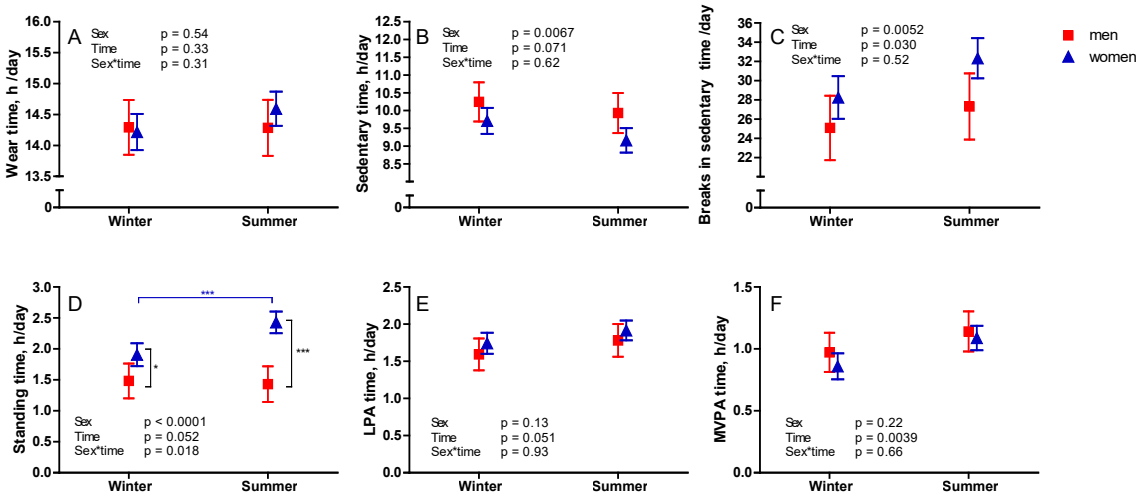

Figure 11. Differences in wear time of the accelerometer (A) and accelerometer-measured sedentary (B) and physical activity time (C–-F) during the winter (November–-March) and the summer (April–-October). The mean results with a 95% confidence interval for women are represented by blue triangles and men by red squares. LPA, light physical activity; MVPA, moderate-to-vigorous physical activity; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. From Original publication II.

The percentage of measured SB time (as percentage of wear time) was significantly lower during the summer months than during the winter months ($p = 0.0076$). Similar to the measured standing time, women had a greater percentage of standing during the summer ($p = 0.0002$), and both sexes had a greater percentage of MVPA during the summer compared to the winter ($p = 0.0078$).

Based on accelerometer data collected during the first week of the four measurement weeks, there was no association between HOMA-IR and SB percentage or LPA, whereas the association between HOMA-IR and MVPA was significant (Table 10). The association between HOMA-IR and SB percentage became significant after two weeks and the LPA time after three weeks of accelerometry, i.e., the associations between HOMA-IR and cumulative means of two and three weeks of accelerometry were significant. The associations of HOMA-IR with SB and standing time, as well as standing and LPA percentages remained non-significant throughout the four weeks of accelerometry. Sex and BMI were significant in all of the models, with BMI being the strongest predictor of HOMA-IR. The models were also tested with the time of the year (summer vs winter) of the data collection included as a categorical variable, but it was not significant in any of the models and it did not essentially change the interpretation of the associations (data not shown).

Table 10. Cumulative means of accelerometer measures (h/%/day) during weeks 1–-4 and associations with HOMA-IR analysed with linear models in which age, sex, and body mass index (BMI) were included in the model. Modified from Original publication II.

	Duration, h/day (SD)	Measurement duration, Days (SD)	estimate, B	p
Sedentary time, h /day				
1 week	9.73 (1.47)	6.7 (0.7)	0.01	0.45
2 weeks	9.68 (1.39)	12.8 (1.8)	0.01	0.34
3 weeks	9.65 (1.36)	19.1 (2.5)	0.01	0.32
4 weeks	9.61 (1.32)	24.9 (3.5)	0.02	0.24
Sedentary proportion,% /day				
1 week	66.6 (8.9)	6.7 (0.7)	0.42	0.055
2 weeks	67.0 (8.6)	12.8 (1.8)	0.46	0.044
3 weeks	67.0 (8.3)	19.1 (2.5)	0.52	0.026
4 weeks	66.8 (8.1)	24.9 (3.5)	0.55	0.023
Standing time, h /day				
1 week	2.02 (0.86)	6.7 (0.7)	−0.03	0.17
2 weeks	1.98 (0.79)	12.8 (1.8)	−0.03	0.21
3 weeks	1.97 (0.76)	19.1 (2.5)	−0.04	0.15
4 weeks	1.97 (0.76)	24.9 (3.5)	−0.03	0.23
LPA time, h /day				
1 week	1.84 (0.59)	6.7 (0.7)	−0.05	0.13
2 weeks	1.80 (0.54)	12.8 (1.8)	−0.06	0.073
3 weeks	1.79 (0.52)	19.1 (2.5)	−0.07	0.041
4 weeks	1.79 (0.50)	24.9 (3.5)	−0.07	0.042
MVPA time, h /day				
1 week	1.01 (0.39)	6.7 (0.7)	−0.12	0.017
2 weeks	0.99 (0.38)	12.8 (1.8)	−0.12	0.017
3 weeks	1.00 (0.38)	19.1 (2.5)	−0.13	0.010
4 weeks	1.00 (0.38)	24.9 (3.5)	−0.14	0.0065

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

5.2 Studies III and IV, the randomised controlled trial

After the screening phase with 144 participants, 64 participants were found eligible for the randomised controlled trial aimed to reduce daily sedentary time by one hour over six months. These 64 participants were randomised into the intervention (n=33) and control (n=31) groups. Of the 64 participants, one participant in the intervention group discontinued the intervention due to personal reasons. Three participants in the control group discontinued the intervention, two due to personal reasons and one due to low back pain. The mean duration of the intervention was 171 (SD 36) days. Accelerometer data from 56 participants was successfully collected during the intervention with a median duration of 117 (Q1 74, Q3 142) days. The data collection from eight participants failed, one due to discontinued participation in the study and seven due to technical errors.

The aim of the intervention was to decrease SB by 1h/day compared to the baseline. During the intervention, accelerometer-measured SB decreased by approximately 40 min/day compared to the baseline in the intervention group, whereas no change was detected in the control group (**Figure 12**). Standing time did not significantly change in either group during the intervention. LPA increased on average by 10 min/day during the intervention, but the difference between groups was not significant. MVPA increased in the intervention group by 20 min/day on average, whereas in the control group the change was not significant. Daily steps increased on average by 3300 steps in the intervention group and by 1600 steps in the control group, and the difference between groups was significant (**Figure 12**).

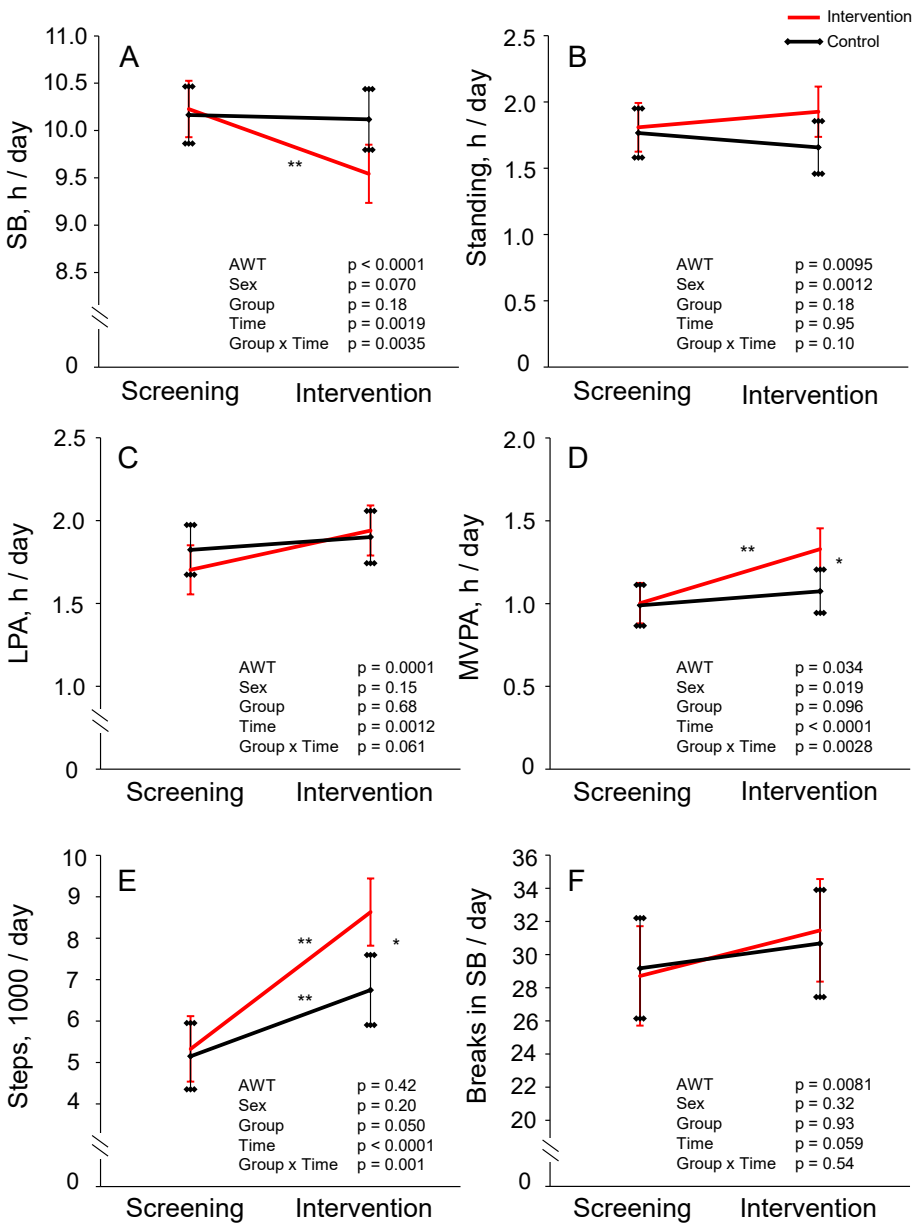

Figure 12. Accelerometer-measured physical activity and sedentary behaviour of the intervention and control groups during a 4–week [mean 26 (SD 4) days of accelerometer data collection] screening phase (**Screening**) and a 6–month [median 117 (Q1 74, Q3 142) days of accelerometer data collection] intervention phase (**Intervention**) presented as model-based means with 95% confidence intervals. A) SB, sedentary behaviour time / day; B) Standing time / day; C) LPA, light physical activity time / day; D) MVPA, moderate to vigorous physical activity time / day; E) Steps / day; F) Breaks in SB / day. AWT, accelerometer wear time; within- or between groups difference * $p < 0.05$; ** $p < 0.01$. From Original publication III.

5.2.1 Changes in whole-body insulin sensitivity and metabolic outcomes, Study III

In Study III body mass, BMI, waist circumference and body fat percentage slightly decreased, but the differences between the groups were not significant (**Figure 13**). The change in body mass was ~ -1 kg on average.

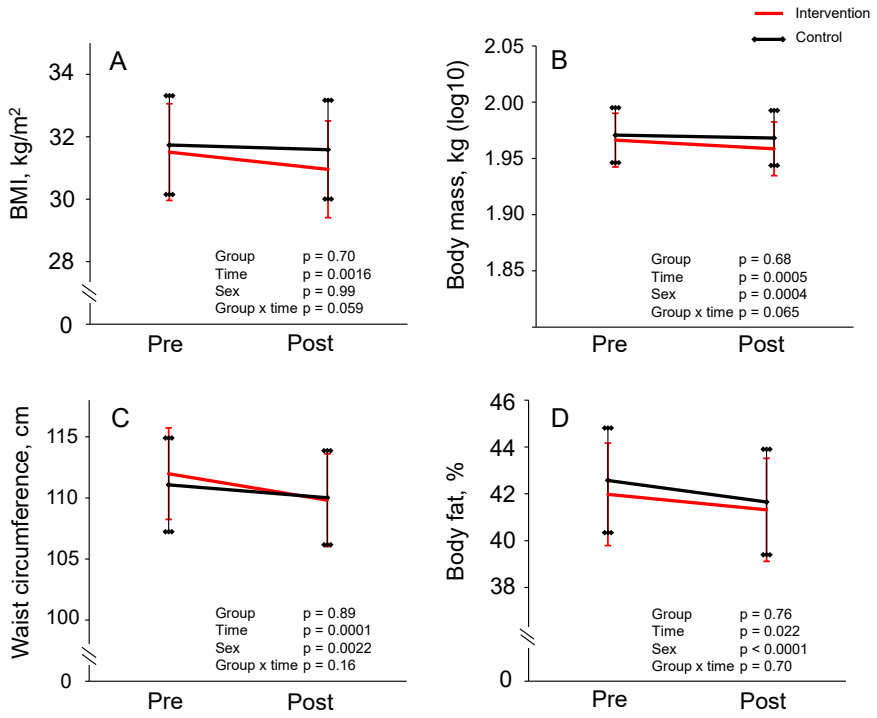

Figure 13. Anthropometric results of the intervention and control groups before and after the intervention presented as model-based means with 95% confidence intervals. A) BMI, Body mass index; B) Body mass; C) waist circumference; D) Body fat percentage measured by air displacement plethysmography. Modified from Original publication III.

As shown in **Figure 14**, whole-body insulin sensitivity (M-value in HEC) and fasting plasma glucose did not significantly change in either group. Additionally, insulin sensitivity relative to lean body mass (measured by air displacement plethysmography) was also calculated, but the results of the linear analyses did not differ from the results of whole-body insulin sensitivity, and thus the results are not reported.

HbA1c increased during the intervention with no difference between groups (**Figure 14**). Fasting insulin decreased in the intervention group compared to the control group (**Figure 14**), as did HOMA-IR (group x time p=0.009). The median

insulin values before and after the intervention were 9 and 8 mU/l in the intervention group and 11 and 12 mU/l in the control group, respectively. However, energy intake measured by food diaries did not change during the intervention in either group (time p = 0.59, time*group p = 0.62).

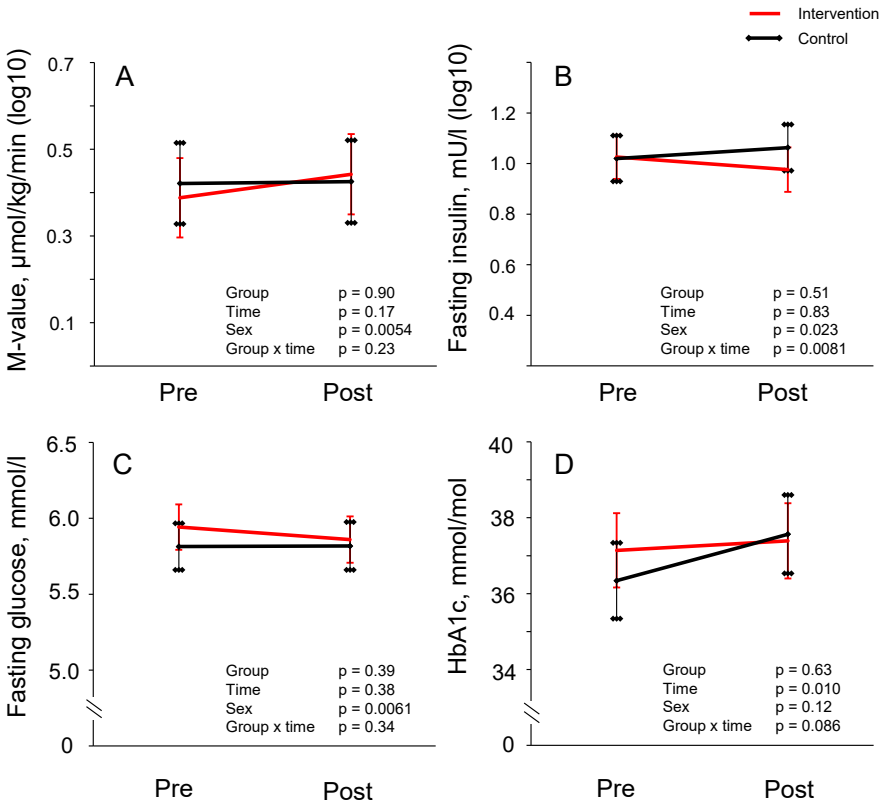

Figure 14. Metabolic results of the intervention and control groups before and after the intervention presented as model-based means with 95% confidence intervals. A) M-value, whole-body insulin-stimulated glucose uptake in hyperinsulinemic euglycemic clamp; B) fasting plasma insulin; C) fasting plasma glucose; D) HbA1c, glycated haemoglobin. Modified from Original publication III.

The change in insulin sensitivity was inversely correlated with the changes in MetS Score, BMI, body mass, fasting glucose, and SB percentage (**Figure 15**). The changes in daily steps and MVPA were inversely correlated with the changes in waist circumference and HbA1c; and the change in LPA was inversely correlated with changes in BMI, body mass, and fat free mass. Additionally, the change in energy intake was positively correlated with the changes in BMI, body mass, and fasting glucose (**Figure 15**).

	Δ MetS Score	Δ WC, cm	Δ BMI, kg/m^2	Δ Body mass, kg	Δ Body fat %	Δ FFM, kg	Δ M-value	Δ fInsulin, mU/l	Δ fGlucose, mmol/l	Δ HbA1c, mmol/l	Δ Energy intake, kJ	Δ SB %	Δ Standing %	Δ LPA %	Δ MVPA %	Δ Steps / day	Δ Breaks in SB /day
Δ MetS Score	**1**	**0.32***	**0.35***	**0.36****	**-0.06**	**0.24**	**-0.30***	**0.51****	**0.55****	**0.10**	**0.21**	**-0.03**	**0.17**	**-0.09**	**-0.12**	**-0.15**	**-0.02**
Δ WC, cm		**1**	**0.53****	**0.54****	**0.13**	**0.16**	**-0.23**	**0.20**	**0.10**	**0.24**	**0.25**	**0.09**	**0.18**	**-0.25**	**-0.29***	**-0.30***	**-0.03**
Δ BMI, kg/m^2			**1**	**1.00****	**0.22**	**0.30***	**-0.45****	**0.21**	**0.23**	**0.31***	**0.32***	**0.27**	**-0.10**	**-0.37****	**-0.19**	**-0.23**	**-0.19**
Δ Body mass, kg				**1**	**0.22**	**0.30***	**-0.44****	**0.23**	**0.23**	**0.32***	**0.33***	**0.27**	**-0.11**	**-0.37****	**-0.17**	**-0.22**	**-0.18**
Δ Body fat %					**1**	**-0.85****	**-0.03**	**-0.02**	**0.01**	**0.15**	**0.01**	**0.12**	**-0.21**	**0.09**	**-0.07**	**-0.12**	**-0.22**
Δ FFM, kg						**1**	**-0.21**	**0.18**	**0.14**	**-0.01**	**0.19**	**0.01**	**0.17**	**-0.28***	**-0.04**	**-0.01**	**0.08**
Δ M-value							**1**	**-0.22**	**-0.32***	**-0.11**	**-0.19**	**-0.31***	**0.24**	**0.26**	**0.17**	**0.19**	**0.02**
Δ fInsulin, mU/l								**1**	**0.23**	**0.18**	**0.15**	**0.17**	**-0.22**	**-0.07**	**-0.02**	**-0.09**	**-0.08**
ΔfGlucose, mmol/l									**1**	**0.11**	**0.49****	**0.00**	**0.13**	**-0.16**	**-0.03**	**-0.17**	**-0.27**
Δ HbA1c, mmol/l										**1**	**0.01**	**0.16**	**0.00**	**-0.14**	**-0.28***	**-0.32***	**-0.17**
Δ Energy intake, kJ											**1**	**-0.08**	**0.12**	**0.05**	**-0.03**	**-0.13**	**-0.09**
Δ SB %												**1**	**-0.79****	**-0.69****	**-0.69****	**-0.55****	**-0.38****
Δ Standing %													**1**	**0.24**	**0.25**	**0.20**	**0.17**
Δ LPA %														**1**	**0.46****	**0.37****	**0.35****
Δ MVPA %															**1**	**0.79****	**0.39****
Δ Steps / day																**1**	**0.48****
Δ Breaks in SB/day																	**1**

◄ **Figure 15.** The Pearson correlation coefficients between changes in different metabolic and physical activity markers during the intervention. Δ, the change from pre-intervention to post-intervention in the metabolic outcomes, and from screening to intervention in the accelerometry outcomes; MetS Score, Metabolic syndrome score (sum of waist circumference, mean blood pressure, fasting plasma glucose, insulin and HDL/triglyceride-ratio); WC, waist circumference; BMI, body mass index; FFM, fat free mass; M-value, whole-body insulin-stimulated glucose uptake measured by hyperinsulinemic euglycemic clamp; fInsulin, fasting plasma insulin, fGlucose, fasting plasma glucose; HbA1c, glycated haemoglobin; SB, sedentary behaviour measured by accelerometry; LPA, light physical activity measured by accelerometry; MVPA, moderate to vigorous physical activity measured by accelerometry. *Significant at the level of $p < 0.05$, ** significant at the level of $p < 0.01$. Modified from Original publication III.

Results of the additional analyses in Study III

Due to the large and overlapping confidence intervals in the accelerometer-measured SB in the intervention- and control groups during the intervention, the individual variation was investigated more closely. Accelerometer wear time increased during the intervention compared to the screening, and it was significant in the model evaluating the change in SB (**Figure 12**). Therefore, the individual changes in SB were evaluated as SB percentage (of the wear time of the accelerometer). Forty participants reduced their SB percentage during the intervention, including 14 participants from the control group. Sixteen participants increased their SB percentage during the intervention, including 3 participants from the intervention group (**Figure 16**).

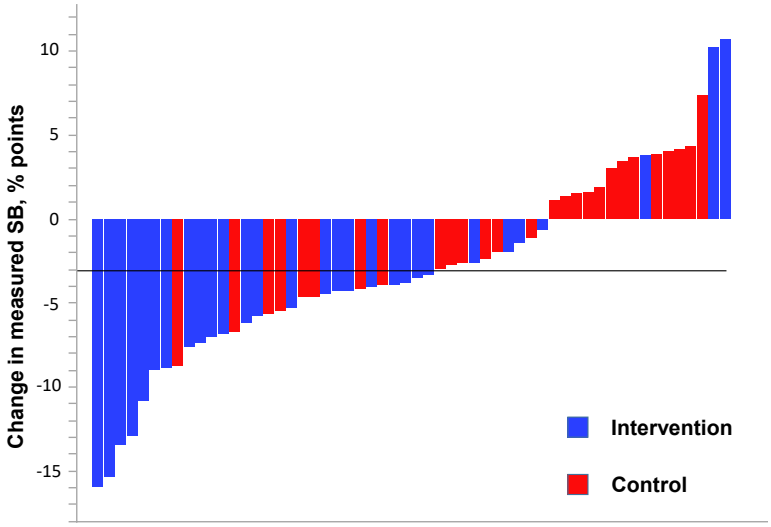

Figure 16. The change in measured sedentary behaviour (SB) of each participant with valid accelerometer data during the intervention (n = 56) as percentage points of accelerometer wear time. The blue colour indicates that the participant was randomised into the intervention group and the red colour the control group. The reference line is set at -3%.

Due to the great variation in the changes in SB in both groups, additional analyses based on the measured SB changes were performed. When the participants were divided into two groups according to the changes in accelerometer-measured SB as explained in the methods Chapter 4.6.5, insulin sensitivity increased in the "more active" group compared to the "continuously sedentary" (**Figure 17**).

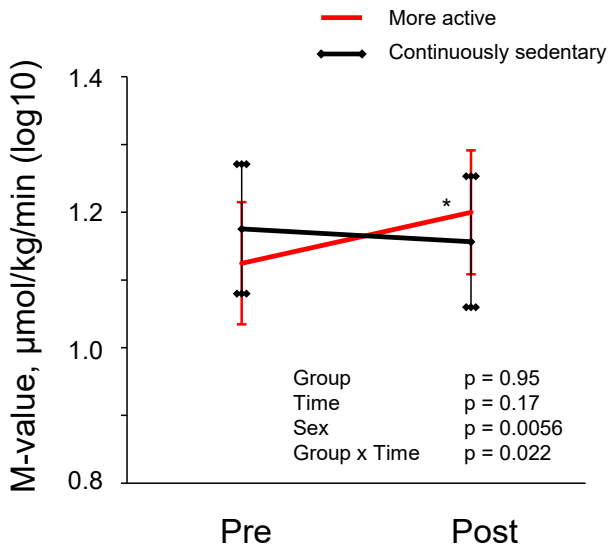

Figure 17. Whole-body insulin-stimulated glucose uptake (M-value) measured by hyperinsulinemic euglycemic clamp in the "more active" (accelerometer-measured sedentary behaviour decreased by at least 3% points ≈ 27 min/day during the intervention compared to screening) and the "continuously sedentary" (accelerometer-measured sedentary behaviour increased, or decreased less than 3% points ≈ 27 min/day during the intervention compared to screening) participants before and after the intervention presented as model-based means with a 95% confidence interval. * Within-group change was significant at the level of $p < 0.05$. Modified from Original publication III.

5.2.2 Changes in skeletal muscle insulin sensitivity, Study IV

A subgroup of the participants (n = 44) in the randomised controlled trial was randomised in the FDG-PET imaging to measure tissue-specific insulin sensitivity. The imaging of the femoral region with the thigh muscles was successfully completed for 43 and 37 participants before and after the intervention, respectively. Parallel to the observed changes in whole-body insulin sensitivity, insulin-stimulated GU in QF or hamstrings did not significantly change during the intervention in either group (**Figure 18**).

Among all the participants, the change in the hamstring GU correlated inversely with the change in SB percentage (% of wear time) and positively with the changes in MVPA, daily steps, whole-body insulin sensitivity and GU in the QF ($r = -0.40$, 0.37, 0.37, 0.69 and 0.85, $p = 0.024$, 0.036, 0.038, < 0.0001 and < 0.0001, respectively). Moreover, whole-body insulin sensitivity correlated inversely with the change in SB percentage and positively with the changes in standing percentage, MVPA and daily steps ($r = -0.47$, 0.40, 0.35 and 0.42, $p = 0.0047$, 0.017, 0.043 and

0.012, respectively). The changes in muscle and whole-body insulin sensitivity had no other significant correlations to the outcomes in this study.

In the additional analyses, when the participants were divided into two groups according to the changes in measured SB, the insulin-stimulated GU in the hamstrings increased among the "more active" participants compared to the "continuously sedentary" participants, but in QF the changes were not significantly different between groups (**Figure 18**).

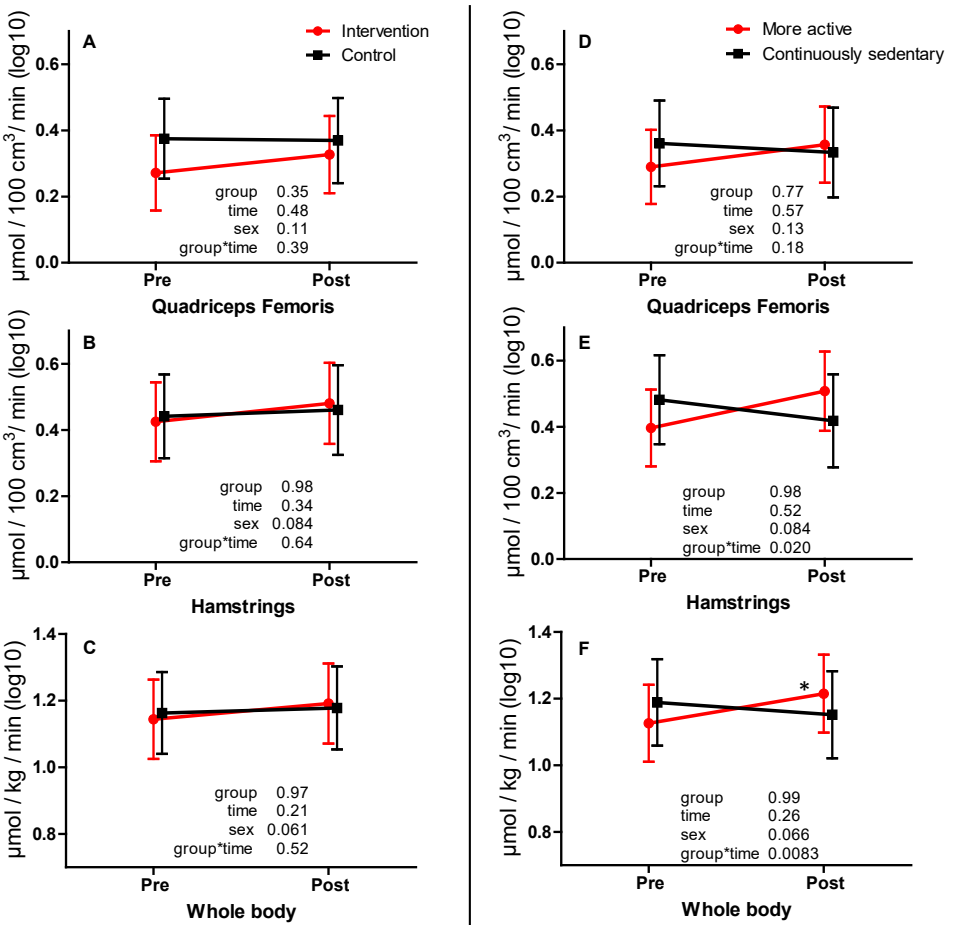

Figure 18. Insulin-stimulated glucose uptake in Quadriceps femoris (A, D) and Hamstring (B, E) muscle groups measured by FDG-PET-imaging and in the whole body (C, F) measured by a hyperinsulinemic euglycemic clamp. The panels A-C represent the log-transformed results in the intervention- (red dots) and control (black squares) groups according to the original randomisation. The panels D-F represent the results of the additional analyses, where the participants were defined as "more active" (red dots) or "continuously sedentary" (black squares) according to the changes in accelerometer-measured sedentary behaviour. The results are presented as model-based means with 95% confidence intervals before and after the intervention. * Within-group change was significant at the level of $p < 0.05$. Modified from Original publication IV.

6 Discussion

In this study, numerous markers of metabolic health, estimated cross-sectionally in the screening phase, were associated with both SB and PA regardless of intensity, in inactive adults with overweight. However, this was partly dependent on the duration of the accelerometer data collection.

The intervention targeted to reduce daily SB for 1h did not improve the primary outcome, i.e. insulin sensitivity measured by HEC in the whole body or in the thigh muscles of previously sedentary and inactive adults with MetS. However, fasting insulin and insulin resistance estimated by the surrogate marker HOMA-IR decreased in the intervention group compared to the control group. Moreover, there was a tendency towards more pronounced decreases in BMI and body mass in favour of the intervention group after the 6-month intervention.

Additionally, the change in SB during the intervention was inversely correlated with the change in insulin sensitivity. Furthermore, there was a large variability and overlap between groups in the changes in measured SB during the intervention. Therefore, the participants were regrouped according to the changes in accelerometer-measured SB to estimate the effects of the actual behaviour change during the intervention. According to these additional analyses, among the participants that successfully reduced their daily SB for at least ~ 27 min/day compared to the baseline, insulin sensitivity increased in the whole body and in the postural hamstring muscles.

6.1 Both SB and PA are associated with metabolic health in cross-sectional settings

Accelerometer-measured SB was associated with the surrogate marker of insulin resistance (HOMA-IR) in participants with overweight and low self-reported PA levels. Moreover, all PA, regardless of the intensity, was associated with lower fasting insulin levels and better insulin sensitivity. Furthermore, all PA, and LPA in particular, was associated with beneficial plasma lipid profile.

In Studies I and II, the associations of metabolic markers with SB and PA were assessed both as units of time and as percentage of the daily wear time of the accelerometer. The differences in the observed associations confirm the earlier

observation that calculating the SB percentage attenuates the effect of variance in wear time of the accelerometer (Aadland & Ylvisåker, 2015). Therefore, the SB percentage can be considered a more accurate estimate of SB than total SB time. Thus, the comparisons to some earlier published findings can become challenging, if the variation in wear time has not been accounted for. For example, the otherwise creditable study of Aadahl et al. (2014) did not weigh the possible wear time effect, which should be taken into consideration when interpreting the results. In the future, it would be advisable to somehow take account of the daily wear time, unless accelerometry is implemented for a full 24 hours.

In Study I, SB percentage was positively associated with fasting insulin, insulin resistance (HOMA-IR), plasma triglycerides and negatively with HDL-cholesterol, when sex, age, BMI, and possible medication for high blood cholesterol were taken into account. If the BMI was not included in the statistical model, the SB percentage was also positively associated with fasting plasma glucose. This suggests that sedentariness plays a role in the regulation of plasma glucose levels, but excessive body adiposity might be a stronger stimulus for elevated plasma glucose than SB per se. Earlier findings have been controversial. For example, similar to our study, sedentary status has associated with HDL but not with HbA1c, when controlled for BMI (Bakrania et al., 2016). SB has also associated with fasting insulin but not with glucose or triglycerides, without including BMI in the analyses (Carson et al., 2014). The observed associations have varied as have the methods and possible confounders included in the analyses. However, in a meta-analysis including 4–15 study reports in the analyses of each outcome, increased amount of SB was associated with small but statistically significant mean increases in waist circumference, fasting plasma glucose, insulin, and triglycerides and a reduction in HDL-cholesterol (Powell et al., 2018). However, the effects were heterogeneous and especially in the case of insulin, the device and quantification method used in the estimation of SB were associated with the effect size (Powell et al., 2018). Based on the observations in Study II of this thesis, possibly a longer duration of accelerometer data collection could enhance the robustness of the associations.

In Study I, MVPA was associated with better insulin sensitivity and a beneficial plasma lipid profile. Generally, the associations of the measured metabolic outcomes with MVPA were moderately stronger than the associations with SB. This is in line with previous studies, that indicate that MVPA can, to some extent, overcome the detrimental effects of SB (Dempsey et al., 2020; Ekelund et al., 2020). However, total PA and step count were the strongest predictors of insulin sensitivity and beneficial lipid profile. This can indicate that the total daily duration of PA, regardless of how it is accumulated, is actually more relevant than the intensity of PA considering the metabolic health of people that are physically inactive, i.e. do not fulfil the current recommendations for PA.

In addition to MVPA, LPA has been recently suggested to be effective in counteracting the detrimental effects of sitting. Generally, the effects of LPA have been considered to be milder compared to the effects of more intensive MPVA. However, in Study I, beneficial blood lipid profile was associated more strongly with LPA than with MVPA. This can be due to the fact that the participants in this study simply had more LPA than MVPA; whereas the old conception of LPA being the best method for enhancing lipid metabolism might also be related to this finding. Even if the superiority of LPA over MVPA in regulating plasma lipids has not previously been confirmed (Debache et al., 2019; Healy et al., 2008; Yates et al., 2020), a recent study employing lipidomic analyses found an association between LPA and HDL particles, whereas the very-low-density lipoprotein particles associated the strongest with MVPA (Henson et al., 2020). Additionally, in a compositional model of total PA and SB time predicting plasma triglycerides, the coefficient vector of LPA was the steepest compared to the other behaviours (Debache et al., 2019). Therefore, staying lightly active throughout the day may help in maintaining a beneficial plasma lipid profile, especially if the recommendations for MVPA are not met.

In Study I, standing was associated with body adiposity, fasting insulin, glucose and triglycerides, but when adjusted with BMI these associations were no longer significant. Studies on the associations between standing and metabolic health markers are scarce, probably due to the challenges in the assessment of standing. Longer standing time has been associated with smaller waist circumference (Husu et al., 2019) and a beneficial plasma lipid profile (Debache et al., 2019). Questionnaire-based estimation of standing has been associated with lower CVD- and all-cause mortality only among the most inactive people (Katzmarzyk, 2014). It seems plausible that increasing standing time could improve metabolic health, but the amount of standing becomes clinically significant only if the level of PA is very low. In our study, the participants were self-reportedly inactive, but cross-sectional associations have only a limited value in estimating the long-term effects of different behaviours. Moreover, the causal relations between standing and health need to be confirmed by experimental studies. However, in the short term, standing breaks can help maintain postprandial glucose homeostasis, but the long-term effects are yet to be confirmed (Henson et al., 2016; Thorp et al., 2014). During our 6-month intervention (Study III), standing time did not significantly change in the intervention group compared to the control group. Therefore, the minor improvement in fasting insulin cannot be considered to result from increased standing time.

Breaks in SB were not significantly associated with metabolic outcomes in Study I, when adjusted for BMI. Neither had the intervention any effect on the number of breaks in Study III. However, previously breaks in SB have had beneficial

associations with metabolic health markers (Husu et al., 2019). Nevertheless, the findings have been controversial. Similar to our findings, the number of sedentary breaks did not associate with glucose metabolism status measured by an oral glucose tolerance test in a comparable study population (van der Berg et al., 2016). Since causation cannot be determined in cross-sectional studies and BMI and waist circumference associate negatively with breaks in SB, it appears that people with overweight or obesity tend to have fewer breaks in their SB during the day. Nevertheless, in the short term, activity breaks during prolonged sitting can have beneficial effects on postprandial glucose homeostasis (Loh et al., 2020). How this can improve metabolic health in the long term is yet to be confirmed and should be the subject of future research.

It is noteworthy, that the coefficients of determination were fairly low in all the models estimating the associations of SB and PA with metabolic health outcomes with age, sex, and possible medication included in the models. This indicates that other lifestyle components, such as a healthy diet and sleep, a healthy body weight, and inherited factors also play important roles in the regulation of metabolic health. The smokers were excluded from this study, but there is also strong evidence that refraining from tobacco is crucially important in maintaining good metabolic health.

It was hypothesized in Study II that there would be systematic changes in the accelerometer-measured SB and PA during the four weeks of data collection, indicating a possible intervention effect caused by wearing the device. However, only minor changes were detected partially depending on the daily wear time of the accelerometer. It is possible that four weeks was too short a period to reveal such changes. However, the sufficient duration of accelerometer data collection has rarely been discussed and investigated (Bergman, 2018). Therefore, future studies with even longer data collection periods are warranted.

The mean estimates of SB and MVPA in Study II were contingent on whether the data was collected during the winter or summer. Depending on the study design, the season of the accelerometer data collection should be considered carefully, especially in countries with substantial seasonal variation in weather (Aadland et al., 2020). However, the season of the data collection was not associated with HOMA-IR in this study. The fasted blood samples were taken during the accelerometer data collection period, and it is possible that both PA behaviours and insulin resistance fluctuate with the season. Therefore, the timing of accelerometer data collection did not confound with the observed association.

Interestingly, even without any clear changes in measured SB or PA during the 4-week data collection, there was no significant association between insulin resistance and SB with the accelerometer data of the first week. However, the association of HOMA-IR with SB became significant after two weeks and with LPA after three weeks of data collection. These strengthened associations can be due to

reduced data variability gained with more measured data points within each individual. Additionally, fasting plasma insulin varies considerably in the general population, and the distribution is often skewed, as was the case in this study. Moreover, the electrochemiluminescence method is sensitive to hemolysis, which can cause even more inconsistency in the results (Chevenne et al., 1998). With such a sensitive measure, a more robust measure as a reference point may be needed.

Based on the findings of Study II, accelerometer data collection for at least three weeks may be needed if the purpose is to find associations with tenuous health outcomes, such as insulin resistance, especially with relatively small study samples. Alternatively, repeated blood sampling could be considered, but currently there is no evidence to support or reject this alternative. Moreover, since plasma insulin cannot be measured without invasive blood sampling, extending the duration of accelerometer data collection is a more pleasant option for the study participants. In population-based large study samples a shorter data collection period may be sufficient to reveal such associations.

6.2 Reducing SB had a minimal effect on insulin sensitivity

The primary outcome of the randomised controlled trial (Study III) was whole-body insulin sensitivity measured by HEC. The intervention, which aimed to reduce daily SB for 1h, did not significantly change the whole-body insulin sensitivity in previously sedentary and inactive participants with MetS.

In Study III, the aim of the tailored intervention was to reduce SB by 1h/day by increasing the amount of standing and habitual PA (including both LPA and MVPA), without adding exercise training. However, the intervention was only successful in reducing SB and increasing MVPA. The intervention resulted in a 40-minute decrease in daily SB and a 20-minute increase in daily MVPA during the six months. The small increase in LPA time during the intervention did not significantly differ from the control group. However, the step count increased significantly in both groups, on average by 3300 steps in the intervention group and by 1600 steps in the control group. By adding up the significant and non-significant changes in LPA and MVPA, the increases in the mean PA duration was 34 min/day in the intervention group and 10 min/day in the control group; this is fairly well in line with the measured step counts. It results in an average 44 steps / PA min in the intervention group and 38 steps / PA min in the control group during the intervention. This indicates, that even if the changes were not significant, the control group also slightly increased the amount of PA during the intervention, which may have partly mixed the intervention effects.

However, there was a difference between the intervention- and control groups regarding the change in fasting insulin after six months, despite there being no significant change in insulin sensitivity. Moreover, a trend towards beneficial changes in the intervention group was consistent across several study parameters. Since there was no primary intervention effect in the main outcome, the correlations of the study outcomes were analysed in the whole study population conjointly. The correlations and the additional analyses suggest, that an actual reduction in measured SB increases insulin sensitivity. However, based on the primary results, it seems that aiming at a 1h reduction in daily SB by replacing it with habitual PA is not sufficient to induce major changes in insulin sensitivity or metabolic health in general.

In Study III, fasting insulin changed in favour of the intervention group. The novel concept about insulin resistance is that it is a protective mechanism against hyperglycemia-induced hyperinsulinemia in plasma and subsequent hyperglycemia-induced tissue damage (Nolan & Prentki, 2019). Therefore, plasma insulin and glucose levels should decrease before any improvements in insulin sensitivity. Otherwise, enhanced insulin sensitivity would increase glucolipotoxicity in the tissues. From that perspective, the minor decrease in fasting insulin in the intervention group of this study can be seen as the first step towards improved glucose metabolism and insulin sensitivity. However, aerobic and resistance exercise, that consist of MVPA, can increase insulin sensitivity very rapidly and even when performed in short bouts (Bird & Hawley, 2017). Possibly more MVPA would have been needed to induce a significant improvement in insulin sensitivity in this study.

Even if the amount of MVPA and daily steps significantly increased in the intervention group, the intervention was unable to enhance insulin sensitivity. However, the MVPA accumulated during the study consisted mainly of moderate-intensity PA, the amount of vigorous PA being marginal (median 0.2 min/day during screening and 0.6 min/day during the intervention). The nearly total lack of vigorous PA may be the reason that MVPA was not effective in improving insulin sensitivity in this study. It is possible that vigorous PA rather than moderate PA is needed to gain health benefits in adults with overweight (Drenowatz et al., 2016).

Additionally, the amount of measured daily MVPA depends on the analysis method used, the epoch length of data collection and possible data smoothing (Banda et al., 2016; Vähä-Ypyä et al., 2021). In this study MVPA was measured in 6s intervals, and the daily increase (20 min in the intervention group) may consist of very short bouts. Even if the length of MVPA bouts is no longer considered essential in gaining health benefits (Jakicic et al., 2019), it is still possible that the duration of bouts of PA play a role in the health promotion of people with overweight and low cardiorespiratory fitness, as in this study. Estimated by personal cut-points according to relative MET values, people with low cardiorespiratory fitness gained more

MVPA in the their daily chores than people with high cardiorespiratory fitness; but only long bouts of vigorous PA and MVPA were positively associated with cardiorespiratory fitness (Vähä-Ypyä et al., 2021). Possibly people with the lowest fitness would need longer bouts of vigorous PA to gain significant health benefits, however, this may not be feasible. This suggests a vicious cycle between low fitness and the ability to be active. However, it is noteworthy that cardiorespiratory fitness relative to body mass can increase considerably simply by losing weight.

A challenge in lengthy behavioural interventions is that committing to the desired behaviour change can become difficult. It has been observed previously, that the intended changes in SB have been more pronounced at the beginning of the intervention (Healy et al., 2016; Pesola et al., 2017). Possible reasons for the lack of commitment include: the lack of perceived health risk in the current behaviour; the preference to adopt more active PA behaviours rather than to sit less; participants may experience social and physical barriers to sitting less; there might also be too few contacts with the researcher and difficulties with the self-monitoring tool (Biddle et al., 2017). Indeed, unofficial discussions with the participants in this study confirm that all of these phenomena might also have occurred in this study. For example, the current intervention aimed at replacing SB with standing and non-exercise PA, but during the intervention the participants were able to sustain only the increase in MVPA. Many of the participants in this study related that they considered walking as the easiest way to increase daily PA, and measured by accelerometers, walking is most often classified as MVPA. It might be easier to add moderate-intensity PA to the daily activities instead of altering daily sedentary chores to lightly active ones. Moreover, social and physical environments are important factors influencing individual SB and PA, and therefore workplace interventions may be more effective in increasing daily standing and LPA (Healy et al., 2016). Individual counselling may have more potential in increasing PA during free time, and MVPA and exercise are possibly the most feasible means to do that.

However, even if the amount of MVPA and daily steps significantly increased in the intervention group, the intervention was unable to enhance insulin sensitivity compared to the control group. There was a disagreement between the results analysed according to the original randomisation compared to the additional analyses with the participants divided into “more active” and “continuously sedentary”. This suggests that the variation in the ability to commit to the intended behaviour change may be the reason for the ineffectiveness of the intervention. Moreover, the majority of the participants reported as their original motivation to participate in this study the desire for a change in behaviour in order to gain health benefits or to lose weight. Therefore, it is possible that some participants in the control group independently also implemented a behaviour change, consciously or unconsciously, with the aid of the accelerometer and application. The increased step count in the control group

supports this prospect. The above-mentioned challenge in the behavioural intervention studies has been previously identified, and for example in the IDES-study the results from the original randomisation differed from the results of re-pooled data (Balducci et al., 2017, 2022), as was the case in the current study.

Even if there was no significant intervention effect on whole-body insulin sensitivity, it was hypothesised that insulin sensitivity in the weight-bearing thigh muscles could still be apparent. The increased whole-body insulin sensitivity induced by PA is considered to predominantly rely on changes in the skeletal muscles (Dela et al., 1992; Reichkendler et al., 2013). However, the average body fat percentage of the participants in this study was 43% and thus a substantial amount of the body mass of the participants comprised of mass other than muscle mass. Therefore, the skeletal muscles may have contributed less to the change in whole-body insulin sensitivity of the participants in this study compared to people with normal weight. Nevertheless, it should be noted that in addition to the outcomes reported in this thesis, insulin sensitivity relative to lean body mass was also calculated; however, the results of the linear analyses did not differ from the results of whole-body insulin sensitivity, and thus the results were not reported.

Pesola and colleagues conducted a similar behavioural counselling intervention to reduce SB among younger and middle-aged adults (Pesola et al., 2014, 2017). Two weeks after the counselling, muscle inactivity time measured by surface-electromyography in the thigh area decreased on average by 33 min/day and light muscle activity time increased on average by 21 min/day (Pesola et al., 2014). Among the same participants, leg lean mass decreased after one year in the control group, but not in the intervention group (Pesola et al., 2017). These findings suggest that the increased muscle activity in the intervention group likely persisted over time and protected against inactivity- and time-induced loss of muscle mass, even if only a small decrease in accelerometer-measured SB during free time was detected after three months (Pesola et al., 2017). Moreover, thigh muscle activity measured by surface-electromyography is greater during quiet standing than during sitting (Gao et al., 2017). Therefore, alterations in thigh muscle insulin sensitivity might occur with relatively light interventions. Subsequently, it was hypothesised that thigh muscle insulin sensitivity could be improved despite no apparent change in whole-body insulin sensitivity being detected.

However, according to the original randomisation, the changes in the QF and hamstring muscle insulin sensitivity were very much in line with the changes in whole-body insulin sensitivity and no differences between intervention- and control groups were observed. Nevertheless, the change in the hamstring insulin sensitivity correlated significantly and inversely with the change in SB percentage and positively with the changes in MVPA percentage and daily steps. Moreover, we reanalysed the results similar that done in Study III by dividing the participants into

"more active" and "continuously sedentary" groups. Interestingly, among the "more active", insulin sensitivity increased in the hamstring muscles, but not in the QF. This seems apprehensible, since hamstrings are considered postural muscles that maintain the upright position of the hip joint against gravity. On the other hand, the QF predominantly consists of phasic fibres that are activated during more vigorous PA (Eskelinen et al., 2015). Additionally, the hamstrings seem to be generally more active than the QF during various tasks with light to moderate intensity (e.g. standing, walking) albeit the difference is not statistically significant (Gao et al., 2017; Pesola et al., 2014). It is therefore conceivable that reducing SB and increasing standing and light to moderate-intensity PA increases insulin sensitivity in the hamstring muscles more than it does in the QF. Probably more strenuous PA (e.g. stair climbing, jogging, muscle strengthening exercises etc.) is needed to significantly improve insulin sensitivity in the phasic fibres of QF. This emphasizes the importance of multimodality-interventions with a focus on both reducing SB and increasing the amount of vigorous PA in improving insulin sensitivity and reducing the risk for developing type 2 diabetes.

In the present study (Study IV), the changes in insulin sensitivity did not differ between the four heads of the QF, and therefore the results are presented as the mean insulin sensitivity in all four heads of the muscle. Previously, vigorous high-intensity interval training increased insulin sensitivity in all the four heads of QF, whereas moderate-intensity exercise increased insulin sensitivity only in three heads of the muscle, but not the RF (Eskelinen et al., 2015). Interestingly, RF is also considered a postural muscle and measured by surface-electromyography during a sit-to-stand transition, both hamstrings and RF are activated simultaneously (Roebroeck et al., 1994). However, whereas the hamstrings shorten and deliver work the RF activation is nearly isometric and it functions as a tendon-like moment transmitter (Roebroeck et al., 1994). It is possible, that the work of RF in light activities requires less energy than the work of the hamstrings, and this could explain why the response in insulin sensitivity of RF did not differ from the responses of the other three heads of the QF in this study. However, to confirm this supposition, further investigations would be needed.

There are a number of potential explanations for the partly conflicting conclusions based on the cross-sectional and longitudinal results of this study. Firstly, the cross-sectional associations may reflect long-term cumulative effects of lifelong SB and PA behaviours. Whereas a 6-month intervention may still be too short a period in which to induce major changes by introducing fairly mild behavioural changes. Moreover, the difficulties of the participants in committing to the desired behaviour during the intervention may have blunted the results. Secondly, the participants in Studies I and II were more heterogeneous considering their daily SB and PA behaviours and metabolic health. Only the participants that met the

criteria of MetS and were sedentary for at least 60% of the daily wear time of the accelerometer were included in the intervention of Studies III and IV leading to a more homogenous study population. It is possible, that among people with several metabolic risk factors more effort may be needed to counteract the detrimental effects of being sedentary, i.e. more vigorous physical activity would be needed. In this study, the majority of the MVPA measured during the intervention consisted of moderate-intensity activities. There is some evidence, that PA intensity rather than duration could be more important in gaining health benefits from PA (Laursen et al., 2012). However, in the cross-sectional study, Study I, the total daily duration of PA was a stronger predictor of better metabolic health than MVPA, which suggests that the duration of PA could be more important in people who do not meet the PA recommendations. Conceivably the total PA volume is essential, and thus with a lower intensity more time is needed to gain some health benefits. With a considerable amount of SB, there might not be enough time (considering the 24 hours of the day) to accumulate a sufficient volume of PA by activities with a low intensity. Therefore, reducing SB by increasing PA of any intensity will induce health benefits, but vigorous PA will do that in relatively shorter time. However, for previously inactive people vigorous PA should be recommended with caution, to prevent possible overstrain injuries.

The discrepancies between interpretations based on cross-sectional and longitudinal data as well as on associations derived from accelerometer data collection periods with different durations highlight the vulnerability of a single assessment. More collected data points will enhance the robustness of the findings. This applies to each individual study as well as the whole evidence base of a topic.

6.3 Strengths and limitations

The key strengths of this study were the randomised controlled design of the intervention, the gold standard method for assessing whole-body insulin sensitivity and the utilisation of validated accelerometry methods for assessing the SB and PA of the participants throughout the six-month intervention. These are clear advantages in this study, since the majority of similar previous studies have used surrogate markers for insulin resistance and markedly shorter periods of accelerometer data collection.

The duration of the intervention of this study was approximately 6 months, which is both a strength and a limitation. Even if it exceeds the duration of the majority of previous SB intervention studies, it still might be too short to reveal actual long-term effects of reducing SB. Additionally, the intervention was conducted in free-living conditions, which on one hand makes it difficult to control the behaviour of the

participants, but on the other hand reflects the actual potential of such interventions to induce desired changes.

Another limitation is that the study participants represented a specific subpopulation, of sedentary inactive adults with MetS. Consequently, the results of this study are valid only in a similar population. Although this is a highly prevalent population in high-income countries, these results cannot be applied to everyone. Additionally, given the behavioural nature of the intervention, the participants could not be blinded to the intervention.

Another element in this study that could be defined as a limitation, is that the study protocol was designed to answer the question "does reducing SB induce a change in insulin sensitivity?" Given the interchangeable nature of different SB and PA behaviours, the novel and perhaps more appropriately formulated question would be "what SB should be replaced with to induce a change?" or "does replacing SB with 'behaviour x' induce a change?" The current study, as designed, cannot give direct answers to such questions, thus the related interpretations remain speculative.

6.4 Future directions

This was one of the first efforts to estimate the effects of reducing SB on insulin sensitivity and the metabolic health risk of adults in a real-life setting over several months. Despite the attention that the associations of SB with mortality and disease incidence have gained in the media, thus far the long-term effects of reducing SB have been rarely studied. Therefore, more controlled intervention studies are warranted to strengthen the evidence base. The results of this study apply only for a similar population: inactive adults with an observed risk of metabolic diseases. Therefore, studies with different study populations are needed.

In this study, we did not estimate the duration of bouts of SB, standing or PA. Even if the breaks in SB had no significance in the current study, previous studies suggest that it is important how different SB and PA behaviours are accumulated during the day. Therefore, in the future, the associations of different bout durations of SB and PA with metabolic outcomes should be estimated more closely. With the available raw accelerometer data, these kinds of analyses are fairly easily achievable.

It should be recognized that SB time cannot be modified without simultaneously altering some other behaviours i.e. PA or sleep. As accelerometer measurements for a full 24 hours are becoming more accessible and frequently used in SB research, new compositional data analysis methods can bring new insights into this research question (Chastin, McGregor, Biddle, et al., 2021; Migueles et al., 2022). To be able to formulate feasible and effective recommendations for healthy behaviours, a better understanding is needed of what SB should be replaced with for the best possible outcome. Estimated by the joint associations of SB, sleep and different intensities of

PA, there is a joint dose-response association between all-cause mortality and the daily balance of time spent in SB and PA of different intensities; however sleep duration does not appear significant (Chastin, McGregor, Palarea-Albaladejo, et al., 2021). Some countries, like Finland and Canada have already published 24-h movement guidelines including recommendations concerning SB, LPA and sleeping time (*Liikkumalla Terveyttä – Askel Kerrallaan. Viikoittainen Liikkumisen Suositus 18–64-Vuotiaille.*, 2019; Ross et al., 2020). The Canadian guidelines also include a maximum recommended duration of 8 h for SB. However, more research is needed before confirmed quantitative guidelines about the recommended durations of these less investigated behaviours SB, standing or LPA can be given.

7 Conclusions

This study demonstrated that a 6-month intervention aimed to reduce daily SB without adding exercise training had a minimal effect on the metabolic health of previously sedentary participants with MetS. However, among the participants that successfully reduced their SB, mainly by increasing moderate-intensity PA, insulin sensitivity modestly increased. This indicates that even if aiming to reduce SB might not be an effective method to improve metabolic health, successfully doing so can induce some small improvements. Therefore, reducing SB could be a good starting point for individuals that find committing to vigorous PA unattainable.

Given the variable nature of individual SB and PA behaviours, future studies to assess the sufficient duration of accelerometer data collection in different study settings are warranted. Accelerometer data collection for at least three weeks may be needed if the purpose is to find associations with tenuous health outcomes, such as insulin resistance, especially within relatively small study samples and specific subpopulations.

Finally, in the light of the current evidence, it can be recommended that everyone should try to avoid excessively long periods of SB, but it is impossible to say how long is ‘too long’. Instead, focusing on regular engagement in moderate-intensity or even vigorous PA is essentially more important in maintaining good metabolic health. If for some reason it is impossible to practice MVPA, breaking up periods of SB with light activities or standing is advisable. However, everyone should be encouraged to gradually increase the duration and intensity of PA to eventually meet the recommended amount of 2.5 h of MVPA per week. However, there is no need to stop after the recommended amount. As expressed by the WHO, for additional health benefits and to reduce the detrimental effects of long periods of SB, adults should aim to do even more than the recommended amount of MVPA.

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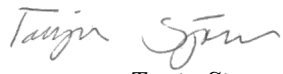

Tanja Sjöros

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