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RESEARCH ARTICLE

Liver Metabolism

Effects of reducing sedentary behavior on liver insulin sensitivity, liver fat content, and liver enzyme levels: a six-month randomized controlled trial

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Abstract

Metabolic syndrome increases the risk of developing noncommunicable diseases such as metabolic dysfunction-associated steatotic liver disease. The aim was to investigate the effects of sedentary behavior (SB) reduction on liver glucose uptake (LGU), endogenous glucose production (EGP), liver fat content (LFC), and liver enzyme levels [alanine aminotransferase (ALT), aspartate aminotransferase, and γ-glutamyltransferase]. Forty-four sedentary (daily SB time ≥ 10 h), physically inactive middle-aged adults with metabolic syndrome were randomized into intervention (INT; $n = 23$, 21 completed) and control (CON; $n = 21$, 19 completed) groups. For 6 mo, INT aimed to limit SB by 1 h/day, whereas CON aimed to maintain usual habits. SB and physical activity (PA) were measured continuously with hip-worn accelerometers. Before and at the end of the intervention, LGU was measured using positron emission tomography during the hyperinsulinemic-euglycemic clamp. EGP was calculated, and LFC was measured by magnetic resonance spectroscopy. INT reduced SB by 51 [95% confidence interval (CI): 22, 78] min/day and increased moderate-to-vigorous physical activity (MVPA) by 22 (95% CI: 12, 33) min/day, with no significant change in CON. Differences in liver health markers between the groups were not significant. However, according to the exploratory analyses among participants who successfully reduced SB, ALT decreased (−1.1 [95% CI: 0.93, 1.36] U/L) compared with the continuously sedentary participants (+0.8 [95% CI: 0.65, 1.05] U/L) (group × time, $P = 0.006$). To enhance liver health, reducing SB for longer durations and/or increasing the intensity of PA may be necessary. However, successfully reducing SB may lead to better levels of circulating ALT liver enzymes.

NEW & NOTEWORTHY Aiming to reduce sedentary behavior (SB) by 1 h/day did not significantly influence liver health markers, suggesting that more substantial reductions or a different approach might be necessary to see improvements. However, achieving the desired behavioral change could lead to improvements in ALT levels. This study is the first to analyze how reducing SB and replacing it with nonguided physical activity impacts liver health in adults with metabolic syndrome, offering insights for future intervention strategies.

endogenous glucose production; liver fat; liver glucose uptake; physical activity; sedentary behavior

INTRODUCTION

Physical inactivity is the fourth leading risk factor for global mortality and causes 3.2 million deaths yearly (1). Based on objectively measured data, adults spend ~8.2 h/day of their waking hours in sedentary behaviors (SBs) (2), such as sitting, lying, or reclining. Emerging evidence shows that in addition to moderate-to-high-intensity exercise also, informal light-intensity physical activity (PA) (so-called non-exercise activity) that replaces sedentary activities such as prolonged sitting likely prevents obesity, diabetes, cardiovascular disease, and cancer risk and mortality (3–6).

Metabolic syndrome (MetS) is defined as a cluster of risk factors, including abdominal obesity, hypertension, high

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blood glucose, and lipid levels (7), which increase the risk of noncommunicable diseases such as type 2 diabetes and metabolic dysfunction-associated steatotic liver disease (MASLD) [formerly known as nonalcoholic fatty liver disease (NAFLD)] (8). SB has been suggested to be an independent risk factor for metabolic syndrome (9), and according to bed rest studies, SB is associated with reduced lipolysis and significant impairments in whole body insulin sensitivity (10, 11). Conversely, exercise training improves whole body and skeletal muscle insulin sensitivity (12, 13) and body composition (14). However, the effects of SB reduction on liver health, such as tissue-specific insulin sensitivity and liver fat, remain unclear.

In the postprandial state, blood glucose levels increase significantly due to the digestion and absorption of carbohydrates. The liver accounts for 34% of glucose uptake with a moderate glucose load, while muscle and adipose tissues collectively absorb 33%, and the remaining glucose is used mostly by the central nervous system and red blood cells (15). This elevation in blood glucose levels triggers insulin secretion from the pancreatic β cells, which enhances glycogen synthesis and lipogenesis in the liver. Conversely, during the fasting state, insulin levels decline, leading the pancreatic α cells to secrete glucagon. This process stimulates endogenous glucose production (EGP) within the liver to fulfill the energy requirements of the brain and red blood cells in particular. EGP is achieved through the breakdown of stored glycogen (glycogenolysis) and the synthesis of glucose from noncarbohydrate sources (gluconeogenesis) (16). The glucose uptake in the liver is regulated by insulin, which enhances glucokinase activity while inhibiting the expression of glucose-6-phosphatase (17–19). After a meal, the liver plays a crucial role in blood glucose regulation by using it for energy or storing glucose as glycogen, with a capacity of ∼100 g. This stored glycogen is essential for providing glucose to other tissues when needed. In addition, effective glycogen storage helps the brain respond to fluctuations in blood sugar levels, contributing to overall metabolic balance (20, 21). During exercise, the energy demands of the working skeletal muscle increase, which increases EGP in the liver via glycogenolysis and gluconeogenesis to maintain glucose homeostasis (22). On the contrary, after exercise, hepatic tissue is more sensitive to insulin, increasing glucose uptake into the liver (23). In healthy humans, liver glucose uptake (LGU) increases, and EGP decreases during insulin stimulation. However, when glucose tolerance is impaired due to insulin resistance, the hepatic tissue does not respond to insulin, which reduces LGU and increases EGP (17, 18). Thus, decreased LGU and increased EGP directly indicate hepatic insulin resistance during insulin stimulation. Fatty liver is associated with peripheral and hepatic insulin resistance (24), which increases the risk of MASLD. Accumulation of excess fat in the liver increases intracellular diacylglyceride content, interfering with insulin signaling by decreasing hepatic glycogen synthesis and increasing hepatic gluconeogenesis and lipid synthesis (25).

Our prior study involving the same cohort determined that an SB reduction intervention did not significantly influence insulin sensitivity at the whole body level or in the thigh muscles, including the quadriceps and hamstrings, during the intervention phase (26). Nevertheless, we identified beneficial associations between the reductions in SB, changes in moderate-to-vigorous physical activity (MVPA), the number of daily steps, and improved insulin sensitivity. Furthermore, successfully reducing daily sitting led to improved insulin sensitivity in the whole body and the hamstring muscles (26). Previous studies have found that moderate-intensity exercise training could increase LGU and decrease EGP (27, 28). Moderate-intensity exercise training may also decrease liver fat content (LFC) in patients with MASLD (29). However, it is unclear how reducing SB without formal exercise training affects these liver-specific markers (LGU, EGP, and LFC). Finding time-efficient doses of physical activity (PA) that can be adopted and accepted by the general population is essential because lack of time is reported to be one of the main reasons people are not engaging in PA (30). However, reducing SB could perhaps be easier than exercise to incorporate into one's daily routines. Understanding the chronic effects of reducing SB on liver insulin resistance and LFC would also be important because these markers reflect an increased risk of developing metabolic diseases such as type 2 diabetes or MASLD. Our cross-sectional findings suggest that especially body weight reduction could improve LFC (31) and liver enzymes (32), and increasing standing could suppress (improve) EGP (33). However, the effects of long-term SB reduction intervention on these variables are currently unknown.

Consequently, our primary aim in the present study was to examine the effects of a 6-mo randomized, parallel-group intervention aiming to reduce SB by 1 h/day on direct liver insulin sensitivity indicators (LGU, EGP), LFC, and liver enzyme levels in inactive adults with MetS. The SB was replaced with nonexercise activities such as standing, walking, and using stairs. LGU, EGP, and LFC were all measured using golden standard methods, positron emission tomography (PET), and magnetic resonance spectroscopy (MRS). SB and PA were measured with validated accelerometers throughout the study. In addition, we examined the correlations between changes in SB and PA, liver insulin sensitivity and fat content and liver enzymes, daily nutrient and energy intake, body composition, and other cardiometabolic health markers.

MATERIALS AND METHODS

Design of the Study

This 6-mo randomized controlled trial (ClinicalTrials.gov ID NCT03101228) took place at the Turku PET Centre, Turku, Finland, from April 2017 to March 2020. The study followed good clinical practice and the Declaration of Helsinki and was approved by the Ethics Committee of the Hospital District of Southwest Finland (16/1801/2017). All participants provided written informed consent.

Study Participants

In total, 263 individuals volunteered. Of these, 155 participated in the screening measurements, and 64 were included in the hyperinsulinemic-euglycemic clamp (HEC) analysis (34). Of these, 44 were included in this imaging study with PET combined with HEC and MRS (Fig. 1, study flow

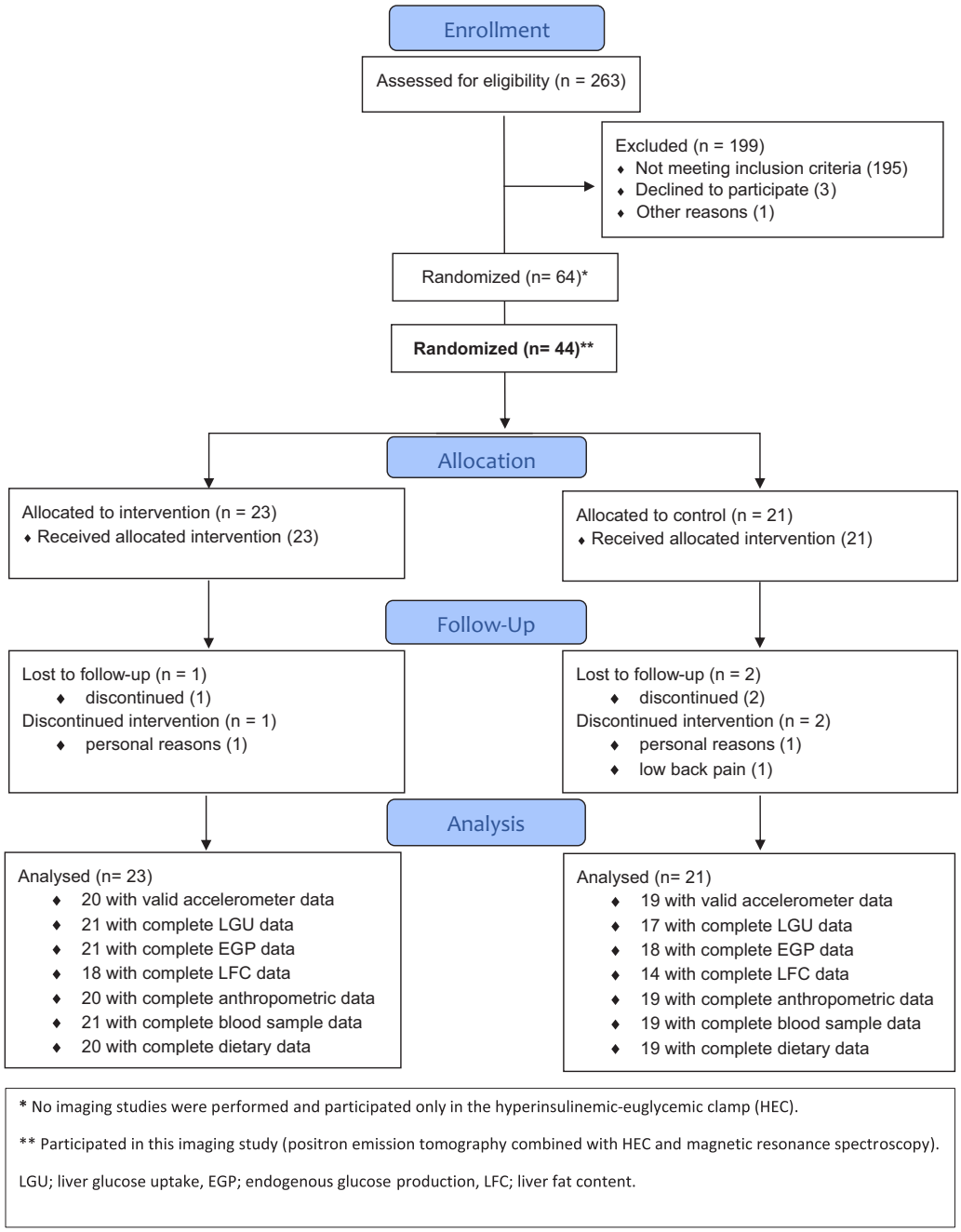

Figure 1. Study flow diagram.

diagram). Participants were sedentary middle-aged adults with MetS (7). Inclusion criteria were as follows: aged 40 to 65 yr, with a body mass index (BMI) ranging from 25 to 40 kg/m^2. Eligible participants must be physically inactive, defined as engaging in less than 120 min of moderate-intensity exercise per week, as reported during telephone screenings and initial physical activity questionnaires. In addition, individuals must demonstrate a sitting time of at least 10 h per day or 60% of the total accelerometer wear time, as measured during screening. Blood pressure values must be below 160/100 mmHg, and fasting plasma glucose levels must be below 7.0 mmol/L. Furthermore, participants must meet at least three of the metabolic syndrome criteria, which include: central obesity (waist circumference of 94 cm or greater for men and 80 cm or greater for women); blood triglycerides of 1.7 mmol/L or greater; HDL cholesterol levels below 1.0 mmol/L for men and below 1.3 mmol/L for women; systolic blood pressure of 130 mmHg or greater and/or diastolic blood pressure of 85 mmHg or greater; and fasting glucose levels exceeding 5.6 mmol/L. Exclusion criteria comprise any history of cardiovascular events, individuals who are insulin-dependent or receiving medical treatment for diabetes, and those with any chronic disease or condition that may compromise the subject's safety, threaten the integrity of the study procedures, or interfere with the interpretation of the study results. Participants with excessive

alcohol consumption, as defined by national guidelines, and those who use narcotics, smoke tobacco, or consume snuff tobacco were also excluded. In addition, individuals with a history of previous positron emission tomography (PET) scans or significant prior exposure to radiation, and those diagnosed with depressive or bipolar disorder did not qualify for participation in this study.

Intervention

The study involved a 1-mo screening phase, followed by a 6-mo intervention period. After the screening phase, participants who satisfied the inclusion criteria were randomly allocated (1:1) into intervention (INT; $n = 23$) and control (CON; $n = 21$) groups using randomized permuted block randomization with stratification for sex, facilitated by a statistician, as previously described (26). A comprehensive account of the intervention has been previously documented (34). Briefly, baseline PA and SB levels were assessed during the 1-mo screening phase. The INT group received instructions to decrease their SB by 1 h/day compared with their individual baseline and to substitute it with nonexercise activities, such as standing, light physical activity (LPA), or moderate-to-vigorous physical activity (MVPA). Conversely, the CON group was advised to uphold their baseline SB and PA. Each participant was provided with tailored guidance and recommendations by the researcher to attain the intervention objectives, with both groups using a specific mobile phone application (ExSed, https://www.exsed.com, UKK Terveyspalvelut Oy, Tampere, Finland) for daily activity monitoring (35).

Sedentary Behavior and Physical Activity

SB, encompassing sitting and lying, and PA were assessed using tri-axial accelerometers worn in the hip. The initial measurement phase spanning 1 mo used UKK AM30 accelerometers (UKK-Institute, Tampere, Finland), followed by continuous monitoring throughout the entire 6-mo intervention period using Movesense accelerometers (Suunto, Vantaa, Finland), as previously reported (34). Data collected from the accelerometers was scrutinized at 6-s epochs (36), with SB, standing, LPA, MVPA, steps taken, and breaks in SB being ascertained through the mean amplitude deviation (MAD) (37) and angle for posture estimation (APE) methodologies (38). Daily averages for SB, breaks in SB, standing, daily steps, LPA, and MVPA were computed. As previously described (26), to account for temporal discrepancies in wear time, SB, standing, LPA, and MVPA were also gauged as percentages in relation to the daily accelerometer wear time. Participants were mandated to adhere to a daily wear-time criterion of 10–19 h and ensure a minimum of four valid measurement days for the collected data to be deemed valid. Any surplus measurement time exceeding 19 h/day was subtracted from the SB timeframe.

Whole Body Insulin Sensitivity

The HEC was performed after at least 10 h of fasting, as previously described (34). A primed-constant insulin infusion using Actrapid (100 U/mL, Novo Nordisk, Bagsvaerd, Denmark) was initiated at a rate of 160 mU/min/m^2 of the participant's body surface area for the first 4 min. This infusion rate was then reduced to 80 mU/m^2/min from 4 to 7 min and maintained at a constant rate of 40 mU/m^2/min from 7 min until the end of the clamp. An exogenous 20% glucose infusion began 4 min after the insulin infusion started at a rate of 0.5 mL/h per kilogram of the participant's body weight. At the 10-min mark, the glucose infusion rate was doubled and subsequently adjusted based on blood glucose concentration to maintain a target level close to 5 mmol/L. Arterialized venous blood samples were collected every 5 min during the first 30 min and then every 10 min at a steady state to determine glucose concentration and adjust the glucose infusion rate accordingly. The whole body insulin-stimulated glucose uptake rate was calculated in 20-min intervals using the steady-state glucose values and the glucose infusion rate, starting from 20 min after the hyperinsulinemic-euglycemic clamp began. The outcome, referred to as the M-value, indicates whole body glucose uptake in mg/kg/min. The equation for the calculation of the M-value is $M = 1/p \times \Sigma_i t_i \times [\Sigma_i(t_i \times v_i \times 1/300) + (G1 - G2) \times 0.19 \times p \times 180]$, in which p = weight of the subject (kg), $\Sigma_i t_i$ = time interval (min), t_i = interval (min), v_i = glucose infusion rate during the interval (mL/min), $G1$ = glucose concentration at beginning of the interval (mmol/L), $G2$ = glucose concentration at end of the interval (mmol/L), 0.19 = glucose distribution volume (l/kg), and 180 = glucose molar mass (mg/mmol).

Liver Glucose Uptake

During HEC, LGU was measured using 2-deoxy-2-[18F] fluoro-D-glucose ([^{18}F]FDG) positron emission tomography (PET) imaging with a PET/computed tomography (CT) scanner (GE D690, GE Healthcare, Milwaukee, WI), as previously described (33). The radiotracer [^{18}F]FDG (39) was produced, and the uptake of [^{18}F]FDG was analyzed according to previously established methods (40). 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 left ventricle of the heart during the first 40 min of PET imaging and from blood samples collected at ~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.

^{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). LGU (μmol/mL/min) was calculated by multiplying the tissue fractional phosphorylation rate (K_i) by the average plasma glucose concentration during scanning. The liver's lumped constant (LC) is 1.0 (41). 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 in., Wallac, Turku, Finland).

Endogenous Glucose Production

EGP was determined by calculating the difference between the exogenous glucose infusion rate, adjusted for space

correction (42), and the glucose rate of disappearance during the HEC (43), as previously described (33).

Liver Fat Content

LFC was measured using magnetic resonance spectroscopy (MRS) with the two-point Dixon (2PD) method, as previously described (31, 33). Initially, measurements were conducted on a Philips 3 Tesla system (Ingenuity TF PET/MR) equipped with a Q-Body coil. However, due to the replacement of the MRI scanner during the study, LFC quantification for seven participants was later performed using a Siemens Magnetom Skyra Fit 3T MRI system (Siemens Healthcare, Erlangen, Germany), which was equipped with Siemens Body 30 and 18-channel coils, and a 32-channel spine coil. It is important to note that pre- and postmeasurements for each participant were always conducted using the same scanner, ensuring that any observed changes in LFC during the intervention were accurately represented.

The spectra were acquired using stimulated echo acquisition mode (STEAM) 1H MRS with the following parameters: repetition time (TR)/echo time (TE)/mixing time (TM) = 2,000/11/17 ms; four averages; 2,048 samples; spectral bandwidth of 2,000 Hz; and an acquisition volume of 20 × 20 × 30 mm^3. Data acquisition occurred during 12 breath holds. Water saturation was achieved using chemical shift selective (CHESS) techniques with a bandwidth of 50 Hz. The total duration of the scan was 3 min and 12 s. In addition, a three-dimensional (3-D) T1-fast field echo sequence was acquired in the axial plane with parameters: TR/TE1/TE2 = 2.8/0.81/1.8 ms, a flip angle of 10°, a field of view of FOV 510 mm × 510 mm, and an imaging matrix of 188 × 188. The data was reconstructed to a voxel size of 2.13 × 2.13 × 4 mm^3. Respiratory gating was applied in the thorax and upper abdomen region. LFC quantification was further conducted with the Siemens Magnetom Skyra Fit 3T MRI system, using Siemens Body 30 and 18-channel coils, and a 32-channel spine coil. The spectra were acquired using point-resolved spectroscopy (PRESS) 1H MRS, with parameters of TR/TE = 4,000/30 ms; 32 averages; 1,024 samples; and an acquisition volume of 20 × 20 × 20 mm^3. Respiratory motion was managed using a navigator. Water saturation was conducted with a bandwidth of 35 Hz, and the duration of the scan was 3 min and 10 s. A 3-D gradient echo volumetric interpolated breath-hold examination (VIBE Dixon) sequence was also acquired in the axial plane, with parameters: TR/TE1/TE2 = 3.97/1.23/2.46, a flip angle of 9°, and a voxel size of 2 × 2 × 2 mm^3. Breath holds were used in the thorax and upper abdomen area, and controlled aliasing in parallel imaging results in higher acceleration (CAIPIRINHA) was also applied. Water signal and fat signal images were used to calculate the fat fraction map (44), which was then used to determine the LFC, with an MRI image serving as an anatomical reference.

The LC Model (v.6.3-0 C) was used to quantify liver fat, using "liver-4" as the spectrum type. Lipid signals at 1.6 ppm, 1.3 ppm, and 0.9 ppm were included in the analysis. Adjustments for fat and water signals were made due to differences in T2 decay and the molar concentrations of 1H nuclei in fat and water, as previously reported (45, 46). Liver fat content was defined as the amount of fat relative to the total weight of the liver tissue (47). MRI images were analyzed using Carimas software version 2.10. Four representative three-dimensional regions of interest (ROIs) were manually delineated on liver sections (left lateral and medial sections, right anterior and posterior sections), carefully avoiding the main portal veins. The results were volume corrected using the formula: mean value of one section × [total volume (mm^3) of one section/total volume of all sections].

Blood Sampling

Venous blood samples were collected after a fasting period of at least 10 h. Plasma glucose levels were measured using an enzymatic reference method with hexokinase (GLUC3), whereas plasma insulin levels were determined using an electrochemiluminescence immunoassay (Cobas 8000 e801, Roche Diagnostics GmbH, Mannheim, Germany). Hemoglobin A1c (HbA1c) was assessed through turbidimetric inhibition immunoassay (Cobas 6000 c501, Roche Diagnostics GmbH, Mannheim, Germany). In addition, plasma triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were analyzed using enzymatic colorimetric tests (Cobas 8000 c702, Roche Diagnostics GmbH, Mannheim, Germany). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured by the photometric International Federation of Clinical Chemistry (IFCC) method using Cobas 8000 c702 and c502 analyzers (Roche Diagnostics GmbH, Mannheim, Germany). Finally, γ-glutamyltransferase (GGT) was determined through enzymatic colorimetric tests (Cobas 8000 c702, Roche Diagnostics GmbH, Mannheim, Germany). All samples were analyzed at the Turku University Hospital Laboratory.

Body Composition and Anthropometry

A validated (48) air displacement plethysmography system with predicted thoracic gas volume, specifically the Bod Pod (COSMED, Inc., Concord, CA), was used to estimate body composition, including body fat percentage after participants fasted for at least 4 h. On the day of measurement, participants were instructed not to exercise or shower beforehand. After emptying their bladders, they entered the measurement chamber wearing a tight cap and either underwear or a swimsuit. Body weight was measured using a scale (Seca 797, Vogel & Halke, Hamburg, Germany) while participants were dressed in light clothing. Their height was recorded barefoot with a wall-mounted stadiometer. Body mass index (BMI) was calculated using the measured weight and height (kg/m^2). Waist circumference was measured with a flexible tape measure positioned midway between the iliac crest and the lowest rib, and the measurement was repeated twice or until two consistent readings were obtained. All anthropometric measurements were taken under standardized conditions, and to ensure consistency, they were performed by the same researcher throughout the study.

Dietary Intake

The study instructed participants to maintain their regular dietary patterns throughout the intervention. The daily

energy intake (EI) of the participants and their consumption levels of various nutrients including fat, protein, carbohydrates (CHO), saccharose, fiber, water-insoluble dietary fiber (WIDF), alcohol, and fatty acids [saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA)] were determined through the collection of 4-day food diaries, which encompassed at least one weekend day. These intake levels were assessed at the commencement and the conclusion of the intervention phase. A nutritionist used computerized software (AivoDiet, Aivo, Turku) based on the Finnish Food Composition Database, Fineli (49), to analyze and calculate the dietary data.

Statistical Methods

Multilevel models for repeated measures were used to analyze the effects of the intervention. In all the models, within-subject factors were sex and time, group was a between-subject factor, and group-by-time interaction (group × time) was used to evaluate whether mean changes over time differed between the groups. We used the Tukey–Kramer method to adjust for multiple comparisons. An unstructured covariance structure or compound symmetry was used for a time, and the model with lower Akaike information criterion (AIC) values was chosen. 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. This analytical approach enables a comprehensive understanding of the strength and direction of associations among changes in the variables during the intervention, thus contributing valuable insights into the interconnections between these changes. INT and CON groups, sexes, and changes during the intervention between sexes were compared with an unpaired t test. Power calculations were made for skeletal muscle insulin sensitivity as the outcome (26). We visually assessed the normal distribution of the residuals with the quantile-quantile (Q-Q) plot. When necessary to fulfill the normal distribution assumption, we used logarithmic transformations, and after the analysis, we back-transformed the values to the original scale (estimates then being geometric means). Missing data were handled by pairwise deletion. The data are presented in terms of mean, standard deviation (SD), and 95% confidence interval (CI) values. A significance level of 5% (two-tailed) was used for all statistical analyses. Correlation analyses were conducted using JMP Pro 16 for Windows (SAS Institute Inc., Cary, NC), whereas other analyses were performed with SAS 9.4 (SAS Institute Inc., Cary, NC). Figures were generated using GraphPad Prism 5.01 (GraphPad Software, San Diego, CA).

Exploratory Analysis

Due to the considerable variation of changes in SB in both groups (see Supplemental Fig. S1 for the change in measured SB for each participant), we conducted an evaluation of the individual changes in SB as hours/day recorded by the accelerometer. We divided all participants who had valid accelerometer data ($n = 39$) into two groups based on whether they *1*) reduced SB ($n = 22$, mean 52 min/day reduction in SB) or *2*) maintained or increased SB ($n = 17$, mean 34 min/day increase in SB).

Finally, it has recently been proposed to lower the upper limit of the LFC to define MASLD from 5% to 1.85% because an LFC of 1.85%–5.56% was associated with reduced insulin sensitivity and increased cardiometabolic risk factors compared with people with an LFC of less than 1.85% (50). Therefore, we also assessed whether the study could result in different outcomes in participants with LFC higher ($n = 19$) or lower ($n = 21$) than 1.85% at the baseline.

RESULTS

Table 1 presents the baseline characteristics of the participants in the INT and CON groups. INT group had 3 participants, and the CON group had 8 participants that met the clinical criteria for MASLD (LFC ≥ 5%) (51), and 29 had normal LFC (<5%) (see Supplemental Fig. S2 for baseline LFC per participant). Twenty-four participants had medication for elevated blood pressure (INT $n = 12$, CON $n = 12$), and nine had medication for elevated blood cholesterol (INT $n = 7$, CON $n = 2$). In addition, seven participants reported using hormonal replacement therapy medication (INT $n = 5$, CON $n = 2$), five using pain medication (INT $n = 3$, CON $n = 2$), five using anticoagulants (INT $n = 4$, CON $n = 1$), four using thyroid medication (INT $n = 3$, CON $n = 1$), four using gastrointestinal medication (INT $n = 3$, CON $n = 1$), four using allergy or asthma medication (INT $n = 2$, CON $n = 2$), three using antidepressants (INT $n = 3$), three using sleep medication (INT $n = 2$, CON $n = 1$), two using medication for urinary problems (CON $n = 2$), one using osteoarthritis medication (INT $n = 1$), and one using medication for restless legs syndrome (INT $n = 1$).

Accelerometry

The accelerometry results have been previously described (26). Briefly, in the INT group, SB decreased by an average of 51 min per day (95% CI from 22 to 78), whereas MVPA increased by 22 min per day (95% CI from 12 to 33) when compared with CON group (group × time, $P = 0.02$ and $P = 0.01$, respectively). During the intervention, all participants increased their SB breaks by three per day and spent 11 min more engaged in LPA daily. There were no differences in these changes between the different groups. The mean step count increased by ∼3,200 steps (95% CI: 2,120–4,192) in the INT group and by ∼1,700 steps (95% CI: 580–2,790) in the CON group. The difference between the groups in step count was found to be significant (group × time, $P = 0.009$) (26).

Intervention Effects on Liver Insulin Sensitivity and Other Liver Health Markers

The intervention had no statistically significant effect: the changes in liver health markers (LGU, EGP, LFC, ALT, AST, or GGT) were not different between the INT and CON groups (group × time $P > 0.05$) (Fig. 2, *A*–*F*). However, LGU and AST increased in both groups during the intervention (time; $P < 0.001$ and $P = 0.02$, respectively). Compared with baseline values, LGU increased by 94% and AST by 10% in the INT group and by 46% and 11% in the CON group, respectively, but the increases were not statistically significantly different between groups (Fig. 2, *A* and *E*). In addition, sex was a statistically significant factor in the models analyzing the

Table 1. *Baseline characteristics of the participants in the INT and CON groups*

	INT	CON	*P*
n#	23	21	
Women, *n* (%)*#	14 (61)	11 (52)	0.76
Age, yr#	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
Waist circumference, 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
f Triglycerides, mmol/L**#	1.4 (1.0, 1.7)	1.1 (0.8, 1.5)	0.06
f Cholesterol, mmol/L#	4.8 (1.2)	4.5 (0.8)	0.39
f 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 sedentary time, *n*/day#	29 (8)	28 (8)	0.79
Steps, *n*/day#	5,272 (2017)	4,860 (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. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CON, control; EGP, endogenous glucose production; f, fasting; GGT, γ-glutamyltransferase; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; INT, intervention; LDL-C, low-density lipoprotein cholesterol; LFC, liver fat content; LGU, liver glucose uptake; LPA, light physical activity; *M*-value, whole body insulin sensitivity; MVPA, moderate-to-vigorous physical activity; NEFA, nonesterified fatty acids. *Median (Q1, Q3); **statistical testing was performed with log10-transformed estimates; #results published previously by Sjöros et al. (26).

intervention effects on LFC ($P = 0.007$) and AST values ($P = 0.01$), which can be explained by the differences in the baseline values between men and women. Men had higher LFC (mean 5.1 [SD 3.8] %) and AST levels (mean 31 [SD 12] U/L) compared with women (mean 2.6 [SD 3.0] %, mean 23 [SD 5] U/L) ($P = 0.03$, $P = 0.01$, respectively). No statistically significant differences between sexes were found in the changes in LFC or AST levels during the intervention ($P = 0.26$, $P = 0.70$, respectively).

Intervention Effects on Cardiometabolic Health Markers

Both groups experienced a statistically significant decrease in BMI, waist circumference, body fat percentage, waist circumference, 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. 3, *A*–*E*). The intervention did not significantly affect fat-free mass (Fig. 3*F*) or the general insulin sensitivity markers: fasting glucose, fasting insulin, and HbA1c (see Supplemental Fig. S3, *A*–*C*). Fasting triglycerides between-group difference was significant (group, $P = 0.04$), but the overall intervention effect was nonsignificant (group × time, $P = 0.95$) (Supplemental Fig. S4*A*). The intervention did not significantly affect other lipid markers: fasting nonesterified fatty acids (NEFA), fasting cholesterol, HDL-C, or LDL-C (Supplemental Fig. S4, *B*–*E*). Changes in weight and body composition were not different between sexes, but sex was a statistically significant factor in the models because women had more adiposity and men had a higher body mass (data not shown).

Correlations between Changes during the Intervention

In addition, we examined the interrelationships among various changes observed during the intervention across all study participants. Notably, we found a positive correlation between the changes in HDL-C and changes in LGU, suggesting that increased HDL-C was associated with improved LGU levels. Conversely, changes in whole body insulin sensitivity (Δ*M*-value) were negatively associated with the changes in EGP, indicating that as whole body insulin sensitivity increased, EGP tended to decrease (improve) (Table 2).

Furthermore, we observed that changes in LFC were positively correlated with changes in fasting insulin levels and changes in HbA1c (Table 3). These results suggest that as LFC rises, corresponding increases in fasting insulin are seen as a marker of insulin resistance, and higher HbA1c levels indicate poor long-term blood sugar control (Table 2). The data also revealed that changes in ALT levels were positively associated with the changes in the amount of time spent in SB (h/day and %/day). In contrast, changes in ALT levels exhibited a negative correlation with changes in standing time, indicating that more time spent in SB was related to higher ALT levels. In addition, changes in AST were positively correlated with the changes in the breaks taken from SB (Table 3). There was no correlation between the change in LFC and the changes in LGU ($r = 0.09$, $P = 0.61$) or EGP ($r = 0.02$, $P = 0.90$).

As for dietary intake, changes in ALT showed positive associations with changes in CHO (g/day and % of daily EI), and changes in AST also exhibited a positive correlation with changes in CHO (% of daily EI). These results indicate that higher daily CHO intake is associated with higher ALT and AST values. The changes in GGT were negatively correlated with changes in protein intake (g/day and % of daily EI), indicating that higher daily protein intake is associated with lower GGT values. Furthermore, changes in saccharose intake also correlated positively with changes in ALT, AST, and GGT (g/day and % of daily EI), indicating that higher daily saccharose intake is associated with higher liver enzyme values (Table 4).

Exploratory Analyses

In those participants who successfully reduced SB, ALT decreased (−1.1 [95% CI: 0.93, 1.36] U/L) compared with the continuously sedentary participants (+0.8 [95% CI: 0.65, 1.05] U/L) (group × time, $P = 0.006$) (Fig. 4*A*). No other group × time effects on liver health markers were observed in the analyses comparing the groups based on measured SB change (data not shown).

The participants whose LFC was ≥1.85% at baseline significantly reduced their LFC (−1.3 [95% CI: 0.95, 1.87]%) when

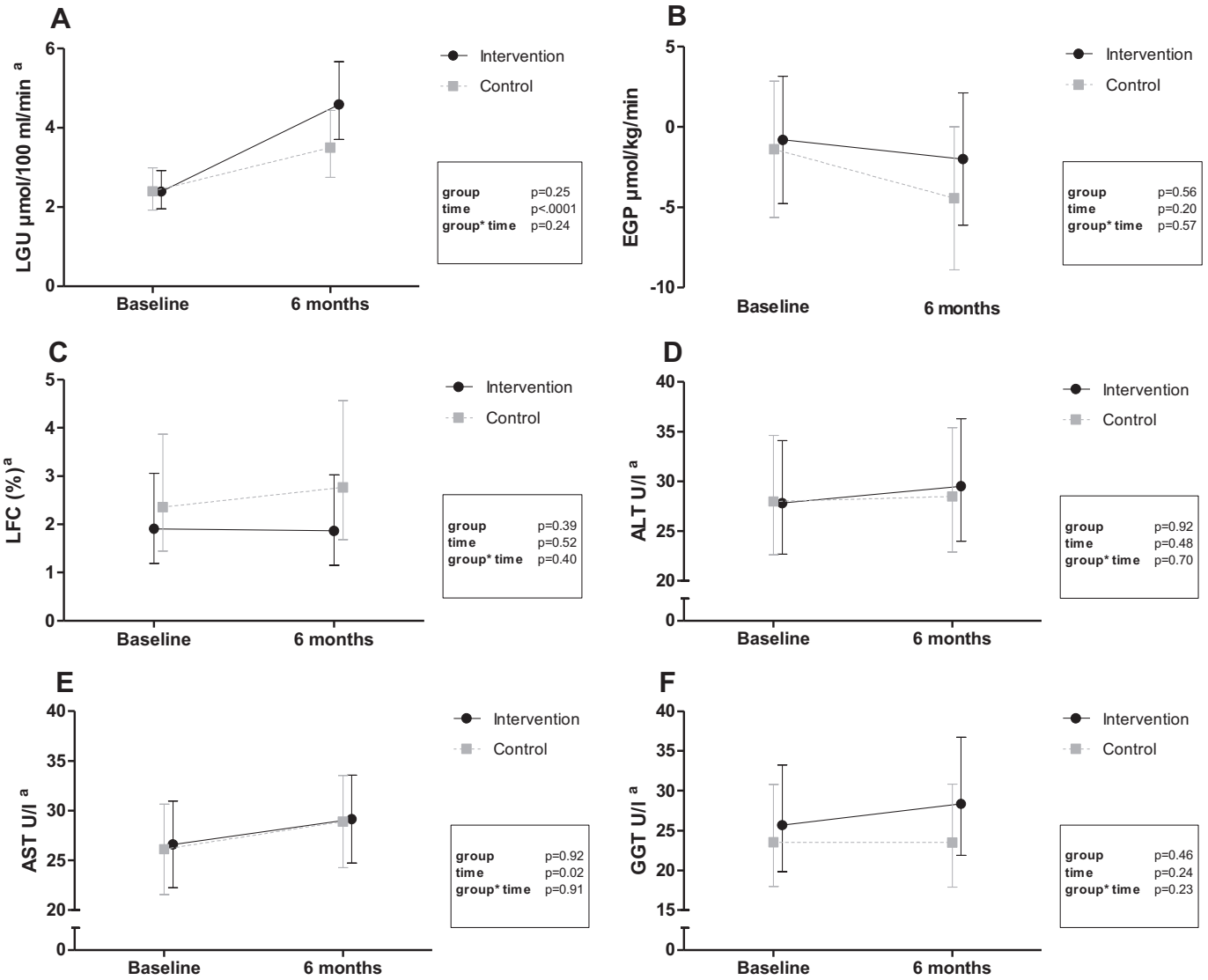

Figure 2. Liver health marker results of the intervention (black line) and control (gray line) groups at baseline and after 6 mo of liver glucose uptake (LGU, *A*); endogenous glucose production (EGP, *B*); liver fat content (LFC, *C*); alanine aminotransferase (ALT, *D*); aspartate aminotransferase (AST, *E*); and γ-glutamyltransferase (GGT, *F*). The values are presented as model-based means (95% CI), [a]log10 transformed (means are back-transformed geometric model-based means [95% CI]).

compared with the participants whose LFC was ≤1.85% at baseline (+0.7 [95% CI: 0.49, 1.00]) % (group × time, P = 0.001) (Fig. 4*B*).

DISCUSSION

Our current study indicates that a 6-mo intervention aimed at reducing SB by 1 h/day did not yield significant effects on the measured liver function markers. These findings are consistent with our previous study involving the same population, which showed that the SB reduction intervention did not significantly affect whole body or thigh muscle insulin sensitivity (26). Our results suggest that to achieve notable improvements in liver health, a longer duration of SB reduction or higher intensity of PA may be necessary. However, our exploratory analyses demonstrated that participants who successfully reduced SB decreased ALT levels compared with continuously sedentary participants. Furthermore, we found a positive correlation between the change in ALT over the 6-mo period and the change in SB, whereas there was an inverse correlation with standing time across all participants. These results suggest that achieving the intended behavior change may improve ALT levels. In addition, we found that LGU, thus a direct measure of liver insulin sensitivity, increased significantly in both groups during the intervention period, which may be explained by increased daily steps and improved body composition in both groups. Furthermore, the changes during the intervention in ALT, AST, and GGT were all positively associated with the changes in daily saccharose intake, although participants were advised against modifying their dietary habits during the study. In addition, the changes in ALT and AST were positively associated with changes in daily CHO, and the changes in GGT were negatively associated

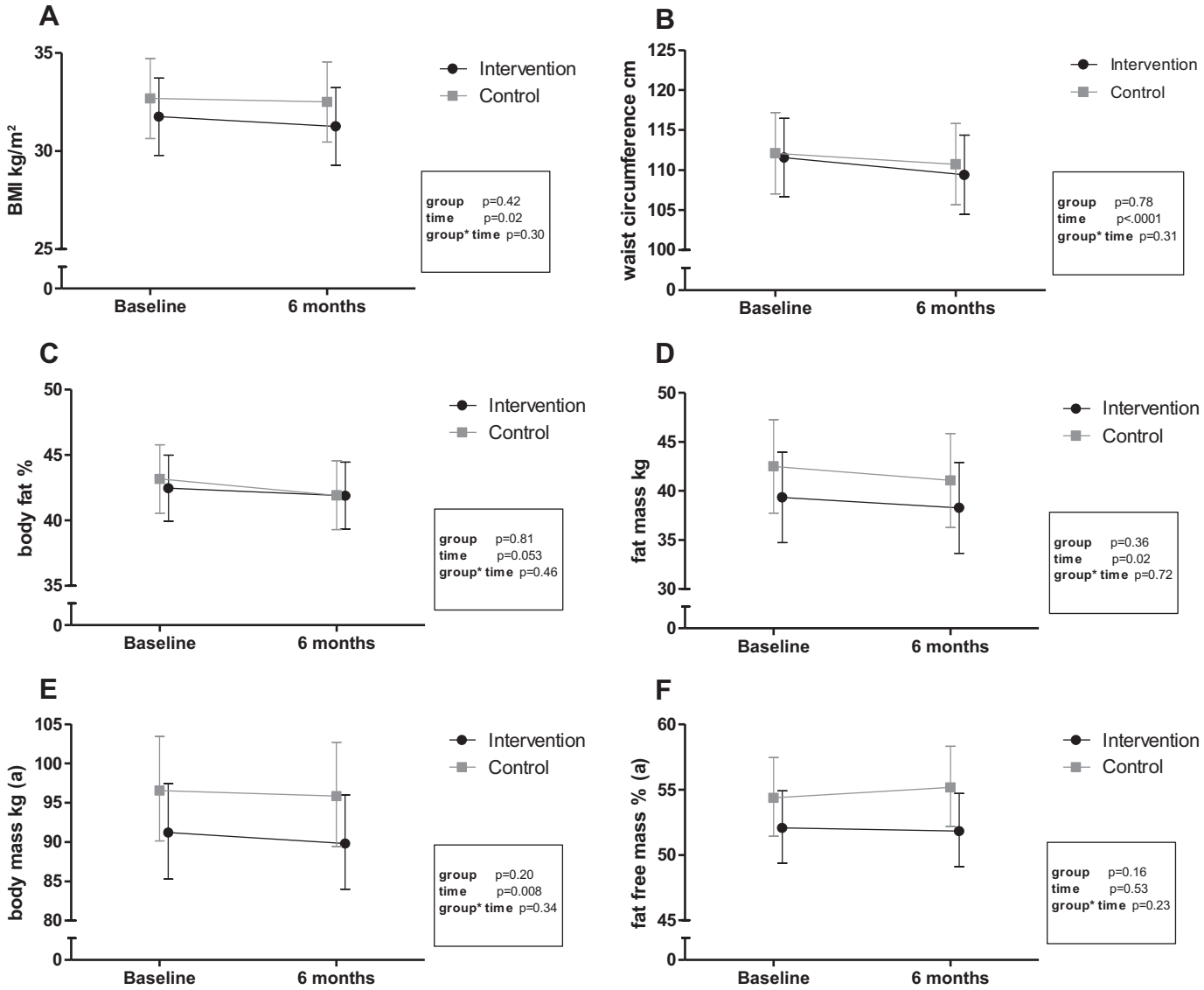

Figure 3. Anthropometric results of the intervention (black line) and control (gray line) groups at baseline and after 6 mo of body mass index (BMI, *A*); waist circumference (*B*); body fat percentage (*C*); fat mass (*D*); fat-free mass (*E*); and body mass (*F*). The values are presented as model-based mean (95% CI), [a]log10 transformed (means are back-transformed geometric model-based means [95% CI]).

with changes in daily protein intake. Thus, our results suggest that replacing some of the daily CHO, especially sugar, with protein sources may benefit liver health. However, all these associations between the changes during the intervention must be confirmed with nutritional intervention studies.

Liver Glucose Uptake

After 6 mo, we found no significant changes in LGU between the two study groups, even though fasting insulin was reduced by this SB intervention, indicating improvements in glucose homeostasis at the whole body level (34). However, we observed increases in LGU from pre- to post-measurements in both INT and CON groups, which indicate improved liver insulin sensitivity. When comparing two different training methods, 2 wk of moderate-intensity continuous training, but not sprint interval training, was found to increase insulin-stimulated LGU in adults with normoglycemia or prediabetes/type 2 diabetes (27). In addition, it has been shown that after 4 mo of resistance training, there were no changes in LGU in older women (52). In our study, both the intervention (INT) and control (CON) groups increased their daily steps and improved their body composition. These improvements likely contributed to increased LGU seen also in both groups. The increase in daily steps may have led to better overall body composition, which could enhance LGU. This enhanced LGU, but unchanged LFC may indicate that the LGU can be improved first before the LFC is effectively reduced. However, this hypothesis requires additional evidence for a clearer understanding, as there was no correlation between the change in LGU and LFC in the present study. Nevertheless, previous cross-sectional research indicates that LGU displays an inverse correlation with LFC in adults with type 2 diabetes with high LFC (53). This finding

Table 2. *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, µmol/ 100 mL/min	ΔEGP, µmol/kg/min	ΔLFC, %	ΔALT, U/L	ΔAST, U/L	ΔGGT, U/L
ΔBMI, kg/m^2	0.04	0.09	0.24	0.05	−0.13	−0.23
ΔWaist, cm	0.09	0.16	0.13	0.25	0.008	−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
Δ*M*-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	0.004	−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***	−0.0002	0.003	−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	−0.007	0.18	0.08
ΔCholesterol, mmol/L	0.21	0.13	0.04	−0.01	0.08	0.21
ΔHDL, mmol/L	**0.43****	0.37	0.03	0.15	0.01	0.08
ΔLDL, mmol/L	0.06	0.03	−0.09	−0.06	0.13	0.18

Δ, change from preintervention to postintervention; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; EGP, endogenous glucose production; f, fasting; GGT, γ-glutamyltransferase; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LFC, liver fat content; LGU, liver glucose uptake; *M*-value, whole body insulin sensitivity; NEFA, nonesterified fatty acids. Significant *P* values; *$P < 0.05$, **$P < 0.01$. Significant values are in bold.

implies that an increase in LGU may be linked to a decrease in liver fat levels, suggesting a potential mechanism for improved metabolic health, although it may also be that higher LFC leads to impaired LGU. However, prior studies have demonstrated that adults with type 2 diabetes who exhibit elevated levels of liver fat have decreased LGU (54). This relationship points to possible disruptions in metabolic processes that may be contributing to the complications associated with type 2 diabetes.

In addition, research has focused on the activation of glucokinase, an enzyme critical for increasing LGU (18). In the context of our results, it is noteworthy that oral administration of a hepatoselective glucokinase activator in the study of Vella et al. (55) led to improvements in glycemic control without affecting triglyceride levels within the liver. This is in contrast with the effects of nonselective glucokinase activation by drugs or genetic mutations, which also activate pancreatic glucokinase, causing enhanced insulin secretion, hyperinsulinemia, and subsequent activation of hepatic lipogenesis (55). In our study, the change in fasting insulin and HbA1c were positively associated with the change in LFC. Thus, it is possible that the improved hepatic insulin sensitivity and glycemic control after 6 mo helped to control LFC by reducing insulin levels and consequently hepatic lipogenesis.

In addition, we found that the changes in LGU during the intervention in both groups were associated positively with the changes in HDL-cholesterol. Previous evidence suggests that HDL may impact glucose metabolism in multiple organs by distinct mechanisms related to insulin secretion, insulin-independent glucose uptake, and insulin sensitivity (56). To the best of our knowledge, there are no studies concerning the effects of HDL on insulin-stimulated LGU in humans. However, raising HDL by inhibiting cholesterol ester transfer protein in animal studies significantly increased LGU in dyslipidemic and insulin-resistant hamsters (57). Thus, an increase in HDL levels may also improve LGU in humans. However, this needs to be confirmed with specific intervention studies.

Endogenous Glucose Production

We found no statistically significantly different changes in EGP between the groups or time effect either. One study (28) found that 6 wk of moderate-to-vigorous exercise (60 to 85% of maximal aerobic capacity) three times per week for at least 20 min decreased EGP and increased whole body insulin

Table 3. *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, µmol/ 100 mL/min	ΔEGP, µmol/kg/min	ΔLFC, %	ΔALT, U/L	ΔAST, U/L	ΔGGT, U/L
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 in SB, 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

Δ, change from preintervention to postintervention; ALT, alanine aminotransferase; AST, aspartate aminotransferase; EGP, endogenous glucose production; GGT, γ-glutamyltransferase; LFC, liver fat content; LGU, liver glucose uptake; LPA, light physical activity, MVPA, moderate-to-vigorous physical activity; SB, sedentary behavior. Significant *P* value; *$P < 0.05$. Significant values are in bold.

Table 4. *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, µmol/ 100 mL/min	ΔEGP, µmol/kg/min	ΔLFC, %	ΔALT, U/L	ΔAST, U/L	ΔGGT, U/L
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, % of daily EI	−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, % of daily EI	−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, % of daily EI	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, % of daily EI	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, % of daily EI	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, % of daily EI	−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, % of daily EI	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, % of daily EI	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

Δ, change from preintervention to postintervention; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHO, carbohydrates; EGP, endogenous glucose production; EI, energy intake; GGT, γ-glutamyltransferase; LFC, liver fat content; LGU, liver glucose uptake; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; WIDF, water-insoluble dietary fiber. Significant *P* values; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Significant values are in bold.

sensitivity in sedentary males. The authors suggested that these improvements in hepatic and peripheral glucose uptake result from decreased circulating NEFA concentrations due to increased insulin sensitivity of lipolysis (28). Our study did not find any significant changes in NEFA or *M*-value, which could mean that to improve EGP and whole body insulin sensitivity, the intensity and duration of PA should be higher. However, previous intervention studies

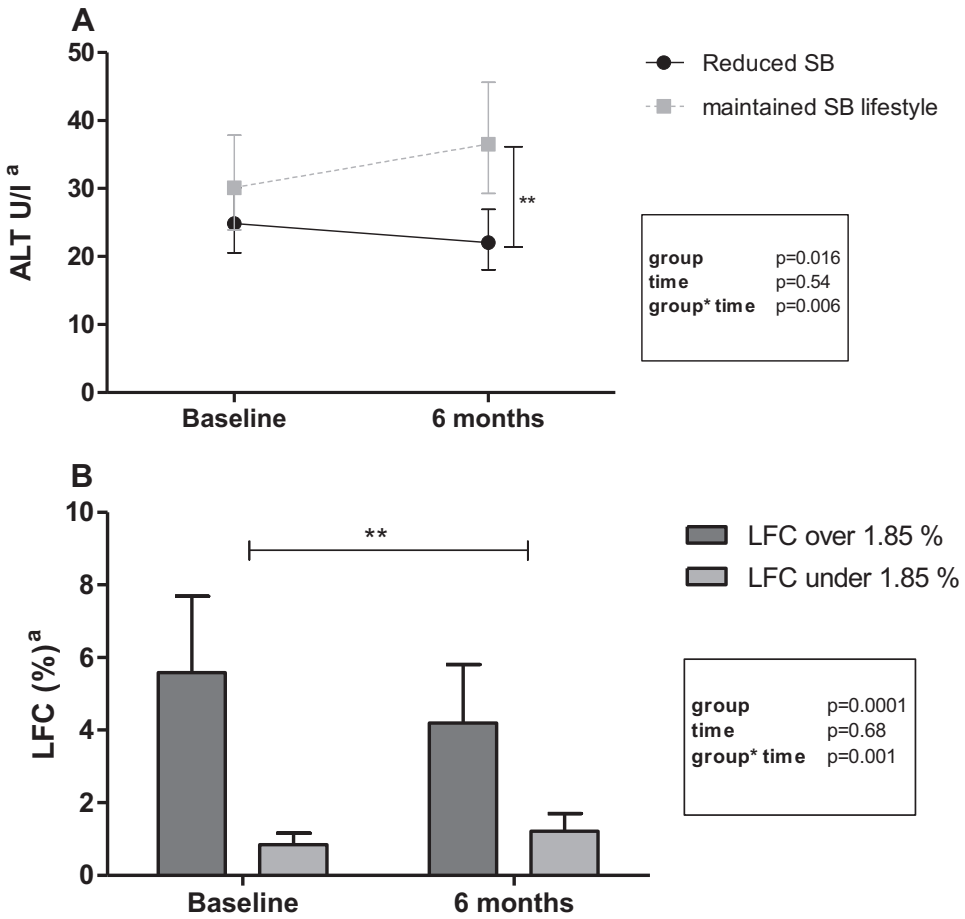

Figure 4. Exploratory analyses results on alanine aminotransferase (ALT, *A*) levels among all participants based on whether they reduced sedentary behavior (SB) (black line) or maintained SB lifestyle (gray line), liver fat content (LFC, *B*) levels among all participants based on whether they had higher (over 1.85%) LFC (black line) or lower (under 1.85%) LFC (gray line) at baseline. The values are presented as model-based means (95% CI), [a]log10 transformed (means are back-transformed geometric model-based means [95% CI]). Significance level; **$P < 0.01$.

have found beneficial effects on EGP from moderate-intensity resistance training and treadmill walking (52, 58). Our previous cross-sectional study with the same study population found that EGP was negatively associated with daily standing time (33). However, as previously reported, there were no significant differences between the groups in daily standing time (26), which could explain why we did not find any significant effects of standing in EGP. Thus, it is still plausible that LPA, such as standing, might also have beneficial effects on EGP. In addition, we found in the present study that the changes in EGP during the intervention in both groups were associated negatively with the changes in whole body insulin sensitivity, which is plausible as the increase in EGP in insulin stimulation is closely associated with insulin resistance (59).

Liver Fat Content

During the 6-mo intervention, we found no statistically significantly different LFC changes between the groups or time effects in LFC. Previously, it has been shown that a supervised progressive, moderate aerobic exercise program (three cycle ergometer sessions, 30–45 min/wk) significantly reduced MRS-measured LFC without weight loss when compared with the placebo group in sedentary adults with obesity. The aforementioned study lasted for 4 wk, and the mean baseline LFC was over 5% (limit to diagnose MASLD) in both intervention and placebo groups (60).

In our study, the baseline mean LFC in both INT and CON groups was within the normal range (lower than 5%), which thus may have complicated finding any significant changes in LFC. In the INT group, three participants had MASLD, and they all managed to decrease LFC to normal or near the normal range during the intervention (baseline mean LFC 9.8%, after 6 mo, mean LFC 4.1%). The mean SB reduction during the intervention was ∼50 min/day. This suggests that individuals with MASLD may benefit from reducing SB. However, due to the limited number of participants in the INT group with MASLD in our study, this must be validated through intervention studies in patients with MASLD.

In addition, it has recently been proposed to lower the upper limit of the LFC to define MASLD from 5% to 1.85% (50). In a large American study ($n = 2{,}331$), an LFC of 1.85%–5.56% was associated with reduced insulin sensitivity and increased cardiometabolic risk factors compared with people with an LFC of less than 1.85% (50). In our study, the participants whose LFC was ≥1.85% significantly reduced their LFC compared with those whose LFC was ≤1.85%. This highlights our suggestion that participants with elevated LFC would especially benefit from the SB intervention.

In patients with MASLD, well-established evidence indicates that physical exercise yields favorable outcomes by reducing intrahepatic lipid content. This is achieved through a decrease in fatty acid synthesis, an increase in fatty acid oxidation, and the inhibition of molecular pathways responsible for mitochondrial and hepatocyte damage (61). A recent meta-analysis showed that exercise can reduce LFC without significant changes in body weight, suggesting that exercise directly affects the liver (29). It is not yet completely clear mechanistically how exercise directly affects the reduction of liver fat. Most research has been done on the part related to insulin resistance, where exercise enhances insulin sensitivity in peripheral tissues, which reduces free fatty acids flux and glucose uptake to the liver (62).

In summary, reducing SB by 51 min/day and replacing it with nonguided nonexercise activities did not lead to a significant reduction in LFC. This could be because the average LFC was already within the normal range or because the intensity of PA and the duration of the SB reduction were too low. However, it could have benefited the participants with higher LFC (>1.85%–5%), but this needs to be confirmed with intervention studies with patients with MASLD. In addition, we found that the changes in LFC during the intervention in both groups were associated positively with the changes in fasting insulin and glycated hemoglobin, which is in line with the previous findings showing that fatty liver is associated with hepatic insulin resistance (63).

Liver Enzymes

The SB reduction intervention did not significantly affect ALT levels. However, in the exploratory analysis, we found that ALT decreased in participants who successfully reduced SB (mean 52 min/day) compared with the continuously sedentary participants. This suggests that even fairly modest reductions in SB could lead to improvements in ALT levels. We also report here that the changes in ALT levels in both groups during the intervention were positively associated with the changes in SB and negatively associated with the changes in standing. This relationship indicates that actively working to reduce periods of inactivity and incorporate standing into daily routines could lead to favorable changes in ALT levels. Our previously published results with a larger study sample (including the current study participants) showed that reducing SB by 50 min/day and increasing LPA and MVPA had beneficial effects on ALT levels after a 3-mo intervention (64). These findings underscore the importance of not only reducing SB but also incorporating more active behaviors into one's daily routine to optimize liver health. To further support our findings, a recent meta-analysis showed that participants with MASLD who engaged in regular exercise for more than 12 wk had significant improvements in ALT levels. Conversely, individuals who managed to maintain an active lifestyle for less than 12 wk did not see similar enhancements in their ALT levels (65). This emphasizes the prominent role of sustained physical activity over an extended period in promoting better liver health, indicating that long-term commitment to an active lifestyle is probably needed for achieving healthier liver enzyme levels.

In our study, the changes in ALT and AST during the intervention were associated positively with the changes in daily CHO and saccharose intake. Some similar results have been reported earlier in cross-sectional settings. A large population-based study ($n = 19{,}749$) showed that the intake of CHO but not fat was associated with elevated aminotransferase levels in Koreans with or without MetS (66), and the consumption of sugar-sweetened beverages was associated with higher serum ALT and AST levels among healthy premenopausal women (67). The latter study concluded that because

fructose is metabolized primarily in the liver, it would increase hepatic lipogenesis and, consequently, the risk for MASLD.

In the present study, AST levels increased in both groups, but there were no significant differences between the groups. We also detected that the changes in AST levels were positively associated with the changes in breaks in SB. AST levels can vary by 5–10% daily in the same individual (68). It has also been shown that exercise (both aerobic and resistance training) (69), and weight loss (particularly in women) (70), can increase AST levels. Thus, the increase in AST could be due to normal fluctuations of the enzyme levels, the overall increase in habitual PA (such as daily steps), or improved body composition, as was seen in both groups in our study.

The intervention did not significantly affect GGT. A previous meta-analysis (71) including adults who were overweight or exhibited MASLD showed that from seven exercise studies with or without dietary intervention, only one study showed significant change in GGT. In addition, when they investigated only the exercise studies (four studies), they did not find any significant reduction in GGT. In our study, the changes during the intervention in GGT levels were negatively associated with the changes in daily protein intake (g/day and %/day) and positively associated with the changes in daily saccharose intake (g/day and %/day). Previously, it has been shown that compared with a low-protein diet, a high-protein diet is associated with a lower risk for fatty liver disease, especially when the protein sources are plant-based (72). It has also been shown that a calorie-reduced high-protein diet reduces hepatic fat more effectively than a low-protein diet by suppressing the genes responsible for fat synthesis, uptake, and storage (73). One study also found that a hypo-caloric high-protein diet decreased ALT and GGT levels in patients with MASLD (74). It has been shown that even moderate doses of regular fructose and sucrose intake increase hepatic lipogenesis (75), increasing the risk of developing fatty liver disease and thus potentially increasing serum liver enzymes. Therefore, nutrition may be more important for regulating serum GGT levels than exercise.

The study's strengths lie in the rigorous methods to assess liver health. Gold standard methods were used to determine *1*) insulin sensitivity with the HEC method (42), combined with *2*) PET imaging to measure LGU and EGP directly in the liver (76) and *3*) LFC measured with MRS (77). Another key strength is the randomized controlled trial and the use of validated accelerometry algorithms for measuring SB and PA during the 6 mo (37, 38), which may provide a more accurate picture of daily behaviors than shorter measurements. Limitations include the medications that participants used, which could have affected the results. Another limitation is the findings from the associations between the changes during the intervention, thus preventing the causal interpretation of these results.

To conclude, intervention aiming to reduce SB by 1 h/day did not significantly affect the measured liver health markers. Reducing SB for extended periods and/or increasing the intensity of PA may be necessary to improve liver health. However, according to exploratory analysis, achieving the intended behavior change may improve ALT levels. Also, replacing some of the daily CHO, especially sugar, with protein sources may benefit liver health via associations between changes during the intervention seen in liver enzymes. LGU increased significantly in both groups, which may be due to increased steps and improved body composition. To our knowledge, this is the first study to show the effects of reducing SB and replacing it with nonguided PA on liver health markers in adults with MetS who are at risk for developing metabolic diseases such as MASLD. Thus, our study provides important information for future intervention studies determining guidelines for the amount of SB reduction potentially required to impact liver health positively.

DATA AVAILABILITY

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author upon reasonable request.

SUPPLEMENTAL MATERIAL

Supplemental Figs. S1–S4: https://doi.org/10.5281/zenodo.15120418.

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AUTHOR CONTRIBUTIONS

T.S., T.V., K.L., K.K.K., V.S., J.K., and I.H.A.H. conceived and designed research; S.L., T.S., T.G., O.E., M.K., J.H., N.H., and V.S. performed experiments; S.L., T.S., T.G., M.-J.H., J.N., E.L., H.V.-Y., H.S., V.S., and I.H.A.H. analyzed data; S.L., T.S., T.G., M.-J.H., J.N., E.L., H.V.-Y., H.S., V.S., and I.H.A.H. interpreted results of experiments; S.L. prepared figures; S.L. drafted manuscript; S.L., T.S., T.G., M.-J.H., E.L., J.N., O.E., M.K., H.V.-Y., H.S., T.V., J.H., K.L., N.H., K.K.K., V.S., J.K., and I.H.A.H. edited and revised manuscript; S.L., T.S., T.G., M.-J.H., E.L., J.N., O.E., M.K., H.V.-Y., H.S., T.V., J.H., K.L., N.H., K.K.K., V.S., J.K., and I.H.A.H. approved final version of manuscript.

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