Effects of short-term sprint interval and moderate-intensity continuous training on liver fat content, lipoprotein profile and substrate uptake: a randomised trial

Kumail K. Motiani (kumail.k.motiani@utu.fi)[1], Anna M. Savolainen (anmsav@utu.fi)[1], Jussi Toivanen (jvatoi@gmail.com)[1], Jari-Joonas Eskelinen (jjvesk@utu.fi)[1], Minna Yli-Karjanmaa (myli@health.sdu.dk) [1], Kirsi A. Virtanen (kianvi@utu.fi) [2], Virva Saunavaara (virva.saunavaara@tyks.fi)[1,3], Marja A. Heiskanen (masink@utu.fi)[1], Riitta Parkkola (Riitta.Parkkola@tyks.fi)[4], Merja Haaparanta Solin (merja.haaparanta-solin@utu.fi)[1,5], Olof Solin (olof.solin@abo.fi)[1,6,7], Nina Savisto (nijosa@utu.fi)[1], Eliisa Löyttyniemi (eliisa.loyttyniemi@utu.fi)[8], Juhani Knuuti (jknuuti@utu.fi)[1], Pirjo Nuutila (pirjo.nuutila@utu.fi)[1,9], Kari K. Kalliokoski (kari.kalliokoski@tyks.fi)[1], Jarna C. Hannukainen (jarna.hannukainen@tyks.fi)[1]

[1]Turku PET Centre, University of Turku, Turku, Finland

[2]Turku PET Centre, Turku University Hospital, Turku, Finland

[3]Department of Medical Physics, Turku University Hospital, Turku, Finland

[4]Department of Radiology, Turku University Hospital, Turku, Finland

[5]MediCity Research Laboratory Turku, University of Turku, Finland

[6]Department of Chemistry, University of Turku, Finland

[7]Turku PET Centre, Åbo Akademi University, Turku, Finland

[8]Department of Biostatistics, University of Turku, Finland

[9]Department of Endocrinology, Turku University Hospital, Turku, Finland

Corresponding author and person to whom reprint requests should be addressed:

Jarna C. Hannukainen, PhD
Turku PET Centre
University of Turku
Turku P.O. Box 52, FI-20521, Finland
Tel: +35823131878
Fax: +35822318191
Email: jarna.hannukainen@tyks.fi

ABSTRACT

Aims/hypothesis: Type 2 diabetes (T2D) and increased liver fat content (LFC) alter lipoprotein profile and composition and impair liver substrate uptake. Exercise training mitigates T2D and reduces LFC, but the benefits of different training intensities on lipoprotein classes and liver substrate uptake are unclear. The aim of this study was to evaluate the effects of moderate-intensity continuous (MICT) or sprint interval training (SIT) on LFC, liver substrate uptake, and lipoprotein profile in subjects with normoglycemia or prediabetes/T2D.

Methods: We randomized fifty-four subjects (normoglycemic n=28, prediabetic/T2D n=26, aged=40-55 years) to perform either MICT or SIT for two-weeks and measured LFC with MRS, lipoprotein composition with NMR, and liver glucose uptake (GU) and fatty acid uptake (FAU) using PET.

Results: At baseline, prediabetic/T2D group had higher LFC, impaired lipoprotein profile and lower whole-body insulin sensitivity and aerobic capacity compared to normoglycemic group. Both training modes improved aerobic capacity ($p<0.001$) and lipoprotein profile (reduced LDL and increased large HDL subclasses) (all $p<0.05$) with no training regimen (SIT/MICT) or group effect (normoglycemic or prediabetic/T2D). LFC tended to reduce in prediabetic/T2D compared to normoglycemic group post-training ($p=0.051$). When subjects were divided according to LFC (High LFC>5.6% and low LFC<5.6%), training reduced LFC in subjects with high LFC ($p=0.009$) and only MICT increased insulin-stimulated liver GU ($p=0.03$).

Conclusion: Short-term SIT and MICT are effective in reducing LFC in subjects with fatty liver and in improving lipoprotein profile regardless of baseline glucose tolerance. Short-term MICT is more efficient in improving liver insulin sensitivity compared to SIT.

Clinical trial number: NCT01344928

Keywords: Liver fat content; liver glucose uptake; sprint interval training; lipoprotein profile and exercise.

List of abbreviations: LFC, liver fat content; T2D, type 2 diabetes; MICT, moderate-intensity continuous training; SIT, sprint interval training, MRS, magnetic resonance spectroscopy; NMR, nuclear magnetic resonance; GU, glucose uptake; FAU, fatty acid uptake; PET, positron emission tomography; LDL, low density lipoprotein; HDL, high density lipoprotein; EGP, endogenous glucose production; MRI, magnetic resonance imaging; OGTT, oral glucose tolerance test; [^{18}F]FTHA, 14(*R,S*)-[^{18}F]fluoro-6-thia-heptadecanoic acid; [^{18}F]FDG, 2-[^{18}F]fluoro-2-deoxy-D-glucose; ALAT, alanine transaminase; ASAT, aspartate transaminase; GT, gamma-glutamyltransferase; CRP, C reactive protein.

New and Noteworthy

- In short-term both SIT and MICT reduce liver fat content and improve lipoprotein profile, however MICT seems to be more preferable in improving liver insulin sensitivity.

INTRODUCTION

Liver is an important determinant of plasma glucose and fatty acid metabolism (34). In obesity and insulin resistance, impairments in hepatic metabolism and endogenous glucose production (EGP) (15; 26) as well as increased hypertriglyceridemia increase the risk for type 2 diabetes (T2D) (4; 47). Moreover, in obese and overweight sedentary subjects, accumulation of excessive triglycerides in liver, known as hepatic steatosis, leads to impaired liver function (decreased hepatic insulin clearance (22) and increased EGP (36)); decreased liver insulin-stimulated glucose uptake (GU) (4) and decreased liver blood flow (33). In obesity and insulin resistance excess visceral fat mass increases the free fatty acid delivery to the liver. The increased free fatty acid delivery to the liver further contributes to the increased liver free fatty acid uptake which has been shown to be associated with hepatic steatosis (47). Furthermore, hepatic steatosis contributes in the development of metabolic syndrome (50) and cardiovascular diseases (43).

The accumulation of fat into the liver has been shown to be associated with dyslipidemia both in normoglycemic (20) and T2D subjects (19). The dyslipidemia associated with hepatic steatosis affects both the lipoprotein subclass profile and composition. In fact, it has been shown by Toledo et al. that in T2D subjects, the severity of dyslipidemia depends on the degree of hepatic steatosis (44). Moreover, recent studies have shown that the distribution of HDL subclasses predict the risk of acquiring T2D, with small HDL having higher risk and large HDL protecting against it (28).

Exercise training reduces liver fat content (LFC) both in subjects with and without T2D even in the absence of weight loss (10; 18). In addition, training improves liver function and the lipoprotein profile independently of weight reduction (11; 18; 23; 41). Several studies have suggested a dose-response relationship between physical activity and health benefits (8), with increasing volume of physical activity achieving the most beneficial outcomes. Even though the health benefits of regular physical exercise training on chronic diseases have been known for long (24), adherence among the general population still remains low as lack of time being one of the main constraints (45). Therefore, many studies are focusing on

93 establishing a time-efficient dose of exercise training, which can be implemented and accepted by the
94 general population on a larger scale.

95 Gibala et al first demonstrated that two weeks of sprint interval training improves exercise performance
96 similarly to moderate-intensity continuous training (MICT). Thereafter, we and others have shown that SIT
97 rapidly induces marked improvements in aerobic capacity (6; 25), skeletal muscle performance (25) and
98 whole-body insulin sensitivity in healthy subjects as well as in patients with cardio-metabolic diseases (3;
99 48). Recently, with the same dataset as in this study, we showed that only MICT improved intestinal insulin
100 sensitivity while both SIT and MICT decreased the FAU in the intestine (29). To our knowledge, it is unclear
101 how SIT challenges liver and whether it leads to positive exercise training-induced responses in liver
102 metabolism and function.

103 The purpose of the current study was to compare the effects of two weeks of SIT and MICT on LFC, liver
104 substrate uptake and lipoprotein subclasses in subjects with normoglycemia and prediabetes/T2D. We
105 hypothesized that there would be impairments in the lipoprotein profile and liver GU in prediabetic/T2D
106 compared to normoglycemic group at baseline and exercise training would reduce LFC and FAU in
107 prediabetic/T2D. In addition, we hypothesized that MICT would induce more significant improvements in
108 liver metabolism due to the higher training volume-induced demands compared to SIT.

109

MATERIALS AND METHODS

The present study is a part of the larger study entitled “The effects of short-term high-intensity interval training on tissue glucose and fat metabolism in healthy subjects and in patients with type 2 diabetes” (NCT01344928). The study was approved by the local ethical committee of the Hospital district of South-Western Finland (decision 95/180/2010 §228) and carried out in compliance with the declaration of Helsinki. The purpose, nature and potential risks involved with the study were explained in detail and written informed consent was obtained before any measurements were performed.

Subjects

The study subjects were recruited in two phases. In the first phase, untrained normoglycemic (healthy) men and in the second phase, untrained prediabetic/T2D subjects (men + women) were recruited. The inclusion criteria for normoglycemic group has been described in detail previously (21). In the second phase (prediabetic/T2D group recruitment), due to the lack of male volunteers also females were included into the study. The inclusion and exclusion criteria for the prediabetic/T2D groups have been explained in detail previously (12). The groups were randomized into SIT and MICT as previously described (12). Given the nature of the intervention, no blinding was used. In total 54 sedentary 40-55 year-old subjects, of whom 28 were normoglycemic men and 26 prediabetic/T2D men or women were recruited in this study (Fig. 1a). Out of 26 prediabetic/T2D subjects (male n=16, female n=10), 17 met the criteria of T2D and 9 had either impaired fasting glucose concentrations and/or impaired glucose tolerance (1). Out of 17 T2D subjects, 13 were treated with oral hypoglycaemic medication (11 metformin; 5 DPP-IV (sitagliptin) and 1 sulphonylurea), while 4 subjects were newly diagnosed and did not take any medication for T2D. None of the prediabetic/T2D subjects were on insulin and two of females were on contraceptives. In addition, 7 prediabetic/T2D subjects were taking statins. In total, seven subjects dropped out during the intervention,

one due to exercise-induced hip pain, one due to training induced migraine, one due to claustrophobic feeling within the MRI scanner, and four due to personal reasons.

Study design

Measurements were performed during three different visits before and after the training intervention as detailed in (Fig. 1b). An overnight fast for at least 10h was required before PET measurements. Participants were also asked to abstain from any caffeinated and alcoholic drinks, avoid strenuous physical exercise and stop all oral hypoglycaemic medication 48 h prior to the measurements. After two weeks of exercise training intervention, follow up studies were repeated, starting on the second day (~48 h) after the last exercise training session. The performed measurements are described in the Fig. 1b.

Exercise interventions

Both SIT and MICT groups exercised three times a week for two weeks. All six training sessions were performed under supervision. The training protocols have been explained previously (21). Briefly, each SIT session consisted of 4-6 x 30s exercise bouts of all-out cycling efforts (Wingate protocol) with 4 min of recovery between the bouts (during the recovery period subjects remained still or continued to do unloaded cycling). Each bout of SIT started with 5 seconds of acceleration to maximal cadence followed by a sudden increase in load which was 7.5% of the whole-body weight in kg for normoglycemic subjects and 10% of fat-free mass in kg for prediabetic/T2D subjects (Monark Ergomedic 828E, Monark, Vansbro, Sweden). MICT training consisted of 40-60 min of cycling at moderate intensity 60% of VO_{2peak} intensity (Tunturi E85, Tunturi Fitness, Almere, Netherlands). The cycling duration was increased by 10 min after every other session until 60 min was reached in last two sessions.

Maximal exercise test

An incremental bicycle ergometer test (Ergoline 800s, VIASYS Healthcare, USA) with direct respiratory measurements using a ventilation and gas exchange (Jaeger Oxycon Pro, VIASYS Healthcare, Germany) was used to measure the maximal oxygen uptake (VO_{2peak}), as described previously (21). Initial exercise intensity was 50 W which was increased by 30 W after every two minutes until volitional exhaustion. Mean oxygen consumption at the highest 1 min was expressed as VO_{2peak}. The workload at the last two minutes of the test was averaged and used as a measure for maximal performance. The peak respiratory exchange ratio was ≥1.15 and peak blood lactate concentration, measured from capillary samples obtained immediately and 1 min after exhaustion (YSI 2300 Stat Plus, YSI Incorporated Life Sciences, USA), was ≥8.0 mmol·L-1 for all the tests. A peak heart rate (HR) (RS800CX, Polar Electro Ltd., Kempele, Finland) within 10 beats of the age-appropriate reference value (220 – age) was true in all except one participant in the both groups and in both pre- and post-training tests. Therefore, the highest value of oxygen consumption was expressed as VO_{2peak} and not VO_{2max}.

Lipoproteins subclasses

Lipid and lipoprotein metabolic biomarkers were quantified from fasting serum samples using high-throughput proton NMR metabolomics (Nightingale Health Ltd, Helsinki, Finland). This technique provides quantification of 14 lipoprotein subclasses. These 14 lipoprotein subclass sizes were defined as follows: extremely-large VLDL (very low density lipoprotein) with particle diameters from 75nm upwards and a possible contribution of chylomicrons, five VLDL subclasses; extra-large, large, medium, small, and extra-small, IDL (intermediate density lipoprotein), three LDL (large density lipoprotein) subclasses; large, medium, and small, and four HDL (high density lipoprotein) subclasses; extra-large, large, medium, and small. The following components of the lipoprotein subclasses were quantified: total lipids, phospholipids, triglycerides, cholesterol, free cholesterol, and cholesterol esters. The details of the experimentation have been described previously (39).

PET scanning

178 PET studies were conducted after an overnight fast. Radiotracers 14(*R,S*)-[^{18}F]fluoro-6-thia-heptadecanoic
179 acid ([^{18}F]FTHA) and 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) were used to measure the liver FAU and
180 GU, respectively. On the first PET scan session, liver FAU was measured using [^{18}F]FTHA PET (35) during
181 fasting state. [^{18}F]FTHA radiotracer (156 [SEM 1.1] MBq) was injected and dynamic imaging of the
182 abdominal region (frames 3 x 300 s) were acquired starting at 46 minutes after the tracer injection. On the
183 second day, liver GU was measured using [^{18}F]FDG under euglycemic hyperinsulinemic clamp. The
184 euglycemic hyperinsulinemic clamp technique was used as previously described (5). On average 91 [SEM 2]
185 minutes after the start of the clamp, and after 47 min of [^{18}F]FDG (157 [SEM 0.9] MBq) injection abdominal
186 region (frames 3 x 300 s) were acquired. Arterialized blood samples were obtained during [^{18}F]FTHA and
187 [^{18}F]FDG scans to measure the plasma radioactivity for calculating the tracer input function. During
188 [^{18}F]FTHA scans blood samples were also collected to measure [^{18}F]FTHA metabolites for correcting the
189 plasma input function (27). Automatic gamma counter (Wizard 1480, Wallac, Turku, Finland) was used to
190 measure the plasma radioactivity. CT imaging was acquired for anatomical references.

191 **Image analysis**

192 The PET imaging data was corrected for dead time, decay and photon attenuation, and was reconstructed
193 using 3D-OSEM method. Carimas 2.7 (www.pet.fi/carimas) software was used for image analysis. Three-
194 dimensional volumes of interest (3-D VOIs) were drawn on the liver, being cautious about the movement
195 of the diaphragm and avoiding major vessels in the liver. Tissue time activity curves were obtained from
196 the 3-D VOIs, and graphical analysis was used to quantify the fractional uptake rate (31). GU and FAU rates
197 were calculated by multiplying corresponding fractional uptake rate values by the mean plasma glucose or
198 FFA level during the imaging period, respectively. Whole liver GU and FAU were obtained by multiplying
199 liver GU and FAU with liver volume, respectively. Lean liver GU and FAU were obtained by subtracting the
200 LFC from liver volume and multiplying it with liver GU and liver FAU. Due to technical problems in the PET

scanner and tracer production, the final number of subjects for liver glucose and free fatty acid uptake analyses were 20 and 17 in the high LFC group and 19 and 16 for the low LFC group, respectively.

MRS and MRI measurements

MRS and MRI studies were performed using Philips Gyroscan Intera 1.5T CV Nova Dual scanner (Philips Medical Systems, the Netherlands). LFC and volume were measured as previously described (32). Abdominal subcutaneous adipose tissue and visceral adipose tissue depots were determined according to the classification by Abate et al. (2). Abdominal fat masses were analysed from the image slice where the xiphoid process was seen to the image slice where both the femur heads were visible using SliceOmatic software v. 4.3 (http://www.tomovision.com/products/sliceomatic.htm). To obtain the mass, the pixel surface area was multiplied by the slice thickness and the density of adipose tissue 0.9196 kg/L (2).

Glycemic status, insulin sensitivity and body composition

Whole-body insulin-stimulated glucose uptake (M-value) was determined during the euglycemic hyperinsulinemic clamp as previously described (5). Insulin was infused at a continuous rate of 1 mU/kg/min (Actrapid; Novo Nordisk, Copenhagen, Denmark) and blood samples were taken every 5-10 min to adjust the exogenous glucose infusion and to maintain the plasma glucose concentration as closely as possible to the level of 5 mmol/L. Insulin (100 U/mL) infusion (Actrapid, Novo Nordisk, Copenhagen, Denmark) was started with the rate of 120 mU/min/m^2 during the first 4 min. After 4 min and up to 7 min, infusion rate was reduced to 80 mU/min/m^2, and, after 7 min to the end of the clamp, it was kept constant at 40 mU/min/m^2. Glucose (20%) infusion was started 4 min after the start of the insulin infusion with a rate of 0.5 x subject's weight kg. At 10 min, glucose infusion was doubled, and after that further adjusted according to plasma glucose levels to maintain the steady state level of 5 mmol/L. Arterialized venous blood samples were collected before the clamp and every 5-10 min to measure the plasma glucose concentration for adjusting the glucose infusion rate. Arterialized plasma glucose was determined in

duplicate by the glucose oxidase method (Analox GM9 Analyzer; Analox Instruments LTD, London, United Kingdom). Whole body insulin-stimulated glucose uptake rate (M-value) was calculated from the measured glucose values collected when the subjects had reached the steady state during the PET scan that was started 91 min [SE 2] after the start of the clamp. [^{18}F]FDG-PET study was performed when the subject had reached the stable glucose concentrations at the level of 5 mmol/L (within 5% range for at least 15 min) after positioning into the PET scanner. EGP was calculated from the PET data (14). Alanine transaminase (ALAT), aspartate aminotransferase (ASAT), total cholesterol, triacylglycerols, and HDL concentrations were measured by automated enzymatic method (Cobus 8000, Roche diagnostics GmbH, Mannheim, Germany). LDL was calculated using the Friedewald equation (7). Finally, whole-body fat percentage was measured using a bio impedance monitor (InBody 720, Mega Electronics, Kuopio, Finland).

Statistics

The sample size for the whole study (NCT01344928) was based on skeletal muscle (quadriceps femoris) glucose uptake (6; 37). No sample size calculation was performed specifically for the parameters of liver. Normal distribution of the variables was tested using Shapiro-Wilk test and evaluated visually. Logarithmic or square root transformations were done when appropriate to achieve the normal distribution. Statistical analyses were performed using hierarchical mixed linear models with compound symmetry covariance structure for repeated measurements. The model included one within-factor (Time; overall mean change between baseline and measurement after intervention), two between-factors (Diabetic status (Dia): Normoglycemic/prediabetic and T2D; Training: SIT/MICT) and all their interactions. In the comparison between normoglycemic/prediabetic and T2D group, women were excluded to avoid mixing of the effects of gender and glucose intolerance (Tables 2, 3 and 4 and Fig. 2). In the comparison between high LFC and low LFC groups, all subjects (men and women) were pooled together using a model that included one within-factor (time), between-factor group (high LFC/low LFC, SIT/MICT) and interaction terms (LFC*time, difference between high LFC and low LFC group and training*time, differences between training modes)

(Table 5 and Fig. 3 and 4). We also took both medication status (taking/not taking oral hypoglycaemic medication; taking/not taking statins) and gender into account in all the analyses. Subjects with one value, but another missing (drop outs, technical problems) are accounted for by restricted maximum likelihood estimation within the linear mixed models. Therefore, model-based means (SAS least square means) and 95% confidence intervals (CI) are reported for all the parameters. Correlations are reported as Pearson's correlation coefficients.

All tests were performed as 2-sided, with a significance level set at 0.05. The analyses were performed using SAS System, version 9.3 for Windows (SAS Institute Inc., Cary, NC, US).

RESULTS

The effects of exercise training were analysed separately between prediabetic and T2D men (Table 1). As most of the changes in the variables were similar, the prediabetic and T2D men were combined into one group. Consequently, the effects of exercise training have been compared between normoglycemic and prediabetic/T2D men. The effects of exercise training between men and women are shown in Supplementary table 1.

At baseline, prediabetic/T2D men were heavier, had higher body adiposity, impaired glucose and lipid profile, and had lower whole-body insulin sensitivity and aerobic capacity than the normoglycemic men (all baseline $p<0.05$, Table 2). Both SIT and MICT improved whole-body insulin sensitivity similarly in both the normoglycemic and prediabetic/T2D men and decreased slightly but significantly HbA_1c, whole-body fat percentage and depot specific adiposity (all time $p<0.05$, Table 2). Both SIT and MICT improved the aerobic capacity (VO_{2peak}) (time $p<0.001$) but the improvement in the SIT group was significantly different compared to the MICT group with SIT group inducing greater increase (training*time $p=0.005$, Table 2).

At baseline the prediabetic/T2D group had a significantly impaired lipoprotein profile, both subclass distribution (VLDL, IDL, LDL and HDL) and composition (lipids, phospholipids, cholesterol, cholesterol

esters, free cholesterol and triglycerides) compared to normoglycemic group (Table 3). Both SIT and MICT improved lipid, phospholipid, free cholesterol, cholesterol and cholesterol esters in extra-large HDL, while there was a significant reduction in various components of IDL, LDL and HDL subclasses (Fig. 2 a-f). There were significant correlations between the lipoprotein subcomponents and M-value, VO_{2peak} and liver parameters (Table 4). After the training intervention, there was no significant change in the VLDL subclasses and composition. In the analyses both diabetic medication and statins were taken as covariate to see if medication affected the training response, but it did not have any effect on the results.

LFC, liver volume, whole-liver GU, EGP and liver enzymes (alanine transaminase (ALAT), aspartate transaminase (ASAT) and gamma-glutamyltranspeptidase (GT)) were higher in the prediabetic/T2D compared to the normoglycemic men (Fig. 3a and Table 2). After training, there was significant reduction in the liver enzymes (ALAT, ASAT and GT) and C-reactive protein (CRP) without any differences between the groups or training modes (Table 2). No training response was observed in liver GU, FAU or EGP in either groups.

Regarding LFC, the training response differed between the normoglycemic and prediabetic/T2D men (Dia*time p=0.03), with a tendency to reduce LFC in the prediabetic/T2D men (p=0.051 time effect for prediabetic/T2D men) (Fig. 3a and b). During further data analysis, we observed that in the normoglycemic group seven subjects had LFC above 5.6%, which has been recommended as the cut-of value for normal LFC (42), whereas seven prediabetic/T2D subjects had LFC below 5.6%. Next, we pooled all subjects (men + women) together and divided them into low (<5.6%) and high (>5.6%) LFC groups. The high LFC group had 522% higher LFC (Fig. 3c) compared to low LFC group. After training LFC reduced by -13% (p=0.009) only in the high LFC group (Fig. 3c). LFC correlated negatively with whole-body insulin sensitivity in all subjects before and after the intervention (Pre: r=-0.67, p<0.001; Post: r=-0.62, p<0.001). Interestingly, in the same comparison with high LFC and low LFC groups we saw that MICT improved insulin-stimulated liver GU by 7%, while no change was observed after SIT (Fig. 4). There were no differences between SIT and MICT in

any other parameters except fasting plasma FFA which reduced significantly only after MICT (Table 5). We found no differences in EGP. In the low LFC group EGP correlated inversely with aerobic capacity ($r=-0.62$, $p<0.01$).

DISCUSSION

We studied the effects of high intensity low volume SIT and low intensity high volume MICT on LFC, lipoprotein subclasses and liver metabolism in untrained, middle-aged subjects with normoglycemia or prediabetes/T2D using MRS, NMR and PET. As expected prediabetic/T2D group had higher LFC and liver enzyme levels and impaired lipoprotein profile compared to normoglycemic subjects (men only) at baseline. However, contrary to our hypothesis no differences were found in the liver substrate uptake between the normoglycemic and prediabetic/T2D groups. After two weeks' training intervention both SIT and MICT reduced LFC, liver enzymes and inflammatory markers in prediabetes/T2D subjects (men only) or subjects with high LFC (men + women). Training improved lipoprotein subclass profile similarly in all subjects regardless of training mode. MICT increased liver insulin stimulated GU and there was a non-significant reduction in liver free fatty acid uptake whereas no changes were found after SIT (men + women). The effects of training on liver substrate uptake were independent of baseline glucose tolerance or LFC.

MICT improves liver insulin sensitivity and leads to a non-significant reduction in liver free fatty acid uptake

Contrary to our hypothesis, we did not find baseline differences in insulin-stimulated liver GU when expressed per 100 g of tissue between the normoglycemic and prediabetic/T2D (men only) and high LFC/low LFC group (men + women). Previous data from our and other groups have shown both similar (47) or impaired insulin-stimulated liver GU in subjects with T2D (15) and increased LFC (15; 33). In the studies showing impaired insulin-stimulated liver GU the subjects have been older than the subjects in this study

and the LFC has been higher than 20%, which may explain discrepancies between this and previous studies (15; 33).

One of the key finding in the present study was the improvement in liver GU (men + women) after the training in MICT but not in the SIT group. This finding agrees with our recent data regarding intestinal insulin sensitivity with the same subjects (29), where the insulin-stimulated colonic glucose uptake improved only after MICT and not after SIT. One explanation for this finding might be the difference in the energy expenditure between SIT and MICT. When we calculated the energy consumption for all training sessions based on the effective training time, MICT had ∼ 691% higher total energy consumption compared to SIT (SIT 392 (355, 429) and MICT 2710 (2474, 2946) kcal). However, according to Skelly et al. the 24-hour energy expenditure is comparable between SIT and MICT due to higher post training energy expenditure after SIT (38). This might explain the similar results we found for most of the parameters in our study.

Another explanation for the difference between the training modes can be the negative association between liver GU and the plasma FFA level (16). It has been shown that increase in the plasma FFA level impairs the insulin-stimulated liver GU (16). This is because plasma FFA has an allosteric inhibitory effect on glucokinase enzyme (which phosphorylates glucose), which leads to less trapping of glucose inside the liver cells resulting in a lower liver GU. Interestingly, in our study, plasma FFA levels reduced only in the MICT group possible explaining the increase in the liver GU in the MICT group only. Moreover, change in fasting plasma FFA correlated inversely with the change in the liver GU only in MICT group ($r=-0.60$, $p=0.01$).

Liver plays a very important role in the whole-body FFA metabolism, each mL of liver has been shown to utilize almost 50 times more FFA compared to 1 g of muscle (17). Therefore, even small changes in liver FFA metabolism warrant attention. There was a non-significant decrease in liver FAU ($p = 0.10$) after MICT but not after SIT in our study (men + women). This decrease in FAU only after MICT is probably due to the

342 greater reduction in the circulating fasting FFA levels due to higher training volumes and energy
343 expenditure than SIT (6). As most of the liver FAU occur during post-prandial period (13; 46), it is possible
344 that the training period was too short to induce training responses great enough to be detectable at fasting
345 conditions. Unfortunately, due to the radiation dose limitations, liver FAU was measured only at fasting
346 conditions in the present study.

347 **Reduction in LFC**

348 Regarding LFC, the training response differed between the normoglycemic and prediabetic/T2D group
349 (men), with a non-significant reduction in LFC in the prediabetic/T2D group ($p=0.051$) while no change was
350 observed in the normoglycemic group. Interestingly, when we further divided the subjects (men + women)
351 into high (LFC >5.6%) and low (LFC <5.6%) LFC groups (42), we saw that just two weeks of exercise training
352 reduced the LFC by -13% in subjects with high LFC to start with. However, there was no reduction in LFC in
353 low LFC group. In the present study the training intervention was short, consisting only of six training
354 sessions and thus probable not long enough to reduce LFC in subjects with LFC already at a normal level.
355 The tendency to decrease LFC in prediabetic/T2D (men) group and the significant decrease in LFC in the
356 high LFC group (men + women) were independent of the training mode in both comparisons. This finding
357 agrees with a recent study done on obese subjects with non-alcoholic fatty liver diseases where they
358 performed either four weeks of high intensity interval training (HIIT) or MICT and showed that both
359 training modes reduced LFC without any differences between training modes (49). Overall notable in this
360 study is that people who are not that obese (BMI below or slightly above 30) already have high LFC and
361 complications of diabetes.

362 **Improvement in lipoprotein profile and protection against diabetes**

363 At baseline, the lipoprotein profile was significantly impaired in prediabetic/T2D compared to
364 normoglycemic group (men only). However, no changes were observed in VLDL subclasses and their

components. This is probably because VLDL has a faster turnover rate compared to LDL and HDL, and the effects of acute and long-term exercise training on VLDL have been shown to be temporary and disappear within a few hours after the last exercise session (30; 40). However, all the IDL (except the IDL triglyceride content) and LDL subclasses and components decreased without any differences between the normoglycemic and prediabetic/T2D (men only) (Fig. 2 a-f). Thus, short-term exercise training improves the lipoprotein profile both in subjects with normal lipoprotein profile but also in subjects with impaired lipoprotein profile regardless of their baseline glucose tolerance. Interestingly, both SIT and MICT had a protective effect for diabetes by efficiently reducing the ones associated with risk of acquiring diabetes and improving the ones associated with diabetes prevention. In our study, the reduction in the small LDL lipoprotein composition is noteworthy as it has been shown that smaller LDL particles are associated with the risk of acquiring diabetes (28). While for HDL subclasses, we saw an improvement in the large HDL subclasses and reduction in the small HDL subclasses. The improvement of large HDL is very vital as shown in previous studies that the larger HDL carries lower risk of acquiring diabetes while the smaller HDL carries high risk of acquiring diabetes (28). The changes in these sub fractions are significant, as Garvey et al. had also demonstrated an association with the progression of insulin resistance and the increase in VLDL, LDL and small HDL concentrations (9). Additionally, we found a positive correlation between extra-large HDL and M-value and negative correlations between extra-large HDL and liver volume and ALAT. While with small HDL we found interesting positive correlation with LFC (Table 4).

Limitations

There are some limitations in this study which warrant consideration. Subjects were only asked to maintain their normal dietary habits and no diet control was performed, thus the effect of diet on weight reduction and body adiposity post training cannot be ruled out when critically interpreting the data. Also, the findings in VO_{2peak} needs to be interpreted in relation to possible measurement error (12). Liver GU and FAU were studied in different metabolic environments, [^{18}F]FDG during euglycemic hyperinsulinemic clamp

and [^{18}F]FTHA during fasting, corresponding to the conditions where GU and FAU are at their highest, respectively. Due to the radiation dose limitations, [^{18}F]FDG and [^{18}F]FTHA studies were not possible to perform both at fast and during clamp. No control subjects were included in the study. The power calculations were made for the whole study (NCT01344928) based on its primary outcome, skeletal muscle glucose uptake and no sample size calculation was performed specifically for the measures of the present study. Finally, the duration of the training intervention was only two weeks and likely more differences would be revealed with longer training period.

Conclusion

In conclusion, training reduced LFC in prediabetic/T2D subjects and subjects with fatty liver but not in subjects with normoglycemia or low liver fat content. Training improved lipoprotein profile, by reducing lipoproteins associated with risk of acquiring T2D and improving the ones associated with diabetes prevention, and liver insulin sensitivity regardless of baseline glucose tolerance. Regarding the training modes, MICT was more effective in improving liver insulin sensitivity compared to SIT, while the training mode had no effect on LFC or lipoprotein profile.

Acknowledgements

The authors would like to thank the staff at Turku PET centre, University of Turku, Åbo Akademi University and Paavo Nurmi Centre for their excellent contributions in the completion of this study.

Duality of interests

The authors declare that there is no duality of interest associated with this study.

411 # Figure legends

412 **Figure 1.** a) Consort flow diagram showing the total number of subjects recruited and analysed. SIT, sprint
413 interval training; MICT, moderate-intensity continuous training. b) Study design: OGTT, oral glucose tolerance
414 test; VO_{2peak}, aerobic capacity; MRS, magnetic resonance spectroscopy; MRI, magnetic resonance imaging;
415 PET, positron emission tomography; [^{18}F]FTHA, 14(*R,S*)-[^{18}F]fluoro-6-thia-heptadecanoic acid; [^{18}F]FDG, 2-
416 [^{18}F]fluoro-2-deoxy-D-glucose; SIT, sprint interval training; MICT, moderate-intensity continuous training.

417 **Figure 2.** Effects of two weeks of exercise training on subcomponents of lipoproteins in (normoglycemic +
418 prediabetic/T2D only men n = 44). a) Lipids, b) Phospholipids, c) Cholesterol, d) Cholesterol esters, e) Free
419 cholesterol and f) Triglycerides. Intermediate density lipoprotein (IDL); LLDL (large low density lipoprotein);
420 MLDL (medium low density lipoprotein); SLDL (small low density lipoprotein); XLHDL (extra-large high density
421 lipoprotein); LHDL (large high density lipoprotein); MHDL (medium high density lipoprotein) and SHDL (small
422 high density lipoprotein). All values are expressed as model-based means and bars are confidence intervals
423 [95% CI]. *p<0.05 value for time interaction (i.e. the groups behaved similarly for the change).

424 **Figure 3.** Liver fat content (LFC) before and after two weeks of intervention. a) effects of two weeks of SIT and
425 MICT on LFC in prediabetic/T2D (normoglycemic + prediabetic/T2D only men n = 44) and, b) effects of SIT and
426 MICT on LFC in high LFC and low LFC group (men + women n = 54). The shaded area in (a) denotes normal liver
427 fat content (≤ 5.6 %). All values are expressed as model-based means and bars are confidence intervals [95%
428 CI]. *p ≤ 0.05 baseline differences between the normoglycemic and prediabetic/T2D and ***p ≤ 0.001
429 baseline differences between low LFC and high LFC groups. †† p ≤ 0.01 time effect for the high LFC group.

430 **Figure 4**. Insulin-stimulated liver glucose uptake in SIT and MICT groups before and after the training
431 intervention in all subjects (men + women, n = 54). All values are expressed as model-based means and bars

are confidence intervals [95% CI]. P-value training*time indicates the change in liver glucose uptake was different between the training groups. † p value the improvement in liver glucose uptake in the MICT group was significant compared to SIT.

1. Classification and diagnosis of diabetes. *Diabetes Care* 38 Suppl: S8-S16, 2015.

2. **Abate N, Burns D, Peshock RM, Garg A and Grundy SM**. Estimation of Adipose-Tissue Mass by Magnetic-Resonance-Imaging - Validation Against Dissection in Human Cadavers. *Journal of Lipid Research* 35: 1490-1496, 1994.

3. **Babraj JA, Vollaard NB, Keast C, Guppy FM, Cottrell G and Timmons JA**. Extremely short duration high intensity interval training substantially improves insulin action in young healthy males. *Bmc Endocrine Disorders* 9: 2009.

4. **Borra R, Lautamaki R, Parkkola R, Komu M, Sijens PE, Hallsten K, Bergman J, Iozzo P and Nuutila P**. Inverse association between liver fat content and hepatic glucose uptake in patients with type 2 diabetes mellitus. *Metabolism-Clinical and Experimental* 57: 1445-1451, 2008.

5. **DeFronzo RA, Tobin JD and Andres R**. Glucose Clamp Technique - Method for Quantifying Insulin-Secretion and Resistance. *American Journal of Physiology* 237: E214-E223, 1979.

6. **Eskelinen JJ, Heinonen I, Loyttyniemi E, Saunavaara V, Kirjavainen A, Virtanen KA, Hannukainen JC and Kalliokoski KK**. Muscle-specific glucose and free fatty acid uptake after sprint interval and moderate-intensity training in healthy middle-aged men. *Journal of Applied Physiology* 118: 1172-1180, 2015.

7. **Friedewald WT, Levy RI and Fredrickson DS**. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18: 499-502, 1972.

8. **Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, Nieman DC and Swain DP**. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining

468 cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing
469 exercise. *Med Sci Sports Exerc* 43: 1334-1359, 2011.

470 9. **Garvey WT, Kwon S, Zheng D, Shaughnessy S, Wallace P, Hutto A, Pugh K, Jenkins AJ, Klein RL and Liao Y**. Effects of
471 insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear
472 magnetic resonance. *Diabetes* 52: 453-462, 2003.

473 10. **Hallsworth K, Fattakhova G, Hollingsworth KG, Thoma C, Moore S, Taylor R, Day CP and Trenell MI**. Resistance
474 exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. *Gut* 60: 1278-
475 1283, 2011.

476 11. **Halverstadt A, Phares DA, Wilund KR, Goldberg AP and Hagberg JM**. Endurance exercise training raises high-density
477 lipoprotein cholesterol and lowers small low-density lipoprotein and very low-density lipoprotein independent of body
478 fat phenotypes in older men and women. *Metabolism* 56: 444-450, 2007.

479 12. **Heiskanen MA, Sjoros TJ, Heinonen IHA, Loyttyniemi E, Koivumaki M, Motiani KK, Eskelinen JJ, Virtanen KA, Knuuti**
480 **J, Hannukainen JC and Kalliokoski KK**. Sprint interval training decreases left-ventricular glucose uptake compared to
481 moderate-intensity continuous training in subjects with type 2 diabetes or prediabetes. *Sci Rep* 7: 10531, 2017.

482 13. **Hsieh J, Hayashi AA, Webb J and Adeli K**. Postprandial dyslipidemia in insulin resistance: mechanisms and role of
483 intestinal insulin sensitivity. *Atheroscler Suppl* 9: 7-13, 2008.

484 14. **Iozzo P, Gastaldelli A, Jarvisalo MJ, Kiss J, Borra R, Buzzigoli E, Viljanen A, Naum G, Viljanen T, Oikonen V, Knuuti J,**
485 **Savunen T, Salvadori PA, Ferrannini E and Nuutila P**. F-18-FDG assessment of glucose disposal and production rates
486 during fasting and insulin stimulation: A validation study. *Journal of Nuclear Medicine* 47: 1016-1022, 2006.

15. **Iozzo P, Hallsten K, Oikonen V, Virtanen KA, Kemppainen J, Solin O, Ferrannini E, Knuuti J and Nuutila P**. Insulin-mediated hepatic glucose uptake is impaired in type 2 diabetes: Evidence for a relationship with glycemic control. *Journal of Clinical Endocrinology & Metabolism* 88: 2055-2060, 2003.

16. **Iozzo P, Lautamaki R, Geisler F, Virtanen KA, Oikonen V, Haaparanta M, Yki-Jarvinen H, Ferrannini E, Knuuti J and Nuutila P**. Non-esterified fatty acids impair insulin-mediated glucose uptake and disposition in the liver. *Diabetologia* 47: 1149-1156, 2004.

17. **Iozzo P, Turpeinen AK, Takala T, Oikonen V, Solin O, Ferrannini E, Nuutila P and Knuuti J**. Liver uptake of free fatty acids in vivo in humans as determined with 14(R, S)-[18F]fluoro-6-thia-heptadecanoic acid and PET. *Eur J Nucl Med Mol Imaging* 30: 1160-1164, 2003.

18. **Johnson NA, Sachinwalla T, Walton DW, Smith K, Armstrong A, Thompson MW and George J**. Aerobic Exercise Training Reduces Hepatic and Visceral Lipids in Obese Individuals Without Weight Loss. *Hepatology* 50: 1105-1112, 2009.

19. **Kelley DE, McKolanis TM, Hegazi RA, Kuller LH and Kalhan SC**. Fatty liver in type 2 diabetes mellitus: relation to regional adiposity, fatty acids, and insulin resistance. *Am J Physiol Endocrinol Metab* 285: E906-E916, 2003.

20. **Kim HJ, Kim HJ, Lee KE, Kim DJ, Kim SK, Ahn CW, Lim SK, Kim KR, Lee HC, Huh KB and Cha BS**. Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. *Arch Intern Med* 164: 2169-2175, 2004.

21. **Kiviniemi AM, Tulppo MP, Eskelinen JJ, Savolainen AM, Kapanen J, Heinonen IHA, Huikuri HV, Hannukainen JC and Kalliokoski KK**. Cardiac Autonomic Function and High-Intensity Interval Training in Middle-Age Men. *Medicine and Science in Sports and Exercise* 46: 1960-1967, 2014.

22. **Kotronen A, Vehkavaara S, Seppala-Lindroos A, Bergholm R and Yki-Jarvinen H**. Effect of liver fat on insulin clearance. *American Journal of Physiology-Endocrinology and Metabolism* 293: E1709-E1715, 2007.

23. **Kraus WE, Houmard JA, Duscha BD, Knetzger KJ, Wharton MB, McCartney JS, Bales CW, Henes S, Samsa GP, Otvos JD, Kulkarni KR and Slentz CA**. Effects of the amount and intensity of exercise on plasma lipoproteins. *N Engl J Med* 347: 1483-1492, 2002.

24. **Kujala UM**. Evidence on the effects of exercise therapy in the treatment of chronic disease. *Br J Sports Med* 43: 550-555, 2009.

25. **Little JP, Safdar A, Wilkin GP, Tarnopolsky MA and Gibala MJ**. A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms. *Journal of Physiology-London* 588: 1011-1022, 2010.

26. **Mackey RH, Greenland P, Goff DC, Jr., Lloyd-Jones D, Sibley CT and Mora S**. High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events: MESA (multi-ethnic study of atherosclerosis). *J Am Coll Cardiol* 60: 508-516, 2012.

27. **Maki MT, Haaparanta M, Nuutila P, Oikonen V, Luotolahti M, Eskola O and Knuuti JM**. Free fatty acid uptake in the myocardium and skeletal muscle using fluorine-18-fluoro-6-thia-heptadecanoic acid. *J Nucl Med* 39: 1320-1327, 1998.

28. **Mora S, Otvos JD, Rosenson RS, Pradhan A, Buring JE and Ridker PM**. Lipoprotein particle size and concentration by nuclear magnetic resonance and incident type 2 diabetes in women. *Diabetes* 59: 1153-1160, 2010.

29. **Motiani KK, Savolainen AM, Eskelinen JJ, Toivanen J, Ishizu T, Yli-Karjanmaa M, Virtanen KA, Parkkola R, Kapanen J, Gronroos TJ, Haaparanta-Solin M, Solin O, Savisto N, Ahotupa M, Loyttyniemi E, Knuuti J, Nuutila P, Kalliokoski KK**

and Hannukainen JC. Two weeks of moderate intensity continuous training, but not high intensity interval training increases insulin-stimulated intestinal glucose uptake. *J Appl Physiol (1985)* jap, 2017.

30. **Nellemann B, Christensen B, Vissing K, Thams L, Sieljacks P, Larsen MS, Jorgensen JO and Nielsen S**. Ten weeks of aerobic training does not result in persistent changes in VLDL triglyceride turnover or oxidation in healthy men. *Eur J Endocrinol* 171: 603-613, 2014.

31. **Patlak CS, Blasberg RG and Fenstermacher JD**. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. *J Cereb Blood Flow Metab* 3: 1-7, 1983.

32. **Rigazio S, Lehto HR, Tuunanen H, Nagren K, Kankaanpaa M, Simi C, Borra R, Naum AG, Parkkola R, Knuuti J, Nuutila P and Iozzo P**. The lowering of hepatic fatty acid uptake improves liver function and insulin sensitivity without affecting hepatic fat content in humans. *American Journal of Physiology-Endocrinology and Metabolism* 295: E413-E419, 2008.

33. **Rijzewijk LJ, van der Meer RW, Lubberink M, Lamb HJ, Romijn JA, de Roos A, Twisk JW, Heine RJ, Lammertsma AA, Smit JWA and Diamant M**. Liver Fat Content in Type 2 Diabetes: Relationship With Hepatic Perfusion and Substrate Metabolism. *Diabetes* 59: 2747-2754, 2010.

34. **Rosenson RS and Underberg JA**. Systematic review: Evaluating the effect of lipid-lowering therapy on lipoprotein and lipid values. *Cardiovasc Drugs Ther* 27: 465-479, 2013.

35. **Savisto N, Viljanen T, Kokkomaki E, Bergman J and Solin O**. Automated production of [(18) F]FTHA according to GMP. *J Labelled Comp Radiopharm* 61: 84-93, 2018.

542 36. **Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A, Halavaara J and Yki-Jarvinen**
543 **H**. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free
544 fatty acids independent of obesity in normal men. *Journal of Clinical Endocrinology & Metabolism* 87: 3023-3028, 2002.

545 37. **Sjoros TJ, Heiskanen MA, Motiani KK, Loyttyniemi E, Eskelinen JJ, Virtanen KA, Savisto NJ, Solin O, Hannukainen JC**
546 **and Kalliokoski KK**. Increased insulin-stimulated glucose uptake in both leg and arm muscles after sprint interval and
547 moderate-intensity training in subjects with type 2 diabetes or prediabetes. *Scand J Med Sci Sports* 2017.

548 38. **Skelly LE, Andrews PC, Gillen JB, Martin BJ, Percival ME and Gibala MJ**. High-intensity interval exercise induces 24-h
549 energy expenditure similar to traditional endurance exercise despite reduced time commitment. *Appl Physiol Nutr*
550 *Metab* 39: 845-848, 2014.

551 39. **Soininen P, Kangas AJ, Wurtz P, Suna T and Ala-Korpela M**. Quantitative serum nuclear magnetic resonance
552 metabolomics in cardiovascular epidemiology and genetics. *Circ Cardiovasc Genet* 8: 192-206, 2015.

553 40. **Sondergaard E, Rahbek I, Sorensen LP, Christiansen JS, Gormsen LC, Jensen MD and Nielsen S**. Effects of exercise
554 on VLDL-triglyceride oxidation and turnover. *Am J Physiol Endocrinol Metab* 300: E939-E944, 2011.

555 41. **St GA, Bauman A, Johnston A, Farrell G, Chey T and George J**. Independent effects of physical activity in patients
556 with nonalcoholic fatty liver disease. *Hepatology* 50: 68-76, 2009.

557 42. **Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH and Dobbins RL**. Magnetic
558 resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general
559 population. *American Journal of Physiology-Endocrinology and Metabolism* 288: E462-E468, 2005.

560 43. **Targher G, Bertolini L, Poli F, Rodella S, Scala L, Tessari R, Zenari L and Falezza G**. Nonalcoholic fatty liver disease
561 and risk of future cardiovascular events among type 2 diabetic patients. *Diabetes* 54: 3541-3546, 2005.

562 44. **Toledo FG, Sniderman AD and Kelley DE**. Influence of hepatic steatosis (fatty liver) on severity and composition of
563 dyslipidemia in type 2 diabetes. *Diabetes Care* 29: 1845-1850, 2006.

564 45. **Trost SG, Owen N, Bauman AE, Sallis JF and Brown W**. Correlates of adults' participation in physical activity: review
565 and update. *Med Sci Sports Exerc* 34: 1996-2001, 2002.

566 46. **van Hees AM, Jans A, Hul GB, Roche HM, Saris WH and Blaak EE**. Skeletal muscle fatty acid handling in insulin
567 resistant men. *Obesity (Silver Spring)* 19: 1350-1359, 2011.

568 47. **Viljanen APM, Iozzo P, Borra R, Kankaanpaa M, Karmi A, Lautamaki R, Jarvisalo M, Parkkola R, Ronnemaa T,**
569 **Guiducci L, Lehtimaki T, Raitakari OT, Mari A and Nuutila P**. Effect of Weight Loss on Liver Free Fatty Acid Uptake and
570 Hepatic Insulin Resistance. *Journal of Clinical Endocrinology & Metabolism* 94: 50-55, 2009.

571 48. **Weston KS, Wisloff U and Coombes JS**. High-intensity interval training in patients with lifestyle-induced
572 cardiometabolic disease: a systematic review and meta-analysis. *British Journal of Sports Medicine* 48: 1227-1U52, 2014.

573 49. **Winn NC, Liu Y, Rector RS, Parks EJ, Ibdah JA and Kanaley JA**. Energy-matched moderate and high intensity exercise
574 training improves nonalcoholic fatty liver disease risk independent of changes in body mass or abdominal adiposity - A
575 randomized trial. *Metabolism* 78: 128-140, 2018.

576 50. **Yki-Jarvinen H**. Fat in the liver and insulin resistance. *Annals of Medicine* 37: 347-356, 2005.

577

578

Fig. 1(a).

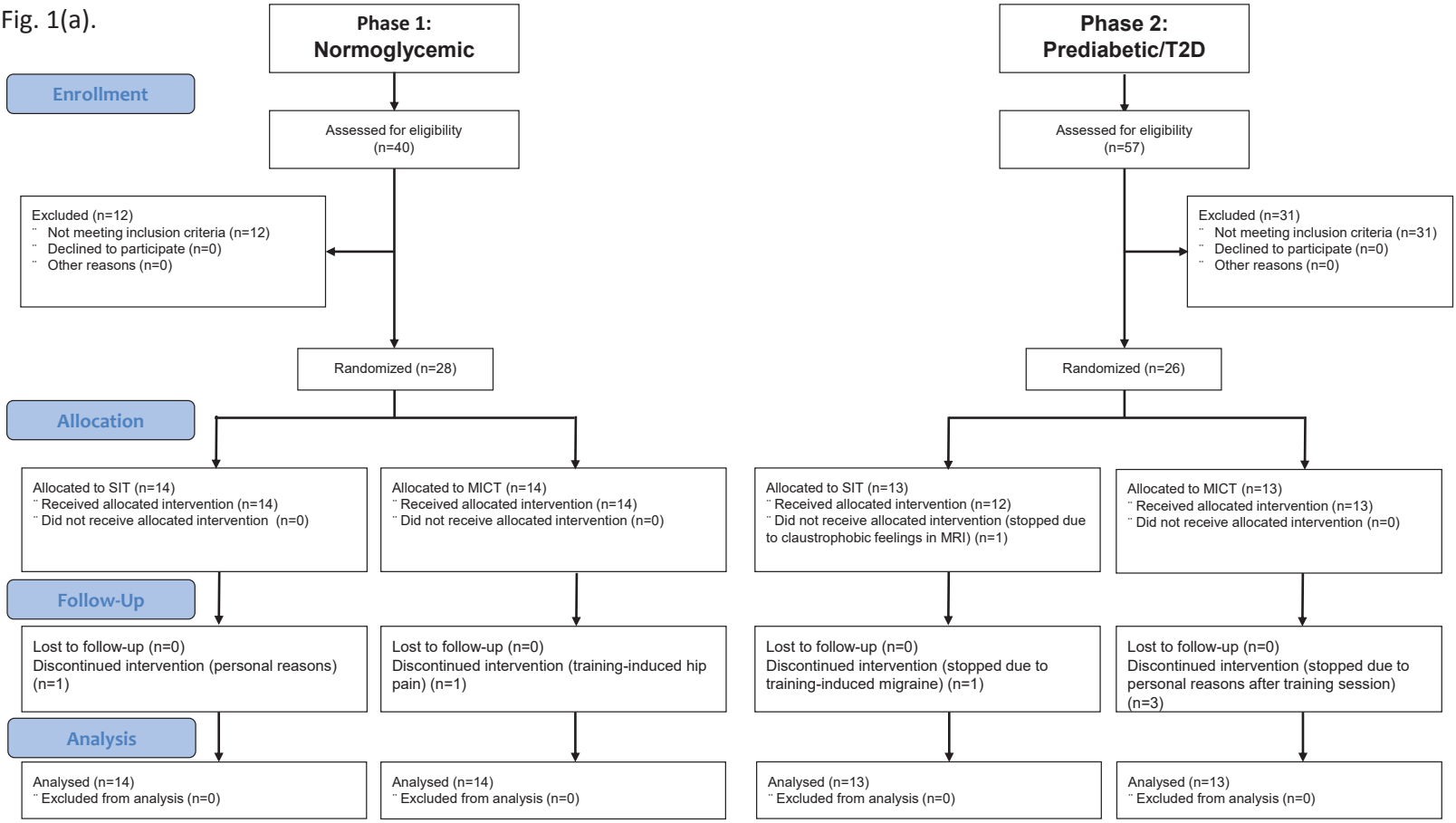
Phase 1:
Normoglycemic
Phase 2:
Prediabetic/T2D
Enrollment
Assessed for eligibility
(n=40)
Assessed for eligibility
(n=57)
Excluded (n=12)
¨ Not meeting inclusion criteria (n=12)
¨ Declined to participate (n=0)
¨ Other reasons (n=0)
Excluded (n=31)
¨ Not meeting inclusion criteria (n=31)
¨ Declined to participate (n=0)
¨ Other reasons (n=0)
Randomized (n=28)
Randomized (n=26)
Allocation
Allocated to SIT (n=14)
¨ Received allocated intervention (n=14)
¨ Did not receive allocated intervention (n=0)
Allocated to MICT (n=14)
¨ Received allocated intervention (n=14)
¨ Did not receive allocated intervention (n=0)
Allocated to SIT (n=13)
¨ Received allocated intervention (n=12)
¨ Did not receive allocated intervention (stopped due to claustrophobic feelings in MRI) (n=1)
Allocated to MICT (n=13)
¨ Received allocated intervention (n=13)
¨ Did not receive allocated intervention (n=0)
Follow-Up
Lost to follow-up (n=0)
Discontinued intervention (personal reasons) (n=1)
Lost to follow-up (n=0)
Discontinued intervention (training-induced hip pain) (n=1)
Lost to follow-up (n=0)
Discontinued intervention (stopped due to training-induced migraine) (n=1)
Lost to follow-up (n=0)
Discontinued intervention (stopped due to personal reasons after training session) (n=3)
Analysis
Analysed (n=14)
¨ Excluded from analysis (n=0)
Analysed (n=14)
¨ Excluded from analysis (n=0)
Analysed (n=13)
¨ Excluded from analysis (n=0)
Analysed (n=13)
¨ Excluded from analysis (n=0)

Fig. 1(b).

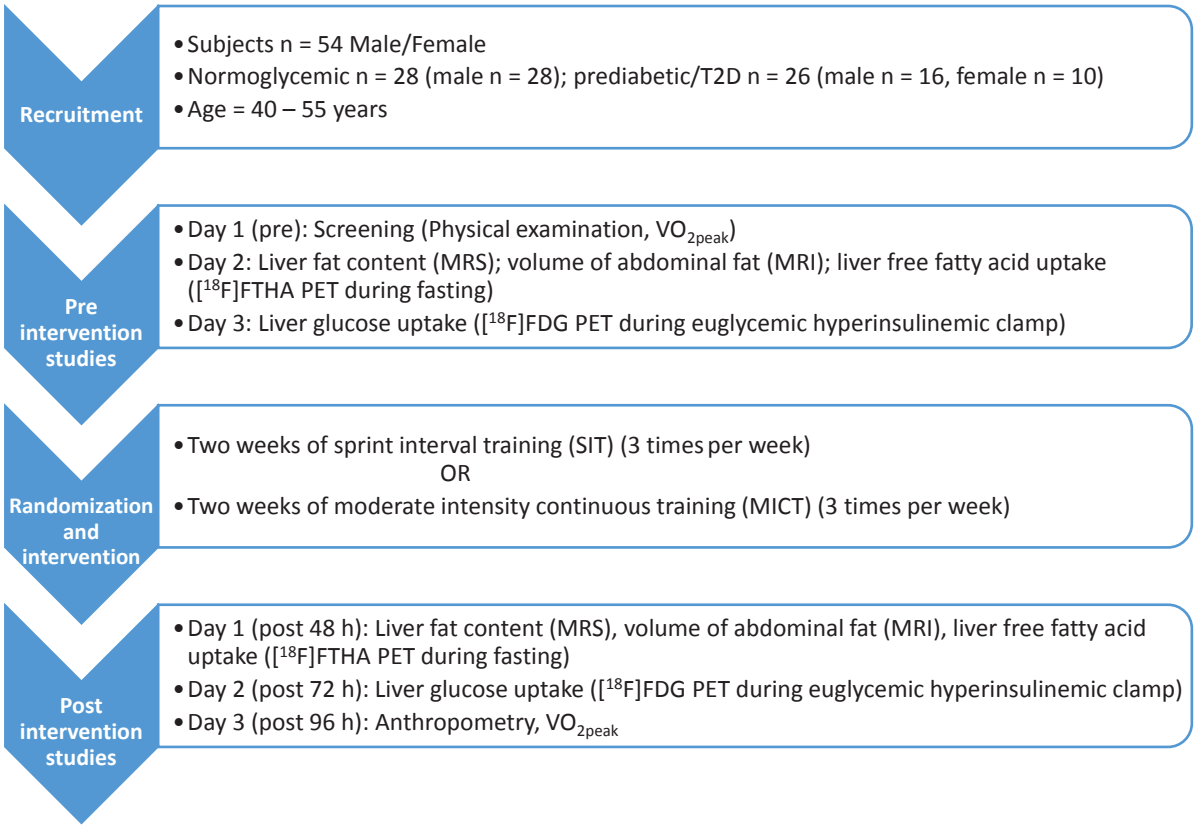
Recruitment
• Subjects n = 54 Male/Female
• Normoglycemic n = 28 (male n = 28); prediabetic/T2D n = 26 (male n = 16, female n = 10)
• Age = 40 – 55 years
Pre intervention studies
• Day 1 (pre): Screening (Physical examination, VO_{2peak})
• Day 2: Liver fat content (MRS); volume of abdominal fat (MRI); liver free fatty acid uptake ([^{18}F]FTHA PET during fasting)
• Day 3: Liver glucose uptake ([^{18}F]FDG PET during euglycemic hyperinsulinemic clamp)
Randomization and intervention
• Two weeks of sprint interval training (SIT) (3 times per week)
OR
• Two weeks of moderate intensity continuous training (MICT) (3 times per week)
Post intervention studies
• Day 1 (post 48 h): Liver fat content (MRS), volume of abdominal fat (MRI), liver free fatty acid uptake ([^{18}F]FTHA PET during fasting)
• Day 2 (post 72 h): Liver glucose uptake ([^{18}F]FDG PET during euglycemic hyperinsulinemic clamp)
• Day 3 (post 96 h): Anthropometry, VO_{2peak}

Fig. 2.

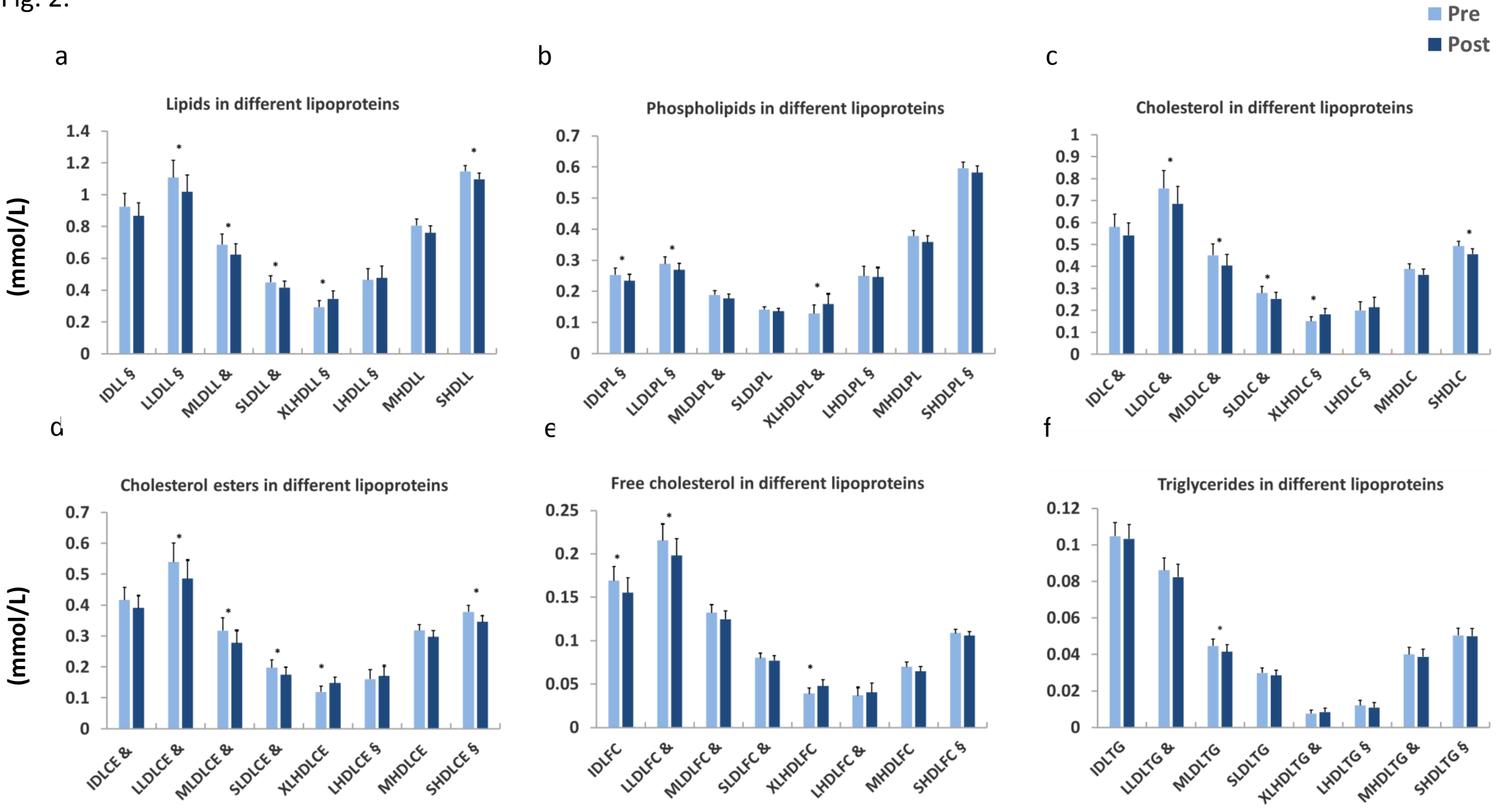
Pre
Post
a
Lipids in different lipoproteins
(mmol/L)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
IDLL §
LLDLL §
MLDLL &
SLDLL &
XLHDLL §
LHDLL §
MHDLL
SHDLL
b
Phospholipids in different lipoproteins
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
IDLPL §
LLDLPL §
MLDLPL &
SLDLPL
XLHDLPL &
LHDLPL §
MHDLPL
SHDLPL §
c
Cholesterol in different lipoproteins
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
IDLC &
LLDLC &
MLDLC &
SLDLC &
XLHDLC §
LHDLC §
MHDLC
SHDLC
d
Cholesterol esters in different lipoproteins
(mmol/L)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
IDLCE &
LLDLCE &
MLDLCE &
SLDLCE &
XLHDLCE
LHDLCE §
MHDLCE
SHDLCE §
e
Free cholesterol in different lipoproteins
0.25
0.2
0.15
0.1
0.05
0
IDLFC
LLDLFC &
MLDLFC &
SLDLFC &
XLHDLFC
LHDLFC &
MHDLFC
SHDLFC §
f
Triglycerides in different lipoproteins
0.12
0.1
0.08
0.06
0.04
0.02
0
IDLTG
LLDLTG &
MLDLTG
SLDLTG
XLHDLTG &
LHDLTG §
MHDLTG &
SHDLTG §

Figure 3

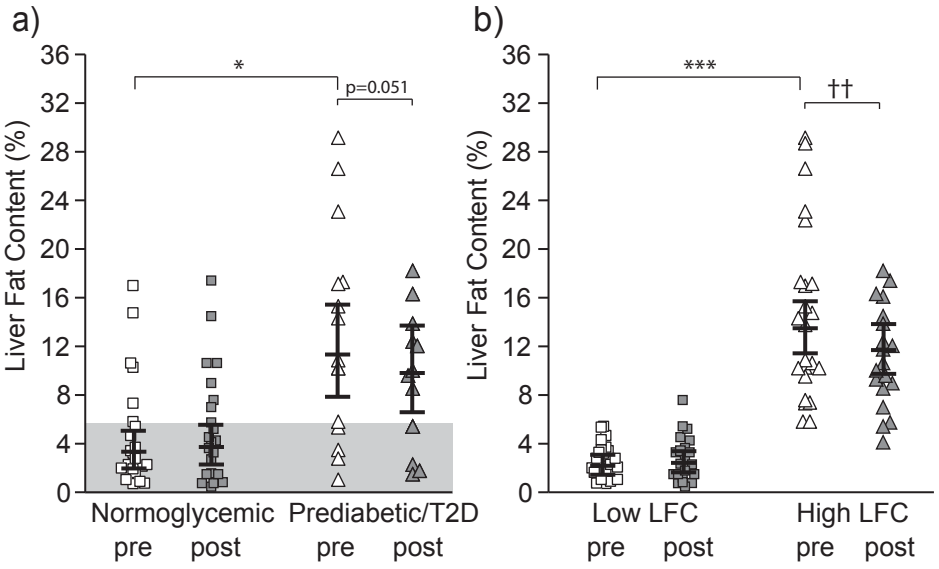
a)
b)
Liver Fat Content (%)
36
32
28
24
20
16
12
8
4
0
*
p=0.051
***
††
Normoglycemic
Prediabetic/T2D
Low LFC
High LFC
pre
post
pre
post
pre
post
pre
post

Figure 4

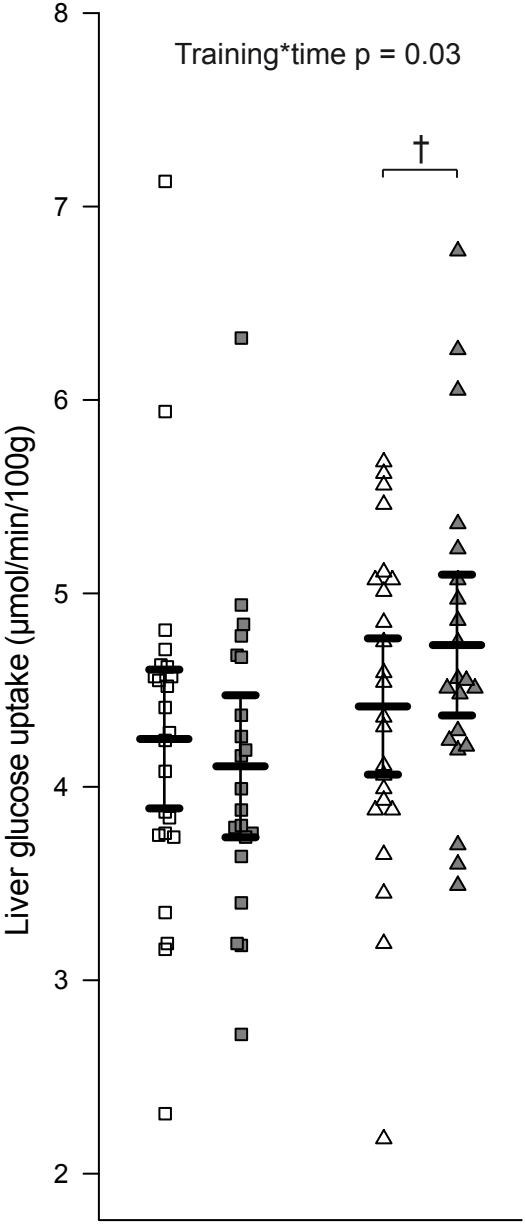
Training*time p = 0.03
†
Liver glucose uptake (µmol/min/100g)
8
7
6
5
4
3
2
SIT
pre
post
MICT
pre
post

Table 1: Effects of exercise training on liver fat content, liver volume, liver substrate uptake and liver enzyme inflammatory profile in prediabetic and T2D men (n = 16).

Parameter	Prediabetic		T2D		Baseline	Time	Dia*time
	Pre	Post	Pre	Post			
n	5	4	11	9			
Men/Women*	5		11				
Anthropometrics							
LFC (%)	15.0 [6.8, 23.1]	14.1 [5.9, 22.2]	11.8 [6.0, 17.5]	9.9 [4.1, 15.7]	0.52	0.06	0.48
Liver volume (mL)	1713 [1370, 2056]	1712 [1367, 2056]	1835 [1592, 2078]	1775 [1532, 2018]	0.55	0.23	0.24
PET data							
Liver GU (µmol/100g/min)	4.3 [3.9, 4.8]	4.2 [3.7, 4.7]	4.2 [3.9, 4.5]	4.4 [4.0, 4.7]	0.64	0.91	0.39
Liver FAU (µmol/100g/min)	10.1 [7.3, 12.9]	10.7 [7.6, 13.8]	8.1 [6.2, 10.0]	8.7 [6.6, 10.9]	0.22	0.52	0.99
EGP (µmol/min/kg)	18.1 [8.5, 27.8]	10.3 [0.4, 20.2]	18.6 [11.4, 25.9]	13.5 [6.1, 20.9]	0.85	**0.01**	0.48
Lean liver GU	78 [58, 99]	83 [67, 98]	76 [55, 97]	83 [68, 99]	0.70	0.84	0.73
Lean liver FAU	185 [134, 236]	157 [121, 193]	192 [137, 248]	156 [116, 195]	0.39	0.88	0.79
Inflammatory markers							
CRP§ (mg/L)	3.1 [1.1, 8.6]	1.1 [0.4, 3.3]	1.5 [0.7, 2.9]	0.7 [0.3, 1.5]	0.17	**0.01**	0.56
ALAT (U/L)	46.8 [32.8, 60.8]	39.7 [24.6, 54.9]	43.9 [34.5, 53.4]	36.5 [26.4, 46.7]	0.74	0.10	0.97
ASAT§ (U/L)	36.0 [26.9, 48.2]	29.4 [21.2, 40.8]	29.8 [24.5, 36.3]	23.4 [18.8, 29.1]	0.41	0.06	0.85
GT§ (U/L)	80.9 [42.7, 153.4]	36.2 [23.5, 55.8]	54.8 [28.1, 106.7]	29.1 [18.6, 45.6]	0.052	0.05	0.54

*Women were excluded from this analysis. All values are model based means [95% confidence intervals]. LFC, liver fat content; GU, glucose uptake; FAU, fatty acid uptake; EGP, endogenous glucose production; CRP, C-reactive protein; ALAT, alanine transaminase; ASAT, aspartate transaminase; GT, gamma-glutamyltranspeptidase. (§) Log transformation was performed to achieve normal distribution. P-value for baseline indicates the differences between the prediabetic and type 2 diabetic men. The p-value for time indicates the change between pre- and post-measurements in the whole study group. The p-value for Dia*time interaction indicates if the change in the parameter was different between prediabetic and type 2 diabetic men. Bolded p values are statically significant ($p \leq 0.05$)

Table 2: Subject characteristics between normoglycemic and prediabetic/T2D groups (all men n = 44) before and after exercise intervention.

Parameter	Normoglycemic		Prediabetic/T2D		Baseline	Time	Dia*time	Training*time
	Pre	Post	Pre	Post				
N	28		26*					
Age	48 [46, 50]		49 [48, 51]					
Men/Women*, n	28/0		16					
Prediabetic/T2D, n			5/11					
SIT/MICT, n	14/14		9/7					
Anthropometrics								
Weight (kg)	83.6 [79.7, 87.5]	83.3 [79.4, 87.2]	96.3 [91.2, 101.5]	96.2 [91.0, 101.3]	**<0.001**	0.22	0.80	0.36
BMI (kg/m^2)	26.1[25.1, 27.1]	26.0 [25.0, 27.0]	30.4 [29.1, 31.8]	30.4 [29.0, 31.7]	**<0.001**	**0.17**	0.70	0.30
Whole body fat§ (%)	22.6 [20.9, 24.3]	21.7 [20.0, 23.3]	28.8 [26.5, 31.2]	28.1 [25.7, 30.4]	**<0.001**	**<0.001**	**<0.001**	0.62
Subcutaneous fat mass§ (kg)	4.1 [3.7, 4.5]	4.0 [3.6, 4.4]	5.6 [4.9, 6.4]	5.5 [4.9, 6.4]	**<0.001**	**0.03**	0.93	0.65
Visceral fat mass& (kg)	3.1 [2.7, 3.4]	3.0 [2.6, 3.4]	4.2 [5.0, 3.6]	4.1 [4.8, 3.5]	**<0.001**	**0.002**	0.54	0.60
VO_{2peak} (mL/kg/min)	34.2 [32.7, 35.7]	35.7 [34.2, 37.2]	29.3 [27.2, 31.4]	30.0 [27.9, 32.1]	**<0.001**	**0.003**	0.23	**0.005**
Liver volume§ (mL)	1366 [1282, 1455]	1373 [1289, 1464]	1773 [1628, 1932]	1730 [1587, 1886]	**<0.001**	0.32	0.12	0.95
Glucose profile								
$Glucose_{fasting}$§ (mmol/L)	5.6 [5.4, 5.8]	5.5 [5.3, 5.7]	6.6 [6.3, 7.0]	6.6 [6.3, 7.0]	**<0.001**	0.86	0.71	0.83
$Glucose_{clamp}$ (mmol/L)	4.9 [4.8, 5.1]	4.9 [4.8, 5.1]	4.8 [4.6, 5.1]	5.0 [4.7, 5.2]	0.40	0.35	0.34	0.86
$Insulin_{fasting}$ FDGday§ (mU/L)	5.5 [4.3, 7.0]	5.4 [4.2, 6.9]	13.1 [9.3, 18.3]	12.0 [8.5, 17.0]	**<0.001**	0.46	0.66	0.14
$Insulin_{clamp}$ (mU/L)	75.4 [69.6, 81.2]	76.5 [70.5, 82.5]	87.6 [79.9, 95.4]	86.0 [77.8, 94.2]	**0.02**	0.92	0.57	0.46

EGP[&] (µmol/min/kg)	5.5 [2.4, 8.5]	4.2 [1.2, 7.2]	18.6 [13.5, 23.5]	13.0 [7.8, 18.4]	**<0.001**	0.38	0.10	0.60
Whole-body insulin sensitivity (M-value)[§] (µmol/min/kg)	35.3 [30.0, 40.6]	38.7 [33.3, 44.1]	17.5 [10.3, 24.8]	21.6 [14.2, 29.0]	**<0.001**	**<0.001**	0.11	0.06
HBA_1c (mmol/mol)	36.9 [35.2, 38.6]	34.8 [33.0, 36.5]	39.6 [37.3, 41.8]	37.5 [35.2, 39.9]	0.08	**<0.001**	0.75	0.38
Lipid profile								
$FFA_{fasting}$ (mmol/L)	0.70 [0.62, 0.77]	0.62 [0.54, 0.70]	0.69 [0.60, 0.78]	0.68 [0.58, 0.78]	0.86	**0.04**	0.11	**0.01**
FFA_{clamp}[&] (mmol/L)	0.065 [0.05, 0.08]	0.060 [0.05, 0.07]	0.093 [0.07, 0.12]	0.082 [0.06, 0.10]	**0.02**	0.15	0.70	0.76
Cholesterol (mmol/L)	5.0 [4.7, 5.3]	4.5 [4.1, 4.8]	4.8 [4.4, 5.3]	4.4 [3.9, 4.9]	0.51	**<0.001**	0.57	0.12
HDL[§] (mmol/L)	1.4 [1.2, 1.5]	1.3 [1.2, 1.4]	1.2 [1.1, 1.4]	1.1 [1.0, 1.2]	0.10	**<0.001**	0.66	0.19
LDL (mmol/L)	3.1 [2.9, 3.4]	2.8 [2.5, 3.1]	2.7 [2.3, 3.1]	2.6 [2.2, 3.0]	0.09	**0.001**	0.16	0.12
Triglycerides[§] (mmol/L)	0.9 [0.8, 1.1]	0.8 [0.7, 1.0]	1.7 [1.4, 2.1]	1.5 [1.2, 1.9]	**<0.001**	0.08	0.96	0.63
Inflammatory markers								
CRP[§] (mg/L)	1.0 [0.6, 1.7]	0.5 [0.3, 0.9]	1.9 [1.1, 3.5]	0.8 [0.4, 1.6]	0.81	**0.001**	0.78	0.75
ALAT[§] (U/L)	27.1 [23.1, 31.9]	23.3 [19.7, 27.6]	42.2 [34.0, 52.3]	34.5 [27.5, 43.2]	**<0.001**	**0.001**	0.62	0.27
ASAT[§] (U/L)	26.0 [23.2, 29.1]	22.7 [20.1, 25.7]	31.8 [27.3, 37.0]	25.3 [21.4, 29.8]	**0.047**	**0.003**	0.40	0.23
GT[§] (U/L)	24.0 [19.0, 30.3]	19.0 [15.0, 24.1]	47.7 [35.0, 65.0]	36.2 [26.3, 49.7]	**<0.001**	**<0.001**	0.70	0.59
PET data								
Lean liver GU	57 [50, 64]	59 [52, 67]	66 [57, 75]	67 [58, 76]	0.10	0.32	0.77	0.09
Lean liver FAU	129 [105, 153]	121 [95, 147]	137 [111, 163]	143 [115, 171]	0.60	0.93	0.50	0.25

* Women were excluded from the analysis to avoid mixing effects of gender. All values are model based means [95% confidence intervals]. T2D, type 2 diabetes; SIT, Sprint interval training; MICT, Moderate intensity continuous training; BMI, body mass index; VO_{2peak}, aerobic capacity; EGP, endogenous glucose production; HbA1c, glycosylated hemoglobin; FFA, free fatty acids; HDL, high density lipoprotein; LDL, low density lipoprotein; CRP, C-reactive protein; ALAT, alanine transaminase; ASAT, aspartate transaminase; GT, gamma-glutamyltranspeptidase; GU, glucose uptake; FAU, fatty acid uptake. (§) Log transformation and ($^{\&}$) square root transformation was performed to achieve normal distribution. P-value for baseline indicates the differences between the normoglycemic and prediabetic/T2D groups. The p-value for time indicates the change between pre- and post-measurements in the whole study group. The p-value for Dia*time interaction indicates if the change in the parameter was different between normoglycemic and prediabetic/T2D groups. The p-value for training* time interaction indicates if the change in the parameter was different between the SIT and MICT training modes.

Table 3: The baseline differences between normoglycemic and prediabetes/T2D (all men n = 44)* in different lipoprotein components and subclasses.

Parameter	Normoglycemic	Prediabetic/T2D*	p value
Lipids (mmol/L)			
Extremely-Large VLDL	0.01 [0.01, 0.02]	0.04 [0.03, 0.05]	<.0001
Extra-large VLDL	0.03 [0.02, 0.05]	0.09 [0.06, 0.13]	<.001
Large VLDL	0.17 [0.13, 0.22]	0.35 [0.26, 0.48]	<.0001
Medium VLDL	0.41 [0.34, 0.50]	0.67 [0.52, 0.87]	<.01
Small VLDL	0.047 [0.42, 0.54]	0.61 [0.51, 0.72]	0.047
IDL	1.06 [0.95, 0.17]	0.81 [0.70, 0.93]	<.01
Large LDL	1.29 [1.15, 1.44]	0.95 [0.82, 1.11]	<.001
Medium LDL	0.79 [0.71, 0.87]	0.59 [0.50, 0.69]	<.01
Small LDL	0.51 [0.46, 0.56]	0.40 [0.34, 0.46]	<.01
Small HDL	1.10 [1.06, 1.14]	1.19 [1.14, 1.25]	<.01
Phospholipids (mmol/L)			
Extremely-Large VLDL	0.001 [0.001, 0.001]	0.004 [0.003, 0.007]	<.0001
Extra-large VLDL	0.005 [0.003, 0.009]	0.012 [0.007, 0.022]	<.0001
Large VLDL	0.03 [0.02, 0.04]	0.06 [0.04, 0.08]	<.0001
Medium VLDL	0.006 [0.004, 0.009]	0.015 [0.009, 0.025]	<.001
Small VLDL	0.11 [0.10, 0.13]	0.15 [0.13, 0.17]	0.01
Extra-small VLDL	0.14 [0.13, 0.15]	0.12 [0.10, 0.13]	0.02
IDL	0.29 [0.27, 0.33]	0.22 [0.19, 0.25]	<.0001
Large LDL	0.33 [0.30, 0.36]	0.25 [0.23, 0.29]	<.001
Medium LDL	0.20 [0.19, 0.22]	0.17 [0.15, 0.19]	0.02
Extra-large HDL	0.16 [0.13, 0.20]	0.10 [0.07, 0.14]	0.02
Small HDL	0.54 [0.52, 0.56]	0.65 [0.62, 0.69]	<.0001
Cholesterol (mmol/L)			
Extremely-Large VLDL	0.001 [0.001, 0.002]	0.004 [0.002, 0.007]	<.001
Extra-large VLDL	0.005 [0.003, 0.008]	0.011 [0.007, 0.018]	<.001
Large VLDL	0.03 [0.02, 0.04]	0.06 [0.04, 0.09]	<.001
Extra-small VLDL	0.21 [0.19, 0.23]	0.17 [0.15, 0.19]	<.001
IDL	0.68 [0.61, 0.75]	0.49 [0.41, 0.57]	<.01
Large LDL	0.90 [0.80, 1.01]	0.62 [0.51, 0.74]	<.0001
Medium LDL	0.54 [0.48, 0.61]	0.37 [0.30, 0.45]	<.01
Small LDL	0.33 [0.29, 0.37]	0.23 [0.19, 0.27]	<.01

Cholesterol esters (mmol/L)

Extra-Large VLDL	0.003 [0.002, 0.004]	0.006 [0.004, 0.010]	<.001
Large VLDL	0.02 [0.01, 0.03]	0.03 [0.02, 0.04]	<.001
Extra-small VLDL	0.14 [0.13, 0.16]	0.11 [0.10, 0.13]	<.001
IDL	0.48 [0.43, 0.53]	0.36 [0.30, 0.42]	<.01
Large LDL	0.64 [0.57, 0.73]	0.44 [0.36, 0.53]	<.001
Medium LDL	0.39 [0.34, 0.45]	0.25 [0.20, 0.31]	<.01
Small LDL	0.24 [0.21, 0.27]	0.16 [0.13, 0.19]	<.001
Small HDL	0.40 [0.38, 0.43]	0.36 [0.33, 0.39]	0.02

Free Cholesterol (mmol/L)

Extremely-Large VLDL	0.0004 [0.0003, 0.0007]	0.0024 [0.0014, 0.0043]	<.0001
Extra-large VLDL	0.002 [0.001, 0.004]	0.005 [0.003, 0.009]	<.001
Large VLDL	0.01 [0.01, 0.02]	0.04 [0.02, 0.05]	<.001
Medium VLDL	0.05 [0.03, 0.06}	0.08 [0.06, 0.10]	<.001
Small VLDL	0.07 [0.06, 0.07]	0.08 [0.07, 0.10]	0.04
Extra-small VLDL	0.07 [0.06, 0.07]	0.06 [0.05, 0.07]	0.03
IDL	0.20 [0.18, 0.22]	0.13 [0.11, 0.16]	<.0001
Large LDL	0.26 [0.23, 0.28]	0.18 [0.15, 0.21]	<.0001
Medium LDL	0.15 [0.14, 0.16]	0.12 [0.10, 0.13]	<.0001
Small LDL	0.09 [0.08, 0.10]	0.07 [0.06, 0.08]	<.01
Extra-large HDL	0.05 [0.04, 0.06]	0.03 [0.02, 0.04]	<.01
Large HDL	0.05 [0.04, 0.06]	0.03 [0.02, 0.04]	0.04
Small HDL	0.10 [0.10, 0.11]	0.12 [0.11, 0.12]	<.0001

Triglycerides (mmol/L)

Extremely-Large VLDL	0.01 [0.008, 0.013]	0.03 [0.020, 0.039]	<.0001
Extra-large VLDL	0.02 [0.02, 0.03]	0.06 [0.04, 0.09]	<.0001
Large VLDL	0.10 [0.08, 0.13]	0.22 [0.17, 0.30]	<.0001
Medium VLDL	0.22 [0.18, 0.27]	0.39 [0.31, 0.51]	<.0001
Small VLDL	0.19 [0.17, 0.22]	0.28 [0.24, 0.34]	<.0001
Extra-small VLDL	0.09 [0.08, 0.10]	0.12 [0.10, 0.13]	0.02
Medium HDL	0.03 [0.03, 0.04]	0.05 [0.04, 0.05]	<.001
Small HDL	0.04 [0.04, 0.05]	0.06 [0.05, 0.07]	<.0001

* Women were excluded from the analysis to avoid mixing effects of gender.T2D, type 2 diabetes; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein and HDL, high density lipoprotein.

Table 4: Correlations at baseline between the subcomponents in lipoprotein subclasses and general parameters (all men n = 44)*.

Components	Lipoprotein components	General parameters	r	p
Lipids	Extra-large HDL[§]	M-value[§]	0.49	<.001
	Extra-large HDL[§]	ALAT[§]	-0.38	0.01
	Extra-large HDL[§]	Liver volume[§]	-0.50	<.001
	Small HDL	M-value[§]	-0.46	<.001
	Small HDL	VO_{2peak}	-0.37	0.01
	Small HDL	LFC[&]	0.35	0.02
	Small HDL	Liver volume[§]	0.36	0.01
Phospholipids	Extra-large HDL[&]	M-value[§]	0.35	0.01
	Extra-large HDL[&]	Liver volume[§]	-0.39	<.001
	Small HDL[§]	M-value[§]	-0.59	<.001
	Small HDL[§]	VO_{2peak}	-0.54	<.001
	Small HDL[§]	LFC[&]	0.50	<.001
	Small HDL[§]	Liver volume[§]	0.46	<.001
Cholesterol	Extra-large HDL[§]	M-value[§]	0.42	<.01
	Extra-large HDL[§]	ALAT[§]	-0.37	0.01
	Extra-large HDL[§]	Liver volume[§]	-0.43	<.001
Cholesterol esters	Extra-large HDL[§]	Liver volume[§]	-0.29	0.03
Free cholesterol	Extra-large HDL[§]	M-value[§]	0.44	<.01
	Extra-large HDL[§]	ALAT[§]	-0.29	0.03
	Extra-large HDL[§]	Liver volume[§]	-0.44	<.001
	Small HDL[§]	M-value[§]	-0.50	<.001
	Small HDL[§]	LFC[&]	0.41	0.01
	Small HDL[§]	Liver volume[§]	0.40	<.01
Triglycerides	Small HDL[§]	M-value[§]	-0.66	<.001
	Small HDL[§]	VO_{2peak}	-0.42	<.001
	Small HDL[§]	ALAT[§]	0.41	<.001
	Small HDL[§]	LFC[&]	0.54	<.001
	Small HDL[§]	Liver volume[§]	0.52	<.001

* Women were excluded from the analysis to avoid mixing effects of gender. HDL, high density lipoprotein; M-value, whole-body insulin sensitivity; ALAT, alanine transaminase; VO_{2peak}, aerobic capacity; LFC, liver fat content. ([§]) Log transformation and ([&]) square root transformation was performed to achieve normal distribution.

Table 5: Subject anthropometrics, glucose lipid profiles and inflammatory markers between SIT and MICT exercise training modes (all men + women n = 54).

Parameter	SIT		MICT		Training*time
	Pre	Post	Pre	Post	
n	27		27		
Men/Women	23/4		21/6		
Anthropometrics					
Weight (kg)	86.6 [82.3, 90.8]	86.1 [81.8, 90.4]	88.1 [83.9, 92.3]	87.8 [83.6, 92.0]	0.63
BMI (kg/m^2)	27.8 [26.6, 29.0]	27.6 [26.4, 28.8]	28.7 [27.5, 29.9]	28.6 [27.4, 29.7]	0.60
Whole body fat[§] (%)	26.2 [23.5, 29.3]	25.1 [22.5, 28.1]	27.1 [24.3, 30.3]	26.2 [23.4, 29.2]	0.65
Subcutaneous fat mass[§] (kg)	5.5 [4.6, 6.4]	5.3 [4.4, 6.3]	5.7 [4.8, 6.7]	5.6 [4.7, 6.6]	0.57
Visceral fat mass[&] (kg)	3.0 [2.4, 3.7]	2.9 [2.3, 3.6]	3.1 [2.5, 3.7]	2.9 [2.3, 3.5]	0.43
VO_{2peak} (ml/kg/min)	31.1 [28.9, 33.2]	32.7 [30.5, 34.9]	30.6 [28.5, 32.8]	31.1 [28.9, 33.3]	0.053
Liver volume[§] (ml)	1417 [1329, 1510]	1398 [1311, 1491]	1547 [1455, 1646]	1543 [1450, 1642]	0.56
Glucose profile					
$Glucose_{fasting}$[§] (mmol/L)	6.1 [5.7, 6.4]	6.0 [5.7, 6.4]	6.0 [5.7 ,6.4]	5.9 [5.6, 6.2]	0.44
$Glucose_{clamp}$ (mmol/L)	4.9 [4.7, 5.1]	4.9 [4.7, 5.1]	4.9 [4.7, 5.1]	5.0 [4.8, 5.2]	0.56
$Insulin_{fasting}$ FDGday[§] (mU/L)	7.9 [6.2, 10.0]	7.0 [5.5, 8.9]	7.4 [5.8, 9.3]	7.6 [6.0, 9.7]	0.19
$Insulin_{clamp}$ (mU/L)	80.7 [74.7, 86.7]	80.5 [74.2, 86.7]	80.7 [74.8, 86.5]	82.8 [76.6, 89.0]	0.56
EGP[&] (µmol/min/kg)	5.8 [4.2, 7.7]	5.9 [4.2, 7.8]	6.6 [4.9, 8.5]	7.1 [5.2, 9.3]	0.76

Whole-body insulin sensitivity (M-value)[§] (µmol/min/kg)	25.0 [20.5, 30.5]	30.6 [25.0, 37.4]	20.6 [16.9, 25.1]	23.6 [19.2, 28.9]	0.47
HBA_1c (mmol/mol)	37.3 [35.7, 38.9]	35.9 [34.3, 37.5]	38.4 [36.9, 40.0]	35.9 [34.2, 37.5]	0.14
Lipid profile					
$FFA_{fasting}$ (mmol/L)	0.69 [0.61, 0.77]	0.68 [0.59, 0.76]	0.81 [0.73, 0.88]	0.71 [0.63, 0.79]*	**0.03**
FFA_{clamp}[&] (mmol/L)	0.071 [0.06, 0.09]	0.063 [0.05, 0.08]	0.082 [0.07, 0.10]	0.067 [0.05, 0.08]	0.58
Cholesterol (mmol/L)	5.0 [4.7, 5.4]	4.4 [4.0, 4.7]	4.9 [4.6, 5.3]	4.6 [4.2, 4.9]	0.07
HDL[§] (mmol/L)	1.3 [1.2, 1.5]	1.2 [1.1, 1.3]	1.3 [1.2, 1.5]	1.3 [1.1, 1.4]	0.23
LDL (mmol/L)	3.0 [2.7, 3.3]	2.6 [2.3, 2.9]	3.0 [2.6, 3.3]	2.7 [2.4, 3.1]	0.09
Triglycerides[§] (mmol/L)	1.2 [1.0, 1.4]	1.1 [0.9, 1.3]	1.2 [1.0, 1.4]	1.0 [0.9, 1.3]	0.83
Inflammatory markers					
CRP[§] (mg/L)	1.2 [0.8, 2.0]	0.6 [0.4, 1.0]	1.6 [1.0, 2.4]	0.7 [0.4, 1.0]	0.64
ALAT[§] (U/L)	31.3 [26.3, 37.2]	25.2 [21.1, 30.0]	29.8 [25.2, 35.3]	26.4 [22.2, 31.5]	0.27
ASAT[§] (U/L)	27.1 [24.0, 30.5]	21.7 [19.2, 24.6]	26.3 [23.4, 29.5]	24.7 [21.8, 28.1]	0.10
GT[§] (U/L)	28.3 [22.0, 36.3]	22.6 [17.5, 29.1]	29.8 [23.3, 38.1]	22.6 [17.6, 29.0]	0.52

All values are model based means [95% confidence intervals]. BMI, body mass index; VO_{2peak}, aerobic capacity; EGP, endogenous glucose production; HbA1c, glycosylated hemoglobin; FFA, free fatty acids; HDL, high density lipoprotein; LDL, low density lipoprotein; CRP, C-reactive protein; ALAT, alanine transaminase; ASAT, aspartate transaminase; GT, gamma-glutamyltranspeptidase. ([§]) Log transformation and ([&]) square root transformation was performed to achieve normal distribution. The p-value for training* time interaction indicates if the change in the parameter was different between the SIT and MICT training modes. * p-value was significant for the MICT group.

Supplementary table 1: Subject characteristics between prediabetic/T2D men and women before and after exercise intervention.

Parameter	Prediabetic/T2D men		Prediabetic/T2D women		Baseline	Time	Sex*time
	Pre	Post	Pre	Post			
N	16		10				
Anthropometrics							
Weight (kg)	96.5 [90.3, 102.7]	96.3 [90.1, 102.5]	84.3 [76.4, 92.2]	83.3 [75.4, 91.2]	**0.02**	**0.03**	0.13
BMI (kg/m^2)	30.5 [29.0, 32.0]	30.4 [28.9, 31.9]	30.4 [28.5, 32.3]	30.0 [28.1, 32.0]	0.97	0.03	0.10
Whole body fat§ (%)	28.5 [26.4, 30.8]	27.7 [25.6, 29.9]	40.7 [36.8, 45.1]	39.4 [35.6, 43.6]	**<.0001**	**0.01**	0.81
Subcutaneous fat mass§ (kg)	6.0 [5.1, 7.1]	5.9 [5.0, 7.4]	9.1 [7.4, 11.2]	8.8 [7.2, 10.8]	**<.001**	**0.03**	0.23
Visceral fat mass& (kg)	4.3 [3.6, 5.2]	4.1 [3.4, 5.0]	2.4 [1.7, 3.2]	2.3 [1.6, 3.1]	**<.001**	**0.01**	0.83
VO_{2peak} (mL/kg/min)	29.3 [27.4, 31.2]	29.9 [28.0, 31.9]	23.7 [21.3, 26.2]	24.3 [21.7, 26.8]	**<.001**	0.15	0.95
Liver volume§ (mL)	1802 [1655, 1950]	1760 [1611, 1908]	1382 [1200, 1564]	1360 [1176, 1543]	**<.001**	0.13	0.62
Liver fat content (%)	11.3 [7.2, 16.4]	9.9 [6.0, 14.7]	9.4 [4.7, 15.6]	8.2 [3.8, 14.1]	0.62	**0.02**	0.91
Glucose profile							
$Glucose_{fasting}$§ (mmol/L)	6.6 [6.2, 7.1]	6.6 [6.2, 7.1]	6.6 [6.1, 7.2]	6.4 [5.8, 6.9]	0.95	0.27	0.21
$Glucose_{clamp}$ (mmol/L)	4.8 [4.7, 4.9]	5.0 [4.8, 5.1]	4.9 [4.8, 5.1]	4.9 [4.7, 5.1]	0.25	0.39	0.30
$Insulin_{fasting}$ FDGday§ (mU/L)	13.1 [9.3, 18.4]	12.1 [8.5, 17.2]	8.5 [5.6, 12.8]	8.2 [5.4, 12.6]	0.09	0.49	0.74
$Insulin_{clamp}$ (mU/L)	87.6 [80.7, 94.6]	85.8 [78.3, 93.2]	85.0 [76.7, 93.2]	90.2 [80.5, 100.0]	0.59	0.61	0.29

EGP[&] (µmol/min/kg)	15.8 [9.5, 23.6]	11.1 [5.9, 17.9]	14.1 [6.9, 23.8]	8.1 [2.9, 16.0]	0.60	**<.01**	0.52
Whole-body insulin sensitivity (M-value)[§] (µmol/min/kg)	17.5 [11.6, 23.5]	21.8 [15.6, 27.9]	19.9 [12.7, 27.0]	22.2 [14.6, 29.9]	0.66	0.07	0.59
HBA_1c (mmol/mol)	39.6 [37.0, 42.1]	37.6 [35.0, 40.2]	39.5 [36.3, 42.8]	37.7 [34.4, 41.0]	0.99	**<.01**	0.88
Lipid profile							
$FFA_{fasting}$ (mmol/L)	0.69 [0.61, 0.77]	0.68 [0.60, 0.77]	0.96 [0.85, 1.07]	0.91 [0.79, 1.04]	**<.0001**	**0.38**	0.56
FFA_{clamp}[&] (mmol/L)	0.09 [0.07, 0.12]	0.08 [0.06, 0.11]	0.08 [0.06, 0.12]	0.06 [0.03, 0.09]	0.78	0.07	0.41
Cholesterol (mmol/L)	4.8 [4.3, 5.3]	4.4 [3.9, 4.9]	5.0 [4.4, 5.7]	4.5 [3.9, 5.2]	0.50	**0.01**	0.67
HDL[§] (mmol/L)	1.2 [1.1, 1.4]	1.1 [0.9, 1.2]	1.5 [1.2, 1.7]	1.4 [1.2, 1.7]	0.052	**0.02**	0.12
LDL (mmol/L)	2.7 [2.3, 3.1]	2.6 [2.2, 3.0]	2.9 [2.4, 3.5]	2.4 [1.9, 3.0]	0.43	**0.01**	0.14
Triglycerides[§] (mmol/L)	1.7 [1.3, 2.2]	1.5 [1.1, 2.0]	1.2 [0.9, 1.7]	1.2 [0.9, 1.7]	0.12	0.55	0.69
Inflammatory markers							
CRP[§] (mg/L)	1.9 [1.1, 3.2]	0.9 [0.5, 1.5]	2.0 [1.0, 3.8]	1.3 [0.6, 2.7]	0.94	<.01	0.26
ALAT[§] (U/L)	41.5 [33.0, 52.3]	33.8 [26.5, 43.2]	26.6 [19.8, 35.6]	22.9 [16.8, 31.2]	**0.01**	**0.04**	0.72
ASAT[§] (U/L)	31.6 [27.1, 36.8]	25.2 [21.3, 29.8]	22.6 [18.6, 27.5]	21.9 [17.7, 27.0]	**0.02**	**0.08**	0.19
GT[§] (U/L)	47.3 [33.6, 66.6]	35.9 [25.3, 51.1]	25.1 [16.2, 38.8]	19.5 [12.4, 30.4]	**0.047**	**<.001**	0.91

All values are model based means [95% confidence intervals]. T2D, type 2 diabetes; BMI, body mass index; VO_{2peak}, aerobic capacity; EGP, endogenous glucose production; HbA1c, glycosylated hemoglobin; FFA, free fatty acids; HDL, high density lipoprotein; LDL, low density lipoprotein; CRP, C-reactive protein; ALAT, alanine transaminase; ASAT, aspartate transaminase; GT, gamma-glutamyltranspeptidase. ([§]) Log

transformation and ([&]) square root transformation was performed to achieve normal distribution. P-value for baseline indicates the differences between the prediabetic/T2D men and women. The p-value for time indicates the change between pre- and post-measurements in the whole study group. The p-value for Sex*time interaction indicates if the change in the parameter was different between men and women in the prediabetic/T2D group.