TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

SEDENTARY BEHAVIOR, PHYSICAL ACTIVITY, AND ENERGY METABOLISM IN METABOLIC SYNDROME

With special reference to insulin sensitivity and metabolic flexibility

Taru Garthwaite

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1808 | MEDICA – ODONTOLOGICA | TURKU 2024

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

SEDENTARY BEHAVIOR, PHYSICAL ACTIVITY, AND ENERGY METABOLISM IN METABOLIC SYNDROME

With special reference to insulin sensitivity and metabolic flexibility

Taru Garthwaite

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1808 | MEDICA – ODONTOLOGICA | TURKU 2024

University of Turku

Faculty of Medicine
Clinical Physiology and Nuclear Medicine
Doctoral Programme in Clinical Research
Turku PET Centre

Supervised by

Adjunct Professor Ilkka Heinonen, PhD
Turku PET Centre
University of Turku
Finland

Professor Juhani Knuuti, MD, PhD
Turku PET Centre
University of Turku
Finland

Reviewed by

Professor Patrick Schrauwen, PhD
Department of Clinical Epidemiology
Leiden University Medical Center
The Netherlands

Academy Research Fellow Laura Karavirta, PhD
Faculty of Sport and Health Sciences
University of Jyväskylä
Finland

Opponent

Professor George A. Brooks, PhD
Department of Integrative Biology
University of California, Berkeley
United States

The originality of this publication has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

ISBN 978-951-29-9841-8 (PRINT)
ISBN 978-951-29-9842-5 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Painosalama, Turku, Finland 2024

It is not the mountain we conquer, but ourselves.
Sir Edmund Hillary

Faculty of Medicine
Clinical Physiology and Nuclear Medicine
Turku PET Centre
TARU GARTHWAITE: Sedentary behavior, physical activity, and energy metabolism in metabolic syndrome – with special reference to insulin sensitivity and metabolic flexibility
Doctoral Dissertation, 159 pp.
Doctoral Programme in Clinical Research
August 2024

ABSTRACT

This study investigated the relationships between physical activity behaviors and energy metabolism in 64 middle-aged, sedentary adults with metabolic syndrome. Associations were examined cross-sectionally, and a 6-month randomized controlled trial was conducted to investigate whether reducing daily sedentary time through increased standing and light-to-moderate-intensity physical activity, without adding intentional exercise training, could improve metabolic health.

The time spent in activity behaviors was measured with hip-worn accelerometers continuously for 6 months. Cardiorespiratory fitness was determined with maximal cycle ergometry. Metabolic outcomes were assessed at baseline, 3 months, and 6 months using hyperinsulinemic euglycemic clamp, positron emission tomography, indirect calorimetry, fasting blood samples, body composition, and anthropometrics.

Activity behaviors and fitness associated with whole-body and skeletal muscle insulin sensitivity, but only associations between standing and insulin sensitivity markers were independent of adiposity. Sedentary time associated adversely and standing and physical activity, of even light-intensity, beneficially with lipid metabolism and metabolic flexibility. A 50-min reduction in daily sedentary time attenuated increases in several cardiometabolic markers at 3 months, but the intervention did not improve metabolic flexibility in 6 months. However, additional analyses showed that successfully reducing daily sedentary time by 30 min or more improved metabolic flexibility compared to continued high sedentary time. Improvements in metabolic flexibility also correlated with increased standing time.

The findings highlight the importance of a healthy body composition and suggest that reducing sedentary time and increasing standing and light-intensity activity might help slow down the progression of metabolic diseases in inactive individuals with an increased cardiometabolic risk.

KEYWORDS: sedentary behavior, physical activity, energy metabolism, insulin sensitivity, metabolic flexibility, metabolic syndrome

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Kliininen fysiologia ja isotooppilääketiede
Valtakunnallinen PET-keskus
TARU GARTHWAITE: Paikallaanolo, fyysinen aktiivisuus ja energia-aineenvaihdunta metabolisessa oireyhtymässä – erityishuomiona insuliiniherkkyys ja aineenvaihdunnallinen joustavuus
Väitöskirja, 159 s.
Turun kliininen tohtoriohjelma
Elokuu 2024

TIIVISTELMÄ

Tässä tutkimuksessa selvitettiin paikallaanolon, liikkumisen ja energia-aineenvaihdunnan välisiä yhteyksiä 64:llä keski-ikäisellä vähän liikkuvalla aikuisella, joilla on metabolinen oireyhtymä. Yhteyksiä tutkittiin poikkileikkausasetelmassa ja 6 kk interventiolla selvitettiin voiko aineenvaihdunnallista terveyttä parantaa vähentämällä paikallaanoloa, ilman varsinaista liikuntaharjoittelua.

Paikallaanoloa, seisomista ja liikkumista mitattiin lantiolla pidettävillä liikemittareilla koko tutkimusjakson ajan. Kestävyyskunto määritettiin maksimaalisella polkupyöräergometritestillä. Aineenvaihdunnallisen terveyden muuttujia tutkittiin lähtötilanteessa, 3 kk ja 6 kk kohdalla käyttämällä hyperinsulineemista euglykeemistä clamp-tutkimusta, positroniemissiotomografiaa, epäsuoraa kalorimetriaa, paastoverinäytteitä, kehonkoostumusta ja antropometriaa.

Paikallaanolo, liikkuminen ja kestävyyskunto olivat yhteydessä koko kehon ja luurankolihasten insuliiniherkkyyteen, mutta vain seisominen oli yhteydessä insuliiniherkkyysmuuttujiin, kun kehon rasvoittuneisuus otettiin huomioon. Runsas paikallaanolo oli yhteydessä huonompaan ja seisominen ja kevytkin liikkuminen parempaan rasva-aineenvaihduntaan ja aineenvaihdunnalliseen joustavuuteen. Interventio vähensi päivittäistä paikallaanoloa 50 min 3 kk aikana, mikä hidasti kontrolliryhmässä havaittua heikkenemistä useissa kardiometabolisissa muuttujissa, mutta interventio ei parantanut aineenvaihdunnallista joustavuutta 6 kk aikana. Aineenvaihdunnallinen joustavuus parani kuitenkin niillä, jotka onnistuneesti vähensivät päivittäistä paikallaanoloa yli 30 min. Aineenvaihdunnallisen joustavuuden muutos korreloi positiivisesti seisomisajan muutoksen kanssa.

Tulokset korostavat terveen kehonkoostumuksen merkitystä ja viittaavat siihen, että paikallaanolon vähentäminen ja kevyenkin liikkumisen lisääminen voi auttaa hidastamaan aineenvaihduntasairauksien etenemistä vähän liikkuvilla riskiryhmillä.

AVAINSANAT: paikallaanolo, fyysinen aktiivisuus, energia-aineenvaihdunta, insuliiniherkkyys, aineenvaihdunnallinen joustavuus, metabolinen oireyhtymä

Table of Contents

Abbreviations

[^{18}F]FDG	^{18}F-fluorodeoxyglucose
APE	Angle for posture estimation
ATP	Adenosine triphosphate
AUC	Area under curve
BMI	Body mass index
CHO	Carbohydrate
CHOox	Carbohydrate oxidation
CI	Confidence interval
CT	Computed tomography
EE	Energy expenditure
FATox	Fat oxidation
FFA	Free fatty acid
FFM	Fat-free mass
FQ	Food quotient
FUR	Fractional uptake rate
GU	Glucose uptake
HbA_{1c}	Glycated hemoglobin
HDL	High-density lipoprotein
HEC	Hyperinsulinemic euglycemic clamp
HOMA-IR	Homeostatic model assessment of insulin resistance
LDL	Low-density lipoprotein
MAD	Mean amplitude deviation
MET	Metabolic equivalent of task
MetFlex	Metabolic flexibility
MFO	Maximal fat oxidation
MVPA	Moderate-to-vigorous physical activity
OGTT	Oral glucose tolerance test
PA	Physical activity
PET	Positron emission tomography
PO	Power output
RER	Respiratory exchange ratio

RQ	Respiratory quotient
Q1	First quartile
Q3	Third quartile
QUICKI	Quantitative insulin sensitivity check index
SD	Standard deviation
VCO_2	Carbon dioxide production
VO_2	Oxygen uptake
VO_{2max}	Maximal oxygen uptake

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 Garthwaite T, Sjöros T, Koivumäki M, Laine S, Vähä-Ypyä H, Saarenhovi M, Kallio P, Löyttyniemi E, Sievänen H, Houttu N, Laitinen K, Kalliokoski K, Vasankari T, Knuuti J & Heinonen I. Standing is associated with insulin sensitivity in adults with metabolic syndrome. *Journal of Science and Medicine in Sport,* 2021;24(12):1255–1260.

II Garthwaite T, Sjöros T, Laine S, Koivumäki M, Vähä-Ypyä H, Eskola O, Rajander J, Kallio P, Saarenhovi M, Löyttyniemi E, Sievänen H, Houttu N, Laitinen K, Kalliokoski K, Vasankari T, Knuuti J & Heinonen I. Associations of sedentary time, physical activity and fitness with muscle glucose uptake in adults with metabolic syndrome. *Scandinavian Journal of Medicine & Science in Sports,* 2023;33:353–358.

III Garthwaite T, Sjöros T, Laine S, Koivumäki M, Vähä-Ypyä H, Verho T, Norha J, Kallio P, Saarenhovi M, Löyttyniemi E, Sievänen H, Houttu N, Laitinen K, Kalliokoski K, Vasankari T, Knuuti J & Heinonen I. Sedentary time associates detrimentally and physical activity beneficially with metabolic flexibility in adults with metabolic syndrome. *American Journal of Physiology-Endocrinology and Metabolism,* 2024;326(4):E503–E514.

IV Garthwaite T, Sjöros T, Laine S, Vähä-Ypyä H, Löyttyniemi E, Sievänen H, Houttu N, Laitinen K, Kalliokoski K, Vasankari T, Knuuti J & Heinonen I. Effects of reduced sedentary time on cardiometabolic health in adults with metabolic syndrome: A three-month randomized controlled trial. *Journal of Science and Medicine in Sport,* 2022;25(7):578–585.

V Garthwaite T, Sjöros T, Laine S, Koivumäki M, Vähä-Ypyä H, Norha J, Kallio P, Saarenhovi M, Löyttyniemi E, Sievänen H, Houttu N, Laitinen K, Kalliokoski K, Vasankari T, Knuuti J & Heinonen I . Sedentary time reduction and metabolic flexibility: A 6-month randomized controlled trial in adults with metabolic syndrome. *Manuscript.*

1 Introduction

Physical activity (PA) used to be a fundamental component of daily life and a necessity for survival for our ancestors. In modern day, however, the demands for PA have significantly reduced. Together with changes in food availability and dietary quality, the increasingly sedentary and inactive lifestyle has doubled the obesity prevalence in the past few decades and now 2.5 billion, or ~ 40 % of adults worldwide are overweight or obese (World Health Organization, 2024). In addition, 463 million people are estimated to have diabetes (Saeedi et al., 2019).

PA plays a key role in the prevention of these lifestyle-related health problems, but despite the well-known health benefits of regular PA (2018 Physical Activity Guidelines Advisory Committee, 2018), nearly a third of adults globally are physically inactive and do not meet the current weekly PA recommendations of at least 150–300 min of moderate-intensity or 75–150 min of vigorous-intensity aerobic activity and muscle-strengthening activities on at least 2 days (Strain et al., 2024; World Health Organization, 2020). Consequently, physical inactivity is an established major health risk and a global public health concern as it is estimated to cause 6–10 % of premature deaths and chronic diseases, levels comparable to obesity and smoking (Katzmarzyk et al., 2021; Lee et al., 2012).

In addition to physical inactivity, sedentary behavior has more recently been identified as an important determinant of health and a modifiable risk factor for adverse health outcomes. Whereas physical inactivity is defined as an insufficient PA level to meet the current recommendations, sedentary behavior covers a broad range of behaviors involving sitting, reclining or lying down with low energy expenditure (EE) (Tremblay et al., 2017). Adults in developed countries now spend on average ~ 9–10 h sedentary daily, of which majority is commonly accumulated through desk-based office work and sedentary leisure-time activities (Bauman et al., 2018; Dohrn et al., 2024; Loyen et al., 2017; Matthews et al., 2021). High levels of sedentary behavior are associated with an increased risk of mortality and chronic diseases, and the risk seems to increase with sedentary time over 9.5 h/day (Bailey et al., 2019; Dempsey et al., 2020; Ekelund, Tarp, et al., 2019; Sagelv et al., 2023). Therefore, a recommendation to limit sedentary time and to replace it with PA of any intensity has recently been included in the activity guidelines (World Health

Organization, 2020). Current evidence is insufficient to provide more specific quantitative recommendations, however (Dempsey et al., 2020).

Although strong observational data supports the relationship between high sedentary time and poor health outcomes, less is known about the impact of reduced sedentary time on health. Meta-analyses have shown that behavioral interventions have potential to reduce daily sedentary time, which might lead to health benefits (Hadgraft et al., 2021; Martin et al., 2015; Nieste et al., 2021; Peachey et al., 2020; Shrestha et al., 2019). However, the evidence on health improvements is limited, studies have mostly targeted healthy populations and occupational sitting, and the majority have lasted for less than 3 months or reported attenuations in sedentary time reductions with longer follow-ups (Hadgraft et al., 2021; Peachey et al., 2020; Shrestha et al., 2018). Moreover, accelerometers are typically used only for a week, which might not accurately reflect habitual activity behaviors over longer periods of time or capture changes in behavior. Considering the high levels of physical inactivity and the rapidly increasing prevalence of lifestyle-related chronic diseases, longer-term free-living interventions and accelerometer-measurements are needed particularly in populations with an increased risk of metabolic diseases.

This PhD study aims to fill some of these research gaps with a 6-month free-living behavioral intervention in adults with metabolic syndrome. Studying the associations and causality between sedentary time, PA, and metabolic outcomes related to energy metabolism and insulin sensitivity will help advance the understanding of the role of lifestyle factors in the progression of metabolic diseases and possibly aid in the development of potential intervention targets.

2 Review of the Literature

2.1 Sedentary behavior and physical activity

Physical activity is defined as 'any bodily movement produced by skeletal muscles that results in energy expenditure' (Caspersen et al., 1985), while not meeting the current PA recommendations is considered physical inactivity (Tremblay et al., 2017). Previously in PA and sedentary behavior research, the term 'sedentary' was often used interchangeably with 'physical inactivity', causing confusion in the field. More recently, the two have been recognized as distinct behaviors with potentially different health consequences, and the Sedentary Behavior Research Network has developed a consensus statement regarding the terminology in an attempt to standardize the definitions (Tremblay et al., 2017). Sedentary behavior is now defined as any waking behavior in a sitting, reclining, or lying posture with an energy expenditure ≤ 1.5 metabolic equivalents (METs) (Tremblay et al., 2017). A MET is a measure of energy expenditure, with 1 MET representing about 3.5 ml/kg/min oxygen consumption (VO_2) while sitting at rest (Ainsworth et al., 1993). Multiples of 1 MET are used to describe the intensity of PA: light-intensity physical activity (LPA) is often defined as > 1.5 to < 3.0 METs, moderate-intensity physical activity (MPA) as 3.0 to < 6.0 METs, and vigorous-intensity physical activity (VPA) as ≥ 6.0 METs.

2.1.1 Assessment of physical activity and sedentary time

Most of the evidence about the health effects of PA and sedentary behavior accrued to date is based on epidemiological studies using self-reported estimates of time spent in specific activity behaviors (World Health Organization, 2020). The estimates can be collected with questionnaires, behavioral logs, diaries, and short-term recalls. The advantages of these methods are that they are relatively inexpensive, implementable on large scale, and they can provide information on the types of activity and the contexts and domains in which the activities take place, separating for example occupational and leisure-time activities (Healy et al., 2011). However, self-reports are prone to measurement error and reporting bias, often overestimating PA and underestimating sedentary time by as much as 1.74 h/day

(Chastin et al., 2018; Prince et al., 2020). They are also unable to record light-intensity and non-exercise, intermittent sporadic bouts of activities in daily living, and in general have poorer accuracy, precision, and validity compared to objective measures (Chastin et al., 2018).

Technical advances have led to an increasing use of objective measuring devices in PA and sedentary behavior research. Devices enable a more accurate quantification of time spent in different activity behaviors compared to self-reports and they can also assess the pattern in which the time is accumulated. The most commonly used objective devices are wearable activity monitors, including accelerometers and inclinometers. The first-mentioned primarily measure accelerations generated by movement and the intensity of such movements, while inclinometers assess the inclination of body parts and provide postural information (Boerema et al., 2020). Consequently, the objective device-based methods are capable of capturing also incidental and light-intensity activity and quantifying the time spent in specific postures, i.e., sitting, standing, and lying. Some disadvantages of devices are the lack of contextual and domain-specific information, a higher cost and participant burden compared to self-report measures, and the inability of some devices to accurately assess certain types of activities (e.g., cycling, resistance training) (Gibbs et al., 2015).

Although activity monitors provide more accurate information on the time spent in different activity behaviors in comparison to subjective self-report measures, the estimates from objective device-based measurements can also vary depending on, for example, monitor placement, the duration of the measurement, daily wear time, and data processing and analysis methods. The most commonly used locations for accelerometers in research settings are the hip, wrist, and thigh. Thigh-worn accelerometers are currently considered the “gold standard” of sedentary behavior measurement because of the capability to accurately identify postures. However, the PA assessment is based on step counts, and a disadvantage of the thigh-worn devices is that they are often attached onto the skin with adhesive and can thus cause skin irritation, a higher participant burden, and possibly a more limited measurement duration in comparison to hip- and wrist-worn devices. Estimates from hip-worn devices in turn are more accurate in estimating whole-body movements than wrist-based measurements due to positioning closer to the center of body mass, whereas wrist-worn devices also capture forearm movements (Rosenberger et al., 2013; Shiroma et al., 2016). Recent technical developments also allow triaxial hip-worn accelerometers to reliably estimate posture with validated algorithms (Vähä-Ypyä et al., 2018).

A typical activity measurement period in studies is 7 days, with a minimum of 10 h/day monitor wear time usually considered sufficient (Atkin et al., 2012). One week may be enough to reliably estimate sedentary time and PA, but a longer

measurement may be needed to detect associations with health outcomes (Aadland & Ylvisåker, 2015; Sjöros et al., 2021). Moreover, analyses should be corrected for individual variation in daily monitor wear time (Aadland & Ylvisåker, 2015; Healy et al., 2011). Making comparisons between devices and studies can also be challenging due to differences in data processing methods and metrics used. Estimates are affected by, for example, the chosen epoch length for acceleration data analysis and the intensity thresholds for activity count-based measurements (Atkin et al., 2012; Sievänen & Kujala, 2017). Although the arbitrary activity counts are a commonly used metric, universal algorithms for raw acceleration data can provide more easily interpretable and physiologically relevant measures that allow comparisons between studies (Vähä-Ypyä, Vasankari, Husu, Suni, et al., 2015).

Overall, although the device-based measures are preferred for a more accurate estimation of total time spent sedentary or in PA and the patterns of accumulation, self-reports can provide complementary, valuable information on the contexts and types of activities. Thus, the combination of the two measurement methods can provide a more comprehensive picture of activity behaviors than either one alone.

2.1.2 Health risks of sedentary behavior

Epidemiological studies have consistently linked high volumes of sitting to an increased risk of premature mortality, cardiovascular disease, type 2 diabetes, certain types of cancer, and metabolic syndrome (Bailey et al., 2019; Biswas et al., 2015; Dempsey et al., 2020; Jingjie et al., 2022; Lynch, 2010; Patterson et al., 2018; Sagelv et al., 2023; Wilmot et al., 2012; Wu et al., 2022). High volumes of sedentary behavior appear to increase particularly the risk of type 2 diabetes, as the risk is approximately doubled in the quartile with the highest self-reported sedentary time in comparison to the lowest quartile (Biswas et al., 2015; Wilmot et al., 2012). Besides an increased risk of mortality and chronic disease incidence, sedentary behavior has also been shown to associate adversely with several cardiometabolic outcomes, including waist circumference, glycemic outcomes and lipid profile (Brocklebank et al., 2015; Powell et al., 2018), as well as mental health and cognitive outcomes (Saunders et al., 2020). These deleterious health effects are likely a result of negative impacts on multiple physiological systems following reduced muscle contractions, blood flow, and energy expenditure in comparison to standing and PA, leading to, for example, insulin resistance, vascular dysfunction, accumulation of lipids and lipid intermediates, a shift in muscle fiber type from oxidative to glycolytic type, and an increased oxidation of carbohydrates (CHO) over fats (Pinto et al., 2023).

The risk of mortality and incident disease has been shown to increase with longer sitting time. The risk increases from self-reported sitting time of 7–8 h/day onwards,

(Chau et al., 2013; Ekelund et al., 2016; Patterson et al., 2018), and in accelerometer studies the risk has been shown to be more pronounced with sitting time over 9.5 h/day (Ekelund, Tarp, et al., 2019). Among the least active even less time spent sedentary might be detrimental. Although sedentary behavior has also been claimed to have detrimental health effects independent of PA (Patterson et al., 2018), PA seems to modify the associations between sedentary time and health outcomes to some extent. The detrimental associations are more pronounced among physically inactive people, and ~ 20–40 min/day of accelerometer-assessed moderate-to-vigorous PA (MVPA) might offset some of the negative effects of sitting (Ekelund, Brown, et al., 2019; Ekelund et al., 2020; Sagelv et al., 2023). In contrast, however, the idea of sedentary behavior as an independent determinant of adverse health effects is supported by bed rest studies showing that even large volumes of exercise do not fully counteract the negative metabolic consequences of bed rest-induced inactivity and sedentariness (Le Roux et al., 2022). This highlights the role of non-exercise activity and LPA in daily living in the maintenance of health.

Not only the total sitting time, but the accumulation patterns of sedentary time may have distinct implications on health outcomes as well, i.e., whether the total sitting time is accrued in prolonged periods or in shorter bouts with frequent breaks. Limited prospective evidence suggests higher rates of chronic diseases and mortality with longer sedentary bouts (Dempsey et al., 2022; Diaz, Howard et al., 2017; Wu et al., 2023), and experimental studies consistently show detrimental effects on cardiometabolic risk markers, including blood pressure, glucose, insulin, and lipid levels (Bellettiere et al., 2017; Dempsey et al., 2018; Diaz, Goldsmith et al., 2017; Dunstan et al., 2012; Grace et al., 2017; Homer et al., 2017; Paterson et al., 2020). Longer sitting bouts may also magnify the deleterious health effects of total sedentary time (Bellettiere et al., 2017). The total volume of sedentary time may, however, be the more detrimental characteristic of sedentary behavior, as the health risks of prolonged sitting bouts do not seem to be independent of total sedentary time (Diaz, Goldsmith et al., 2017; Diaz, Howard et al., 2017).

Besides the total sedentary time and the pattern of accumulation, the relationships between sedentary behaviors and health outcomes may be influenced by the domain in which sedentary time is accrued, and the type of sedentary behavior. For example, the deleterious effects of sitting seem to be more pronounced with sitting during leisure time and with mentally passive sedentary behaviors (e.g., TV viewing), rather than with sitting time accrued at work or during mentally active behaviors (e.g., reading) (Hallgren et al., 2020; Ketels et al., 2020; Kitano et al., 2022; Saidj et al., 2016). It is worth noting, however, that other lifestyle factors related to TV viewing, including eating habits, may contribute to the associations with adverse outcomes (Heinonen et al., 2013).

As lifestyle-related cardiometabolic diseases result in a great disease burden globally and high volumes of sedentary time are associated with adverse metabolic outcomes, reducing the time spent sedentary could be a potential strategy to improve, or maintain, health. The health risks appear to be the highest with the combination of high total sedentary time and its accrual in prolonged bouts, particularly during leisure time, thus targeting both reductions and interruptions in leisure-time sitting is likely to yield the most health benefits.

2.1.3 Effects of reduced sedentary time on health

Acute experimental studies and short-term crossover interventions have suggested potential cardiometabolic health benefits for reducing and breaking up prolonged sitting (Buffey et al., 2022; Loh et al., 2020), and an increasing number of interventions are now targeting reductions in sedentary time to investigate the potential health benefits in longer term. Meta-analyses show that sedentary behavior interventions can lead to reductions in daily sitting time ranging from ~ 0.5 h to 1.5 h with varying settings, behavior change techniques, and intervention durations (Compernolle et al., 2019; Martin et al., 2015; Murtagh et al., 2020; Neuhaus et al., 2014; Nieste et al., 2021; Peachey et al., 2020; Prince et al., 2020; Shrestha et al., 2018, 2019). Self-monitoring and environmental restructuring (e.g., sit–to–stand workstations at workplaces) appear to be particularly useful strategies (Gardner et al., 2016), and targeting sedentary behavior alone is more effective in reducing sedentary time than targeting changes in PA alone or in combination with sedentary behavior (Nguyen et al., 2020). Most sedentary behavior interventions have targeted workplaces, significantly reducing occupational sitting time (Shrestha et al., 2018) and showing promise for improved cardiometabolic risk (Brierley et al., 2019). However, from public health perspective it is also important to note that there might be a compensatory effect by increased sitting and reduced activity outside of work hours (Mansoubi et al., 2016). Although a large amount of sitting is accrued in leisure time, fewer interventions have targeted specifically non-occupational sedentary behavior. Non-worksite interventions seem to be effective in reducing total sedentary time (Thraen-Borowski et al., 2017), and targeted interventions can also reduce leisure-time sitting on average by 30 min/day in medium-term (4–12 months), and TV viewing by 61 min/day in short-term (≤ 4 months) and 11 min/day in medium-term (Shrestha et al., 2019). The sustainability and potential for health improvements over longer term remain unclear, however.

The evidence on the health effects of reduced sitting is still limited. In short term, reducing sitting by increased standing and light-intensity walking might improve insulin sensitivity and circulating lipids, as suggested by 4-day free-living crossover trials (Duvivier et al., 2013, 2016, 2017). Meta-analyses of acute, experimental

studies also show consistent glycemic benefits with frequent interruptions to prolonged sitting by either light- or moderate-intensity PA breaks, with greater benefits for those with underlying metabolic impairments (Buffey et al., 2022; Dempsey et al., 2018; Loh et al., 2020; Saunders et al., 2018). Breaking up sitting by standing may provide benefits as well, but the evidence is more inconsistent. Short, frequent PA breaks seem slightly more beneficial than one continuous bout (Chastin et al., 2015; Loh et al., 2020), likely due to increased, more frequent muscle contractions (Gao et al., 2024). Although the optimal frequency, type and intensity of activity breaks remain unclear, breaking up sitting every 30 min has been suggested as an optimal and feasible target (Diaz, Howard et al., 2017; Dong et al., 2024; Duran et al., 2023).

Meta-analyses of longer-term interventions targeting sedentary behavior, alone or in combination with increases in PA, show small improvements in weight, waist circumference, body fat-%, glucose and lipid metabolism, and blood pressure (Hadgraft et al., 2021; Nieste et al., 2021). Interventions reducing sedentary behavior may also have an influence on cardiorespiratory fitness, which is a strong predictor of health outcomes (Prince et al., 2024). Most sedentary behavior interventions have lasted for less than 3 months, however, or reported attenuations in sedentary time reduction with longer follow-ups (Blackburn et al., 2020; Peachey et al., 2020). Three months might not be long enough to see health effects, as more promising cardiometabolic benefits have been observed at 6 months (Bodker et al., 2021) and 12 months, despite a greater reduction in sedentary time at 3 months (Healy et al., 2017; Winkler et al., 2018). Moreover, the effects of sedentary behavior interventions on health are also dependent on what activity sedentary time is replaced by, as a reduction in one type of behavior inherently leads to an increase in some other behavior (standing, LPA, MVPA, sleep) within a finite 24-h day (Segura-Jiménez et al., 2022).

Isotemporal substitution modeling and compositional data analysis take into account this co-dependency of activity behaviors. Studies using these methods show that the greatest health benefits are achieved by reallocating sedentary time to MVPA, but replacing some of the daily sitting with LPA and standing can also have beneficial health effects (Blodgett et al., 2024; Brakenridge et al., 2024; Chastin et al., 2015; del Pozo-Cruz et al., 2018; Miatke et al., 2023). Reallocating sedentary time to either LPA or MVPA is beneficially associated with cardiometabolic outcomes and adiposity, and reduced mortality risk, and the least active are likely to benefit the most from reduced sedentary time (del Pozo-Cruz et al., 2018; Miatke et al., 2023). The most effective and time-efficient way to achieve cardiometabolic benefits is the reallocation of time from other behaviors to MVPA, theoretically with even as little as 4–12 min/day (Blodgett et al., 2024; Miatke et al., 2023). A 24-h behavioral time-use composition with less time spent sitting and more standing, PA,

and sleep has the most beneficial associations with cardiometabolic health, and the average optimal composition for health was recently suggested to be 6 h sitting, 5 h 10 min standing, 2 h 10 min LPA, 2 h 10 min MVPA, and 8 h 20 min sleeping (Brakenridge et al., 2024). The beneficial associations of compositions with greater PA and less sitting with cardiometabolic health also seem to be stronger in those with impaired glucose metabolism or type 2 diabetes compared to normoglycemia, suggesting that especially individuals with poorer metabolic health would benefit from reallocating sitting time to PA (Brakenridge et al., 2024). In addition to those with existing metabolic impairments, the least active, previously sedentary individuals are likely to gain the greatest benefits from reduced sitting and increased activity of any intensity, even if the amount is still below PA recommendations (Greenwalt et al., 2023).

In summary, it is possible to intervene on activity behaviors and to reduce daily sedentary time, although long-term sustainability remains unclear. The majority of sedentary behavior interventions have targeted occupational sitting time and healthy populations, used self-reports or short accelerometer measurements to assess sedentary time, and reported limited health benefits. Free-living, longer-term interventions investigating the health effects of reduced habitual sedentary behavior in populations with existing metabolic impairments are lacking. As inactive and sedentary individuals with an increased risk of metabolic diseases are likely to benefit the most from reduced sedentary time, studying the causality and the potential of reduced sedentary time as a health-enhancing strategy is particularly important in this population.

2.2 Energy metabolism

Energy to fuel all processes in the body is generated through the breakdown of energy substrates, primarily glucose and fatty acids. The contribution of each to the production of adenosine triphosphate (ATP) depends on several factors influencing the availability, uptake, transport, and utilization of substrates. A few central features in the regulation of these aspects of energy metabolism are described in the following section.

2.2.1 Blood glucose regulation

Glucose is the major energy source in human metabolism. The maintenance of normal blood glucose level within a physiological range (~ 4–9 mmol/L) is essential to ensure a constant energy supply to tissues, particularly to the glucose-dependent brain (Dimitriadis et al., 2021). The regulation of glucose homeostasis is primarily dependent on the dynamic interactions between the liver, muscle, and adipose tissue

and the effects of pancreatic hormones, particularly glucagon and insulin (Röder et al., 2016). When blood glucose levels are low, the pancreas secretes glucagon to stimulate hepatic glucose production. In contrast, elevated blood glucose levels stimulate increased insulin secretion. Insulin then lowers blood glucose levels by increasing insulin-dependent glucose uptake (GU) by muscle and adipose tissue and the promotion of glycogen synthesis for storage (Röder et al., 2016).

The liver is in a critical role in glucose metabolism and the regulation of blood glucose levels as it is a major site for postprandial glucose storage and primarily responsible for glucose production via gluconeogenesis and glycogenolysis in the fasting state (Petersen et al., 2017). Skeletal muscle also has a central role in glycemic control since lactate released from muscle is the major gluconeogenic substrate (Brooks 2018), and ~ 80–85 % of total insulin-stimulated glucose uptake is by muscle tissue (DeFronzo et al., 1985). Muscle contractions also function as an insulin-independent mechanism to increase glucose uptake. Although the order in which tissues develop insulin resistance is uncertain, insulin resistance originating from skeletal muscle has been proposed as the primary defect in the development of type 2 diabetes (DeFronzo & Tripathy, 2009). Adipose tissue, in turn, contributes to the blood glucose homeostasis by increased glucose uptake and suppression of lipolysis in response to insulin, and by providing free fatty acids (FFA) as an alternative fuel source when glycogen stores and blood glucose levels are low (Dimitriadis et al., 2021).

2.2.2 Insulin sensitivity

As mentioned above, insulin is a key hormone in blood glucose regulation, exerting direct and indirect effects on the primary target tissues of muscle, liver, and adipose tissue (Figure 1). In insulin-sensitive state, insulin promotes glucose uptake and storage in skeletal muscle by stimulating glucose transport and glycogen synthesis. In the liver, insulin increases glucose uptake and inhibits gluconeogenesis and glycogenolysis to maintain and regulate blood glucose levels. A major function of insulin in adipose tissue is to suppress lipolysis and prevent the hydrolysis and release of triglycerides into the circulation as FFA (Petersen & Shulman, 2018).

The gold standard of insulin sensitivity measurement is the hyperinsulinemic euglycemic clamp (HEC) (DeFronzo et al., 1979). HEC is based on raising plasma insulin to a supraphysiological level by infusion, together with a constant glucose infusion to maintain euglycemia. The aim is to suppress endogenous glucose production by the liver, so that the amount of infused glucose needed to maintain steady plasma glucose level would reflect the amount taken up by tissues in response to insulin stimulation. HEC can also be combined with ^{18}F-fluorodeoxyglucose positron emission tomography ([^{18}F]FDG-PET imaging) to quantify tissue-specific

insulin sensitivity by assessing the uptake of glucose analogue [^{18}F]FDG tracer. However, HEC is a labor-intensive, costly, and impractical method to use in clinical settings and epidemiological studies. Therefore, simple surrogate measures from fasting blood samples are often used to estimate insulin sensitivity, such as homeostatic model assessment of insulin resistance (HOMA-IR) index, and quantitative insulin sensitivity check index (QUICKI), which primarily reflect hepatic insulin sensitivity (Muniyappa et al., 2008). Oral glucose tolerance test (OGTT) is another commonly used method. Although OGTT is not a measure of insulin sensitivity per se, it allows a more physiological assessment of the efficiency of the body to dispose of glucose after an oral glucose load in comparison to HEC (Muniyappa et al., 2008).

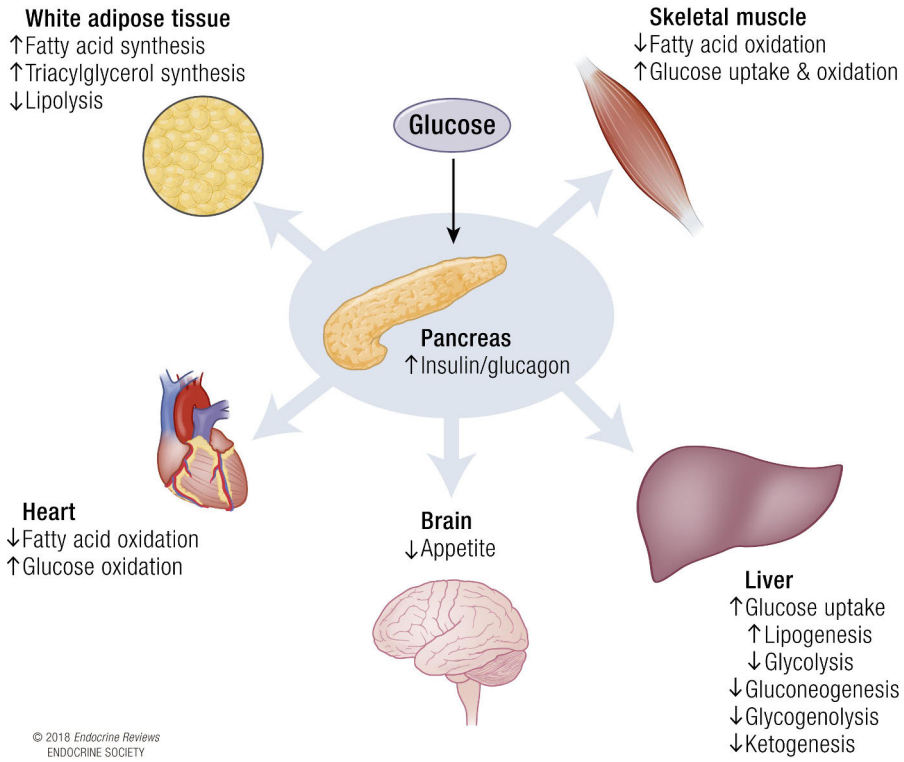

Figure 1. Systemic response to insulin. Upon glucose sensing, the pancreas increases the insulin/glucagon ratio. The rise in insulin stimulates many metabolic processes in the key metabolic organs: the liver, heart, brain, white adipose tissue, and skeletal muscle. Collectively, these metabolic processes switch metabolism from a preference of fatty acid oxidation to glucose uptake and oxidation. Reprinted from Smith et al., Metabolic Flexibility as an Adaptation to Energy Resources and Requirements in Health and Disease, Endocrine Reviews, Volume 39, Issue 4, August 2018, pages 489–517, https://doi.org/10.1210/er.2017-00211. Copyright © 2018 Endocrine Society. Published under the Creative Commons Attribution License (CC BY; https://creativecommons.org/licenses/by/4.0/).

Proper insulin action is essential for the maintenance of glucose homeostasis. Disruptions in the secretion and/or action of insulin resulting primarily from an imbalance between energy intake and expenditure eventually give rise to insulin resistance (Roden & Shulman, 2019). As a result of blunted response to insulin by target tissues, blood glucose levels rise and insulin secretion is further increased as a compensatory effort to lower glucose, leading to hyperinsulinemia and hyperglycemia if not managed. Central features of insulin resistance are impaired glucose uptake by tissues, decreased glycogen synthesis, and an impaired inhibition of hepatic glucose production and adipose tissue lipolysis (Petersen & Shulman, 2018).

Decreased insulin sensitivity precedes type 2 diabetes and is one of the earliest defects in the pathogenic events leading to the onset of type 2 diabetes (Roden & Shulman, 2019). Although the mechanisms underlying the development of insulin resistance are not entirely understood yet, lipotoxicity and impaired insulin signalling resulting from the accumulation of lipids and lipid metabolites in muscle and liver are proposed as some primary characteristics and relatively early events in the causal pathway (Corpeleijn et al., 2009; Roden & Shulman, 2019; Silva Rosa et al., 2020). Lipid oversupply and increased relative fat oxidation also lead to an increased production of reactive oxygen species, which can damage mitochondria and further promote the accumulation of lipids and contribute to insulin resistance (Petersen & Shulman, 2018). Ectopic lipid accumulation is thus a result of an imbalance between lipid availability and oxidation, and therefore dysregulated fuel switching and the concept of metabolic inflexibility are implicated as important contributors to the onset of insulin resistance (Corpeleijn et al., 2009; Silva Rosa et al., 2020; Smith et al., 2018).

2.2.3 Metabolic flexibility

The capacity to match fuel oxidation to fuel availability and changing energy demands is defined as metabolic flexibility (MetFlex) (Galgani & Fernández-Verdejo, 2021). MetFlex plays an essential role in maintaining energy homeostasis and overall metabolic health by ensuring a constant and sufficient energy supply to tissues and cells when transitioning from fasting to feeding, or rest to exercise, for example. On the other hand, dysregulated lipid and CHO metabolism and a blunted ability to switch between substrates characterizes metabolic inflexibility, which is a common feature in obesity, insulin resistance, metabolic syndrome and type 2 diabetes (Kelley et al., 1999; Kelley & Simoneau, 1994; San-Millán & Brooks, 2018; Smith et al., 2018).

MetFlex is an adaptive, systemic response coordinated by multiple organs and tissues. The determinants and regulatory mechanisms are thus complex and

multifactorial, and not entirely clear yet. A few proposed determinants include energy balance and dietary macronutrient composition, plasma substrate concentration, glucose disposal rate, and mitochondrial oxidative capacity (Galgani et al., 2008). The identification of primary factors influencing MetFlex also depends on the level at which fuel availability, a central feature in the concept of MetFlex, is assessed (Galgani & Fernández-Verdejo, 2021). For example, fuel availability on the intake level is determined primarily by dietary composition and energy balance, which in turn affect the circulating level of substrates. The capacity for glucose and fatty acid uptake from circulation determine the fuel availability on the tissue/cellular level, and lastly, fuel availability and MetFlex can also be considered at the mitochondrial level (Galgani & Fernández-Verdejo, 2021). Mitochondrial function is suggested to be at the core of MetFlex, since mitochondria are major regulators of glucose and fat metabolism as oxidation sites (San-Millán, 2023; Smith et al., 2018). Reduced skeletal muscle glucose disposal and impaired suppression of adipose tissue lipolysis, together with mitochondrial dysfunction, favor the accumulation of lipids and lipid intermediates ectopically, which is proposed as a key contributor to the development of metabolic inflexibility (Galgani et al., 2008; Sparks et al., 2009). Endocrine regulation together with metabolic signalling and pathways involving key regulatory enzymes, such as pyruvate dehydrogenase, malonyl-CoA, and carnitine palmitoyltransferase, also play an essential role in fuel sensing, uptake, transport, storage, and expenditure (Smith et al., 2018).

MetFlex can be assessed in response to various metabolic or physiological challenges, with insulin stimulation by HEC, high-fat diet, and exercise being some of the most commonly used methods. Originally MetFlex was assessed with the arteriovenous leg balance technique during insulin stimulation by HEC and expressed as the change in respiratory quotient (ΔRQ = insulin-stimulated RQ − fasting RQ) (Kelley et al., 1999). RQ is the ratio of produced carbon dioxide (VCO_2) to consumed oxygen (VO_2) (VCO_2/VO_2) and it reflects the fuel mix being oxidized for energy production. RQ measured with the leg balance technique represents substrate metabolism at the tissue level, while an indirect measurement of gas exchange from breath allows a less invasive measurement and an estimation of whole-body substrate oxidation with respiratory exchange ratio (RER). RER is an approximate measure of RQ and equals RQ over time, thus it is often used interchangeably with RQ to represent whole-body fuel metabolism. Fasting RQ or RER is also often used as a marker of MetFlex, and more recently alternative indicators have been proposed as well, for example, an index of postprandial RQ and insulin variability (Bergouignan, Antoun et al., 2013), 24-h RQ kinetics (Carnero et al., 2021), and the rates of glucose clearance and fractional gluconeogenesis during OGTT (Curl et al., 2024).

2.2.4 Substrate oxidation

The relative contribution of each of the primary oxidative substrates, carbohydrates and fatty acids, to energy production depends primarily on fuel availability and metabolic demand. Despite the large fat stores in the body and the virtually unlimited potential of fat as an energy source, fatty acids are less readily available for oxidation than CHO, because triglycerides, the major storage form of fat, must first be broken down to FFA by hydrolysis. Carbohydrates are available as glucose in the blood and as glycogen in skeletal muscle and the liver, but the stores are limited. The availability is also affected by constant transitions between fasting and fed states and consequent fluctuations in fuel supply. For example, healthy individuals are normally able to increase fat oxidation (FATox) in a fasting state in response to abundant availability of fatty acids released from adipose tissue by lipolysis following glycogen depletion, whereas CHO oxidation increases after a carbohydrate-containing meal and the subsequent increase in blood glucose availability (Figure 2). In obesity, insulin resistance, and type 2 diabetes, however, fat oxidation during fasting is impaired, together with a blunted ability to switch from the oxidation of fat to CHO after a meal or insulin stimulation (Corpeleijn et al., 2008; Kelley et al., 1999). In comparison to healthy counterparts, the ability to increase fat oxidation in response to a high-fat diet is also impaired in obesity (Battaglia et al., 2012).

Besides in the transition from fasting to a fed state or insulin stimulation, the ability to alternate oxidized fuels is required during exercise in order to increase energy supply to match the increased energy demands of working muscles. Fat and CHO are oxidized simultaneously during exercise, but their source and relative contribution to energy production is largely determined by the intensity and duration of exercise (Egan & Zierath, 2013). During low-intensity and prolonged exercise, plasma fatty acids are the preferred fuel, while the contribution of intramuscular triglycerides increases towards moderate-intensity exercise. With further increases in exercise intensity, fat oxidation is suppressed and the use of CHO, particularly from muscle glycogen, increases (Brooks & Mercier, 1994; Romijn et al., 1993). In impaired glucose tolerance, type 2 diabetes and metabolic syndrome, impaired fat oxidation rates are often reported already at (both absolute and relative) low-to-moderate exercise intensities (Blaak et al., 2000; Mensink et al., 2001; San-Millán & Brooks, 2018).

The relative contribution of CHO and fat to total fuel oxidation can be estimated from respiratory gas exchange based on the different chemical compositions of the substrates and the amount of oxygen needed for oxidation (Livesey & Elia, 1988). The oxidation of glucose requires less oxygen than the oxidation of fat, thus low RQ or RER values (i.e., close to 0.7) indicate higher oxidation of fats, and high values (i.e., close to 1.0) indicate higher CHO oxidation (CHOox).

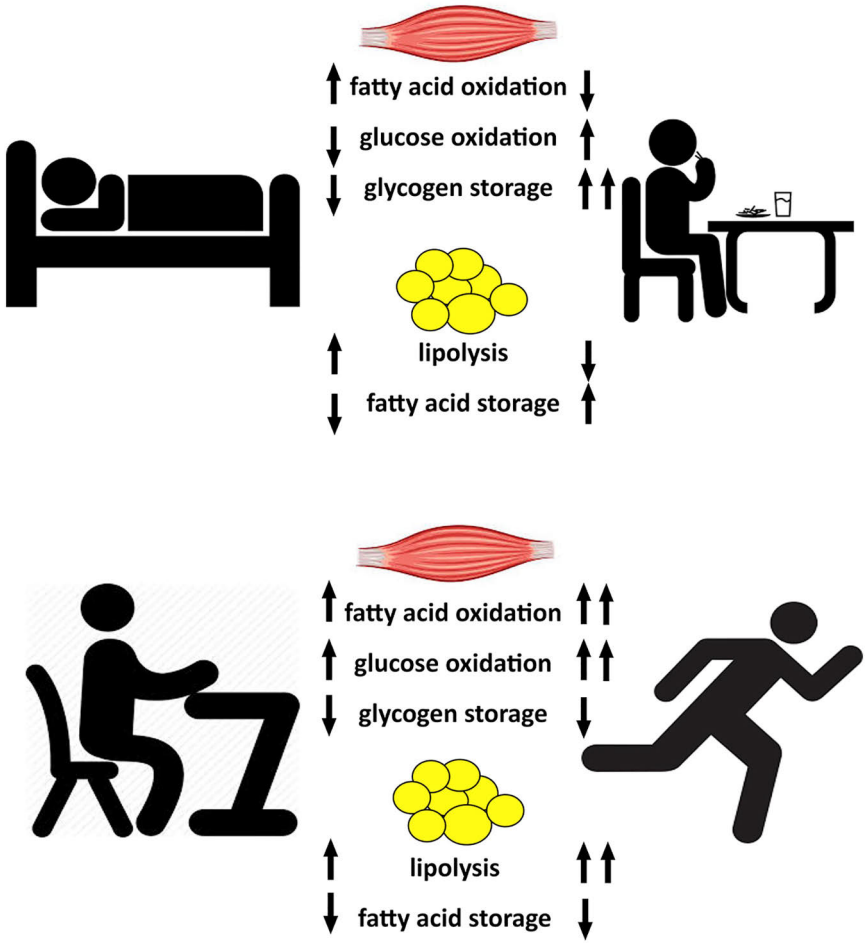

Figure 2. Summary of fuel metabolism changes within skeletal muscle and adipose tissue during periods of sleeping, fasting, feeding, rest, and exercise. Skeletal muscle switches from higher rates of fatty acid oxidation during sleeping/post-absorptive conditions to greater oxidation and storage of glucose after feeding, and reduced fatty acid oxidation. Adipose tissue shifts from higher rates of lipolysis to suppression of lipolysis and fat storage during the fasting to feeding transition. From rest to exercise, skeletal muscle increases rates of both fatty acid and glucose oxidation to support higher energy demands, while lipolysis in adipose tissue is drastically enhanced. Reprinted from Cell Metabolism, Vol 25, Goodpaster, B. & Sparks, L., Metabolic Flexibility in Health and Disease, Pages 1027–1036, Copyright (2017), with permission from Elsevier.

2.3 Physical activity and metabolic flexibility

Lifestyle changes, including dietary modifications and exercise training, are well-established strategies in the maintenance of healthy weight and the prevention and management of metabolic syndrome, insulin resistance, and type 2 diabetes. The onset of these metabolic disorders might be preceded by metabolic inflexibility, but

less is known about the effects of habitual activity behaviors and lifestyle interventions on the regulation of MetFlex per se.

2.3.1 Effects of exercise training

Regular exercise is well-known to improve classical cardiometabolic risk factors, including markers of glycemic control, triglycerides, blood pressure, and waist circumference (Chudyk & Petrella, 2011). Improvements in several key components of MetFlex have been reported following weight loss, including insulin sensitivity, insulin-mediated suppression of fat oxidation, and the ability to switch from fat to CHO oxidation after a meal (Corpeleijn et al., 2008; Kelley et al., 1999). However, weight loss achieved by dietary changes alone appears to be insufficient to induce changes in mitochondrial capacity, fasting fat oxidation, and insulin-stimulated MetFlex, despite improved insulin sensitivity (Goodpaster et al., 2003; Kelley et al., 1999; Menshikova et al., 2018; Toledo et al., 2008). When dietary modifications are combined with exercise, however, these aspects of MetFlex can also be improved (Goodpaster et al., 2003; Ryan & Ortmeyer, 2019; Toledo et al., 2007). Exercise and caloric restriction have differential effects on mitochondria (Menshikova et al., 2018), and MetFlex and insulin sensitivity have also been shown to improve following weight loss by exercise training alone, without dietary intervention (Malin et al., 2013).

Weight loss itself might not even be necessary for enhanced MetFlex and fatty acid oxidation capacity (Fritzen et al., 2020). For example, a 3-month exercise program combining aerobic and resistance exercise [30 min cycling at 55 % of maximal power output (PO) twice a week, and 3 sets of 8 repetitions of large muscle group exercises at 55–75 % of maximal voluntary contraction once a week] improved insulin sensitivity, mitochondrial function, and MetFlex in adults with type 2 diabetes, without changes in weight (Meex et al., 2010). Notably, mitochondrial function and MetFlex were completely restored to the level of age- and BMI-matched control subjects without diabetes. Furthermore, MetFlex improved only in those with type 2 diabetes and not in control subjects (Meex et al., 2010). Other studies similarly suggest that existing metabolic impairments influence the capacity of exercise to improve MetFlex and insulin sensitivity, so that those with more severe disturbances are likely to benefit the most (Goodpaster et al., 2003; Malin et al., 2013).

Exercise-induced improvements in MetFlex have often been linked to concurrent improvements in insulin sensitivity (Meex et al., 2010; Ryan & Ortmeyer, 2019; Malin et al., 2013). Improvements in fatty acid mobilization, uptake and oxidation, in turn, have been proposed as key contributors to improvements in insulin sensitivity following exercise (Goodpaster et al., 2003; Schenk et al., 2009), and

exercise interventions have often reported beneficial effects specifically on fat metabolism and oxidation, either at rest or during exercise (Atakan et al., 2022; Gaitán et al., 2019; Goodpaster et al., 2003; Mensink et al., 2005; Nancekievill et al., 2023; Schrauwen et al., 2002). Moreover, it seems that it is possible to improve fat oxidation already with exercise interventions of very short duration, volume, and intensity. For example, just 10 consecutive days of aerobic exercise (1 h/day at 70 % of VO_{2peak}) was enough to increase fat oxidation in response to a dietary high fat challenge in obese adults (Battaglia et al., 2012), and 3 months of low-volume and low-intensity exercise (~ 45–50 min cycling at 40 % of VO_{2max} three times per week) increased the utilization of fat for fuel during exercise in both healthy and obese men (Schrauwen et al., 2002; Van Aggel-Leijssen et al., 2002). A 2-month intervention that aimed to increase daily walking by 45 min led to improvements in fat oxidation in a fasting state as well (Trenell et al., 2008). Fasting fat oxidation was also enhanced with a combined 4-month exercise and diet intervention, and particularly the intensity and duration of PA predicted improvements in fat oxidation, not changes in body composition (Goodpaster et al., 2003). Overall, increased PA and associated increases in energy expenditure and muscle contractions appear to be essential in improving or maintaining MetFlex and oxidative capacity since dietary changes are not able to induce similar changes, despite improvements in weight and insulin sensitivity.

2.3.2 Effects of physical inactivity and sedentary behavior

Physical inactivity and sedentary behavior are now recognized to play a fundamental part in the development of metabolic diseases. They have also been proposed as the main determinants of metabolic inflexibility, while a higher level of PA predicts better MetFlex (Bergouignan et al., 2011; Rynders et al., 2018). Changes in habitual PA level by training, detraining or bed rest have been shown to modify MetFlex (Bergouignan, Antoun, et al., 2013), and, importantly, the adverse effects of detraining seem to be more pronounced than the beneficial effects of exercise (Bergouignan, Momken, et al., 2013). Furthermore, the negative metabolic consequences of inactivity and sedentary behavior may not be fully counteracted by PA either, since the shift in substrate use towards CHO during 2 months of bed rest was only partially prevented by a combined aerobic and resistance exercise program (Bergouignan et al., 2009). A 3-week bed rest study also showed that inactivity and sedentariness trigger metabolic inflexibility and muscle lipid accumulation despite maintained energy balance and fat mass (Rudwill et al., 2018).

Although not representative of daily living conditions, bed rest studies have provided valuable mechanistic insights into how physical inactivity and sedentary behavior may be primary causes in the development of metabolic inflexibility. The

evidence gained from these studies suggests that, independent of energy balance, inactivity induces insulin resistance; impaired fatty acid trafficking; reduced fat oxidation in favor of increased CHO oxidation, and ectopic fat accumulation (Bergouignan et al., 2011). The bed rest-induced reduction in muscle contractions per se has also been shown to be an important contributor to the blunted shift in substrate use associated with inactivity (Shur et al., 2022). The key role of muscle activation in glucose and lipid metabolism is also highlighted by recent studies showing that sustained, low-intensity local muscle activity can improve systemic metabolic regulation quickly (Hamilton et al., 2022), and that increased muscle contractions are likely the primary mechanism underlying the more pronounced glycemic benefits gained with more frequent activity breaks in comparison to a single bout (Gao et al., 2024).

Meta-analyses of experimental studies suggest that breaking up prolonged periods of muscle inactivity (sitting) can have beneficial effects and attenuate some of the metabolic disturbances associated with sedentary behavior. Short breaks with either light- or moderate-intensity PA have consistently led to improvements in postprandial glucose and insulin levels, with some evidence indicating improvements in blood lipids as well (Buffey et al., 2022; Loh et al., 2020; Saunders et al., 2018). Breaking up sitting with standing may also induce similar benefits, but the evidence is more inconsistent, and higher intensity activity likely yields greater benefits (Buffey et al., 2022; Saunders et al., 2018). Substantial evidence thus indicates benefits to postprandial circulating levels of substrates with interrupted sitting, but studies considering the effects on substrate utilization and switching are more limited. Some experimental trials suggest increases in postprandial fat oxidation in comparison to uninterrupted sitting with a single acute 2-h standing bout (Gao et al., 2017) and with more frequent light-intensity walking breaks over 8 hours (Thorsen et al., 2019). More frequent short standing breaks have also been shown to increase total fat oxidation over an 8-h period in comparison to prolonged sitting and longer standing bouts less frequently (Hawari et al., 2016). Frequent breaks over a day with moderate-intensity activity, however, increased 24-h CHO oxidation, while an energy-matched single exercise bout increased reliance on fat (De Jong et al., 2019). Interestingly, these activity-induced metabolic effects relating to substrate use seem to be independent of EE, again supporting the crucial contribution of muscle contractions to metabolic health (De Jong et al., 2019; Thorsen et al., 2019).

Although the relationship between lifestyle factors and MetFlex has only recently started to gain research interest, it seems increasingly clear that physical inactivity, sedentary behavior and prolonged bouts of sitting as distinct health behaviors have detrimental consequences on metabolic regulation and fuel utilization. However, as most of the current evidence of the effects of inactivity and sedentary behavior on MetFlex is from experimental settings and bed rest studies,

there is a lack of knowledge regarding the effects of free-living habitual activity behaviors. Moreover, the potential of reduced daily sedentary time as a health-enhancing strategy in the context of substrate metabolism and MetFlex in longer term remains to be thoroughly investigated. Interventions focusing on this are needed to better understand how the increasingly prevalent sedentary lifestyles may contribute to the progression of metabolic diseases. Particularly studies in inactive individuals with existing metabolic impairments could provide important insights due to an increased risk of developing metabolic diseases and potential to gain the greatest benefits. The findings may aid in the development of intervention targets for the prevention of chronic diseases and thus have important public health implications.

2.4 Metabolic syndrome

Metabolic syndrome refers to a cluster of several known cardiometabolic risk factors, including central obesity, dyslipidemia, elevated blood pressure, and elevated blood glucose. Several clinical definitions with variation in required components and cut points exist, but according to a consensus statement developed by several major organizations (International Diabetes Federation; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society and International Association for the Study of Obesity) metabolic syndrome is defined by meeting three of the following five criteria (Alberti et al., 2009):

- Elevated waist circumference (according to population- and country-specific definitions; for people of European origin the cut points are ≥ 94 cm for men and ≥ 80 cm for women)
- Elevated triglycerides (≥ 1.7 mmol/L, or drug treatment for elevated triglycerides)
- Reduced high-density lipoprotein (HDL) cholesterol (< 1.0 mmol/L for men and < 1.3 mmol/L for women, or drug treatment for reduced HDL)
- Elevated blood pressure (systolic ≥ 130 mmHg and/or diastolic ≥ 85 mmHg, or antihypertensive drug treatment)
- Elevated fasting glucose (≥ 5.6 mmol/L, or drug treatment for elevated glucose)

An imbalance between energy intake and expenditure as a result of overnutrition and physical inactivity is considered to be one of the primary underlying reasons behind metabolic syndrome. The prevalence of metabolic syndrome is rising simultaneously with increases in sedentary lifestyles and obesity, and although

varying definitions make accurate assessments challenging, ~ 30 % of global adult population is estimated to have metabolic syndrome (Noubiap et al., 2022). Considering the high prevalence and a significantly increased risk of cardiovascular disease and type 2 diabetes associated with metabolic syndrome (Ford et al., 2008; Mottillo et al., 2010), it is a serious public health concern worldwide.

3 Aims

The overall aim of this PhD study was to investigate the associations of sedentary time and PA with energy metabolism and insulin sensitivity, and to investigate whether reducing daily sedentary time could improve metabolic health in a sedentary population at an increased risk of metabolic diseases. It was hypothesized that sedentary time is adversely and PA beneficially associated with metabolic outcomes, and that reducing sedentary time, without adding exercise per se, has a favorable impact on metabolic health.

The specific aims were:

1) To investigate the associations of accelerometer-assessed activity behaviors with whole-body and skeletal muscle insulin sensitivity (Studies I and II).
2) To investigate the associations of accelerometer-assessed activity behaviors with MetFlex and substrate oxidation (Study III).
3) To investigate the effects of a randomized controlled trial aiming to reduce daily sedentary time on cardiometabolic risk factors in 3 months (Study IV).
4) To investigate the effects of a randomized controlled trial aiming to reduce daily sedentary time on MetFlex and substrate oxidation in 6 months (Study V).

4 Materials and Methods

4.1 Study design

The study design is a parallel-group randomized controlled trial, consisting of a 1-month screening phase and a 6-month intervention phase. Participants wore accelerometers continuously through both phases, and cardiometabolic outcomes were assessed at baseline after screening, at 3 months, and at 6 months. Anthropometrics and blood sample-derived outcomes were assessed at all timepoints, while HEC, PET imaging, and calorimetry were performed at baseline and at 6 months. Cross-sectional studies I-III used accelerometer data from the screening phase and baseline outcome measurements. The data was collected at the Turku PET Centre (Turku, Finland) between April 2017 and March 2020 according to good clinical practice and the Declaration of Helsinki. The study was approved by the Ethics Committee of the Hospital District of Southwest Finland (16/1801/2017) and is registered at Clinicialtrials.gov (NCT03101228). All participants gave written informed consent before entering the study.

4.2 Participants

The target population was sedentary and physically inactive adults with metabolic syndrome. Participants were recruited from the local community with newspaper advertisements and bulletin leaflets. The inclusion criteria were age 40–65 years, physical inactivity (< 120 min/week of self-reported MVPA), sedentary time ≥ 10 h/day or ≥ 60 % of accelerometer wear time/day during screening, body mass index (BMI) 25–40 kg/m^2, blood pressure < 160/100 mmHg, fasting glucose < 7.0 mmol/L, and fulfilment of metabolic syndrome criteria (Alberti et al., 2009). The exclusion criteria were a history of any cardiac disease; diagnosed diabetes, abundant alcohol consumption [according to Finnish guidelines: > 23 and > 12 units (1 unit = 12 g of pure alcohol) per week for men and women, respectively]; cigarette smoking; use of snuff tobacco or narcotics; a depressive or bipolar disorder; inability to understand written Finnish; previous PET imaging or other considerable exposure to radiation; and any chronic disease or condition that could endanger participant safety or study procedures, or interfere with the interpretation of results.

4.3 Intervention

After the screening phase, the participants fulfilling the inclusion and exclusion criteria were randomized into the intervention and control groups using random permuted blocks with a 1:1 ratio, a block size of 44, and stratification for sex. A 14:30 allocation ratio was used to further randomize the participants into a subsample undergoing HEC with PET imaging (n=44), or HEC only (n=20). The randomization code was generated by a statistician with SAS 9.4 (SAS Institute Inc., Cary, NC, USA) in advance, and the sealed envelopes containing the group allocation information for each participant were opened after the baseline measurements.

The aim of the behavioral intervention was to reduce sedentary time by 1 h/day compared to the individually determined baseline during screening. The participants in the intervention group were guided by a researcher in 1-hour personal counselling sessions to sit less by increasing standing and non-exercise PA in their daily lives, without intentionally adding exercise training per se. The ways to reduce sedentary time and increase activity were individually discussed according to each participant's preferences, and could include, for example, using standing desks, taking stairs instead of an elevator, and taking walks. The control group was guided to maintain their usual activity habits.

Both groups used accelerometers continuously throughout the intervention phase. The accelerometers were connected to a mobile application (ExSed, UKK Terveyspalvelut Oy, Tampere, Finland) to enable self-monitoring of the individually set goals for daily sedentary time and PA (Vasankari et al., 2019). For the intervention group, 1 h was subtracted from the baseline sedentary time and added to standing and PA according to the individual preferences (with a maximum of 20 min added to MVPA), and for the control group the goals were equal to the baseline values. The application provided a visual summary of the daily accumulation of time spent sedentary and in PA of different intensities (Figure 3).

The participants were contacted by phone two to three times during the intervention, and they visited the research center at the midpoint of the intervention for the 3-month outcome assessments and to assure that the accelerometers and the mobile application were working properly. After the outcome assessments at 6 months, the control group participants had an opportunity to receive guidance for behavior change in similar personal counselling sessions as the intervention group participants had at baseline.

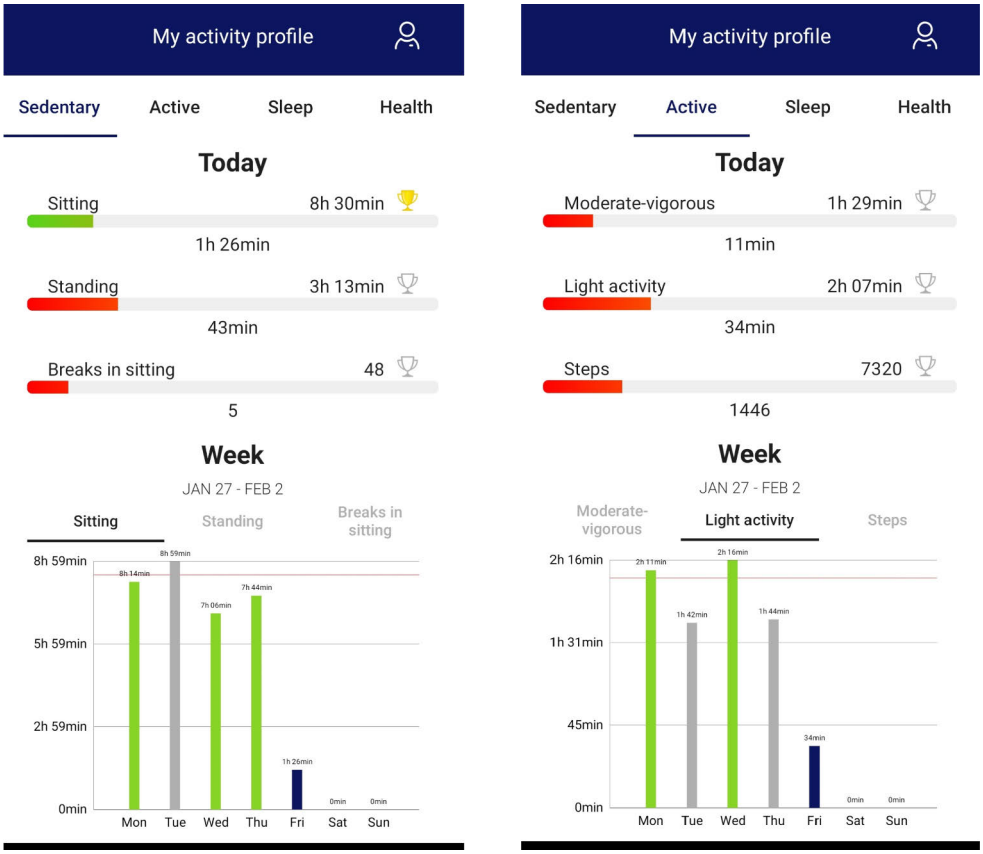

Figure 3. Examples of the visual feedback provided by the ExSed-application (UKK Terveyspalvelut Oy, Tampere, Finland).

4.4 Physical activity and cardiorespiratory fitness

4.4.1 Accelerometry

Triaxial hip-worn accelerometers were used during waking hours to assess sedentary time, PA, standing, and breaks in sedentary time throughout the screening (UKK AM30, UKK Institute, Tampere Finland) and intervention phases (Movesense Suunto, Vantaa, Finland).

The raw accelerometer data (recorded at a sampling frequency of 100 Hz during screening and 52 Hz during the intervention) was analyzed by a collaborating researcher at the UKK Institute in 6-s epochs with the validated mean amplitude deviation (MAD) method (Vähä-Ypyä, Vasankari, Husu, Mänttäri, et al., 2015).

Epoch-wise MAD values were calculated as:

$$\mathrm{MAD} = \frac{1}{\mathrm{N}} \sum_{\mathrm{i=j}}^{\mathrm{j+N-1}} |\mathrm{r_i} - \mathrm{R_{ave}}|$$

where N is the number of samples in the epoch, j is the starting point of the epoch, r_i is the acceleration signal of the three axes at each time point (i), and R_{ave} is the mean resultant acceleration value for each 6-s epoch. The unit of the MAD is milligravity (mg); i.e., the Earth's gravity 1 *g* is equal to 1000 m*g* (Vähä-Ypyä, Vasankari, Husu, Mänttäri, et al., 2015). The epoch-wise MAD values were converted to METs: sedentary time and standing were defined as < 1.5 METs (MAD < 22.5 m*g*), LPA as 1.5 – < 3.0 METs (MAD 22.5 – < 91.5 m*g*), and MVPA as ≥ 3.0 METs (MAD ≥ 91.5 m*g*). Moderate- and vigorous-intensity activities are combined as MVPA due to a negligible amount of vigorous PA accrued by the participants.

In order to differentiate between the time spent sedentary and standing during epochs with < 1.5 METs, body posture was assessed with the validated angle for posture estimation (APE) method, which identifies postures with 90 % accuracy in free-living conditions (Vähä-Ypyä et al., 2018). APE represents the angle between the incident epoch-wise accelerometer orientation and a reference vector determined during walking (Earth's gravity vector, i.e., zero degrees). A sedentary posture is classified by APE ≥ 11.6° and a standing posture by APE < 11.6°.

The number of steps/day was determined by an algorithm splitting 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, a step is detected. The algorithm requires a walking speed of ~ 3 km/h to detect every step (Vähä-Ypyä et al., 2018). Breaks in sedentary time (=sit-to-stand transitions) were determined as sedentary periods with a 1-min exponential moving average < 1.5 METs ending in vertical acceleration and a subsequent standing posture or movement (≥ 1.5 METs) (Vähä-Ypyä et al., 2018).

A wear time of 10-19 h/day and a minimum of 4 days were considered valid. Daily measurement time ≥ 19 h likely indicates that the accelerometer was also worn while sleeping and therefore the exceeding hours were subtracted from sedentary time on the days with wear time ≥ 19 h. Periods with acceleration of each axis remaining within 187.5 m*g* range for ≥ 30 min were considered non-wear time. In addition to the absolute time (h/day), the proportions of time spent in different activities (% of accelerometer wear time/day) were calculated.

4.4.2 Maximal cycle ergometry test

In Studies I, II, III, and V, cardiorespiratory fitness was assessed with a graded maximal cycle ergometer test (eBike EL Ergometer with CASE v6.7, GE Medical

Systems Information Technologies Inc., Milwaukee, WI, USA). After an unloaded warm-up, the test started at 25 W and the load was increased by 25 W every 3 min until volitional exhaustion. Maximal PO was calculated as $W_{last} + (t / 180 \times 25)$, where W_{last} is the last completed load (W) and t is the number of seconds on the last uncompleted load. The rate of perceived exertion (Borg scale 6-20), any physical symptoms, and blood pressure were assessed after 1 min on each load. The test was stopped by a physician before reaching volitional exhaustion if there was a medical reason for termination (e.g., chest pain, abnormal blood pressure response). A metabolic cart with a facemask (Vyntus CPX, CareFusion, Yorba Linda, CA, USA) was used for a direct breath-by-breath measurement of respiratory gases, and the values were averaged over 20-s periods. Maximal oxygen uptake (VO_{2max}) was defined as the highest 1-min VO_2 average per body weight (mL/kg/min) and per fat-free mass (FFM) (mL/kg_{FFM}/min). The test was considered maximal if at least one criterion was met: RER > 1.0, a plateau in VO_2, or heart rate within ± 10 bpm of age-predicted maximum.

4.5 Insulin sensitivity

4.5.1 Hyperinsulinemic euglycemic clamp

In Studies I, II, III, and V, whole-body glucose uptake was measured with HEC after an overnight fast. The participants were instructed to avoid strenuous PA, caffeine, and alcohol on the previous day before the research visit and to minimize PA on the morning of measurement by arriving at the research center by car or by bus. The participants rested in a lying position for ~ 1 h before the measurement and during HEC. Antecubital veins of both arms were cannulated for blood sampling and insulin and glucose infusions. Venous blood of the arm used for blood sampling was arterialized with a hot water bottle. Insulin (Actrapid, 100 U/mL, Novo Nordisk, Bagsvaerd, Denmark) was infused at a steady 40 mU/m^2 body surface area/min rate after 7 min of priming with higher doses (first 4 min at 160 mU/m^2/min, and then 3 min at 80 mU/m^2/min). A 20 % glucose infusion was started ~ 4 min after starting the insulin infusion, and the rate was adjusted according to blood sampling every 5-10 min to maintain an ~ 5 mmol/L plasma glucose concentration. Whole-body GU (per kg body weight) was calculated as a measure of whole-body insulin sensitivity from the measured glucose values and changes in the glucose infusion rate in 20-min intervals, starting at 20 min after the start of HEC.

4.5.2 Positron emission tomography

In Study II, HEC was combined with [^{18}F]FDG-PET imaging to quantify skeletal muscle GU. Imaging was performed with a combined PET/computed tomography (CT) scanner (Discovery 690 PET/CT, GE Healthcare, Milwaukee, WI, USA) as previously described (Eskelinen et al., 2015; Sjöros et al., 2018). A positron-emitting glucose analog [^{18}F]FDG-tracer was injected into the antecubital vein 75 [standard deviation (SD) 12] min after starting HEC, and cardiac region imaging was started simultaneously. The femoral region (quadriceps femoris and hamstring muscle groups) was imaged in 3 × 300 s time frames after cardiac and abdominal regions, starting at 57 (SD 13) min after the tracer injection. The cumulative tracer availability in plasma (time-activity curve) was determined from radioactivity in the left ventricle of the heart during the first 40 min of imaging, and from blood samples collected at ~ 50 and ~ 70 min after the injection (1480 Wizard 3; Wallac, Turku, Finland). The calculated radiation dose was 10.4 mSv, which is approximately twice the average annual amount of natural background radiation in Finland [Siiskonen (ed.), 2020].

The images were analyzed with Carimas software v. 2.9 (Turku PET Centre, Turku, Finland). Regions of interest were manually drawn to quadriceps femoris (rectus femoris, vastus lateralis, vastus medialis, vastus intermedius) and hamstring muscle groups (biceps femoris, semitendinosus, semimembranosus) on fused PET/CT images in five cross-sectional mid-thigh planes (5 × 3.3 mm), using the CT images for anatomical reference. The regions of interest were used to extract tissue time-activity curves from the PET data. Figure 4 shows an example of a PET image.

The plasma and tissue time-activity curves and graphical analyses were used to calculate the fractional uptake rate (FUR) of the tracer (Patlak et al., 1983; Rutland et al., 2000; Thie, 1995). Skeletal muscle GU (per kg tissue weight) was then calculated by multiplying FUR by the mean plasma glucose concentration during the PET scan and dividing by the skeletal muscle tissue density 1.0414 g/mL (ICRP, 1975) and a lumped constant value 1.2, which corrects for differences between the transport and phosphorylation of [^{18}F]FDG and glucose in skeletal muscle (Peltoniemi et al., 2000).

4.5.3 Surrogate markers

Fasting insulin, HOMA-IR index [calculated as: glucose (mmol/L) × insulin (mU/L) / 22.5], and triglyceride/HDL ratio were used as surrogate measures of insulin sensitivity.

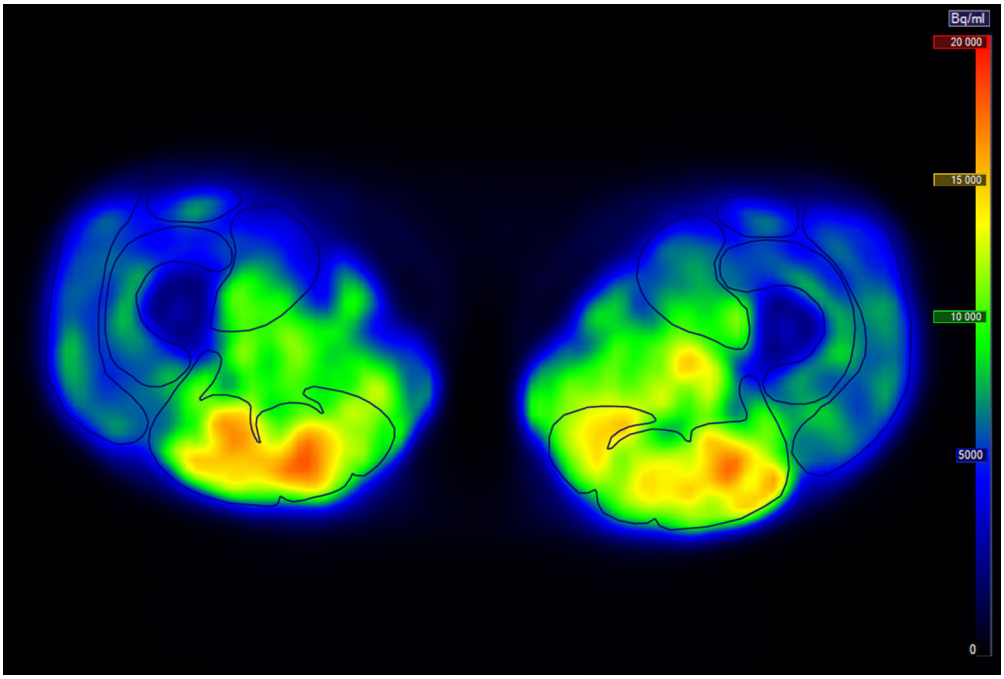

Figure 4. An example of a [^{18}F]FDG-PET image in one transaxial slice. Regions of interest were manually drawn to quadriceps femoris (rectus femoris, vastus lateralis, vastus medialis, vastus intermedius) and hamstring muscle groups using a computed tomography image as anatomical reference. Color bar on the right shows the glucose uptake rate (Bq/mL), with red and yellow colors indicating the highest uptake. [^{18}F]FDG-PET, ^{18}F-fluorodeoxyglucose positron emission tomography. From Original publication II.

4.6 Metabolic flexibility

4.6.1 Indirect calorimetry

In Studies III and V, indirect calorimetry measurements were performed during HEC and the exercise test to measure respiratory gas exchange for the assessment of resting, and insulin- and exercise-stimulated EE, substrate metabolism, and MetFlex.

4.6.1.1 Insulin stimulation

A metabolic cart with a ventilated canopy hood (Quark RMR with OMNIA, COSMED, Rome, Italy) was used at rest after an overnight fast and during HEC. The metabolic cart was calibrated according to the manufacturer's instructions. The participants rested in a lying position for a minimum of 20 min before the measurement. VO_2 and VCO_2 were measured for 20 (SD 1) min in the fasting state, and for 15 (SD 2) min during HEC, starting at 29 (SD 8) min after the initiation of

HEC. The calorimeter software recorded the gas exchange, RER, and EE values in 10-s intervals. The first 4 min were discarded, and steady state was determined by < 10 % coefficient of variation in VO_2 and VCO_2 and/or < 5 % coefficient of variation in RER for ≥ 4 min (Fullmer et al., 2015). ΔRER from fasting to insulin stimulation (=RER during HEC − fasting RER) was calculated from averaged steady-state RER values and defined as the measure of insulin-stimulated MetFlex.

4.6.1.2 Exercise

Respiratory gas exchange was measured breath-by-breath with a facemask (Vyntus CPX, CareFusion, Yorba Linda, CA, USA) during the maximal exercise test. The autocalibrated metabolic cart averaged VO_2, VCO_2, and RER values over 20-s periods, and the minimum and maximum RER during the test were used to calculate ΔRER from the starting load (25 W) to maximal exercise as the measure of exercise-stimulated MetFlex. The starting load 25 W corresponded to < 42 % of maximal PO of all participants and was therefore considered low-intensity exercise. EE at low- and maximal-intensity exercise was calculated from VO_2 and VCO_2, taking into account the increased use of muscle glycogen as an energy source at higher intensities (Jeukendrup & Wallis, 2005).

4.6.2 Fat and carbohydrate oxidation

Fasting and insulin-stimulated fat and CHO oxidation were calculated from VO_2 and VCO_2 measured at rest and during HEC, with the assumption of negligible protein oxidation (Frayn, 1983):

$$\text{FATox (g/min)} = 1.67 \times VO_2\,\text{(L/min)} - 1.67 \times VCO_2\,\text{(L/min)}$$
$$\text{CHOox (g/min)} = 4.55 \times VCO_2\,\text{(L/min)} - 3.21 \times VO_2\,\text{(L/min)}$$

The CHOox rate was subtracted from the glucose infusion rate to estimate non-oxidative glucose disposal, and negative values were interpreted as zero. CHOox exceeding the amount of exogenous glucose was assumed to represent the oxidation of other CHO sources, i.e., endogenous glucose, glycogen, and lactate.

To calculate substrate oxidation rates during exercise, the following formulas were used, accounting for the increased contribution of muscle glycogen to CHOox with increasing exercise intensity (Jeukendrup & Wallis, 2005):

$$\text{Low-intensity: CHOox (g/min)} = 4.344 \times VCO_2\,\text{(L/min)} - 3.061 \times VO_2\,\text{(L/min)}$$
$$\text{Maximal intensity: CHOox (g/min)} = 4.210 \times VCO_2\,\text{(L/min)} - 2.962 \times VO_2\,\text{(L/min)}$$

FATox (g/min) was calculated as 1.695 × VO_2 (L/min) – 1.701 × VCO_2 (L/min) at both intensities, and all calculations assumed negligible protein oxidation. Negative FATox values at maximal exercise intensity were interpreted as zero and assumed to represent a complete suppression of FATox. Substrate oxidation rates at intensities > 75 % VO_{2max} should be interpreted with caution, however, as the shift in acid-base balance and subsequent increases in bicarbonate buffering of hydrogen ions at high-intensity exercise will result in the production of (non-oxidative) CO_2 and elevated VCO_2 (Jeukendrup & Wallis, 2005). Delta exercise efficiency was also calculated from exercise PO and EE (kcal/min) as ΔPO/ΔEE × 100 to represent the ability to transfer consumed energy to mechanical work (Gaesser & Brooks, 1975). PO was converted from watts to kcal/min using a conversion factor of 0.014. For women, the efficiency was calculated between 25 and 75 W and for men between 25 and 100 W to represent efficiency at moderate-intensity exercise, as the chosen upper limits correspond to 65 % and 66 % of mean maximal PO, respectively.

4.7 Other measurements

4.7.1 Anthropometrics and body composition

Body weight was measured, and body fat-% and FFM estimated by air displacement plethysmography (Bod Pod, COSMED USA, Inc., Concord, CA, USA) after at least a 4-h fast. Height was measured with a wall-mounted stadiometer. BMI was calculated from weight and height (kg/m^2). Waist circumference was measured with a flexible measuring tape midway between the iliac crest and the lowest rib.

4.7.2 Blood samples

Venous blood samples were drawn after fasting at least 10 h and analyzed at the Turku University Hospital Laboratory. Plasma insulin was measured by electrochemiluminescence immunoassay (Cobas 8000 e801), plasma glucose by enzymatic reference method with hexokinase GLUC3, and cholesterol [total, HDL, and low-density lipoprotein (LDL)], triglycerides, FFA and lactate by enzymatic colorimetric tests (Cobas 8000 c702). Glycated hemoglobin (HbA_{1c}) was determined by turbidimetric inhibition immunoassay (Cobas 6000 c501) and liver enzymes by the photometric IFCC method (Cobas 8000 c702); all analyzers by Roche Diagnostics, Mannheim, Germany. Blood samples were also collected from all participants during HEC (~ 80 min from the start) to determine the insulin-stimulated FFA suppression and lactate increase. Additional blood samples were drawn at timepoints ~ 115 min, ~ 135 min and ~ 155 min from the 44 participants in the PET subsample, which were used to calculate the area under curve (AUC) for FFA and

lactate during HEC with the trapezoidal rule. Blood pressure was measured in a seated position with a digital blood pressure monitor (Apteq AE701f, Rossmax International Ltd, Taipei, Taiwan) after at least a 5-min seated rest.

4.7.3 Dietary intake

Structured 4-day food diaries (including one weekend day) were used to assess dietary intake in Studies III and V. The participants were instructed to not alter their diet during the intervention and to log all consumed food and drink (excluding water) in the diary. A researcher checked the diaries for reliable reporting with a portion picture booklet. The mean daily intakes of total energy and macronutrients were calculated with a software (AivoDiet 2.2.0.1, Aivo, Turku, Finland).

Food quotient (FQ; theoretical expected RER if dietary macronutrients were completely oxidized) was calculated with the following formula, with macronutrients expressed as percentages of total energy intake (%) (Toubro et al., 1998):

$$FQ = (0.207 \times CHO + 0.159 \times fat + 0.193 \times protein + 0.137 \times alcohol) / (0.207 \times CHO + 0.226 \times fat + 0.243 \times protein + 0.206 \times alcohol)$$

RER/FQ ratio was calculated to represent substrate oxidation relative to macronutrient intake, e.g., a value > 1 suggests lower FATox relative to fat intake (Carstens et al., 2013).

4.8 Statistical analyses

In all studies, the normal distribution of variables was evaluated visually and by Shapiro-Wilk test. Logarithmic (log10) transformations were performed when necessary to fulfil the assumption of normality, but the results are presented with values backtransformed to the original scale for easier interpretation, when applicable. The level of statistical significance was set at $p < 0.05$ (two-tailed) for all analyses. IBM SPSS Statistics 27.0 (IBM Corp., Armonk, NY, USA) was used for correlation analyses, and SAS 9.4 and JMP Pro 16.0.0 (SAS Institute Inc., Cary, NC, USA) for all other analyses.

Sample size

The sample size was determined by power calculations for the primary outcome of the whole research project that this study is a part of (whole-body insulin sensitivity; M value) (Sjöros, Laine, Garthwaite, Vähä-Ypyä, Löyttyniemi, et al., 2023). Based

on an earlier finding of a 2.4 μmol/kg/min increase after 2 weeks of moderate-intensity exercise (Eskelinen et al., 2015), the sedentary time reduction intervention was estimated to increase insulin sensitivity by 1.9 (SD 1.8) μmol/kg/min in the intervention group and by 0.2 μmol/kg/min in the control group in 6 months. To detect a statistically significant intervention effect in comparison to the control group, it was calculated that 24 participants are needed in each group ($\alpha = 0.05$, $1 - \beta = 0.9$). To allow possible dropouts and technical problems in the measurements, 64 participants were recruited in total, of which 44 were allocated to the PET subsample for the measurement of tissue-specific insulin sensitivity.

4.8.1 Cross-sectional analyses

The associations of sedentary time, PA, and fitness with whole-body insulin sensitivity (Study I), skeletal muscle insulin sensitivity (Study II), MetFlex and substrate oxidation (Study III) were examined with multivariable linear models. The linear model always included one metabolic outcome as the dependent variable and one accelerometer or fitness outcome as the independent variable. All models were adjusted for sex and age, and the models with accelerometry outcomes were also adjusted for accelerometer wear time. Pearson partial correlation analysis was used to examine the correlations with secondary metabolic, anthropometric, and dietary outcomes. PA outcomes expressed as % of accelerometer wear time were used in correlation analyses to account for differences in wear time.

Due to correlations between body fat-% and insulin sensitivity in Studies I and II, additional analyses were performed with body fat-% included as a covariate in the model to adjust for confounding adiposity. In Study III, the models were further adjusted for the use of lipid-lowering medication (statins) and blood pressure medication due to associations between medication use and energy metabolism outcomes. Total PA, sedentary time, and fitness were also used as covariates to further analyze the independence of observed significant associations between metabolic and PA outcomes. Additional analyses including the interaction between sex and each accelerometer variable were also performed to see if the associations between metabolic and PA outcomes were different between sexes. Differences in metabolic outcomes between groups with varying levels of sedentary time and standing were examined with unpaired t-test or one-way ANOVA. To account for differences in accelerometer wear time, the group stratification was done using proportions (%) of accelerometer wear time/day but is presented in h/day for easier interpretation. The results for continuous independent variables are reported as β-coefficients with 95 % confidence intervals (CI).

4.8.2 Longitudinal analyses

In Studies IV and V, the intervention effects and within- and between-group changes over time were assessed with a linear mixed model for repeated measurements (including group, time, and group × time interaction). All models were adjusted for sex, and those with accelerometer outcomes additionally for accelerometer wear time. The Tukey-Kramer method was used for multiple comparisons, and the results are reported as model-based means with 95 % CI. Correlations between changes in metabolic and PA outcomes during the intervention were analyzed with Spearman's rank correlation. PA outcomes expressed as % of accelerometer wear time were used in correlation analyses to account for differences in wear time.

Additional analyses

Additional analyses were performed by dividing the participants into two groups according to the measured change in sedentary time during the intervention. The 'reducers' included participants who reduced their mean daily sedentary time (% of accelerometer wear time/day) by ≥ 3 percentage points compared to the baseline (n=30), and the participants with a smaller reduction or an increase in sedentary time were defined as 'continuously sedentary' (n=26). The cut-point 3 percentage points corresponds to ~ 27 min/day with 15 h wear time and was chosen due to relatively equally sized groups. Participants with missing accelerometer data (n=8) were allocated according to the original randomization, resulting in 34 participants (26 from the intervention group and 8 from the control group) in the 'reducers' and 30 (7 from the intervention group and 23 from the control group) in the 'continuously sedentary' group.

Sample size for the intervention was calculated for whole-body insulin sensitivity but based on a previous finding of a ~ 0.03 increase in insulin-stimulated ΔRER in adults with type 2 diabetes after a 3-month moderate-intensity exercise program (Meex et al., 2010), it can be speculated that the above-mentioned group sizes are adequate for the additional analyses as well. To detect a similar statistically significant 0.03 (SD 0.03) group difference in ΔRER ($\alpha = 0.05$, $1 - \beta = 0.9$) following 6 months of sedentary time reduction, 23 participants would be needed in each group. The linear mixed model analyses were repeated with these groups.

5 Results

5.1 Participant characteristics

A total of 263 people volunteered, of which 144 completed the screening phase to fulfil the target of 64 eligible intervention participants. Most of the participants (58 %) were women. Forty-one % of the participants were overweight and 59 % obese.

Table 1. Participant characteristics.

	All	Men	Women
N (%)	64	27 (42)	37 (58)
Age, years	58.3 (6.8)	58.6 (6.0)	58.0 (7.4)
Weight, kg	93.2 (16.1)	101.2 (16.5)	87.4 (13.1)***
BMI, kg/m^2	31.6 (4.3)	31.6 (4.5)	31.6 (4.2)
Waist circumference, cm	110.9 (11.3)	115.5 (12.5)	107.5 (9.0)**
Body fat-%	43.1 (7.9)	37.2 (7.6)	47.4 (4.7)***
Systolic blood pressure, mmHg	143 (16)	141 (16)	144 (16)
Diastolic blood pressure, mmHg	88 (8)	88 (10)	88 (7)
Blood pressure medication, n (%)	34 (53)	20 (74)	14 (38)**
Lipid profile			
Free fatty acids, mmol/L	0.60 (0.20)	0.53 (0.18)	0.66 (0.20)*
Triglycerides, mmol/L	1.2 (0.9, 1.7)	1.3 (1.1, 1.7)	1.2 (0.8, 1.5)
Total cholesterol, mmol/L	4.7 (4.1, 5.2)	4.3 (4.1, 4.7)	4.8 (4.5, 5.4)**
LDL cholesterol, mmol/L	3.0 (2.6, 3.5)	2.9 (2.6, 3.2)	3.1 (2.7, 3.8)
HDL cholesterol, mmol/L	1.4 (0.3)	1.2 (0.3)	1.5 (0.3)**
Lipid-lowering medication, n (%)	14 (22)	7 (26)	7 (19)
Glucose regulation			
Fasting glucose, mmol/L	5.9 (0.4)	6.0 (0.5)	5.7 (0.3)*
Fasting insulin, pmol/L	69.5 (48.6, 104.2)	90.3 (55.6, 173.6)	55.6 (48.6, 83.3)*
HOMA-IR	2.4 (1.7, 3.8)	3.6 (2.3, 6.8)	2.1 (1.7, 3.1)**

Table 1 continued.

	All	Men	Women
Whole-body glucose uptake, mg/kg/min	2.5 (1.9, 3.8)	1.9 (1.3, 3.6)	2.8 (2.1, 4.3)**
HbA_{1c}, mmol/mol	37 (3)	37 (3)	36 (3)
Fasting lactate, mmol/L	1.0 (0.8, 1.3)	1.0 (0.9, 1.2)	1.0 (0.8, 1.3)
Energy metabolism			
REE, kcal/day	1697 (297)	1912 (298)	1552 (191)***
Fasting CHO oxidation, mg/kg/min	2.5 (0.9)	2.4 (0.9)	2.6 (0.9)
Fasting fat oxidation, mg/kg/min	0.4 (0.4)	0.4 (0.4)	0.3 (0.4)
Fasting RER	0.91 (0.85, 0.98)	0.87 (0.83, 0.97)	0.93 (0.86, 0.99)
ΔRER HEC	0.00 (−0.04, 0.03)	−0.01 (−0.03, 0.01)	0.00 (−0.05, 0.05)
ΔRER exercise	0.39 (0.07)	0.36 (0.06)	0.40 (0.07)*
Physical activity and fitness			
Sedentary time, h/day	10.04 (1.01)	10.20 (1.08)	9.93 (0.95)
Standing, h/day	1.79 (0.59)	1.47 (0.44)	2.02 (0.58)***
LPA, h/day	1.74 (0.44)	1.63 (0.50)	1.82 (0.38)
MVPA, h/day	0.97 (0.32)	1.03 (0.39)	0.92 (0.26)
Total PA, h/day	2.70 (0.62)	2.66 (0.74)	2.74 (0.53)
Steps/day	5149 (1825)	5329 (2084)	5018 (1629)
Breaks in sedentary time/day	29 (8)	26 (7)	30 (9)*
VO_{2max}, ml/kg/min	22.7 (4.7)	25.0 (4.9)	21.1 (3.7)**
Dietary intake			
Energy intake, kcal/day	1797 (399)	1910 (444)	1715 (346)
CHO, % of energy intake/day	39.2 (7.6)	38.7 (7.4)	39.5 (7.8)
Fat, % of energy intake/day	38.8 (6.5)	38.4 (6.1)	39.1 (6.8)
Protein, % of energy intake/day	17.8 (2.8)	18.1 (3.1)	17.6 (2.7)
FQ	0.83 (0.02)	0.83 (0.02)	0.83 (0.02)
RER/FQ	1.11 (0.11)	1.09 (0.10)	1.12 (0.11)

BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; HbA_{1c}, glycated hemoglobin; REE, resting energy expenditure; CHO, carbohydrate; RER, respiratory exchange ratio; HEC, hyperinsulinemic euglycemic clamp; LPA, light-intensity physical activity; MVPA, moderate-to-vigorous physical activity, PA, physical activity; VO_{2max}, maximal oxygen uptake; FQ, food quotient. Values are means with standard deviation (SD) or medians with first and third quartiles (Q1, Q3). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ between sexes (unpaired t-test, Mann-Whitney U-test, or Fisher's exact test). Modified from Original publications I-III.

5.2 Cross-sectional analyses (Studies I-III)

During the 1-month screening phase, the accelerometers were worn on average for 26 (SD 4) days and for 14.54 (SD 0.97) h/day for the determination of baseline sedentary time and PA. The participants spent on average 10.04 (SD 1.01) h sedentary, 1.79 (SD 0.59) h standing, and 2.70 (SD 0.62) h physically active, taking 5149 (SD 1825) steps daily. Besides being sedentary, the participants had low cardiorespiratory fitness as the mean VO_{2max} was 22.7 (SD 4.7) ml/kg/min.

5.2.1 Associations with whole-body insulin sensitivity

In Study I, sedentary time was inversely, and standing, steps, and VO_{2max} (ml/kg/min) positively associated with insulin sensitivity markers (whole-body GU, HOMA-IR, fasting insulin) (Table 2). Breaks in sedentary time associated only with whole-body GU, while LPA, MVPA and total PA did not associate with any of the insulin sensitivity markers. Fitness did not associate with any markers either when scaled to FFM instead of body weight. When whole-body GU was scaled to FFM instead of body weight, however, the results were unchanged (data not shown). None of the activity and fitness outcomes associated with fasting glucose or HbA_{1c} (data not shown).

Adding body fat-% to the model turned all associations with whole-body GU non-significant (Table 2). Except for standing, all other associations of activity and fitness outcomes with HOMA-IR and fasting insulin also turned non-significant. The body fat-%-adjusted association between standing and whole-body GU was nearly statistically significant as well ($p = 0.08$) and remained significant when adjusted for weight or BMI instead of body fat-% (data not shown). Additionally, both whole-body GU and HOMA-IR were better with more standing time, as illustrated by the differences in both insulin sensitivity markers between the tertiles of standing time (Figure 5).

►**Table 2.** Associations of sedentary time, physical activity, and cardiorespiratory fitness with insulin sensitivity markers. GU, glucose uptake; HOMA-IR, homeostatic model assessment of insulin resistance; LPA, light-intensity physical activity; MVPA, moderate-to-vigorous physical activity; PA, physical activity; VO_{2max}, maximal oxygen uptake; FFM, fat-free mass. [a] = log10 transformed. Values are β-coefficients with 95 % confidence intervals. Model 1 adjusted for sex, age, and accelerometer wear time. Model 2 adjusted for sex, age, body fat-%, and accelerometer wear time. * $p < 0.05$, statistical significance. Modified from Original publication I.

	Whole-body GU (mg/kg/min) [a]				HOMA-IR [a]				Fasting insulin (pmol/L) [a]			
	Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
	β	p	β	p	β	p	β	p	β	p	β	p
Sedentary time, h/day	-0.38 (-0.67, -0.10)	0.01*	-0.17 (-0.42, 0.08)	0.18	0.42 (0.14, 0.70)	0.004*	0.21 (-0.03, 0.46)	0.09	0.43 (0.15, 0.72)	0.003*	0.22 (-0.03, 0.47)	0.08
Standing, h/day	0.40 (0.13, 0.67)	0.004*	0.21 (-0.02, 0.45)	0.08	-0.55 (-0.79, -0.30)	<0.001*	-0.38 (-0.60, -0.17)	<0.001*	-0.54 (-0.79, -0.29)	<0.001*	-0.37 (-0.59, -0.15)	0.001*
LPA, h/day	0.06 (-0.22, 0.34)	0.67	0.05 (-0.17, 0.27)	0.64	0.00 (-0.27, 0.27)	0.99	0.01 (-0.21, 0.23)	0.93	-0.03 (-0.31, 0.24)	0.82	-0.02 (-0.24, 0.20)	0.86
MVPA, h/day	0.22 (-0.04, 0.48)	0.10	0.00 (-0.23, 0.23)	0.99	-0.14 (-0.41, 0.12)	0.26	0.08 (-0.14, 0.31)	0.46	-0.15 (-0.41, 0.12)	0.27	0.09 (-0.14, 0.31)	0.45
Total PA, h/day	0.16 (-0.11, 0.43)	0.24	0.04 (-0.18, 0.26)	0.74	-0.08 (-0.34, 0.19)	0.57	0.05 (-0.17, 0.27)	0.66	-0.10 (-0.37, 0.17)	0.46	0.03 (-0.19, 0.25)	0.80
Steps/day	0.35 (0.11, 0.59)	0.01*	0.11 (-0.12, 0.34)	0.35	-0.25 (-0.50, -0.00)	0.047*	0.01 (-0.22, 0.23)	0.96	-0.25 (-0.50, -0.00)	0.049*	0.01 (-0.21, 0.24)	0.90
Breaks in sedentary time/day	0.28 (0.02, 0.54)	0.04*	0.13 (-0.10, 0.35)	0.26	-0.21 (-0.47, 0.06)	0.12	-0.05 (-0.27, 0.18)	0.67	-0.22 (-0.48, 0.05)	0.11	-0.05 (-0.28, 0.17)	0.63
VO_{2max}, ml/kg/min	0.61 (0.35, 0.87)	<0.001*	0.28 (-0.02, 0.58)	0.06	-0.45 (-0.72, -0.18)	0.002*	-0.05 (-0.35, 0.25)	0.74	-0.45 (-0.73, -0.18)	0.002*	-0.03 (-0.33, 0.27)	0.83
VO_{2max}, $ml/kg_{FFM}/min$	0.24 (-0.05, 0.52)	0.10	0.22 (-0.01, 0.44)	0.06	-0.06 (-0.34, 0.23)	0.70	-0.03 (-0.26, 0.19)	0.77	-0.05 (-0.34, 0.24)	0.72	-0.03 (-0.25, 0.20)	0.80

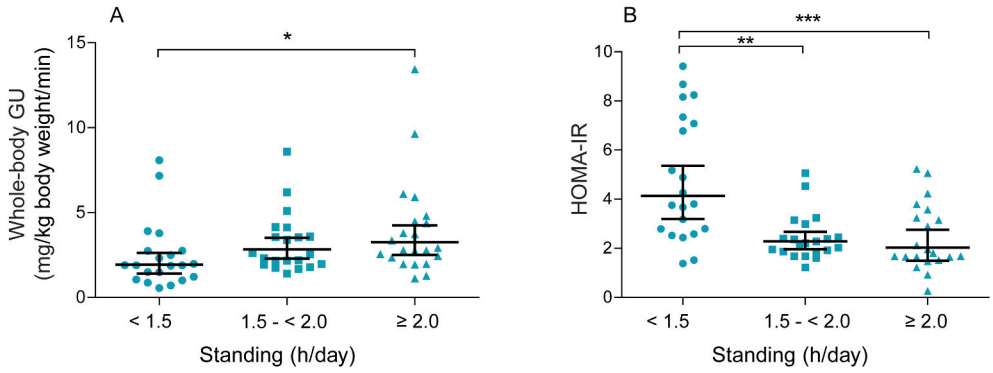

Figure 5. Differences in **A)** whole-body glucose uptake and **B)** HOMA-IR between tertiles of standing time (h/day). Symbols represent individual participants and black lines with error bars indicate geometric means backtransformed from log10-scale with 95 % confidence intervals. GU, glucose uptake; HOMA-IR, homeostatic model assessment of insulin resistance. * = $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ between groups. Modified from Original publication I.

The associations of standing with HOMA-IR and fasting insulin remained significant after further adjustment for sedentary time, total PA, and fitness level (Table 3).

Table 3. Associations of standing with insulin sensitivity markers when adjusted for sedentary time, physical activity, and fitness.

	Whole-body GU (mg/kg/min) [a]		**HOMA-IR [a]**		**Fasting insulin (pmol/L) [a]**	
	β	p	β	p	β	p
Standing (h/day) adjusted for:						
Sex, age, body fat-%, wear time, sedentary time (h/day)	0.20 (-0.14, 0.53)	0.24	− 0.47 (-0.78, -0.17)	0.003*	− 0.44 (-0.75, -0.13)	0.01*
Sex, age, body fat-%, wear time, total PA (h/day)	0.21 (-0.03, 0.45)	0.08	− 0.39 (-0.61, -0.17)	<0.001*	− 0.37 (-0.60, -0.15)	0.001*
Sex, age, body fat-%, wear time, VO_{2max} (ml/kg/min)	0.22 (-0.03, 0.46)	0.09	− 0.35 (-0.59, -0.12)	0.005*	− 0.33 (-0.56, -0.09)	0.01*
Sex, age, body fat-%, sedentary time (h/day), total PA (h/day), VO_{2max} (ml/kg/min)	0.27 (-0.02, 0.55)	0.07	− 0.35 (-0.62, -0.07)	0.01*	− 0.29 (-0.57, -0.02)	0.04*

GU, glucose uptake; HOMA-IR, homeostatic model assessment of insulin resistance; PA, physical activity; VO_{2max}, maximal oxygen uptake. [a] = log10 transformed. Values are β-coefficients with 95 % confidence intervals. * $p < 0.05$, statistical significance. Modified from Original publication I.

When the interaction between sex and each activity outcome was included in the models in additional analyses, the associations of standing with HOMA-IR and fasting insulin appeared to be stronger in men compared to women (men: $\beta = -0.753$ and -0.763, respectively; women: $\beta = -0.401$ and -0.377; $p < 0.05$), but no other sex differences were found.

5.2.2 Associations with muscle insulin sensitivity

Skeletal muscle insulin sensitivity was assessed with PET imaging in a subsample of 44 participants (Study II). In this subsample, the median GU with first (Q1) and third quartiles (Q3) in quadriceps femoris was 1.9 (Q1 1.3, Q3 2.9) mg/kg tissue/min, in hamstrings 3.0 (Q1 1.5, Q3 4.6) mg/kg tissue/min, and in the whole body 2.6 (Q1 1.9, Q3 3.9) mg/kg body weight/min.

Muscle insulin sensitivity correlated strongly with whole-body insulin sensitivity (quadriceps femoris $r = 0.85$ and hamstrings $r = 0.91$, $p < 0.001$ for both). Sedentary time associated detrimentally and standing and steps beneficially with hamstring and whole-body insulin sensitivity in the sex-, age- and accelerometer wear time-adjusted model (Table 4). Breaks in sedentary time associated positively with whole-body insulin sensitivity. Higher fitness level associated with better insulin sensitivity in quadriceps, hamstrings, and the whole body when adjusted for sex and age. The associations were not different between men and women (data not shown). Additional adjustment for body fat-% turned all above-mentioned associations non-significant (Table 4).

Both hamstring and whole-body insulin sensitivity were better with sedentary time < 10.5 h/day in comparison to more sedentary time (Figure 6). Similar to the total sample in Study I, whole-body insulin sensitivity was also better with standing time over 2.0 h/day compared to less than 1.5 h/day, but there was no difference in muscle insulin sensitivity between the tertiles of standing time. The difference in hamstring insulin sensitivity between standing time < 1.5 h/day and ≥ 2.0 h/day was only marginally non-significant, however (p=0.053) (Figure 7).

►**Table 4.** Associations of sedentary time, physical activity, and cardiorespiratory fitness with skeletal muscle and whole-body insulin sensitivity. GU, glucose uptake; LPA, light-intensity physical activity; MVPA, moderate-to-vigorous physical activity; PA, physical activity; VO_{2max}, maximal oxygen uptake; FFM, fat-free mass. [a] = log10 transformed. Values are β-coefficients with 95 % confidence intervals. Model 1 adjusted for sex, age, and accelerometer wear time. Model 2 adjusted for sex, age, body fat-%, and accelerometer wear time. * $p < 0.05$, statistical significance. Modified from Original publication II.

	Quadriceps femoris GU (mg/kg tissue/min) [a]				Hamstrings GU (mg/kg tissue/min) [a]				Whole-body GU (mg/kg body weight/min) [a]			
	Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
	β	p	β	p	β	p	β	p	β	p	β	p
Sedentary time, h/day	−0.27 (−0.63, 0.08)	0.13	−0.11 (−0.45, 0.23)	0.52	−0.37 (−0.73, −0.02)	0.04*	−0.20 (−0.53, 0.13)	0.23	−0.36 (−0.71, −0.01)	0.04*	−0.18 (−0.50, 0.14)	0.26
Standing, h/day	0.30 (−0.06, 0.65)	0.10	0.16 (−0.18, 0.49)	0.35	0.38 (0.02, 0.73)	0.04*	0.22 (−0.11, 0.55)	0.18	0.41 (0.06, 0.75)	0.02*	0.25 (−0.05, 0.56)	0.10
LPA, h/day	0.04 (−0.32, 0.41)	0.81	0.02 (−0.31, 0.34)	0.92	0.12 (−0.25, 0.49)	0.52	0.09 (−0.23, 0.41)	0.58	0.09 (−0.27, 0.44)	0.63	0.06 (−0.23, 0.36)	0.67
MVPA, h/day	0.21 (−0.11, 0.54)	0.19	0.03 (−0.29, 0.35)	0.84	0.27 (−0.06, 0.59)	0.11	0.07 (−0.25, 0.38)	0.68	0.22 (−0.10, 0.54)	0.17	0.00 (−0.30, 0.30)	0.99
Total PA, h/day	0.16 (−0.19, 0.50)	0.36	0.03 (−0.29, 0.35)	0.85	0.24 (−0.10, 0.59)	0.16	0.10 (−0.21, 0.42)	0.51	0.20 (−0.14, 0.53)	0.25	0.05 (−0.25, 0.34)	0.74
Steps/day	0.29 (−0.03, 0.61)	0.08	0.09 (−0.24, 0.41)	0.59	0.35 (0.03, 0.67)	0.03*	0.13 (−0.19, 0.46)	0.41	0.34 (0.03, 0.65)	0.03*	0.11 (−0.20, 0.41)	0.48
Breaks in sedentary time/day	0.21 (−0.16, 0.58)	0.26	0.10 (−0.24, 0.44)	0.55	0.35 (−0.01, 0.72)	0.06	0.23 (−0.09, 0.56)	0.16	0.36 (0.01, 0.72)	0.04*	0.24 (−0.06, 0.55)	0.12
VO_{2max}, ml/kg/min	0.57 (0.22, 0.92)	0.002*	0.27 (−0.19, 0.72)	0.25	0.60 (0.26, 0.94)	0.001*	0.29 (−0.16, 0.73)	0.20	0.61 (0.30, 0.93)	<0.001*	0.33 (−0.12, 0.79)	0.15
VO_{2max}, ml/kg_{FFM}/min	0.24 (−0.14, 0.61)	0.21	0.21 (−0.12, 0.53)	0.20	0.25 (−0.12, 0.63)	0.18	0.22 (−0.10, 0.54)	0.16	0.36 (−0.02, 0.73)	0.06	0.24 (−0.08, 0.57)	0.14

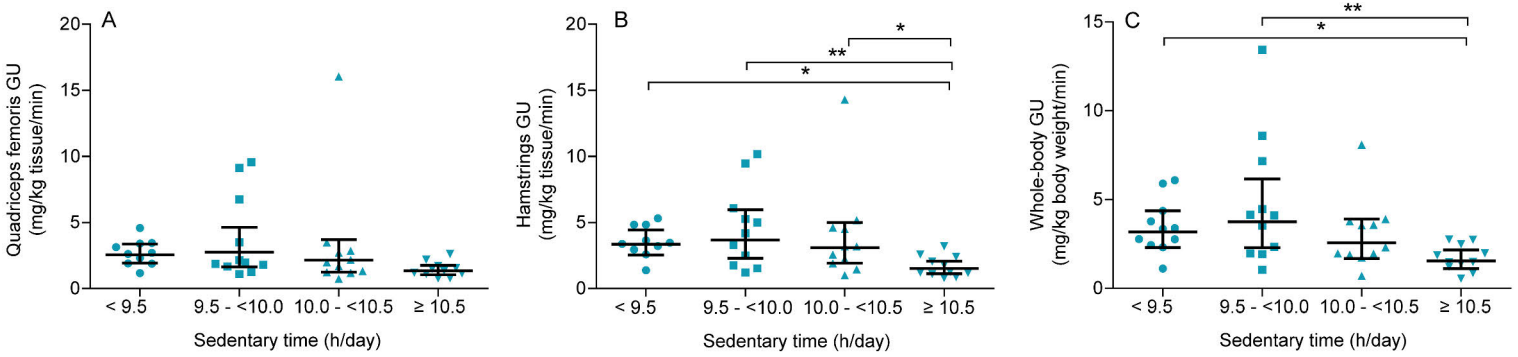

Figure 6. Differences in **A)** quadriceps femoris glucose uptake, **B)** hamstrings glucose uptake, and **C)** whole-body glucose uptake between quartiles of sedentary time (h/day). Symbols represent individual participants and black lines with error bars indicate geometric means backtransformed from log10-scale with 95 % confidence intervals. GU, glucose uptake. * $p < 0.05$, ** $p < 0.01$ between groups. Modified from Original publication II.

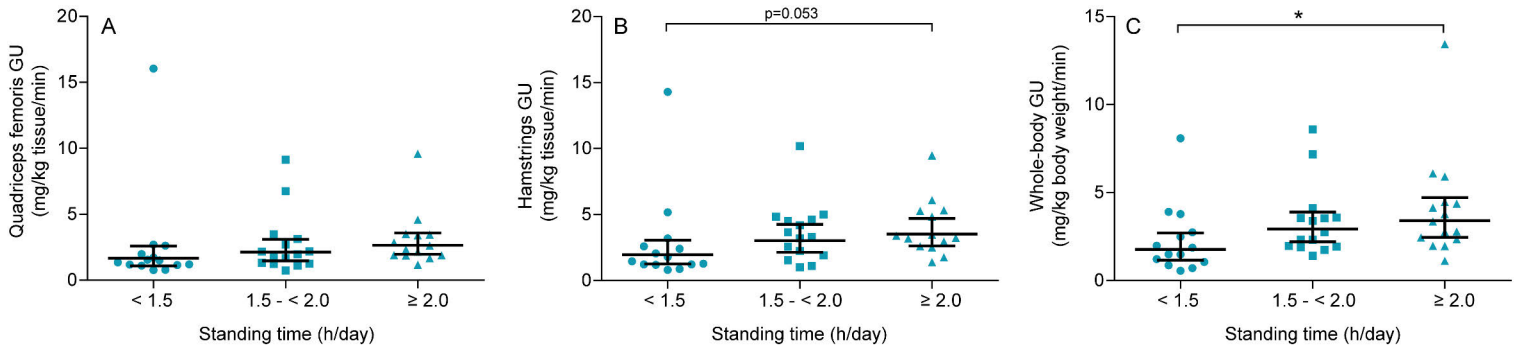

Figure 7. Differences in **A)** quadriceps femoris glucose uptake, **B)** hamstrings glucose uptake, and **C)** whole-body glucose uptake between tertiles of standing time (h/day). Symbols represent individual participants and black lines with error bars indicate geometric means backtransformed from log10-scale with 95 % confidence intervals. GU, glucose uptake. * $p < 0.05$ between groups. Modified from Original publication II.

5.2.3 Associations with metabolic flexibility

Study III indicated impaired MetFlex by a low capacity for fasting fat oxidation (low FATox rate and high fasting RER) and a blunted shift in substrate use in response to insulin stimulation by HEC. The median fasting RER was 0.91 (Q1 0.85, Q3 0.98), and the mean FATox rate was 0.4 (SD 0.4) mg/kg/min. CHO was the predominant substrate already in the fasting state, thus the changes in RER and substrate oxidation from fasting to insulin stimulation were not statistically significant ($p > 0.05$) despite a slight further increase in CHOox [2.5 (SD 0.9) to 2.6 (SD 1.1) mg/kg/min] (Table 5). The total insulin-stimulated CHOox was calculated to consist of 76 % of exogenous glucose disposal and 24 % from other CHO sources, and CHOox represented the majority of insulin-stimulated glucose disposal, as the calculated estimate of non-oxidative glucose disposal was 0.6 (SD 1.3) mg/kg/min. FFA decreased by 0.48 (SD 0.20) mmol/L and plasma lactate increased by 0.18 (SD 0.30) mmol/L on average during HEC ($p < 0.001$ for both) (Table 5).

Table 5. Metabolic outcomes during fasting and insulin stimulation.

	Fasting	HEC
Insulin, pmol/L	69.5 (48.6, 104.2)	503.7 (100.9)***
Glucose, mmol/L	5.9 (0.4)	5.3 (0.3)***
Free fatty acids, mmol/L	0.60 (0.20)	0.12 (0.06, 0.18)***
Lactate, mmol/L	1.0 (0.8, 1.3)	1.2 (1.1, 1.4)***
VO_2, mL/min	240 (44)	253 (44)***
VCO_2, mL/min	215 (198, 240)	224 (204, 253)***
EE, kcal/day	1697 (297)	1788 (300)***
CHO oxidation, mg/kg/min	2.5 (0.9)	2.6 (1.1)
Fat oxidation, mg/kg/min	0.4 (0.4)	0.4 (0.4)
RER	0.91 (0.85, 0.98)	0.92 (0.10)
ΔRER (HEC – fasting)	0.00 (− 0.04, 0.03)	

HEC, hyperinsulinemic euglycemic clamp; VO_2, oxygen uptake; VCO_2, carbon dioxide production; EE, energy expenditure; CHO, carbohydrate; RER, respiratory exchange ratio. Values are means with standard deviation (SD) or medians with first and third quartiles (Q1, Q3). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ between fasting and insulin stimulation (paired t-test or Wilcoxon signed rank test). Modified from Original publication III.

During exercise, the mean RER increased from 0.74 (SD 0.05) at low intensity (25 W) to 1.12 (SD 0.06) at maximal intensity (mean 135 W) ($p < 0.001$) (Table 6).

The incremental increase in RER with increasing exercise loads is illustrated in Figure 8. At low-intensity exercise fat was the primary substrate [2.4 (SD 1.0) mg/kg/min vs. CHO 2.2 (SD 1.5) mg/kg/min]. At maximal intensity FATox was entirely suppressed (negative values were interpreted as zero) and CHOox increased to 39.2 (SD 8.9) mg/kg/min ($p < 0.05$ for both) (Table 6).

Table 6. Metabolic outcomes during exercise.

	Low-intensity	Maximal intensity
Power output, W	25	135 (103, 151)***
VO_2, mL/min	601 (140)	2127 (482)***
VCO_2, mL/min	471 (110)	2345 (524)***
EE, kcal/min	2.9 (0.7)	10.8 (2.4)***
CHO oxidation, mg/kg/min	2.2 (1.5)	39.2 (8.9)***
Fat oxidation, mg/kg/min	2.4 (1.0)	− 4.2 (2.2)***
RER	0.74 (0.05)	1.12 (0.06)***
ΔRER (Maximal – low-intensity exercise)	0.39 (0.07)	
Delta exercise efficiency, %	16.7 (2.5)	

VO_2, oxygen uptake; VCO_2, carbon dioxide production; EE, energy expenditure; CHO, carbohydrate; RER, respiratory exchange ratio. Values are means with standard deviation (SD). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ between low-intensity and maximal exercise (paired t-test). Modified from Original publication III.

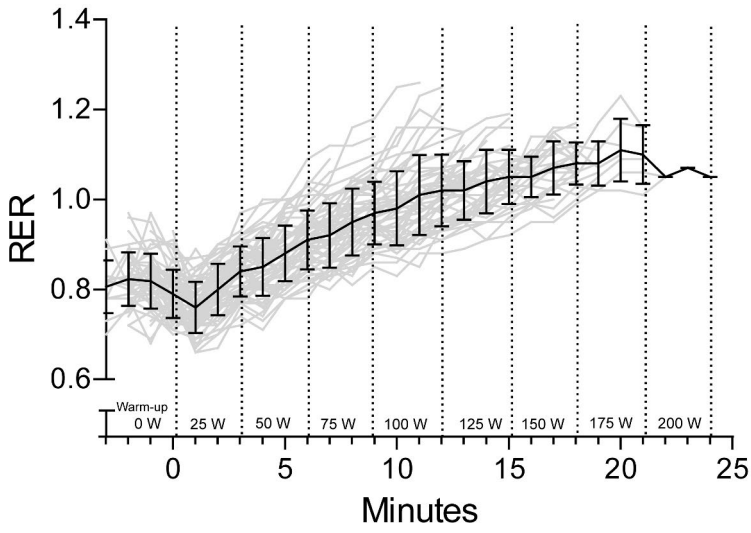

Figure 8. Respiratory exchange ratio at incrementally increasing exercise loads during a graded maximal cycle ergometer test. Gray lines represent individual participants and black line with error bars indicates the mean with standard deviation. RER, respiratory exchange ratio. From Original publication III.

Sedentary time associated positively with fasting RER and inversely with MetFlex (ΔRER) from fasting to insulin stimulation (Table 7). Additionally, MetFlex was better with sedentary time ≤ 10.0 h/day compared to > 10.0 h/day [ΔRER + 0.01 (95 % CI: − 0.02, 0.04) vs. – 0.03 (95 % CI: – 0.05, 0.00)], respectively, p = 0.04] (Figure 9). Standing time associated negatively with fasting RER, and both LPA and total PA associated positively with insulin-stimulated MetFlex (Table 7).

Table 7. Associations of sedentary time, physical activity, and cardiorespiratory fitness with metabolic flexibility from fasting to insulin stimulation.

	Fasting RER		**Insulin-stimulated RER**		**ΔRER HEC**	
	β	p	β	p	β	p
Sedentary time, h/day	0.35 (0.04, 0.67)	0.03*	−0.09 (−0.40, 0.23)	0.59	−0.41 (−0.72, −0.09)	0.01*
Standing, h/day	−0.32 (−0.62, −0.02)	0.04*	−0.04 (−0.34, 0.25)	0.77	0.21 (−0.10, 0.52)	0.18
LPA, h/day	−0.09 (−0.38, 0.20)	0.53	0.15 (−0.14, 0.43)	0.31	0. 33 (0.05, 0.61)	0.02*
MVPA, h/day	−0.21 (−0.48, 0.06)	0.13	0.09 (−0.19, 0.36)	0.53	0.18 (−0.09, 0.46)	0.19
Total PA, h/day	−0.17 (−0.45, 0.11)	0.23	0.15 (−0.13, 0.42)	0.30	0.33 (0.05, 0.60)	0.02*
Steps/day	−0.24 (−0.50, 0.02)	0.07	0.09 (−0.18, 0.36)	0.51	0.20 (−0.06, 0.47)	0.13
Breaks in sedentary time/day	−0.14 (−0.42, 0.15)	0.34	−0.08 (−0.36, 0.21)	0.59	0.06 (−0.24, 0.35)	0.71
VO_{2max}, ml/kg/min	0.07 (−0.23, 0.38)	0.63	0.28 (−0.03, 0.59)	0.07	0.05 (−0.27, 0.37)	0.74
VO_{2max}, ml/kg_{FFM}/min	0.08 (-0.22, 0.38)	0.61	0.13 (-0.17, 0.44)	0.38	0.00 (-0.32, 0.31)	0.98

RER, respiratory exchange ratio, HEC, hyperinsulinemic euglycemic clamp; LPA, light-intensity physical activity; MVPA, moderate-to-vigorous physical activity; PA, physical activity; VO_{2max}, maximal oxygen uptake; FFM, fat-free mass. Values are β-coefficients with 95 % confidence intervals. Model adjusted for sex, age, and accelerometer wear time. * p < 0.05, statistical significance. From Original publication III.

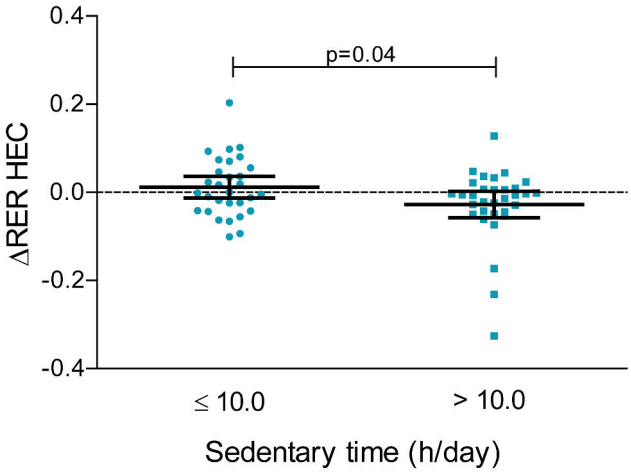

Figure 9. Difference in insulin-stimulated metabolic flexibility between participants with sedentary time ≤ 10.0 h/day and > 10.0 h/day. Symbols represent individual participants and black lines with error bars indicate means with 95 % confidence intervals. RER, respiratory exchange ratio; HEC, hyperinsulinemic euglycemic clamp. Modified from Original publication III.

Sedentary time also associated with lower fasting FATox, while higher standing time and number of daily steps associated with higher fasting FATox (Table 8). Figure 10 illustrates the scatterplots. When adjusted for total PA, the associations of sedentary time and standing with fasting RER remained statistically significant ($p < 0.05$). The association of sedentary time with ΔRER, as well as the associations of sedentary time, standing, and steps with fasting FATox, turned non-significant ($p > 0.05$).

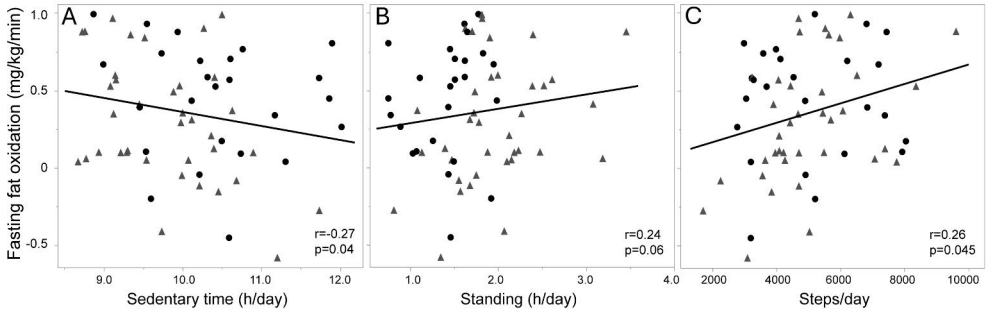

Figure 10. Correlations between fasting fat oxidation and **A)** sedentary time, **B)** standing time, and **C)** daily steps. Correlation coefficients are adjusted for sex; solid black circles represent men, and gray triangles women. Modified from Original Publication III.

Table 8. Associations of sedentary time, physical activity, and cardiorespiratory fitness with fasting and insulin-stimulated substrate oxidation.

	Fasting CHOox (mg/kg/min)		**Fasting FATox (mg/kg/min)**		**HEC CHOox (mg/kg/min)**		**HEC FATox (mg/kg/min)**	
	β	p	β	p	β	p	β	p
Sedentary time, h/day	0.24 (−0.08, 0.57)	0.14	−0.36 (−0.67, −0.04)	0.03*	-0.21 (−0.52, 0.10)	0.19	0.04 (−0.28, 0.35)	0.81
Standing, h/day	−0.29 (−0.59, 0.02)	0.06	0.31 (0.01, 0.61)	0.04*	0.04 (−0.26, 0.33)	0.82	0.06 (−0.24, 0.35)	0.71
LPA, h/day	−0.02 (−0.31, 0.28)	0.90	0.10 (−0.19, 0.39)	0.49	0.18 (−0.10, 0.46)	0.20	−0.11 (−0.39, 0.17)	0.45
MVPA, h/day	−0.10 (−0.38, 0.18)	0.50	0.22 (−0.05, 0.49)	0.11	0.21 (−0.06, 0.48)	0.13	−0.04 (−0.31, 0.24)	0.80
Total PA, h/day	−0.06 (−0.35, 0.23)	0.67	0.18 (−0.10, 0.47)	0.20	0.24 (−0.04, 0.51)	0.09	−0.09 (−0.37, 0.19)	0.51
Steps/day	−0.12 (−0.39, 0.16)	0.39	0.26 (0.00, 0.53)	0.047*	0.22 (−0.04, 0.49)	0.10	−0.03 (−0.29, 0.24)	0.85
Breaks in sedentary time/day	−0.09 (−0.38, 0.20)	0.55	0.17 (−0.12, 0.45)	0.24	0.02 (−0.26, 0.30)	0.88	0.11 (−0.17, 0.39)	0.42
VO_{2max}, ml/kg/min	0.19 (−0.12, 0.50)	0.22	−0.02 (−0.33, 0.28)	0.88	0.43 (0.13, 0.73)	0.01*	−0.24 (−0.55, 0.07)	0.12
VO_{2max}, ml/kg_{FFM}/min	0.08 (-0.23, 0.38)	0.62	-0.04 (-0.34, 0.26)	0.79	0.16 (-0.14, 0.47)	0.29	-0.13 (-0.43, 0.18)	0.41

CHOox, carbohydrate oxidation; FATox, fat oxidation; HEC, hyperinsulinemic euglycemic clamp; LPA, light-intensity physical activity; MVPA, moderate-to-vigorous physical activity; PA, physical activity; VO_{2max}, maximal oxygen uptake; FFM, fat-free mass. Values are β-coefficients with 95 % confidence intervals. Model adjusted for sex, age, and accelerometer wear time. * $p < 0.05$, statistical significance. From Original publication III.

Sedentary time nor PA associated with MetFlex during exercise, but higher cardiorespiratory fitness (VO_{2max}, ml/kg/min) associated with higher CHOox during HEC (Table 8), lower RER at low-intensity exercise (Table 9), and higher CHOox during maximal exercise (Table 10). When VO_{2max} was expressed per FFM, however, the associations with insulin-stimulated CHOox and low-intensity RER turned non-significant.

Table 9. Associations of sedentary time, physical activity, and cardiorespiratory fitness with metabolic flexibility from low-intensity to maximal exercise.

	Low-intensity exercise RER		Maximal exercise RER		ΔRER Exercise	
	β	p	β	p	β	p
Sedentary time, h/day	0.11 (−0.20, 0.42)	0.47	−0.01 (−0.34, 0.32)	0.94	−0.07 (−0.39, 0.24)	0.64
Standing, h/day	0.02 (−0.28, 0.31)	0.92	−0.08 (−0.40, 0.24)	0.61	−0.06 (−0.37, 0.25)	0.71
LPA, h/day	−0.06 (−0.35, 0.23)	0.67	0.20 (−0.11, 0.51)	0.20	0. 17 (−0.13, 0.47)	0.27
MVPA, h/day	−0.22 (−0.50, 0.05)	0.11	−0.09 (−0.38, 0.21)	0.57	0.07 (−0.22, 0.36)	0.64
Total PA, h/day	−0.16 (−0.44, 0.12)	0.26	0.09 (−0.22, 0.39)	0.57	0.15 (−0.14, 0.44)	0.31
Steps/day	−0.20 (−0.47, 0.07)	0.14	−0.11 (−0.40, 0.18)	0.44	0.03 (−0.25, 0.32)	0.75
Breaks in sedentary time/day	0.06 (−0.23, 0.34)	0.70	0.04 (−0.26, 0.35)	0.79	−0.03 (−0.33, 0.27)	0.85
VO_{2max}, ml/kg/min	−0.34 (−0.65, −0.04)	0.03*	−0.05 (−0.37, 0.27)	0.75	0.19 (−0.12, 0.49)	0.23
VO_{2max}, $ml/kg_{FFM}/min$	-0.24 (-0.54, 0.06)	0.11	0.10 (-0.20, 0.41)	0.50	0.25 (-0.04, 0.54)	0.09

RER, respiratory exchange ratio, LPA, light-intensity physical activity; MVPA, moderate-to-vigorous physical activity; PA, physical activity; VO_{2max}, maximal oxygen uptake; FFM, fat-free mass. Values are β-coefficients with 95 % confidence intervals. Model adjusted for sex, age, and accelerometer wear time. * $p < 0.05$, statistical significance. From Original publication III.

Sedentary time, LPA, MVPA, total PA, and steps all associated with CHOox at maximal exercise intensity, and sedentary time, MVPA, total PA additionally with FATox at low-intensity exercise (Table 10). Cardiorespiratory fitness was a more important determinant of CHOox at maximal exercise, however, as the associations between activity outcomes and CHOox turned non-significant when VO_{2max} was included as a covariate (due to a strong relationship with CHOox during maximal exercise) (data not shown). The mean exercise efficiency at moderate-intensity exercise was 16.7 (SD 2.5 %), and sedentary time associated inversely with exercise efficiency [β = − 0.31 (95 % CI: −0.60, − 0.02), p = 0.03]. Higher efficiency correlated with better insulin-stimulated MetFlex, although marginally non-significantly (r = 0.26, p = 0.051).

Table 10. Associations of sedentary time, physical activity, and cardiorespiratory fitness with substrate oxidation during exercise.

	Low-intensity exercise CHOox (mg/kg/min		Low-intensity exercise FATox (mg/kg/min)		Maximal exercise CHOox (mg/kg/min)		Maximal exercise FATox (mg/kg/min)	
	β	p	β	p	β	p	β	p
Sedentary time, h/day	0.09 (−0.19, 0.38)	0.52	−0.32 (−0.61, −0.03)	0.03*	−0.31 (−0.60, −0.03)	0.03*	0.12 (−0.21, 0.44)	0.48
Standing, h/day	0.02 (−0.26, 0.30)	0.88	0.11 (−0.18, 0.40)	0.44	0.07 (−0.22, 0.36)	0.64	0.09 (−0.23, 0.41)	0.59
LPA, h/day	−0.13 (−0.40, 0.14)	0.33	0.26 (−0.01, 0.53)	0.06	0.31 (0.03, 0.58)	0.03*	−0.29 (−0.59, 0.02)	0.06
MVPA, h/day	−0.10 (−0.36, 0.16)	0.46	0.27 (0.01, 0.53)	0.04*	0.29 (0.03, 0.55)	0.03*	−0.07 (−0.37, 0.23)	0.64
Total PA, h/day	−0.14 (−0.40, 0.12)	0.28	0.32 (0.07, 0.58)	0.02*	0.36 (0.10, 0.62)	0.01*	−0.23 (−0.53, 0.07)	0.13
Steps/day	−0.04 (−0.29, 0.21)	0.75	0.22 (−0.03, 0.48)	0.09	0.29 (0.04, 0.55)	0.03*	−0.03 (−0.32, 0.26)	0.83
Breaks in sedentary time/day	0.00 (−0.26, 0.26)	0.99	0.13 (−0.14, 0.40)	0.34	0.23 (−0.04, 0.51)	0.09	−0.13 (−0.44, 0.17)	0.39
VO_{2max}, ml/kg/min	−0.09 (−0.38, 0.19)	0.51	0.22 (−0.07, 0.52)	0.13	0.81 (0.62, 1.00)	<0.001*	−0.27 (−0.59, 0.04)	0.08
VO_{2max}, $ml/kg_{FFM}/min$	-0.08 (-0.35, 0.20)	0.58	0.05 (-0.24, 0.33)	0.73	0.69 (0.48, 0.90)	<0.001*	-0.35 (-0.64, -0.05)	0.02*

CHOox, carbohydrate oxidation; FATox, fat oxidation; LPA, light-intensity physical activity; MVPA, moderate-to-vigorous physical activity; PA, physical activity; VO_{2max}, maximal oxygen uptake; FFM, fat-free mass. Values are β-coefficients with 95 % confidence intervals. Model adjusted for sex, age, and accelerometer wear time. * $p < 0.05$, statistical significance. From Original publication III.

In additional analyses, no sex differences in the associations between metabolic and PA outcomes were seen when the analyses were repeated with the interaction term between sex and each activity outcome included in the model (data not shown). Additional adjustments for the use of statins (n = 14) and blood pressure medication (n = 34) did not change the main results and conclusions either (data not shown).

Correlations between MetFlex and metabolic variables

Insulin- and exercise-stimulated MetFlex did not correlate with each other ($p > 0.05$). Better insulin-stimulated MetFlex did, however, correlate with higher FATox and lower CHOox both in a fasting state ($r = 0.40$ and $r = -0.33$, respectively) and during low-intensity exercise ($r = 0.29$ and $r = -0.34$). Insulin-stimulated MetFlex also correlated with FATox and CHOox during HEC ($r = -0.40$ and $r = 0.46$), and exercise-stimulated MetFlex correlated with FATox and CHOox at maximal exercise intensity ($r = -0.68$ and $r = 0.51$) ($p < 0.05$ for all).

MetFlex and substrate oxidation variables did not correlate with fasting glucose or FFA levels, but they did correlate with insulin sensitivity markers. Whole-body GU correlated with CHOox at maximal exercise intensity ($r = 0.33$), and HOMA-IR and fasting insulin both correlated with insulin-stimulated CHOox ($r = -0.26$ for both). Fasting insulin also correlated with FATox at low-intensity exercise ($r = -0.28$). Triglyceride/HDL ratio correlated with fasting RER ($r = 0.29$), as well as fasting CHO and fat oxidation ($r = 0.34$ and $r = -0.32$, respectively) ($p < 0.05$ for all).

Substrate oxidation and insulin sensitivity were related to non-oxidative glucose disposal as well. Higher non-oxidative glucose disposal correlated with better whole-body GU ($r = 0.61$), lower fasting and insulin-stimulated CHOox ($r = -0.29$ and $r = -0.26$, respectively), and higher fasting and insulin-stimulated FATox ($r = 0.31$ and $r = 0.41$). Non-oxidative glucose disposal also correlated with standing time ($r = 0.30$) ($p < 0.05$ for all).

MetFlex and substrate oxidation also correlated with plasma lactate levels. Fasting lactate correlated inversely with insulin-stimulated MetFlex and fasting FATox ($r = -0.35$ and $r = -0.36$), and positively with fasting RER and CHOox ($r = 0.36$ and $r = 0.43$). Additionally, lower fasting lactate correlated with lower sedentary time ($r = 0.35$) and higher standing time ($r = -0.40$). Greater insulin-stimulated increases in lactate correlated with greater FFA suppression ($r = 0.35$), and both correlated with higher standing time ($r = 0.47$ and $r = 0.28$) ($p < 0.05$ for all). Lower fasting lactate, and greater insulin-stimulated changes in lactate and FFA also correlated with better whole-body insulin sensitivity and lower adiposity (data not shown).

Habitual food intake did not appear to be a key determinant of MetFlex in this study, as neither FQ, nor the intake of total energy and primary energy substrates CHO and fats correlated with MetFlex outcomes.

5.2.4 Secondary outcomes

The associations of activity and fitness outcomes, whole-body insulin sensitivity (Study I), muscle insulin sensitivity (Study II), and energy metabolism outcomes

(Study III) with adiposity, lipid profile, and dietary intake were also examined to investigate the possible determinants of metabolic outcomes.

Sedentary time correlated positively and MVPA, steps, breaks in sedentary time, and VO_{2max} (ml/kg/min) inversely with weight, BMI, body fat-%, and waist circumference. Total PA correlated inversely with weight and waist circumference and standing correlated inversely with body fat-% (Table 11). Better whole-body and muscle insulin sensitivity also correlated with lower weight and adiposity. Neither MetFlex nor fasting substrate oxidation correlated with any adiposity measures (Table 11), but CHOox during insulin stimulation and maximal exercise correlated inversely with weight and adiposity (data not shown).

Table 11. Correlations of activity and fitness outcomes, insulin sensitivity, and energy metabolism outcomes with adiposity measures.

	Weight (kg)	BMI (kg/m^2)	Body fat-%	Waist circumference (cm)
Physical activity and fitness				
Sedentary time	0.39**	0.32*	0.34**	0.35**
Standing	-0.23	-0.24	-0.31*	-0.24
LPA	-0.21	-0.09	-0.05	-0.11
MVPA	-0.42**	-0.35**	-0.35**	-0.42**
Total PA	-0.37**	-0.25	-0.22	-0.30*
Steps/day	-0.51***	-0.46***	-0.44***	-0.48***
Breaks in sedentary time/day	-0.29*	-0.36**	-0.27*	-0.32*
VO_{2max}, ml/kg/min	-0.61***	-0.64***	-0.64***	-0.61***
Insulin sensitivity				
Whole-body GU, mg/kg/min [a]	-0.54***	-0.59***	-0.61***	-0.67***
Quadriceps femoris GU, mg/kg/min [a]	-0.40*	-0.51**	-0.51**	-0.58***
Hamstrings GU, mg/kg/min [a]	-0.54***	-0.58***	-0.53***	-0.66***
Energy metabolism				
ΔRER HEC	-0.14	-0.01	-0.06	-0.10
ΔRER exercise	-0.15	-0.12	0.15	-0.14
Fasting RER [a]	0.02	-0.05	0.02	0.02
Fasting CHO oxidation, mg/kg/min	-0.13	-0.20	-0.12	-0.10
Fasting fat oxidation, mg/kg/min	-0.08	-0.02	-0.07	-0.07

◄ BMI, body mass index, LPA, light-intensity physical activity; MVPA, moderate-to-vigorous physical activity; PA, physical activity; VO_{2max}, maximal oxygen uptake; GU, glucose uptake; RER, respiratory exchange ratio; HEC, hyperinsulinemic euglycemic clamp; CHO, carbohydrate. Accelerometer outcomes as % of accelerometer wear time. [a] = log10 transformed. Sex- and age-adjusted Pearson partial correlation analysis, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Modified from Original publications I–III.

LPA correlated with HDL, but none of the other correlations between activity and lipid outcomes were statistically significant (Table 12). Better insulin sensitivity correlated with a better lipid profile, and fasting substrate metabolism correlated with fasting triglycerides (Table 12). Insulin-stimulated CHOox also correlated with fasting triglycerides, but none of the other insulin- and exercise-stimulated substrate oxidation variables correlated with lipid profile, or dietary intake (data not shown). Protein intake correlated with steps ($r = -0.27$, $p = 0.04$) and insulin-stimulated MetFlex ($r = 0.30$, $p = 0.02$), but no other statistically significant correlations were seen between the primary outcomes and dietary outcomes.

Table 12. Correlations of activity and fitness outcomes, insulin sensitivity, and energy metabolism outcomes with lipid profile.

	Free fatty acids (mmol/L)	Triglycerides (mmol/L)[a]	Total cholesterol (mmol/L)[a]	LDL (mmol/L)[a]	HDL (mmol/L)
Physical activity and fitness					
Sedentary time	0.10	0.21	-0.11	-0.08	-0.10
Standing	-0.13	-0.18	-0.02	-0.01	-0.11
LPA	-0.06	-0.20	0.19	0.10	0.32*
MVPA	0.03	0.00	0.10	0.09	0.05
Total PA	-0.02	-0.14	0.18	0.12	0.25
Steps/day	-0.05	-0.06	0.08	0.06	0.04
Breaks in sedentary time/day	-0.05	-0.10	0.24	0.24	0.18
VO_{2max}, ml/kg/min	-0.19	0.01	0.07	-0.02	0.07
Insulin sensitivity					
Whole-body GU, mg/kg/min [a]	-0.38**	-0.31*	0.12	0.01	0.26*
Quadriceps femoris GU, mg/kg/min [a]	-0.31	-0.38*	-0.11	-0.22	0.34*
Hamstrings GU, mg/kg/min [a]	-0.30	-0.41**	-0.05	-0.14	0.36*

Table 12 continued.

	Free fatty acids (mmol/L)	Triglycerides (mmol/L)[a]	Total cholesterol (mmol/L)[a]	LDL (mmol/L)[a]	HDL (mmol/L)
Energy metabolism					
ΔRER HEC	-0.05	-0.18	-0.01	0.01	0.03
ΔRER exercise	0.07	0.09	0.04	-0.04	0.10
Fasting RER [a]	-0.07	0.38**	0.20	0.16	0.03
Fasting CHO oxidation, mg/kg/min	-0.14	0.44**	0.19	0.12	0.01
Fasting fat oxidation, mg/kg/min	0.09	-0.39**	-0.19	-0.16	0.02

LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol, LPA, light-intensity physical activity; MVPA, moderate-to-vigorous physical activity; PA, physical activity; VO_{2max}, maximal oxygen uptake; GU, glucose uptake; RER, respiratory exchange ratio; HEC, hyperinsulinemic euglycemic clamp; CHO, carbohydrate. Accelerometer outcomes as % of accelerometer wear time. [a] = log10 transformed. Sex- age, and lipid-lowering medication-adjusted Pearson partial correlation analysis, * $p < 0.05$, ** $p < 0.01$. Modified from Original publications I-III.

5.3 Intervention effects (Studies IV-V)

Sixty-three participants participated in the midpoint outcome assessments at 3 months (Study IV) and 60 completed the intervention and participated in the 6-month follow-up measurements (Study V). One participant dropped out during the intervention due to low back pain and three due to personal reasons. The mean intervention duration was 171 (SD 36) days. Valid accelerometer data was successfully collected from the intervention period from 56 participants with a median duration of 117 (Q1 74, Q3 142) days and mean wear time of 14.98 (SD 0.82) h/day. The missing data is due to discontinued participation and technical issues.

5.3.1 Effects on cardiometabolic outcomes

The changes in activity behaviors and effects on cardiometabolic outcomes during the first half of the intervention period were investigated in Study IV. The intervention group reduced sedentary time from baseline to 3 months by ~ 50 (95 % CI: 24, 73) min/day primarily by increasing MVPA [24 (95 % CI: 14, 34) min/day] and LPA [19 (95 % CI: 8, 30) min/day], with no changes in the control group (group × time $p < 0.05$) (Figure 11). The change in standing was also different between groups as standing time increased in the intervention group [6 (95 % CI: − 11, 23)

min/day] and decreased in the control group [− 13 (95 % CI: − 30, 5) min/day] (group × time p = 0.04). Both groups increased steps, but the increase was greater in the intervention group compared to the control group: 3800 (95 % CI: 2685, 4195) vs. 1900 (95 % CI: 801, 3036) steps/day. Breaks in sedentary time did not change.

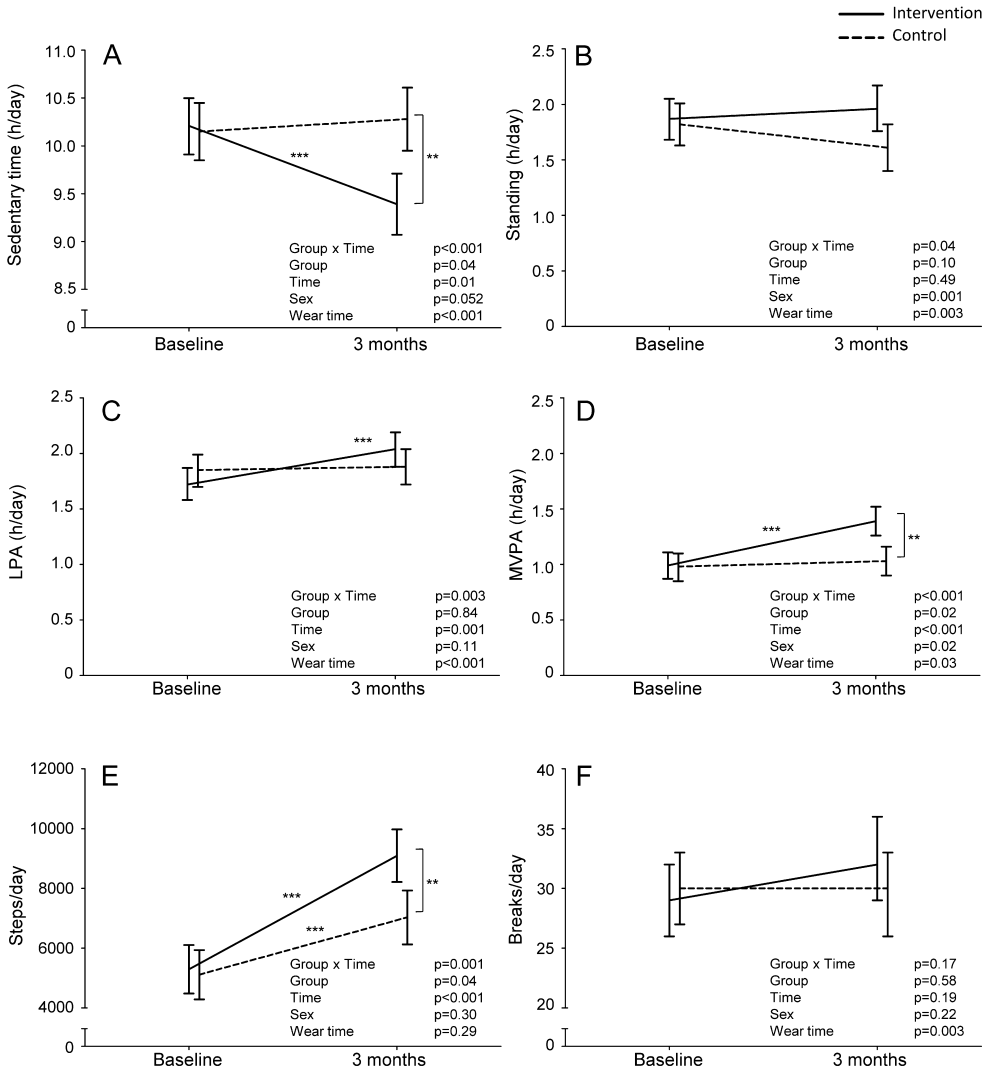

Figure 11. Intervention effects on activity outcomes in 3 months. **A)** Sedentary time, **B)** standing, **C)** LPA, light-intensity physical activity, **D)** MVPA, moderate-to-vigorous physical activity, **E)** steps/day, and **F)** breaks in sedentary time/day. Baseline indicates model-based daily means of a continuous 1-month screening accelerometer measurement with 95 % confidence intervals, and the value at 3 months indicates model-based daily means of a continuous accelerometer measurement throughout the 3-month intervention with 95 % confidence intervals. ** $p < 0.01$; *** $p < 0.001$ within or between groups (Tukey's post hoc test). Modified from Original publication IV.

Statistically significant intervention effects favoring the intervention group were seen in fasting insulin, HOMA-IR, HbA_{1c} (Figure 12), triglycerides, resting heart rate, and liver enzyme alanine aminotransferase (Table 13). The effects occurred mainly due to increases from baseline to 3 months in the control group that exceeded any changes in the intervention group.

Waist circumference, body fat-%, fat mass, and blood pressure decreased slightly during the intervention with no difference between groups (Table 13). Fasting glucose (Figure 12), FFM, total, LDL and HDL cholesterol, and liver enzymes aspartate aminotransferase and gamma-glutamyl transferase increased similarly in both groups (Table 13). Weight or BMI did not change in either group.

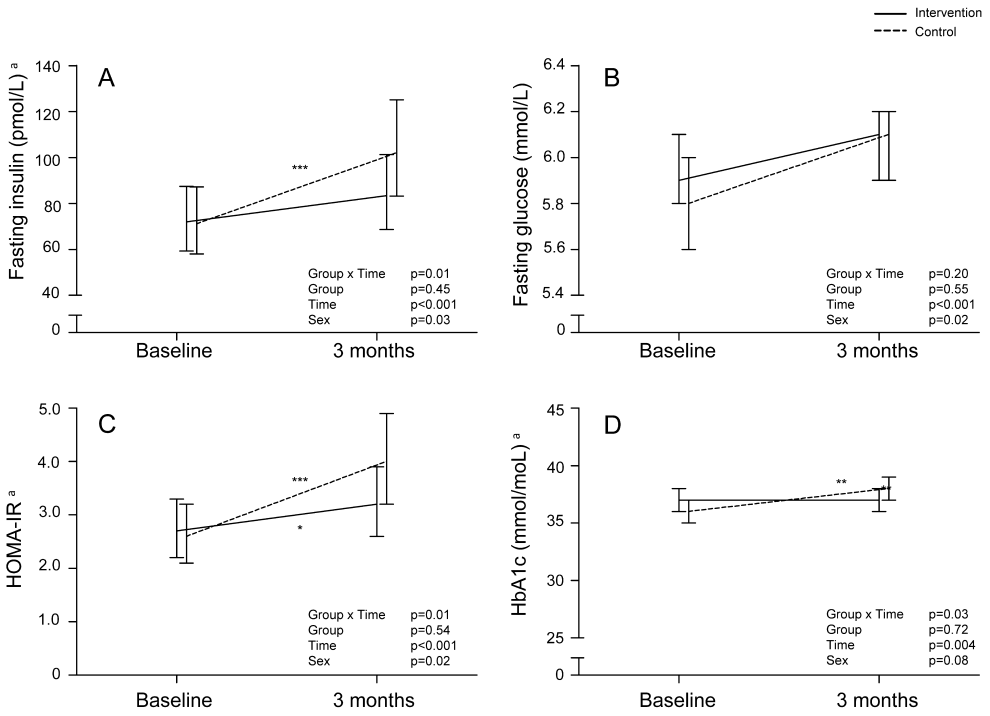

Figure 12. Intervention effects on glycemic outcomes in 3 months. **A)** Fasting insulin, **B)** fasting glucose, **C)** HOMA-IR, homeostatic model assessment of insulin resistance, and **D)** HbA_{1c}, glycated hemoglobin. Values are model-based means with 95 % confidence intervals. [a] = log10 transformed; means are backtransformed geometric means. ** $p < 0.01$; *** $p < 0.001$ within or between groups (Tukey's post hoc test). Modified from Original publication IV.

Table 13. The intervention effects on cardiometabolic outcomes within and between groups from baseline to 3 months.

	Intervention		Control		Difference between groups from baseline to 3 months	p values [a]		
	Baseline	3 months	Baseline	3 months		Group	Time	Group × Time
Weight, kg	92.8 (87.6, 98.0)	92.3 (87.1, 97.5)	93.7 (88.4, 99.1)	93.7 (88.3, 99.1)	− 0.5 (− 1.6, 0.6)	0.76	0.29	0.34
BMI, kg/m^2	31.5 (30.0, 33.0)	31.3 (29.8, 32.8)	31.7 (30.2, 33.3)	31.7 (30.1, 33.3)	− 0.2 (− 0.5, 0.2)	0.78	0.22	0.35
Waist circumference, cm	111.3 (107.5,115)	109.7 (105.9,113.5)	110.3 (106.5,114.2)	109.5 (105.6,113.4)	− 0.7 (−2.6, 1.1)	0.83	0.01*	0.42
Body fat-%	42.8 (40.6,44.9)	41.8 (39.6,43.9)	43.4 (41.1,45.6)	42.4 (40.1,44.6)	0.0 (− 1.3, 1.3)	0.70	0.004*	0.99
Fat mass, kg	39.7 (36.0, 43.5)	38.6 (34.8, 42.4)	40.9 (37.1, 44.8)	40.0 (36.2, 43.9)	− 0.2 (− 1.7, 1.3)	0.62	0.01*	0.78
FFM, kg	53.1 (50.8, 55.5)	53.7 (51.4, 56.0)	52.8 (50.4, 55.2)	53.7 (51.2, 56.1)	− 0.3 (− 1.5, 0.9)	0.91	0.02*	0.61
Systolic blood pressure, mmHg	146 (140, 152)	141 (136, 147)	139 (133, 145)	136 (130, 142)	− 2 (− 9, 6)	0.11	0.03*	0.64
Diastolic blood pressure, mmHg	89 (86, 92)	87 (84, 90)	88 (85, 91)	84 (81, 87)	1 (− 3, 5)	0.22	0.01*	0.51
Resting heart rate, bpm [b]	68 (65, 71)	67 (64, 70)	66 (63, 69)	68 (65, 72)	0.95 (0.90, 1.00)	0.82	0.49	0.03*

Table 13 continued.

	Intervention		Control		Difference between groups from baseline to 3 months	p values [a]		
	Baseline	3 months	Baseline	3 months		Group	Time	Group × Time
Total cholesterol, mmol/L [b]	4.7 (4.4, 5.0)	5.0 (4.7, 5.3)	4.6 (4.3, 4.9)	5.0 (4.7, 5.3)	0.98 (0.93, 1.03)	0.71	<0.001*	0.51
LDL cholesterol, mmol/L [b]	3.0 (2.7, 3.3)	3.2 (2.9, 3.5)	2.9 (2.7, 3.2)	3.3 (3.0, 3.6)	0.97 (0.92, 1.03)	0.91	<0.001*	0.37
HDL cholesterol, mmol/L	1.3 (1.2, 1.4)	1.4 (1.3, 1.6)	1.4 (1.3, 1.5)	1.5 (1.4, 1.6)	0.0 (− 0.1, 0.1)	0.45	<0.001*	0.47
Triglycerides, mmol/L [b]	1.4 (1.2, 1.6)	1.4 (1.2, 1.6)	1.0 (0.9, 1.2)	1.2 (1.1, 1.5)	0.82 (0.68, 0.99)	0.06	0.10	0.04*
Alanine aminotransferase, U/L [b]	28 (24, 33)	28 (24, 33)	27 (23, 31)	33 [†] (28, 38)	0.81 (0.69, 0.95)	0.67	0.02*	0.01*
Aspartate aminotransferase, U/L [b]	25 (23, 28)	28 (25, 31)	25 (22, 27)	30 (27, 34)	0.90 (0.81, 1.00)	0.66	<0.001*	0.06
Gamma-glutamyl transferase, U/L [b]	29 (23, 35)	29 (24, 36)	26 (21, 32)	31 (25, 38)	0.87 (0.74, 1.01)	0.89	0.03*	0.07

BMI, body mass index; FFM, fat-free mass; LDL, low-density lipoprotein; HDL, high-density lipoprotein. [a] = Group, the main effect of group differences; Time, the main effect of time; Group × time, the interaction between the two main effects. Values are model-based means with 95 % confidence intervals. [b] = log10 transformed, means are backtransformed geometric model-based means, and the difference is the ratio of geometric population means (intervention/control) with 95 % confidence intervals. * $p < 0.05$, statistical significance. [†] $p < 0.05$ within-group difference between baseline and 3 months (Tukey's post hoc test). From Original publication IV.

5.3.2 Effects on metabolic flexibility

Study V investigated the effects of the 6-month intervention period on energy metabolism outcomes. From baseline to 6 months, the average reduction in daily sedentary time in the intervention group was ~ 40 min/day, and the increase in MVPA 20 min/day, with no change in the control group (group × time $p < 0.05$) (Sjöros, Laine, Garthwaite, Vähä-Ypyä, Löyttyniemi, et al., 2023). The intervention group also increased LPA (14 min/day) and standing (7 min/day), but these changes were not statistically significantly different from the control group (group × time $p > 0.05$). Both groups increased steps, but similar to the midpoint of the study, the increase was greater in the intervention group (3300 vs. 1600 steps/day) (Sjöros, Laine, Garthwaite, Vähä-Ypyä, Löyttyniemi, et al., 2023).

Neither insulin- nor exercise-stimulated MetFlex changed statistically significantly during the intervention in the intervention or control group, nor were any significant between-group differences observed (Figure 13). Changes in insulin- and exercise-stimulated MetFlex did not correlate with each other either (Table 14).

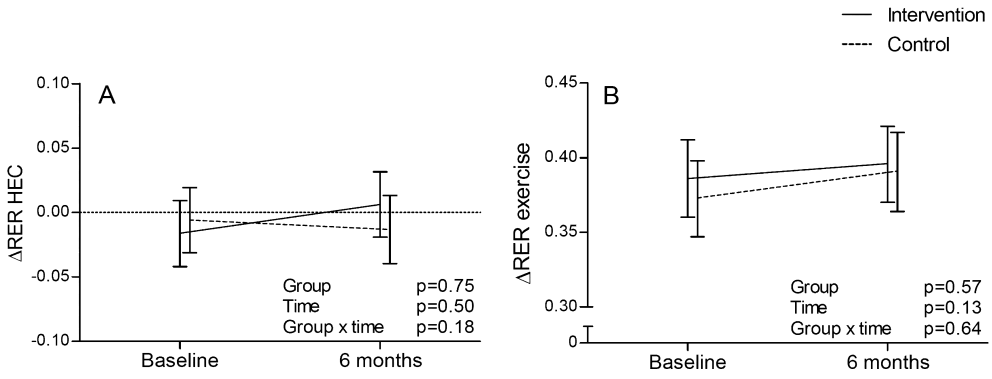

Figure 13. Intervention effects on metabolic flexibility in 6 months. **A)** Insulin-stimulated metabolic flexibility, **B)** metabolic flexibility during exercise. Values are model-based means with 95 % confidence intervals. RER, respiratory exchange ratio; HEC, hyperinsulinemic euglycemic clamp. Modified from Original publication V.

A trend towards increased fasting FATox and decreased fasting CHOox was observed in the intervention group, but the changes or between-group differences in fasting and insulin-stimulated substrate oxidation and RER were not statistically significant (Figure 14). Changes in fasting FATox and CHOox correlated with changes in insulin-stimulated MetFlex ($r = 0.36$ and $r = -0.35$, respectively; $p = 0.01$ for both).

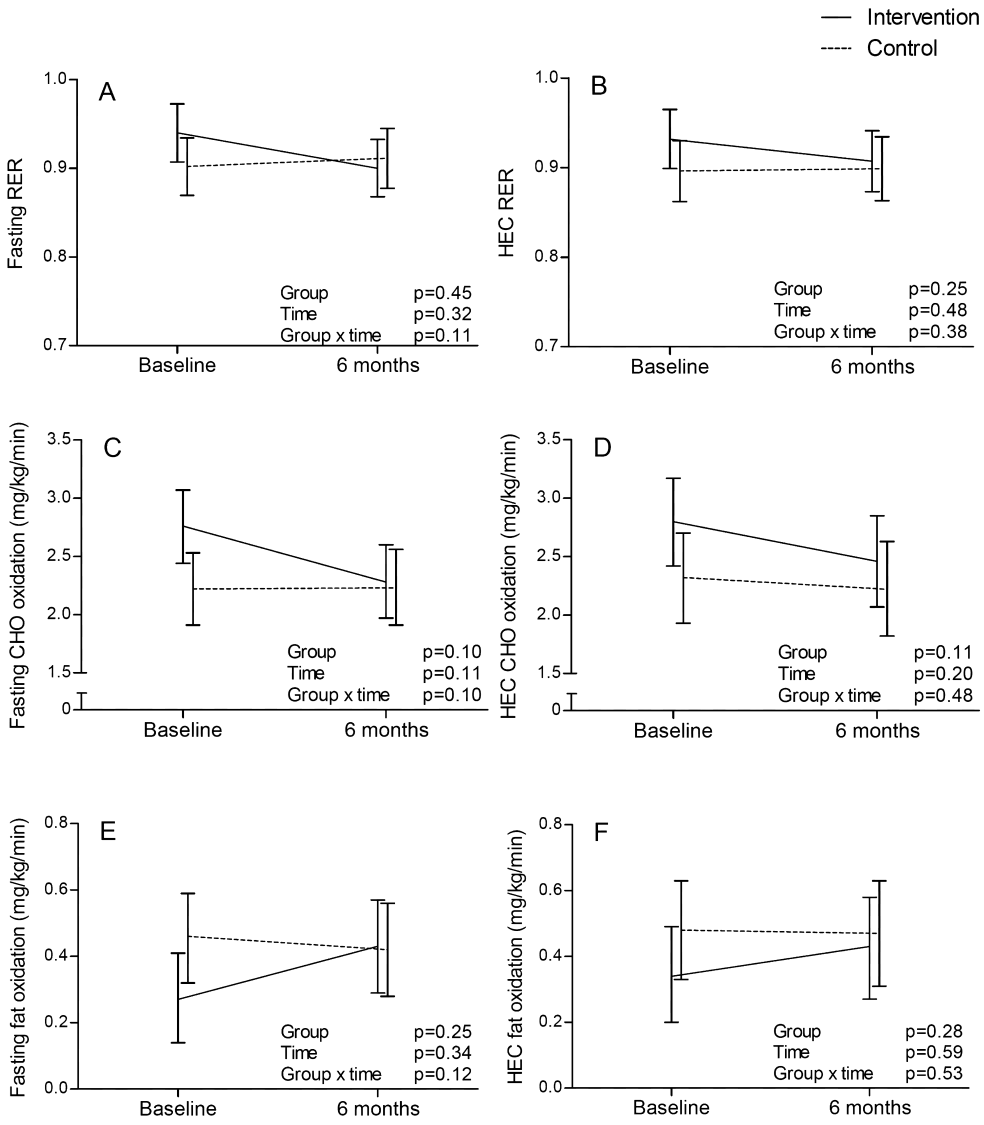

Figure 14. Intervention effects on fasting and insulin-stimulated substrate metabolism in 6 months. **A)** Fasting RER, **B)** insulin-stimulated RER, **C)** fasting carbohydrate oxidation, **D)** insulin-stimulated carbohydrate oxidation, **E)** fasting fat oxidation, **F)** insulin-stimulated fat oxidation. Values are model-based means with 95 % confidence intervals. RER, respiratory exchange ratio; HEC, hyperinsulinemic euglycemic clamp, CHO, carbohydrate. From Original publication V.

The change in CHOox at maximal exercise intensity was statistically significantly different between groups (group × time p = 0.03). During the intervention, the intervention group increased, and the control group decreased CHOox at maximal exercise intensity by 2.6 (95 % CI: − 0.8, 6.1) mg/kg/min and

1.4 (95 % CI: − 4.9, 2.1) mg/kg/min, respectively. No other changes in RER and substrate oxidation during exercise were observed.

The intervention had no effect on fasting plasma glucose or FFA concentration. Fasting lactate decreased on average by 0.11 (95 % CI: − 0.19, − 0.03) mmol/L during the intervention in both groups (time p = 0.01). The decrease appeared greater in the intervention group (− 0.19 mmol/L) than the control group (− 0.03 mmol/L), but the difference was marginally non-significant (group × time p = 0.054). The changes in fasting lactate correlated with changes in insulin-stimulated MetFlex and fasting RER (Table 14), as well as with changes in fasting FATox and CHOox ($r = -0.38$, $p = 0.001$, and $r = 0.45$, $p = 0.004$, respectively). No changes or differences between groups were observed in FFA and lactate concentrations or AUC during HEC.

Additional analyses

Due to large interindividual variation in sedentary time changes and no intervention effects between the intervention and control groups, additional analyses were performed between two groups based on the measured change in sedentary time as described in chapter 4.8.2.

The groups had similar descriptive characteristics but differed in baseline activity levels. Those who successfully reduced daily sedentary time by 30 min or more during the intervention (‘reducers’) spent at baseline 38 (95 % CI: 9, 67) min more sedentary, 18 (95 % CI: 0, 35) min less standing, and 11 (95 % CI: 2, 20) min less in MVPA daily than the ‘continuously sedentary’ group ($p < 0.05$).

During the intervention, the mean sedentary time reduction among the ‘reducers’ was 60 (95 % CI: 42, 76) min/day, achieved through a statistically significant ~ 20 min/day increase of each MVPA, LPA, and standing time, while there were no changes in sedentary time, LPA or MVPA in the ‘continuously sedentary’ group (group × time $p < 0.001$). Standing time was reduced by 18 (95 % CI: 5, 31) min/day in the ‘continuously sedentary’ group, and both groups increased steps but ‘reducers’ more so: 3450 (95 % CI: 2531, 4350) vs. 1500 (95 % CI: 555, 2450) (group × time $p < 0.001$). The results remained virtually unchanged after adjusting for the baseline differences, and the ~ 60-min difference in daily sedentary time at the 6-month timepoint between groups was statistically significant ($p < 0.001$).

During the intervention, the change in insulin-stimulated MetFlex was different between groups in favor of the ‘reducers’ compared to the ‘continuously sedentary’ (Figure 15). Those who reduced sedentary time also improved FATox at low-intensity exercise compared to the continuously sedentary (Figure 15). A statistically significant group × time-effect ($p = 0.04$) was also observed in fasting RER, which was driven primarily by a higher baseline value in the ‘reducers’ group. Adjustment

for baseline fasting RER slightly mitigated the intervention effect in MetFlex (group × time p = 0.05). Low-intensity exercise RER decreased by – 0.01 (95 % CI: − 0.03, 0.00) on average during the intervention, with no difference between the groups.

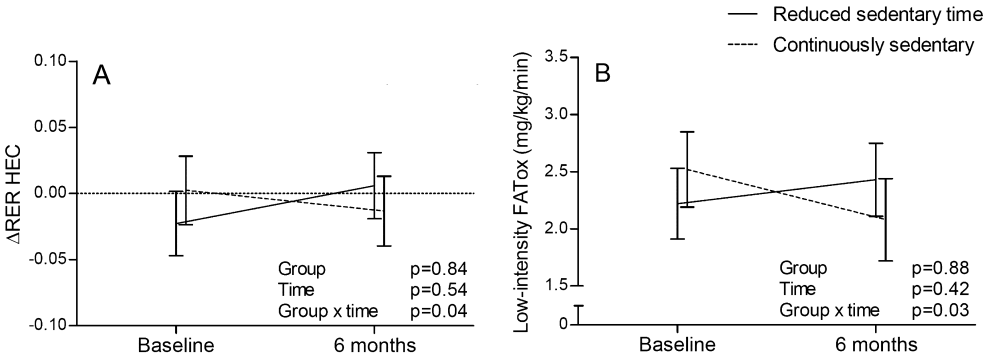

Figure 15. Intervention effects in 6 months on **A)** metabolic flexibility and **B)** low-intensity exercise fat oxidation in those who successfully reduced daily sedentary time by ≥ 30 min and the continuously sedentary participants. Values are model-based means with 95 % confidence intervals. RER, respiratory exchange ratio; HEC, hyperinsulinemic euglycemic clamp; FATox, fat oxidation. Modified from Original publication V.

Correlations between changes

Sedentary time and physical activity

Improvements in insulin-stimulated MetFlex correlated with increased standing, and a reverse trend was observed with sedentary time (Figure 16). Neither changes in MetFlex during exercise (Table 14), nor changes in any substrate oxidation variables (data not shown) correlated with changes in activity outcomes. The correlation between changes in standing and in low-intensity exercise FATox was nearly significant, however (r = 0.27, p = 0.06).

Insulin sensitivity

Changes in whole-body insulin sensitivity correlated positively with changes in insulin-stimulated MetFlex (Figure 16) and RER (Table 14). Changes in insulin sensitivity also correlated with changes in insulin-stimulated CHOox (r = 0.42, p = 0.001), FATox (r = − 0.38, p = 0.004), FFA suppression (r = 0.30, p = 0.02), lactate increase (r = 0.34, p = 0.01), and estimated non-oxidative glucose disposal (r = 0.39, p = 0.01). Changes in insulin sensitivity also correlated with changes in sedentary time, standing, LPA, steps, weight, and body composition (Table 14).

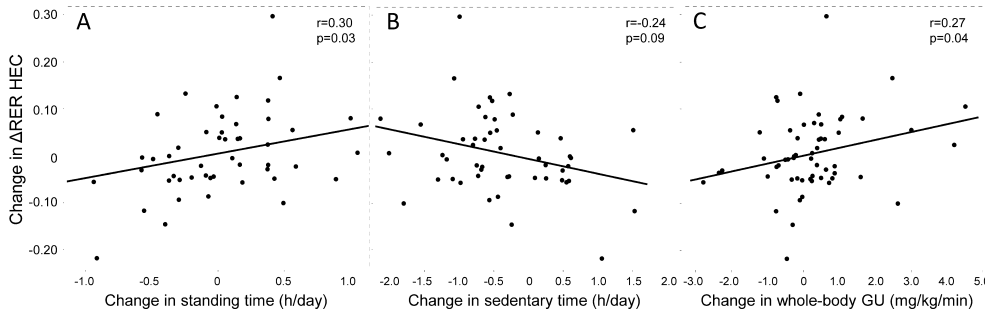

Figure 16. Correlations between changes during the intervention in insulin-stimulated metabolic flexibility and **A)** standing time, **B)** sedentary time, and **C)** whole-body glucose uptake. RER = respiratory exchange ratio; HEC = hyperinsulinemic euglycemic clamp; GU = glucose uptake. From Original publication V.

Weight and body composition

Changes in weight and body composition did not correlate with changes in insulin-stimulated MetFlex or substrate oxidation, but they did correlate with changes in MetFlex and RER during exercise (Table 14) and with changes in substrate oxidation at maximal exercise intensity (data not shown). Changes in weight and adiposity also correlated with changes in sedentary time, LPA, and steps (Table 14).

Diet

Changes in the intake (g/day) of total fat, saturated and polyunsaturated fatty acids, and protein correlated positively, and changes in the RER/FQ ratio inversely, with changes in insulin-stimulated MetFlex ($r = 0.31$, $r = 0.29$, $r = 0.28$, $r = 0.31$, and $r = -0.32$, respectively; $p < 0.05$ for all). FQ did not change during the intervention, nor did changes in FQ correlate with changes in MetFlex or substrate oxidation.

► **Table 14.** Correlations between changes (Δ) in metabolic flexibility, anthropometrics, fasting metabolic outcomes, and physical activity during the intervention. RER, respiratory exchange ratio; HEC, hyperinsulinemic euglycemic clamp; MetF, metabolic flexibility; Fat%, body fat-%; Insu, insulin; Gluc, glucose; GU, whole-body glucose uptake; FFA, free fatty acids; Lact; lactate; Trigly, triglycerides; Sed%, sedentary time as % of accelerometer wear time; Stand%, standing time as % of accelerometer wear time; LPA%, light-intensity physical activity as % of accelerometer wear time; MVPA%, moderate-to-vigorous intensity physical activity as % of accelerometer wear time. Bold p-values indicate statistical significance; * = $p < 0.05$, ** = $p < 0.01$. Modified from Original publication V.

	ΔFasting RER	ΔHEC RER	ΔHEC MetF	ΔLow exercise RER	ΔMax exercise RER	ΔExercise MetF	Δ Weight	Δ Fat%	Δ Insu	Δ Gluc	Δ GU	Δ FFA	Δ Lact	Δ Trigly	Δ Sed%	Δ Stand%	Δ LPA%	Δ MVPA%
ΔHEC RER	**0.77** **	--																
ΔHEC MetF	**-0.33***	0.26	--															
ΔLow exercise RER	0.12	0.09	-0.10	--														
ΔMax exercise RER	**0.29** *	**0.41** **	0.14	**0.33** *	--													
ΔExercise MetF	0.08	0.27	0.21	**-0.47****	**0.60****	--												
ΔWeight	-0.10	-0.23	-0.08	-0.06	**-0.37****	**-0.34***	--											
ΔFat%	-0.07	-0.17	-0.09	0.15	-0.20	**-0.34***	**0.28***	--										
ΔInsu	-0.13	-0.05	0.05	-0.23	-0.06	0.10	**0.31***	-0.04	--									
ΔGluc	-0.01	0.02	0.11	-0.16	-0.09	0.00	0.14	-0.03	**0.28** *	--								
ΔGU	0.11	**0.32** *	**0.27** *	-0.03	0.16	0.16	**-0.53** **	-0.09	**-0.29** *	**-0.26** *	--							
ΔFFA	-0.19	**-0.28** *	-0.14	-0.12	-0.26	-0.11	**0.28** *	0.02	-0.08	-0.20	**-0.27** *	--						
ΔLact	**0.37** **	0.13	**-0.28** *	-0.19	0.09	0.21	**0.27** *	-0.00	0.17	0.18	-0.16	-0.13	--					
ΔTrigly	0.16	-0.05	-0.22	-0.15	0.05	0.13	**0.26***	-0.09	0.15	0.01	-0.24	0.15	0.21	--				
ΔSed%	0.15	-0.02	-0.24	-0.15	-0.21	-0.04	**0.36** **	0.13	0.22	-0.09	**-0.38** **	-0.03	0.14	0.07	--			
ΔStand%	-0.18	0.07	**0.30** *	0.20	0.23	0.03	-0.24	-0.18	-0.20	0.20	**0.32** *	-0.04	-0.17	-0.04	**-0.76** **	--		
ΔLPA%	0.00	0.03	0.19	-0.08	0.06	0.06	**-0.32** *	0.08	-0.20	-0.09	**0.31** *	0.08	-0.17	-0.09	**-0.65** **	0.20	--	
ΔMVPA%	-0.10	-0.13	0.04	0.22	0.12	-0.12	-0.25	-0.09	-0.07	-0.05	0.20	0.20	-0.18	-0.16	**-0.66** **	0.25	**0.52** **	--
ΔSteps/day	0.02	0.00	0.00	0.15	0.14	-0.05	**-0.36** **	**-0.29** *	-0.22	-0.15	**0.30** *	0.11	-0.15	-0.26	**-0.60** **	**0.29** *	**0.43** **	**0.73** **

6 Discussion

This study aimed to elucidate the relationships between physical activity behaviors and energy metabolism in a population with an increased risk of lifestyle-related metabolic diseases. Activity behaviors and cardiorespiratory fitness were found to associate with whole-body and muscle insulin sensitivity, but some of these associations appeared to be mediated by adiposity. Only standing was associated with insulin sensitivity markers independent of adiposity. Standing and PA of even light-intensity also associated beneficially with fasting FATox and MetFlex, while high sedentary time associated with impaired lipid metabolism and metabolic inflexibility.

The sedentary time reduction intervention was able to reduce daily sedentary time, which led to beneficial effects in several cardiometabolic risk markers in 3 months. The intervention itself did not improve MetFlex according to the original group allocation, but additional analyses showed that successfully reducing daily sedentary time by 30 min or more may improve MetFlex in 6 months, compared to a continuously sedentary lifestyle. Improvements in MetFlex also correlated with increased standing time and improved insulin sensitivity.

6.1 Sedentary time, standing, and physical activity associate with insulin sensitivity and metabolic flexibility

As per study design, the participants were highly sedentary; in comparison to a population-based sample of Finnish adults of similar age, they spent ~ 1.5 h more sedentary and had ~ 1 h less LPA and ~ 30 min less MVPA daily (Husu et al., 2016). Sedentary time was adversely associated with whole-body and muscle insulin sensitivity, and both systemic and tissue-specific insulin resistance was more pronounced in those with sedentary time over 10.5 h/day compared to less sedentary time. The findings are in line with the consistent evidence of an adverse association between objectively measured sedentary time and insulin sensitivity, as well as other markers of glycemic control, including fasting insulin and glucose (Brocklebank et al., 2015; Powell et al., 2018).

Few studies have assessed insulin sensitivity with the gold standard hyperinsulinemic euglycemic clamp, however. One that has, similarly indicated that accelerometer-assessed activity outcomes are related to whole-body insulin sensitivity, showing an inverse association with sedentary time and a positive association with MVPA (Lahjibi et al., 2013). In the current study, more time spent standing, a higher number of steps, and better cardiorespiratory fitness were also associated with better insulin sensitivity both in the whole body and in skeletal muscle, while breaks in sedentary time associated only with whole-body insulin sensitivity.

Interestingly, the associations between activity outcomes and skeletal muscle insulin sensitivity varied between muscle groups. Sedentary time, standing, and steps were all statistically significantly associated with glucose uptake only in the hamstrings, and not in the quadriceps femoris. This could be partly explained by the localized effects of muscular activity, as exercise studies have shown improved glucose uptake only in contracting muscles, both acutely and after 2 weeks of training (Eskelinen et al., 2015; Gondoh et al., 2009). Quadriceps are primarily activated by more intense PA, while postural muscles such as hamstrings are predominantly active during standing and walking. Moreover, high body mass requires increased activation of postural muscles to support upright positions (Pesola et al., 2016). It is thus logical that glucose uptake was 55 % greater in the hamstrings than in quadriceps femoris and more hamstring-related associations were observed in this sedentary and overweight/obese population, who did virtually no vigorous activity.

Body composition appears to at least partially mediate the relationship between activity outcomes and insulin sensitivity, however, since adjustment for adiposity turned the associations of sedentary time, steps, breaks, and fitness with insulin sensitivity non-significant, both on the whole body and skeletal muscle level. Similarly, adjustment for waist circumference attenuated the association of sedentary time with insulin sensitivity in adults with newly diagnosed type 2 diabetes (Cooper et al., 2011), and in healthy adults adjustment for BMI turned the association between MVPA and insulin sensitivity non-significant (Lahjibi et al., 2013). Adiposity has been suggested to be a mediating factor in the associations of PA and fitness with insulin sensitivity previously as well (Chartrand et al., 2020; García-Hermoso et al., 2016).

Moreover, adiposity and fitness level may both influence the ability to engage in PA. It is easier for normal-weight, high-fit individuals to engage in, and maintain for longer, more vigorous activity, while daily living activities of low absolute intensity may require most of the aerobic capacity of low-fit individuals and thus be perceived as strenuous (Vähä-Ypyä et al., 2021). Weight reduction even without changes in absolute VO_{2max} (mL/min) could in turn improve fitness level and ease the physical

demands of daily routines (Vähä-Ypyä et al., 2021). Although the causal direction of the relationship between sedentary behavior and adiposity is not clear, i.e., obesity might lead to higher sedentary time and not the other way around (Pedisic et al., 2014), the findings from the current study further highlight the importance of a healthy body composition for metabolic health.

Body composition may not be a key modulator in the relationship between standing and metabolic health, however, since standing remained significantly associated with markers of insulin sensitivity even after adjustment for adiposity. The associations appeared to be independent of PA, sedentary time, and fitness as well. Previous evidence regarding the health effects of standing is limited, likely due to challenges in the assessment of postures and the identification of static, non-ambulatory standing as a separate behavior from sitting. Recent technical developments have enabled the investigation of specific postures and their impact on health, but few have assessed insulin sensitivity per se.

In line with the current results, a cross-sectional study in adults with a high risk of type 2 diabetes showed an association between better insulin sensitivity and a 30-min reallocation of sitting to standing (Edwardson et al., 2017). In healthy adults, however, total standing time was only related to better lipid profile, not blood glucose (Debache et al., 2019). Recent studies assessing optimal amounts and compositions of specific activity types and postures have also shown more favorable cardiometabolic outcomes with greater total standing time and with compositions including more time spent standing (Ahmadi et al., 2024; Blodgett et al., 2024; Brakenridge et al., 2024). The glycemic benefits of compositions with lower sitting time and more standing, PA, and sleep are particularly pronounced in those with type 2 diabetes (Brakenridge et al., 2024).

Experimental studies show conflicting results regarding the health effects of standing, however. Short, 5-min standing breaks every 30 min in prolonged sitting were shown to be beneficial to glucose and insulin metabolism in a similar high-risk population (Henson et al., 2016) and frequent breaks by standing improved postprandial glycemic response in healthy adults as well (Benatti et al., 2017). Some studies, in turn, show improved glucose and insulin responses only with light-intensity walking breaks, not standing (Bailey & Locke, 2015; Pulsford et al., 2017). The discrepancies are likely related to the frequency and duration of breaks, as some studies utilized 2-min breaks as frequently as every 20 min (Bailey & Locke, 2015), and others explored the effects of 5-min (Henson et al., 2016) or 15-min (Benatti et al., 2017) breaks every 30 min. In the current study, a higher number of breaks (sit-to-stand transitions) was associated with better whole-body insulin sensitivity, but the patterns were not assessed and adjustment for adiposity attenuated the association. The study population seems to influence the findings as well, as increased standing time and frequent standing breaks appear to provide potential

benefits to those with metabolic dysfunctions, whereas for healthy individuals they might not be enough to elicit changes in insulin and glucose metabolism.

Before this study, evidence on the relationship between accelerometer-assessed habitual free-living activity behaviors and HEC-assessed MetFlex was lacking. Bed rest studies, however, provide compelling evidence of the detrimental consequences of physical inactivity and sedentary behavior on MetFlex (Bergouignan et al., 2011), and modifying the level of PA by training and detraining has been shown to influence MetFlex (Bergouignan, Antoun et al., 2013). The findings from the current study extend the evidence to habitual behaviors in the daily living environment and to a population with existing metabolic dysfunctions, supporting the previous conclusions. Both lower sedentary time and a higher amount of light-intensity and total PA associated with better MetFlex, and MetFlex was better with daily sedentary time below 10 h compared to over 10 h. Lower fat oxidation capacity, both in a fasting state and during low-intensity exercise, was also associated with higher sedentary time. Standing and steps, on the other hand, associated with higher fasting FATox; all of these together indicating that activity behaviors are likely related to MetFlex particularly through effects on lipid metabolism. None of the activity outcomes associated with MetFlex during exercise, but interestingly higher sedentary time associated with lower exercise efficiency. The association was not significant after adjustment for body composition, however.

Two previous accelerometer studies have also examined the associations of sedentary time and PA with MetFlex by using maximal fat oxidation (MFO) or FAT_{max} (intensity that elicits MFO) during exercise as indicators of MetFlex (Amaro-Gahete et al., 2020; Corral-Pérez et al., 2022). They showed that sedentary behavior and PA may be related to MetFlex, depending on the study cohort. Sedentary behavior was associated with MFO in young active adults (Corral-Pérez et al., 2022), but not in young or middle-aged sedentary adults (Amaro-Gahete et al., 2020). Light and vigorous PA, on the other hand, associated with MFO in young active adults independent of confounders (Corral-Pérez et al., 2022). Among young and middle-aged sedentary women, however, cardiorespiratory fitness was a more important determinant of MFO since the association with PA disappeared after controlling for VO_{2max} (Amaro-Gahete et al., 2020). Training status and exercise capacity have indeed been shown to be important regulators of substrate metabolism and MetFlex during exercise (San-Millán & Brooks, 2018). This is supported by the current study as well, as higher fitness associated with lower RER at low exercise intensity, indicating a preference for fatty acids over CHO, as well as a better ability to utilize CHO at maximal exercise intensity. Furthermore, the adjustment for VO_{2max} similarly turned the associations between PA outcomes and substrate oxidation during exercise non-significant.

The role of fitness in modulating MetFlex responses to physiological or metabolic challenges other than exercise is unclear, however. Fitness did not associate with insulin-stimulated MetFlex in this study, and the previous evidence is inconsistent (Apostolopoulou et al., 2016; Ryan & Ortmeyer, 2019; Ukropcova et al., 2005). MetFlex responses to varying challenges might also be regulated by different underlying mechanisms since insulin- and exercise-stimulated MetFlex did not correlate, and the associations with activity outcomes were different. Adiposity might play a role and be a more important determinant of MetFlex during exercise as a limiting factor to exercise capacity.

Besides the total time spent in activity behaviors and fitness status, experimental evidence suggests that the patterns of behaviors and time accumulation may impact fuel oxidation as well. Although there was no association between breaks and MetFlex or substrate oxidation in this study, more frequent breaks by standing (Hawari et al., 2016) or LPA (Thorsen et al., 2019) have been shown to increase fat oxidation over a day. The associations with LPA and total PA, but not MVPA in this study suggest that, in the context of MetFlex, the total volume might be a more important characteristic of PA than intensity. The adjustment for total PA also turned several associations between other activity outcomes and MetFlex or fasting FATox non-significant. Sedentary time and standing remained associated with fasting RER also independent of total PA, however.

Overall, the accumulating evidence indicates that physical (in)activity indeed plays a role in the regulation of fuel metabolism, but the effects and mechanisms through which the effects are elicited might vary depending on several factors. The frequency, intensity, and volume of muscle contractions are likely to contribute, and the existing activity and fitness level might modulate the relationship.

6.2 Reducing sedentary time has beneficial effects on metabolic health

The intervention was successful in reducing daily sedentary time by 50 min in the first 3 months. By 6 months, the reduction was attenuated by 10 min, but was still significant. At both timepoints, half of the reduction in sedentary time was through increased MVPA, and the other half through a combination of LPA and standing. Only the increase in MVPA was statistically significantly different from the control group throughout the study, however. Both groups increased daily steps, but the increase was greater in the intervention group (+ 3300 steps in the intervention group and + 1600 steps in the control group from baseline to 6 months).

6.2.1 Cardiometabolic benefits in three months

At the midpoint of the study, benefits were mainly seen in cardiometabolic markers related to insulin sensitivity and glycemic control (fasting insulin, HOMA-IR, HbA_{1c}). The intervention was able to attenuate the increases in these outcomes that were seen in the control group, which is an important finding in terms of preventing the progression towards incident type 2 diabetes in a high-risk group. Slight improvements were seen in body composition and HDL as well, although similar changes were also seen in the control group.

The beneficial effect on liver enzyme alanine aminotransferase was a novel finding, together with the borderline significant intervention effects in aspartate aminotransferase and gamma-glutamyl transferase. These enzymes are markers of liver dysfunction or injury, and most often elevated levels are a result of non-alcoholic fatty liver disease (Oh et al., 2017). Sedentary behavior has been associated with fatty liver (Helajärvi et al., 2015), but sedentary time reduction interventions have not traditionally studied the effects on liver health markers. The beneficial intervention effects indicating attenuated increases in liver enzymes also suggest that reduced sedentary time might have potential as a preventive measure, as elevated aminotransferase levels are associated with obesity and dyslipidemia (Chalasani et al., 2012) and they independently predict type 2 diabetes (Hanley et al., 2004).

The reduction in sedentary time was not able to prevent the worsening of all cardiometabolic outcomes, however, including fasting glucose and total cholesterol, which increased in both groups. The overall trend indicating increases in the control group across most outcomes is noteworthy, considering that the control group also increased their daily steps. This suggests that the levels of cardiometabolic health markers may keep rising steadily over time in metabolic syndrome as it often precedes the onset of cardiometabolic diseases, and a considerable amount of PA might be required to see actual improvements in the outcomes, instead of maintenance of the current levels.

6.2.2 Changes in metabolic flexibility in six months

Several experimental studies, acute laboratory-based trials, and exercise training interventions have studied the effects of modified activity behaviors on components of MetFlex. Before this study, no long-term free-living accelerometer studies targeting reductions in daily sedentary time without intentional exercise training had investigated the effects of habitual PA on MetFlex, however. Despite the achieved behavior change and the reduction in mean daily sedentary time, the intervention itself was not able to improve MetFlex in 6 months. Additional analyses, however, suggested that a successful sedentary time reduction daily by 30 min or more might improve MetFlex in comparison to continued high volumes of sitting.

Although not statistically significant, there was a trend towards increased fat oxidation and decreased CHO oxidation in a fasting state in the intervention group. Sedentary time might influence substrate oxidation during exercise as well, as the intervention showed a beneficial effect on CHO utilization at maximal exercise intensity in the intervention group compared to the control group. However, the changes in CHOox also correlated with increased fitness, as well as reduced weight and adiposity, which in turn correlated with reduced sedentary time. It is thus likely that sedentary time itself does not elicit changes in substrate oxidation during maximal exercise, but it might indirectly affect the use of substrates through effects on body composition.

In contrast, activity behaviors may influence low-intensity exercise substrate oxidation, and the effects do not seem to be similarly dependent on changes in weight and body composition. Both sedentary time and PA associated with FATox at low-intensity exercise cross-sectionally, and in additional analyses the ≥ 30-min reduction in sedentary time was shown to improve low-intensity exercise FATox over 6 months in comparison to continued high sedentary time. Moreover, increased standing time during the intervention tended to correlate with improvements in FATox at low-intensity exercise. Increased standing time also correlated with improved insulin-stimulated MetFlex, unlike other activity outcomes. Notably, changes in neither standing or low-intensity exercise FATox, nor in insulin-stimulated MetFlex, correlated with changes in weight and body composition.

Previous interventions with low-intensity and low-volume exercise programs have also improved the utilization of fat for fuel during exercise (Schrauwen et al., 2002; Van Aggel-Leijssen et al., 2002), while a low-intensity intervention aiming to only increase daily walking by 45 min improved fat oxidation in a fasting state as well (Trenell et al., 2008). CHOox, on the other hand, appears unaffected by low-intensity exercise training (Schrauwen et al., 2002). Altogether, these findings suggest that reducing sedentary time and increasing even very light-intensity activity may favorably impact substrate oxidation and MetFlex, through effects on lipid metabolism in particular. Moreover, the beneficial effects do not seem to be primarily modulated by changes in weight and adiposity.

The central role of lipid metabolism is also indicated by changes in MetFlex correlating with changes in fat intake. Cross-sectionally neither fat intake, nor FQ, correlated with MetFlex, however, indicating that habitual fat intake or macronutrient composition was not the primary determinant of MetFlex. Concurrently with the correlation between changes in fat intake and MetFlex, changes in RER/FQ ratio also correlated with changes in MetFlex. This reflects an improved ability to also utilize fats relative to intake, suggesting a more prominent role for the oxidative capacity, rather than fat intake itself, in determining MetFlex. Interestingly, higher protein intake also correlated with better MetFlex, both cross-

sectionally and in the intervention setting. The reasons for this remain unclear, but the effects of protein intake on circulating insulin and glucagon levels have been proposed as a contributing factor to altered substrate metabolism (Forslund et al., 1999).

The intervention effects on whole-body and muscle insulin sensitivity have been published previously outside of this thesis (Sjöros, Laine, Garthwaite, Vähä-Ypyä, Koivumäki, et al., 2023; Sjöros, Laine, Garthwaite, Vähä-Ypyä, Löyttyniemi, et al., 2023). Similar to MetFlex, no group differences were seen according to the original randomization, but similarly performed additional analyses also showed improvements in whole-body and muscle insulin sensitivity with a successful ≥ 30 min/day sedentary time reduction. Moreover, the beneficial effects on fasting insulin and HOMA-IR observed at 3 months remained statistically significant at 6 months (Sjöros, Laine, Garthwaite, Vähä-Ypyä, Löyttyniemi, et al., 2023). Indicated by the correlation between the changes during the intervention as well, improvements in MetFlex indeed appear to be paralleled by improvements in insulin sensitivity, as also shown in previous exercise studies (Meex et al., 2010; Ryan & Ortmeyer, 2019; Malin et al., 2013). Not all studies have reported effects on MetFlex by exercise training, however, despite improved insulin sensitivity (Amador et al., 2020; Lefai et al., 2017). Unlike the first-mentioned studies in populations with type 2 diabetes or an increased risk of it, the latter two were conducted in healthy individuals, indicating that existing metabolic impairments may influence the capacity of exercise to improve MetFlex and oxidative capacity. The same is also suggested by more pronounced benefits to those with more severe metabolic impairments (Malin et al., 2013).

The improvements in insulin sensitivity and MetFlex following 3 months of exercise were at least partially accounted for by improved mitochondrial function in adults with type 2 diabetes (Meex et al., 2010). Although not directly assessed, the same could be speculated to have contributed to the findings in this study as well, as the intervention also led to decreased levels of blood lactate, which has been proposed as an indirect measure of mitochondrial function (San-Millán & Brooks, 2018). Fasting lactate decreased in both groups, but the decrease was more pronounced in the intervention group, and the improvements in fasting lactate correlated with improved MetFlex and substrate oxidation, favoring fat oxidation. Moreover, fasting and insulin-stimulated levels of lactate also correlated with sedentary time and standing, possibly indirectly indicating mitochondrial function as a contributor to the relationship between activity behaviors and MetFlex. Altogether, the interventional and cross-sectional findings in combination with previous exercise and bed rest studies support the role of activity behaviors as important regulators of MetFlex, particularly through effects on lipid metabolism.

Although the intervention was successful in reducing sedentary time, metabolic changes were modest. Besides reduced sedentary time, the increases in standing, LPA, and MVPA all were also significantly different from the control group at 3 months, and a 20-min MVPA increase was maintained through 6 months. The results thus appear to be somewhat in contrast to the findings from epidemiological and observational studies that consistently indicate improvements in health outcomes with reallocations of sedentary time to other behaviors, particularly to MVPA and even with as little as 4–12 min/day (Miatke et al., 2023; Blodgett et al., 2024). However, the majority of benefits reported with the reallocation of sedentary time to PA in compositional data analyses are only theoretical and based on cross-sectional data (Miatke et al., 2023). Evidence is lacking from longitudinal studies and intervention settings reflecting changes in activity behaviors in real life, and the cross-sectional associations between different compositions and cardiometabolic benefits have not always transferred to longitudinal data (Miatke et al., 2023). In line with the current findings, a meta-analysis of ≥ 7-day free-living sedentary time reduction interventions also reported only small improvements in fasting insulin (− 1.4 pmol/L), HDL (+ 0.04 mmol/L), weight (− 0.6 kg), waist circumference (− 0.7 cm), body fat-% (− 0.3 %), and systolic blood pressure (− 1.1 mmHg) (Hadgraft et al., 2021). How the reported changes occurred in response to changes in sedentary time and PA remains unclear, however.

It cannot be concluded in this study either how much the reduction in sedentary time and/or increases in different intensity activities contributed to changes in health outcomes. Although MVPA is a strong determinant of cardiorespiratory fitness and increased fitness level predicts improvements in health (Ross et al., 2016), fitness did not improve despite the significant 20-min MVPA increase in 6 months (Norha et al., 2023). Changes in fitness did not correlate with changes in metabolic outcomes either. On the other hand, changes in sedentary time, standing, and LPA, but not changes in MVPA, correlated with changes in whole-body insulin sensitivity, and changes in standing correlated with changes in MetFlex. This suggests an important role for reduced sedentary time and increased low-intensity activity in improving or maintaining metabolic health in highly sedentary and inactive individuals, even without changes in fitness through higher intensity activities.

The limited changes in health outcomes may be partly explained by the unintended behavioral changes in the control group participants as well, since they also increased daily steps by 1600 and MVPA by 5 min/day in 6 months (although the latter was not statistically significant). This may also have contributed to the similar ~ 1 kg weight loss in both groups in 6 months (Sjöros, Laine, Garthwaite, Vähä-Ypyä, Löyttyniemi, et al., 2023), and the lack of differences in weight and body composition between groups might thus have blunted some of the intervention effects in metabolic outcomes.

Since already the baseline amount of MVPA in this study (~ 1 h/day) may seem relatively high in light of the weekly recommendation of 2.5 h moderate-intensity activity for health benefits, it is worth noting that the PA guidelines are mainly based on self-reported activity data. The accelerometer data in this study was analyzed in 6-second epochs and thus even short bouts and incidental movements contribute to the daily accumulation of MVPA. Despite the changes in activity behaviors, even the intervention group was still more sedentary and had less PA than a comparable population-based sample of Finnish adults analyzed using the same method (Husu et al., 2016). Additionally, the MVPA increase consisted practically entirely of moderate-intensity activity, with the amount of vigorous activity being marginal (median < 1 min/day).

All in all, it can be expected that a larger reduction in sedentary time together with a greater increase in the volume and/or intensity of PA would yield greater improvements in metabolic health, and potential increases in cardiorespiratory fitness as well.

6.3 Physiological considerations

The low FATox and a preference of CHO already at a fasting state, together with a blunted shift in substrate use from fasting to insulin stimulation characterizes the participants in this study as metabolically inflexible. Fat oxidation capacity during exercise appeared to be impaired as well, since CHO was utilized at a similar rate already at the lowest exercise intensity.

The high CHOox rate and RER in a fasting state might represent a compensatory effort against rising blood glucose levels that are associated with metabolic syndrome in the progression towards type 2 diabetes, suggested by the midpoint results of the intervention as well. Since blood glucose and FFA did not correlate with MetFlex, substrate availability on the circulating level does not seem to be the primary determinant of metabolic inflexibility in this study. Defects in insulin sensitivity and transport of substrates are more likely explanations, as indicated by the associations between markers of MetFlex, substrate oxidation and insulin sensitivity, as well as by improvements in MetFlex coinciding with improvements in insulin sensitivity. Improved MetFlex correlated with a decreased level of fasting lactate as well, which could indirectly indicate mitochondrial function as a determinant.

Several metabolic outcomes were beneficially associated with standing and PA, and more pronouncedly with LPA than MVPA. The results also suggest total PA as a more important predictor of MetFlex than intensity per se. This could be partly explained by a higher amount of LPA and a negligible amount of vigorous activity. The relatively small variation in PA in this specific, inactive, and sedentary population could have limited the detection of some significant associations as well.

However, the beneficial associations of standing and LPA specifically with outcomes related to lipid metabolism are physiologically plausible, and they align with previous findings. For example, similar to this study, LPA, but not higher-intensity activities, had the most beneficial associations with HDL in individuals at a high risk of type 2 diabetes (Henson et al., 2020). Standing was also previously shown to associate with better lipid profile, but not with blood glucose levels or adiposity measures (Debache et al., 2019). More pronounced effects on circulating lipids, insulin sensitivity markers, and glycemic control were also observed when sitting was replaced by high-volumes of standing and LPA in free-living conditions, in comparison to one bout of EE-matched exercise (Duvivier et al., 2013, 2016, 2017).

Muscle contractions per se seem to be an important regulator of the PA-induced health benefits (Bergouignan et al., 2016; Gao et al., 2024; Hamilton et al., 2022). The beneficial associations observed between light-, but not high-, intensity activities and health outcomes in this study might be related to differences in muscle groups that are activated by specific behaviors. Hamstrings, which typically include more type I oxidative muscle fibers that favor fat metabolism, are primarily activated by standing and walking, whereas higher intensity activities recruit predominantly glycolytic type II fibers (Sale, 1987; Tirrell et al., 2012). In line with this, standing and steps associated with glucose uptake in the hamstrings, but not in the more glycolytic quadriceps femoris in this study. A successful sedentary time reduction through increased standing, light- and moderate-intensity activity also increased glucose uptake only in the hamstrings (Sjöros, Laine, Garthwaite, Vähä-Ypyä, Koivumäki, et al., 2023). Moreover, the positive association between standing and non-oxidative glucose disposal, not CHOox, suggests that the glucose that is taken up may be directed more towards storage instead of oxidation, which might contribute to the beneficial associations between standing and insulin sensitivity.

Skeletal muscle lipoprotein lipase activity might also partly explain the more pronounced associations of lipid metabolism and MetFlex with (in)activity outcomes other than MVPA since it is more sensitive to inactivity and low-intensity muscle contractions than higher intensity activities (Hamilton, 2018). Particularly standing appeared to associate with outcomes related to lipid metabolism, both cross-sectionally and during the intervention. The associations of lactate with standing, substrate oxidation, insulin sensitivity, and MetFlex are possibly indicative of mitochondrial function mediating the effects of standing on fat metabolism and insulin sensitivity to an extent. Lactate is known to contribute to MetFlex by influencing glucose availability as a major gluconeogenic precursor, and by downregulating FATox by effects on malonyl-CoA and carnitine palmitoyltransferase, key regulators of fatty acid transport to mitochondria (Brooks, 2018). The underlying physiological mechanisms of health benefits of low-intensity

activity are not thoroughly understood yet, particularly in long-term, but short-term studies have indeed proposed alterations in the delivery and uptake of fatty acids (Newsom et al., 2013), and molecular metabolic changes similar to exercise (Remie et al., 2021) as likely contributing factors.

Although the mechanisms are yet to be clarified, LPA seems to have potential to influence health outcomes. The benefits of regular exercise and MVPA are well-established and higher-intensity activity is likely to provide greater health improvements in a more time-efficient way. For example, the volume of LPA needed to induce similar reductions in mortality risk may be 3–4 times the amount of MVPA (Ekelund et al., 2024), and 2.6 h/day of standing has similar beneficial associations with cardiometabolic outcomes than 1 h/day of walking (Ahmadi et al., 2024). It is also worth noting that in addition to the potential health benefits of standing, prolonged standing may have detrimental health consequences as well, such as musculoskeletal symptoms, adverse effects on vasculature, leg swelling, discomfort and fatigue (Chester et al., 2002; Coenen et al., 2018; Huo Yung Kai et al., 2021). It has been proposed, however, that musculoskeletal adaptation to changed standing behavior may alleviate short-term symptoms in longer term (Dzakpasu et al., 2023; Edwardson et al., 2022).

Considering that increased MVPA despite its known benefits may not feasible or attractive to all, at least as a first step from previous inactivity, replacing sedentary time by standing and LPA may be a promising complementary health-enhancing strategy. The greatest benefits are also achieved initially when previously sedentary individuals increase the activity of any intensity, even if the amount is still below the recommendations (Greenwalt et al., 2023).

6.4 Strengths and limitations

The key strength of this study is the combination of rigorous methodology, including a 6-month free-living randomized controlled trial setting with continuous accelerometry, the gold standard method of whole-body insulin sensitivity measurement together with [^{18}F]FDG-PET imaging to assess tissue-specific insulin sensitivity, and indirect calorimetry. It is worth noting, however, that the indirect calorimetry measurement during HEC, as classically done, does not represent physiological conditions. Therefore, another strength is the respiratory gas exchange measurement and MetFlex assessment during exercise as well. The 6-month continuous accelerometry measurement is a particularly novel feature of this study, since most studies collect accelerometer data only for short periods (typically ≤ 7 days) at the beginning and end of the intervention. The 6-month accelerometer measurement throughout the intervention is likely to be more representative of habitual behaviors over longer term.

The considerable number of outcomes may have increased the chance of type I errors in the cross-sectional analyses. However, the main associations and key conclusions (e.g., associations of standing with insulin sensitivity, and of sedentary time, LPA, and total PA with MetFlex) remain even at a lower statistical significance level ($p \leq 0.02$). The unintended behavioral changes in the control group, possibly due to a participation effect and an increased awareness of health behaviors, may have blunted the detection of some intervention effects. Beneficial effects on health outcomes favoring the intervention were still seen, however. Not controlling for diet on the days preceding research visits can be considered a limitation as well. The participants were instructed to fast overnight and avoid strenuous physical exertion, caffeine, and alcohol for 24 h before research visits. Moreover, the potential effect of nutritional differences on circulating substrate availability is minimized by controlling the glucose and insulin concentrations during HEC.

It should be noted that the very specific population of inactive and sedentary, middle-aged adults with metabolic syndrome may limit the generalizability of the findings to other populations. Given the prevalence of sedentary lifestyles and overweight and obesity, however, the findings may be applicable on a wider scale as well.

6.5 Future directions

Strong epidemiological evidence shows that high volumes of sitting are indeed a health risk. Consequently, a growing number of intervention studies have aimed to investigate the effects of reduced sedentary time on health, showing modest improvements in anthropometric and cardiometabolic outcomes. The current evidence is mostly from studies including primarily healthy people, and future studies should focus more on inactive, at-risk individuals or populations with chronic diseases. More research is also needed to investigate the long-term sustainability of the achieved behavioral changes, as well as the clinical significance of the modest health improvements. The optimal amount and pattern of sedentary time reduction for health benefits, and activities replacing sedentary time, also remain to be clarified.

Moreover, studies should continue to explore the potential of increased standing and LPA as feasible, low-barrier health-enhancing strategies in addition to the established role of MVPA. Overall, although the field of sedentary behavior research has evolved rapidly, evidence is still insufficient to inform the development of quantitative guidelines, which remains the goal for the future.

Emerging evidence mainly from experimental studies and exercise interventions suggests a role for lifestyle factors in the regulation of MetFlex, but evidence is lacking regarding the effects of habitual free-living activity behaviors in longer-term. This study aimed to address this with a 6-month randomized controlled trial targeting

reductions in daily sedentary time, without adding intentional exercise training. The findings support the conclusions of previous studies, indicating that activity behaviors do play a part in MetFlex regulation, and modest benefits could be achieved with successful behavior changes. The causality of the relationship must be confirmed in future studies, since this conclusion was mainly drawn from the cross-sectional findings and additional analyses based on the measured behavior change, not the primary intervention analyses and randomization per se.

More studies are also needed in varying populations, including those with a high risk of metabolic diseases since metabolic inflexibility is a common feature of lifestyle-related metabolic disorders and possibly in the causal pathway. Experimental and mechanistic studies are needed to better understand the regulatory mechanisms behind inactivity-induced metabolic inflexibility and MetFlex responses to varying metabolic and physiological challenges. The mechanisms likely differ between challenges, as also implied by the lack of correlation between insulin- and exercise-stimulated MetFlex and the different associations of the two with activity and metabolic outcomes in this study. A previous study in obese adults at high risk of type 2 diabetes similarly found no association between MetFlex responses to insulin and exercise (Prior et al., 2014), and a 10-week exercise intervention improved MetFlex only in response to a meal challenge, but not to insulin stimulation (Carnero et al., 2021). One proposed explanation is differences in insulin- and contraction-mediated glucose uptake pathways (Prior et al., 2014), but more research is needed to understand the underlying reasons for different MetFlex responses.

7 Conclusions

This study shows that habitual sedentary time, standing, and PA play a part in energy metabolism regulation in sedentary adults with metabolic syndrome, and highlights the importance of a healthy body composition to metabolic health. Successfully reducing daily sedentary time by 30 min or more had a favorable impact on MetFlex, compared to a continuously sedentary lifestyle. The inverse associations of sedentary time, and beneficial associations of standing and LPA with insulin sensitivity and MetFlex support the idea of even light-intensity activity as a potential health-enhancing behavior, particularly through effects on lipid metabolism. Standing alone is not likely to directly lead to major improvements in health, but reducing sedentary time through the combination of standing and even light-intensity activity might be beneficial to energy metabolism and help slow down the progression of metabolic diseases in inactive individuals with metabolic impairments. The current PA guidelines emphasize the benefits of MVPA, and more substantial improvements in overall metabolic health are indeed likely to be achieved through an increased volume and intensity of PA. Especially for inactive individuals with an increased risk of cardiometabolic diseases, however, promoting changes in habitual daily activity behaviors through lower intensity activities might be a useful and likely a more achievable, complementary approach to improve health and a first step towards increased PA levels.

Acknowledgements

The data for this thesis was collected at the Turku PET Centre and the Department of Clinical Physiology and Nuclear Medicine, University of Turku and Turku University Hospital during the years 2017–2020. The study was conducted within the Centre of Excellence in Cardiovascular and Metabolic Disease supported by the Research Council of Finland, University of Turku, Turku University Hospital, and Åbo Akademi University. I want to sincerely thank the University of Turku Graduate School and the Doctoral Programme of Clinical Research for the opportunity to work full-time as a Doctoral Researcher, allowing me to focus completely on research throughout this thesis project.

Besides world-class research facilities and funding, this thesis was made possible by contributions from several parties. First, thank you to Professor Juhani Knuuti, the Director of Turku PET Centre, for forwarding my inquiry about an internship and Master's Thesis topic to Adjunct Professor Ilkka Heinonen many years ago. At the time, I had no plans for a PhD, but from the first time setting foot at the PET Centre, the enthusiasm about research was inspirational and contagious – and here we are. I was lucky to eventually get both of you as supervisors for my thesis. Thank you for allowing a great deal of independence, challenging me to learn and figure things out myself, but pointing into the right direction and helping always when needed. I want to thank Ilkka for warm and always positive guidance and support; and Juhani for sharing valuable advice about science and research gained from extensive experience. Thank you also to the members of the follow-up committee, Kari Kalliokoski and Marja Heiskanen, for your support during this process.

I appreciate the feedback, insightful comments and suggestions for improvements from the reviewers Professor Patrick Schrauwen and Academy Research Fellow Laura Karavirta. Moreover, I am truly thankful, and honored, to have the opportunity to discuss my thesis with Professor George Brooks as an opponent and a highly distinguished expert in the field of exercise physiology and metabolism.

I want to thank all the staff at the PET Centre that contributed to this project, measurements, and a great work environment, as well as the coauthors and collaborators. The collection and analysis of accelerometer data was made possible

by Tommi Vasankari, Henri Vähä-Ypyä, and Harri Sievänen at the UKK Institute. Thank you to Kirsi Laitinen and Noora Houttu at the Institute of Biomedicine for the collection of dietary and body composition data, and the staff at the Department of Clinical Physiology at the University Hospital for conducting the clinical exercise tests. Many thanks to Eliisa Löyttyniemi at the Department of Biostatistics for the invaluable help with statistical analyses. And of course a big thank you goes to all the participants without whom this study would not have been possible.

I've also been lucky to have great people to share the PhD journey, and all joys and sorrows of it, with. Thank you Tanja Sjöros for your help as the first Doctoral Researcher in the group in getting the rest of us started on our paths, and all the (long) lunches and good times. Thank you Saara Laine, Jooa Norha, and Tiia Koivula for your enjoyable company at work as well, and outside of it. Hiking in the Rocky Mountains, enjoying live music at Scottish pubs, and exploring the local food culture with friends on conference trips are some of the best memories from this journey. Special thanks to my roommate on all trips, and now one of my closest friends, Saara.

I want to also thank my friends outside of work, as well as my basketball teams over the years. You have had an important role in my well-being, both mental and physical, during this process. Moreover, I want to thank my family - my parents Olli and Tuula and siblings Tiia and Ville, for a warm family environment where everybody is always there for each other. Mom and Dad, thank you for your never-ending support, and encouragement to go chase challenges and achieve dreams – even if it means moving across the ocean and thousands of kilometers away. Without that, I would not be where I am today.

Finally, thank you from the bottom of my heart to my biggest supporter, my best friend, my adventure partner, and my husband Daniel. The amount of support and patience you have shown during this project, with everything else that we have gone through at the same time, is unbelievable. Thank you for always believing in me, especially when I don't myself. Molly, our lovely dog, also deserves a thankyou for bringing joy to every day at the home office. Through winding paths and ups and downs, we have reached the top of another mountain to add to the list of adventures, together.

August 27, 2024

Taru Garthwaite

References

2018 Physical Activity Guidelines Advisory Committee. (2018). *2018 Physical Activity Guidelines Advisory Committee Scientific Report*. U.S. Department of Health and Human Services.

Aadland, E. & Ylvisåker, E. (2015). Reliability of Objectively Measured Sedentary Time and Physical Activity in Adults. *PLoS One*, *10*(7), e0133296.

Ahmadi, M. N., Blodgett, J. M., Atkin, A. J., Chan, H.-W., Del Pozo Cruz, B., Suorsa, K., Bakker, E. A., Pulsford, R. M., Mielke, G. I., Johansson, P. J., Hettiarachchi, P., Thijssen, D. H. J., Stenholm, S., Mishra, G. D., Teixeira-Pinot, A., Rangul, V., Sherar, L. B., Ekelund, U., Hughes, A. D., … Stamatakis, E. (2024). Relationship of device measured physical activity type and posture with cardiometabolic health markers: Pooled dose–response associations from the Prospective Physical Activity, Sitting and Sleep Consortium. *Diabetologia*, 67(6):1051–1065.

Ainsworth, B. E., Haskell, W. L., Leon, A. S., Jacobs, D. R., Montoye, H. J., Sallis, J. F. & Paffenbarger, R. S. (1993). Compendium of Physical Activities: Classification of energy costs of human physical activities: *Medicine & Science in Sports & Exercise*, *25*(1), 71–80.

Alberti, K. G., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., Fruchart, J. C., James, W. P., Loria, C. M. & Smith, S. C. (2009). Harmonizing the Metabolic Syndrome. *Circulation*, *120*(16), 1640–1645.

Amador, M., Meza, C. A., McAinch, A. J., King, G. A., Covington, J. D. & Bajpeyi, S. (2020). Exercise-Induced Improvements in Insulin Sensitivity Are Not Attenuated by a Family History of Type 2 Diabetes. *Frontiers in Endocrinology*, *11*, 120.

Amaro-Gahete, F. J., Acosta, F. M., Migueles, J. H., Ponce González, J. G. & Ruiz, J. R. (2020). Association of sedentary and physical activity time with maximal fat oxidation during exercise in sedentary adults. *Scandinavian Journal of Medicine & Science in Sports*, *30*(9), 1605–1614.

Apostolopoulou, M., Strassburger, K., Herder, C., Knebel, B., Kotzka, J., Szendroedi, J. & Roden, M. (2016). Metabolic flexibility and oxidative capacity independently associate with insulin sensitivity in individuals with newly diagnosed type 2 diabetes. *Diabetologia*, *59*(10), 2203–2207.

Atakan, M. M., Guzel, Y., Shrestha, N., Kosar, S. N., Grgic, J., Astorino, T. A., Turnagol, H. H. & Pedisic, Z. (2022). Effects of high-intensity interval training (HIIT) and sprint interval training (SIT) on fat oxidation during exercise: A systematic review and meta-analysis. *British Journal of Sports Medicine*, *56*(17), 988–996.

Atkin, A. J., Gorely, T., Clemes, S. A., Yates, T., Edwardson, C., Brage, S., Salmon, J., Marshall, S. J. & Biddle, S. J. (2012). Methods of Measurement in epidemiology: Sedentary Behaviour. *International Journal of Epidemiology*, *41*(5), 1460–1471.

Bailey, D. P., Hewson, D. J., Champion, R. B. & Sayegh, S. M. (2019). Sitting Time and Risk of Cardiovascular Disease and Diabetes: A Systematic Review and Meta-Analysis. *American Journal of Preventive Medicine*, *57*(3), 408–416.

Bailey, D. P. & Locke, C. D. (2015). Breaking up prolonged sitting with light-intensity walking improves postprandial glycemia, but breaking up sitting with standing does not. *Journal of Science and Medicine in Sport*, *18*(3), 294–298.

Battaglia G. M., Zheng D, Hickner R. C. & Houmard J. A. (2012). Effect of exercise training on metabolic flexibility in response to a high-fat diet in obese individuals. *American Journal of Physiology - Endocrinology and Metabolism*, *303*(12), 1440–1445.

Bauman, A. E., Petersen, C. B., Blond, K., Rangul, V. & Hardy, L. L. (2018). The descriptive epidemiology of sedentary behavior. In M. F. Leitzmann, C. Jochem, & D. Schmid (Eds.), *Sedentary Behavior Epidemiology* (1–Book, Section, pp. 73–106). Springer International Publishing.

Bellettiere, J., Winkler, E. A., Chastin, S. F., Kerr, J., Owen, N., Dunstan, D. W. & Healy, G. N. (2017). Associations of sitting accumulation patterns with cardio-metabolic risk biomarkers in Australian adults. *PloS One*, *12*(6), e0180119.

Benatti, F. B., Larsen, S. A., Kofoed, K., Nielsen, S. T., Harder-Lauridsen, N. M., Lyngbæk, M. P., Eriksen, D., Karstoft, K., Krogh-Madsen, R., Pedersen, B. K. & Ried-Larsen, M. (2017). Intermittent Standing but not a

Moderate Exercise Bout Reduces Postprandial Glycemia. *Medicine and Science in Sports and Exercise*, *49*(11), 2305–2314.

Bergouignan A., Antoun E., Momken I., Schoeller D.A., Gauquelin-Koch G., Simon C. & Blanc S. (2013). Effect of contrasted levels of habitual physical activity on metabolic flexibility. *Journal of Applied Physiology*, *114*(3), 371–379.

Bergouignan, A., Latouche, C., Heywood, S., Grace, M. S., Reddy-Luthmoodoo, M., Natoli, A. K., Owen, N., Dunstan, D. W. & Kingwell, B. A. (2016). Frequent interruptions of sedentary time modulates contraction- and insulin-stimulated glucose uptake pathways in muscle: Ancillary analysis from randomized clinical trials. *Scientific Reports*, *6*(1), 32044.

Bergouignan, A., Momken, I., Lefai, E., Antoun, E., Schoeller, D. A., Platat, C., Chery, I., Zahariev, A., Vidal, H., Gabert, L., Normand, S., Freyssenet, D., Laville, M., Simon, C. & Blanc, S. (2013). Activity energy expenditure is a major determinant of dietary fat oxidation and trafficking, but the deleterious effect of detraining is more marked than the beneficial effect of training at current recommendations. *The American Journal of Clinical Nutrition*, *98*(3), 648–658.

Bergouignan A., Rudwill F., Simon C. & Blanc S. (2011). Physical inactivity as the culprit of metabolic inflexibility: Evidence from bed-rest studies. *Journal of Applied Physiology*, *111*(4), 1201–1210.

Bergouignan, A., Trudel, G., Simon, C., Chopard, A., Schoeller, D. A., Momken, I., Votruba, S. B., Desage, M., Burdge, G. C., Gauquelin-Koch, G., Normand, S. & Blanc, S. (2009). Physical Inactivity Differentially Alters Dietary Oleate and Palmitate Trafficking. *Diabetes*, *58*(2), 367–376.

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

Blaak, E. E., van Aggel-Leijssen, D. P., Wagenmakers, A. J., Saris, W. H. M. & van Baak, M. A. (2000). Impaired oxidation of plasma-derived fatty acids in type 2 diabetic subjects during moderate-intensity exercise. *Diabetes*, *49*(12), 2102–2107.

Blackburn, N. E., Wilson, J. J., McMullan, I. I., Caserotti, P., Giné-Garriga, M., Wirth, K., Coll-Planas, L., Alias, S. B., Roqué, M., Deidda, M., Kunzmann, A. T., Dallmeier, D. & Tully, M. A. (2020). The effectiveness and complexity of interventions targeting sedentary behaviour across the lifespan: A systematic review and meta-analysis. *International Journal of Behavioral Nutrition and Physical Activity*, *17*(1), 53.

Blodgett, J. M., Ahmadi, M. N., Atkin, A. J., Chastin, S. F., Chan, H.-W., Suorsa, K., Bakker, E. A., Hettiarcachchi, P., Johansson, P. J., Sherar, L. B., Rangul, V., Pulsford, R. M., Mishra, G., Eijsvogels, T. M. H., Stenholm, S., Hughes, A. D., Teixeira-Pinto, A. M., Ekelund, U., Lee, I-M., … ProPASS Collaboration. (2024). Device-measured physical activity and cardiometabolic health: The Prospective Physical Activity, Sitting, and Sleep (ProPASS) consortium. *European Heart Journal*, *45*(6), 458–471.

Bodker, A., Visotcky, A., Gutterman, D., Widlansky, M. E. & Kulinski, J. (2021). The impact of standing desks on cardiometabolic and vascular health. *Vascular Medicine*, *26*(4), 374–382.

Boerema, S. T., van Velsen, L., Vollenbroek, M. M. & Hermens, H. J. (2020). Pattern measures of sedentary behaviour in adults: A literature review. *Digital Health 6*, 2055207620905418.

Brakenridge, C. J., Koster, A., De Galan, B. E., Carver, A., Dumuid, D., Dzakpasu, F. Q. S., Eussen, S. J. P. M., Savelberg, H. H. C. M., Bosma, H., Owen, N., Schaper, N. C., Healy, G. N. & Dunstan, D. W. (2024). Associations of 24 h time-use compositions of sitting, standing, physical activity and sleeping with optimal cardiometabolic risk and glycaemic control: The Maastricht Study. *Diabetologia*, *67*(7), 1356–1367.

Brierley, M. L., Chater, A. M., Smith, L. R. & Bailey, D. P. (2019). The Effectiveness of Sedentary Behaviour Reduction Workplace Interventions on Cardiometabolic Risk Markers: A Systematic Review. *Sports Medicine, 49*(11), 1739–1767.

Brocklebank, L. A., Falconer, C. L., Page, A. S., Perry, R. & Cooper, A. R. (2015). Accelerometer-measured sedentary time and cardiometabolic biomarkers: A systematic review. *Preventive Medicine*, *76*, 92–102.

Brooks, G. A. (2018). The Science and Translation of Lactate Shuttle Theory. *Cell Metabolism*, *27*(4), 757-785.

Brooks, G. A. & Mercier, J. (1994). Balance of carbohydrate and lipid utilization during exercise: The "crossover" concept. *Journal of Applied Physiology*, *76*(6), 2253–2261.

Buffey, A. J., Herring, M. P., Langley, C. K., Donnelly, A. E. & Carson, B. P. (2022). The Acute Effects of Interrupting Prolonged Sitting Time in Adults with Standing and Light-Intensity Walking on Biomarkers of Cardiometabolic Health in Adults: A Systematic Review and Meta-analysis. *Sports Medicine*, *52*(8),1765–1787.

Carnero, E. A., Bock, C. P., Distefano, G., Corbin, K. D., Stephens, N. A., Pratley, R. E., Smith, S. R., Goodpaster, B. H. & Sparks, L. M. (2021). Twenty-four hour assessments of substrate oxidation reveal differences in metabolic flexibility in type 2 diabetes that are improved with aerobic training. *Diabetologia*, *64*(10), 2322–2333.

Carstens, M. T., Goedecke, J. H., Dugas, L., Evans, J., Kroff, J., Levitt, N. S. & Lambert, E. V. (2013). Fasting substrate oxidation in relation to habitual dietary fat intake and insulin resistance in non-diabetic women: A case for metabolic flexibility? *Nutrition & Metabolism*, *10*(1), 8.

Caspersen, C. J., Powell, K. E. & Christenson, G. M. (1985). Physical activity, exercise, and physical fitness: Definitions and distinctions for health-related research. *Public Health Reports*, *100*(2), 126–131.

Chalasani, N., Younossi, Z., Lavine, J. E., Diehl, A. M., Brunt, E. M., Cusi, K., Charlton, M., Sanyal, A. J., American Gastroenterological Association, American Association for the Study of Liver Diseases & American College of Gastroenterologyh. (2012). The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology*, *142*(7), 1592–1609.

Chartrand, D. J., Larose, E., Poirier, P., Mathieu, P., Alméras, N., Pibarot, P., Lamarche, B., Rhéaume, C. & Després, J.-P. (2020). Visceral adiposity and liver fat as mediators of the association between cardiorespiratory fitness and plasma glucose-insulin homeostasis. *American Journal of Physiology: Endocrinology and Metabolism*, *319*(3), E548–E556.

Chastin, S. F., Egerton, T., Leask, C. & Stamatakis, E. (2015). Meta-analysis of the relationship between breaks in sedentary behavior and cardiometabolic health. *Obesity*, *23*(9), 1800–1810.

Chastin, S. F., Dontje, M. L., Skelton, D. A., Cukic, I., Shaw, R. J., Gill, J. M. R., Greig, C. A., Gale, C. R., Deary, I. J., Der, G., Dall, P. M. & Seniors USP team. (2018). Systematic comparative validation of self-report measures of sedentary time against an objective measure of postural sitting (activPAL). *The International Journal of Behavioral Nutrition and Physical Activity*, *15*(1), 21.

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

Chau, J. Y., Grunseit, A. C., Chey, T., Stamatakis, E., Brown, W. J., Matthews, C. E., Bauman, A. E. & van der Ploeg, H. P. (2013). Daily sitting time and all-cause mortality: A meta-analysis. *PloS One*, *8*(11), e80000.

Chester, M. R., Rys, M. J. & Konz, S. A. (2002). Leg swelling, comfort and fatigue when sitting, standing, and sit/standing. *International Journal of Industrial Ergonomics*, *29*(5), 289–296.

Chudyk, A. & Petrella, R. J. (2011). Effects of Exercise on Cardiovascular Risk Factors in Type 2 Diabetes. *Diabetes Care*, *34*(5), 1228–1237.

Coenen, P., Willenberg, L., Parry, S., Shi, J. W., Romero, L., Blackwood, D. M., Maher, C. G., Healy, G. N., Dunstan, D. W. & Straker, L. M. (2018). Associations of occupational standing with musculoskeletal symptoms: A systematic review with meta-analysis. *British Journal of Sports Medicine*, *52*(3), 176–183.

Compernolle, S., DeSmet, A., Poppe, L., Crombez, G., De Bourdeaudhuij, I., Cardon, G., van der Ploeg, H. P. & Van Dyck, D. (2019). Effectiveness of interventions using self-monitoring to reduce sedentary behavior in adults: A systematic review and meta-analysis. *The International Journal of Behavioral Nutrition and Physical Activity*, *16*(1), 63–3.

Cooper, A. R., Sebire, S., Montgomery, A. A., Peters, T. J., Sharp, D. J., Jackson, N., Fitzsimons, K., Dayan, C. M. & Andrews, R. C. (2011). Sedentary time, breaks in sedentary time and metabolic variables in people with newly diagnosed type 2 diabetes. *Diabetologia*, *55*(3), 589–599.

Corpeleijn, E., Mensink, M., Kooi, M. E., Roekaerts, P. M. H. J., Saris, W. H. M. & Blaak, E. E. (2008). Impaired Skeletal Muscle Substrate Oxidation in Glucose-intolerant Men Improves After Weight Loss. *Obesity*, *16*(5), 1025–1032.

Corpeleijn, E., Saris, W. H. M. & Blaak, E. E. (2009). Metabolic flexibility in the development of insulin resistance and type 2 diabetes: Effects of lifestyle. *Obesity Reviews*, *10*(2), 178–193.

Corral-Pérez, J., Velázquez-Díaz, D., Perez-Bey, A., Montes-de-Oca-García, A., Fernandez-Santos, J. R., Amaro-Gahete, F. J., Jiménez-Pavón, D., Casals, C. & Ponce-González, J. G. (2022). Accelerometer-measured physical activity and sedentary time are associated with maximal fat oxidation in young adults. *European Journal of Sport Science*, *22*(10), 1595–1604.

Curl, C., Leija, R., Arevalo, J., Osmond, A., Duong, J., Huie, M., Masharani, U., Horning, M. & Brooks, G.A. (2024). Altered glucose kinetics occurs with aging: a new outlook on metabolic flexibility. *American Journal of Physiology-Endocrinology and Metabolism, 327*(2), E127–E228.

De Jong, N. P., Rynders, C. A., Goldstrohm, D. A., Pan, Z., Lange, A. H., Mendez, C., Melanson, E. L., Bessesen, D. H. & Bergouignan, A. (2019). Effect of frequent interruptions of sedentary time on nutrient metabolism in sedentary overweight male and female adults. *Journal of Applied Physiology*, *126*(4), 984–992.

Debache, I., Bergouignan, A., Chaix, B., Sneekes, E. M., Thomas, F. & Sueur, C. (2019). Associations of Sensor-Derived Physical Behavior with Metabolic Health: A Compositional Analysis in the Record Multisensor Study. *International Journal of Environmental Research and Public Health*, *16*(5), 741.

DeFronzo, R. A., Gunnarsson, R., Björkman, O., Olsson, M. & Wahren, J. (1985). Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. *Journal of Clinical Investigation*, *76*(1), 149–155.

DeFronzo, R. A., Tobin, J. D. & Andres, R. (1979). Glucose clamp technique: A method for quantifying insulin secretion and resistance. *The American Journal of Physiology*, *237*(3), 214.

DeFronzo, R. A. & Tripathy, D. (2009). Skeletal Muscle Insulin Resistance Is the Primary Defect in Type 2 Diabetes. *Diabetes Care*, *32*(suppl_2), S157–S163.

del Pozo-Cruz, J., García-Hermoso, A., Alfonso-Rosa, R. M., Alvarez-Barbosa, F., Owen, N., Chastin, S. F. & del Pozo-Cruz, B. (2018). Replacing Sedentary Time: Meta-analysis of Objective-Assessment Studies. *American Journal of Preventive Medicine*, *55*(3), 395–402.

Dempsey, P. C., Biddle, S. J. H., Buman, M. P., Chastin, S. F., Ekelund, U., Friedenreich, C. M., Katzmarzyk, P. T., Leitzmann, M. F., Stamatakis, E., Van Der Ploeg, H. P., Willumsen, J. & Bull, F. (2020). New global guidelines on sedentary behaviour and health for adults: Broadening the behavioural targets. *International Journal of Behavioral Nutrition and Physical Activity*, *17*(1), 151.

Dempsey, P. C., Larsen, R. N., Winkler, E. A., Owen, N., Kingwell, B. A. & Dunstan, D. W. (2018). Prolonged uninterrupted sitting elevates postprandial hyperglycaemia proportional to degree of insulin resistance. *Diabetes, Obesity & Metabolism*, *20*(6), 1526–1530.

Dempsey, P. C., Strain, T., Winkler, E. A., Westgate, K., Rennie, K. L., Wareham, N. J., Brage, S. & Wijndaele, K. (2022). Association of Accelerometer-Measured Sedentary Accumulation Patterns With Incident Cardiovascular Disease, Cancer, and All-Cause Mortality. *Journal of the American Heart Association*, *11*(9), e023845.

Dempsey, P.C., Strain, T., Khaw, K.-T., Wareham, N., Brage, S. & Wijndaele, K. (2020). Prospective Associations of Accelerometer-Measured Physical Activity and Sedentary Time With Incident Cardiovascular Disease, Cancer, and All-Cause Mortality. *Circulation*, *141*(13), 1113–1115.

Diaz, K. M., Goldsmith, J., Greenlee, H., Strizich, G., Qi, Q., Mossavar-Rahmani, Y., Vidot, D. C., Buelna, C., Brintz, C. E., Elfassy, T., Gallo, L. C., Daviglus, M. L., Sotres-Alvarez, D. & Kaplan, R. C. (2017). Prolonged, Uninterrupted Sedentary Behavior and Glycemic Biomarkers Among US Hispanic/Latino Adults: The HCHS/SOL (Hispanic Community Health Study/Study of Latinos). *Circulation*, *136*(15), 1362–1373.

Diaz, K. M., Howard, V. J., Hutto, B., Colabianchi, N., Vena, J. E., Safford, M. M., Blair, S. N. & Hooker, S. P. (2017). Patterns of Sedentary Behavior and Mortality in U.S. Middle-Aged and Older Adults: A National Cohort Study. *Annals of Internal Medicine*, *167*(7), 465–475.

Dimitriadis, G. D., Maratou, E., Kountouri, A., Board, M. & Lambadiari, V. (2021). Regulation of Postabsorptive and Postprandial Glucose Metabolism by Insulin-Dependent and Insulin-Independent Mechanisms: An Integrative Approach. *Nutrients*, *13*(1), 159.

Dohrn, I-M., Tarp, J., Steene-Johannessen, J., Vasankari, T. & Hagströmer, M. (2024). Device-measured physical activity and sedentary time in the Nordic countries: A scoping review of population-based studies. *Journal of Sport and Health Science, 13*(5):650–660.

Dong, Y., Pan, Y., Zhang, X., He, Q., Chen, S., Du, L. & Yin, S. (2024). Impact of Prolonged Sitting Interruption on Blood Glucose, Insulin and Triacylglycerol in Adults: A Systematic Review and Meta-Analysis. *Applied Sciences*, *14*(8), 3201.

Dunstan, D. W., Kingwell, B. A., Larsen, R., Healy, G. N., Cerin, E., Hamilton, M. T., Shaw, J. E., Bertovic, D. A., Zimmet, P. Z., Salmon, J. & Owen, N. (2012). Breaking up prolonged sitting reduces postprandial glucose and insulin responses. *Diabetes Care*, *35*(5), 976–983.

Duran, A. T., Friel, C. P., Serafini, M. A., Ensari, I., Cheung, Y. K. & Diaz, K. M. (2023). Breaking Up Prolonged Sitting to Improve Cardiometabolic Risk: Dose–Response Analysis of a Randomized Crossover Trial. *Medicine & Science in Sports & Exercise*, *55*(5), 847–855.

Duvivier, B. M. F. M., Schaper, N. C., Bremers, M. A., Van Crombrugge, G., Menheere, P. P. C. A., Kars, M. & Savelberg, H. H. C. M. (2013). Minimal Intensity Physical Activity (Standing and Walking) of Longer Duration Improves Insulin Action and Plasma Lipids More than Shorter Periods of Moderate to Vigorous Exercise (Cycling) in Sedentary Subjects When Energy Expenditure Is Comparable. *PLoS One*, *8*(2), e55542.

Duvivier, B. M. F. M., Schaper, N. C., Hesselink, M. K. C., van Kan, L., Stienen, N., Winkens, B., Koster, A. & Savelberg, H. H. C. M. (2016). Breaking sitting with light activities vs structured exercise: A randomised crossover study demonstrating benefits for glycaemic control and insulin sensitivity in type 2 diabetes. *Diabetologia*, *60*(3), 490–498.

Duvivier, B. M. F. M., Schaper, N. C., Koster, A., Van Kan, L., Peters, H. P. F., Adam, J. J., Giesbrecht, T., Kornips, E., Hulsbosch, M., Willems, P., Hesselink, M. K. C., Schrauwen, P. & Savelberg, H. H. C. M. (2017). Benefits of Substituting Sitting with Standing and Walking in Free-Living Conditions for Cardiometabolic Risk Markers, Cognition and Mood in Overweight Adults. *Frontiers in Physiology*, *8*, 353.

Dzakpasu, F. Q. S., Owen, N., Carver, A., Brakenridge, C. J., Eakin, E. G., Healy, G. N., Lamontagne, A. D., Moodie, M., Coenen, P., Straker, L. & Dunstan, D. W. (2023). Changes in Desk-Based Workers' Sitting, Standing, and Stepping Time: Short- and Longer-Term Effects on Musculoskeletal Pain. *Medicine & Science in Sports & Exercise*, *55*(12), 2241.

Edwardson, C. L., Biddle, S. J. H., Clemes, S. A., Davies, M. J., Dunstan, D. W., Eborall, H., Granat, M. H., Gray, L. J., Healy, G. N., Jaicim, N. B., Lawton, S., Maylor, B. D., Munir, F., Richardson, G., Yates, T. & Clarke-Cornwell, A. M. (2022). Effectiveness of an intervention for reducing sitting time and improving health in office workers: Three arm cluster randomised controlled trial. *BMJ*, *378*, e069288.

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

Egan, B. & Zierath, J. R. (2013). Exercise Metabolism and the Molecular Regulation of Skeletal Muscle Adaptation. *Cell Metabolism*, *17*(2), 162–184.

Ekelund, U., Brown, W. J., Steene-Johannessen, J., Fagerland, M. W., Owen, N., Powell, K. E., Bauman, A. E., & Lee, I-M. (2019). Do the associations of sedentary behaviour with cardiovascular disease mortality and cancer mortality differ by physical activity level? A systematic review and harmonised meta-analysis of data from 850 060 participants. *British Journal of Sports Medicine*, *53*(14), 886–894.

Ekelund, U., Sanchez-Lastra, M. A., Dalene, K. E., & Tarp, J. (2024). Dose–response associations, physical activity intensity and mortality risk: A narrative review. *Journal of Sport and Health Science*, *13*(1), 24–29.

Ekelund, U., Steene-Johannessen, J., Brown, W. J., Fagerland, M. W., Owen, N., Powell, K. E., Bauman, A., Lee, I-M., Lancet Physical Activity Series 2 Executive Committee & Lancet Sedentary Behaviour Working Group. (2016). Does physical activity attenuate, or even eliminate, the detrimental association of sitting time with mortality? A harmonised meta-analysis of data from more than 1 million men and women. *Lancet, 388*(10051), 1302–1310.

Ekelund, U., Tarp, J., Fagerland, M. W., Johannessen, J. S., Hansen, B. H., Jefferis, B. J., Whincup, P. H., Diaz, K. M., Hooker, S., Howard, V. J., Chernofsky, A., Larson, M. G., Spartano, N., Vasan, R. S., Dohrn, I.-M., Hagströmer, M., Edwardson, C., Yates, T., Shiroma, E. J., … Lee, I-M. (2020). Joint associations of accelerometer-measured physical activity and sedentary time with all-cause mortality: A harmonised meta-analysis in more than 44 000 middle-aged and older individuals. *British Journal of Sports Medicine*, *54*(24), 1499–1506.

Ekelund, U., Tarp, J., Steene-Johannessen, J., Hansen, B. H., Jefferis, B., Fagerland, M. W., Whincup, P., Diaz, K. M., Hooker, S. P., Chernofsky, A., Larson, M. G., Spartano, N., Vasan, R. S., Dohrn, I. M., Hagstromer, M., Edwardson, C., Yates, T., Shiroma, E., Anderssen, S. A. & Lee, I-M. (2019). Dose-response associations between accelerometry measured physical activity and sedentary time and all cause mortality: Systematic review and harmonised meta-analysis. *BMJ, 366*, I4570.

Eskelinen, J.-J., Heinonen, I., Löyttyniemi, E., Saunavaara, V., Kirjavainen, A., Virtanen, K. A., Hannukainen, J. C. & Kalliokoski, K. (2015). Muscle-specific glucose and free fatty acid uptake after sprint interval and moderate-intensity training in healthy middle-aged men. *Journal of Applied Physiology*, *118*(9), 1172–1180.

Ford, E. S., Li, C. & Sattar, N. (2008). Metabolic syndrome and incident diabetes: Current state of the evidence. *Diabetes Care*, *31*(9), 1898–1904.

Forslund, A. H., El-Khoury, A. E., Olsson, R. M., Sjödin, A. M., Hambraeus, L. & Young, V. R. (1999). Effect of protein intake and physical activity on 24-h pattern and rate of macronutrient utilization. *American Journal of Physiology-Endocrinology and Metabolism*, *276*(5), E964–E976.

Frayn, K. N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology*, *55*(2), 628–634.

Fritzen, A. M., Lundsgaard, A.-M. & Kiens, B. (2020). Tuning fatty acid oxidation in skeletal muscle with dietary fat and exercise. *Nature Reviews Endocrinology*, *16*(12), 683–696.

Fullmer, S., Benson-Davies, S., Earthman, C. P., Frankenfield, D. C., Gradwell, E., Lee, P. S. P., Piemonte, T. & Trabulsi, J. (2015). Evidence Analysis Library Review of Best Practices for Performing Indirect Calorimetry in Healthy and Non–Critically Ill Individuals. *Journal of the Academy of Nutrition and Dietetics*, *115*(9), 1417–1446.e2.

Gaesser, G. A., & Brooks, G. A. (1975). Muscular efficiency during steady-rate exercise: Effects of speed and work rate. *Journal of Applied Physiology*, *38*(6), 1132–1139.

Gaitán, J. M., Eichner, N. Z. M., Gilbertson, N. M., Heiston, E. M., Weltman, A. & Malin, S. K. (2019). Two Weeks of Interval Training Enhances Fat Oxidation during Exercise in Obese Adults with Prediabetes. *Journal of Sports Science & Medicine*, *18*(4), 636–644.

Galgani, J. E. & Fernández-Verdejo, R. (2021). Pathophysiological role of metabolic flexibility on metabolic health. *Obesity Reviews*, *22*(2), e13131.

Galgani, J. E., Heilbronn, L. K., Azuma, K., Kelley, D. E., Albu, J. B., Pi-Sunyer, X., Smith, S. R., Ravussin, E. & and the Look AHEAD Adipose Research Group. (2008). Metabolic Flexibility in Response to Glucose Is Not Impaired in People With Type 2 Diabetes After Controlling for Glucose Disposal Rate. *Diabetes*, *57*(4), 841–845.

Galgani J.E., Moro C. & Ravussin E. (2008). Metabolic flexibility and insulin resistance. *American Journal of Physiology - Endocrinology And Metabolism*, *295*(5), 1009–1017.

Gao, Y., Li, Q.-Y., Finni, T. & Pesola, A. J. (2024). Enhanced muscle activity during interrupted sitting improves glycemic control in overweight and obese men. *Scandinavian Journal of Medicine & Science in Sports*, *34*(4), e14628.

Gao, Y., Silvennoinen, M., Pesola, A. J., Kainulainen, H., Cronin, N. J. & Finni, T. (2017). Acute Metabolic Response, Energy Expenditure, and EMG Activity in Sitting and Standing. *Medicine & Science in Sports & Exercise*, *49*(9), 1927–1934.

García-Hermoso, A., Martínez-Vizcaíno, V., Recio-Rodriguez, J. I., Díez-Fernández, A., Gómez-Marcos, M. A., García-Ortiz, L. & for the EVIDENT Group. (2016). Abdominal obesity as a mediator of the influence of physical activity on insulin resistance in Spanish adults. *Preventive Medicine*, *82*, 59–64.

Gardner, B., Smith, L., Lorencatto, F., Hamer, M. & Biddle, S. J. (2016). How to reduce sitting time? A review of behaviour change strategies used in sedentary behaviour reduction interventions among adults. *Health Psychology Review*, *10*(1), 89–112.

Gibbs, B. B., Hergenroeder, A. L., Katzmarzyk, P. T., Lee, I. M. & Jakicic, J. M. (2015). Definition, measurement, and health risks associated with sedentary behavior. *Medicine and Science in Sports and Exercise*, *47*(6), 1295–1300.

Gondoh, Y., Tashiro, M., Itoh, M., Masud, M. M., Sensui, H., Watanuki, S., Ishii, K., Takekura, H., Nagatomi, R. & Fujimoto, T. (2009). Evaluation of individual skeletal muscle activity by glucose uptake during pedaling exercise at different workloads using positron emission tomography. *Journal of Applied Physiology*, *107*(2), 599–604.

Goodpaster, B. H., Katsiaras, A. & Kelley, D. E. (2003). Enhanced Fat Oxidation Through Physical Activity Is Associated With Improvements in Insulin Sensitivity in Obesity. *Diabetes*, *52*(9), 2191–2197.

Grace, M. S., Dempsey, P. C., Sethi, P., Mundra, P. A., Mellett, N. A., Weir, J. M., Owen, N., Dunstan, D. W., Meikle, P. J. & Kingwell, B. A. (2017). Breaking Up Prolonged Sitting Alters the Postprandial Plasma Lipidomic Profile of Adults With Type 2 Diabetes. *The Journal of Clinical Endocrinology & Metabolism*, *102*(6), 1991–1999.

Greenwalt, D., Phillips, S., Ozemek, C., Arena, R. & Sabbahi, A. (2023). The Impact of Light Physical Activity, Sedentary Behavior and Cardiorespiratory Fitness in Extending Lifespan and Healthspan Outcomes: How Little is Still Significant? A Narrative Review. *Current Problems in Cardiology*, *48*(10), 101871.

Hadgraft, N. T., Winkler, E. A., Climie, R. E., Grace, M. S., Romero, L., Owen, N., Dunstan, D., Healy, G. & Dempsey, P. C. (2021). Effects of sedentary behaviour interventions on biomarkers of cardiometabolic risk in adults: Systematic review with meta-analyses. *British Journal of Sports Medicine*, *55*(3), 144–154.

Hallgren, M., Dunstan, D. W. & Owen, N. (2020). Passive Versus Mentally Active Sedentary Behaviors and Depression. *Exercise and Sport Sciences Reviews*, *48*(1), 20–27.

Hamilton, M. T. (2018). The role of skeletal muscle contractile duration throughout the whole day: Reducing sedentary time and promoting universal physical activity in all people: *The Journal of Physiology*, *596*(8), 1331–1340.

Hamilton, M. T., Hamilton, D. G. & Zderic, T. W. (2022). A potent physiological method to magnify and sustain soleus oxidative metabolism improves glucose and lipid regulation. *iScience*, *25*(9), 104869.

Hanley, A. J., Williams, K., Festa, A., Wagenknecht, L. E., D'Agostino, R. B., Kempf, J., Zinman, B., Haffner, S. M. & The Insulin Resistance Atherosclerosis Study. (2004). Elevations in markers of liver injury and risk of type 2 diabetes: The insulin resistance atherosclerosis study. *Diabetes*, *53*(10), 2623–2632.

Hawari, N. S. A., Al-Shayji, I., Wilson, J. & Gill, J. M. (2016). Frequency of Breaks in Sedentary Time and Postprandial Metabolic Responses. *Medicine and Science in Sports and Exercise*, *48*(12), 2495–2502.

Healy, G. N., Clark, B. K., Winkler, E. A., Gardiner, P. A., Brown, W. J. & Matthews, C. E. (2011). Measurement of adults' sedentary time in population-based studies. *American Journal of Preventive Medicine*, *41*(2), 216–227.

Healy, G. N., Winkler, E. A., Eakin, E. G., Owen, N., Lamontagne, A. D., Moodie, M. & Dunstan, D. W. (2017). A Cluster RCT to Reduce Workers' Sitting Time: Impact on Cardiometabolic Biomarkers. *Medicine and Science in Sports and Exercise*, *49*(10), 2032–2039.

Heinonen, I., Helajärvi, H., Pahkala, K., Heinonen, O. J., Hirvensalo, M., Palve, K., Tammelin, T., Yang, X., Juonala, M., Mikkila, V., Kahonen, M., Lehtimaki, T., Viikari, J. & Raitakari, O. T. (2013). Sedentary behaviours and obesity in adults: The Cardiovascular Risk in Young Finns Study. *BMJ Open*, *3*(6), e002901.

Helajärvi, H., Pahkala, K., Heinonen, O. J., Juonala, M., Oikonen, M., Tammelin, T., Hutri-Kahonen, N., Kahonen, M., Lehtimaki, T., Mikkila, V., Viikari, J. & Raitakari, O. T. (2015). Television viewing and fatty liver in early midlife. The Cardiovascular Risk in Young Finns Study. *Annals of Medicine*, *47*(6), 519–526.

Henson, J., Davies, M. J., Bodicoat, D. H., Edwardson, C. L., Gill, J. M. R., Stensel, D. J., Tolfrey, K., Dunstan, D. W., Khunti, K. & Yates, T. (2016). Breaking Up Prolonged Sitting With Standing or Walking Attenuates the Postprandial Metabolic Response in Postmenopausal Women: A Randomized Acute Study. *Diabetes Care*, *39*(1), 130–138.

Henson, J., Edwardson, C. L., Davies, M. J., Gill, J. M. R., Heaney, L. M., Khunti, K., Ng, L., Sattar, N., Zaccardi, F. & Yates, T. (2020). Physical activity and lipidomics in a population at high risk of type 2 diabetes mellitus. *Journal of Sports Sciences*, *38*(10), 1150–1160.

Homer, A. R., Fenemor, S. P., Perry, T. L., Rehrer, N. J., Cameron, C. M., Skeaff, C. M. & Peddie, M. C. (2017). Regular activity breaks combined with physical activity improve postprandial plasma triglyceride,

nonesterified fatty acid, and insulin responses in healthy, normal weight adults: A randomized crossover trial. *Journal of Clinical Lipidology*, *11*(5), 1268–1279.e1.

Huo Yung Kai, S., Ferrières, J., Carles, C., Turpin, M., Lapébie, F.-X., Dutheil, F., Bura-Rivière, A. & Esquirol, Y. (2021). Lower limb venous and arterial peripheral diseases and work conditions: Systematic review. *Occupational and Environmental Medicine*, *78*(1), 4–14.

Husu, P., Suni, J., Vähä-Ypyä, H., Sievänen, H., Tokola, K., Valkeinen, H., Mäki-Opas, T. & Vasankari, T. (2016). Objectively measured sedentary behavior and physical activity in a sample of Finnish adults: A cross-sectional study. *BMC Public Health*, *16*(1), 920.

ICRP. (1975). *Report of the Task Group on Reference Man.* (ICRP Publication 23). Pergamon Press.

Jeukendrup, A. E. & Wallis, G. A. (2005). Measurement of Substrate Oxidation During Exercise by Means of Gas Exchange Measurements. *International Journal of Sports Medicine*, *26*, S28–S37.

Jingjie, W., Yang, L., Jing, Y., Ran, L., Yiqing, X. & Zhou, N. (2022). Sedentary time and its association with risk of cardiovascular diseases in adults: An updated systematic review and meta-analysis of observational studies. *BMC Public Health*, *22*(1), 286.

Katzmarzyk, P. T., Friedenreich, C., Shiroma, E. J. & Lee, I.-M. (2021). Physical inactivity and non-communicable disease burden in low-income, middle-income and high-income countries. *British Journal of Sports Medicine*, *56*(2), 101–106.

Kelley, D. E., Goodpaster, B. H., Wing, R. R. & Simoneau, J-A. (1999). Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *American Journal of Physiology-Endocrinology and Metabolism*, *277*(6), E1130–E1141.

Kelley, D. E. & Simoneau, J-A. (1994). Impaired free fatty acid utilization by skeletal muscle in non-insulin-dependent diabetes mellitus. *Journal of Clinical Investigation*, *94*(6), 2349–2356.

Ketels, M., Rasmussen, C. L., Korshøj, M., Gupta, N., De Bacquer, D., Holtermann, A. & Clays, E. (2020). The Relation between Domain-Specific Physical Behaviour and Cardiorespiratory Fitness: A Cross-Sectional Compositional Data Analysis on the Physical Activity Health Paradox Using Accelerometer-Assessed Data. *International Journal of Environmental Research and Public Health*, *17*(21), 7929.

Kitano, N., Kai, Y., Jindo, T., Fujii, Y., Tsunoda, K. & Arao, T. (2022). Association of domain-specific physical activity and sedentary behavior with cardiometabolic health among office workers. *Scandinavian Journal of Medicine & Science in Sports*, *32*(8), 1224–1235.

Lahjibi, E., Heude, B., Dekker, J. M., Hojlund, K., Laville, M., Nolan, J., Oppert, J. M., Balkau, B. & RISC Study Group. (2013). Impact of objectively measured sedentary behaviour on changes in insulin resistance and secretion over 3 years in the RISC study: Interaction with weight gain. *Diabetes & Metabolism*, *39*(3), 217–225.

Le Roux, E., De Jong, N. P., Blanc, S., Simon, C., Bessesen, D. H. & Bergouignan, A. (2022). Physiology of physical inactivity, sedentary behaviours and non-exercise activity: Insights from the space bedrest model. *The Journal of Physiology*, *600*(5), 1037–1051.

Lee, I-M., Shiroma, E. J., Lobelo, F., Puska, P., Blair, S. N., Katzmarzyk, P. T. & Lancet Physical Activity Series Working Group. (2012). Effect of physical inactivity on major non-communicable diseases worldwide: An analysis of burden of disease and life expectancy. *Lancet*, *380*(9838), 219–229.

Lefai, E., Blanc, S., Momken, I., Antoun, E., Chery, I., Zahariev, A., Gabert, L., Bergouignan, A. & Simon, C. (2017). Exercise training improves fat metabolism independent of total energy expenditure in sedentary overweight men, but does not restore lean metabolic phenotype. *International Journal of Obesity*, *41*(12), 1728–1736.

Livesey, G. & Elia, M. (1988). Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: Evaluation of errors with special reference to the detailed composition of fuels. *The American Journal of Clinical Nutrition*, *47*(4), 608–628.

Loh, R., Stamatakis, E., Folkerts, D., Allgrove, J. E. & Moir, H. J. (2020). Effects of Interrupting Prolonged Sitting with Physical Activity Breaks on Blood Glucose, Insulin and Triacylglycerol Measures: A Systematic Review and Meta-analysis. *Sports Medicine*, *50*(2), 295–330.

Loyen, A., Clarke-Cornwell, A. M., Anderssen, S. A., Hagstromer, M., Sardinha, L. B., Sundquist, K., Ekelund, U., Steene-Johannessen, J., Baptista, F., Hansen, B. H., Wijndaele, K., Brage, S., Lakerveld, J., Brug, J. & van der Ploeg, H. P. (2017). Sedentary Time and Physical Activity Surveillance Through Accelerometer Pooling in Four European Countries. *Sports Medicine*, *47*(7), 1421–1435.

Lynch, B. M. (2010). Sedentary Behavior and Cancer: A Systematic Review of the Literature and Proposed Biological Mechanisms. *Cancer Epidemiology, Biomarkers & Prevention*, *19*(11), 2691–2709.

Malin S.K., Haus J.M., Solomon T.P.J, Blaszczak A., Kashyap S.R. & Kirwan J.P. (2013). Insulin sensitivity and metabolic flexibility following exercise training among different obese insulin-resistant phenotypes. *American Journal of Physiology - Endocrinology and Metabolism*, *305*(10), 1292–1298.

Mansoubi, M., Pearson, N., Biddle, S. J. & Clemes, S. A. (2016). Using Sit-to-Stand Workstations in Offices: Is There a Compensation Effect? *Medicine and Science in Sports and Exercise*, *48*(4), 720–725.

Martin, A., Fitzsimons, C., Jepson, R., Saunders, D. H., van der Ploeg, H. P., Teixeira, P. J., Gray, C. M., Mutrie, N. & EuroFIT consortium. (2015). Interventions with potential to reduce sedentary time in adults: Systematic review and meta-analysis. *British Journal of Sports Medicine*, *49*(16), 1056–1063.

Matthews, C. E., Carlson, S. A., Saint-Maurice, P. F., Patel, S., Salerno, E. A., Loftfield, E., Troiano, R. P., Fulton, J. E., Sampson, J. N., Tribby, C., Keadle, S. K. & Berrigan, D. (2021). Sedentary Behavior in U.S. Adults: Fall 2019. *Medicine & Science in Sports & Exercise*, *53*(12), 2512–2519.

Meex, R. C., Schrauwen-Hinderling, V. B., Kornips, E., Schaart, G., Mensink, M., Phielix, E., van de Weijer, T., Sels, J. P., Schrauwen, P. & Hesselink, M. K. (2010). Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity. *Diabetes*, *59*(3), 572–579.

Menshikova, E. V., Ritov, V. B., Dube, J. J., Amati, F., Stefanovic-Racic, M., Toledo, F. G. S., Coen, P. M. & Goodpaster, B. H. (2018). Calorie Restriction-induced Weight Loss and Exercise Have Differential Effects on Skeletal Muscle Mitochondria Despite Similar Effects on Insulin Sensitivity. *The Journals of Gerontology: Series A*, *73*(1), 81–87.

Mensink, M., Blaak, E. E., van Baak, M. A., Wagenmakers, A. J. & Saris, W. H. M. (2001). Plasma Free Fatty Acid Uptake and Oxidation Are Already Diminished in Subjects at High Risk for Developing Type 2 Diabetes. *Diabetes*, *50*(11), 2548–2554.

Mensink, M., Blaak, E. E., Wagenmakers, A. J. & Saris, W. H. M. (2005). Lifestyle Intervention and Fatty Acid Metabolism in Glucose-Intolerant Subjects. *Obesity Research*, *13*(8), 1354–1362.

Miatke, A., Olds, T., Maher, C., Fraysse, F., Mellow, M. L., Smith, A. E., Pedisic, Z., Grgic, J. & Dumuid, D. (2023). The association between reallocations of time and health using compositional data analysis: A systematic scoping review with an interactive data exploration interface. *International Journal of Behavioral Nutrition and Physical Activity*, *20*(1), 127.

Mottillo, S., Filion, K. B., Genest, J., Joseph, L., Pilote, L., Poirier, P., Rinfret, S., Schiffrin, E. L. & Eisenberg, M. J. (2010). The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. *Journal of the American College of Cardiology*, *56*(14), 1113–1132.

Muniyappa R., Lee S., Chen H. & Quon M.J. (2008). Current approaches for assessing insulin sensitivity and resistance in vivo: Advantages, limitations, and appropriate usage. *American Journal of Physiology - Endocrinology and Metabolism*, *294*(1), 15–26.

Murtagh, E. M., Murphy, M. H., Milton, K., Roberts, N. W., O'Gorman, C. S. & Foster, C. (2020). Interventions outside the workplace for reducing sedentary behaviour in adults under 60 years of age. *Cochrane Database of Systematic Reviews*, *2020*(7).

Nancekievill, D., Colpitts, B. H., Seaman, K., Girard, M. & Sénéchal, M. (2023). The impact of sprint interval training with or without weight loss on substrate oxidation in adults: A secondary analysis of the i- FLEX study. *Physiological Reports*, *11*(9), e15684.

Neuhaus, M., Eakin, E. G., Straker, L., Owen, N., Dunstan, D. W., Reid, N. & Healy, G. N. (2014). Reducing occupational sedentary time: A systematic review and meta-analysis of evidence on activity-permissive workstations. *Obesity Reviews*, *15*(10), 822–838.

Newsom, S. A., Everett, A. C., Hinko, A. & Horowitz, J. F. (2013). A Single Session of Low-Intensity Exercise Is Sufficient to Enhance Insulin Sensitivity Into the Next Day in Obese Adults. *Diabetes Care*, *36*(9), 2516–2522.

Nguyen, P., Le, L. K.-D., Nguyen, D., Gao, L., Dunstan, D. W. & Moodie, M. (2020). The effectiveness of sedentary behaviour interventions on sitting time and screen time in children and adults: An umbrella review of systematic reviews. *International Journal of Behavioral Nutrition and Physical Activity*, *17*(1), 117.

Nieste, I., Franssen, W. M. A., Spaas, J., Bruckers, L., Savelberg, H. H. C. M. & Eijnde, B. O. (2021). Lifestyle interventions to reduce sedentary behaviour in clinical populations: A systematic review and meta-analysis of different strategies and effects on cardiometabolic health. *Preventive Medicine*, *148*, 106593.

Norha, J., Sjöros, T., Garthwaite, T., Laine, S., Saarenhovi, M., Kallio, P., Laitinen, K., Houttu, N., Vähä-Ypyä, H., Sievänen, H., Löyttyniemi, E., Vasankari, T., Knuuti, J., Kalliokoski, K. & Heinonen, I. (2023). Effects of reducing sedentary behavior on cardiorespiratory fitness in adults with metabolic syndrome: A 6-month RCT. *Scandinavian Journal of Medicine & Science in Sports, 33*(8), 1452–1461.

Noubiap, J. J., Nansseu, J. R., Lontchi-Yimagou, E., Nkeck, J. R., Nyaga, U. F., Ngouo, A. T., Tounouga, D. N., Tianyi, F.-L., Foka, A. J., Ndoadoumgue, A. L. & Bigna, J. J. (2022). Geographic distribution of metabolic syndrome and its components in the general adult population: A meta-analysis of global data from 28 million individuals. *Diabetes Research and Clinical Practice*, *188*, 109924.

Oh, R. C., Hustead, T. R., Ali, S. M. & Pantsari, M. W. (2017). Mildly Elevated Liver Transaminase Levels: Causes and Evaluation. *American Family Physician*, *96*(11), 709–715.

Paterson, C., Fryer, S., Zieff, G., Stone, K., Credeur, D. P., Barone Gibbs, B., Padilla, J., Parker, J. K. & Stoner, L. (2020). The Effects of Acute Exposure to Prolonged Sitting, With and Without Interruption, on Vascular Function Among Adults: A Meta-analysis. *Sports Medicine*, *50*(11), 1929–1942.

Patlak, C. S., Blasberg, R. G. & Fenstermacher, J. D. (1983). Graphical Evaluation of Blood-to-Brain Transfer Constants from Multiple-Time Uptake Data. *Journal of Cerebral Blood Flow & Metabolism*, *3*(1), 1–7.

Patterson, R., McNamara, E., Tainio, M., de Sa, T. H., Smith, A. D., Sharp, S. J., Edwards, P., Woodcock, J., Brage, S. & Wijndaele, K. (2018). Sedentary behaviour and risk of all-cause, cardiovascular and cancer mortality, and incident type 2 diabetes: A systematic review and dose response meta-analysis. *European Journal of Epidemiology*, *33*(9), 811–829.

Peachey, M. M., Richardson, J., V Tang, A., Dal-Bello Haas, V. & Gravesande, J. (2020). Environmental, behavioural and multicomponent interventions to reduce adults' sitting time: A systematic review and meta-analysis. *British Journal of Sports Medicine*, *54*(6), 315–325.

Pedisic, Z., Grunseit, A., Ding, D., Chau, J. Y., Banks, E., Stamatakis, E., Jalaludin, B. B. & Bauman, A. E. (2014). High sitting time or obesity: Which came first? Bidirectional association in a longitudinal study of 31,787 Australian adults. *Obesity*, *22*(10), 2126–2130.

Peltoniemi, P., Lonnroth, P., Laine, H., Oikonen, V., Tolvanen, T., Gronroos, T., Strindberg, L., Knuuti, J. & Nuutila, P. (2000). Lumped constant for [18F]fluorodeoxyglucose in skeletal muscles of obese and nonobese humans. *American Journal of Physiology - Endocrinology and Metabolism*, *279*(5), 1122–1130.

Pesola, A. J., Laukkanen, A., Tikkanen, O. & Finni, T. (2016). Heterogeneity of muscle activity during sedentary behavior. *Applied Physiology, Nutrition, and Metabolism*, *41*(11), 1155–1162.

Petersen, M. C. & Shulman, G. I. (2018). Mechanisms of Insulin Action and Insulin Resistance. *Physiological Reviews*, *98*(4), 2133–2223.

Petersen, M. C., Vatner, D. F., & Shulman, G. I. (2017). Regulation of hepatic glucose metabolism in health and disease. *Nature Reviews Endocrinology*, *13*(10), 572–587.

Pinto, A. J., Bergouignan, A., Dempsey, P. C., Roschel, H., Owen, N., Gualano, B. & Dunstan, D. W. (2023). Physiology of sedentary behavior. *Physiological Reviews*, *103*(4), 2561–2622.

Powell, C., Herring, M. P., Dowd, K. P., Donnelly, A. E, & Carson, B. P. (2018). The cross-sectional associations between objectively measured sedentary time and cardiometabolic health markers in adults – a systematic review with meta-analysis component. *Obesity Reviews*, *19*(3), 381–395.

Prince, S. A., Cardilli, L., Reed, J. L., Saunders, T. J., Kite, C., Douillette, K., Fournier, K. & Buckley, J. P. (2020). A comparison of self-reported and device measured sedentary behaviour in adults: A systematic review and meta-analysis. *The International Journal of Behavioral Nutrition and Physical Activity*, *17*(1), 31–33.

Prince, S. A., Dempsey, P. C., Reed, J. L., Rubin, L., Saunders, T. J., Ta, J., Tomkinson, G. R., Merucci, K. & Lang, J. J. (2024). The Effect of Sedentary Behaviour on Cardiorespiratory Fitness: A Systematic Review and Meta-Analysis. *Sports Medicine*, *54*(4), 997–1013.

Prior, S. J., Ryan, A. S., Stevenson, T. G. & Goldberg, A. P. (2014). Metabolic inflexibility during submaximal aerobic exercise is associated with glucose intolerance in obese older adults. *Obesity, 22*(2), 451–457.

Pulsford, R. M., Blackwell, J., Hillsdon, M. & Kos, K. (2017). Intermittent walking, but not standing, improves postprandial insulin and glucose relative to sustained sitting: A randomised cross-over study in inactive middle-aged men. *Journal of Science and Medicine in Sport*, *20*(3), 278–283.

Remie, C. M. E., Janssens, G. E., Bilet, L., van Weeghel, M., Duvivier, B. M. F. M., de Wit, V. H. W., Connell, N. J., Jörgensen, J. A., Schomakers, B. V., Schrauwen-Hinderling, V. B., Hoeks, J., Hesselink, M. K. C., Phielix, E., Houtkooper, R. H. & Schrauwen, P. (2021). Sitting less elicits metabolic responses similar to exercise and enhances insulin sensitivity in postmenopausal women. *Diabetologia*, *64*(12), 2817–2828.

Roden, M. & Shulman, G. I. (2019). The integrative biology of type 2 diabetes. *Nature*, *576*(7785), 51–60. Röder, P. V., Wu, B., Liu, Y., & Han, W. (2016). Pancreatic regulation of glucose homeostasis. *Experimental & Molecular Medicine*, *48*(3), e219–e219.

Romijn, J. A., Coyle, E. F., Sidossis, L. S., Gastaldelli, A., Horowitz, J. F., Endert, E., & Wolfe, R. R. (1993). Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *American Journal of Physiology-Endocrinology and Metabolism*, *265*(3), E380–E391.

Rosenberger, M. E., Haskell, W. L., Albinali, F., Mota, S., Nawyn, J. & Intille, S. (2013). Estimating Activity and Sedentary Behavior from an Accelerometer on the Hip or Wrist. *Medicine & Science in Sports & Exercise*, *45*(5), 964–975.

Ross, R., Blair, S., Arena, R., Church, T., Després, J-P., Franklin, B., Haskell, W., Kaminsky, L., Levine, B., Lavie, C., Myers, J., Niebauer, J., Sallis, R., Sawada, S., Sui, X., Wisløff, U., on behalf of the American Heart Association Physical Activity Committee of the Council on Lifestyle and Cardiometabolic Health; Council on Clinical Cardiology; Council on Epidemiology and Prevention; Council on Cardiovascular and Stroke Nursing; Council on Functional Genomics and Translational Biology; and Stroke Council. (2016). Importance of Assessing Cardiorespiratory Fitness in Clinical Practice: A Case for Fitness as a Clinical Vital Sign: A Scientific Statement From the American Heart Association. *Circulation, 134*(24), e653–e699.

Rudwill, F., O'Gorman, D., Lefai, E., Chery, I., Zahariev, A., Normand, S., Pagano, A. F., Chopard, A., Damiot, A., Laurens, C., Hodson, L., Canet-Soulas, E., Heer, M., Meuthen, P. F., Buehlmeier, J., Baecker, N., Meiller, L., Gauquelin-Koch, G., Blanc, S., … Bergouignan, A. (2018). Metabolic Inflexibility Is an Early Marker of Bed-Rest–Induced Glucose Intolerance Even When Fat Mass Is Stable. *The Journal of Clinical Endocrinology and Metabolism*, *103*(5), 1910–1920.

Rutland, M., Que, L. & Hassan, I. M. (2000). "FUR" – one size suits all. *European Journal of Nuclear Medicine*, *27*(11), 1708–1713.

Ryan, A. S. & Ortmeyer, H. K. (2019). Insulin suppression of fatty acid skeletal muscle enzyme activity in postmenopausal women, and improvements in metabolic flexibility and lipoprotein lipase with aerobic exercise and weight loss. *International Journal of Obesity (2005)*, *43*(2), 276–284.

Rynders, C. A., Blanc, S., DeJong, N., Bessesen, D. H. & Bergouignan, A. (2018). Sedentary behaviour is a key determinant of metabolic inflexibility. *The Journal of Physiology*, *596*(8), 1319–1330.

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., Ogurtsova, K., Shaw, J. E., Bright, D., Williams, R. & IDF Diabetes Atlas Committee. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9(th) edition. *Diabetes Research and Clinical Practice*, *157*, 107843.

Sagelv, E. H., Hopstock, L. A., Morseth, B., Hansen, B. H., Steene-Johannessen, J., Johansson, J., Nordström, A., Saint-Maurice, P. F., Løvsletten, O., Wilsgaard, T., Ekelund, U. & Tarp, J. (2023). Device-measured physical activity, sedentary time, and risk of all-cause mortality: An individual participant data analysis of four prospective cohort studies. *British Journal of Sports Medicine*, *57*(22), 1457–1463.

Saidj, M., Jørgensen, T., Jacobsen, R. K., Linneberg, A., Oppert, J.-M. & Aadahl, M. (2016). Work and leisure time sitting and inactivity: Effects on cardiorespiratory and metabolic health. *European Journal of Preventive Cardiology*, *23*(12), 1321–1329.

Sale, D. (1987). Influence of Exercise and Training on Motor Unit Activation. *Exercise and Sport Sciences Reviews*, *15*(1), 95–152.

San-Millán, I. (2023). The Key Role of Mitochondrial Function in Health and Disease. *Antioxidants*, *12*(4), 782.

San-Millán, I. & Brooks, G.A. (2018). Assessment of Metabolic Flexibility by Means of Measuring Blood Lactate, Fat, and Carbohydrate Oxidation Responses to Exercise in Professional Endurance Athletes and Less-Fit Individuals. *Sports Medicine*, *48*(2), 467–479.

Saunders, T. J., Atkinson, H. F., Burr, J., MacEwen, B., Skeaff, C. M. & Peddie, M. C. (2018). The Acute Metabolic and Vascular Impact of Interrupting Prolonged Sitting: A Systematic Review and Meta-Analysis. *Sports Medicine*, *48*(10), 2347–2366.

Saunders, T. J., McIsaac, T., Douillette, K., Gaulton, N., Hunter, S., Rhodes, R. E., Prince, S. A., Carson, V., Chaput, J.-P., Chastin, S. F., Giangregorio, L., Janssen, I., Katzmarzyk, P. T., Kho, M. E., Poitras, V. J., Powell, K. E., Ross, R., Ross-White, A., Tremblay, M. S. & Healy, G. N. (2020). Sedentary behaviour and health in adults: An overview of systematic reviews. *Applied Physiology, Nutrition, and Metabolism*, *45*(10 (Suppl. 2)), S197–S217.

Schenk, S., Harber, M. P., Shrivastava, C. R., Burant, C. F. & Horowitz, J. F. (2009). Improved insulin sensitivity after weight loss and exercise training is mediated by a reduction in plasma fatty acid mobilization, not enhanced oxidative capacity. *The Journal of Physiology*, *587*(20), 4949–4961.

Schrauwen, P., Van Aggel-Leijssen, D. P. C., Hul, G., Wagenmakers, A. J. M., Vidal, H., Saris, W. H. M. & Van Baak, M. A. (2002). The Effect of a 3-Month Low-Intensity Endurance Training Program on Fat Oxidation and Acetyl-CoA Carboxylase-2 Expression. *Diabetes*, *51*(7), 2220–2226.

Segura-Jiménez, V., Biddle, S. J. H., De Cocker, K., Khan, S. & Gavilán-Carrera, B. (2022). Where Does the Time Go? Displacement of Device-Measured Sedentary Time in Effective Sedentary Behaviour Interventions: Systematic Review and Meta-Analysis. *Sports Medicine*, *52*(9), 2177–2207.

Shiroma, E. J., Schepps, M. A., Harezlak, J., Chen, K. Y., Matthews, C. E., Koster, A., Caserotti, P., Glynn, N. W. & Harris, T. B. (2016). Daily physical activity patterns from hip- and wrist-worn accelerometers. *Physiological Measurement*, *37*(10), 1852–1861.

Shrestha, N., Grgic, J., Wiesner, G., Parker, A., Podnar, H., Bennie, J. A., Biddle, S. J. H. & Pedisic, Z. (2019). Effectiveness of interventions for reducing non-occupational sedentary behaviour in adults and older adults: A systematic review and meta-analysis. *British Journal of Sports Medicine*, *53*(19), 1206–1213.

Shrestha, N., Kukkonen-Harjula, K. T., Verbeek, J. H., Ijaz, S., Hermans, V. & Pedisic, Z. (2018). Workplace interventions for reducing sitting at work. *The Cochrane Database of Systematic Reviews*, *12*, CD010912.

Shur, N. F., Simpson, E. J., Crossland, H., Chivaka, P. K., Constantin, D., Cordon, S. M., Constantin-Teodosiu, D., Stephens, F. B., Lobo, D. N., Szewczyk, N., Narici, M., Prats, C., Macdonald, I. A. & Greenhaff, P. L. (2022). Human adaptation to immobilization: Novel insights of impacts on glucose disposal and fuel utilization. *Journal of Cachexia, Sarcopenia and Muscle*, *13*(6), 2999–3013.

Sievänen, H. & Kujala, U. M. (2017). Accelerometry-Simple, but challenging. *Scandinavian Journal of Medicine & Science in Sports*, *27*(6), 574–578.

Siiskonen T. (ed.). (2020). |*The average effective dose of Finns in 2018*| (STUK-A263).

Silva Rosa, S. C., Nayak, N., Caymo, A. M. & Gordon, J. W. (2020). Mechanisms of muscle insulin resistance and the cross-talk with liver and adipose tissue. *Physiological Reports*, *8*(19).

Sjöros, T., Heiskanen, M. A., Motiani, K. K., Löyttyniemi, E., Eskelinen, J., Virtanen, K. A., Savisto, N. J., Solin, O., Hannukainen, J. C. & Kalliokoski, K. (2018). Increased insulin-stimulated glucose uptake in both leg and

arm muscles after sprint interval and moderate-intensity training in subjects with type 2 diabetes or prediabetes. *Scandinavian Journal of Medicine & Science in Sports*, *28*(1), 77–87.

Sjöros, T., Laine, S., Garthwaite, T., Vähä-Ypyä, H., Koivumäki, M., Eskola, O., Löyttyniemi, E., Houttu, N., Laitinen, K., Kalliokoski, K., Sievänen, H., Vasankari, T., Knuuti, J. & Heinonen, I. (2023). The effects of a 6-month intervention aimed to reduce sedentary time on skeletal muscle insulin sensitivity: A randomized controlled trial. *American Journal of Physiology-Endocrinology and Metabolism*, *325*(2), E152–E162.

Sjöros, T., Laine, S., Garthwaite, T., Vähä-Ypyä, H., Löyttyniemi, E., Koivumäki, M., Houttu, N., Laitinen, K., Kalliokoski, K., Sievänen, H., Vasankari, T., Knuuti, J. & Heinonen, I. (2023). Reducing Sedentary Time and Whole-Body Insulin Sensitivity in Metabolic Syndrome – A 6-Month Randomized Controlled Trial. *Medicine & Science in Sports & Exercise*, *55*(3), 342–353.

Sjöros, T., Vähä-Ypyä, H., Laine, S., Garthwaite, T., Löyttyniemi, E., Sievänen, H., Kalliokoski, K., Knuuti, J., Vasankari, T. & Heinonen, I. (2021). 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*, *18*(9), 4950.

Smith, R. L., Soeters, M. R., Wüst, R. C. I. & Houtkooper, R. H. (2018). Metabolic flexibility as an adaptation to energy resources and requirements in health and disease. *Endocrine Reviews*, *39*(4), 489–517.

Sparks, L. M., Ukropcova, B., Smith, J., Pasarica, M., Hymel, D., Xie, H., Bray, G. A., Miles, J. M. & Smith, S. R. (2009). Relation of adipose tissue to metabolic flexibility. *Diabetes Research and Clinical Practice*, *83*(1), 32–43.

Strain, T., Flaxman S., Guthold R., Semenova E., Cowan M., Riley L. M., Bull F. C. & Stevens G.A (2024). National, Regional, and Global Trends in Insufficient Physical Activity among Adults from 2000 to 2022: A Pooled Analysis of 507 Population-Based Surveys with 5·7 Million Participants. *Lancet Global Health, 12(*8):e1232–1243.

Thie, J. A. (1995). Clarification of a Fractional Uptake Concept. *Journal of Nuclear Medicine*, *36*(4), 711–712.

Thorsen, I. K., Johansen, M. Y., Pilmark, N. S., Jespersen, N. Z., Brinkløv, C. F., Benatti, F. B., Dunstan, D. W., Karstoft, K., Pedersen, B. K. & Ried-Larsen, M. (2019). The effect of frequency of activity interruptions in prolonged sitting on postprandial glucose metabolism: A randomized crossover trial. *Metabolism, Clinical and Experimental*, *96*, 1–7.

Thraen-Borowski, K. M., Ellingson, L. D., Meyer, J. D. & Cadmus-Bertram, L. (2017). Nonworksite Interventions to Reduce Sedentary Behavior among Adults: A Systematic Review. *Translational Journal of the American College of Sports Medicine*, *2*(12), 68–78.

Tirrell, T. F., Cook, M. S., Carr, J. A., Lin, E., Ward, S. R. & Lieber, R. L. (2012). Human skeletal muscle biochemical diversity. *Journal of Experimental Biology*, *215*(15), 2551–2559.

Toledo, F. G. S., Menshikova, E. V., Azuma, K., Radiková, Z., Kelley, C. A., Ritov, V. B. & Kelley, D. E. (2008). Mitochondrial Capacity in Skeletal Muscle Is Not Stimulated by Weight Loss Despite Increases in Insulin Action and Decreases in Intramyocellular Lipid Content. *Diabetes*, *57*(4), 987–994.

Toledo, F. G. S., Menshikova, E. V., Ritov, V. B., Azuma, K., Radikova, Z., DeLany, J. & Kelley, D. E. (2007). Effects of Physical Activity and Weight Loss on Skeletal Muscle Mitochondria and Relationship With Glucose Control in Type 2 Diabetes. *Diabetes*, *56*(8), 2142–2147.

Toubro, S., Sørensen, T. I. A., Hindsberger, C., Christensen, N. J. & Astrup, A. (1998). Twenty-Four-Hour Respiratory Quotient: The Role of Diet and Familial Resemblance. *The Journal of Clinical Endocrinology & Metabolism*, *83*(8), 2758–2764.

Tremblay, M. S., Aubert, S., Barnes, J. D., Saunders, T. J., Carson, V., Latimer-Cheung, A. E., Chastin, S. F. M., Altenburg, T. M., Chinapaw, M. J. M. & SBRN Terminology Consensus Project Participants. (2017). Sedentary Behavior Research Network (SBRN)—Terminology Consensus Project process and outcome. *The International Journal of Behavioral Nutrition and Physical Activity*, *14*(1), 75–78.

Trenell, M. I., Hollingsworth, K. G., Lim, E. L. & Taylor, R. (2008). Increased Daily Walking Improves Lipid Oxidation Without Changes in Mitochondrial Function in Type 2 Diabetes. *Diabetes Care*, *31*(8), 1644–1649.

Ukropcova, B., McNeil, M., Sereda, O., de Jonge, L., Xie, H., Bray, G. A. & Smith, S. R. (2005). Dynamic changes in fat oxidation in human primary myocytes mirror metabolic characteristics of the donor. *Journal of Clinical Investigation*, *115*(7), 1934–1941.

Van Aggel-Leijssen, D. P. C., Saris, W. H. M., Wagenmakers, A. J. M., Senden, J. M. & Van Baak, M. A. (2002). Effect of exercise training at different intensities on fat metabolism of obese men. *Journal of Applied Physiology*, *92*(3), 1300–1309.

Vasankari, V., Halonen, J., Husu, P., Vähä-Ypyä, H., Tokola, K., Suni, J., Sievänen, H., Anttila, V., Airaksinen, J., Vasankari, T. & Hartikainen, J. (2019). Personalised eHealth intervention to increase physical activity and reduce sedentary behaviour in rehabiliation after cardiac operations: study protocol for the PACO randomised controlled trial (NCT03470246). *BMJ Open Sport & Exercise Medicine, 5*(1), e000539.

Vähä-Ypyä, H., Husu, P., Suni, J., Vasankari, T. & Sievänen, H. (2018). Reliable recognition of lying, sitting, and standing with a hip-worn accelerometer. *Scandinavian Journal of Medicine & Science in Sports*, *28*(3), 1092–1102.

Vähä-Ypyä, H., Sievänen, H., Husu, P., Tokola, K. & Vasankari, T. (2021). Intensity Paradox—Low-Fit People Are Physically Most Active in Terms of Their Fitness. *Sensors 21*(6), 2063.

Vähä-Ypyä, H., Vasankari, T., Husu, P., Mänttäri, A., Vuorimaa, T., Suni, J. & Sievänen, H. (2015). Validation of Cut-Points for Evaluating the Intensity of Physical Activity with Accelerometry-Based Mean Amplitude Deviation (MAD). *PLoS One*, *10*(8), e0134813.

Vähä-Ypyä, H., Vasankari, T., Husu, P., Suni, J. & Sievänen, H. (2015). A universal, accurate intensity-based classification of different physical activities using raw data of accelerometer. *Clinical Physiology and Functional Imaging*, *35*(1), 64–70.

Wilmot, E. G., Edwardson, C. L., Achana, F. A., Davies, M. J., Gorely, T., Gray, L. J., Khunti, K., Yates, T. & Biddle, S. J. (2012). Sedentary time in adults and the association with diabetes, cardiovascular disease and death: Systematic review and meta-analysis. *Diabetologia*, *55*(11), 2895–2905.

Winkler, E. A., Chastin, S. F., Eakin, E. G., Owen, N., Lamontagne, A. D., Moodie, M., Dempsey, P. C., Kingwell, B. A., Dunstan, D. W. & Healy, G. N. (2018). Cardiometabolic Impact of Changing Sitting, Standing, and Stepping in the Workplace. *Medicine and Science in Sports and Exercise*, *50*(3), 516–524.

World Health Organization. (2020). *WHO guidelines on physical activity and sedentary behaviour*. Geneva, World Health Organization. Available online https://www.who.int/publications/i/item/9789240015128. Accessed May 1, 2024.

World Health Organization. (2024). *Obesity and overweight*. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. Accessed May 1, 2024.

Wu, J., Fu, Y., Chen, D., Zhang, H., Xue, E., Shao, J., Tang, L., Zhao, B., Lai, C. & Ye, Z. (2023). Sedentary behavior patterns and the risk of non-communicable diseases and all-cause mortality: A systematic review and meta-analysis. *International Journal of Nursing Studies*, *146*, 104563.

Wu, J., Zhang, H., Yang, L., Shao, J., Chen, D., Cui, N., Tang, L., Fu, Y., Xue, E., Lai, C. & Ye, Z. (2022). Sedentary time and the risk of metabolic syndrome: A systematic review and dose–response meta-analysis. *Obesity Reviews*, *23*(12), e13510.

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

ISBN 978-951-29-9841-8 (PRINT)
ISBN 978-951-29-9842-5 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)

Painosalama, Turku, Finland 2024