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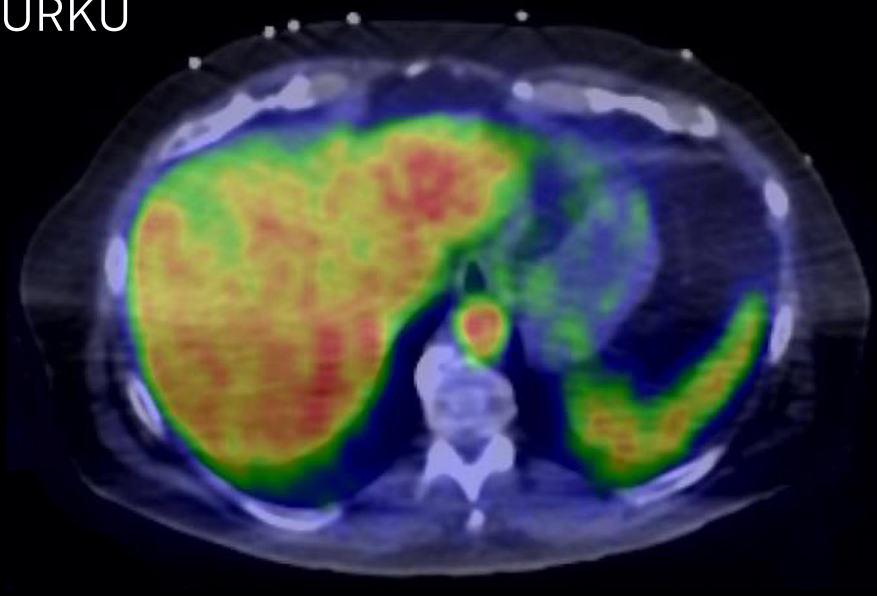

LIFESTYLE FACTORS, LIVER INSULIN SENSITIVITY, LIVER FAT, AND LIVER ENZYMES IN ADULTS WITH METABOLIC SYNDROME

Saara Laine

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"Curiosity is one of the great secrets of happiness."

Bryant H. McGill

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SAARA LAINE: Lifestyle factors, liver insulin sensitivity, liver fat, and liver enzymes in adults with metabolic syndrome
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ABSTRACT

This PhD work aims to address the rising rates of physical inactivity and obesity-related liver diseases by exploring the relationships between sedentary behavior, physical activity, dietary intake, fitness, liver insulin sensitivity, liver fat content, and liver enzyme levels in 44 middle-aged, sedentary adults with metabolic syndrome. The work is structured around four interconnected studies, all framed within a cohesive research design. It has two primary objectives: to investigate these relationships and to assess the effects of reducing sedentary behavior on liver health over a six-month randomized controlled trial.

Sedentary behavior and physical activity were measured using hip-worn accelerometers, liver insulin sensitivity by positron emission tomography during a hyperinsulinemic-euglycemic clamp, and liver fat content by magnetic resonance spectroscopy. Additional measurements included fasting blood samples for liver enzyme levels, maximal bicycle ergometry for fitness assessment, air displacement plethysmography for body composition analysis, and food diaries for dietary tracking.

The results indicated that sedentary behavior, physical activity, and fitness levels were not significantly associated with liver fat or liver enzyme levels. Instead, overall body adiposity demonstrated a strong correlation, highlighting the importance of maintaining a healthy body composition. Dietary factors also played a significant role; higher protein intake, reduced carbohydrate and sugar consumption, and increased unsaturated fatty acids and fiber were beneficially associated with liver insulin sensitivity.

Although increasing daily standing time enhanced liver insulin sensitivity, the intervention aimed at reducing sedentary behavior by one hour daily did not result in notable changes in liver health markers. Suggesting that a further reduction in sedentary time and/or more extensive or intense physical activity may be necessary. However, we demonstrated that successfully reducing sedentary behavior reduces liver enzyme levels, particularly alanine aminotransferase. This study offers new insights into how reducing sedentary behavior impacts liver health, contributing to future intervention strategies for metabolic syndrome.

KEYWORDS: sedentary behavior, physical activity, nutrition, liver fat, liver glucose uptake, endogenous glucose production, liver enzymes, metabolic syndrome.

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
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Väitöskirja, 194 s.
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TIIVISTELMÄ

Fyysinen inaktiivisuus ja ylipainoon liittyvät maksasairaudet ovat hälyttävästi kasvussa. Tämän väitöstutkimuksen tavoitteena oli tutkia paikallaanolon, fyysisen aktiivisuuden, ravinnon saannin, kestävyyskunnon ja maksan terveysmarkkereiden välisiä yhteyksiä 44:llä keski-ikäisellä vähän liikkuvilla aikuisilla, joilla on metabolinen oireyhtymä. Tutkimuksella oli kaksi päätavoitetta: tutkia näitä yhteyksiä sekä arvioida paikallaanolon vähentämisen vaikutuksia maksaterveyteen 6kk satunnaistetussa kontrolloidussa tutkimuksessa. Paikallaanoloa ja fyysistä aktiivisuutta mitattiin lantiolla pidettävillä kiihtyvyysanturimittareilla, maksan insuliiniherkkyys määritettiin positroniemissiotomografialla hyperinsulineemis-euglykeemisen clamp-tutkimuksen aikana sekä maksan rasvapitoisuus määritettiin magneettiresonanssispektroskopialla. Maksaentsyymitasot määritettiin paastoverinäytteistä, kestävyyskunto maksimaalisella polkupyöräergometrialla, kehonkoostumus ilman syrjäyttämiseen perustuvalla pletysmografialla ja ruokavaliota seurattiin ruokapäiväkirjojen avulla.

Paikallaanolo, fyysinen aktiivisuus ja kestävyyskunto eivät olleet merkittävästi yhteydessä maksan rasva- tai maksaentsyymitasoihin. Sen sijaan kehon rasvaisuus osoitti vahvan korrelaation, korostaen terveen kehonkoostumuksen osuutta maksaterveyden kannalta. Ruokavaliotekijöillä oli myös merkittävä rooli; suurempi proteiinin saanti, vähäinen hiilihydraattien ja sokerin kulutus sekä lisääntynyt tyydyttymättömien rasvahappojen sekä kuitujen saanti olivat hyödyllisiä maksaterveydelle. Päivittäinen seisomisaika oli positiivisesti yhteydessä maksan insuliiniherkkyyteen, mutta, interventio, jonka tarkoituksena oli vähentää istumista tunnin päivässä, ei johtanut merkittäviin muutoksiin maksan terveysmarkkereissa. Näin ollen hyödyllisten muutosten aikaansaamiseksi fyysisen aktiivisuuden keston ja/tai intensiteetin tulisi olla suurempaa. Istumisen vähentäminen voi kuitenkin johtaa parempiin maksaentsyymitasoihin, erityisesti alaniini aminotransferaasin. Tämä tutkimus tarjoaa uusia näkemyksiä siitä, kuinka paikallaanolon vähentäminen vaikuttaa maksaterveyteen, edistäen näin metabolisen oireyhtymään liittyviä tulevia interventiostrategioita.

AVAINSANAT: paikallaanolo, fyysinen aktiivisuus, maksan rasvoittuminen, maksan glukoosinotto, endogeeninen glukoosintuotto, maksaentsyymit, metabolinen oireyhtymä

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Abbreviations

[18F] FDG	2-deoxy-2-(18F) fluoro-D-glucose
2PD	two-point Dixon
ALT	alanine aminotransferase
APE	angle for posture estimation
AST	aspartate aminotransferase
BMI	body mass index
CON	control
CT	computed tomography
DBP	diastolic blood pressure
EGP	endogenous glucose production
EI	energy intake
FFM	fat free mass
FFQ	food frequency questionnaire
GGT	γ-glutamyltransferase
HDL-C	high-density lipoprotein cholesterol
HEC	hyperinsulinemic-euglycemic clamp
HOMA-IR	homeostatic model assessment for insulin resistance
INT	intervention
LDL-C	low-density lipoprotein cholesterol
LPA	light physical activity
LPA (%)	daily proportion of light physical activity
MAD	mean amplitude deviation
MASLD	metabolic dysfunction-associated steatotic liver disease
MET	metabolic equivalent
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MUFA	monounsaturated fatty acids
M-value	whole-body insulin sensitivity
MVPA	moderate-to-vigorous physical activity
MVPA (%)	daily proportion of moderate-to-vigorous physical activity
NAFLD	non-alcoholic fatty liver disease

NEFA	non-esterified fatty acids
PA	physical activity
PET	positron emission tomography
PUFA	polyunsaturated fatty acids
ROI	region of interest
ROS	reactive oxygen species
SB	sedentary behavior
SBP	systolic blood pressure
SFA	saturated fatty acids
VLDL	very low-density lipoprotein
VO2max	maximal oxygen consumption
WC	waist circumference
WIDF	water-insoluble dietary fiber
Wmax	maximal load

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 Laine S, Sjöros T, Vähä-Ypyä H, Garthwaite T, Löyttyniemi E, Sievänen H, Vasankari T, Knuuti J, and Heinonen I. **Body adiposity, but not elements of objectively measured sedentary behavior or physical activity, is associated with circulating liver enzymes in adults with overweight and obesity**. *Frontiers in Endocrinology,* 2021; 12: 655756.

II Laine S, Sjöros T, Garthwaite T, Saarenhovi M, Kallio P, Löyttyniemi E, Vähä-Ypyä H, Sievänen H, Vasankari T, Laitinen K, Houttu N, Saukko E, Knuuti J, Saunavaara V, and Heinonen I. **Relationship between liver fat content and lifestyle factors in adults with metabolic syndrome.** *Scientific Reports*, 2022; 12: 17428.

III Laine S, Sjöros T, Garthwaite T, Honka M-J, Löyttyniemi E, Eskola O, Saarenhovi M, Kallio P, Koivumäki M, Vähä-Ypyä H, Sievänen H, Vasankari T, Hirvonen J, Laitinen K, Houttu N, Kalliokoski K, Saunavaara V, Knuuti J, and Heinonen I. **Daily standing time, dietary fiber, and intake of unsaturated fatty acids are beneficially associated with hepatic insulin sensitivity in adults with metabolic syndrome**. *Frontiers in Endocrinology*, 2024; 15: 1272886.

IV Laine S, Sjöros T, Garthwaite T, Honka M-J, Löyttyniemi E, Eskola O, Koivumäki M, Vähä-Ypyä H, Sievänen H, Vasankari T, Hirvonen J, Laitinen K, Houttu N, Kalliokoski K, Saunavaara V, Knuuti J, and Heinonen I. **Effects of sedentary behavior reduction on liver insulin sensitivity, liver fat content, and liver enzymes levels – A six-month randomized controlled trial**. *American Journal of Physiology-Endocrinology and Metabolism,* 2025; 328(6): 00406.

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1 Introduction

The decreasing levels of physical activity represent a significant global public health challenge. The World Health Organization (WHO) has established an ambitious target to reduce the prevalence of insufficient physical activity by 10% by 2025. However, data indicate this goal is unlikely to be achieved without significant changes to current habits and policies (Guthold et al., 2018). Therefore, it is essential to prioritize and implement effective strategies that promote increased physical activity across various populations. In Finland, alarming statistics reveal that adults spend over half their waking hours engaged in sedentary behaviors, such as sitting or lying down. Moreover, only about 20% of adults meet the recommended guidelines for physical exercise (Husu et al., 2018), which typically include at least 150 minutes of moderate-intensity aerobic activity per week. Emerging research has begun to highlight that, in addition to structured moderate or high-intensity exercise, light-intensity physical activity that replaces sedentary behaviors could reduce the risk for obesity, diabetes, cardiovascular disease, certain types of cancer, and even premature mortality (Dunstan et al., 2010, 2012; Dunstan & Owen, 2012; Schmid & Leitzmann, 2014).

Research has shown an association between prolonged sedentary time and higher liver fat (Bowden Davies et al., 2018; Helajärvi et al., 2015; Henson et al., 2015, 2018), visceral fat, and pericardial and intrathoracic fat (Henson et al., 2015, 2018; Larsen et al., 2014), particularly in individuals at increased risk for obesity and diabetes. Additionally, a healthy lifestyle is closely linked to a lower risk of increased liver fat content (Yuan et al., 2024). On the other hand, individuals with higher liver fat content who maintain an unhealthy lifestyle have been shown to have a significantly higher risk of major cardiovascular events and all-cause mortality (Wu et al., 2023). Several studies have demonstrated that substituting sedentary time with moderate-to-vigorous physical activity (MVPA) is associated with beneficial cardiometabolic health, including improvements in overall body composition, body mass index (BMI), waist circumference (WC), body fat percentage, and favorable lipid profiles (Grgic et al., 2018; Peterson et al., 2014; Scheers et al., 2013; Wood et al., 1985). Similar benefits have been observed when light-intensity physical activity (non-exercise activities) replaces sedentary behaviors (Aadahl et al., 2014; Danquah

et al., 2017; E F Graves et al., 2015; Grgic et al., 2018; Jin et al., 2018; Wiseman et al., 2014). However, such findings predominantly come from cross-sectional studies or short-term laboratory interventions. This limitation hampers our ability to draw clear causal conclusions about the impact of these changes on long-term health outcomes.

Metabolic syndrome is characterized by a cluster of interconnected risk factors, including abdominal obesity, hypertension, elevated blood glucose levels, and dyslipidemia (Alberti et al., 2009), all of which contribute to a heightened risk of developing obesity-related liver diseases, such as metabolic dysfunction-associated steatotic liver disease (MASLD). MASLD has been identified as the most prevalent chronic liver disease worldwide, impacting over 30% of the adult population (Miao et al., 2024), and can lead to severe health complications, including liver inflammation, fibrosis, and even liver cancer (National Institute of Diabetes and Digestive and Kidney Diseases, 2021a). Previous meta-analysis suggests that sedentary behavior can independently contribute to the risk of developing metabolic syndrome (Edwardson et al., 2012). For instance, bed rest studies have shown prolonged sedentary behavior is associated with decreased lipolysis and significant impairments in whole-body insulin sensitivity (Alibegovic et al., 2009; Højbjerre et al., 2010). In contrast, regular physical activity and exercise training have enhanced insulin sensitivity across the body and skeletal muscles (Eskelinen et al., 2015; Fisher et al., 2019) and positively affect body composition (Bellicha et al., 2021). However, further investigation is needed to understand how reductions in sedentary behavior specifically impact liver health, including organ-specific insulin sensitivity and liver fat accumulation.

Consequently, this PhD work seeks to address critical gaps in the existing literature by investigating the effects of reducing sedentary behavior on liver health (liver fat, liver insulin sensitivity, and liver enzymes) among inactive, sedentary adults with metabolic syndrome. The work is structured around four interconnected studies, all framed within a cohesive research design. The first study was executed cross-sectionally during the initial screening, while the second and third studies were conducted at the baseline of the intervention. The final study represented the sedentary behavior reduction intervention. The intervention was designed to promote increased engagement in light-intensity physical activities such as standing, walking, and household chores, encouraging participants to adopt a more active lifestyle. In addition to examining the primary effects on liver health, the work also explores the relationships between liver health markers, sedentary behavior, levels of physical activity, dietary habits (such as daily dietary intakes of nutrients and total energy intake), body composition metrics (such as body fat percentage, BMI, and WC), cardiorespiratory fitness and various metabolic indicators (such as blood glucose levels and lipid profiles). Consequently, this research aims to evaluate how lifestyle

modifications affect liver health, providing insights that could improve treatment strategies for individuals with metabolic syndrome. By investigating interconnected factors, the study seeks to enhance our understanding of the relationship between lifestyle choices and liver health. Ultimately, this knowledge may lead to better intervention strategies for metabolic syndrome and address the risks of sedentary lifestyles in today's inactive world.

2 Review of the Literature

2.1 Lifestyle factors: sedentary behavior, physical activity, and nutrition

2.1.1 Definition of sedentary behavior, physical activity, and nutrition

Sedentary behavior is any waking activity requiring an energy expenditure of 1.5 metabolic equivalents (METs) or lower. This behavior usually occurs while a person is sitting, reclining, or lying down (Tremblay et al., 2017). In very light-intensity activities, the maximum heart rate is below 57% (American College of Sports Medicine, 2000). The MET is a standardized measurement used to quantify the oxygen consumption rate during various physical activities. One MET is a unit used to quantify the energy expenditure associated with physical activity. It is defined as the energy cost of physical activity that amounts to 1 kilocalorie per kilogram of body weight per hour (1 kcal/kg/hour), estimating the energy expenditure of a wide range of activities compared to a state of rest, represented by sitting quietly (Ainsworth et al., 2000). Additionally, one MET corresponds to an oxygen uptake of 3.5 milliliters per kilogram of body weight per minute (mL/kg/min) (Jetté et al., 1990). This measurement allows a consistent way to assess and compare the energy expenditure of different activities across individuals of varying body weights.

Sedentary behavior encompasses many common activities that often dominate daily routines. Essentially, any time a person is seated or horizontal, they are considered engaging in sedentary behavior. These behaviors can vary widely and include watching television and playing video games, which can be both immersive and lengthy, and using computers, whether for work, communication, or leisure, collectively referred to as "screen time." Additionally, sedentary behavior includes driving automobiles, where individuals remain stationary for extended periods, and reading books or articles, which, although mentally engaging, still involve remaining sedentary (Tremblay et al., 2017).

Physical activity is any bodily movement that the skeletal muscles generate that results in energy expenditure (Caspersen et al., 1985). This broad definition encompasses a wide range of activities, including those performed during leisure

time, commuting to and from various locations, and engaging in tasks associated with work or household duties. Light physical activity encompasses any form of movement or exercise that results in an energy expenditure of less than 3.0 METs (Ainsworth et al., 2000) and a maximal heart rate between 57–63% (American College of Sports Medicine, 2000). This includes activities such as walking at a leisurely pace, light gardening, and, for example, standing and performing household chores such as washing dishes. These activities are generally low-intensity and can be easily integrated into daily routines, making them accessible for individuals looking to maintain or improve their overall health without strenuous exercise. Moderate physical activity is any exercise or movement resulting in an energy expenditure ranging from 3.0 to 6.0 METs (Ainsworth et al., 2000) and a maximal heart rate between 64–76% (American College of Sports Medicine, 2000). This activity level typically includes, for example, brisk walking and cycling at a leisurely pace. During moderate physical activity, individuals can generally talk but not sing comfortably, indicating that the intensity is enough to elevate the heart rate and breathing without being overly strenuous. Engaging in such activities regularly is beneficial for maintaining overall health and fitness. Lastly, vigorous physical activity is defined as any form of exercise or movement that involves a significant level of exertion, resulting in an energy expenditure that exceeds 6.0 METs (Ainsworth et al., 2000) and a maximal heart rate between 77–95% (American College of Sports Medicine, 2000). Activities categorized as vigorous typically include high-intensity sports such as running, cycling at a fast pace, and swimming vigorously. Such activities can improve cardiovascular fitness, build strength, and enhance overall health.

Figure 1 offers an overview of the different MET values and percentages of maximal heart rate associated with various intensities of sedentary behavior and physical activity. It categorizes activities into four levels: sedentary, light, moderate, and vigorous. This categorization highlights how each intensity level corresponds to specific MET values and maximal heart rate percentages.

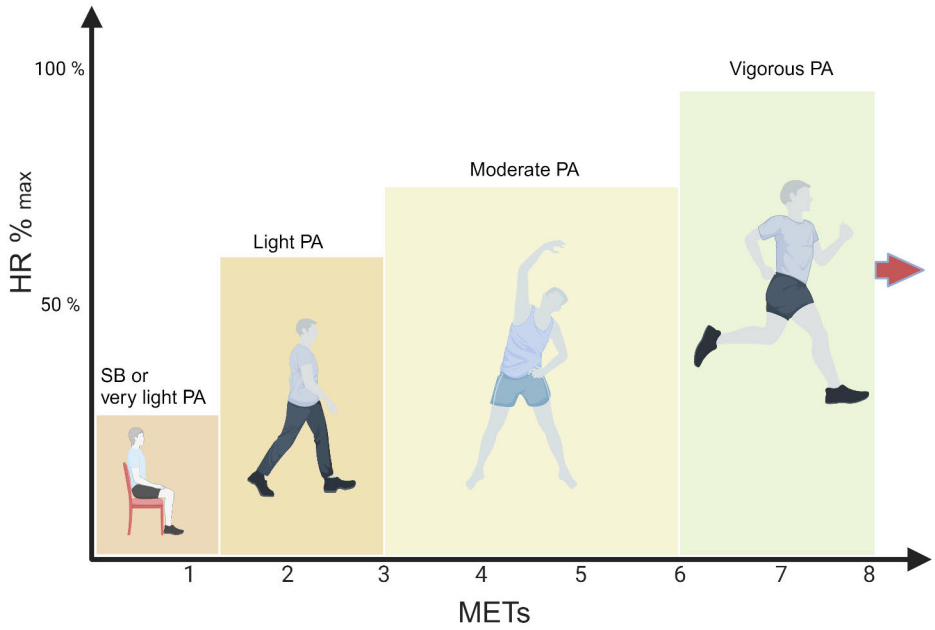

Figure 1. Overview of MET values and maximal heart rate percentages for different intensities of sedentary behavior (SB) and physical activity (PA). Activities are categorized into four levels: sedentary, light, moderate, and vigorous. This figure illustrates the association between each intensity level, its corresponding MET values, and maximal heart rate percentages. The figure is made with BioRender and modified from the image made by SBRN (Sedentary Behaviour Research Network). Accessed Dec. 25, 2024: sedentarybehaviour.org/.

Physical inactivity is a level of physical engagement that does not meet established guidelines for physical activity (Bull et al., 2020). In Finland, adults (aged 18 to 64) are encouraged to adhere to several key recommendations to promote overall health and well-being (The UKK Institute for Health Promotion Research, 2019). Foremost, it is essential to prioritize restorative sleep by aiming for 7 to 9 hours per night. Establishing a consistent sleep schedule and creating a conducive sleep environment can significantly enhance the quality of rest. It is advisable to incorporate breaks from sedentary behavior by standing, stretching, or walking every 30 to 60 minutes. Such practices contribute to improved circulation and the reduction of fatigue. Furthermore, individuals should engage in light physical activities, such as walking or gardening, throughout the day. The recommendation is to achieve at least 150 minutes of moderate-intensity aerobic activity each week, which may include brisk walking or swimming or 75 minutes of vigorous-intensity activity, such as running. Additionally, muscle-strengthening exercises targeting all major muscle groups should be performed at least twice weekly. Suitable activities may include weight lifting, resistance training, and bodyweight exercises. Moreover, balance-

enhancing activities, such as yoga or tai chi, are recommended to improve stability and mitigate the risk of falls (The UKK Institute for Health Promotion Research, 2019).

Nutrition refers to how we eat, digest, and utilize food to support essential growth, development, and health maintenance functions. Macronutrients are the main nutrients that provide energy. They include carbohydrates, proteins, and fats (National Institute of Environmental Health Sciences, 2025). Carbohydrates are important, serving as a key energy source for various bodily functions. When consumed, carbohydrates are broken down into glucose, which is then utilized by the body's cells for energy or stored in the liver and muscle tissue. In addition to providing energy, carbohydrates are vital in managing blood glucose levels, ensuring that insulin is released appropriately to maintain balance in the bloodstream (Holes et al., 2023). Proteins are vital for growth, tissue repair, and the production of enzymes and hormones. They are made up of amino acids, which are the building blocks of the body's tissues. Humans utilize twenty amino acids, of which nine are essential, as they cannot be synthesized by the body. Consequently, dietary protein is a crucial source of these amino acids, integral to synthesizing various hormones and other vital molecules (LaPelusa & Kaushik, 2022).

Fats serve a critical function in the body, including storing energy, producing hormones, and absorbing fat-soluble vitamins (Field & Robinson, 2019). Fats can be categorized into three main types: saturated, unsaturated, and trans fats. Saturated fats, commonly found in animal products, can raise "bad" LDL-C levels in the blood when consumed excessively, which may increase the risk of heart disease (Silverman et al., 2016). Unsaturated fats, which include monounsaturated and polyunsaturated fats found in foods like olive oil, nuts, and fatty fish, are generally considered beneficial for health. These fats can help to lower LDL-C levels (Mensink & Katan, 1989). Trans fats, on the other hand, are artificially created through hydrogenation and are found in some processed foods. They are known to have a detrimental effect on cardiovascular health and should be limited in the diet (de Souza et al., 2015). The human body requires two essential fatty acids: omega-3 and omega-6. These fatty acids are polyunsaturated fats that play crucial roles in various bodily functions, including brain health, inflammation regulation, and heart health. However, since the body cannot produce these fatty acids on its own, they must be obtained from dietary sources (National Institutes of Health, 2024)

Micronutrients include vitamins and minerals, which are essential for various biochemical processes in the body. Although required in smaller amounts, they are crucial for maintaining health, supporting immune function, and aiding energy production from macronutrients (National Institute of Environmental Health Sciences, 2025). Proper water intake is also important for staying hydrated, regulating body temperature, and aiding physiological processes like digestion

(Popkin et al., 2010). National dietary guidelines have tailored instructions for different age groups and individuals with specific dietary needs to promote healthy eating habits and lower the risk of chronic diseases.

2.1.2 Measuring methods of physical activity, sedentary behavior, and dietary intake

Self-report instruments, such as surveys and questionnaires, remain among the most widely used methods for assessing physical activity and sedentary behavior (Atkin et al., 2012; Castillo-Retamal & Hinckson, 2011). Commonly utilized tools include the International Physical Activity Questionnaire (IPAQ) and the Global Physical Activity Questionnaire (GPAQ). These tools facilitate data collection across diverse populations and serve various cultural contexts. Self-report surveys are cost-effective, easy to administer, and capable of capturing multiple activities, including frequency and duration. They enable researchers to gather contextual information about activity types, enhancing their understanding of lifestyle patterns. However, despite their practicality, self-reports are subject to biases. Participants often struggle with recall accuracy and may overestimate physical activities while underreporting sedentary behavior, leading to potential inaccuracies in data (Sallis & Saelens, 2000).

Objective measurement methods, such as accelerometers, provide valuable insights into physical activity intensity, duration, and frequency. These devices, worn on the body, record real-time movement, offering a precise account of activity levels. Accelerometers are recognized for their accuracy in quantifying physical activity and can capture subtle variations in movement patterns. They allow continuous monitoring over extended periods, providing a more representative view of an individual's activity behavior. However, the effectiveness of objective measurements can vary based on several factors, including the type of accelerometer used, the placement of the device, and the population being studied. Common placements for accelerometers include the wrist, hip, ankle, thigh, and even embedded in clothing. Each placement has its advantages and disadvantages. For example, while a wrist placement may be more comfortable and convenient for users, it may not be as effective in capturing lower body movements as a hip placement.

One of the challenges of using hip-worn accelerometers is their difficulty distinguishing between standing and sitting postures. These positions can produce similar movement patterns, especially when a person is relatively still. However, recent technological advancements have demonstrated that body posture can be measured with a single tri-axial accelerometer placed on the hip using validated algorithms (Sievänen & Kujala, 2017; Vähä-Ypyä et al., 2018). The Angle for Posture Estimation (APE) method enables the assessment of body posture by

examining the orientation of an accelerometer in relation to the gravitational vector. This method assumes the gravitational vector is constant and that individuals maintain an upright posture while walking. The APE method detects walking patterns, eliminating the need for precise orientation control of the accelerometer on the hip (Vähä-Ypyä et al., 2018). This approach offers a significant advantage over traditional methods that often require multiple devices on the body.

Additionally, thigh-worn accelerometers have shown promise in improving the differentiation of movement postures due to their proximity to the body's center of mass. By placing the accelerometer higher on the thigh, researchers may capture leg movements more effectively, aiding data collection on activities such as walking, running, or transitioning between sitting and standing postures (Matthew L Stevens et al., 2020). However, the use of thigh accelerometers is often limited by practicality; these devices may require specific clothing or fastening methods that are not as widely accepted or convenient for participants compared to waist-worn devices. Furthermore, all accelerometers can also struggle to differentiate between certain activity types, such as cycling and resistance training, which may involve minimal movement but significant exertion (Gibbs et al., 2015). These factors highlight the importance of careful consideration when selecting a monitoring device and its placement to ensure accurate data collection. Despite their benefits, accelerometers can be costly and require ongoing maintenance and technical expertise for data analysis. Additionally, adherence issues may arise if participants do not wear the device consistently, which can impact the reliability of the data obtained.

The 24-hour dietary recall method is widely used to assess dietary intake by asking participants to report all foods and beverages consumed in the past 24 hours through a structured interview. It provides a detailed account of food consumption and is easy to administer with minimal training. This method is effective for diverse populations and clinical settings but relies on the participant's ability to recall their intake, which can introduce bias. Daily dietary patterns can vary, so researchers often use multiple recalls, which can increase time and resource demands. Food frequency questionnaires (FFQ) assess habitual nutritional intake over time, typically from a month to a year. Participants report how often they consume specific food items, enabling the capture of long-term dietary patterns and nutritional status. FFQs are easy to administer to large populations and can be tailored for specific research needs or cultural contexts. They allow for comparability across studies; however, they may not accurately reflect recent dietary changes or portion sizes, as participants often find it difficult to estimate these. Regular updates to the FFQ content are essential to keep pace with changing nutritional trends (Dao et al., 2019).

Food diaries involve participants recording their food and beverage intake over a specific period, varying from several days to a week. This method requires

participants to document their consumption in real-time. Dietary records facilitate detailed dietary assessments, providing quantitative food intake and portion size data. Compared to recall methods, they reduce recall bias, as participants log their intake immediately after consumption. This method can yield rich, context-specific data about eating behaviors. The success of dietary records relies on participant compliance, which can be challenging. Participants may underreport intake due to the perceived burden of recording or modifying their behavior because they monitor their diet. Additionally, processing and analyzing dietary records can be time-consuming for researchers (Dao et al., 2019).

Direct observation involves trained observers recording physical activity, sedentary behavior, and dietary intake within specific contexts, such as schools, workplaces, or homes. This method provides in-depth qualitative insights, capturing detailed contextual factors influencing individuals' behaviors. Direct observation allows for a nuanced understanding of activity patterns for physical activity, highlighting specific behaviors and environmental influences that contribute to physical inactivity. In the context of dietary assessment, it offers accurate data, particularly regarding portion sizes, without relying on self-reporting. However, this method can be time-intensive and requires significant resources, making it challenging to apply across larger populations (Sylvia et al., 2014). Observer bias may also affect data reliability, and the presence of observers could alter participants' behaviors.

As technology advances, digital self-monitoring tools and mobile applications for tracking physical activity and dietary intake have become popular. These user-friendly platforms enable individuals to log their activities and meals easily, often incorporating features such as real-time feedback, barcode scanning for nutritional information, and social networking capabilities to foster community engagement. While these innovations provide personalized insights and motivation to maintain or increase activity levels and improve dietary habits, accessibility and privacy concerns persist. Not everyone has access to smartphones or the internet, leading to disparities in who benefits from these technologies. Additionally, the accuracy of the data collected is not standardized and may vary depending on the device used (Negreiros et al., 2022).

In summary, assessing physical activity, sedentary behavior, and dietary intake requires a combined approach to understand these behaviors comprehensively. Self-reported measures, though widely accessible, are susceptible to bias and inaccuracies. In contrast, objective methods like direct observation and digital tools offer more precise data but may involve significant resource commitments. Integrating diverse measurement approaches will enhance intervention effectiveness and contribute to a more profound understanding of the interplay between physical activity, sedentary behavior, and dietary practices in various populations.

2.1.3 The impact of sedentary behavior, physical activity, and nutrition on overall health and well-being

Sedentary lifestyles have become a significant public health concern due to their detrimental impact on overall health and well-being. People who engage in little to no physical activity are at a considerably higher risk of experiencing various health issues and are at risk of developing non-communicable diseases. Specifically, studies show that sedentary behavior can lead to increased mortality rates from all causes, effectively increasing the risk of developing severe conditions such as cardiovascular diseases and type 2 diabetes (Onagbiye et al., 2024; Patterson et al., 2018). Furthermore, individuals who lead sedentary lives are more likely to suffer from obesity, which itself is a risk factor for a range of other health problems, such as metabolic syndrome (Engin, 2017) and obesity-related liver diseases: metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD) and metabolic dysfunction-associated steatohepatitis (MASH), formerly known as non-alcoholic steatohepatitis (NASH) (Ong & Younossi, 2007).

Research also indicates that prolonged inactivity is associated with a higher likelihood of developing specific types of cancer, such as colon cancer (Cong et al., 2014). In addition, sedentary behavior can contribute to high blood pressure (P. H. Lee & Wong, 2015), osteoporosis, and lipid disorders (Park et al., 2020), further complicating an individual's health profile. Mental health is also affected, with increased rates of depression observed among those who lead sedentary lives (Teychenne et al., 2010). A recent study has indicated that approximately one-third (31%) of the global adult population is physically inactive, equating to 1.8 billion individuals (Strain et al., 2024). This finding highlights sedentary behavior as one of the most significant public health challenges facing society today, and the concern is not limited to adults; most adolescents globally are also insufficiently active (Guthold et al., 2020), which can have profound long-term implications for their health. Lack of physical activity in childhood is linked to a higher risk of obesity, poor physical fitness, and an increased likelihood of chronic diseases later in life (WHO Consultation on Obesity (1999: Geneva & World Health Organization, 2000).

It is well known that regular physical activity is vital for preventing and managing non-communicable diseases. Consistent exercise reduces the risk of these conditions and can help individuals who have already been diagnosed manage their symptoms more effectively. In addition to its physical health benefits, regular exercise is essential for maintaining a healthy body weight. It helps burn calories and build muscle, which can lead to weight loss or weight maintenance. Beyond physical health, exercise is linked to improved mental health by reducing symptoms of anxiety and depression, enhancing mood, and promoting cognitive function

(Anderson & Shivakumar, 2013; Kvam et al., 2016). Participants often report an overall improvement in quality of life and a greater sense of well-being when they include physical activity in their daily routines (Marquez et al., 2020).

Popular activities for staying active include, for example, brisk walking, cycling, sports, dancing, hiking, and playing games. These can be tailored to any skill level and are enjoyable for people of all ages. Every day tasks like cleaning, gardening, and grocery shopping also contribute to physical activity. Additionally, jobs involving physical labor provide additional opportunities to stay active. Incorporating these movements into daily life can greatly enhance health and well-being.

Research consistently demonstrates that nutritious, healthy eating significantly influences overall well-being and health. Studies have shown that a balanced diet, which includes fruits, vegetables, whole grains, lean proteins, and healthy fats, is linked to numerous health benefits. For instance, a review from 2020 summarized that a Mediterranean diet—rich in olive oil, fish, and fresh produce—improves cardiovascular, metabolic, and mental health (Ventriglio et al., 2020). Moreover, another review found that diets high in processed foods and sugars are associated with increased risks of depression and anxiety (Kris-Etherton et al., 2021). In contrast, diets rich in omega-3 fatty acids, such as those found in fish, can improve cognitive functions, such as memory and learning (Dighriri et al., 2022). Furthermore, maintaining a healthy diet is vital for effective weight management. Research has shown that a well-rounded and balanced diet not only aids in achieving and sustaining a healthy weight but also significantly reduces the risk for non-communicable diseases such as metabolic syndrome, type 2 diabetes, hypertension, and certain cancers (Cena & Calder, 2020).

The World Health Organization highlights that poor dietary habits and a lack of physical activity pose significant health risks, greatly contributing to the global disease burden (World Health Organization, 2020). This finding underscores the importance of promoting healthy eating habits and encouraging more physical activity among individuals of all ages. Improving these behaviors is essential for enhancing health outcomes and preventing non-communicable diseases in future generations.

2.2 Metabolic syndrome

Metabolic syndrome is a combination of conditions that includes abdominal obesity, elevated blood pressure, high blood sugar levels, and abnormal cholesterol or triglyceride levels (Alberti et al., 2009). These factors increase the risk of developing cardiovascular diseases, type 2 diabetes (Després & Lemieux, 2006), and MASLD (W.-K. Chan et al., 2023). MASLD is characterized by an excessive accumulation

of fat in the liver. If left untreated, this excess fat can lead to liver inflammation, fibrosis, and even cirrhosis (W.-K. Chan et al., 2023).

Several risk factors contribute to the development of metabolic syndrome. One of the primary factors is obesity, particularly central obesity, which is when excess fat is concentrated around the abdomen. This significantly increases the risk of developing the syndrome (Després & Lemieux, 2006). Physical inactivity also plays a crucial role; a lack of regular exercise and a sedentary lifestyle can lead to weight gain and reduced insulin sensitivity (Venables & Jeukendrup, 2009). Additionally, the risk of metabolic syndrome increases with age, as hormonal changes and various lifestyle factors can impact health over time (Pataky et al., 2021). Genetics is another important factor, as a family history of diabetes or heart disease can predispose individuals to metabolic syndrome (Dunkley et al., 2009).

Furthermore, certain ethnic groups have been found to have higher occurrences of metabolic syndrome. It has been shown that women from ethnic minority backgrounds exhibit higher rates of metabolic syndrome than their male counterparts (Adjei et al., 2024). Diet is also a significant contributor; diets high in processed foods, sugars, and unhealthy fats can lead to obesity and other metabolic risk factors (Poti et al., 2017). Lastly, insulin resistance, a condition in which the body's cells do not respond effectively to insulin, is central to the development of metabolic syndrome (Roberts et al., 2013).

2.3 Liver

The liver is the heaviest internal organ (average weight 1,5kg – 1.8 kg) (Simon et al., 2020) and the largest gland in the body, located in the right upper quadrant of the abdominal cavity (Sumadewi, 2023). The liver is crucial in maintaining metabolic balance and overall health through various functions. It regulates carbohydrate, protein, and fat metabolism, helping control blood sugar levels, and is a storage site for essential nutrients. One of the liver's critical roles is detoxification, as it filters out toxins from the blood and metabolizes harmful substances, including various drugs that enter the body. Additionally, the liver produces bile, which is necessary for digesting and absorbing fats. During the early stages of development, the liver produces blood cells. It also acts as a storage depot for various vitamins, minerals, and glycogen, which the body utilizes for energy. The liver synthesizes proteins essential for blood clotting, which is crucial for wound healing. Moreover, the liver contributes to the immune system by producing immune factors and filtering pathogens from the blood. It interacts with various organ systems, influencing digestion, cholesterol management, hormone metabolism, and immune responses (Kalra et al., 2023). This intricate interplay underscores the liver's status as a central hub for maintaining the body’s metabolic and overall health.

2.4 Liver enzymes

2.4.1 Common liver enzymes and their functions

In general, enzymes are biological catalysts that accelerate chemical reactions in the body. They do this by binding to specific molecules known as substrates, lowering the energy required for the reactions. The relationship between an enzyme and its substrate is often described using the analogy of a "key" and a "lock" (Fischer, 1894). Only the correctly shaped and sized substrate can fit into a specific enzyme. Once the substrate binds to the enzyme's active site, it facilitates the conversion into products. After releasing the products, the enzyme can bind to another substrate and repeat the process. Liver enzymes are important indicators of liver health and functionality. They are frequently used in clinical settings to diagnose and monitor liver diseases. The commonly measured liver enzymes in routine blood tests are alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT). Each of these enzymes provides specific insights into the condition of the liver.

ALT is an enzyme predominantly located in the liver. AST is also concentrated in the liver and distributed throughout other vital tissues, including the heart and skeletal muscles (Kalas et al., 2021). The molecular mechanisms of ALT and AST center around their crucial roles in amino acid metabolism, where they act as important catalysts in transferring amino groups. ALT catalyzes the reversible reaction between the amino acid alanine and alpha-ketoglutarate, resulting in the production of pyruvate and glutamate (Z. Liu et al., 2014), and AST facilitates the transfer of an amino group from aspartate to alpha-ketoglutarate, yielding oxaloacetate and glutamate (Sookoian & Pirola, 2012). These reactions are essential for gluconeogenesis, where the body generates glucose from non-carbohydrate sources, and the urea cycle, eliminating excess nitrogen. These aminotransferases are critical for maintaining the body's balance of amino acids and energy (McGill, 2016).

Clinically, their activity levels are frequently measured to diagnose liver conditions, as elevated levels of ALT and AST can indicate liver damage or dysfunction. However, since AST is found in various tissues, high levels in the bloodstream may indicate problems in other organs, complicating the diagnostic process (Giannini et al., 2005). Therefore, analysis of the AST-to-ALT ratio can improve understanding of liver pathology, providing more insights into liver health (Williams & Hoofnagle, 1988).

GGT is a pivotal enzyme in the body that is integral to the metabolism of glutathione, a powerful antioxidant, and the transport of amino acids across cell membranes (Kunutsor, 2016). GGT catalyzes the transfer of a gamma-glutamyl

group from glutathione to other molecules. This process is essential for both the breakdown of glutathione and the conjugation of drugs and other xenobiotics, aiding in detoxifying and eliminating harmful substances from the body (Han et al., 2007). This enzymatic action occurs primarily in the liver, which serves as the body's detoxification center; however, GGT is also found in significant amounts in the kidneys, pancreas, and other tissues (Xing et al., 2022). By facilitating the removal of potentially harmful substances and aiding in cellular protection against oxidative stress and inflammation, GGT plays a critical role in maintaining overall metabolic balance (Kunutsor, 2016). Elevated GGT levels can indicate metabolic issues such as obesity, which may lead to fatty liver disease. Additionally, high GGT levels are associated with excessive alcohol consumption and can serve as a marker for alcohol-related liver dysfunction (Kunutsor, 2016).

Regular monitoring of liver enzymes is commonly used for early detection of liver inflammation, damage, or disease progression. Routine blood tests that assess these enzymes enable clinicians to identify potential liver issues, track the progression of existing conditions, and evaluate the effectiveness of ongoing treatments (T. H. Lee et al., 2012). However, it is important to note that liver enzyme levels can differ among various age groups and between sexes and that exercise can also cause these levels to increase (Giannini et al., 2005). Therefore, standard liver tests do not always indicate the presence of liver diseases, such as MASLD, and additional diagnostic methods, such as liver biopsy or magnetic resonance spectroscopy (MRS), may be necessary for accurate detection (Obika & Noguchi, 2012).

2.4.2 Lifestyle factors and liver enzymes

Sedentary behavior, physical activity, and liver enzymes

Elevated liver enzyme levels, particularly ALT, are important markers for MASLD (Ekstedt et al., 2006). Research indicates that individuals afflicted with MASLD typically lead more sedentary lifestyles and simultaneously engage in significantly lower physical activity levels (Hallsworth et al., 2015). A thorough meta-analysis (Ma et al., 2022) revealed that participants with MASLD who adhered to a consistent exercise routine exceeding 12 weeks exhibited substantial improvements in their ALT levels, reflecting a positive shift in liver health. This reduction in ALT levels suggests that sustained physical activity can be an important factor in mitigating liver damage and enhancing overall metabolic health. Conversely, the analysis highlighted a concerning trend: no significant improvements in ALT levels were observed when active lifestyle habits were maintained for less than 12 weeks (Ma et al., 2022). These results underscore the importance of committing to long-term

exercise routines to achieve optimal health outcomes and effectively combat the adverse effects of MASLD.

Additionally, one study has revealed a significant correlation between a lack of regular physical activity and elevated levels of ALT in newly diagnosed adult patients with type 2 diabetes (Mor et al., 2014). This association persisted even after adjustments for confounding variables such as abdominal obesity and alcohol consumption, highlighting the critical role of physical activity in supporting liver health within this population. However, the findings of this study relied on self-reported measures of physical activity, which are known to be prone to certain limitations. Existing literature indicates that individuals often overestimate both the frequency and intensity of their physical exercise, leading to potential biases in data reporting (Hukkanen et al., 2018; Sallis & Saelens, 2000).

However, recent research presents more robust evidence supporting the health benefits of increased physical activity in reducing liver enzyme levels in individuals with type 2 diabetes. Specifically, the study reported that reducing sedentary behavior while concurrently elevating physical activity levels can yield significant improvements in ALT and GGT levels (Haxhi et al., 2024). These conclusions were derived from a comprehensive *post hoc* analysis of a three-year randomized clinical trial, where the participants' physical activity and sedentary behavior were measured using accelerometry. During the initial four-month period, participants wore accelerometers continuously, followed by assessments conducted every four months for one week. The analysis indicated that the most significant reductions in liver enzyme levels were observed in conjunction with MVPA engagement (Haxhi et al., 2024), suggesting the importance of promoting higher levels of physical activity to enhance liver health among individuals with type 2 diabetes.

Research examining the relationship between liver enzyme levels and objectively measured physical activity, as well as sedentary behavior in individuals with metabolic syndrome and conditions such as obesity or being overweight, is notably limited. Most existing studies primarily focus on children and adolescents, which restricts the generalizability of their findings to adult populations. The results of these studies are also often conflicting.

For instance, one study found that adolescents who met the physical activity recommendation of engaging in at least 60 minutes of MVPA each day exhibited elevated levels of AST and a higher AST/ALT ratio. This finding was consistent regardless of the sedentary behavior or overall body fat levels, both central and total (Ruiz et al., 2014). Another investigation indicated that improved cardiorespiratory fitness in children was associated with lower GGT levels and a higher AST/ALT ratio (Medrano et al., 2020). Research suggests that physical activities such as aerobic and resistance training can also lead to increased AST levels in adult populations (Fragala et al., 2017). Furthermore, weight loss has been shown to affect

AST levels, particularly with increases observed, especially in women (Gasteyger et al., 2008). Additionally, a study focusing on obese children aged 11 to 13 found that self-reported screen time was positively correlated with elevated ALT and AST levels, even after controlling for MVPA (Norman et al., 2017). Additionally, a large cross-sectional study involving 10,385 Hispanic/Latino adults in the United States with an average BMI of approximately 30 found a significant relationship between increased sedentary time and higher levels of both ALT and GGT. This association remained significant even after accounting for key cardiometabolic markers, including BMI (J. Li et al., 2020). However, some studies have indicated that sedentary behavior, measured by accelerometers, is not associated with ALT and AST levels (Keating et al., 2016; Norman et al., 2017).

These mixed findings highlight a complex relationship between physical activity, sedentary behavior, body weight, and liver enzymes. Furthermore, one limitation across these studies is the brief duration for which physical activity was monitored, typically spanning only 3 to 7 days. This relatively short monitoring period raises important questions regarding the reliability and validity of the findings. Consequently, there is an urgent need for more comprehensive and longitudinal research to fully understand these associations within adult populations.

Nutrition and liver enzymes

Regarding nutrition and liver enzymes, a comprehensive population-based study (Kwon et al., 2012) involving 19,749 participants investigated the relationship between dietary intake and liver enzyme levels among Koreans, focusing on individuals with and without metabolic syndrome. The findings indicated that an increased intake of carbohydrates was significantly linked to elevated levels of ALT and AST, whereas fat intake did not exhibit a similar association (Kwon et al., 2012). Additionally, one study assessed the impact of sugar-sweetened beverage consumption on liver health. It was determined that healthy premenopausal women who regularly consumed these beverages displayed markedly higher serum ALT and AST levels (Shimony et al., 2016). The study's authors posited that this effect could be largely attributed to the metabolic processing of fructose in the liver. They postulated that fructose significantly contributes to hepatic lipogenesis—the process through which the liver converts excess carbohydrates into fat—thereby increasing the risk of developing MASLD (Shimony et al., 2016).

Furthermore, another pertinent study highlighted the effects of dietary modifications on liver enzyme levels in individuals diagnosed with MASLD. The research demonstrated that adherence to a hypocaloric high-protein diet reduced ALT and GGT levels (Haidari et al., 2020), suggesting that targeted dietary interventions effectively manage liver health. Additional evidence indicated that

even a moderate daily fructose and sucrose intake could lead to increased hepatic lipogenesis (Geidl-Flueck et al., 2021), reinforcing the potential risks associated with these sugars that may elevate the likelihood of fatty liver disease and increase serum liver enzyme levels. Finally, a meta-analysis (Smart et al., 2018) encompassing several exercise studies involving adults who were either overweight or diagnosed with MASLD provided further insights into the role of physical activity and diet in liver health management. Seven studies were examined, integrating exercise interventions and dietary changes; however, only one reported a significant reduction in GGT levels. Notably, when the analysis was confined to the four studies focused solely on exercise interventions, no significant changes in GGT levels were observed (Smart et al., 2018). This indicates that while physical activity may contribute to overall health, its influence on liver enzyme levels may be limited without concurrent dietary modifications.

2.5 Liver fat

2.5.1 Overview of liver fat accumulation

Fatty liver disease, clinically known as hepatic steatosis, refers to abnormal fat accumulation within the liver cells. In a healthy individual, the liver normally contains a small percentage of fat, essential for various metabolic processes, including energy storage, the synthesis of proteins, and the regulation of cholesterol levels (González-Manchón et al., 1990; Trapani et al., 2012). However, when the fat content in the liver exceeds 5 % of its total weight, it may indicate underlying health concerns that could lead to liver diseases, such as MASLD (Nassir et al., 2015).

The liver is an essential organ critical to the body's overall metabolism. In the postprandial state, after food consumption, macronutrients—carbohydrates, fats, and proteins—are metabolized into glucose, fatty acids, and amino acids, which are absorbed in the gastrointestinal tract and transported to the liver via the hepatic portal vein. In the liver, glucose can be stored as glycogen or converted into fatty or amino acids. Fatty acids are synthesized into triacylglycerols (TAGs), which may be stored or released into circulation as very low-density lipoprotein (VLDL). Additionally, amino acids are utilized for energy production or synthesizing proteins, glucose, or other essential molecules. During fasting or increased physical activity, the liver releases glucose and TAGs into the bloodstream to provide energy for other tissues, such as skeletal muscle (Rui, 2014). However, this metabolic balance in the liver can be disrupted by various factors, including obesity, metabolic syndrome, a diet rich in sugars, insulin resistance, and chronic alcohol consumption, leading to excessive fat accumulation and increasing the risk of developing MASLD (National Institute of Diabetes and Digestive and Kidney Diseases, 2021c).

The accumulation of fat in the liver can also trigger a cascade of inflammatory responses, which can lead to a condition known as metabolic dysfunction-associated steatohepatitis (MASH). This inflammation can cause cellular damage and fibrosis (the formation of scar tissue) and, if left untreated, may progress to cirrhosis, which is the advanced scarring of the liver that can impair its function significantly and lead to life-threatening complications, such as liver cancer (National Institute of Diabetes and Digestive and Kidney Diseases, 2021a). Excessive accumulation of liver fat is a critical indicator of overall metabolic health. It can signal an increased risk of developing various associated conditions, including type 2 diabetes, cardiovascular diseases, and different types of cancers beyond the liver (Duell et al., 2022; J. A. Thomas et al., 2024).

2.5.2 Mechanisms of liver fat accumulation

One of the primary factors contributing to fat accumulation in the liver is the increased delivery of fatty acids to the organ. This delivery can occur through three principal mechanisms: 1) the breakdown of stored fat via lipolysis, 2) the direct intake of fat from dietary sources, or 3) the synthesis of new fat from excess carbohydrates or proteins (glucose, fructose, amino acids) through a process known as *de novo* lipogenesis. When the body breaks down stored fats in adipose tissue through lipolysis, free fatty acids (or non-esterified fatty acids, NEFAs) are released into the bloodstream. Elevated levels of these fatty acids can overwhelm the liver, especially when combined with a diet high in fatty foods. This can exceed the liver's ability to process them efficiently. The liver can also synthesize fatty acids from excess carbohydrates and proteins—a process known as *de novo* lipogenesis. When a person consumes more carbohydrates and protein than can be utilized for immediate energy, the liver converts these surplus macronutrients into fatty acids, increasing liver fat storage (Roumans et al., 2021).

Under normal conditions, the liver efficiently metabolizes fatty acids through a process called β-oxidation, which takes place in the mitochondria of liver cells and produces energy in the form of adenosine triphosphate (ATP). However, when there is excessive fat accumulation in the liver, fatty acid oxidation increases to the extent that it can overwhelm this pathway. This results in the production of harmful reactive oxygen species (ROS), leading to oxidative stress. Excessive ROS can damage various cellular components, notably the mitochondrial membranes and DNA (Prasun et al., 2021). The accumulation of oxidative stress plays a significant role in developing several liver conditions. It is particularly linked to hepatocyte apoptosis, the programmed cell death process that occurs when liver cells are damaged. This dysfunction often triggers an inflammatory response in the liver, leading to hepatic inflammation. Over time, persistent oxidative stress and

inflammation can contribute to the progression of liver fibrosis (Bessone et al., 2019; Masarone et al., 2018).

Insulin resistance is a condition where cells in the liver, muscle, and adipose tissue become increasingly unresponsive to the effects of insulin, a hormone critical for regulating blood sugar levels and fat metabolism. Insulin resistance is strongly associated with developing hepatic steatosis and the disease's progression (Nogueira & Cusi, 2024). Low insulin levels trigger lipolysis in fasting, breaking down fat stores for energy. In the postprandial state, rising insulin levels inhibit lipolysis and promote glucose uptake by the liver and muscles, showcasing metabolic flexibility. This ability to switch between fatty acids and glucose is crucial for energy balance and metabolic health (Nogueira & Cusi, 2024). However, in insulin resistance, insulin fails to suppress the breakdown of fat in adipose tissue, causing free fatty acids to spill into the bloodstream and the liver while also failing to suppress hepatic gluconeogenesis, even during fed states (Hatting et al., 2018). In the liver, hyperinsulinemia and hyperglycemia trigger the production of fatty acids and cholesterol, leading to a rise in triglyceride production and the formation and release of VLDL. When the rate of triglyceride production exceeds the secretion of VLDL, excess triglycerides build up, which can result in the development of a fatty liver (Fon Tacer & Rozman, 2011). Therefore, excess free fatty acids can overwhelm the liver's capacity to process them, leading to increased fat accumulation in the liver and causing dyslipidemia (abnormal blood lipid levels), which increases the risk of cardiovascular disease, such as atherosclerosis and heart disease (Pappan et al., 2024). Finally, insulin resistance is not just a malfunction of insulin signaling but also a catalyst for broader metabolic disturbances, including dysregulation of fat metabolism and increased risk of liver disease and cardiovascular complications. **Figure 2** summarizes the mechanisms of liver fat accumulation and the associated pathological processes.

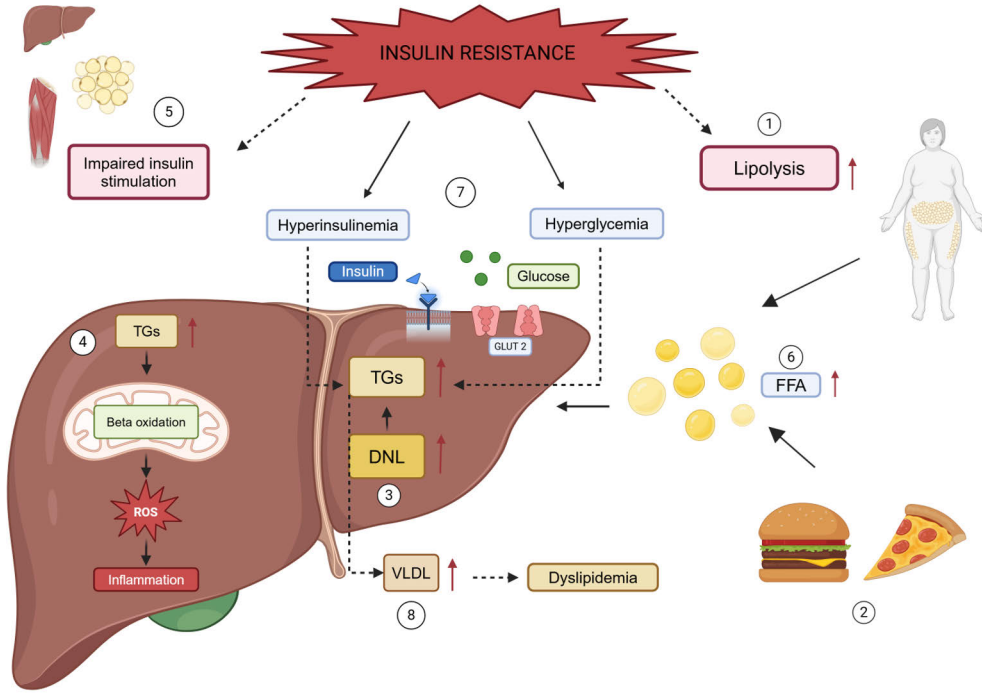

Figure 2. This figure illustrates the mechanisms involved in fat accumulation in the liver and the associated pathophysiological processes. The primary sources of fatty acids delivered to the liver include **(1)** lipolysis of stored fat, **(2)** direct dietary intake, and **(3)** *de novo* lipogenesis (DNL) from excess carbohydrates and proteins. Under normal conditions, fatty acids are metabolized through β-oxidation in liver mitochondria, yielding ATP. However, excessive triglyceride (TG) accumulation **(4)** triggers heightened fatty acid oxidation, leading to increased production of reactive oxygen species (ROS) and oxidative stress, which damages liver cells, promotes hepatocyte apoptosis, and induces hepatic inflammation, contributing to the progression of liver fibrosis. Insulin resistance is characterized by reduced cellular responsiveness to insulin in the liver, muscle, and adipose tissue **(5)**, impairing the hormone's ability to regulate fat metabolism and blood sugar levels. This condition leads to unregulated fat breakdown in adipose tissue, releasing free fatty acids **(6)** into the bloodstream and liver. In the liver, hyperinsulinemia and hyperglycemia **(7)** stimulate the synthesis of fatty acids and cholesterol, increasing triglyceride production and the secretion of very low-density lipoprotein (VLDL) **(8)**. When triglyceride production surpasses VLDL secretion, excess triglycerides accumulate, potentially resulting in fatty liver. Consequently, an overflow of free fatty acids can exceed the liver's processing capacity, culminating in dyslipidemia **(9)**, which heightens the risk of cardiovascular diseases, including atherosclerosis and heart disease. This figure was created with BioRender.com.

2.5.3 Lifestyle factors and liver fat accumulation

Sedentary behavior, physical activity, and liver fat accumulation

Research findings have shown that sedentary behavior has shown to have a negative impact on body composition, often characterized by increased body fat, BMI, and

WC (Bellettiere et al., 2017; Heinonen et al., 2013; Swartz et al., 2012). Conversely, consistent engagement in physical activity correlates with enhanced body composition (Bradbury et al., 2017; Cárdenas Fuentes et al., 2018). Furthermore, exercise promotes the breakdown of lipids in the liver (Smart et al., 2018), which is important for maintaining optimal liver function and preventing fat accumulation within the liver. Additionally, regular exercise contributes to managing body weight, which is critical in mitigating the risk of obesity-related liver diseases and enhancing liver health.

Research indicates prolonged sedentary behavior is significantly linked to metabolic dysfunctions, particularly in developing conditions such as MASLD (D. Kim et al., 2020; Ryu et al., 2015). Moreover, evidence suggests that sedentary behavior may be an independent predictor of MASLD (D. Kim et al., 2020). This finding implies that individuals who engage in extended periods of inactivity face an increased risk of developing this condition, irrespective of other influencing factors. Conversely, increasing levels of regular physical activity are associated with a lower risk of developing MASLD (D. Kim et al., 2020). For instance, one study found that a supervised and progressive aerobic exercise program, consisting of three weekly sessions on a cycle ergometer lasting between 30 to 45 minutes each, effectively reduced liver fat content in obese sedentary adults (Johnson et al., 2009). This study took place over four weeks and involved participants with an average baseline liver fat content exceeding 5%, the threshold for diagnosing MASLD.

Specifically, previous studies have demonstrated a positive association between liver fat content and sedentary behavior, while an inverse relationship has been established with habitual physical activity. Notably, this association appears to be strengthened in a dose-dependent manner, indicating that greater levels of physical activity correspond with lower liver fat content (Gianluca Perseghin et al., 2007; Kistler et al., 2011; Y. Li et al., 2019; Ryu et al., 2015; Wei et al., 2016a; Zelber-Sagi et al., 2008). A recent meta-analysis supports this, finding that high and moderate physical activity levels are better for reducing the risk of MASLD than light physical activity (Qiu et al., 2017). Furthermore, it suggests that to lower the risk of MASLD significantly, individuals should aim to exceed the recommended minimum levels of activity, specifically, engaging in at least 150 minutes of moderate-intensity exercise or 75 minutes of vigorous-intensity activity per week (Qiu et al., 2017). Notably, one study found that the association between liver fat content and physical activity diminished when WC was included as a covariate in the analysis (Zelber-Sagi et al., 2008). This finding suggests that body composition may significantly influence the nature of this relationship. However, it is important to note that many of these studies relied on self-reported measures, which may lead to an overestimation of actual levels of sedentary behavior and physical activity, thereby affecting the reliability of the findings (Sallis & Saelens, 2000). Moreover,

a significant limitation of the existing literature is that assessing liver fat content has predominantly employed methods such as ultrasound or fatty liver index (FLI) rather than utilizing the gold-standard assessment techniques of liver biopsy and MRS. This lack of rigorous assessment methods raises questions regarding the accuracy of the reported associations.

Research employing accelerometers to assess physical activity levels has yielded inconsistent results regarding the relationship between physical activity, sedentary behavior, and liver fat accumulation. One study identified a positive correlation between sedentary behavior and liver fat percentage, as measured by MRS, specifically in consistently habitual active individuals aged from young adulthood through middle age (Bowden Davies et al., 2019a). This relationship was observed across both groups—those with and those without metabolic syndrome—suggesting that even in actively engaged individuals, prolonged periods of inactivity may adversely affect liver health. Furthermore, another study indicated that higher levels of sedentary behavior are associated with increased liver fat, particularly among populations that are at a greater risk for metabolic diseases, such as type 2 diabetes (Henson et al., 2015). Conversely, some findings have failed to find significant correlations between habitual physical activity, sedentary behavior, and liver fat content in adults who are overweight or obese (Keating et al., 2016). This lack of consistent findings points to the complexity of the underlying relationships and suggests that more research is necessary to understand these dynamics fully.

Discrepancies in the results across various studies may arise from various factors, including differences in the demographic characteristics of participants, the methodologies employed for measuring physical activity and liver fat, and genetic variability that can impact individual fitness levels and metabolic health (Chung et al., 2021). In addition, most studies that have employed accelerometers have utilized measurement durations ranging from only 3 to 7 days, which may not adequately reflect habitual activity and behavior over longer timeframes (Sjöros et al., 2021). Such short measurement periods can lead to incomplete assessments of individuals' typical physical activity patterns and sedentary behaviors, further complicating the understanding of their true impact on liver health.

However, evidence highlights the beneficial effects of physical exercise on individuals diagnosed with MASLD, particularly regarding its ability to reduce intrahepatic lipid content significantly. This reduction is achieved through a variety of complex mechanisms. Notably, exercise leads to a decrease in fatty acid synthesis, an increase in the oxidation of fatty acids, and the inhibition of specific molecular pathways that can cause damage to mitochondria and hepatocytes (van der Windt et al., 2018). A comprehensive meta-analysis has supported these beneficial effects, demonstrating that regular exercise can effectively lower liver fat content without causing substantial changes in overall body weight (Stine et al., 2023). This finding

suggests that the positive impact of exercise on liver health operates independently of weight reduction, pointing to a direct physiological effect on liver function.

The precise mechanistic pathways through which physical activity contributes to reduced liver fat are not fully understood. Current research predominantly focuses on the interplay between exercise and insulin resistance. Studies have revealed that physical activity enhances insulin sensitivity in peripheral tissues, improving metabolic regulation. This enhancement in insulin sensitivity is crucial because it reduces the flux of free fatty acids into the liver and decreases glucose uptake by hepatic cells (Cigrovski Berkovic et al., 2021). As a result, these processes help mitigate the accumulation of liver fat, promoting better overall liver health in individuals with MASLD. Further research is necessary to explore and clarify these mechanisms in detail, as a deeper understanding could lead to more targeted interventions for managing MASLD and improving liver health through exercise.

Nutrition and liver fat

It has been shown that a well-balanced diet that includes a variety of whole foods, such as colorful fruits, nutrient-rich vegetables, whole grains, lean proteins, and healthy fats, greatly supports liver health (National Institute of Diabetes and Digestive and Kidney Diseases, 2021b). These dietary choices significantly enhance liver health by providing essential vitamins, minerals, and antioxidants, which help reduce inflammation and oxidative stress that can lead to cellular damage over time (Lemasters & Jaeschke, 2020). In contrast, diets high in processed foods, added sugars, and unhealthy fats could worsen liver conditions (National Institute of Diabetes and Digestive and Kidney Diseases, 2021b). These negative dietary patterns contribute to fat accumulation in the liver and increase inflammatory responses, creating a higher risk of chronic liver diseases.

Excessive consumption of sugars, particularly from sugary beverages and processed snacks, has been associated with increased hepatic fat accumulation and the development of insulin resistance (Huneault et al., 2023). This progression can significantly complicate liver health outcomes and lead to a range of metabolic disorders. A recent study indicated that a diet characterized by high protein content, especially from plant-based sources such as legumes, nuts, and whole grains, is linked to a reduced risk of developing MASLD (Khazaei et al., 2023). These findings underscore the potential benefits of integrating plant-based protein sources into dietary regimens to promote liver health. Additionally, research has demonstrated that even moderate intakes of fructose and sucrose can enhance *de novo* lipogenesis (Geidl-Flueck et al., 2021). Consequently, this elevates the risk for MASLD and highlights the importance of regulating sugar consumption within dietary practices (31).

It is also important to acknowledge that various types of fats and carbohydrates exert differing effects on liver fat accumulation, contingent upon the specific macronutrients consumed (Hydes et al., 2021; Winters-van Eekelen et al., 2021). Studies have established that saturated fatty acids—commonly found in fatty meats, full-fat dairy products, and certain oils—and fructose, a sugar prevalent in sugary beverages and processed foods, can significantly elevate intrahepatic triglyceride levels (Hydes et al., 2021; Jensen et al., 2018; Winters-van Eekelen et al., 2021). Such increases may contribute to the progression of MASLD. Conversely, unsaturated fatty acids, prevalent in foods such as olive oil, avocados, and fatty fish, are generally associated with positive outcomes for liver health (Winters-van Eekelen et al., 2021). These healthy fats enhance metabolic functions and possess anti-inflammatory properties that can mitigate hepatic damage (Coniglio et al., 2023). Consequently, incorporating unsaturated fats while reducing saturated fats and added sugars is important for promoting healthier liver function and improving overall metabolic health.

As mentioned, previous studies underscore the importance of the quality and quantity of various macronutrients in their impact on liver fat accumulation. These macronutrients are integral to metabolic functions and the mechanism by which the body stores fat. However, evidence also highlights that overall daily caloric intake may significantly affect liver fat levels more than the specific ratios of these macronutrients (Hydes et al., 2021; Parry & Hodson, 2017). This suggests that managing overall energy consumption may be more vital for controlling liver fat content than solely concentrating on the dietary composition of macronutrients.

2.6 Liver insulin sensitivity

The liver is an essential organ recognized for its high insulin sensitivity, a crucial hormone that regulates metabolism and maintains energy balance in the body. Among its many functions, the liver plays a key role in controlling glucose levels in the bloodstream. This continuous regulatory process ensures that the body's energy needs are effectively met while maintaining stable blood glucose levels, thereby supporting metabolic homeostasis (Meshkani & Adeli, 2009).

2.6.1 Liver glucose uptake and endogenous glucose production

Liver glucose uptake is a vital process that involves the liver’s ability to absorb glucose from the bloodstream. This process is regulated by insulin, which promotes glucokinase activity while simultaneously inhibiting the expression of glucose-6-phosphatase (Iozzo et al., 2003; Nozaki et al., 2020; Rita Basu et al., 2004). Liver

glucose uptake occurs primarily through specialized transport proteins, particularly GLUT2, which enable glucose molecules to enter hepatocytes (Sun et al., 2023). In the postprandial state, blood glucose levels rise significantly due to the digestion and absorption of carbohydrates. The liver plays a key role in regulating blood sugar levels by capturing this excess glucose and utilizing it for energy production or converting it into glycogen, a stored form of energy (a capacity of around 100g) (Wills, 1985).

On the other hand, endogenous glucose production (EGP) is a crucial adaptive mechanism that occurs primarily in the liver during periods of fasting or when dietary carbohydrate intake is low. This process involves two key pathways: gluconeogenesis and glycogenolysis (Al-Yousif et al., 2021). Gluconeogenesis is a process through which glucose is synthesized from non-carbohydrate sources like amino acids, resulting from protein breakdown, and glycerol derived from fat metabolism (Chourpiliadis & Mohiuddin, 2023). Conversely, glycogenolysis involves the degradation of stored glycogen into glucose, which is subsequently released into the bloodstream to meet the body's increased energy demands during fasting or increased physical activity (Paredes-Flores et al., 2024). Moreover, efficient glycogen storage significantly aids the brain in responding to fluctuations in blood sugar levels, contributing to a better overall metabolic balance (Klein et al., 2021; Warner et al., 2023).

2.6.2 Liver insulin resistance

Liver insulin resistance is a metabolic condition marked by the impaired ability of hepatocytes to respond effectively to insulin. This condition is primarily associated with chronic inflammation, which is often linked to visceral obesity. In individuals with significant abdominal fat accumulation, inflammatory markers such as cytokines are released into the bloodstream and interfere with the insulin signaling pathways in hepatocytes (Meshkani & Adeli, 2009). As the liver becomes insulin-resistant, its capacity to manage glucose production is severely compromised. In a healthy metabolic state, insulin prompts the liver to reduce glucose output, particularly after meals when blood sugar levels are elevated. However, in insulin resistance, the liver fails to respond appropriately and continues to produce glucose via EGP. This persistent glucose synthesis contributes to consistently high blood sugar levels, known as hyperglycemia (Meshkani & Adeli, 2009).

Moreover, liver insulin resistance can have a further impact beyond glucose metabolism. It can stimulate the liver to increase the synthesis and release of lipids, particularly VLDL. This process occurs because insulin normally plays a role in inhibiting fat production in the liver; when this mechanism is disrupted, fat accumulates, leading to elevated levels of triglycerides in the bloodstream—a

condition known as hypertriglyceridemia (Uehara et al., 2023). Hyperglycemia and hypertriglyceridemia are both prominent features of metabolic disorders such as type 2 diabetes, metabolic syndrome, and MASLD (Barrera et al., 2024; Goyal et al., 2023; Deprince et al., 2020; Subramanian & Chait, 2012). The interplay between these conditions is significant, as insulin resistance can create a cycle that exacerbates other metabolic complications.

The hyperinsulinaemic-euglycaemic clamp method is the gold standard for measuring insulin resistance (J. K. Kim, 2009). In this method, plasma glucose levels are maintained during intravenous insulin infusion by frequently measuring plasma glucose and adjusting the rate of intravenous glucose infusion to prevent insulin-induced glucose lowering (DeFronzo et al., 1979). Insulin administration typically stimulates the liver to enhance glucose uptake while decreasing EGP in healthy individuals, effectively regulating blood sugar levels. However, in an insulin-resistant state, the hepatic tissue fails to adequately increase glucose uptake, which results in lower glucose absorption by the liver. Conversely, there is a failure to decrease EGP, leading to increased glucose production by the liver (Iozzo et al., 2003; Rita Basu et al., 2004). As a result, the combination of decreased liver glucose uptake and increased EGP indicates liver insulin resistance during periods of insulin stimulation, highlighting the dysfunction in glucose metabolism associated with insulin resistance.

2.6.3 Lifestyle factors and liver insulin sensitivity

Sedentary behavior, physical activity, and liver insulin sensitivity

Studies have demonstrated that individuals who engage in excessive sedentary behavior are at an increased risk of heightened insulin resistance and disturbances in glucose metabolism and, thus, at risk for developing metabolic disorders such as type 2 diabetes and MASLD (Eaton & Eaton, 2017; Nogueira & Cusi, 2024; Yaribeygi et al., 2021). In contrast, exercise has been shown to enhance overall insulin sensitivity (Bird & Hawley, 2016), a vital factor in regulating blood glucose levels. Exercise improves insulin responsiveness, contributing to more effective glucose management and mitigating the risk of metabolic disorders. Despite these findings, a notable gap exists in comprehensive research regarding the specific relationship between objectively measured sedentary behavior, levels of physical activity, and liver insulin sensitivity. Furthermore, studies investigating the effects of reducing sedentary time while simultaneously increasing physical activity are limited, which constrains our understanding of how lifestyle modifications impact liver health.

Few intervention studies using positron emission tomography (PET) have provided insights into how varying intensities and durations of different exercises

impact liver glucose uptake. For instance, a recent investigation revealed that moderate-intensity training significantly improved liver glucose uptake compared to sprint interval training in adults categorized as normoglycemic or those with prediabetes/type 2 diabetes (Motiani et al., 2019). This finding indicates that it might be that not all exercise modalities yield equivalent benefits for liver health. Conversely, another study indicated that resistance training did not significantly impact liver glucose uptake in elderly females (Honka et al., 2016), suggesting that the type of exercise may play a role in determining outcomes related to liver glucose uptake.

Furthermore, past intervention studies have emphasized the beneficial effects of specific exercise modalities, such as resistance training and treadmill walking, on EGP. In one aforementioned study, elderly females participated in medium-intensity resistance training thrice per week over four months, resulting in a 28% suppression of EGP compared to baseline values (Honka et al., 2016). Similarly, in another research effort, adults with type 2 diabetes engaged in a treadmill walking program at medium intensity for 15 weeks, attending sessions four to five days per week. This regimen reduced EGP, even when splanchnic glucose uptake was decreased (Gregory et al., 2019).

Additionally, a study focusing on exercise protocols found that adherence to a six-week moderate-to-vigorous exercise program (ranging from 60 to 85% of maximal aerobic capacity), performed three times per week for a minimum of 20 minutes, led to both reduced EGP and increased whole-body insulin sensitivity in sedentary males (Shojaee-Moradie et al., 2007). The authors suggested that these enhancements in both hepatic and peripheral glucose uptake were due to decreased circulating levels of non-esterified fatty acids (NEFA), resulting from increased insulin sensitivity related to lipolysis (Shojaee-Moradie et al., 2007). In conclusion, it seems that EGP can be positively influenced by various forms of exercise, encompassing low-intensity and moderate-to-vigorous physical activity and resistance training.

Further studies are required to elucidate the effects of different exercise intensities on liver insulin sensitivity and overall metabolic health, particularly within populations at risk for developing metabolic disorders.

Nutrition and liver insulin sensitivity

Previous research has established that consuming sugars, particularly fructose, plays a significant role in the progression of MASLD (Jensen et al., 2018). Fructose is involved in the pathogenesis of fatty liver through two critical metabolic pathways: *de novo* lipogenesis, which involves the synthesis of fatty acids from non-fat sources,

and β-oxidation, responsible for the breakdown of fatty acids to generate energy. These processes can ultimately lead to liver insulin resistance (Jensen et al., 2018).

Evidence indicates that diets enriched with monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) can benefit glucose metabolism and overall liver function. These fats have been shown to reduce HbA1c levels (a marker of long-term blood glucose control), lower blood glucose concentrations, and decrease liver fat accumulation (Silva Figueiredo et al., 2017). In contrast, diets high in saturated fatty acids (SFA) have been found to influence the inflammatory response within the body. These diets are associated with an increase in the infiltration of M2 macrophages, which are considered pro-inflammatory, while simultaneously reducing the presence of M1 macrophages, which possess anti-inflammatory properties. This transformation, along with heightened levels of inflammatory cytokines and circulating endotoxins, contributes to the development of insulin resistance (Silva Figueiredo et al., 2017). Additionally, one study showed that short-term consumption of a diet high in SFA can lead to temporary insulin resistance (Koska et al., 2016). This study found that after just 24 hours on the saturated fatty acids-enriched diet, participants exhibited a significant decline in insulin effectiveness. While most of this impairment resolved after switching to a healthy diet for one day, some effects persisted. Additionally, individuals with normal and impaired glucose tolerance showed similar responses to the SFA diet (Koska et al., 2016). This highlights that poor dietary choices can adversely affect insulin sensitivity, regardless of initial metabolic health status.

Furthermore, extensive prospective studies have confirmed a robust correlation between high dietary fiber intake, particularly from insoluble cereal fibers, and a reduced risk of developing type 2 diabetes (Weickert & Pfeiffer, 2008). This association is likely attributable to fiber's ability to enhance insulin sensitivity, diminish inflammation, and positively impact gut microbiota composition (Weickert & Pfeiffer, 2008). Dietary fiber also demonstrates beneficial effects on numerous metabolic markers related to liver disease and liver cancer, including elevated blood glucose levels, insulin resistance, fatty liver conditions, and indicators of metabolic syndrome (X. Liu et al., 2021).

In conclusion, diet plays an important role in liver health and metabolism. High fructose and saturated fatty acid consumption increases the risk of MASLD and insulin resistance. In contrast, diets rich in monounsaturated and polyunsaturated fatty acids and high fiber intake offer protective benefits by improving glucose metabolism and liver function. Nevertheless, the precise mechanisms through which specific dietary choices influence liver insulin sensitivity, particularly concerning liver glucose uptake and EGP, remain inadequately understood. One previous study reported that liver glucose uptake did not display significant alterations after a six-week very low-calorie diet in individuals with obesity. Participants replaced all

meals with specially formulated dietary products comprising 53% carbohydrates, 44% protein, and only 3% fat (Viljanen et al., 2009). This specific macronutrient composition may have influenced the observed stability in liver glucose uptake, highlighting the necessity of evaluating various combinations of macronutrients when assessing metabolic outcomes.

Continued research is essential to elucidate the direct relationships between dietary patterns and their impact on metabolic pathways within the liver, thereby enhancing our understanding of liver health management.

3 Aims

I. To study the associations between liver enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltransferase (GGT) and sedentary behavior and physical activity in sedentary, inactive adults with overweight or obesity. Additionally, we aimed to examine the associations between liver enzymes and markers of overall body adiposity and other cardiometabolic markers.

II. To examine the associations between liver fat content and sedentary behavior, physical activity, daily nutrient and energy intake, fitness, and common markers of cardiometabolic health in sedentary, inactive adults with metabolic syndrome. Lastly, we aim to evaluate the correlation and agreement between the two liver fat content quantification methods, magnetic resonance spectroscopy and magnetic resonance imaging.

III. To study the associations between liver insulin sensitivity markers (liver glucose uptake and endogenous glucose production [EGP]) and sedentary behavior, physical activity, daily nutrient and energy intake, fitness, body composition, liver fat content, and common markers of cardiometabolic risk in sedentary, inactive adults with metabolic syndrome.

IV. To investigate the effects of a 6-month randomized, parallel-group intervention to reduce sedentary behavior by 1 h/day on liver insulin sensitivity markers, liver fat content, and liver enzyme levels in sedentary adults with metabolic syndrome. Additionally, we aim to examine the associations between changes during the intervention in sedentary behavior, physical activity, liver insulin sensitivity, liver fat content, liver enzymes, daily nutrient and energy intake, body composition, and other cardiometabolic health markers.

4 Materials and Methods

4.1 Study design

The study was conducted in two phases: a 1-month screening phase and a 6-month (5 months minimum duration) randomized controlled trial (ClinicalTrials.gov ID NCT03101228), and it was performed at the Turku PET Centre, Turku, Finland, between April 2017 and March 2020. It was conducted in accordance with good clinical practice and the Declaration of Helsinki and received ethical approval from the Ethics Committee of the Hospital District of Southwest Finland (16/1801/2017). Importantly, all participants provided written informed consent before enrollment, ensuring their full understanding and agreement to participate.

4.2 Study timeline

Figure 3 delineates the timeline of the study. The initial measurements (Measurement 1) were conducted during the screening phase and included assessments of anthropometric variables, blood pressure, and blood samples. The subsequent measurements (Measurement 2) were performed at the baseline of the intervention study and comprised positron emission tomography (PET) imaging with euglycemic hyperinsulinemic clamp, magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), anthropometric measurements, body composition analysis, blood samples, maximal oxygen consumption tests, blood pressure evaluations, and 4 days food diaries. The final measurements (Measurement 3) were collected at the end of the intervention and mirrored those taken at baseline.

Accelerometer data were acquired during both the screening and intervention phases. The screening phase involved a consistent one-month period of blinded monitoring. Following this period, eligible participants were randomized into either an intervention group or a control group, with their activity monitored via accelerometry for six months and supervised through a mobile application. The intervention group aimed to reduce sedentary behavior by one hour per day, while the control group sought to maintain their typical sedentary habits. A comprehensive description of the methods and measurements will be provided later in this chapter.

This thesis encompasses four interconnected studies (I-IV) structured within a unified research framework. Study I was conducted as a cross-sectional study during the screening phase (Measurement 1). Studies II and III were also cross-sectional investigations carried out at the baseline of the intervention study (Measurement 2). Finally, Study IV represented the intervention study, incorporating Measurements 2 and 3.

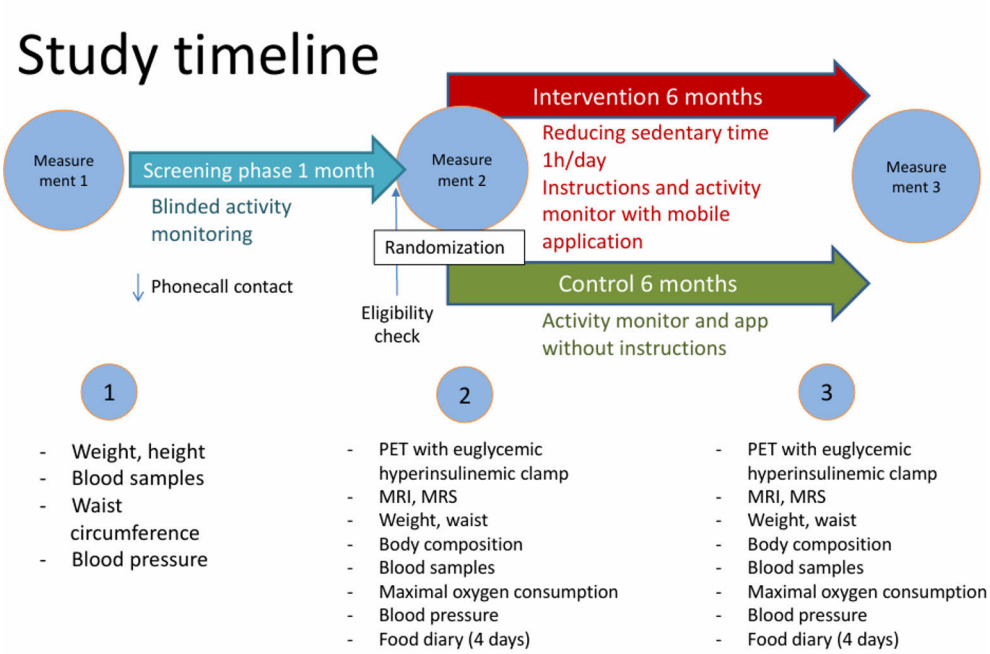

Figure 3. Overview of the study timeline and the conducted measurements. Initial measurements (Measurement 1) were conducted during the screening phase, including assessments of anthropometric variables, blood pressure, and blood samples. Subsequent measurements (Measurement 2) were performed at the baseline of the intervention study, incorporating positron emission tomography (PET) imaging with euglycemic hyperinsulinemic clamp, magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), anthropometric measurements, body composition analysis, blood samples, maximal oxygen consumption tests, blood pressure evaluations and 4 day food diaries. Final measurements (Measurement 3) were collected at the end of the intervention, replicating the assessments done at baseline. Accelerometer data were obtained during both the screening and intervention phases. The screening phase included a blinded one-month monitoring period, after which eligible participants were randomized into an intervention group or a control group, with activity monitored via accelerometry for six months using a mobile application. The intervention group focused on reducing sedentary behavior by one hour per day, while the control group maintained their typical sedentary habits. The figure has been modified from the original work created by Tanja Sjöros, who has provided permission for its utilization.

4.3 Study participants (I–IV)

The participants were recruited from the local community through newspaper advertisements and bulletin leaflets. **Table 1** presents the inclusion and exclusion criteria for entering the study. In the screening phase, inclusion and exclusion criteria included points 1–6, and intervention phase points 1–7.

Table 1. Inclusion and exclusion criteria. Screening phase (1–6), and intervention phase (1–7).

Inclusion criteria	**Exclusion criteria**
1) Age 40-65 years	1) History of a cardiac event
2) BMI 25-40 kg/m^2	2) Insulin or medically treated diabetes
3) Physically inactive (less than 120 minutes of moderate-intensity exercise per week reported during phone screening and initial physical activity questionnaires)	3) Any chronic disease or condition that could create a hazard to the participant's safety, endanger the study procedures, or interfere with the interpretation of study results
4) Sitting time ≥ 10 h /day or 60% of accelerometer wear time (measured by the accelerometer during screening)	4) Abundant use of alcohol. Alcohol consumption was determined by a questionnaire as units/week. One unit contains about 10 to 14 grams of alcohol. Abundant use was considered consumption higher than the national limits for high risk in Finland (more than 12 units for women and 23 units for men).
5) Blood pressure < 160/100 mmHg	6) Use of narcotics, smoking of tobacco, or consuming snuff tobacco
6) Fasting plasma glucose < 7.0 mmol/l	7) Diagnosed depressive or bipolar disorder
7) Fulfilment of the metabolic syndrome criteria (Alberti et al., 2009), including three of the following symptoms: - Central obesity (WC ≥ 94 cm for men and ≥ 80 cm for women) - Blood triglycerides ≥ 1.7 mmol/l - HDL-C < 1.0 mmol/l for men and < 1.3 mmol/l for women - Systolic blood pressure ≥130 and/or diastolic blood pressure ≥85 mmHg - Fasting glucose > 5.6 mmol/l	8) Previous positron emission tomography (PET) or considerable exposure to radiation*
*Inclusion criteria to enter the intervention phase	*Exclusion criteria for the intervention phase *The table has been modified from the original study II.*

4.3 Screening

The eligible volunteers were interviewed, and their BMI, WC, and blood pressure were measured. Subsequently, they were given a UKK AM30 accelerometer (UKK Institute, Tampere, Finland), with instructions to affix it to a flexible belt and wear it on their right hip for four consecutive weeks, beginning the following day. Participants were directed to wear the accelerometer at all waking hours, except for water-immersive activities, due to the device's lack of waterproofing. Furthermore, they were advised to maintain their usual activities and lifestyle during the measurement period. Owing to the limited storage capacity and battery life of the UKK AM30 sensor, participants were required to revisit the PET Centre after two weeks to obtain a replacement sensor with fresh batteries. Following this exchange, accelerometer measurements continued for an additional two weeks. Throughout the four-week measurement period, participants were directed to visit the nearest or most conveniently located Turku University Hospital Laboratory unit at a mutually agreeable time to collect fasting venous blood samples.

4.4 Intervention

After the screening phase, the participants who fulfilled the inclusion criteria were randomized (1:1) by a statistician into intervention (INT; n = 23) and control (CON; n = 21) groups by randomly permuted block randomization with stratification for sex (code generated with SAS 9.4 [SAS Institute Inc., Cary, NC, USA]). Baseline physical activity and sedentary behavior levels were measured during the 1-month screening phase. Next, the INT group was instructed to reduce their sedentary behavior by 1 h/day compared to the individual baseline and to replace it with non-exercise activities (e.g., standing, light physical activity [LPA], or moderate-to-vigorous physical activity [MVPA]), and the CON group was asked to maintain their baseline sedentary behavior and physical activity levels. Each participant received personal guidance and tips to achieve the goals, and both groups could monitor their daily activity on a mobile phone application (ExSed, www.exsed.com, UKK Terveyspalvelut Oy, Tampere, Finland) (Vasankari et al., 2019).

The mobile application displayed a visual summary of the collected data, showing the time spent in sedentary behavior, standing, LPA, and MVPA, as well as the number of steps and breaks in sedentary time for each day. This allowed participants to monitor their activity levels. In the application, target levels for sedentary behavior and different physical activity categories (standing, LPA, MVPA) were set based on baseline measurements during screening. One hour of sedentary behavior measured during screening was reduced and added to standing,

LPA, and MVPA, either equally or according to participant preferences. A maximum of 1/3 (20 minutes) was added to MVPA. The **figure 4** provides examples of the visual feedback provided by the mobile phone application.

Figure 4. Two illustrative examples of the visual feedback features offered by the mobile application developed by UKK Terveyspalvelut Oy (Tampere, Finland).

Participants were not recommended to add formal exercise but were encouraged to increase physical activity during work and free time and to reduce sitting time. Incorporating more walking into daily activities was accepted, as most participants found this to be the easiest and most convenient way to replace sitting with physical activity. They were contacted by telephone about once a month, and they visited the research center halfway through the intervention to support them in achieving intervention goals and ensuring the proper functioning of the accelerometer and mobile application.

The control group participants were asked to continue their normal daily routines and given the same accelerometer with a mobile application as the intervention group. The target levels of physical activity and sedentary behavior were determined based on the accelerometry results from the screening phase. They were contacted by telephone approximately once a month and visited the research center at the midpoint of the intervention to ensure the accelerometer was functioning properly. After measurements at the end of the study period, the control group participants could participate in a counseling session similar to the one initially received by the intervention group participants.

4.5 Methods

4.5.1 Accelerometry

Sedentary behavior and physical activity, standing, and breaks in sedentary time were measured during waking hours with validated hip-worn tri-axial accelerometers in the 1-month screening phase (UKK AM30, UKK-Institute, Tampere, Finland) and during the whole six-month intervention period (ExSed Movesense, Suunto, Vantaa, Finland) (**Figure 5**). Both accelerometers were equipped with digital tri-axial acceleration sensors. The UKK AM30 accelerometer (ADXL345; Analog Devices, Norwood MA, USA) records acceleration signals with a 100 Hz sampling frequency, a measurement range of ±16 gravitational units (g), and a resolution of 4 milligravities (mg). On the other hand, the Movesense accelerometer (LSM6DS3; STMicroelectronics, Geneva, Switzerland) operates at a 52 Hz sampling frequency, a measurement range of ±8 g, and a resolution of 4 mg. The Movesense device was linked to the ExSed application on the participant's mobile phone. Participants without a smartphone were provided with a suitable phone for the study. Collected acceleration data during the intervention were securely stored in a cloud server and later transferred for analysis. The analysis of the data was conducted by a researcher at the UKK Institute using a program in Microsoft Excel 2010 Visual Basic for Applications. Non-wear time was defined as a period during which the acceleration from all three measurement axes remained within a range of 187.5 mg for at least 30 minutes. A day with 10–19 hours of wear time was considered valid, whereas a measurement time exceeding 19 hours implied that the participant had likely slept with the accelerometer. The excess measurement hours were deducted from sedentary behavior time in such instances because it could distort the analysis by showing excessive immobility, particularly when lying down. Furthermore, the study calculated the proportions of different activity intensities (e.g., sedentary behavior, standing, LPA, MVPA) as a percentage of the wear time.

Figure 5. A visual presentation of the hip-worn tri-axial accelerometer used throughout the six-month intervention period (ExSed Movesense, Suunto, Vantaa, Finland).

Analysis of accelerometry data

In studies I–IV, the collected accelerometer data were analyzed in six-second epochs (Vähä-Ypyä, Vasankari, Husu, Suni, et al., 2015) using the validated mean amplitude deviation (MAD) method (Vähä-Ypyä, Vasankari, Husu, Mänttäri, et al., 2015). MAD values were further converted to metabolic equivalents (METs). LPA was defined as 1.5–2.9 METs (MAD 22.5–91.5 mg), and MVPA as ≥ 3.0 METs (MAD > 91.5 mg). Additionally, the proportions of different activity intensities (LPA and MVPA) per day were calculated and presented as a percentage of wear time. Total physical activity was calculated by adding LPA to MVPA. The number of participants who gained vigorous physical activity was very low, and the duration of such activity was very short (only a few minutes). Therefore, moderate and vigorous physical activity values were added and presented as MVPA.

Body posture was determined with the angle for posture estimation (APE) method only for the epochs when the estimated MET value was lower than the commonly considered 1.5 MET cut point for sedentary behavior (MAD less than 22.5 mg) (Vähä-Ypyä et al., 2018). The epochs with APE values less than 11.6° were classified as standing, and those with APE values at least 11.6° as sedentary behavior, including sitting and lying. The APE value for separating sitting from lying was 73° (Vähä-Ypyä et al., 2018). In reference (Vähä-Ypyä et al., 2018), the optimal cut-off point for separating sitting from lying is 64.9 degrees. However, in the

reference, the smallest measured APE value for lying is 73.9 degrees, and the highest APE value for sitting is 55.9 degrees. We decided to use 73 degrees as a cut-off point. Thus, reclining is more likely classified as sitting. In addition to actual time (h/day) spent sedentary, standing, and in physical activity, proportions of accelerometer wear time in sedentary behavior (sedentary behavior %) and standing (standing %) were calculated.

The number of breaks in sedentary time denoted the number of sedentary periods during which the one-minute exponential moving average of the MAD value was less than 22.5 mg and which ended up with a clear vertical acceleration and subsequent standing position or movement (Vähä-Ypyä et al., 2018). The step detection algorithm splits the measured acceleration into vertical and horizontal components. The vertical component is band-pass filtered (1–4 Hz), and positive values are integrated. When the integral value exceeds the specified limit, a step is detected. The step algorithm requires about a 3 km/h walking speed to detect every step (Vähä-Ypyä et al., 2018). A period was classified as non-wear time if the raw acceleration of each three-measurement axis remained within the 187.5 mg range for at least 30 minutes. For valid data collection, a wear time of 10–19 h/day on at least four days was required.

4.5.2 Whole-body insulin sensitivity and HOMA-IR

In studies II-IV, the hyperinsulinemic-euglycemic clamp was conducted following a minimum 10-hour fasting period. A primed-constant insulin infusion of Actrapid (100 U/ml, Novo Nordisk, Bagsvaerd, Denmark) at a rate of 160 mU/min/m2 of the participant's body surface area was administered for the initial 4 minutes. Subsequently, the infusion rate was reduced to 80 mU/m2 /min between 4 and 7 minutes and then maintained at 40 mU/m2 /min until the clamp was completed. An exogenous 20% glucose infusion, starting 4 minutes after the onset of the insulin infusion, was administered at a rate of ml/h per participant's body mass (kg) × 0.5. For instance, for an individual weighing 80 kg, the rate was 40 ml/h, equivalent to approximately 8 g of glucose per hour. At 10 minutes, the glucose infusion was doubled and further adjusted based on blood glucose concentration to closely maintain it at 5 mmol/L. Arterialized venous blood samples were collected every 5 minutes within the initial 30 minutes and at steady state every 10 minutes to ascertain glucose concentration and adjust the glucose infusion rate. The whole-body insulin-stimulated glucose uptake rate was derived from measured steady-state glucose values and glucose infusion rate, beginning at 20 minutes post-commencement of the hyperinsulinemic-euglycemic clamp. The outcome, M-value, represents whole-body glucose uptake as mg/kg/min. In studies II and III, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated utilizing the formula: fasting glucose (mmol/l) x fasting insulin (µmol/l) / 22.5.

4.5.3 Liver glucose uptake

In studies III and IV, liver glucose uptake was measured during a hyperinsulinemic-euglycemic clamp combined with 2-deoxy-2-[^{18}F] fluoro-D-glucose ([^{18}F]FDG) positron emission tomography (PET) imaging using a PET/computed tomography (CT) scanner (GE D690, GE Healthcare, Milwaukee, US) (**Figure 6**). Radiotracer [^{18}F]FDG (Hamacher et al., 1986) was produced, and [^{18}F]FDG uptake was analyzed as previously described (Sjöros, Laine, Garthwaite, Vähä-Ypyä, Löyttyniemi, et al., 2023). Hepatic region imaging started simultaneously with the tracer (168 [SD 11] MBq) injection into the antecubital vein 75 (SD 12) min after starting the clamp. The cumulative availability of the tracer in plasma (input function) was determined from the radioactivity in the heart's left ventricle during the first 40 min of PET imaging and from blood samples collected at approximately 50 and 70 min after the injection. All data were corrected for dead-time, decay, and measured photon attenuation. Dynamic PET scan was reconstructed using an iterative reconstruction method.

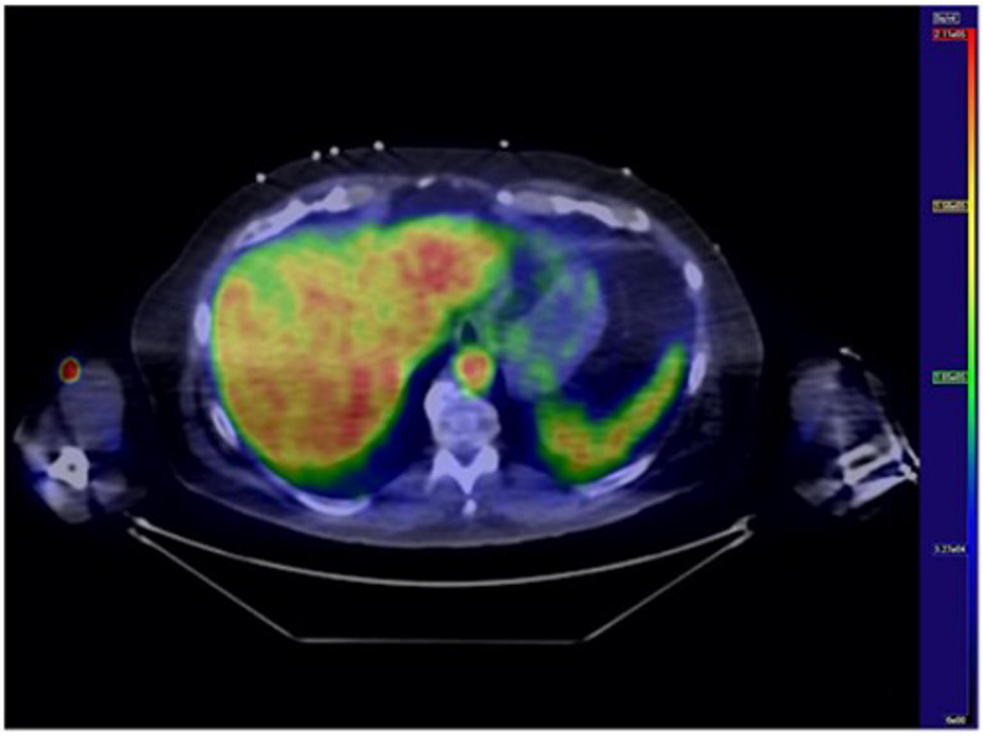

Figure 6. Liver glucose uptake. This transaxial [^{18}F]FDG PET/CT image of the liver illustrates the variation in glucose uptake. Areas depicted in blue signify low [^{18}F]FDG uptake, whereas regions shown in red indicate high uptake levels. *The figure is from the original publication III.*

^{18}F-FDG activity in the hepatic tissue was measured by drawing a region of interest (ROI) in the right lobe of the liver using a CT image as an anatomic reference. The PET/CT images were analyzed with Carimas (v.2.71, Turku PET Centre, Turku, Finland). Liver glucose uptake (µmol/ml/min) was calculated by multiplying the tissue fractional phosphorylation rate (Ki) by the average plasma glucose concentration and dividing by a lumped constant (LC) during scanning. The liver's LC is 1.0 (Iozzo et al., 2007). The software Carimas (v.2.71, Turku PET Centre, Turku, Finland) was used to analyze PET/CT images of the liver. Plasma radioactivity was measured with an automatic gamma counter (Wizard 1480 3", Wallac, Turku, Finland).

4.5.4 Endogenous glucose production

In studies III and IV, EGP was calculated as previously described (Honka, 2019) by subtracting the exogenous glucose infusion rate (GIR) corrected by space correction (DeFronzo et al., 1979) from the glucose rate of disappearance (Rd) during the hyperinsulinemic-euglycemic clamp (Honka et al., 2018), according to the following equation;

$$\mathbf{EGP = Rd + V_{glucose}\ x\ \frac{\Delta\ glucose}{\Delta T}\ -\ GIR}$$

Rd = rate of disappearance (µmol/kg/min)

$\mathbf{V_{glucose}}$ = estimated glucose distribution volume (0.19 l/kg)

Δglucose = change in glucose from [^{18}F]FDG injection to the end of blood sampling (mmol/l)

ΔT = time from [^{18}F]FDG injection to the end of blood sampling (min)

GIR = glucose infusion rate (µmol/kg/min)

Rd was calculated using [^{18}F]FDG clearance corrected by tracer lost to urine (Iozzo et al., 2006);

$$\mathbf{Rd} = \frac{dose_{FDG} - urine_{FDG}}{AUC_{FDG}}\ \mathbf{x}\ glucose_{avg}$$

$\mathbf{Dose_{FDG}}$ = radioactivity of the injected [^{18}F]FDG

$\mathbf{Urine_{FDG}}$ = secreted [^{18}F]FDG to urine from the tracer injection until voiding bladder at the end of the study

$\mathbf{AUC_{FDG}}$ = area under the curve representing [^{18}F]FDG from the tracer injection to infinity

$\mathbf{Glucose_{avg}}$ = average glycemia during the interval between the time of [^{18}F]FDG injection and the end of blood sampling.

4.5.5 Cardiorespiratory fitness

In studies II and III, we measured the participants' maximal oxygen consumption (VO_{2max}) after they underwent a thorough physical examination and electrocardiographical measurements. To measure VO_{2max}, we used a bicycle ergometer (eBike EL Ergometer + CASE v6.7, GE Medical Systems Information Technologies, Inc. Milwaukee, WI, USA) with direct respiratory measurements (Vyntus CPX, CareFusion, Yorba Linda, CA, USA). We also calculated VO_{2max} per fat-free mass (FFM) (ml/min/kgFFM) and maximal load (Wmax). The exercise workload started at 25 W and increased by 25 W every three minutes until exhaustion. Participants were instructed to maintain a 60–65 rpm pace throughout the test. We measured blood pressure and perceived exertion on the Borg scale (Borg, 1982) one minute after each increase in workload. We considered VO_{2max} to be achieved if one of the following criteria was met: a respiratory exchange ratio > 1.0, a plateau in VO2, or a heart rate within ±10 bpm of the age-predicted maximum. VO_{2max} was defined as the highest one-minute average in ml/min/kg.

4.5.6 Liver fat content

In studies II–IV, liver fat content was measured by magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI), based on the two-point Dixon [2PD] method using a Philips 3 Tesla system (Ingenuity TF PET/MR) with a Q-Body coil. The spectra were acquired using stimulated echo acquisition mode (STEAM) 1H MRS with parameters: repetition time (TR)/echo time (TE)/mixing time (TM) = 2000/11/17 ms, 4 averages, 2048 samples, spectral bandwidth 2000 Hz, and acquisition volume 20 × 20 × 30 mm^3. Data were acquired during 12 breath holds. Water saturation was done with chemical shift selective (CHESS) pulses with 50 Hz bandwidth. The duration of the scan was 3:12. The 3D T1-fast field echo sequence was acquired in the axial plane with parameters: TR/TE1/TE2 = 2.8 / 0.81 / 1.8 ms, flip angle 10°, FOV 510 mm × 510 mm, imaging matrix 188 × 188. Data was reconstructed to voxel size 2.13 × 2.13 × 4 mm^3. Respiratory gating was used in the thorax–upper abdomen area.

Because of the MRI scanner replacement during these studies, MRS and MRI quantification of the liver fat content of seven participants was conducted with Siemens Magnetom Skyra fit 3 T MRI system (Siemens Healthcare, Erlangen, Germany) with Siemens Body 30 and 18 channel coils and 32 channel Spine coil. The spectra were acquired with point resolved spectroscopy (PRESS) 1H MRS with parameters TR/TE = 4000/30 ms, averages 32, 1024 samples, acquisition volume 20 × 20 × 20 mm^3. Respiratory motion was controlled using a navigator. Water saturation was done with a 35 Hz bandwidth. The duration of the scan was 3:10. The 3D gradient echo volumetric interpolated breath-hold examination (VIBE Dixon)

sequence was acquired in the axial plane with parameters: TR/TE1/TE2 = 3.97 / 1.23 / 2.46, flip angle 9°, and voxel size 2 × 2 × 2 mm3. Breath holds were used in the thorax–upper abdomen area. Controlled aliasing in parallel imaging results in higher acceleration (CAIPIRINHA) and was used as a data acquisition technique to reduce breath-holds without affecting image resolution, coverage, or contrast. (https://www.siemens-healthineers.com/en-us/magnetic-resonance-imaging/options and-upgrades/clinical-applications/caipirinha). Water signal and fat signal images were used to calculate the fat fraction map (Bray et al., 2018), from which the liver fat content was determined; an MRI image was used as an anatomical reference.

Image analysis

The LC Model (Version 6.3-0C) was used to quantify liver fat, with 'liver-4' as a spectrum type. Lipid signals at 1.6 ppm, 1.3 ppm, and 0.9 ppm were used. The fat and water signals were corrected due to the difference in T2 decay (Bray et al., 2018; Rigazio et al., 2008) and molar concentrations of 1H nuclei in fat and water, as reported before (Rigazio et al., 2008; Szczepaniak et al., 1999). Liver fat content was defined as fat in relation to the total weight of liver tissue (Thomsen et al., 1994).

MRI images were analyzed using Carimas software version 2.10 (http://turkupetcentre.fi/). Four representative three-dimensional regions of interest (ROIs) were drawn manually on the liver sections (left lateral and medial section, right anterior and posterior section), avoiding the main portal veins. The results were volume corrected with the formula: mean value of one section x (total volume (mm3) of one section / total volume of all sections).

4.5.7 Body composition, anthropometry, and blood sampling

In studies II-IV, we utilized validated (Fields et al., 2002) air displacement plethysmography (the Bod Pod system, COSMED, Inc., Concord, CA, USA) (**Figure 7**) with predicted thoracic gas volume to estimate body composition (body fat%) following a minimum four-hour fast. Before the measurement, participants were instructed to abstain from exercise and showering. Upon emptying their bladders, participants entered the measurement chamber wearing a tight cap, underwear, or a swimming suit. In studies I–IV, body weight was recorded using a scale (Seca 797, Vogel & Halke, Hamburg, Germany) with participants in light clothing, and body height was measured barefoot using a wall-mounted stadiometer. Body mass index (BMI) was derived from the measured weight and height in kg/m^2. Waist circumference (WC) was measured using a flexible measuring tape placed midway between the iliac crest and the lowest rib, with the measurement repeated twice or until the same measure was obtained twice. The anthropometric variables

were assessed under standardized conditions, with all measurements performed by the same researcher to ensure consistency.

Figure 7. Air displacement plethysmography (the Bod Pod system, COSMED, Inc., Concord, CA, USA) with predicted thoracic gas volume was used to estimate body composition.

In studies I–IV, the plasma glucose levels were analyzed using the enzymatic reference method with hexokinase GLUC3, while plasma insulin levels were determined using electrochemiluminescence immunoassay (Cobas 8000 e801, Roche Diagnostics GmbH, Mannheim, Germany). Hemoglobin A1c (HbA1c) levels were assessed using a turbidimetric inhibition immunoassay (Cobas 6000 c501, Roche Diagnostics GmbH, Mannheim, Germany). Additionally, plasma triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured using enzymatic colorimetric tests (Cobas 8000 c702, Roche Diagnostics GmbH, Mannheim, Germany). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined using the photometric IFCC (International Federation of Clinical Chemistry) method (Cobas 8000 c702 and c 502 Analyzer, Roche

Diagnostics GmbH, Mannheim, Germany), and γ-glutamyltransferase (GGT) levels were assessed using enzymatic colorimetric tests and assay (Cobas 8000 c702, Roche Diagnostics GmbH, Mannheim, Germany). All sample analyses took place at the Turku University Hospital Laboratory.

4.5.8 Dietary intake

In studies II–IV, participants were instructed to adhere to their regular eating habits during the intervention. Daily total energy intake (EI) and intakes of carbohydrates (CHO), protein, fat, alcohol, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), saccharose, fiber, and water-insoluble dietary fiber (WIDF) were computed from 4-day food diaries, which included one weekend day. These calculations were performed before and following the intervention and analyzed by a nutritionist utilizing the computerized AivoDiet software (AivoDiet 2.2.0.1, Aivo, Turku), which utilizes the Finnish Food Composition Database Fineli (Health & Welfare, 2019).

4.5.9 Statistical methods

In all studies (I–IV), the normality of distribution was assessed by visual evaluation, the Shapiro-Wilk test, and logarithmic transformations. Log variables were back-transformed to the original scale (estimates then being geometric means). All the associations were examined using Pearson correlation coefficient analysis and linear mixed models. Intervention effects were analyzed with linear mixed models for repeated measures (IV). An unpaired t-test was used to test differences between sexes (I-IV) or INT and CON groups (IV). In all studies (I–IV), missing data were handled by pairwise deletion, and multicollinearity was controlled for with the variance inflation factor in all the models. All the values were below five and, thus, were considered not to have multicollinearity issues.

The specific sample size was not determined for study (I) because it consists of the screening phase of a clinical trial. A power calculation was performed to determine the sample size for the primary outcome (whole-body insulin sensitivity) of the sedentary behavior reduction intervention study (NCT03101228), from which the imaging measurements form the data of studies II- IV. In all the studies, data are expressed as mean and standard deviation (SD), standardized β coefficients, and 95% CI values. The level of statistical significance was set at 5% (two-tailed).

Correlation analyses were carried out with the JMP pro 13.1 for Windows (SAS Institute Inc., Cary, NC, USA), IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp, Armonk, NY, USA), JMP pro 16 for Windows (SAS Institute Inc., Cary, NC, USA) and GraphPad Prism version 5.01 for Windows (GraphPad Software, La

Jolla California USA). Intervention effects were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). All the figures were created with GraphPad Prism 5.01 (GraphPad Software, San Diego, CA, USA) and with JMP®, pro 16.0 (SAS Institute Inc., Cary, NC, 1989–2023).

Statistical methods (I)

Study I examined the associations between ALT, AST, and GGT and health markers, sedentary behavior (SB), and physical activity (PA) measures. There was a significant difference in liver enzyme levels between men and women, and therefore, sex was included as a covariate in all analyses. In the linear model, BMI was added to the model to adjust for confounding overweight and obesity. For GTT (a marker of alcohol use) related outcomes, the use of alcohol consumption was added to the model. Additionally, we added the accelerometer wear time to the model. We ran the following linear mixed model for outcomes (liver enzymes) = sex + age + SB/PA measure + age × SB/PA + sex × SB/PA. Logarithmic (log10) transformations were performed on ALT, AST, GGT, and HOMA-IR. Four of the 144 participants had missing values: fasting blood samples were missing for two participants as they failed to visit the laboratory, and resting heart rate values were missing for two participants due to incomplete documentation.

Statistical methods (II)

Study II examined the associations between liver fat content and sedentary behavior, physical activity measures, fitness, health markers, and nutrient intake. Because of a significant between-sex difference in liver fat content values, sex was included as an explanatory variable in all analyses (model 1). Body fat % was added to the linear model to adjust for confounding overweight and obesity (model 2). Linear regression model, Tukey mean difference test, and Bland–Altman analysis were used to analyze the correlation and the agreement between MRS and MRI (2PD). Three participants' MRS-measured liver fat content was missing due to image artifacts, and one participant's MRS and MRI-measured liver fat content was missing due to technical challenges with the scanner. The VO_{2max} measures of two participants were missing because they interrupted the test before exhaustion (knee pain or breathing difficulties), and the results of one participant were lost due to technical difficulties. The fasting plasma glucose value of one participant and the resting heart rate values of two participants were missing due to incomplete documentation.

Statistical methods (III)

In study III, we examined the associations of liver glucose uptake and EGP with sedentary behavior and physical activity measures, nutrient intake, fitness, and cardiometabolic health markers. The first model was adjusted for sex and age (model 1), and the second model additionally for body fat % (model 2). Additionally, all the models with sedentary behavior and physical activity outcomes were adjusted for accelerometer wear time. One participant's liver glucose uptake and EGP measures were missing due to technical difficulties. VO_{2max} measures of two participants were excluded because the test was stopped before reaching volitional exhaustion (due to knee pain or difficulties in breathing), and the results of one participant were lost due to technical problems. The MRS-measured liver fat content of three participants was missing due to image artifacts, and the MRS and MRI-measured liver fat content of one participant was missing due to technical challenges with the scanner.

Statistical methods (IV)

Intervention effects were analyzed with multilevel models for repeated measures. We examined the effects of reducing sedentary behavior on liver insulin sensitivity (liver glucose uptake, EGP), liver fat content, and liver enzymes. Models included sex and time as within-subject factors, a group as a between-subject factor, and group-by-time interaction (group * time), which evaluates whether mean changes over time differ between the groups. Multiple comparisons were adjusted with the Tukey-Kramer method. An unstructured covariance structure or compound symmetry was used for a time, and the model with lower Akaike information criterion (AIC) values was chosen. We visually assessed the normal distribution of the residuals with the quantile-quantile (Q-Q) plot. A Pearson correlation analysis was performed to investigate the relationships between the changes (Δ) observed during the intervention. Our correlation analyses were comprehensive and included all participants from the study, regardless of their group assignments. INT and CON groups, sexes, and changes during the intervention between sexes were compared with an unpaired t-test.

Secondary analysis (III and IV)

In the study III, we used an unpaired t-test to compare EGP according to standing time (≤1h 45min [n=22] vs. >1h 45 min [n=21], and daily sedentary time (≤ 10.0 h/day [n=21] vs. >10.0 h/day [n=22]), and liver glucose uptake according to daily fiber consumption (≤ 18 g/day [n=21] vs. >18 g/day [n=22]) and water-insoluble fiber consumption (≤ 13 g/day [n=21] vs. >13 g/day [n=22]).

In study IV, there was considerable variation in changes in sedentary behavior in both groups. Therefore, we evaluated each participant's individual changes in measured sedentary behavior (hours/day) with valid accelerometry data (n=39) (**Figure 8**). We divided all participants into two groups based on whether they A) reduced sedentary behavior (n=22, mean 52 min/day [from 5 to 151 min] reduction in sedentary behavior) or B) maintained or increased sedentary behavior (n=17, mean 34 min/day [4–94 min] increase in sedentary behavior).

Additionally, an adjustment to the upper limit of the liver fat content to define MASLD from 5% to 1.85% has recently been proposed due to the association of a liver fat content of 1.85–5.56% with reduced insulin sensitivity and increased cardiometabolic risk factors compared to individuals with a liver fat content of less than 1.85% (Petersen et al., 2022). Consequently, an assessment was also conducted to determine whether the reduction in sedentary behavior could yield varied outcomes in participants with an MRS-measured liver fat content higher or lower than 1.85%.

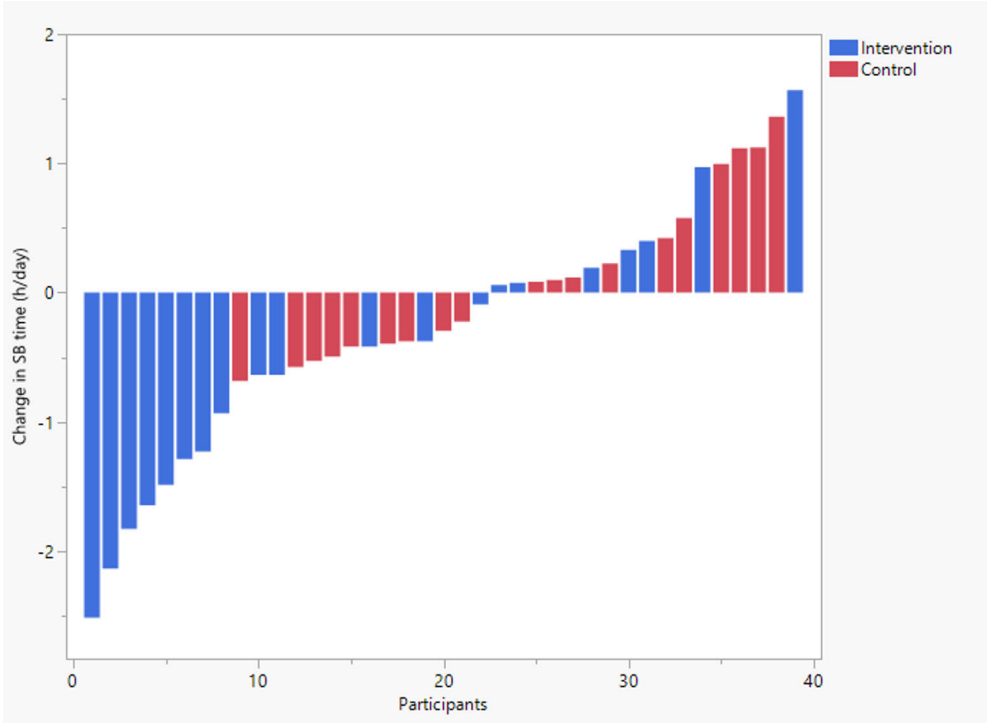

Figure 8. The individual change in measured sedentary behavior (SB) time (hours/day) of each participant with valid accelerometer data (n=39) during the intervention. Intervention group (blue bar) and control group (red bar). *The figure is from the original publication IV supplementary material.*

5 Results

5.1 Baseline characteristics of the screening phase (I)

The **Table 2** illustrates the baseline characteristics of participants in the screening phase categorized by sex. Of the 263 individuals screened, 102 women and 42 men met the inclusion criteria, completed the accelerometer measurements, and were consequently included in the analyses. The average accelerometer wear time was 14.4 (SD 1.0) hours per day, and the average measurement duration was 25 (SD 4) days. Sixty-two percent of the participants were classified as obese (BMI > 30 kg/m^2), while 38% were categorized as overweight (BMI 25.0 to < 30). Half of the participants were on medication for elevated blood pressure, and 11% for elevated blood cholesterol. A portion of the participants disclosed their use of various medications, including hormonal medication (14.6%), thyroid medication (13.8%), antidepressants (13.2%), gastrointestinal medication (11.1%), pain medication (9.7%), rheumatoid or osteoarthritis medication (6.9%), allergy medication (5.6%), asthma medication (3.5%), medication for urinary problems (4.2%), anticoagulants (4.2%), sleep medication (3.5%), medication for vision and hearing related issues (2.8%), migraine medication (2.1%), medication for restless legs syndrome (1.4%), psoriasis medication (0.7%), and epilepsy medication (0.7%).

In comparison, male participants exhibited significantly higher daily lying time, sedentary behavior time, and sedentary behavior proportion. Conversely, women demonstrated more interruptions in daily sedentary time, with higher standing time and standing proportion than men. Additionally, there were no differences between the sexes in sitting time, daily steps, LPA, LPA percentage, MVPA, MVPA percentage, overall physical activity, and physical activity percentage. In terms of health measurements, men showcased significantly higher alcohol consumption, as well as elevated levels of ALT, GGT, fasting insulin, and HOMA-IR. Conversely, women exhibited higher cholesterol and HDL-C levels. No significant variations between the sexes were observed in AST, systolic blood pressure (SBP), diastolic blood pressure (DBP), resting heart rate, fasting glucose, triglycerides, LDL-C, and HbA1c.

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

	Men	Women
n, (% of total)	42 (29)	102 (71)
Age, years	58.0 (6.0)	56.4 (6.7)
Anthropometrics		
BMI, kg/m^2	31.8 (3.6)	31.7 (4.2)
Waist, cm	116.3 (11.0)***	106.7 (10.4) ***
Health measurements		
ALT, U/l	37 (20) **	28 (14) **
AST, U/l	29 (10)	27 (7)
GGT, U/l	40 (19)**	33 (33) **
Alcohol consumption, units (10–14g of alcohol)/week	5 (6)**	2 (2)**
SBP, mmHg	149 (19)	147 (20)
DBP, mmHg	91 (11)	90 (12)
Resting heart rate, bpm	70 (11)	71 (11)
BP medication, n (%)	23 (55)*	34 (33) *
Cholesterol medication, n (%)	8 (11)	11 (11)
f-Glucose, mmol/l	5.9 (0.7)	5.8 (0.9)
f-Insulin, µmol/l	16 (10)**	12 (7) **
HOMA-IR	4.2 (3.0)*	3.2 (2.4) *
Triglycerides, mmol/l	1.6 (0.9)	1.4 (0.8)
Cholesterol, mmol/l	5.0 (0.7)*	5.4 (0.9) *
HDL-C, mmol/l	1.3 (0.3)***	1.7 (0.4) ***
LDL-C, mmol/l	3.4 (0.7)	3.5 (0.9)
HbA_{1c}, mmol/mol	38 (5)	37 (6)
Sedentary behavior		
Lying time, h/days	2.0 (1.1)***	1.3 (0.7) ***
Sitting time, h/day	8.1 (1.4)	8.1 (1.1)
Sedentary time, h/day	10.1 (1.2)**	9.4 (1.3) **
Sedentary proportion, %/day	71.0 (7.3)***	65.4 (8.1) ***
Physical activity		
Accelerometry, days	24 (5)	26 (4)
Wear time, h/day	14.3 (1.1)	14.4 (1.0)
Breaks in SB, time/day	26 (7)**	30 (8) **
Standing, h/day	1.4 (0.4)***	2.2 (0.8) ***
Standing proportion, %/day	10.1 (2.9)***	15.0 (5.0) ***
Daily steps	5408 (2288)	5206 (2046)
LPA, h/day	1.7 (0.6)	1.8 (0.5)
LPA proportion, %/day	11.7 (3.9)	12.8 (3.1)
MVPA, h/day	1.0 (0.4)	0.98 (0.4)
MVPA proportion, %/day	7.3 (2.9)	6.8 (2.5)
PA, h/day	2.7 (0.9)	2.8 (0.7)
PA proportion, %/day	19.0 (5.8)	19.6 (4.8)

Significant p-values; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Sex difference in t-test (or Fisher's exact test, when applicable). Abbreviations; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BP = blood pressure, DBP = diastolic blood pressure; GGT = γ-glutamyltransferase; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; LPA = light physical activity; MVPA = moderate to vigorous physical activity; PA = physical activity (LPA and MVPA together); SBP = systolic blood pressure. *The table is from the original publication I.*

5.2 Baseline characteristics of cross-sectional studies (II & III) and the intervention (IV)

In total, 263 individuals volunteered. Of these, 155 participated in the screening measurements, and 64 were included in the hyperinsulinemic-euglycemic clamp (HEC) analysis (Sjöros, Laine, Garthwaite, Vähä-Ypyä, Löyttyniemi, et al., 2023). Of these, 44 were included in this imaging study with PET combined with HEC and MRS. Participants were sedentary middle-aged adults with metabolic syndrome. **Figure 9** shows the study flow diagram.

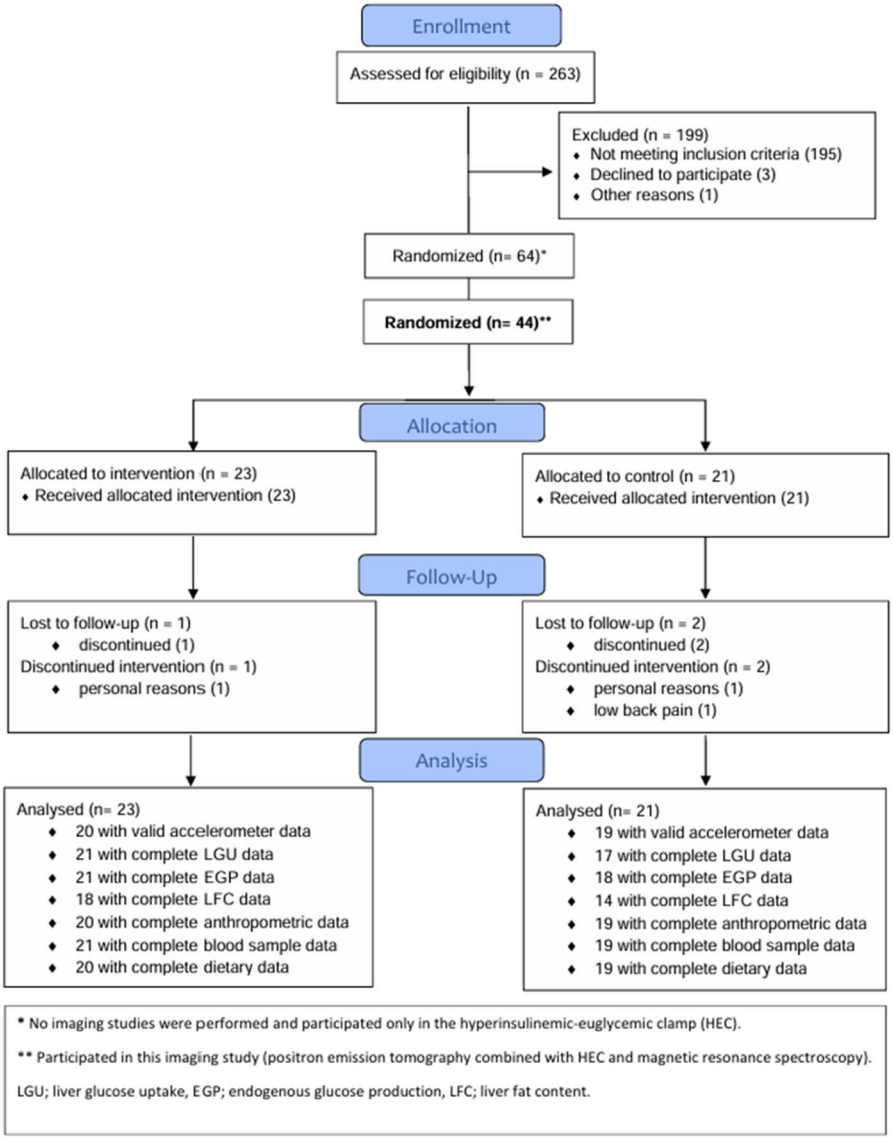

Figure 9. Study flow diagram. *The figure is from the original publication IV.*

The baseline characteristics of the participants are shown in **Table 3**, categorized by sex. Sixty-six percent of the participants were obese (BMI > 30 kg/m^2), and 34% were overweight (BMI 25.0 to < 30). Men exhibited significantly higher liver fat content, AST, HOMA-IR, and fasting insulin levels than women. Conversely, women had higher body fat % and HDL-C levels. Women also had slightly longer daily accelerometer wear time, standing time, daily standing %, LPA and LPA %, and breaks in sedentary behavior. However, men had higher daily sedentary behavior % and VO_{2max} levels compared to women. Participants in the study were taking medication for elevated blood pressure (n=24) and elevated blood cholesterol (n=9). Additionally, some participants reported using hormonal replacement therapy medication (n=7), pain medication (n=5), anticoagulants (n=5), thyroid medication (n=4), gastrointestinal medication (n=4), allergy or asthma medication (n=4), antidepressants (n=3), sleep medication (n=3), medication for urinary problems (n=2), osteoarthritis medication (n=1), and medication for restless legs syndrome (n=1).

Table 3. Characteristics of the study participants by sex. The results are reported as mean (SD) if not otherwise stated.

	Men	**Women**
n, (% of total)	19 (43)	25 (57)
Age, years	58 (6.0)	57 (7.3)
Anthropometrics		
BMI, kg/m^2	31.8 (4.7)	32.5 (4.1)
Waist circumference, cm	115.2 (13.2)	108.4 (9.5)
Body fat %	37.5 (7.8)	48.1 (3.8) *
Liver fat content		
MRS, fat fraction %	5.1 (3.8)	2.6 (3.0) *
MRI, fat fraction %	11.9 (5.8)	8.0 (3.9) *
LGU and EGP		
LGU, µmol/100 ml/min	2.2 (1.0)	3.0 (1.4)
EGP, µmol/kg/min	-0.2 (8.4)	-1.9 (7.7)
Health measurements		
SBP, mmHg	140 (14)	146 (13)
DBP, mmHg	89 (9)	89 (6)
BP medication, n (%)	13 (68)	9 (36)
Cholesterol medication, n (%)	4 (21)	4 (16)
Resting heart rate, bpm	67 (6)	68 (10)
f-Glucose, mmol/l	5.9 (0.5)	5.7 (0.2)
f-Insulin, µmol/l	16.2 (9.4)	10.4 (4.3)*
HbA_{1c}, mmol/mol	37.8 (2.5)	36.8 (2.7)
HOMA-IR	4.3 (2.7)	2.7 (1.1) *
M-value, mg/kg/min	2.9 (2.8)	3.6 (2.1)

	Men	Women
Triglycerides, mmol/l	1.4 (0.5)	1.3 (0.7)
Cholesterol, mmol/l	4.4 (0.7)	4.9 (1.1)
HDL-C, mmol/l	1.1 (0.3)	1.4 (0.3) **
LDL-C, mmol/l	2.9 (0.7)	3.2 (1.0)
ALT, U/l	36 (18)	27 (12)
AST, U/l	31 (12)	23 (5) *
GGT, U/l	34 (21)	25 (17)
Accelerometry & fitness		
Lying time, h/days	1.9 (0.9)	1.6 (0.5)
Sitting time, h/day	8.3 (1.1)	8.5 (1.0)
SB time, h/day	10.3 (0.9)	10.1 (0.9)
SB time, % of daily wear time	72 (7)	68 (5) *
Accelerometry, days	26 (2)	27 (3)
Wear time, h/day	14.3 (1.0)	14.9 (0.8) *
Breaks in SB, time/day	24 (5)	32 (8) **
Standing, h/day	1.5 (0.4)	2.0 (0.5) ***
Standing, % of daily wear time	10.1 (2.8)	13.3 (3.2) **
Daily steps	5194 (2134)	4986 (1382)
LPA, h/day	1.6 (0.5)	1.9 (0.3) *
LPA, % of daily wear time	10.9 (3.2)	12.7 (2.1) *
MVPA, h/day	1.0 (0.4)	0.9 (0.2)
MVPA, % of daily wear time	6.9 (2.7)	6.2 (1.6)
PA, h/day	2.6 (0.7)	2.8 (0.4)
PA, % of daily wear time	17.8 (4.5)	18.9 (2.7)
VO_{2max}, ml/min/kg	24.7 (5.6)	21.3 (3.4) *
VO_{2max}, ml/min/kg_{FFM}	39.5 (7.4)	41.0 (5.6)
Maximal load, W	145.7 (36.6)	120.1 (28.4)
Nutrition		
Total EI, kcal/day	1884.0 (377.8)	1742.5 (341.9)
Protein, % of daily EI	17.9 (2.9)	17.8 (2.8)
Carbohydrates, % of daily EI	39.7 (7.8)	40.7 (6.0)
Fat, % of daily EI	38.8 (6.2)	38.0 (5.2)
Alcohol, % of daily EI	1.7 (3.5)	1.4 (1.6)
SFA, % of daily EI	14.5 (3.3)	13.7 (2.4)
MUFA, % of daily EI	13.5 (3.7)	12.9 (2.0)
PUFA, % of daily EI	5.6 (1.5)	5.9 (1.2)
Saccharose, % of daily EI	7.3 (3.2)	8.3 (4.2)

Significant p-values; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, vs men. Sex difference in t-test (or Fisher's exact test, when applicable). Abbreviations: ALT = alanine aminotransferase, AST = aspartate aminotransferase, DBP = diastolic blood pressure, EGP = endogenous glucose uptake, EI = energy intake, f = fasting, FFM = fat-free mass, GGT = γ-glutamyltransferase, HbA_{1c} = hemoglobin A_{1c}, HDL-C = high-density lipoprotein cholesterol, HOMA-IR = homeostatic model assessment for insulin resistance, LDL-C = low-density lipoprotein cholesterol, LGU = liver glucose uptake, LPA = light physical activity, MRI = magnetic resonance imaging, MRS = magnetic resonance spectroscopy, MUFA = monounsaturated fatty acids, M-value = whole-body insulin sensitivity, MVPA = moderate to vigorous physical activity, PA = physical activity (LPA + MVPA), SB = sedentary behavior (sitting + lying), SBP = systolic blood pressure, SFA = saturated fatty acids, PUFA = polyunsaturated fatty acids, VO_{2max} = maximal oxygen consumption. *The table is modified from the original publications II and III.*

5.3 Cross-sectional studies (I, II & III)

5.3.1 Associations between liver enzymes, sedentary behavior, physical activity, body composition, and other metabolic markers (I)

Associations between overall body adiposity with physical activity and sedentary behavior

BMI was associated negatively with MVPA (r= -0.23), daily steps (r=-0.30), and breaks in sedentary behavior time (r=-0.32), and positively with sedentary behavior % (r=0.21) but not with total sedentary time (r=-0.04). WC was associated with breaks in sedentary behavior (r=-0.35), MVPA (r=-0.26), daily steps (r=-0.31), sedentary behavior % (r=0.27), and standing time (r=-0.27), (all p-values < 0.05), whereas with LPA, there was no association (r=-0.13, p=0.13). All associations were adjusted for sex and age.

Association between liver enzymes, overall body adiposity, and other health markers

Upon adjusting for age and sex, ALT, AST, and GGT were associated positively with BMI and WC. Furthermore, these enzymes were associated positively with SBP, resting heart rate, fasting insulin, HOMA-IR, and HDL-C. Additionally, ALT and GGT were found to be positively associated with fasting glucose and triglycerides, while ALT and AST demonstrated positive associations with HbA1c. However, ALT, AST, and GGT did not exhibit statistically significant associations with LDL-C or total cholesterol **(Table 3)**.

Table 3. Age and sex-adjusted Pearson partial correlation coefficients between circulating liver enzymes (ALT, AST, GGT), anthropometrics, and health measurements.

	ALT[a], U/l	**AST[a], U/l**	**GGT[a], U/l**
Anthropometrics			
BMI, kg/m^2	**0.34 *****	**0.17 ***	**0.29 *****
Waist, cm	**0.41 *****	**0.26 ****	**0.32 *****
Health measurements			
SBP, mmHg	**0.27 ****	**0.18 ***	**0.25 ***
DBP, mmHg	**0.27 ****	**0.23 ****	**0.27 ****
Resting heart rate, bpm	**0.20 ***	**0.27 ****	**0.25 ****
f-Glucose, mmol/l	**0.30 *****	**0.16**	**0.18 ***
f-Insulin, mU/l	**0.48 *****	**0.35 *****	**0.31 *****
HOMA-IR[a]	**0.46 *****	**0.30 *****	**0.31 *****
Triglycerides, mmol/l	**0.23 ****	**0.08**	**0.22 ****
Cholesterol, mmol/l	-0.04	-0.04	0.04
HDL-C, mmol/l	**-0.24 ****	**-0.19 ***	**-0.18 ***
LDL-C, mmol/l	-0.02	0.04	0.04
HbA_{1c}, mmol/mol	**0.30 *****	**0.20 ***	0.09

Significant p-values; * < 0.05, ** < 0.01, *** < 0.001. Significant values are in bold. Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; DBP = diastolic blood pressure; GGT = γ-glutamyltransferase; HDL-C = high-density lipoprotein cholesterol; HOMA-IR = homeostatic model assessment for insulin resistance; LDL-C = low-density lipoprotein cholesterol; SBP = systolic blood pressure; [a] = log10 transformed variables. *The table is from the original publication I.*

Associations between liver enzymes with sedentary behavior and physical activity

No statistically significant associations were observed between the liver enzymes ALT, AST, and GGT and various aspects of sedentary behavior such as lying time, sitting time, sedentary behavior time, and sedentary behavior percentage. Similarly, no statistically significant associations were found between the liver enzymes and measures of physical activity, including standing time, standing %, LPA, LPA %, MVPA, MVPA %, total physical activity (combined LPA and MVPA), total physical activity %, breaks in sedentary behavior, and daily steps when adjusted for age and sex **(Table 4)**.

Table 4. Age and sex-adjusted Pearson partial correlation coefficients between circulating liver enzymes (ALT, AST, GGT), sedentary behavior, and physical activity.

	ALT[a], U/l	**AST[a], U/l**	**GGT[a], U/l**
Sedentary behavior			
Lying time, h/day	-0.06	-0.06	-0.03
Sitting time, h/day	0.05	0.03	-0.03
SB time, h/day	0.002	-0.01	-0.05
SB proportion, %/day	0.04	-0.008	0.03
Physical activity			
Breaks in SB time/day	-0.14	-0.07	-0.16
Standing, h/day	-0.08	-0.06	-0.12
Standing proportion, %/day	-0.07	-0.07	-0.11
Steps, number/day	-0.15	-0.03	-0.07
LPA, h/day	0.07	0.1	0.04
LPA proportion, %/day	0.09	0.11	0.06
MVPA, h/day	-0.13	-0.01	-0.02
MVPA proportion, %/day	-0.11	-0.009	-0.005
PA, h/day	0.02	0.14	0.16
PA proportion, %/day	0.001	0.14	0.16

Abbreviations; ALT= alanine aminotransferase; AST = aspartate aminotransferase; GGT = γ-glutamyltransferase; LPA = light physical activity; MVPA = moderate-to-vigorous physical activity; SB = sedentary behavior, PA = physical activity (LPA and MVPA together). [a] = log10 transformed variables. *The table is from the original publication I.*

Associations between liver enzymes and health markers based on multivariable models

Most associations between liver enzymes and health variables observed in the sex and age-adjusted correlation analysis remained significant when BMI was added to the multivariable model. ALT was positively associated with WC, SBP, DBP, resting heart rate, fasting glucose, fasting insulin, HOMA-IR, and HbA_{1c}. However, the associations between ALT and triglycerides and HDL-C turned non-significant. AST was associated with WC, DBP, resting heart rate, fasting insulin, HOMA-IR, and HbA_{1c}. However, the association between SBP and HDL-C turned non-significant. GGT was associated with SBP, resting heart rate, fasting insulin, and HOMA-IR. However, the associations between GGT and WC, fasting glucose, triglycerides, and HDL-C turned non-significant **(Table 5)**.

Table 5. Age, sex, and BMI-adjusted linear mixed regression estimates (standardized β coefficients) between circulating liver enzymes (ALT, AST, GGT), anthropometrics, and cardiometabolic risk factors.

	ALT[a] (U/l) **β**	**AST[a] (U/l)** **β**	**GGT[a] (U/l)** **β**
Anthropometrics			
Waist (cm)	**0.42** **	**0.37** *	0.22
Cardiometabolic biomarkers			
SBP (mmHg)	**0.20** *	0.14	**0.19** *
DBP (mmHg)	**0.22** **	**0.23** **	**0.23** **
Heart rate (bpm)	**0.17** *	**0.25** **	**0.23** **
BP medication	-0.07	-0.05	-0.07
Cholesterol medication	-0.10	-0.001	-0.01
Glucose (mmol/l)	**0.19** *	0.10	0.08
Insulin (mU/l)	**0.41** ***	**0.36** ***	**0.20** *
HOMA-IR	**0.42** ***	**0.36** ***	**0.21** *
Triglycerides (mmol/l)	0.15	0.04	0.15
Cholesterol (mmol/l)	0.003	-0.007	0.08
HDL-C (mmol/l)	-0.16	-0.15	-0.10
LDL-C (mmol/l)	0.001	0.05	0.06
HbA_{1c} (mmol/mol)	**0.23** **	**0.17** *	0.03

Significant p-values; * < 0.05, ** < 0.01, *** < 0.001.Significant values are in bold. Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; BP = blood pressure, DBP = diastolic blood pressure, GGT = γ-glutamyltransferase; HDL-C = high-density lipoprotein cholesterol, HOMA-IR = homeostatic model assessment for insulin resistance; LDL-C = low-density lipoprotein cholesterol, SBP = systolic blood pressure. [a] = log10 transformed variables. *The table is from the original publication I.*

When BMI was added to the model, the associations between sedentary behavior, physical activity measures, and liver enzymes remained non-significant **(Table 6)**.

Table 6. Age, sex, and BMI-adjusted linear mixed regression estimates (standardized β coefficients) between circulating liver enzymes (ALT, AST, GGT) and sedentary behavior and physical activity.

	ALT[a], U/l β	**AST[a], U/l** β	**GGT[a], U/l** β
Sedentary behavior			
Lying time, h/day	-0.12	-0.09	-0.09
Sitting time, h/day	0.03	0.01	-0.05
SB time, h/day	-0.05	-0.04	-0.10
Physical activity			
Breaks in SB, times/day	-0.02	-0.005	-0.06
Standing, h/day	0.02	0.02	-0.03
Standing proportion, %/day	0.02	0.0002	-0.02
Steps, number/day	-0.06	0.03	0.03
LPA, h/day	0.10	0.13	0.07
LPA proportion, %/day	0.11	0.13	0.09
MVPA, h/day	-0.05	0.03	0.05
MVPA proportion, %/day	-0.05	0.02	0.06
PA, h/day	0.008	0.14	0.15
PA proportion, %/day	-0.002	0.14	0.15

Abbreviations; ALT= alanine aminotransferase; AST = aspartate aminotransferase; GGT = γ-glutamyltransferase; LPA = light physical activity; MVPA = moderate-to-vigorous physical activity; SB = sedentary behavior. [a] = log10 transformed variables. *The table is from the original publication I.*

Additional analysis

We conducted several tests related to liver enzymes and various factors, including medications, physical activity, alcohol consumption, and health markers.

We found no association between liver enzymes (ALT, AST, and GGT) and blood pressure medication (ALT β = -0.07, 95% CI [-0.04–0.02], p = 0.42; AST β = -0.05, 95% CI [-0.02–0.01], p = 0.57; GGT β = -0.07, 95% CI [-0.06–0.03], p = 0.42), cholesterol medication (ALT β = -0.10, 95% CI [-0.07–0.02], p = 0.22, AST β = -0.001, 95% CI [-0.03–0.03], p = 0.99; GGT β = -0.01, 955 CI [-0.07–0.06], p = 0.87), or other medications *(data not shown)* when adjusted for sex, age, and BMI.

Regarding physical activity, we observed different associations between liver enzymes and physical activity based on gender and age. ALT showed a negative association with total physical activity and physical activity percentage in males, while in females, it showed a positive association. GGT demonstrated a negative association with MVPA, MVPA percentage, and steps in individuals under 50, alongside a positive association in those aged 50 or above. Other significant interactions in variables were not identified. When we analyzed accelerometer data and adjusted for

wear time and valid accelerometer days, we found a significant positive association between GGT and total physical activity (β = 0.15, 95% CI [0.001–0.11], p = 0.046, β = 0.16, 95% CI [0.001–0.11], p = 0.047, respectively). However, no significant interactions were observed with other variables *(data not shown).*

Regarding alcohol consumption, after adjusting for age, sex, and BMI, we found no significant associations between ALT and AST and alcohol consumption. The associations between GGT and cardiometabolic markers remained significant even when accounting for age, sex, BMI, and alcohol consumption. Lastly, the AST/ALT ratio, after adjustment for sex, age, and BMI, showed significant negative associations with several health markers, including SBP (β = -0.17, 95% CI [-0.002–0.0001], p = 0.03), fasting glucose (β = -0.19, 95% CI [-0.05–-0.005], p = 0.02), fasting insulin (β = -0.30, 95% CI [-0.007–-0.002], p = 0.001), HOMA-IR (β = -0.32, 95% CI [-0.02–-0.007], p = 0.001), triglycerides (β = -0.19, 95% CI [-0.05–-0.005], p = 0.02), and HbA1c (β = -0.20, 95% CI [-0.007–-0.001], p = 0.01). However, no significant association was found between the AST/ALT ratio and sedentary behavior or physical activity *(data not shown)*.

5.3.2 Associations between liver fat content, sedentary behavior, physical activity, body composition, fitness, and other metabolic markers (II)

Associations of body composition with sedentary behavior, physical activity, and fitness

The relationships between body fat %, WC, BMI, sedentary behavior, physical activity, and fitness are shown in **Table 7**. After adjusting for age and sex, body fat % was positively associated with time spent lying down and engaging in sedentary behavior (%/day) and negatively associated with time spent standing (h/day), daily steps, MVPA, MVPA %, total physical activity, and VO_{2max}. WC was positively associated with time spent lying down and the percentage spent in sedentary behavior and negatively associated with time spent standing, standing %, MVPA, MVPA %, total physical activity, daily steps, and breaks in sedentary behavior. BMI was positively associated with time spent lying down and negatively associated with time spent standing, MVPA, daily steps, breaks in sedentary behavior, and VO_{2max}.

Table 7. Age -and sex-adjusted linear mixed regression estimates (standardized β coefficients) between body composition, sedentary behavior, physical activity, and fitness.

	Body fat % **β**	**WC** **β**	**BMI** **β**
Lying time, h/day	**0.25** *	**0.39** **	**0.49** **
Sitting time, h/day	-0.12	-0.08	-0.21
SB time, h/day	0.05	0.20	0.13
SB time, %/day	**0.27** *	**0.38** *	0.32
Breaks in SB, times/day	-0.24	**-0.39** *	**-0.50** **
Standing, h/day	**-0.28** *	**-0.39** *	**-0.36** *
Standing, %	-0.22	**-0.35** *	-0.30
Steps, number/day	**-0.38****	**-0.48** **	**-0.44** **
LPA, h/day	-0.12	-0.12	-0.14
LPA, % of	-0.08	-0.09	-0.09
MVPA, h/day	**-0.33** **	**-0.42****	**-0.35** *
MVPA, %	**-0.3** *	**-0.4****	-0.31
PA, h/day	**-0.26** *	**-0.31***	-0.28
PA, %	-0.22	-0.28	0.24
VO_{2max}, ml/min/kg	**-0.62** ***	**-0.65** ***	**-0.76** ***

Significant p-values; * < 0.05, ** < 0.01, *** < 0.001. Significant values are in bold. Abbreviations: BMI = body mass index, LPA = light physical activity; MVPA = moderate-to-vigorous physical activity; PA = physical activity (LPA and MVPA together), SB = sedentary behavior (sitting + lying), VO_{2max} = maximal oxygen consumption, WC = waist circumference, % = percentage of the daily wear time. *The table is modified from the original publication II.*

Associations of liver fat content with sedentary behavior and physical activity

When adjusted for age and sex, MRS-measured liver fat content was not associated with any sedentary behavior or physical activity variables; however, MRI-measured liver fat content was negatively associated with daily steps **(model 1, Table 8)**. When body fat % was added to the model, all the associations remained or turned non-significant **(model 2, Table 8)**.

Table 8. Age-, sex- and body fat % -adjusted linear mixed regression estimates (standardized β coefficients [95% CI]) between MRS- and MRI-measured LFC, sedentary behavior, and physical activity.

	LFC MRS[a] (%)		LFC MRI (%)	
	Model 1 β	**Model 2 β**	**Model 1 β**	**Model 2 β**
Lying time, h/day	0.08	0.01	0.03	-0.13
Sitting time, h/day	-0.09	-0.09	-0.03	0.05
SB time, h/day	-0.04	-0.09	-0.02	-0.04
SB time, %	0.03	-0.14	0.10	-0.07
Breaks times/day	-0.25	-0.17	-0.15	-0.01
Standing, h/day	-0.02	0.08	-0.07	0.10
Standing, %	-0.01	0.06	-0.06	0.08
Steps, number/day	-0.29	-0.11	**-0.31 ***	-0.10
LPA, h/day	0.17	0.26	0.02	0.09
LPA, %	0.17	0.23	0.04	0.08
MVPA, h/day	-0.22	-0.03	-0.24	-0.05
MVPA, %	-0.21	-0.02	-0.23	-0.06
PA, h/day	-0.04	0.17	-0.11	0.05
PA, %	-0.02	0.15	-0.11	0.03

Significant p-values; * < 0.05, ** < 0.01, *** < 0.001. Significant values are in bold. Abbreviations: LFC = liver fat content, LPA = light physical activity, MUFA = monounsaturated fatty acids, MRI = magnetic resonance imaging, MRS = magnetic resonance spectroscopy, MVPA = moderate to vigorous physical activity, PA = physical activity (LPA and MVPA together), SB =sedentary behavior (sitting and lying), % = percentage of the daily wear time. [a] = log10 transformed variables. Model 1 adjusted for age and sex. Model 2 adjusted for age, sex, and body fat %. *The table is modified from the original publication II.*

Associations of liver fat content with fitness and nutrient intake

In the sex- and age-adjusted model, MRS-measured liver fat content was not associated with fitness or nutrient intake variables **(model 1, table 9)**. When body fat % was added to the model, all other associations were non-significant except for the negative association between MRS-measured liver fat content and protein intake **(model 2, table 9)**. In the sex- and age-adjusted model, MRI-measured liver fat content was negatively associated with VO_{2max} and positively associated with daily MUFA intake **(model 1, table 9)**. However, both associations turned non-significant when body fat % was added to the model **(model 2, table 9)**, and the association between MRI-measured liver fat content and daily protein intake turned significant **(model 2, table 9)**.

Table 9. Age-, sex- and body fat % -adjusted linear mixed regression estimates (standardized β coefficients) between MRS- and MRI-measured LFC, fitness, and dietary intake.

	LFC MRS[a] (%)		LFC MRI (%)	
	Model 1 β	**Model 2 β**	**Model 1 β**	**Model 2 β**
VO_{2max}, ml $min^{-1}kg^{-1}$	-0.39	-0.07	**-0.50 ****	-0.15
VO_{2max}, ml/min/kg_{FFM}	-0.10	-0.13	-0.08	-0.06
Maximal load, W	-0.14	-0.02	-0.21	0.03
Total EI, kcal/day	0.14	0.14	0.17	0.16
Protein, %	-0.25	**-0.31 ***	-0.23	**-0.30 ***
CHO, %	-0.05	0.08	-0.11	0.03
Fat, %	-0.05	0.06	0.21	0.09
Alcohol, %	-0.02	0.01	0.11	0.12
SFA, %	0.05	0.10	-0.13	-0.05
MUFA, %	0.16	0.01	**0.30 ***	0.14
PUFA, %	0.1	-0.03	0.25	0.10
Saccharose, %	0.11	0.11	0.09	0.16

Significant p-values; * < 0.05, ** < 0.01, *** < 0.001. Significant values are in bold. Abbreviations: CHO = carbohydrates, EI = energy intake, FFM = fat-free mass, LFC = liver fat content, MUFA = monounsaturated fatty acids, MRI = magnetic resonance imaging, MRS = magnetic resonance spectroscopy, PUFA = polyunsaturated fatty acids, SFA = saturated fatty acids, VO_{2max} = maximal oxygen consumption. % = percentage of the daily energy intake. [a] = log10 transformed variables. Model 1 adjusted for age and sex. Model 2 adjusted for age, sex, and body fat %. *The table is modified from the original publication II.*

Additionally, we examined the relationship between liver fat content as measured by MRS and MRI and the daily intake of various nutrients, including protein, carbohydrates, fat, alcohol, SFA, MUFA, PUFA, saccharose, and fiber in grams. We found that MRS-measured liver fat content did not show any significant association with the nutrient variables after adjusting for sex, age, and further with body fat percentage *(data not shown)*. MRI-measured liver fat content was initially associated with MUFA intake (g) (β=0.35, 95% CI [0.05, 0.64], p=0.02). However, when body fat percentage was included in the model, this association became non-significant (β=0.22, 95% CI [-0.007, 0.50], p=0.13).

Associations of liver fat content with overall body adiposity and other cardiometabolic health markers

When adjusted for age and sex, MRS-measured liver fat content was associated positively with body fat %, BMI, WC, triglycerides, ALT, fasting insulin, HOMA-IR, M-value (whole-body insulin sensitivity), and HbA1c **(model 1, table 10)**. After further adjustment for body fat %, MRS-measured liver fat content remained positively associated with WC, M-value, HbA1c, triglycerides, and ALT, and the

association between MRS-measured liver fat content and GGT turned significant. On the other hand, the associations between MRS-measured liver fat content and fasting insulin and HOMA-IR turned non-significant **(model 2, table 10)**.

Most associations between the MRI-measured liver fat content with overall body adiposity and other health markers showed similar results to the MRS-measured liver fat content **(model 1-2, table 10)**. Only in the age- and sex-adjusted model was the association between MRI-measured liver fat content and BMI not statistically significant, and the association between AST became significant **(model 1, table 10)**. When body fat % was added to the model, the association between MRI-measured liver fat content and WC and GGT turned non-significant, while the association between AST remained significant **(model 2, table 10).**

Table 10. Age-, sex- and body fat %-adjusted linear mixed regression estimates (standardized β coefficients) between MRS- and MRI-measured LFC, body composition, and cardiometabolic risk factors.

	LFC MRS[a] (%)		LFC MRI (%)	
	Model 1 β	Model 2 β	Model 1 β	Model 2 β
Body fat, %	**0.50 ***		**0.62 ****	
Waist, cm	**0.58 *****	**0.55 ****	**0.43 ****	0.20
BMI, kg/m2	**0.34 ***	0.17	0.26	-0.14
SBP, mmHg	-0.08	-0.11	-0.09	-0.19
DBP, mmHg	0.13	0.08	0.18	0.08
Resting heart rate, bpm	-0.21	-0.19	-0.04	0.04
BP medication	0.08	0.02	-0.03	-0.03
Cholesterol medication	-0.06	0.02	-0.03	0.07
f-Glucose, mmol/l	-0.11	-0.13	-0.11	0.05
f-Insulin, mU/l	**0.41 ***	0.31	**0.39 ***	0.22
HOMA-IR	**0.38 ***	0.28	**0.38 ***	0.21
M-value, mg/kg/min	**-0.43 ****	**-0.34 ***	**-0.39 ****	-0.19
HbA1c, mmol/mol	**0.54 *****	**0.48 ****	**0.55 *****	**0.46 *****
Triglycerides, mmol/l	**0.38 ***	**0.38 ****	**0.32 ***	**0.30 ***
Cholesterol, mmol/l	0.22	0.22	0.18	0.20
HDL-C, mmol/l	-0.18	-0.18	-0.14	-0.09
LDL-C, mmol/l	0.18	0.18	0.16	0.16
ALT, U/l	**0.50 *****	**0.46 ****	**0.51 *****	**0.41 ****
AST, U/l	0.24	0.32	**0.39 ***	**0.33 ***
GGT, U/l	0.30	**0.29 ***	0.29	0.23

Significant p-values; * < 0.05, ** < 0.01, *** < 0.001. Significant values are in bold. Abbreviations: ALT = alanine aminotransferase, AST =aspartate aminotransferase, BP =blood pressure, DBP =diastolic blood pressure, GGT = γ-glutamyltransferase, HbA_{1c} = hemoglobin A_{1c}, HDL-C = high-density lipoprotein cholesterol, HOMA-IR = homeostatic model assessment for insulin resistance, LDL-C = low-density lipoprotein cholesterol, LFC = liver fat content, MRI = magnetic resonance imaging and MRS = magnetic resonance spectroscopy, M-value = whole-body insulin sensitivity, SBP = systolic blood pressure. [a] = log10 transformed variables. Model 1 adjusted for age and sex. Model 2 adjusted for age, sex, and body fat %. *The table is modified from the original publication II.*

5.3.3 Correlation and the agreement between MRS and MRI (II)

We additionally investigated the correlation and agreement between the two methods for measuring liver fat content: magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI). **Figure 10** shows the linear regression plot with significant positive correlation (r = 0.76, 95% CI [0.59–0.87], p<0.0001) between MRS-measured liver fat content and MRI-measured liver fat content. **Figure 11** represents the results from the Bland-Altman analysis.

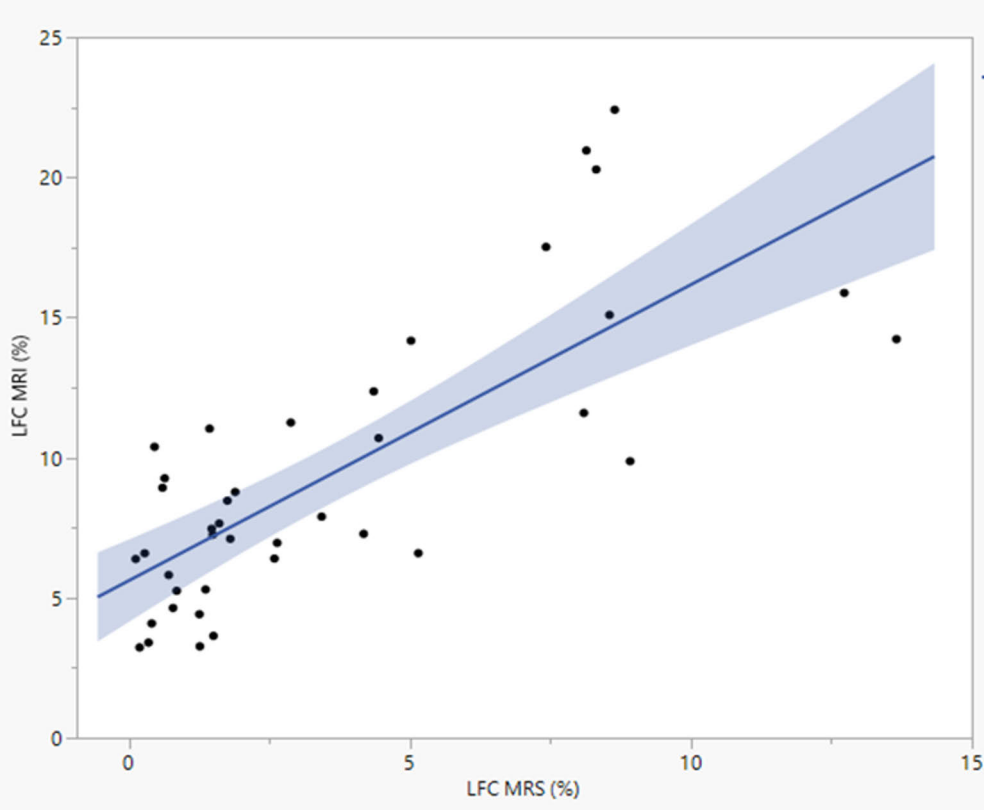

Figure 10. Correlation between MRS-measured liver fat content (LFC) and MRI-measured LFC. *The figure is from the original publication II supplementary file 2.*

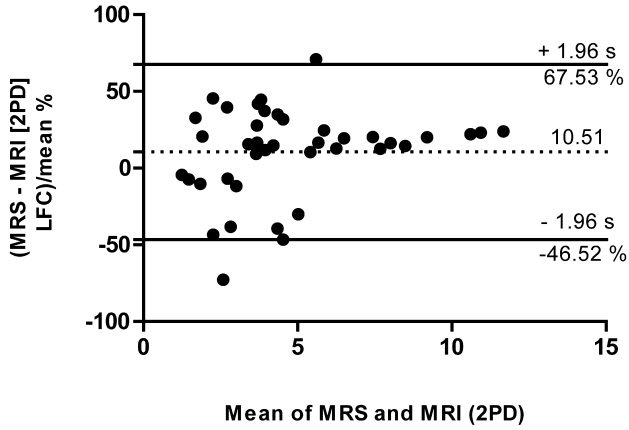

Figure 11. Result of Bland-Altman analysis. Solid lines delineate 95% CIs, and dashed lines show central bias. MRS = magnetic resonance spectroscopy, MRI = magnetic resonance imaging, 2PD = 2-point Dixon method. *The figure is from the original publication II supplementary file 2.*

5.3.4 Associations between liver insulin sensitivity, sedentary behavior, physical activity, and other lifestyle factors (III)

Associations of liver glucose uptake and EGP with sedentary behavior, physical activity, and fitness

After adjusting for age, sex, and accelerometer wear time, no significant associations were found between liver glucose uptake and sedentary behavior, physical activity, or fitness variables **(model 1, Table 11)**. These non-significant associations persisted even when body fat % was added to the model **(model 2, Table 11)**. In the age-, sex-, and accelerometer wear time-adjusted model **(model 1)**, EGP was negatively associated with standing time (h/day) and positively associated with sedentary behavior time (h/day). **In model 2**, when body fat % was included, the association between EGP and standing time remained significant, but the association between EGP and sedentary behavior time became non-significant **(model 1-2, table 11)**. Furthermore, EGP was better when daily standing time was more than 1 hour and 45 minutes compared to 1 hour and 45 minutes or less [EGP -4.8 (-8.1, -1.6) vs. +2.4 (−0.8, 5.5), respectively, p = 0.003] **(Figure 12A)**, and when sedentary time was 10.0 h/day or less compared to more than 10.0 h/day [EGP -5.4 (-9.3, -1.6) vs. +3.0 (0.6, 5.3), respectively, p = 0.0005] **(Figure 12B)**. In the age- and sex-adjusted model **(model 1, Table 11)**, EGP was negatively associated with VO_{2max} (ml/min/kg). However, when body fat % was included in the model, this association became non-significant **(model 2, Table 11).**

Table 11. Age-, sex- accelerometry wear time, and body fat % -adjusted linear mixed regression estimates (standardized β coefficients) between liver glucose uptake, EGP, sedentary behavior, physical activity, and cardiorespiratory fitness.

	LGU[a] (µmol/ 100 ml/min)		EGP (µmol/kg/min)	
	Model 1 β	**Model 2 β**	**Model 1 β**	**Model 2 β**
SB time, h/day	0.07	0.18	**0.39 ***	0.26
Breaks in SB, times/day	0.16	0.1	-0.24	-0.15
Standing, h/day	-0.02	-0.11	**-0.53 ****	**-0.43 ***
Steps, number/day	0.14	0.02	-0.08	0.14
LPA, h/day	-0.15	-0.16	-0.16	-0.13
MVPA, h/day	0.03	-0.08	-0.05	0.13
PA, h/day	-0.09	-0.17	-0.14	-0.03
VO_{2max}, ml/min/kg	0.13	-0.21	**-0.42 ***	-0.12
VO_{2max}, ml/min/kgFFM	-0.19	-0.2	-0.02	-0.01
Maximal load, W	0.03	-0.13	-0.16	0.03

Significant p-values;* < 0.05, ** < 0.01, *** < 0.001. Significant values are in bold. Abbreviations: LGU = liver glucose uptake, EGP = endogenous glucose production, LPA = light physical activity, MVPA = moderate to vigorous physical activity, PA = physical activity (LPA and MVPA together), VO_{2max} = maximal oxygen consumption, FFM = fat-free mass. [a] = log10 transformed variables. Model 1 adjusted for age, sex, and accelerometry wear time. Model 2 adjusted for age, sex, accelerometry wear time, and body fat %. *The table is modified from the original publication III.*

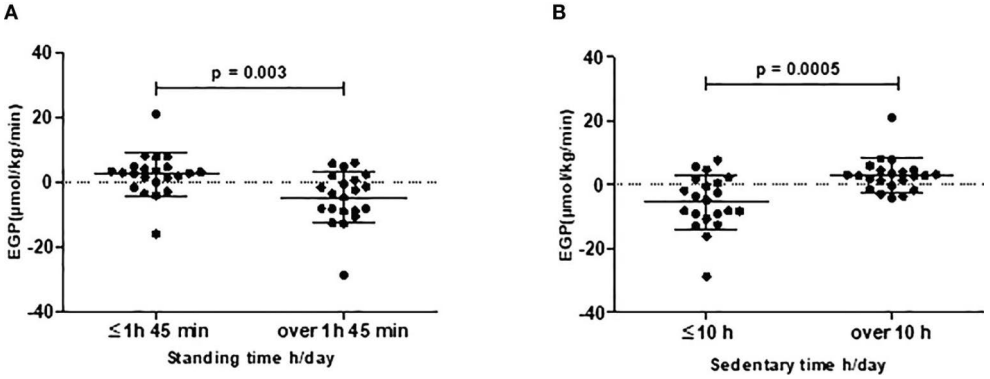

Figure 12. Endogenous glucose production (EGP) is better with (A) standing time >1h 45 min (n=21) vs ≤1h 45 min (n=22) and with (B) sedentary time >10.0 h/day (n=22) vs. ≤10.0 h/day (n = 21). Black dots represent individual participants, and black lines with error bars indicate means (SD). *The figure is from the original publication III.*

Associations of liver glucose uptake and EGP with nutrient intake

In a model adjusted for age and sex, there was a positive correlation between liver glucose uptake and daily total fiber intake (g/day) as well as WIDF (g/day**) (model 1, table 12)**. The association between liver glucose uptake and WIDF remained statistically significant even after adjusting for body fat % **(model 2, table 12)**. Liver

glucose uptake was also found to be higher with a fiber consumption exceeding 18 g/day compared to 18 g/day or less [liver glucose uptake 3.0 (± 0.3) vs. 2.2 (± 0.2), respectively, p = 0.03] **(Figure 13A)**, and with consumption of WIDF exceeding 13 g/day compared to 13g/day or less [liver glucose uptake 3.0 (± 0.3) vs. 2.2 (± 0.2), respectively, p = 0.03] **(Figure 13B)**. Furthermore, in the model adjusted for age, sex, and body fat %, liver glucose uptake exhibited a negative association with carbohydrate intake (CHO, EI%) and saccharose (EI%) and a positive association with the proportion of monounsaturated and polyunsaturated fatty acids (MUFA, EI%; PUFA, EI%; respectively) **(model 2, table 12)**. In the model adjusted for sex and age, EGP did not show any significant association with the dietary variables **(model 1, table 12)**, and these non-significant associations persisted when body fat % was included in the model **(model 2, table 12).**

Table 12. Age- sex- and body fat % -adjusted linear mixed regression estimates (standardized β coefficients [95% CI]) between liver glucose uptake, EGP, and dietary intake.

	LGU[a] (µmol/100 ml/min)		**EGP (µmol/kg/min)**	
	Model 1 β	**Model 2** β	**Model 1** β	**Model 2** β
Total EI, kcal/day	0.06	0.07	0.02	0.01
Protein, %	0.03	0.09	0.02	-0.06
CHO, %	-0.17	**-0.32 ***	-0.04	0.12
Fat, %	0.16	0.3	0.02	-0.14
Alcohol, %	-0.02	-0.01	0.07	0.06
SFA, %	0.01	-0.03	0.04	0.09
MUFA, %	0.17	**0.35 ***	0.02	-0.17
PUFA, %	0.24	**0.41 ***	-0.06	-0.25
Saccharose, %	-0.21	**-0.32 ***	-0.04	0.08
Fiber all, g/day	**0.32 ***	0.26	-0.16	-0.07
WIDF, g/day	**0.35 ***	**0.29 ***	-0.13	-0.02

Significant p-values; * < 0.05, ** < 0.01, *** < 0.001. Significant values are in bold. Abbreviations: LGU = liver glucose uptake, EGP =endogenous glucose production, EI = energy intake, CHO = carbohydrates, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, WIDF = water-insoluble dietary fiber, % = percentage of the daily energy intake. [a] = log10 transformed variables. Model 1 adjusted for age and sex. Model 2 adjusted for age, sex, and body fat %. *The table is modified from the original publication III.*

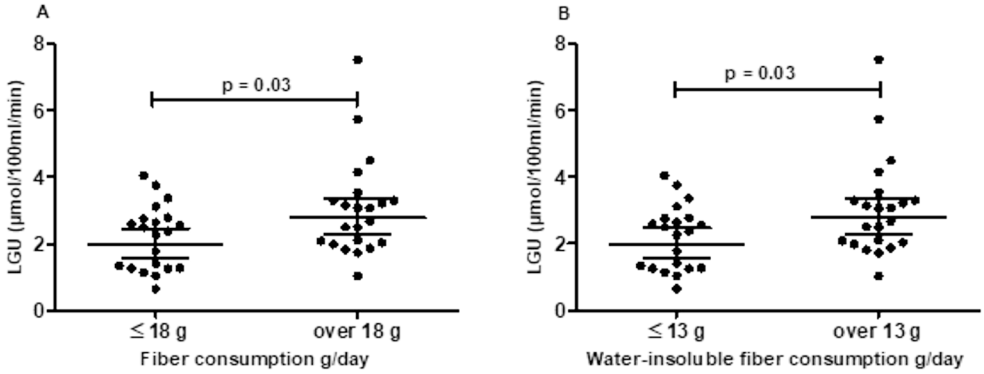

Figure 13. Liver glucose uptake (LGU) is better with A) fiber consumption >18 g/day (n=22) vs. ≤ 18 g/day (n=21) and B) water-insoluble fiber consumption >13 g/day (n=22) vs. ≤ 13 g/day (n=21). Black dots represent individual participants, and black lines with error bars indicate means (SD). LGU values are log10 transformed (means are back-transformed geometric model-based means [95 % CI]). *The figure is modified from the original publication III.*

Associations of liver glucose uptake and EGP with cardiometabolic health markers and liver fat content

Upon adjusting for age and sex, the study revealed that liver glucose uptake exhibited negative associations with body fat %, fasting insulin, and HOMA-IR and a positive association with M-value **(model 1, table 13)**. However, subsequent adjustment for body fat percentage showed that only the association between liver glucose uptake and M-value remained statistically significant **(model 2, table 13)**. After adjusting for age and sex, EGP demonstrated positive associations with body fat %, WC, fasting insulin, and HOMA-IR and a negative association with M-value and LDL-C **(model 1, table 13)**. Following further adjustment for body fat %, the associations between EGP and M-value and EGP and LDL-C remained significant **(model 2, table 13)**. Neither liver glucose uptake nor EGP displayed any association with liver enzymes ALT, AST, or GGT, or with MRS- and MRI-measured liver fat content **(model 1-2, table 13)**.

Table 13. Age-, sex-, and body fat % -adjusted linear mixed regression estimates (standardized β coefficients) between liver glucose uptake, EGP, cardiometabolic health markers, and liver fat content.

	LGU[a] (µmol/ 100 ml/min)		EGP (µmol/kg/min)	
	Model 1 β	**Model 2 β**	**Model 1 β**	**Model 2 β**
Body fat, %	**-0.42 ***		**0.56 ***	
weight, kg	-0.15	0.06	0.20	-0.06
BMI, kg/m2	-0.22	-0.01	0.27	-0.04
WC, cm	-0.13	0.15	**0.36 ***	0.12
SBP, mmHg	-0.1	-0.04	0.19	0.11
DBP, mmHg	0.17	0.24	0.18	0.1
Resting HR, bpm	0.21	0.21	0.08	0.08
f-Glucose, mmol/l	-0.14	-0.09	0.32	0.26
f-Insulin, mU/l	**-0.34 ***	-0.23	**0.38 ***	0.22
HOMA-IR	**-0.33 ***	-0.23	**0.38 ***	0.24
M-value	**0.55 *****	**0.58 ****	**-0.59 *****	**-0.53 ****
HbA1c, mmol/mol	0.04	0.13	0.04	-0.07
Triglycerides, mmol/l	-0.02	-0.01	0.02	0.004
Cholesterol, mmol/l	0.27	0.25	-0.29	-0.26
HDL-C, mmol/l	0.26	0.22	0.03	0.09
LDL-C, mmol/l	0.18	0.17	**-0.32 ***	**-0.31 ***
ALT, U/l	0.02	0.13	0.27	0.15
AST, U/l	0.21	0.27	0.01	-0.06
GGT, U/l	0.13	0.18	0.12	0.06
MRS-measured LFC, %	-0.06	-0.003	0.13	0.09
MRI-measured LFC, %	-0.38	0.18	0.21	-0.02

Significant p-values; * < 0.05, ** < 0.01, *** < 0.001. Significant values are in bold. Abbreviations; WC = waist circumference, LGU = liver glucose uptake, EGP = endogenous glucose production, BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, f = fasting, HOMA-IR = homeostatic model assessment for insulin resistance, M-value = whole-body insulin sensitivity, HbA_{1c} = hemoglobin A_{1c}, HDL-C =high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, ALT = alanine aminotransferase, AST = aspartate aminotransferase, GGT = γ-glutamyltransferase, MRS = magnetic resonance spectroscopy, LFC = liver fat content, MRI = magnetic resonance imaging. [a] = log10 transformed variables. Model 1 adjusted for age and sex. Model 2 adjusted for age, sex, and body fat-%. *The table is modified from the original publication III.*

5.4 The intervention study (IV)

5.4.1 Baseline characteristics of the participants in the INT and CON groups

The baseline characteristics of the participants in the INT and CON groups are presented in **Table 14.** Within the INT group, three participants and within the CON group 8 participants met the clinical criteria for MASLD (liver fat content ≥ 5%)

(Rinella et al., 2023). Twenty-nine had normal liver fat content (< 5%) (**Figure 14.** Baseline liver fat content per participant).

Table 14. Baseline characteristics of the participants in the INT (intervention) and CON (control) groups.

	INT	CON	p
n #	23	21	
Women, n (%) *#	14 (61)	11 (52)	0.76
Age, years#	59.9 (6.0)	56.3 (7.1)	0.08
LGU, µmol/100 ml/min **	2.5 (1.8, 3.3)	2.5 (1.7, 3.1)	0.94
EGP, µmol/kg/min	-1.1 (5.9)	-1.2 (10.2)	0.94
LFC, % **	1.7 (1.3, 3.2)	3.9 (0.4, 8.2)	0.82
BMI, kg/m^2	31.8 (4.5)	32.7 (4.5)	0.51
WC, cm#	110.7 (12.4)	111.9 (11.6)	0.74
Body fat, %#	43.6 (7.8)	43.4 (8.3)	0.94
Fat-free mass, %**	48.1 (40.9, 60.9)	52.9 (47.7, 60.9)	0.24
Body mass, kg**#	88.7 (77.6, 102.4)	93.2 (85.6,109.3)	0.18
M-value,µmol/kg/min**#	15.4 (10.7, 21.7)	12.9 (8.3, 22.0)	0.95
f Glucose, mmol/l#	5.8 (0.5)	5.8 (0.3)	0.49
f Insulin, mU/l**#	10.0 (7.0, 16.0)	11.0 (6.5, 17.5)	1.0
HbA1c, mmol/mol **#	37.5 (2.6)	37.0 (2.7)	0.48
Triglycerides, mmol/l**#	1.4 (1.0, 1.7)	1.1 (0.8, 1.5)	0.06
Cholesterol, mmol/l#	4.8 (1.2)	4.5 (0.8)	0.39
NEFA, mmol/l	0.6 (0.2)	0.6 (0.2)	0.51
HDL-C, mmol/l #	1.3 (0.3)	1.3 (0.4)	0.81
LDL-C, mmol/l #	3.2 (1.0)	3.0 (0.8)	0.50
ALT, U/l **	27 (21, 35)	29 (20, 40)	0.82
AST, U/l	26 (8)	26 (12)	0.95
GGT, U/l**	27 (15, 39)	22 (16, 35)	0.75
Accelerometry duration, days#	26.1 (3.2)	26.4 (2.0)	0.71
Accelerometry duration, h/day#	14.56 (0.93)	14.69 (0.98)	0.65
Sedentary time, h/day#	10.11 (1.0)	10.24 (0.94)	0.66
Breaks in SB, n/day#	29 (8)	28 (8)	0.79
Steps, n/day#	5272 (2017)	4860 (1473)	0.45
Standing, h/day#	1.78 (0.59)	1.7 (0.46)	0.63
LPA, h/day#	1.71 (0.44)	1.81 (0.47)	0.45
MVPA, h/day#	0.96 (0.33)	0.94 (0.33)	0.80

Results are presented as mean (SD). Group differences were tested using T-tests or Fisher's exact test = *. Median (Q1, Q3) = **. Statistical testing was performed with log10-transformed estimates. # Results published previously by Sjöros, Laine et al. (Sjöros, Laine, Garthwaite, Vähä-Ypyä, Koivumäki, et al., 2023). Abbreviations: LGU = liver glucose uptake, EGP = endogenous glucose production, LFC = liver fat content, BMI = body mass index, M-value = whole-body insulin sensitivity, HbA1c = hemoglobin A1c, NEFA = non-esterified fatty acids, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, ALT = alanine aminotransferase, AST = aspartate aminotransferase, GGT = γ-glutamyltransferase, SB = sedentary behavior, LPA = light physical activity, MVPA = moderate-to-vigorous physical activity. *The table is from the original publication IV.*

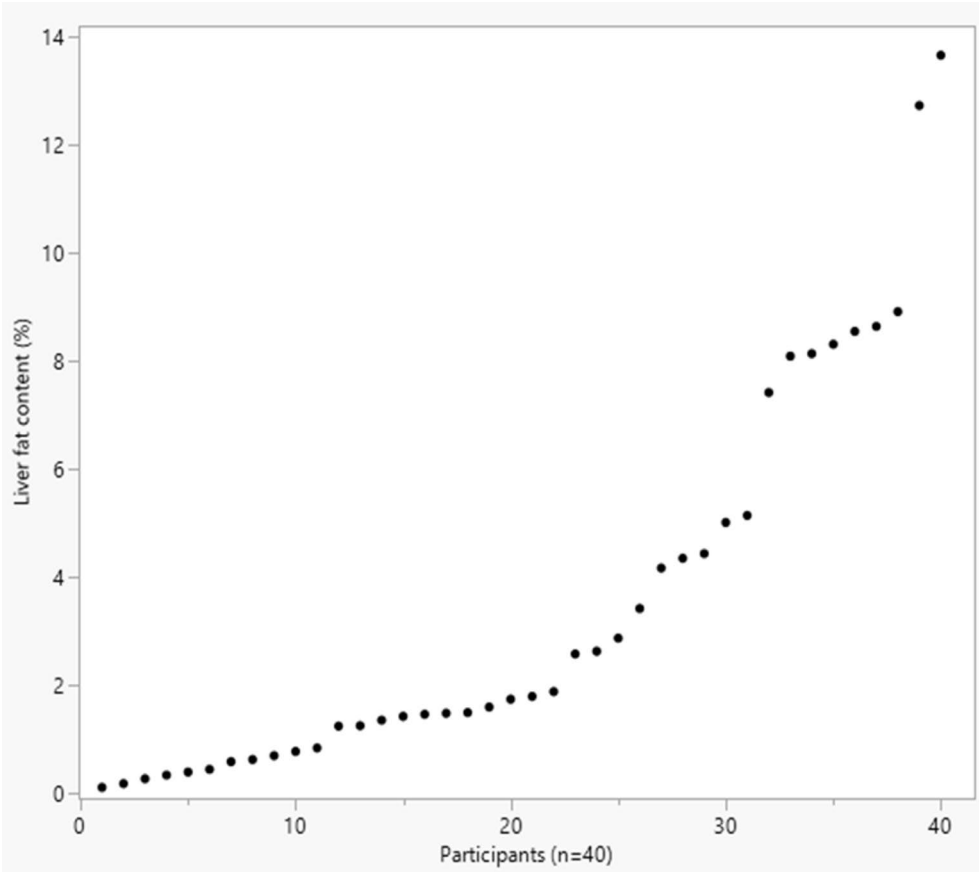

Figure 14. MRS-measured liver fat content (%) at baseline for each participant (n=40). *The figure is from the original publication IV supplementary material.*

5.4.2 Accelerometry

The findings from the accelerometry assessment have been previously documented (Sjöros, Laine, Garthwaite, Vähä-Ypyä, Koivumäki, et al., 2023). In the INT group, sedentary behavior decreased by an average of 51 minutes per day (95% CI: 22 to 78), while MVPA increased by 22 minutes per day (95% CI: 12 to 33) compared to the CON group (group*time, p=0.02 and p=0.01, respectively). Throughout the intervention, all participants increased their breaks in sedentary behavior by three per day and spent an additional 11 minutes engaged in LPA daily. The changes were consistent across all groups, with no differences observed. The mean step count increased by approximately 3200 steps (95% CI: 2120 to 4192) in the INT group and by approximately 1700 steps (95% CI: 580 to 2790) in the CON group. The difference in step count between the groups was statistically significant (group*time, p=0.009) (Sjöros, Laine, Garthwaite, Vähä-Ypyä, Koivumäki, et al., 2023).

5.4.3 Intervention effects on liver insulin sensitivity and other liver health markers

The intervention did not significantly affect liver function markers (liver glucose uptake, EGP, liver fat content, ALT, AST, or GGT) **(Fig. 15 A-F).** However, both liver glucose uptake and AST increased in both groups (time; $p < 0.001$ and $p = 0.02$, respectively). Liver glucose uptake increased by 94% and AST by 10% in the INT group and 46% and 11% in the CON group, respectively **(Fig. 15 A and E)**.

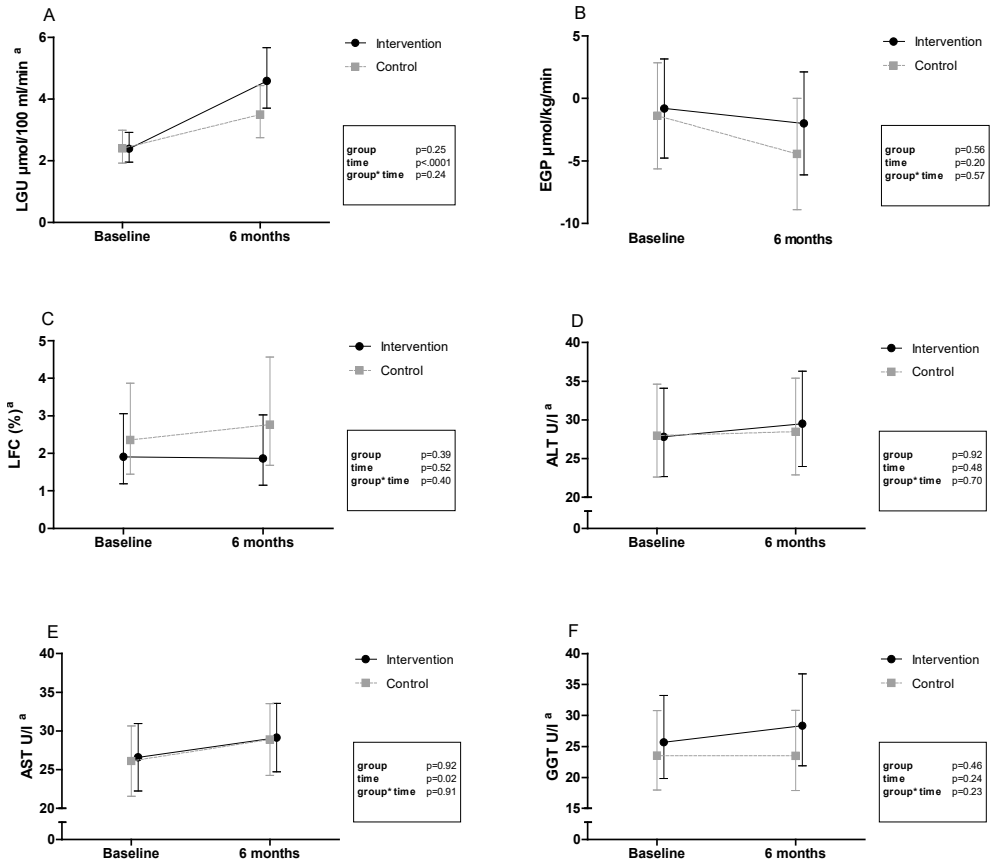

Figure 15. Liver health marker results of the intervention (black line) and control (grey line) groups at baseline and after six months of A) LGU, liver glucose uptake; B) EGP, endogenous glucose production; C) LFC, liver fat content; D) ALT, alanine aminotransferase; E) AST, aspartate aminotransferase and F) GGT, γ-glutamyltransferase. The values are presented as model-based means (95% CI), [a] = log10 transformed (means are back-transformed geometric model-based means [95% CI]). *The figure is from the original publication IV.*

5.4.4 Intervention effects on cardiometabolic health markers

Both groups experienced a significant decrease in BMI, WC, body fat %, fat mass, and body mass during the intervention (time; p=0.02, p<0.001, p=0.05, p=0.02, p=0.01, respectively), with no statistically significant difference in mean changes between the groups **(Fig. 16 A-E)**. The intervention did not notably impact fat-free mass **(Fig. 16 F)** or the general insulin sensitivity markers, including fasting glucose, fasting insulin, and HbA1c **(Fig. 17 A-C)**. While within-group changes in fasting triglycerides were significant (group, p=0.04), the overall intervention effect was not significant (group*time, p=0.95) (**Fig. 18 A**). Furthermore, the intervention did not significantly influence other lipid markers: fasting NEFA, fasting cholesterol, HDL-C, or LDL-C **(Fig. 18 B-E)**.

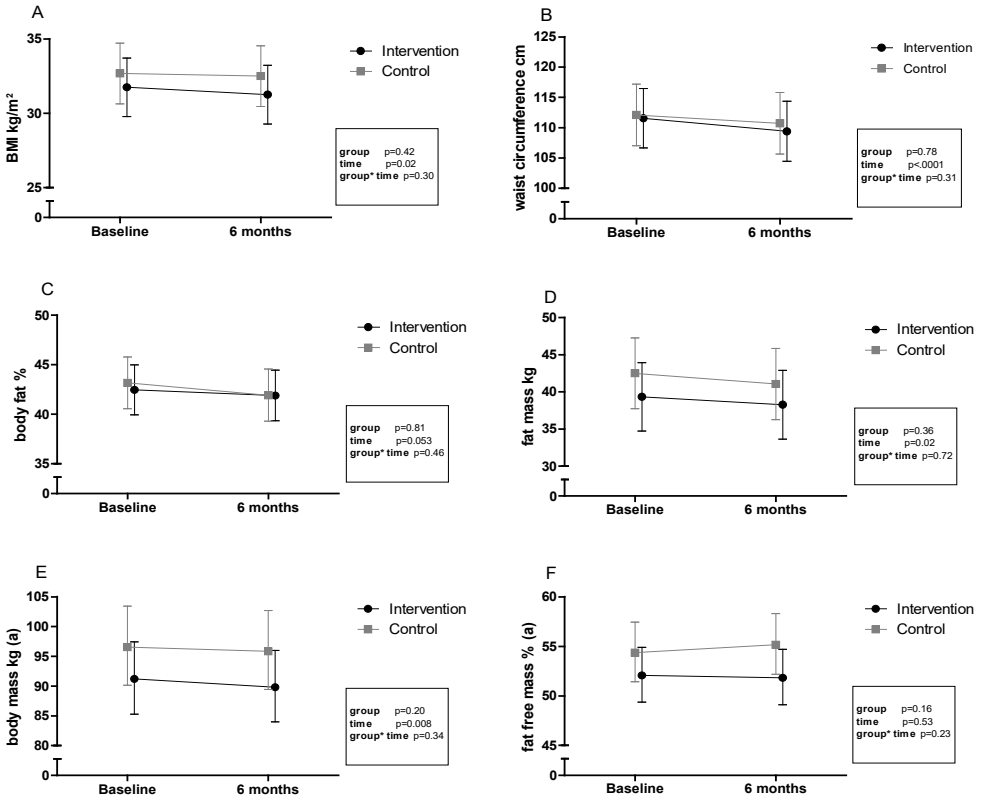

Figure 16. Anthropometric results of the intervention (black line) and control (grey line) groups at baseline and after six months of A) BMI, body mass index; B) waist circumference; C) body fat percentage; D) fat mass; E) fat-free mass, and F) body mass. The values are presented as model-based mean (95% CI), [a] = log10 transformed (means are back-transformed geometric model-based means [95% CI]). *The figure is from the original publication IV.*

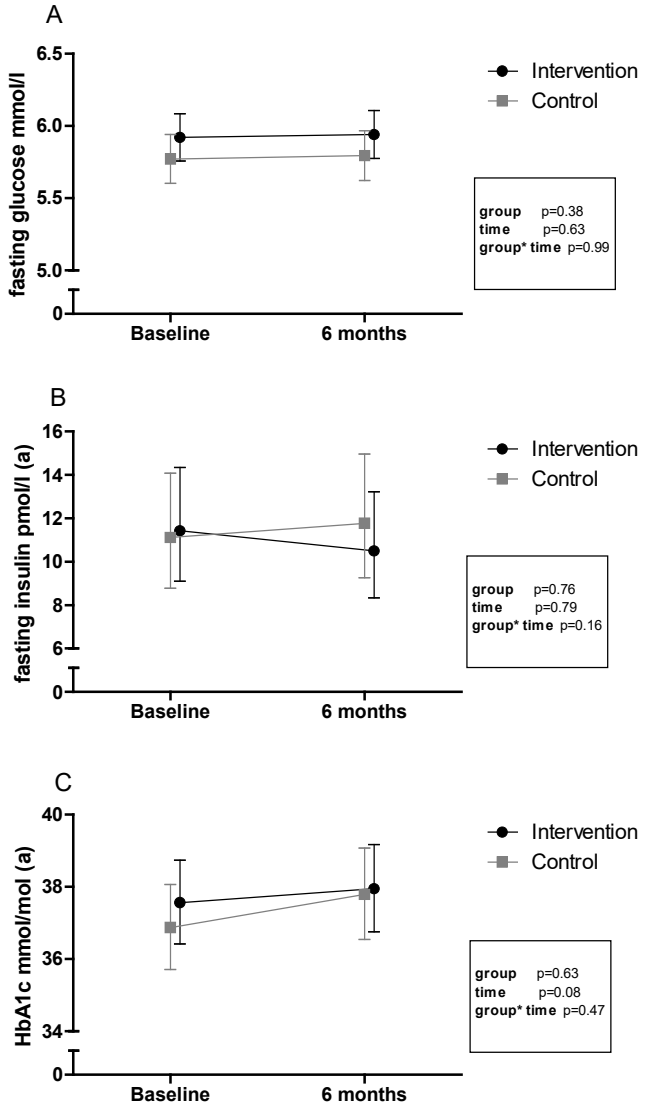

Figure 17. Insulin sensitivity marker results of the intervention (black line) and control (grey line) groups at baseline and after six months of A) fasting glucose, B) fasting insulin, and C) HbA1c (glycated hemoglobin). The values are presented as model-based means (95% CI), [a] = log10 transformed (means are back-transformed geometric model-based means). *The figure is from the original publication IV supplementary materials.*

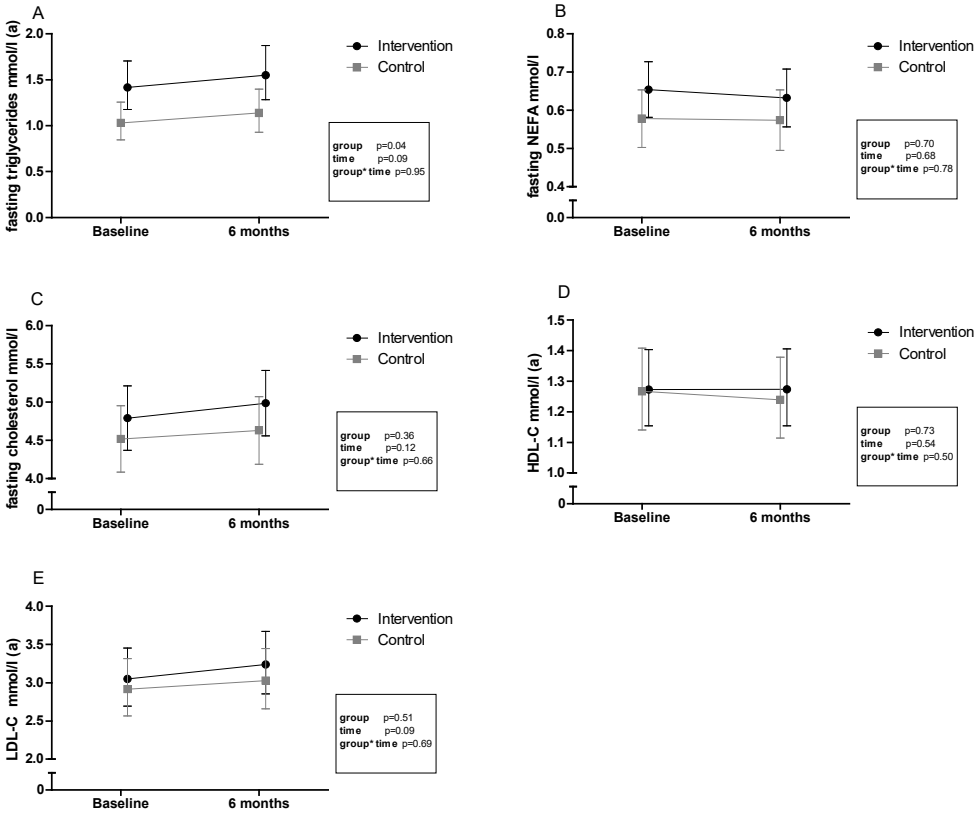

Figure 18. Lipid marker results of the intervention (black line) and control (grey line) groups at baseline and after six months of A) fasting triglycerides; B) NEFA, non-esterified fatty acids; C) cholesterol; D) HDL-C, high-density lipoprotein cholesterol, and E) LDL-C, low-density lipoprotein cholesterol. The values are presented as model-based means (95% CI), [a] = log10 transformed (means are back-transformed geometric model-based means). *The figure is from the original publication IV supplementary materials.*

5.4.5 Correlations between changes during the intervention

We also investigated the associations between the changes (Δ) that occurred during the intervention among all the participants in the study. We found that Δliver glucose uptake had a positive correlation with ΔHDL-C, while ΔEGP had an inverse correlation with ΔM-value. Furthermore, Δliver fat content was positively correlated with Δfasting insulin and ΔHbA1c **(Table 15)**. ΔALT showed a positive correlation with Δsedentary behavior time (h/day and %/day) and a negative correlation with standing time (h/day and %/day). At the same time, ΔAST was positively correlated with Δbreaks in sedentary behavior **(Table 16)**. Additionally, ΔALT and ΔAST were positively correlated with ΔCHO (g/day and % of daily energy intake), and ΔAST was only correlated with % of daily energy intake and Δsaccharose (g/day and % of daily energy intake) **(Table 17)**. Lastly, ΔGGT had an inverse correlation with

Δprotein (g/day and % of daily energy intake) and a positive correlation with Δsaccharose (g/day and % of daily energy intake) **(Table 17)**.

Table 15. Associations between the changes (post – pre Δ values) in liver glucose uptake, endogenous glucose production, liver fat content, liver enzymes, and different metabolic health markers during the intervention.

	ΔLGU	ΔEGP	ΔLFC	ΔALT	ΔAST	ΔGGT
ΔBMI, kg/m^2	0.04	0.09	0.24	0.05	-0.13	-0.23
ΔWC, cm	0.09	0.16	0.13	0.25	8^{*10-3}	-0.10
ΔBody fat, %	-0.03	-0.19	0.13	0.30	0.03	-0.04
ΔFat-free mass, %	0.07	0.23	0.03	-0.24	-0.08	-0.04
ΔBody mass, kg	0.02	0.09	0.25	0.05	-0.12	-0.22
ΔM-value, μmol/kg/min	0.06	**-0.42** **	-0.05	-0.02	0.08	-0.04
Δf-Glucose, mmol/l	0.08	0.18	0.03	4^{*10-3}	-0.05	0.11
Δf-Insulin, mU/l	-0.13	0.12	**0.54** *	0.16	0.16	0.02
ΔHbA1c, mmol/mol	-0.07	-0.19	**0.42** *	-2^{*10-4}	3^{*10-3}	-0.07
ΔTriglycerides, mmol/l	0.20	0.14	0.29	-0.20	-0.26	0.06
ΔNEFA, mmol/l	0.03	0.14	0.21	-7^{*10-3}	0.18	0.08
ΔCholesterol, mmol/l	0.21	0.13	0.04	-0.01	0.08	0.21
ΔHDL-C, mmol/l	**0.43** **	0.37	0.03	0.15	0.01	0.08
ΔLDL-C, mmol/l	0.06	0.03	-0.09	-0.06	0.13	0.18

Significant p-values; * < 0.05, ** < 0.01, *** < 0.001. Significant values are in bold. Abbreviations: Δ = change from pre-intervention to post-intervention, LGU = liver glucose uptake (μmol/ 100 ml/min), EGP = endogenous glucose production (μmol/kg/min), LFC = liver fat content (%), ALT= alanine aminotransferase (U/l), AST = aspartate aminotransferase (U/l), GGT = γ-glutamyltransferase (U/l), BMI = body mass index, M-value = whole-body insulin sensitivity, f = fasting, HbA1c = hemoglobin A1c, NEFA = non-esterified fatty acids, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol. *The table is from the original publication IV.*

Table 16. Associations between the changes (post – pre Δ values) in liver glucose uptake, endogenous glucose production, liver fat content, liver enzymes, and accelerometry measured sedentary behavior and physical activity during the intervention.

	ΔLGU	ΔEGP	ΔLFC	ΔALT	ΔAST	ΔGGT
ΔSB time, h/day	-0.04	0.20	0.16	**0.42** *	0.18	0.03
ΔSB time, %/day	-0.20	0.15	0.25	**0.39** *	0.12	0.09
ΔStanding time, h/day	0.23	-0.18	-0.35	**-0.40** *	-0.25	-0.17
ΔStanding time, %/day	0.21	-0.22	-0.33	**-0.41** *	-0.25	-0.16
ΔSteps, number/day	-0.13	-0.08	-0.16	-0.07	0.21	0.09
ΔBreaks, number/day	-0.36	-0.11	-0.13	0.01	**0.41** *	0.28
ΔLPA, h/day	0.20	-0.01	-0.08	-0.23	-0.01	-0.03
ΔLPA, %/day	0.19	-0.05	-0.04	-0.27	-0.07	-0.03
ΔMVPA, h/day	0.03	0.03	-0.16	-0.14	0.15	0.03
ΔMVPA, %/day	0.01	0.01	-0.15	-0.15	0.13	0.04

Abbreviations: Δ = change from pre-intervention to post-intervention, LGU= liver glucose uptake (μmol/ 100 ml/min), EGP = endogenous glucose production (μmol/kg/min), LFC = liver fat content (%), ALT= alanine aminotransferase (U/l), AST = aspartate aminotransferase (U/l), GGT = γ-glutamyltransferase (U/l), SB = sedentary behavior, LPA = light physical activity, MVPA = moderate-to-vigorous physical activity. *The table is from the original publication IV.*

Table 17. Associations between the changes (post – pre Δ values) in liver glucose uptake, endogenous glucose production, liver fat content, liver enzymes, and dietary intake during the intervention.

	ΔLGU	ΔEGP	ΔLFC	ΔALT	ΔAST	ΔGGT
ΔTotal EI, kcal/day	-0.04	0.13	0.04	0.14	0.06	-0.004
ΔProtein, g/day	-0.10	-0.12	-0.12	-0.14	-0.20	**-0.37** *
ΔProtein, %	-0.09	-0.29	-0.14	-0.29	-0.23	**-0.41** **
ΔCHO, g/day	-0.05	0.19	0.18	0.34*	0.27	0.17
ΔCHO, %	-0.06	0.17	0.14	0.38*	0.33*	0.26
ΔFat, g/day	0.02	0.02	-0.03	-0.04	-0.15	-0.17
ΔFat, %	0.07	-0.11	-0.17	-0.19	-0.25	-0.23
ΔAlcohol, g/day	0.04	0.13	-0.21	-0.17	0.01	0.29
ΔAlcohol, %	0.07	0.09	0.11	-0.26	0.002	0.29
ΔSFA, g/day	0.16	0.11	-0.08	-0.11	-0.17	-0.14
ΔSFA, %	0.19	-0.002	-0.25	-0.25	-0.21	-0.15
ΔMUFA, g/day	-0.05	0.02	0.06	0.10	-0.04	-0.10
ΔMUFA, %	-0.01	-0.09	-0.02	0.02	-0.08	-0.11
ΔPUFA, g/day	0.02	0.02	0.24	-0.002	-0.13	-0.11
ΔPUFA, %	0.03	-0.05	0.28	-0.02	-0.15	-0.12
ΔSaccharose, g/day	0.03	0.09	0.26	**0.49** **	**0.56** ***	**0.38** *
ΔSaccharose, %	0.07	0.03	0.20	**0.47** **	**0.52** ***	**0.38** *
ΔFiber, g/day	0.02	0.08	0.26	0.15	-0.08	-0.16
ΔWIDF, g/day	0.04	0.05	0.26	0.21	-0.002	-0.17

Significant p-values; * < 0.05, ** < 0.01, *** < 0.001. Significant values are in bold. Abbreviations: Δ = change from pre-intervention to post-intervention, LGU = liver glucose uptake (µmol/ 100 ml/min), EGP = endogenous glucose production (µmol/kg/min), LFC = liver fat content (%), ALT = alanine aminotransferase (U/l), AST = aspartate aminotransferase (U/l), GGT = γ-glutamyltransferase (U/l), EI = energy intake; CHO = carbohydrates; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; WIDF = water-insoluble dietary fiber, % = percentage of the daily energy intake. *The table is from the original publication IV.*

5.4.6 Exploratory analyses

Analysis of sedentary behavior changes and their impact on liver health

Due to the considerable variation of changes in sedentary time in both INT and CON groups, we evaluated the individual changes in sedentary time recorded by the accelerometer to examine the per-protocol effects. Regardless of the original group allocation, we divided all participants with valid accelerometer data (n=39) into two groups based on whether they A) reduced sedentary behavior (n=22) or B) maintained or increased sedentary behavior (n=17). In the study, it was observed that participants who successfully reduced their sedentary behavior experienced a decrease in ALT levels (-1.1 [95% CI: 0.93, 1.36]) compared to those who remained sedentary (+0.8 [95% CI: 0.65, 1.05]) (group*time, p=0.006) **(Fig. 19 A)**. No other significant group*time effects on liver health markers were observed when comparing the groups based on measured sedentary behavior change *(data not shown)*.

Analysis of sedentary behaviour reduction on liver fat content below or higher than 1.85%

Recent research (Petersen et al., 2022) has questioned the traditional threshold of 5% liver fat content used to define MASLD. It suggests a lower threshold of 1.85% may better indicate insulin sensitivity issues and increased cardiometabolic risks. The study found that individuals with liver fat content between 1.85% and 5.56% exhibit significant metabolic impairments compared to those below 1.85%. This study, therefore, sought to explore whether a reduction in sedentary behavior could yield varied outcomes in participants regardless of the original group allocation, with an MRS-measured liver fat content higher or lower than 1.85%. We found that participants with a liver fat content of ≥1.85% (n=19) significantly reduced their liver fat content (-1.3 [95% CI: 0.95, 1.87]) compared to those with a liver fat content of ≤1.85% 8 (n=21) (+0.7 [95% CI: 0.49, 1.00]) (group*time, p=0.001) **(Fig. 19 B)**.

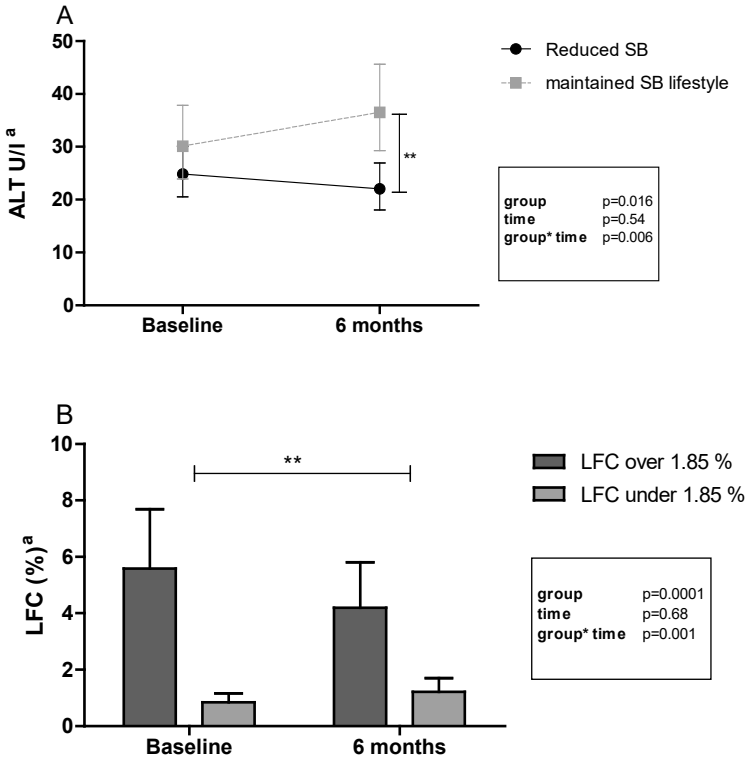

Figure 19. Exploratory analyses results on A) alanine aminotransferase (ALT) levels among all participants based on whether they reduced sedentary behavior (SB) (black line) or maintained SB lifestyle (grey line), B) liver fat content (LFC) levels among all participants based on whether they had higher (over 1.85%) LFC (black line) or lower (under 1.85%) LFC (grey line). The values are presented as model-based means (95 % CI), [a] = log10 transformed (means are back-transformed geometric model-based means). Significance levels; $p < 0.05$ = *; $p < 0.01$= ** and $p < 0.001$ = ***. *The figure is from the original publication IV*

6 Discussion

The studies showed that liver enzymes and liver fat content were strongly associated with overall body adiposity and other health risk markers, independent of sedentary behavior and physical activity levels. Liver fat content was also shown to be beneficially associated with daily protein intake. Furthermore, the studies suggested that increasing daily standing time, dietary fiber intake, and replacing some daily carbohydrates and sugars with unsaturated fat sources and protein might potentially improve hepatic insulin sensitivity and liver health. Additionally, aiming to reduce sedentary behavior by 1 hour per day did not yield significant effects on the measured liver function markers, suggesting that a longer duration of sedentary behavior reduction or higher intensity of physical activity may be necessary to achieve notable improvements in liver health. However, participants who successfully reduced sedentary behavior showed decreased ALT levels, and beneficial associations were observed between changes in liver health markers and dietary intake.

6.1 Body adiposity, but not sedentary behavior or physical activity, is associated with circulating liver enzymes (I)

In the first study, which was part of the screening phase of the intervention study (IV), we investigated the associations between liver enzymes ALT, AST, and GGT and sedentary behavior and physical activity in sedentary, inactive adults with overweight or obesity. Additionally, we examined the associations between liver enzymes and markers of overall body adiposity and other cardiometabolic markers. In general, elevated liver enzyme levels are established risk factors associated with various forms of liver disease and have been correlated with liver-related mortality (Hyeon et al., 2004; T. H. Lee et al., 2008; Ruhl & Everhart, 2009). This association makes the measurement of circulating liver enzymes an important tool for assessing liver injury within clinical practice.

Relationship with liver enzymes and sedentary behavior and physical activity

The initial hypothesis posited that there would be a positive association between liver enzyme levels and sedentary time and a negative association with both standing time and breaks taken from sedentary activities. However, the results of this study did not reveal any significant associations between liver enzyme levels and sedentary behavior metrics, including total sedentary time, standing, or breaks in sedentary behavior. Other studies have also reported no significant associations between accelerometer-measured sedentary time and circulating liver enzymes (Keating et al., 2016; Norman et al., 2017). Additionally, we found no significant associations between liver enzyme levels and LPA and MVPA once adjusted for confounding factors, including age, sex, and BMI. Similar outcomes have been observed among participants diagnosed with MASLD and type 2 diabetes (Bacchi et al., 2013; Hallsworth et al., 2015).

In contrast, previous research has also highlighted a different perspective. One notable study discovered that a lack of regular physical activity was linked to elevated ALT levels in newly diagnosed adult patients with type 2 diabetes (Mor et al., 2014). However, it is crucial to note that this study relied on self-reported data regarding exercise frequency. Self-reported measures often carry inherent limitations, such as the potential for participants to over-report the frequency and intensity of their physical activities (Hukkanen et al., 2018), which could skew the results. Additionally, another study indicated that accelerometer-measured sedentary time was independently associated with increased levels of ALT and GGT after controlling for fundamental cardiometabolic markers (J. Li et al., 2020). These findings underscore the complex relationship between physical inactivity and liver enzyme levels. The discrepancies observed between various studies may stem from differences in the demographic characteristics of the study populations, such as ethnicity, age range, and health status.

Other research has reported varied findings regarding the impact of structured exercise training on liver enzyme levels. Some studies suggest that exercise training can increase (Fragala et al., 2017; Pettersson et al., 2008) and decrease (Fragala et al., 2017; Slentz et al., 2011; Sullivan et al., 2012) certain liver enzyme levels. These conflicting outcomes can be attributed to multiple factors, including the participant's age, gender, and the specific type of exercise undertaken. Moreover, genetic differences in liver enzyme levels (Whitfiled & Martin, 1985) and variability in individual levels of physical activity (Koutedakis et al, 1993) may complicate the interpretation of these findings. Overall, the inconsistent results from previous studies imply that definitive conclusions regarding the relationships between liver enzymes, sedentary behavior, and physical activity cannot be firmly established. However, based on the findings from our research, it appears that a longer duration

of reducing sedentary behavior or engaging in higher-intensity physical activity may be necessary to achieve a positive effect on liver enzyme levels, particularly among adults with overweight and obesity.

Relationship with liver enzymes and cardiometabolic risk markers

In our investigation, all liver enzymes were strongly and positively associated with various cardiometabolic risk factors, such as obesity, blood pressure, fasting insulin, and HOMA-IR. Our findings align with prior research documenting similar associations (Ahn et al., 2015; Clark et al., 2003; T. H. Lee et al., 2008; Marchesini et al., 2005; Rahman et al., 2020; Ruhl & Everhart, 2009). For instance, one study highlighted that in a population of adults classified as obese, both ALT, AST, and GGT levels exhibited a positive correlation with HOMA-IR. Notably, it was found that ALT alone was positively correlated with fasting glucose levels and HbA1c (Marchesini et al., 2005) —a marker used to assess long-term glucose control. This research further supports the notion that elevated levels of ALT may be indicative of an increased risk for metabolic conditions, particularly type 2 diabetes (Vozarova et al., 2002). Interestingly, our results imply a stronger link between liver enzymes and markers of glucose metabolism rather than lipid profiles. Our study did not find significant correlations between liver enzymes and lipid metrics such as triglycerides, LDL-C, and total cholesterol levels. This lack of association suggests that the influence of liver enzymes may be more pronounced in the context of glucose regulation.

In addition, our analysis revealed a positive correlation between resting heart rate and liver enzyme levels, even after adjusting for BMI. The significance of this relationship is underscored by previous studies involving participants diagnosed with obesity and metabolic syndrome, where similar positive associations were reported between ALT and GGT levels and resting heart rate (Straznicky et al., 2012). Moreover, a notable study indicated a positive link between resting heart rate and MASLD, specifically within a population of post-menopausal women (H.-B. Kim & Lee, 2020). These findings suggest a potential interplay between liver health, chronic stress levels, autonomic nervous system imbalances, and the dysregulation of glucose metabolism. Given these associations, our research proposes that monitoring resting heart rate could serve as an additional tool for assessing the risk of liver diseases among individuals with excess weight. However, it is important to note that our analysis did not incorporate other significant cardiometabolic risk factors—including fasting insulin levels and elevated blood pressure—into the statistical model employed. The absence of these variables may obscure some underlying mechanisms that explain the associations observed between liver enzymes and resting heart rate. Further investigative studies are warranted to validate

these findings and explore other possible explanations for the connections between liver enzyme levels and resting heart rate dynamics.

6.2 Liver fat content is associated with body adiposity and protein intake, not sedentary behavior or physical activity (II)

The second study utilized the baseline data from the intervention study (IV). We aimed to investigate the associations between liver fat content, sedentary behavior, and physical activity in sedentary, inactive adults with metabolic syndrome. Additionally, we wanted to examine the associations between liver fat content and daily nutrient and energy intake, fitness, and common markers of cardiometabolic health. Lastly, we evaluated the correlation and agreement between the two different liver fat content quantification methods: magnetic resonance spectroscopy and magnetic resonance imaging.

Relationship with liver fat content and sedentary behavior, physical activity, fitness, and cardiometabolic health markers

Our analysis revealed that liver fat content is positively associated with several important health markers, including obesity, insulin resistance, fasting plasma triglycerides, and circulating liver enzymes. These findings support previous evidence that liver fat content is linked to various cardiometabolic risk factors (De Chiara et al., 2019; Gastaldelli et al., 2009; Jakobsen et al., 2007; L. Li et al., 2016; Riquelme et al., 2009; Yki-Järvinen, 2005). Interestingly, our study did not uncover significant associations between liver fat content and the objectively measured components of sedentary behavior, physical activity, or fitness levels. However, we found a noteworthy correlation between MRI-measured liver fat content and the number of daily steps taken and VO_{2max}, a measure of cardiovascular fitness. Nonetheless, when we adjusted for body fat percentage in our analyses, the significance of these associations diminished. This indicates that while there may be a relationship between liver fat content and physical activity and fitness, an individual's overall body adiposity could heavily influence this connection. Similar results have been reported regarding the relationship between liver fat content and fitness levels (Church et al., 2006).

Most previous investigations relied on self-reported data to determine participants' sedentary behavior and physical activity levels. As a result, they often reported a positive correlation between liver fat content and sedentary behavior (Ryu et al., 2015; Wei et al., 2016b), along with a negative correlation regarding physical activity (Gianluca Perseghin et al., 2007; Kistler et al., 2011; Y. Li et al., 2019; Wei

et al., 2016b). Notably, studies have indicated that these associations grow stronger with increased amounts and intensity of physical activity (Gianluca Perseghin et al., 2007; Kistler et al., 2011; Y. Li et al., 2019; Zelber-Sagi et al., 2008). In contrast, some research has shown that the relationship between liver fat content and physical activity becomes less pronounced when WC is included in the statistical model (Zelber-Sagi et al., 2008). This supports our findings that body composition may play a crucial role in modulating liver fat content.

Sedentary behavior has been identified as a potential independent predictor of MASLD (D. Kim et al., 2020). Furthermore, enhancements in habitual and transportation-related physical activity have been correlated with a decreased risk of developing MASLD in a dose-dependent manner (Henson et al., 2015). However, studies that employed accelerometers to measure physical activity and sedentary behavior yielded conflicting results. In contrast to our findings, one study indicated a positive correlation between sedentary behavior and MRI-assessed liver fat percentage among subjects both with and without metabolic syndrome, as well as among those at an elevated risk for type 2 diabetes (Bowden Davies et al., 2019b). On the other hand, evidence supports our results, such as a study involving adults with overweight or obesity that found no association between liver fat content and measured habitual physical activity or sedentary behavior (Keating et al., 2016).

The disparities in the research outcomes may be attributed to differences in study demographics, methodologies employed, and genetic factors influencing fitness levels (Chung et al., 2021). Additionally, variations in the techniques used to assess liver fat, the timing of data collection, and the positioning of accelerometers could all impact results. Our study adopted an extensive data collection period and utilized hip-worn accelerometers to monitor activity levels. This may provide a more accurate and complete representation of daily behavior patterns. Crucially, the intensity and duration of physical activity appeared to be significant factors in defining meaningful associations between liver fat content and physical activity levels. For instance, a more recent meta-analysis has suggested that higher levels of physical activity correspond with a reduced risk of MASLD in a dose-dependent manner (Qiu et al., 2017). It also proposed that to achieve a substantial reduction in MASLD risk, individuals might need to engage in physical activity beyond the recommended guidelines, which suggest a minimum of 150 minutes of moderate physical activity or 75 minutes of vigorous physical activity per week (Qiu et al., 2017). In summary, our findings suggest that neither sedentary behavior nor habitual physical activity is independently linked to liver fat content among inactive adults with metabolic syndrome. Furthermore, a greater volume and/or intensity of physical activity may be necessary to improve liver fat content in this population effectively.

Relationship between liver fat content with nutrient and energy intake

In our investigation of the relationship between liver fat content and daily nutrient and energy intake, we found that an increased daily protein intake correlates with lower liver fat levels, suggesting a beneficial impact on liver health. This aligns with findings from a recent review that advocates for a dietary pattern emphasizing high protein intake, particularly from plant-based sources, while concurrently minimizing carbohydrates and sugars (De Chiara et al., 2019). Such an approach may be an effective strategy for reducing liver fat and enhancing insulin sensitivity (De Chiara et al., 2019), making it a valuable option for individuals aiming to improve metabolic health. Our study did not uncover significant associations between liver fat content and consuming carbohydrates, sugars, or specific fatty acids. However, previous studies indicate that certain dietary modifications can benefit liver health. For instance, reducing fructose intake has been shown to have positive effects (Jensen et al., 2018), as has replacing saturated fatty acids with unsaturated ones (Winters-van Eekelen et al., 2021). Furthermore, adopting a low-carbohydrate diet has also been linked to improved liver health (Haghighatdoost et al., 2016). Overall, it's clear that the quality and quantity of various macronutrients are critical factors influencing liver fat accumulation (Hydes et al., 2021; Winters-van Eekelen et al., 2021). However, total daily caloric intake may be a more influential factor (Hydes et al., 2021; Parry & Hodson, 2017). Therefore, focusing on the types of nutrients consumed and overall energy balance is essential for effectively managing liver fat and promoting better metabolic health.

Correlation and agreement between two different liver fat content assessment methods

Finally, we compared two methods for quantifying liver fat content: magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI), specifically utilizing the 2-point Dixon (2PD) technique. Our findings demonstrated a strong correlation between the two methods; however, we observed a statistically significant difference in their mean values. MRI consistently reported liver fat content values more than 2.5 times higher than those obtained through MRS. Previous studies have highlighted a robust correlation between the data derived from MRI and MRS (D. C. Chan et al., 2006; Schuchmann et al., 2007). However, despite this correlation, it has been established that the fat content measurements obtained from MRI can vary substantially due to the chosen technical parameters during imaging. These variations may limit the clinical applicability of MRI in assessing liver fat content accurately, prompting experts to suggest the development of protocol-specific cutoffs for liver fat quantification (H. Kim et al., 2008).

Furthermore, studies indicate that liver biopsy yields significantly higher liver fat content values compared to what is reported through MR techniques (Parente et al., 2014). Liver biopsy is often regarded as the gold standard for accurately assessing liver fat content (Berger et al., 2019). However, this method is subject to sampling errors, as only about 1/50000th of the liver is analyzed histologically (Maharaj et al., 1986), and liver fat distribution can be heterogenous (Arun et al., 2007). Additionally, lipid quantification is often based on subjective assessments by pathologists rather than objective chemical analyses, which diminishes the accuracy of determining the liver fat content (Borra, 2009; Hussain et al., 2005; Petersen et al., 1996; E. L. Thomas et al., 2005). A liver biopsy involves the invasive procedure of extracting a small sample of liver tissue for histopathological analysis. Although this method provides highly precise results, its invasive nature poses several challenges, including potential complications, patient discomfort, and limitations in its routine use for monitoring liver fat. Given the invasive nature and associated patient risks of liver biopsies, there is an urgent need to explore non-invasive alternatives. Techniques such as MRI and MRS hold promise for more frequent and accessible assessments of liver fat content, aligning with patient-centric approaches in clinical practice. Both MRS and MRI are employed in clinical settings to measure liver fat content effectively. While MRS has been found to demonstrate greater accuracy compared to other non-invasive methods (Pasanta et al., 2021a), it also has notable limitations. For example, MRS can potentially misestimate liver fat content due to the small size of the sampled liver section, particularly in cases with heterogeneous fat distribution across the liver tissue. Furthermore, the procedure for conducting MRS is generally more time-consuming and technically demanding than MRI (Parente et al., 2014). Considering these factors, further research is essential to ascertain the most accurate and reliable method for quantifying liver fat content. This knowledge is crucial for accurately diagnosing MASLD. Underestimating liver fat content through MRS might result in important clinical implications, such as the risk of overlooking or not addressing underlying MASLD.

6.3 Daily standing time and fiber intake are associated positively with liver insulin sensitivity (III)

In the third study, we also utilized the baseline data from the intervention study (IV). We aimed to examine the associations between liver insulin sensitivity markers (liver glucose uptake, EGP), sedentary behavior, and physical activity in sedentary adults with metabolic syndrome. Additionally, we examined the associations of liver glucose uptake and EGP with daily nutrient and energy intake, fitness, body composition, liver fat content, and common markers of cardiometabolic risk factors.

In general, liver glucose uptake increases in insulin stimulation in healthy individuals, and EGP decreases compared to fasting (Moore et al., 2012). However, in individuals with insulin resistance, the liver fails to appropriately increase liver glucose uptake and decrease EGP in response to insulin stimulation (Titchenell et al., 2017). Thus, liver glucose uptake and EGP can be described as liver insulin sensitivity markers.

Relationship with liver insulin sensitivity and sedentary behavior, physical activity, and fitness

We did not identify a significant association between liver glucose uptake and habitual sedentary behavior and physical activity levels, as quantified by accelerometers. Despite these findings, some PET intervention studies have provided valuable insights into how different exercise intensities and durations can distinctly influence liver glucose uptake. For instance, one study found that moderate-intensity aerobic exercise led to a more pronounced liver glucose uptake enhancement than sprint interval training (Motiani et al., 2019). This finding suggests that the steady-state nature of moderate-intensity workouts may contribute to a more beneficial metabolic response in the liver. In contrast, another investigation revealed that resistance training did not produce a statistically significant effect on liver glucose uptake (Honka et al., 2016), indicating that strength training alone may not be sufficient to enhance this particular aspect of glucose metabolism.

These results suggest that moderate-intensity aerobic exercise could offer more advantages for improving liver glucose uptake than other training forms. However, it is essential to consider that the overall intensity and duration of physical activity observed in the current study may have been inadequate to demonstrate any meaningful correlations with liver glucose uptake. Furthermore, the study's findings might have been influenced by the limited variation in physical activity levels among participants, most of whom belonged to a relatively homogenous group characterized by sedentary and inactive lifestyles. This lack of diversity in activity levels could impede the ability to detect any potential associations between physical activity and liver glucose uptake.

Our results revealed a noteworthy beneficial relationship between EGP and the amount of daily standing time, indicating that even when body fat percentage was controlled, increased standing time correlated with suppressed EGP levels. Previous intervention studies have demonstrated that different forms of physical activity, including resistance training (Honka et al., 2018), treadmill walking (Gregory et al., 2019), and moderate to vigorous physical activities (Shojaee-Moradie et al., 2007), contribute to improvements in EGP. Additionally, our previous research has provided insights into the beneficial connection between standing and whole-body

insulin sensitivity (Garthwaite et al., 2021), further highlighting the importance of non-sedentary behaviors. Given these observations, it is reasonable to hypothesize that replacing sedentary activities, particularly sitting with standing, could positively affect insulin sensitivity at the whole-body level and within hepatic tissues. Moreover, our current study observed a positive association between EGP and sedentary time. However, this relationship lost significance when we incorporated body fat percentage into our statistical model. This suggests that sedentary behavior may not independently influence EGP and underscores body fat's potentially critical role in regulating EGP levels. However, it is also crucial for future studies to examine the locations of fat (e.g., visceral fat, subcutaneous fat, etc.) and how they might affect EGP.

The link between aerobic fitness and whole-body insulin sensitivity has been documented in previous research, establishing a connection between improved aerobic fitness and enhanced metabolic health (Messier et al., 2008; Solomon et al., 2015). However, the effects of aerobic fitness on liver insulin sensitivity remain less definitively established in the literature. Our study did not identify a significant association between liver glucose uptake and cardiorespiratory fitness. Upon adjusting for variables such as sex and age, we found a negative correlation between EGP and fitness levels. Nevertheless, this association diminished when we further accounted for body fat percentage. This suggests that while aerobic fitness may influence glucose metabolism to some extent, it may not provide an independent correlation with liver insulin sensitivity as much as overall body adiposity does, implying that the composition of body fat may exert a more substantial impact on the regulation of liver glucose metabolism. Thus, future research should further explore the intricate interactions between physical activity, body composition, and insulin sensitivity to understand these relationships better.

Relationship with liver insulin sensitivity and nutrient and energy intake

Previous research has established a significant link between sugar consumption, particularly fructose, and the onset of MASLD. Fructose contributes to the development of fatty liver by promoting de novo lipogenesis—converting excess carbohydrates into fatty acids—and enhancing β-oxidation, which can ultimately lead to liver insulin resistance (Jensen et al., 2018). This dysfunction is critical, as it can pave the way for more severe metabolic disorders. Our study observed that liver glucose uptake exhibited an inverse relationship with the intake of carbohydrates and saccharose as a percentage of total energy consumption. This finding implies that a dietary regimen with lower carbohydrate and sugar intake, compared to other macronutrients, could enhance liver insulin sensitivity in individuals with metabolic syndrome. Interestingly, a previous study with adults with obesity revealed that liver

glucose uptake remained unchanged after participants followed a 6-week very low-calorie diet (Viljanen et al., 2009). However, in this study, the effects of varying combinations of macronutrients on liver glucose uptake were not fully explored.

Moreover, our research uncovered a positive association between liver glucose uptake and the consumption of unsaturated fatty acids, particularly MUFAs and PUFAs. Prior studies have suggested that these unsaturated fats can positively influence glucose metabolism and hepatic function, reducing key metabolic markers such as HbA1c, glycemia, and hepatic fat accumulation (Silva Figueiredo et al., 2017). In contrast, dietary patterns high in saturated fatty acids were associated with an increased accumulation of intrahepatic triglycerides and elevated adipose tissue lipolysis (Luukkonen et al., 2018), which may exacerbate metabolic dysfunction. Conversely, diets rich in unsaturated fatty acids did not increase liver fat content and lipolysis as much as a diet with saturated fatty acids (Luukkonen et al., 2018), indicating a potential protective effect against liver-related conditions.

In addition, our findings illustrated a positive correlation between liver glucose uptake and dietary fiber consumption, especially focusing on WIDF. Previous extensive prospective studies have linked a high dietary fiber intake, including insoluble cereal, with a significantly lowered risk of developing type 2 diabetes. This protective effect is thought to arise from improvements in insulin sensitivity, inflammatory markers, and positive changes in the intestinal microbiota (Weickert & Pfeiffer, 2008). Furthermore, dietary fiber may benefit various metabolic markers associated with liver disease and liver cancer, including elevated blood glucose levels, insulin resistance, and features of fatty liver and metabolic syndrome (X. Liu et al., 2021). In summary, the outcomes of our study suggest that adopting a dietary approach that emphasizes reducing carbohydrate and sugar intake while simultaneously increasing the consumption of unsaturated fatty acids and daily fiber may contribute positively to improving liver insulin sensitivity in adults with metabolic syndrome.

Relationship with liver insulin sensitivity, liver fat content, and other metabolic markers

The accumulation of excess fat within the liver can significantly hinder the liver's ability to take up glucose effectively. This occurs primarily because fat can physically occupy space within hepatocytes, disrupting normal cellular functions. Additionally, fat metabolism produces various intermediates, one of which is diacylglyceride, which has been shown to interfere with insulin signaling pathways, further complicating glucose metabolism (Erion & Shulman, 2010). Overnutrition—characterized by an intake of calories exceeding the body's energy requirements—combined with excess body weight, often leads to higher concentrations of

diacylglycerides in the liver. This increase can stem from two major sources: the influx of free fatty acids into the liver from the bloodstream and a process known as de novo lipogenesis, where the liver synthesizes fatty acids from carbohydrates. As these processes culminate in increased intrahepatocellular lipid content, they contribute to developing insulin resistance within the liver (Erion & Shulman, 2010; Weiss et al., 2003).

While a previous study evaluating both healthy individuals and those with type 2 diabetes established an inverse relationship between liver fat content—assessed through MRS—and liver glucose uptake (Borra et al., 2008), our current investigation did not replicate this significant correlation when examining liver glucose uptake or EGP against liver fat content as measured by MRS or MRI techniques. This discrepancy raises the possibility that it may not be the quantity of liver fat directly impeding glucose metabolism but rather the metabolic intermediates resulting from fatty acid breakdown that could be responsible for disrupting liver insulin sensitivity.

In addition to these findings, we noted an inverse relationship between EGP and LDL-C. This association might be counterintuitive, especially considering the well-documented correlation between elevated plasma LDL-C levels and a heightened risk of cardiovascular diseases (Carson et al., 2020). However, emerging research suggests a nuanced relationship between LDL-C and glucose metabolism. Notably, increasing LDL-C levels have been positively correlated with insulin secretion and inversely correlated with the risk of developing type 2 diabetes (Dannecker et al., 2021; Merino & Rotter, 2020). Furthermore, a comprehensive meta-analysis indicated that the use of cholesterol-lowering medications carries a slight increase in the risk of developing type 2 diabetes (Sattar et al., 2010). These observations lead us to hypothesize that the inverse relationship between EGP and LDL-C could imply that elevated LDL-C levels might reduce EGP, thereby promoting liver insulin sensitivity. This effect could potentially lead to a paradoxical decrease in the risk of type 2 diabetes among adults who are classified as having metabolic syndrome. Nonetheless, further controlled intervention studies are essential to establish definitive mechanisms that could clarify how LDL-C might influence diabetes risk.

Lastly, our analysis revealed that liver glucose uptake was negatively correlated with overall body adiposity, fasting insulin levels, and HOMA-IR. Conversely, EGP demonstrated a positive association with all these factors. Liver glucose uptake was positively linked to whole-body insulin sensitivity, as assessed through the hyperinsulinemic-euglycemic clamp method. In contrast, EGP was found to be inversely associated with this measure of insulin sensitivity. Our results underscore the connections between obesity, markers of insulin resistance, and hepatic insulin resistance, strengthening the existing body of evidence.

6.4 The effects of sedentary behavior reduction on liver health markers (IV)

In the fourth study, we investigated the effects of a 6-month randomized intervention aimed at reducing sedentary behavior by 1 h/day on liver insulin sensitivity (liver glucose uptake, EGP), liver fat content, and liver enzyme levels in sedentary, inactive adults with metabolic syndrome. Additionally, we wanted to examine the correlations between changes in sedentary behavior, physical activity, liver insulin sensitivity, liver fat content, liver enzymes, daily nutrient and energy intake, body composition, and other cardiometabolic health markers.

Effects of reducing sedentary behavior on liver glucose uptake

Our previous research demonstrated that an intervention designed to reduce sedentary behavior effectively lowered fasting insulin levels, which could enhance overall insulin sensitivity (Sjöros, Laine, Garthwaite, Vähä-Ypyä, Löyttyniemi, et al., 2023). Despite these promising findings, the current study did not reveal statistically significant differences in liver glucose uptake between the INT and CON groups. However, a noteworthy trend emerged: both the intervention and control groups showed a significant increase in liver glucose uptake from the initial measurements to those taken after the intervention. This indicates a possible improvement in liver insulin sensitivity across both groups. This enhancement in liver glucose uptake may stem from both groups increasing their daily step counts and improving their overall body composition. Existing literature has highlighted that the intensity of physical exercise can produce varying effects on liver glucose uptake. For example, one study found that moderate-intensity training was linked to increased insulin-stimulated liver glucose uptake in adults with normal glucose levels or who were classified as prediabetic or with type 2 diabetes. In contrast, sprint interval training did not yield the same benefits (Motiani et al., 2019). Furthermore, a thorough analysis of a four-month resistance training program conducted with older women revealed no significant changes in liver glucose uptake (Honka et al., 2016), suggesting that the type of exercise employed may significantly influence the outcomes.

Additionally, our investigation uncovered a positive correlation between changes in liver glucose uptake during the intervention and changes in HDL-C cholesterol levels. Prior studies have indicated that HDL-C could play a crucial role in glucose metabolism, potentially affecting insulin secretion, insulin-independent glucose uptake, and overall insulin sensitivity (Siebel et al., 2015). While a limited number of studies have explored the relationship between HDL-C and insulin-stimulated liver glucose uptake in humans, research involving animal models has revealed significant findings. For example, experiments showed that elevating HDL-

C levels considerably increased liver glucose uptake in insulin-resistant and dyslipidemic hamsters, primarily by inhibiting the function of cholesterol ester transfer protein (Briand et al., 2014). These findings suggest a potential connection between higher HDL-C levels and improved liver glucose uptake in humans. However, further research and targeted intervention studies are needed to validate this hypothesis effectively.

Effects of reducing sedentary behavior on endogenous glucose production

Our study found no significant changes in EGP between the groups or time effect either. This outcome contrasts with findings from a previous study among sedentary males, which indicated that a regimen of moderate-to-vigorous exercise—specifically, engaging in physical activity at 60 to 85% of maximal aerobic capacity for at least 20 minutes on three separate occasions each week—resulted in a noteworthy decrease in EGP and increase in whole-body insulin sensitivity (Shojaee-Moradie et al., 2007). The authors proposed that these improvements could be attributed to reduced levels of circulating non-esterified fatty acids (NEFA), likely due to enhanced insulin sensitivity impacting lipolysis (Shojaee-Moradie et al., 2007). In our investigations, however, we did not observe any significant alterations in NEFA concentrations or whole-body insulin sensitivity. This raises the possibility that greater intensity and longer duration of physical activity may be necessary to achieve meaningful improvements in EGP and whole-body insulin sensitivity.

It is worth noting that prior intervention studies have documented positive effects on EGP stemming from moderate-intensity resistance training and treadmill walking (Gregory et al., 2019; Honka et al., 2016), highlighting the potential for varied exercise modalities to influence glucose metabolism. Additionally, in our prior cross-sectional study that utilized the same participant population, we discovered a negative association between EGP and daily standing time (Laine et al., 2024), suggesting that greater amounts of time spent standing could be linked to lower levels of EGP. However, as we previously reported, there were no significant differences in daily standing time between the groups (Sjöros, Laine, Garthwaite, Vähä-Ypyä, Koivumäki, et al., 2023), which may account for our failure to observe any substantial effects of standing on EGP. Despite this, it remains possible that light physical activity, such as standing, could benefit EGP. Furthermore, this study (IV) identified that the changes in EGP observed during the intervention were negatively correlated with changes in whole-body insulin sensitivity across both groups. This finding is understandable, given that an increase in EGP during insulin stimulus is often closely associated with higher levels of insulin resistance (DeFronzo, 2009). Overall, while our results did not align with previous findings, they underscore the

need for further research to delineate the specific conditions under which physical activity can effectively modulate EGP and insulin sensitivity.

Effects of reducing sedentary behavior on liver fat content

No significant changes in liver fat content were observed between the INT and CON groups throughout the six-month intervention. In contrast, previous research demonstrated that a four-week supervised, progressive, moderate aerobic exercise program effectively reduced liver fat content, as measured by MRS, without accompanying weight loss (Johnson et al., 2009). This earlier study specifically focused on sedentary adults with obesity, who exhibited a mean baseline liver fat content exceeding 5%—the diagnostic threshold for MASLD (Johnson et al., 2009). In our study, however, the baseline mean liver fat content for the intervention and control groups fell within the normal range, specifically below 5%. This aspect may have complicated our ability to detect significant changes in liver fat content over the course of the intervention. Additionally, it is noteworthy that the intensity and duration of physical activity in our study were significantly lower compared to the previously mentioned study.

However, among the participants in the intervention group, three individuals had liver fat content levels that exceeded 5%. Remarkably, each of these participants reduced their liver fat content to normal or nearly normal levels by the end of the intervention period, with their baseline mean liver fat content recorded at 9.8%, which dropped to a mean of 4.1% after six months. The intervention resulted in an average reduction in sedentary behavior of approximately 50 minutes daily for all participants. This reduction signals a potential benefit for individuals with MASLD, as minimizing sedentary time is an important lifestyle change. However, it is essential to note that the limited number of participants with MASLD in the intervention group necessitates further validation through more targeted intervention studies that specifically involve MASLD patients.

Furthermore, a recent proposal suggests revising the upper limit for defining MASLD by lowering it from 5% to 1.85% (Petersen et al., 2022). In a sizable American study involving 2,331 participants, findings indicated that liver fat content ranging from 1.85% to 5.56% was linked to decreased insulin sensitivity and heightened cardiometabolic risk factors compared to individuals with liver fat content below 1.85% (Petersen et al., 2022). Within our study's dataset, participants with liver fat content equal to or greater than 1.85% exhibited significant reductions in their liver fat levels compared to those whose liver fat content was lower than 1.85%. This observation underscores the potential effectiveness of reducing sedentary behavior as an intervention for individuals with elevated liver fat content.

Prior studies have shown a positive impact of physical exercise on patients with MASLD, demonstrating that such activity can lead to significantly improved outcomes by decreasing intrahepatic lipid content. These improvements are achieved through a multi-faceted approach that includes reduced fatty acid synthesis, increased fatty acid oxidation, and inhibition of the molecular pathways responsible for damage to both mitochondria and hepatocytes (van der Windt et al., 2018). Additionally, a recent meta-analysis reveals that exercise can reduce liver fat content independent of significant changes in body weight, suggesting a direct beneficial effect on liver health (Stine et al., 2023). However, the specific mechanisms by which exercise exerts this effect are still not fully understood. Much of the existing research has emphasized the impact of exercise on enhancing insulin sensitivity in peripheral tissues, which consequently reduces the flow of free fatty acids and glucose uptake in the liver (Cigrovski Berkovic et al., 2021).

In summary, our efforts to minimize daily sedentary behavior through non-guided, non-exercise activities did not yield significant reductions in liver fat content. One reason for this outcome may be that the average liver fat content was already within the normal range at the study's outset. Additionally, the intensity of the physical activity and the extent of sedentary behavior reduction may have been inadequate to trigger meaningful changes. Nevertheless, our findings suggest that the intervention may have benefited participants with higher liver fat content levels. However, these observations must be confirmed through further studies focusing specifically on patients with MASLD. Future research should also integrate the analysis of body fat distribution because different compartments, such as subcutaneous and visceral fat, exhibit varying responses to exercise based on individual characteristics. Furthermore, we documented a positive association between changes in liver fat content during the intervention in both groups and changes in fasting insulin levels and glycated hemoglobin, aligning with previous studies that indicate a connection between fatty liver and liver insulin resistance (Paschos & Paletas, 2009).

Effects of reducing sedentary behavior on liver enzymes

The intervention did not yield a statistically significant impact on ALT levels between the groups. Despite this result, additional analysis revealed that all participants who successfully reduced their sedentary behavior (mean 52 minutes per day) did experience a notable decrease in ALT levels compared to those who maintained a consistently sedentary lifestyle. Furthermore, our analysis demonstrated a positive correlation between changes in ALT levels and changes in sedentary behavior and a negative correlation between changes in standing time.

These results suggest that reducing sedentary time and increasing daily standing may improve ALT levels.

In our previous three-month study involving a subset of the same population, we observed that participants who reduced their sedentary behavior by an average of 50 minutes per day while increasing their LPA and MVPA demonstrated favorable effects on ALT levels (Garthwaite et al., 2022). Supporting our findings, a recent meta-analysis indicated that individuals diagnosed with MASLD who exercised regularly for more than 12 weeks showed significant improvements in their ALT levels. Conversely, those who maintained an active lifestyle for fewer than 12 weeks did not experience any changes in ALT levels (Ma et al., 2022), highlighting the importance of sustained physical activity over time.

In addition to sedentary behavior, our study identified a positive correlation between changes in both ALT and AST levels and changes in daily carbohydrate and sugar intake throughout the intervention. This aligns with previous cross-sectional studies that reported similar associations. For instance, a large-scale population-based study involving 19,749 individuals revealed that increased carbohydrate intake—but not fat intake—was linked to higher aminotransferase levels in Koreans, both with and without metabolic syndrome (Kwon et al., 2012). Additionally, research indicated that consuming sugar-sweetened beverages positively correlated with elevated serum ALT and AST levels among healthy premenopausal women (Shimony et al., 2016). They suggested that fructose, primarily metabolized in the liver, may induce increased hepatic lipogenesis (Shimony et al., 2016), subsequently raising the risk of MASLD.

Our research noted that AST levels increased in both intervention and control groups, but no significant differences were found between them. Moreover, changes in AST levels demonstrated a positive correlation with the frequency of breaks taken from sedentary activities. It is essential to recognize that AST levels can regularly fluctuate by 5–10% within the same individual daily (Cooper, 2019). Previous studies have indicated that engagement in aerobic and resistance training (Fragala et al., 2017) alongside weight loss, particularly in women (Gasteyger et al., 2008), can contribute to elevated AST levels. Thus, the increase in AST levels observed in our study may be attributed to normal fluctuations, an overall rise in habitual physical activity (such as increased daily step counts), or improved body composition across both groups.

Regarding the GGT levels, our intervention did not result in any significant changes. A prior meta-analysis focusing on overweight individuals or those with MASLD found that among seven exercise-based studies, both with and without dietary interventions, only one study noted a significant alteration in GGT levels (Smart et al., 2018). Furthermore, when concentrating exclusively on the four exercise studies examined, there was no significant decrease in GGT levels (Smart

et al., 2018). In our research, we discovered that changes in GGT levels during the intervention were negatively associated with changes in daily protein intake (both in grams and as a proportion of total daily intake) while showing a positive association with changes in daily sugar consumption (also in grams and as a percentage of total intake). Earlier research has indicated that adhering to a high-protein diet, especially one derived from plant sources, correlates with a reduced risk of developing fatty liver disease compared to a low-protein diet (Khazaei et al., 2023). Additionally, calorie-restricted high-protein diets have proven more effective in decreasing hepatic fat than low-protein diets, achieved by inhibiting the genes responsible for fat synthesis, uptake, and storage (Xu et al., 2020). A previous study also demonstrated that a hypocaloric high-protein diet could decrease ALT and GGT levels in patients diagnosed with MASLD (Haidari et al., 2020). Furthermore, consistent moderate fructose and sucrose consumption has been associated with increased hepatic lipogenesis (Geidl-Flueck et al., 2021), which may elevate the risk of fatty liver disease and potentially lead to higher serum liver enzyme levels. Thus, these findings suggest that dietary factors may play a more pivotal role than exercise in regulating serum GGT levels.

6.5 Strengths and limitations

One of the most significant strengths of the fourth study's design is the use of a randomized controlled trial, which enhances the study's credibility and ensures that the results may be more reliable and valid. Moreover, the strengths of studies II, III, and IV are emphasized by their robust methodologies for assessing liver health. To assess insulin sensitivity, we employed the euglycemic-hyperinsulinemic clamp method, which is considered the gold standard in clinical research (DeFronzo et al., 1979). This technique allows for precise control of blood glucose levels while administering insulin, providing a reliable measure of how effectively tissues respond to insulin. Additionally, the study employed PET imaging to measure liver glucose uptake and EGP directly in the liver. PET imaging is esteemed for its non-invasive capabilities and offers detailed insights into tissue-specific glucose metabolism in vivo (Honka et al., 2022), making it an excellent tool for understanding glucose dynamics within the body. By integrating these two advanced techniques, the study enhances the reliability and validity of its findings on insulin sensitivity and glucose regulation. Specifically, we measured liver fat content using two differentiated and advanced imaging techniques: MRI and MRS. MRS is the gold standard for non-invasively quantifying liver fat, providing highly accurate results (Pasanta et al., 2021b).

One major strength of our studies was using accelerometers alongside validated algorithms (Vähä-Ypyä et al., 2018; Vähä-Ypyä, Vasankari, Husu, Mänttäri, et al.,

2015) to measure sedentary behavior and physical activity across all studies. This method offers a high level of precision compared to self-reported questionnaires, which often rely on individuals' subjective perceptions and may not accurately reflect their true experiences (Hukkanen et al., 2018). The accuracy of the accelerometers provides a more reliable dataset, which enhances the validity of our findings. To evaluate physical activity and sedentary behavior, we used validated hip-worn accelerometers. Placing the accelerometer on the hip improves motion detection and distinguishes between different postures more effectively than wrist-worn devices (Yang & Hsu, 2010). Furthermore, the relatively extended measurement duration of four weeks in studies I, II, and III and six months in study IV enhances the robustness of our results, especially when compared to other studies that utilized much shorter tracking periods, typically ranging from a few days to a week. Additionally, our participants operated with accelerometers in a free-living environment, another key strength of our research. This setting may allow for a more authentic representation of daily behaviors compared to controlled laboratory environments, where participants may alter their behavior due to the artificiality of the situation. Participants had the opportunity to choose how to compensate for the reduction in sitting time. This approach aimed to enhance their motivation to increase physical activity by allowing them to influence their own behavior. Additionally, it better reflected their actual activity levels, which was the goal of the intervention, compared to a strictly defined physical activity target. However, if physical activity had been more precisely defined, the results could have varied in different ways. On one hand, it might have led to an increase in physical activity, potentially resulting in greater improvements in liver health markers. On the other hand, strict guidelines could have decreased physical activity levels by demotivating sedentary participants. Furthermore, the cardiorespiratory fitness assessment was further enhanced by employing direct respiratory gas measurements, providing valuable insights into the participants' overall health and aerobic capacity.

Despite these strengths, our study does have several limitations. It is important to acknowledge that all techniques for measuring physical activity carry inherent limitations. For instance, most available accelerometers may not accurately capture the intensity of certain physical activities, such as swimming, resistance training, or carrying heavy loads. As a result, our analysis might have systematically underestimated the overall level of strenuousness associated with physical activity. One concern arose from replacing the MRI scanner during the study, necessitating the use of a different scanner for MRS and MRI to quantify liver fat content in seven participants. This change in equipment could potentially introduce variability in the results, thereby impacting the comparability and accuracy of the findings. However, it is essential to highlight that pre- and post-intervention measurements were consistently taken with the same imaging scanner for each participant. This practice

ensures that the comparisons accurately represent any potential changes in liver fat content during the intervention period. Using the same equipment minimizes variability and bias, allowing for a more reliable assessment of the intervention's impact on liver fat levels. Additionally, our research would have been enhanced by the inclusion of data concerning body fat distribution, which would provide a clearer understanding of the observed lack of response in liver fat content to the intervention.

Our research specifically targeted individuals who were not only physically inactive but also classified as sedentary and diagnosed with metabolic syndrome. The outcomes we observed may not be generalizable to a broader population, including individuals exhibiting various sedentary behaviors and varying degrees of physical activity. Additionally, results could differ significantly for those with more severe health issues beyond metabolic syndrome. Considering a more diverse sample might provide insights into how varying levels of activity and different health conditions influence outcomes. However, we specifically wanted to investigate participants at risk for cardiometabolic diseases due to the global prevalence of sedentary lifestyles and obesity in high-income countries, making the results applicable to larger populations. Understanding these factors can help inform public health strategies to mitigate these risks on a broader scale. We also acknowledge the possibility that participants may have either underestimated their food intake or altered their eating behaviors while completing the food diary. Despite this potential bias, food diaries, when analyzed at the group level, tend to provide reliable data regarding the intake of energy-yielding nutrients. Moreover, obtaining more nuanced data regarding the relationship between resting heart rate, liver enzymes, and dietary factors, beyond alcohol intake, could have provided valuable insights into our results (I). Further, conducting detailed analyses on the sources of protein consumed (distinguishing between animal-based and plant-based proteins) would have enriched our understanding of the relationship between liver fat content and daily protein intake (II). However, such a detailed examination was beyond the scope of these studies and should be regarded as an additional limitation worth addressing in future research.

One limitation of this study is that the power calculations were specifically conducted for the primary outcome, whole-body insulin sensitivity. This means that the statistical strength of the study was primarily focused on this measure, potentially overlooking other relevant outcomes. As a result, the findings related to other aspects of insulin sensitivity or associated variables may not be as robust or reliable. Additionally, our sample size was relatively small, which could limit the generalizability of our results. However, PET imaging is inherently resource-intensive, limiting the possibility of including larger groups of participants. Furthermore, it’s worth noting that the employed methodologies, including PET, MRS, accelerometry, and direct respiratory gas measurements during fitness testing,

are known for their high sensitivity in detecting small variations in outcomes. Therefore, these tools can be effectively utilized even in studies with smaller sample sizes.

In Study III, we identified a significant relationship between time spent standing and EGP after controlling for age, sex, body fat percentage, and the duration of accelerometer wear time. However, it is important to note that the analysis did not include additional adjustments for physical activity variables, sedentary behavior, and fitness levels. Incorporating these factors could have provided a more comprehensive understanding of their potential influence on the observed relationship. Additionally, in Study IV, the analysis of individual variations in sedentary behavior included all participants with complete accelerometer data, regardless of original group allocation. While this per-protocol approach allowed for a focused examination of the direct effects of reduced sedentary behavior on liver health markers, it also has limitations. Specifically, combining participants from different groups may undermine the benefits of randomization, potentially leading to inaccurate estimates of the intervention's effects. Additionally, some individuals in the study were using medications that could have influenced the outcomes, introducing variability that might affect the results. The most significant limitation, however, is the cross-sectional design of studies I, II, and III. This framework restricts the ability to establish causal relationships between the variables examined. As a result, interpretations derived from these studies should be cautiously approached.

6.6 Future directions

All associations between liver health markers, lifestyle factors, and metabolic markers should be confirmed through longitudinal studies. Such studies can provide valuable insights into how changes in sedentary behavior, physical activity levels, and nutrition affect liver health over time. By monitoring liver enzyme levels, liver fat content, liver insulin sensitivity, body composition, and lifestyle habits, researchers can identify causal relationships and potential timeframes for observing significant health changes.

Our intervention results indicated that aiming to reduce sedentary behavior by one hour per day and replacing it with non-guided physical activity was insufficient to impact liver health markers significantly. Therefore, it is essential to develop targeted interventions that reduce sedentary behavior and increase the intensity and duration of physical activities. Such approaches may result in more noticeable improvements in liver health in adults with metabolic syndrome. These interventions should aim to extend the time spent reducing sedentary behavior and potentially

incorporate various forms of physical activity, including structured exercise programs, to determine which methods yield the best outcomes.

However, it must be noted that in the INT group, all participants with liver fat percentages exceeding 5 %—the diagnostic threshold for MASLD—demonstrated a significant reduction in liver fat content during the intervention, decreasing from a mean of 9.8% to 4.1%. This outcome emphasizes the potential efficacy of lifestyle modifications, specifically reducing sedentary behavior by an average of 50 minutes per day, which was substituted with light physical activity for individuals exhibiting higher liver fat levels. Future studies must validate these findings with a larger cohort of MASLD patients, as integrating light physical activity into daily routines may serve as a feasible approach to initiate a more active lifestyle.

It is also important to deepen our understanding of how specific dietary patterns and their components affect liver health. Future research could involve randomized controlled trials to assess the impacts of diets, such as Mediterranean, low-carb, or high-fiber diets, on liver health markers. Additionally, discovering the optimal balance of macronutrients (fats, carbohydrates, and proteins) that promote liver health would be essential. Exploring the mechanisms influencing liver insulin sensitivity in relation to lifestyle factors is a promising area for future research. Studies could focus on how varying levels of standing time, dietary fats (particularly monounsaturated and polyunsaturated fats), and overall diet quality impact insulin sensitivity and liver health. Understanding these relationships could lead to more targeted dietary and physical activity recommendations for improving metabolic health. Future research should prioritize high-risk populations, including those with existing metabolic syndrome and varying demographic backgrounds. By examining how socio-economic factors, cultural dietary practices, and lifestyle differences influence liver health outcomes, researchers can develop tailored interventions that address the needs of these groups.

In general, developing effective behavior change strategies is essential for encouraging individuals to decrease sedentary time and sustainably increase physical activity. This could involve creating community programs, workshops, or online platforms that promote lifestyle modifications and provide ongoing support for individuals looking to improve their liver health. Additionally, wearable fitness trackers and mobile health applications could promote physical activity and reduce sedentary behavior. These technologies could empower individuals to take active steps toward improving their liver health by providing real-time feedback, goal-setting features, and personalized recommendations.

Lastly, advocating for policy changes that support public health initiatives aimed at promoting active lifestyles and healthier dietary options is crucial. This could involve encouraging schools, workplaces, and communities to create environments that facilitate physical activity, such as safe walking paths, accessible gym facilities,

and health education programs, ultimately leading to better liver and overall health outcomes for the population. Doing this can advance our understanding of the complex relationships between lifestyle factors, body composition, and liver health and lead to more effective prevention and intervention strategies, ultimately improving public health.

7 Conclusions

Our findings indicated that in inactive individuals who are overweight or obese, objectively measured sedentary behavior and physical activity were not significantly associated with liver enzyme levels (ALT, AST, or GGT). In contrast, various measures of overall body adiposity—including BMI and WC —showed a strong association with these liver enzymes. This suggests that maintaining healthy body fat levels might be more crucial for liver health than simply adhering to current physical activity guidelines. While neither sedentary behavior nor habitual physical activity appeared to be the primary factors influencing liver fat content, they did have an indirect relationship through their effects on body composition. Our results highlighted that obesity markers, like body fat percentage and BMI, were positively associated with daily sedentary behavior and negatively associated with habitual physical activity. Therefore, while sedentary behavior and physical activity alone may not directly regulate liver fat content, they can still affect liver health by influencing body composition.

Furthermore, dietary factors emerged as potentially more impactful than physical activity in promoting liver health. Higher daily protein intake was associated with lower liver fat content, suggesting a beneficial impact on liver health. Additionally, the negative associations found between liver glucose uptake and daily carbohydrate and sugar intake, alongside positive correlations with MUFAs, PUFAs, and fiber intake, suggest that dietary modifications, such as substituting some carbohydrates and sugars with healthier fats and adding fiber, could lead to improved liver condition. We also found that increasing daily standing time was beneficially associated with liver sensitivity via suppressed (improved) EGP. Notably, unhealthy body composition was linked to impaired hepatic insulin sensitivity, emphasizing the importance of body fat distribution in metabolic health. Additionally, plasma LDL-C levels demonstrated a beneficial relation to EGP, suggesting that optimizing LDL-C could help enhance hepatic insulin sensitivity and possibly, paradoxically, reduce the risk of type 2 diabetes. However, all of these associations warrant further verification through targeted intervention studies.

An intervention aimed at reducing sedentary behavior by one hour per day and replacing it with non-exercise physical activity showed no significant change in liver

health markers, indicating that more extended periods of reduced sedentary behavior or increased intensity or duration of physical activity may be necessary for noticeable improvements in liver health. However, exploratory analyses among all participants suggested that achieving specific behavior changes could lead to better ALT levels. The study also revealed a significant increase in liver glucose uptake in both the INT and CON groups, possibly due to increased daily steps and improved body composition. This research is noteworthy as it is one of the first to examine the effects of reducing sedentary behavior and replacing it with non-exercise physical activity on liver health markers in adults with metabolic syndrome at risk for developing MASLD. Overall, the study provides critical insights and groundwork for future interventions focused on determining effective guidelines for reducing sedentary behavior to positively impact liver health.

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