Two weeks of moderate intensity continuous training, but not high intensity interval training increases insulin-stimulated intestinal glucose uptake

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Short Title: Exercise training and intestinal metabolism

Financial support:

This study was conducted within the Centre of Excellence in Cardiovascular and Metabolic Diseases and supported by the Academy of Finland, the University of Turku, Turku University Hospital, and Åbo Akademi University. The study was financially supported by the European Foundation for the Study of Diabetes, the Emil Aaltonen Foundation, the Hospital District of Southwest Finland, the Orion Research Foundation, the Finnish Diabetes Foundation, the Ministry of Education of the State of Finland, the Academy of Finland (grants 251399, 251572, 256470, 281440, and 283319), the Paavo Nurmi Foundation, the Novo Nordisk Foundation and the Centre of Excellence funding.

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***Keywords**:* Intestine, intestinal metabolism, high intensity interval training, moderate intensity continuous training, exercise, positron emission tomography.

***Conflict of interest**:* No conflict of interest

ABSTRACT

Similar to muscles, the intestine is also insulin resistant in obese subjects and subjects with impaired glucose tolerance. Exercise training improves muscle insulin sensitivity, but its effects on intestinal metabolism are not known. We studied the effects of high intensity interval training (HIIT) and moderate intensity continuous training (MICT) on intestinal glucose and free fatty acid uptake from circulation in humans. Twenty-eight healthy middle-aged sedentary men were randomized for two weeks of HIIT or MICT. Intestinal insulin-stimulated glucose uptake and fasting free fatty acid uptake from circulation were measured using positron emission tomography and [^{18}F]FDG and [^{18}F]FTHA. In addition, effects of HIIT and MICT on intestinal Glut2 and CD36 protein expression were studied in rats. Training improved aerobic capacity ($p=0.001$) and whole-body insulin sensitivity ($p=0.04$), but not differently between HIIT and MICT. Insulin-stimulated glucose uptake increased only after the MICT in the colon [HIIT=0%; MICT=37%] ($p=0.02$ for time*training) and tended to increase in the jejunum [HIIT=-4%; MICT=13%] ($p=0.08$ for time*training). Fasting free fatty acid uptake decreased in the duodenum in both groups [HIIT=-6%; MICT=-48%] ($p=0.001$ time) and tended to decrease in the colon in the MICT group [HIIT=0%; MICT=-38%] ($p=0.08$ for time*training). In rats, both training groups had higher Glut2 and CD36 expression compared to control animals. This study shows that already two weeks of MICT enhances insulin-stimulated glucose uptake while both training modes reduce fasting free fatty acid uptake in the intestine in healthy middle-aged men, providing an additional mechanism by which exercise training can improve whole body metabolism.

New & Noteworthy

This is the first study where the effects of exercise training on the intestinal substrate uptake have been investigated using the most advanced techniques available. We also show the importance of exercise intensity in inducing these changes.

INTRODUCTION

The intestine is a large organ and a major determinant of whole body energy homeostasis through its control over nutrient absorption and release of gut hormones during digestion (6). Evidence demonstrating the potential role of the intestine in the pathogenesis of obesity and insulin resistance is rapidly increasing. In type 2 diabetes, there is a continuous deterioration of intestinal endocrine function (16) and alterations in the intestinal microbiota content have been shown to be associated with the development of insulin resistance in humans and animals (8; 9; 26). Splanchnic glucose uptake (SGU) accounts up to 60 % of total glucose metabolism after an oral glucose load. In insulin resistance splanchnic glucose uptake is impaired and plays a role in the pathogenesis of hyperglycaemia in type 2 diabetes.(10; 27)We have previously shown that tissue-specific intestinal glucose uptake from circulation into enterocytes is impaired in insulin stimulated state, i.e. intestinal insulin resistance exists, in obese and type 2 diabetic subjects (29). The role of intestinal insulin resistance in the pathology of type 2 diabetes is unclear, however, it has been suggested that intestinal insulin resistance leads to abnormalities in the signalling mechanism responsible for the Glut2 mediated glucose uptake in the small intestine, particularly in the jejunum, leading to increased transepithelial or lumen to blood glucose exchange, causing hyperglycemia (3).

Regular exercise training enhances skeletal muscle insulin sensitivity (11; 20; 23; 35) in working muscles. Exercise training also enhances the regulation and utilization of lipids in the skeletal muscle (13; 19; 22; 42). The training-induced adaptations in muscle substrate metabolism and oxidative capacity lead to improvements in the whole body metabolism and insulin sensitivity. Although muscle is widely studied, previous data about the effects of exercise on abdominal organs concerns mainly on liver and pancreas and data is limited about the effects of exercise on intestine (28; 33; 33; 36). Thus, it is not known whether exercise training could enhance intestinal substrate metabolism, and whether any possible changes would be reflected in the insulin sensitivity of the whole body.

We have previously shown that two weeks of low volume high intensity interval training (HIIT) and moderate intensity training (MICT) increases both aerobic capacity and whole body and main working skeletal muscle insulin-stimulated glucose uptake (GU) in sedentary middle-aged men (7). In the present study, using the intestine data from

this same clinical trial (NCT01344928) our aim was to quantify the effects of exercise on tissue-specific insulin-stimulated glucose and fasting free fatty acid uptake (FAU) from circulation into the intestine (duodenum, jejunum and colon) using positron emission tomography (PET) and radiotracers 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) and 14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid (FTHA) before and after HIIT and MICT. We hypothesized that the higher training volume instead of the intensity would strain the intestinal metabolism more and thus lead to the increased intestinal insulin-stimulated GU and decreased FAU after MICT compared to HIIT. Additionally, to explore possible mechanisms behind the changes in intestinal GU and FFAU, we also studied healthy Wistar rats, which underwent corresponding HIIT and MICT interventions and analysed the intestinal protein expression of Glut2 and CD36. We hypothesized that training would increase the expression of Glut2 and CD 36 in enterocytes more after MICT than HIIT.

MATERIALS AND METHODS

Subjects

Twenty-eight, middle-aged sedentary individuals were recruited and randomized into two groups; one with two weeks of high intensity interval training (HIIT) and the other with two weeks of moderate intensity continuous training (MICT). The subjects were non-obese (aged 40-55 years, VO_{2peak} < 40 ml·kg^{-1}·min^{-1}) and had no previous experience of active exercise training. The inclusion and exclusion criteria of the recruitment process have been described in detail previously (24). Two of the subjects withdrew during the intervention, one from the HIIT group due to exercise-induced hip pain and one from MICT group due to personal reasons; this left thirteen subjects in each group. The purpose, nature, and potential risks involved in participating in the study were explained in detail and informed consent was obtained before any measurements were performed. The study was approved (NCT01344928) 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.

Study design

Initial screening included a physical examination, an oral glucose tolerance test (OGTT), and a VO_{2peak} test to assess the participant's health, glycemic status, and aerobic capacity. The participants then underwent two PET imaging sessions on two different days. On the first day 14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid ([^{18}F]FTHA) and PET was used to measure, under a fasting state, the free fatty acid uptake in different intestinal regions (duodenum, jejunum, and colon) and the quadriceps femoris (QF) and deltoid muscles (the muscle results were taken from our previous publication (10)). On the second day 2-[^{18}F] fluoro-2-deoxy-D-glucose ([^{18}F]FDG) and PET was used to measure the insulin-stimulated glucose uptake in the intestine and the muscles during hyperinsulinemia. Once again the muscle results used were from our previous publication (10). An overnight fast of at least 10 hours was required before the OGTT and PET measurements. Participants were also asked to abstain from any caffeinated and alcoholic beverages, and to avoid strenuous exercise 48 hours prior to these studies. After the two weeks exercise training intervention, all measurements were repeated starting with [^{18}F]FTHA PET 48 hours after the last exercise session and continuing with a [^{18}F]FDG PET post 72 hours and finally an OGTT and VO_{2peak} test were done post 96 hours (Fig. 1).

Exercise interventions

Participants were randomized into HIIT and MICT exercise groups and both training groups had six supervised training sessions within two weeks. Each HIIT session consisted of 4-6 x 30 s exercise bouts of all out cycling efforts (Wingate protocol, load 7.5 % of the whole body weight, Monark Ergomedic 828E, Monark, Vansbro, Sweden) with 4 mins of recovery in between the exercise bouts (5). All the participants were familiarized with the HIIT training protocol (2 x 30 s bouts) before they were randomized into training groups. MICT training consisted of 40-60 min of cycling at a moderate intensity (60 % of VO_{2peak} intensity). In both groups, the training was progressive and in the HIIT group the number of cycling bouts increased from 4 to 5 and finally to 6, and in the MICT group the training time increased from 40 to 50 and then to 60 min in every second training session.

PET scans

Participants underwent four PET sessions: one [^{18}F]FTHA PET and one [^{18}F]FDG PET before and after the training intervention. Antecubital veins of both arms were cannulated for the PET studies. One catheter was used to inject the radiotracers [^{18}F]FTHA and [^{18}F]FDG while the other one was for blood sampling. To arterialize the venous blood samples the arm was heated using an electronically powered cushion. On the first PET scan session, intestinal free fatty acid uptake was measured using [^{18}F]FTHA PET in a fasting state. [^{18}F]FTHA radiotracer (155 [SEM 0.4] MBq) was injected and dynamic imaging of the abdominal region (frames 3x300 sec) was acquired starting on average at 46 minutes after the tracer injection. This was followed by a femoral region scanning (quadriceps femoris) (frames 3x300sec), starting approximately 65 min after the tracer injection. Finally, the shoulder region (deltoid) (frames 3x300 sec) was scanned starting approximately 90 min after the tracer injection. On the second day intestinal glucose uptake was measured using [^{18}F]FDG under euglycemic hyperinsulinemic clamp. On average 87 [SEM 1] minutes after the start of the clamp [^{18}F]FDG (156[SEM 0.5] MBq) was injected and similar time frames were acquired as described earlier for [^{18}F]FTHA scans, starting at 49, 70, and 90 minutes after the tracer injection. Arterialized blood samples were obtained at regular intervals during both the [^{18}F]FTHA and [^{18}F]FDG scans to measure the plasma radioactivity in order to calculate the tracer input function. . An automatic gamma counter (Wizard 1480, Wallac, Turku, Finland) was used to measure the plasma radioactivity. A GE Discovery TM ST system (General Electric Medical Systems, Milwaukee, WI, USA) was used to acquire the PET/CT images. CT images were acquired for anatomical references.

Image analysis

The imaging data obtained from the PET scanner was corrected for dead time, decay, and photon attenuation and the images were reconstructed using the 3D-OSEM method. Carimas 2.7 (www.pet.fi/carimas) was used to manually draw the regional tubular three-dimensional regions of interest (ROIs) on sections of the descending duodenum, the jejunum, and the transverse colon, using CT images as anatomical reference. The tubular ROIs were carefully drawn to outline the

intestinal wall while avoiding the intestinal contents and external metabolically active tissues (17). From these regional (duodenum, jejunum, and colon) ROI's time activity curves (TAC) were extracted.

The rate constant (K_i) for the uptake of the radiotracer ([^{18}F]FTHA, [^{18}F]FDG) into the cells was calculated using tissue TACs obtained from the duodenum, jejunum, and colon and a tracer input function using a fractional uptake rate (FUR) method as previously described (17). Regional glucose and free fatty acid uptakes were calculated by multiplying region specific K_i by the corresponding plasma glucose or free fatty acid concentration respectively. For glucose uptake the products were further divided by a lumped constant (LC) of 1.15 (17) and a recovery coefficient of 2.5 (17) was applied for the colonic glucose uptake to take into account the partial volume effect (4; 25). For the duodenal and jejunal glucose uptake, no recovery coefficient was needed. The ROI's for the deltoid and quadriceps femoris muscles were drawn as explained previously (7).

Maximal exercise test

As previously described (24) the maximal oxygen uptake (VO_{2peak}) was determined by performing an incremental bicycle ergometer test (Ergoline 800s, VIASYS Healthcare, USA) with direct respiratory measurements using a ventilation and gas exchange (Jaeger Oxycon Pro, VIASYS Helthcare, Germany) at the Paavo Nurmi Centre (Turku, Finland). Initial exercise intensity was 50 W and after every two minutes the exercise intensity was increased by 30 W until volitional exhaustion. VO_{2peak} was expressed as the highest 1 min mean oxygen consumption. 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}.

The euglycemic hyperinsulinemic clamp

The euglycemic hyperinsulinemic clamp technique was used as previously described (7; 39). Insulin was infused at a rate of 1mU/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. Euglycemic hyperinsulinemic clamp was performed after the subjects had fasted at least for 10 h. Insulin (Actrapid, 100 U/ml, Novo Nordisk, Bagsvaerd, Denmark) infusion was started with the rate of 40 mU/min/m^2 during the first 4 min. After 4 min and up to 7 min, infusion rate was reduced to 20 mU/min/m^2, and, after 7 min to the end of the clamp, it was kept constant at 10 mU/min/m^2. Glucose infusion was started 4 min after the start of the insulin infusion with a rate of subject's weight (kg)$\cdot 0.1^{-1} \cdot g^{-1} \cdot h^{-1}$. 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 the steady state during the PET scan that was started 87 min (SEM 1) after the start of the clamp. 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.

MRI

Adipose tissue depot masses were measured with MRI. MRI scans were performed using Philips Gyroscan Intera 1.5 T CV Nova Dual scanner (Philips Medical Systems, the Netherlands). Abdominal area axial T1 weighted dual fast field echo images (TE 2.3 and 4.7 ms, TR 120ms, slice thickness 10mm without gap) were obtained. To measure different adipose tissue masses the images were analyzed using SliceOmatic software v. 4.3

(http://www.tomovision.com/products/sliceomatic.htm). To obtain the mass the pixel surface area was multiplied with the slice thickness and the density of adipose tissue 0.9196 kg/l (1).

Other measurements

A two hour 75 g oral glucose tolerance test (OGTT) was conducted after the subjects had fasted for 12-hours. Blood samples were collected at 0, 15, 30, 60, 90, and 120 minutes after the glucose ingestion to determine the glucose and insulin levels. Measurements of oxidized LDL and oxidized HDL were based on spectrophotometric analyses of oxidized lipids in lipoproteins isolated by precipitation methods (2). Whole body fat percentage was measured at the Paavo Nurmi Centre using a bioimpedance monitor (InBody 720, Mega Electronics, Kuopio, Finland).

Animal study design

Twenty-four male Wistar rats were randomly divided into three groups: HIIT (n=8), MICT (n=8) and control (CON) (n=8). At the central animal laboratory of the University of Turku, the animals (aged between 8 to 12 weeks) were housed under standard conditions (temperature 21°C, humidity 55±5%, lights on from 6:00 a.m. to 6:00 p.m.) with free access to food and tap water. Before the exercise intervention rodents' body weight, body fat mass, and lean tissue mass were measured using EchoMRI-700 (Echo Medical Systems LLC, Houston, TX, USA), and OGTT and VO_{2max} test were performed, and free living energy consumption measured. Animals in the HIIT and MICT groups had 10 exercise sessions within two weeks. Each HIIT exercise session comprised of 8-10 x about 30 sec swimming bouts with 1 min resting period after each bout. Animals in the HIIT group had extra weights of 30 - 50 grams tied to the waist to force them to make all-out efforts. Animals in the MICT group started with 40 min swimming exercise and thereafter the exercise duration was increased by 10 minutes every second session until 80 min was reached in the last two sessions. In the MICT group, the rats did not bear any additional weights. One day after the last training session OGTT was performed which followed VO_{2max} tests on the second and third day after the last exercise session. Thereafter the animals were kept in the metabolic gages for two days. Animals were sacrificed five days after from the last exercise session and

intestinal samples from duodenum were collected for protein expression analyses. All animal procedures were approved by the National Animal Experimental Board (ESAVI/5053/04.10.03/2011) and performed in accordance with the guidelines of the European Community Council Directives 86/609/EEC.

Western blot

The frozen duodenal tissue pieces were homogenized on ice in a lysis buffer (150 mM NaCl, 1% NP-40, 0,5% Na-deoxycholate, 0,1% SDS, 50 mM Tris-HCl pH 8,0), supplemented with a protease inhibitor cocktail with an Ultra-Turrax T25 (Ika® -Werke GmbH & Co. KG). The protein concentration was then quantified with the Thermo Scientific Pierce™ BCA protein assay kit (Thermo Fisher Scientific) prior to the sample denaturation with SDS loading buffer containing β-mercaptoethanol (Sigma-Aldrich) in +95°C for 5 min. Samples were run on a 10% SDS–polyacrylamide gel and, after electrophoresis, transferred onto a nitrocellulose membrane (Santa Cruz Biotechnology, Inc.). An incubation with 5% (w/v) milk diluted in TBS-T (0,02 M Tris-buffered saline, 0,1% Tween-20) was used to block the unspecific binding sites prior to the overnight incubation in +4°C with the following primary antibodies: Glut2 (#07-1402, Millipore), CD36 (#sc-9154, Santa Cruz Biotechnology, Inc.), vascular endothelial growth factor 2 (VEGFR2) (#NB-100-530, Novus Biologicals) and β-actin (#sc-8432, Santa Cruz Biotechnology, Inc.). The fluorescent signal from the secondary antibodies IRDye® 800CW Donkey anti-Rabbit lgG (H+L) and IRDye® 800CW Donkey anti-Mouse lgG (H+L) (LI-COR Biosciences) was detected by using the LI-COR Odyssey® CLx Imager (LI-COR,Inc.). The intensities were normalized to a reference band in each membrane and the relative values were used for fold-change calculations.

Other measurements in rats

Body composition was measured using EchoMRI-700 (Echo Medical Systems LLC, Houston, TX, USA). Each animal was scanned before and after the exercise intervention and body fat mass and lean tissue mass was measured. The aerobic capacity was studied by measuring the VO_{2max} with rat single lane treadmill (Panlab- Harvard Apparatus, Spain). Animals were familiarized to the rat single lane treadmill (Panlab- Harvard Apparatus) for three days before the VO_{2max} test. The

test started after a warm up period. During the test the angle of the treadmill was 25° degrees and the speed was increased by 3 cm/s after every other minute until exhaustion. Oral glucose tolerance test (OGTT) was performed after 6 hours fast. Glucose (20%, wt/vol, 1 ml /100g) was administered orally and tail vein glucose was measured at 0, 30, 60, 90 and 120 min with a Precision Xceed Glucose Monitoring Device (Abbott Diabetes Care Ltd, Abbot Park, IL, USA). Whole body energy expenditure was measured with a metabolic gage (Oxylet system, Panlab, Harvard Apparatus, Spain) over 48 hours. The energy expenditure was calculated according to the measured carbon dioxide (CO_2) production and oxygen (O_2) consumption and averaged over 24 hours.

Statistics

Descriptive statistics shown in the tables and the figures are based on model based means [95 % confidence intervals, CI]. Association between the anthropometrics, glucose profile, and the lipid profile and the training groups, time points, and time*training interaction were performed with hierarchical linear mixed model, using the compound symmetry covariance structure for time. Transformations (logarithmic or square root) were done to ($insulin_{fasting}$, HDL, colonic, quadriceps femoris and deltoid glucose uptake; duodenal, jejunal, colonic and quadriceps femoris free fatty acid uptake) to achieve the normal distribution assumption. All tests were performed as 2-sided, with a significance level set at 0.05. Correlations were calculated using Pearson r. In the animal study, one-way analysis of variance was used. All the analyses were performed using SAS System, version 9.3 for Windows (SAS Institute Inc., Cary, NC, US).

RESULTS

Characteristics

The effects of exercise on whole-body fat percentage, aerobic capacity (VO_{2peak}), and whole body insulin sensitivity (M-value) have been published in our previous study (5). Total, LDL, and HDL cholesterol levels decreased significantly after

training (Table 1). In the cholesterols the only difference between the training modes was the greater decrease in LDL cholesterol in the HIIT group compared with the MICT group [p = 0.03, time*training].

Intestinal substrate uptake

Colonic insulin-stimulated glucose uptake improved in the MICT group (+ 37%) while no response was observed in the HIIT group (+/- 0%) (p = 0.02 time*training) (Fig. 2). Jejunal glucose uptake tended to respond differently between the training modes, with only MICT increasing the uptake (HIIT - 4%, MICT + 13 % p = 0.08 time*training) (Fig.2). Both exercise modes decreased the free fatty acid uptake in the duodenum (p = 0.001 time, Fig. 2) and MICT tended to also decrease the uptake in the colon (HIIT 0%, MICT -38%, p = 0.08 time*training, Fig. 2). The jejunal glucose uptake associated positively with aerobic capacity (VO_{2peak}) [Pre: r = 0.46, p = 0.03; Post: r = 0.45, p = 0.03] and negatively with visceral fat mass [Pre: r = - 0.42, p = 0.05; Post: r = - 0.45, p = 0.03]. Glucose uptake both in the jejunum [Pre: r = - 0.31, p = 0.15; Post: r = - 0.50, p = 0.02] and duodenum [Pre: r = - 0.12, p = 0.59; Post: r = - 0.53, p = 0.02] associated negatively with HcA1c levels. In the MICT group, the glucose uptake in the colon associated positively [Pre: r = 0.17, p = 0.63; Post: 0.68, p = 0.03] (Fig. 3) and the duodenal free fatty acid uptake negatively [Pre: r = -0.38, p = 0.31; Post: r= -0.94, p = 0.01] with the whole body glucose uptake after the training. Quadriceps femoris (QF) and deltoid muscle results in these subjects have been published elsewhere (10). For comparison purposes those results have been added to Fig. 2.

Animal results

There was a significant increase in the body weight and fat free mass of all the animal groups indicating to the age-related growth during the study intervention. (Table 2) While the fat percentage increased in the CON group, it significantly decreased in both HIIT and MICT groups after the training. There were no differences in glucose values at time points 0’ and 120’ or in the glucose AUC in any of the group*s. The aerobic capacity (VO_2 max) tended to improve significantly in both HIIT and MICT groups compared to the CON group (Pre: HIIT: 70.07 [66.2, 74.0]; MICT: 71.2 [67.3, 75.1]; CON: 69.0 [65.1, 72.9] (ml/min/kg^0.75); Post: HIIT: 72.9 [69.0, 76.8]; MICT: 72.8 [68.9, 76.7]; CON: 68.9 [65.0,

72.8] (ml/min/kg^0.75) [95 % CI], p = 0.05). Glut2 protein expression in the rat intestine was significantly higher in the HIIT and MICT groups compared to CON group (HIIT: 19090 [12930, 28190]; MICT: 11606 [7651, 17604]; CON: 4141 [2730, 2141] [95 % CI] (arbitrary units), $p < 0.01$). Also CD36 expression was higher in the HIIT and MICT groups compared to CON group (HIIT: 635 [366, 1100]; MICT: 696 [387, 558]; CON: 79 [44, 63] [95 % CI] (arbitrary units), $p < 0.05$). While VEGFR2 was only higher in the HIIT group compared to MICT and CON group (HIIT: 704 [477, 976]; MICT: 345 [193, 541]; CON: 294 [147, 491] [95 % CI] (arbitrary units), $p < 0.05$). No significant differences were observed in Glut2, CD36 or VEGFR2 expression between the HIIT and the MICT groups.

DISCUSSION

In the present study, the effects of two weeks of exercise training, HIIT and MICT, on intestinal substrate uptake from circulation were studied in healthy, untrained, middle-aged men. The data shows that MICT increases insulin-stimulated glucose uptake while both training modes decrease fasting free fatty acid uptake in the intestine and that intestinal insulin-stimulated glucose uptake correlates positively with aerobic capacity and negatively with visceral fat and HbA1c. In addition both training modes increased Glut2 and CD36 protein expressions in rat enterocytes. To our knowledge, this is the first study that provides evidence about the beneficial effects of exercise training on the intestinal substrate metabolism and an additional mechanism by which exercise improves whole body metabolism.

The intestinal glucose uptake values during hyperinsulinemia in the present study agree with our recent data in healthy lean controls and obese subjects (17; 29). Studies by Honka et al. (2014) and Mäkinen et al (2015) show that insulin increases the intestinal glucose uptake compared to fasting state in healthy lean controls but the increase is blunted in obese subjects. This means that the intestine is an insulin sensitive organ and intestinal insulin resistance exists in obesity. Furthermore, it was shown that in obese subject's intestinal insulin resistance is ameliorated after rapid weight loss (17; 29). In enterocytes, glucose is transported from blood to lumen by Glut2 transporter proteins (40). In obesity and intestinal insulin resistance there is an impairment in the insulin stimulated Glut2 internalisation in the enterocyte; which has been suggested to restrain the normal glucose uptake in the intestine (41). In the present study,

the insulin-stimulated intestinal glucose uptake before the training intervention was at the same level as the healthy controls in our previous study (29). Insulin-stimulated glucose uptake improved in the colon (+37%) and tended to improve in the jejunum in the MICT group after the training, while it remained essentially unchanged in the HIIT group. To study the mechanisms behind the exercise-induced improvements in intestinal glucose uptake in our human data, we performed corresponding short HIIT and MICT training interventions in healthy rats. As Glut2 is responsible for the uptake of glucose from basolateral membrane in the intestine (21) we hypothesized that exercise would increase the expression of Glut2 in enterocytes to enhance the intestinal glucose uptake and that the increase would be higher in MICT compared to HIIT due to higher training volume. We found that both HIIT and MICT increased intestinal Glut 2 expression in rats with no differences between the groups. The reason why the increased GU was seen only after MICT in humans, while Glut 2 expression increased in both training groups in rats is unclear. However, it might be that although two weeks of low volume HIIT was enough to induce changes in protein level in rats, longer time is need to be able to detect a change in tissue level non-invasively in humans.

The discrepancy in glucose uptake in different parts of the intestine agrees with the findings of Mäkinen and co-workers, and may be due to the differences in the location of Glut2 receptor in the enterocytes (41). In humans, Glut2 has been observed in the apical membrane of an enterocyte in the jejunum but not in the duodenum (3). The discrepancy in substrate uptake in different parts of the intestine is possibly also related to the different digestive tasks between the small and large intestines and how exercise training strains these mechanisms.

The results in this study demonstrate a decreased free fatty acid uptake in the duodenum after the training intervention in both training groups. The digestion and delivery of dietary fats throughout the body is mediated by the small intestine. In the small intestine, inside the enterocytes, the dietary fats are resynthesized into triacylglycerols (TAG) and secreted into the circulation or stored in cytoplasmic lipid droplets. Postprandially, the increased secretion of TAG from the small intestine leads to an increment in the circulating TAG levels; however, during a fast the levels decrease as a result of clearance by peripheral tissues (30). Recently, Hung and co-workers showed that in rodent's endurance training leads to enhanced lipid turnover and more efficient fatty acid oxidation for energy utilization within the enterocytes (18). Our data regarding the higher CD36 expression, in both HIIT and MICT trained rats, is in agreement

with the results of Hung et al. (18). In spite of the higher CD36 expression the reduced intestinal FFAU after training in the present study could be due to the more efficient fatty acid oxidation. This is because enhanced fatty acid oxidation means that less fatty acids are needed to produce the same amount of energy.

Another possible mechanism for the decreased intestinal FFAU could be the reduced free fatty acid flux in the intestine. In fact, we found in the present study an almost significant ($p = 0.052$, Table 1.) drop in the levels of circulating plasma free fatty acids after the training during the FTHA-PET study (fasting). The lower free fatty acid levels can be explained by decreased visceral fat mass and increased whole body insulin sensitivity post training, as both reduce the adipose tissue lipolysis and thereby circulating FFAs (Table 1) (31; 34; 38).

At the moment little is known about the different mechanisms how exercise training could strain the intestinal metabolism, yet some data exists about exercise and splanchnic bed. Splanchnic blood flow reduces during dynamic training and as a function of exercise intensity. However it has been shown that the reduction in splanchnic blood flow during exercise attenuates as a response to long term training. (32; 33) The smaller reduction in splanchnic blood flow during exercise after regular training seems to be related to the enhanced vasodilation and reduced vasoconstriction of splanchnic and renal vasculature which further could indicate improved nutrient supply and utilization during exercise in a trained state. (33) In the present study we did not measure intestinal blood flow in humans. In rodents we found higher VEGFR2 (a marker of angiogenesis) expression level in enterocytes in HIIT compared to MICT and CON group (Fig. 4). Thus angiogenesis could be also one factor explaining the attenuated reduction in the intestinal blood flow shown after exercise training (33). The difference in VEGFR2 levels between the groups in the present study might be due to higher transient reduction of flow into the splanchnic area during HIIT compared to MICT. HIIT is extremely intense exercise mode and during the intervals body concentrates to supply blood mainly to the working muscles which may induce hypoxic condition in splanchnic area and further stimulate intestinal angiogenesis. Other possible factors regulating intestinal metabolism could be peristaltic movements and colon transit time (37; 43).

We used two different training modes in this study. These both included six training sessions within an intervention period of two weeks. Both the time spent during the training (time HIIT 15 vs. MICT 300 minutes) and the average calculated energy consumption during the training (403 and 2680 kcal, respectively (7)) were much less in HIIT

than MICT. Despite this difference, both training modes improved whole body insulin sensitivity (M-value, HIIT 12% and MICT 7%) and aerobic capacity (VO_{2peak}, HIIT 6% and MICT 3%) without significantly different responses between the training modes. In contrast to this, intestinal metabolism seems to be more sensitive to MICT than HIIT. As intestine mediates the delivery of nutrients throughout the body, it may be that the aerobic training mode and longer exercise time per session in MICT compared to HIIT challenges the intestinal metabolism more and thus may be a more rapid and effective way to improve intestinal metabolism.

It is also possible that the difference in the daily habitual physical activity levels or in dietary intake affects to the observed findings. In the present study subjects were instructed not to perform any additional physical activity except daily normal living and they reported having done so. However no pedometer or any other device was used to follow the activity. Thus we cannot completely rule out the possible effect of habitual physical activity on our results. Subjects were also instructed to maintain their normal dietary habits and they kept dietary logs for three days before and during the exercise intervention. According to the dietary logs there were no changes in the total caloric intake or in the caloric content before and after the intervention in either study group (data not shown).

Most of the beneficial effects of exercise on the whole body are attributed to skeletal muscles and thus it is interesting to compare these intestinal findings to our previous findings concerning skeletal muscles in these same subjects (10). In skeletal muscles, both training modes increased insulin-stimulated GU in the main working muscle, the quadriceps femoris (QF), while no changes were observed in deltoid and other upper body muscles (Fig. 2). In addition, no significant changes were observed in the FFAU in any of the studied muscles. (10) Adding the findings from the present study to the overall picture, it is interesting to note that intestinal metabolism seems to respond more readily to MICT than the metabolism in the non-working upper body muscles (Fig. 2).

Previously intestinal insulin-stimulated glucose uptake has been shown to be associated with whole body glucose uptake (M-value), both in healthy and obese subjects (23). Our data is in line with these previous findings showing that whole body glucose uptake associates positively with insulin-stimulated glucose uptake in the colon and inversely with the duodenal free fatty acid uptake. Furthermore, the jejunal glucose uptake correlated positively with the VO_{2peak} and negatively with visceral fat mass and HbA1c, which are both known risk markers for metabolic diseases.

Thus, although exercise training induces major health benefits through the body's muscular system, also its effects on the intestine, with an average weight of 3-4 kg and surface of 200-300 m^2, warrants further research.

There are some limitations in this study. Firstly, the location of the intestine; this is because even though the duodenum has a relatively fixed location in the abdomen, the distal segments of the intestine move within the abdomen. This issue was addressed by confirming the drawn ROIs with a CT scan. Secondly, the results might have been affected by spill-over and partial volume effects due to the trans-axial resolution of the PET scanner and the thinness of the intestinal mucosal wall. However, this effect was demonstrated to be minimal in our previous validation study (17). Thirdly, in this study, we measured the substrate uptake from the circulation into the enterocytes without knowing the release from the enterocytes into the circulation (i.e. from lumen to circulation). Fourth, due to the radiation dose limits we could not perform the [^{18}F]FDG and [^{18}F]FTHA PET scans both at fast and during euglycemic hyperinsulinemic clamp. Thus we studied the FFAU at fasting state and GU during euglycemic hyperinsulinemic clamp, in situations when the FFAU and GU, respectively, are at highest. Finally, the exercise duration in this study was only two weeks. Although this kind of intervention has been shown to be effective (7; 12; 14; 15; 44), it must be emphasized that the findings show only the early training response and, therefore, the long term effects of these training modes on intestinal metabolism should be studied further in future experiments.

In conclusion, this study shows that intestinal insulin sensitivity associates positively with aerobic capacity and inversely with the metabolic risk markers visceral adiposity and HbA1C. Two weeks of regular training (HIIT and MICT) was shown to already improve aerobic capacity and whole body insulin sensitivity, and specifically MICT to induce positive changes in intestinal substrate metabolism in middle-aged, healthy men. The changes in intestinal substrate uptake seem to be related to improvements in Glut2 and CD36 protein levels. It is likely that regular long term training has pronounced effects on intestine and whole body metabolism and thus the role of exercise training on intestinal substrate uptake in patient populations warrant further studies.

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Figure 1. Study design: Subjects were studied on three separate days before and after the exercise intervention. OGTT, oral glucose tolerance test; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; PET, positron emission tomography; FTHA, 14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid ([^{18}F]FTHA); PET-FDG,[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG).

Figure 2. Insulin stimulated glucose uptake a) and fasting free fatty acid uptake b) in different tissues before and after two weeks of either high intensity interval training (HIIT) (▲ black) and moderate intensity continuous training (MICT) (▬ grey). The muscle (QF + Deltoid) results have been adapted from the Eskelinen et al 2015 (7). All values are expressed as model-based means and bars are confidence intervals [95 % CI]. P-value for time interaction (i.e. the groups behaved similarly for the change in parameter with no differences between the training modes). P-value for time*training interaction (i.e. the groups behaved differently for the change in parameter with significant difference between them). QF, quadriceps femoris; HIIT, high intensity interval training; MICT, moderate intensity continuous training.

Figure 3. Correlation between insulin-stimulated jejunal glucose uptake and VO_{2peak} a) and visceral fat mass b) in pooled analysis of MICT (▬ grey) and HIIT (▲ black) subjects'. In figure c) correlation between insulin-stimulated colonic glucose uptake and whole body glucose uptake (M-value) in MICT (▬ grey) subjects. VO_{2peak}, aerobic capacity; HIIT, high intensity interval training; MICT, moderate intensity continuous training.

Figure 4. a) Relative expression of CD36, Glut2 and VEGFR2 on in duodenum where *n* is 6-8. All values are expressed as model-based means with error bars representing the confidence intervals [95 % CI]* p-value <0.05. b) Western blots of CD36 (75kDa), Glut2 (55kDa) and VEGFR2 (105 kDa). Animals without detectable band were excluded from the analysis. HIIT, high intensity interval training; MICT, moderate intensity continuous training; CON, control group.

Figure 1

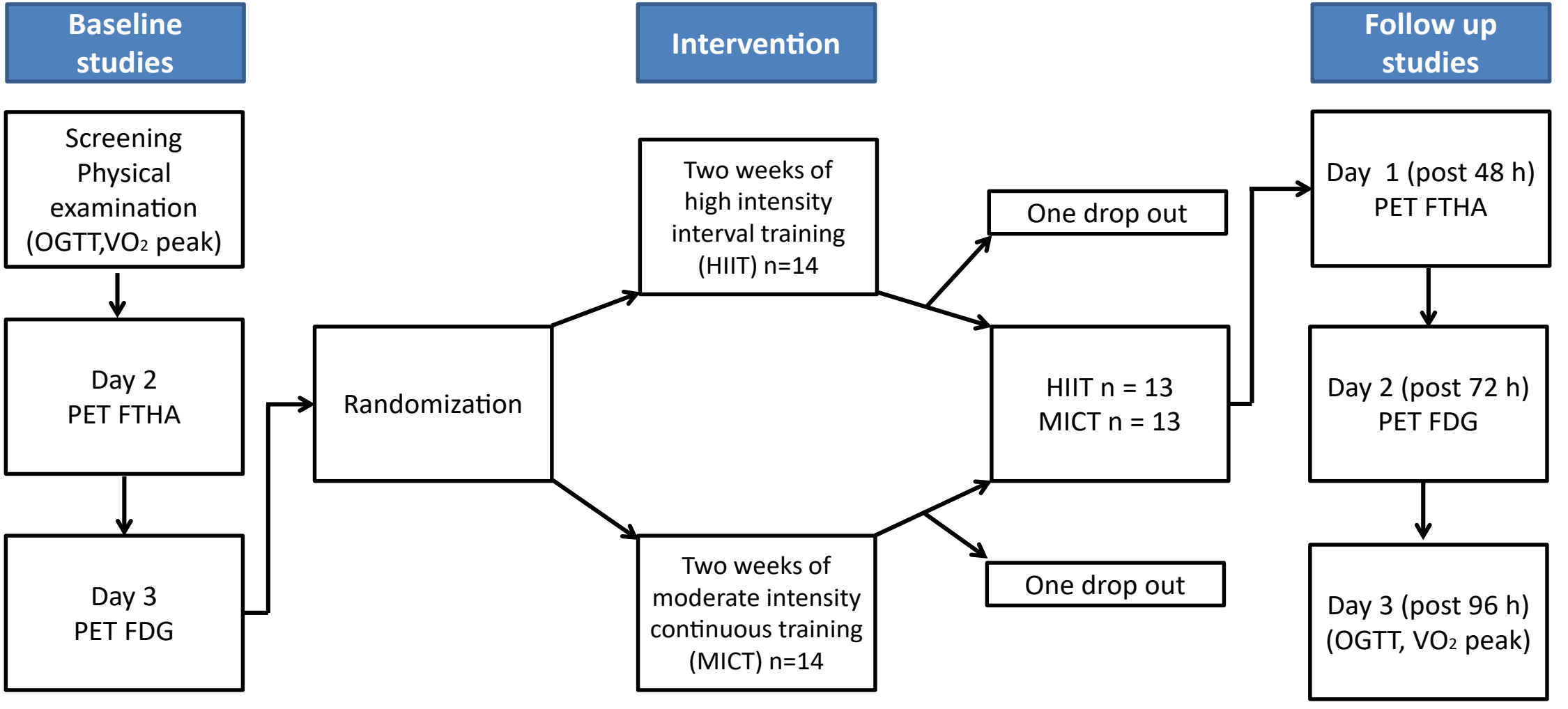

Baseline studies
Intervention
Follow up studies
Screening Physical examination (OGTT,VO2 peak)
Day 2 PET FTHA
Day 3 PET FDG
Randomization
Two weeks of high intensity interval training (HIIT) n=14
Two weeks of moderate intensity continuous training (MICT) n=14
One drop out
One drop out
HIIT n = 13
MICT n = 13
Day 1 (post 48 h) PET FTHA
Day 2 (post 72 h) PET FDG
Day 3 (post 96 h) (OGTT, VO2 peak)

Figure 2

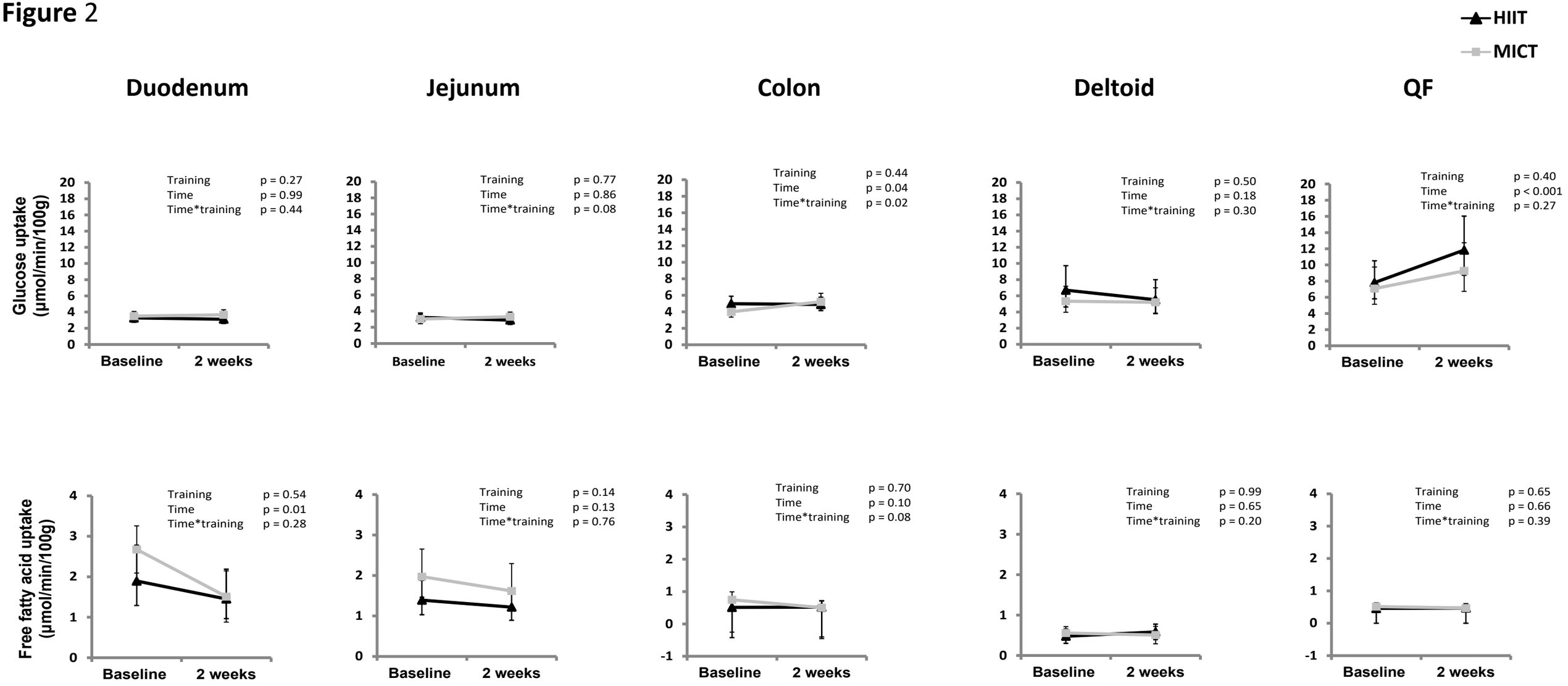
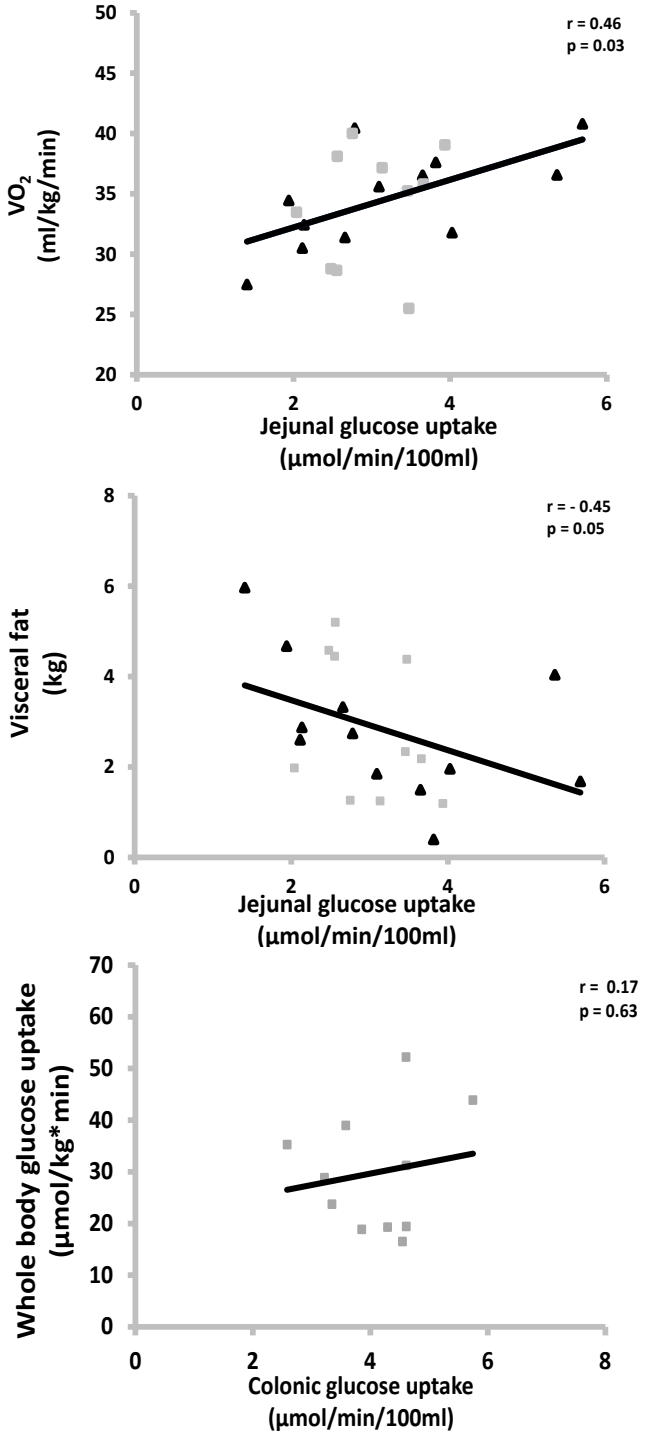
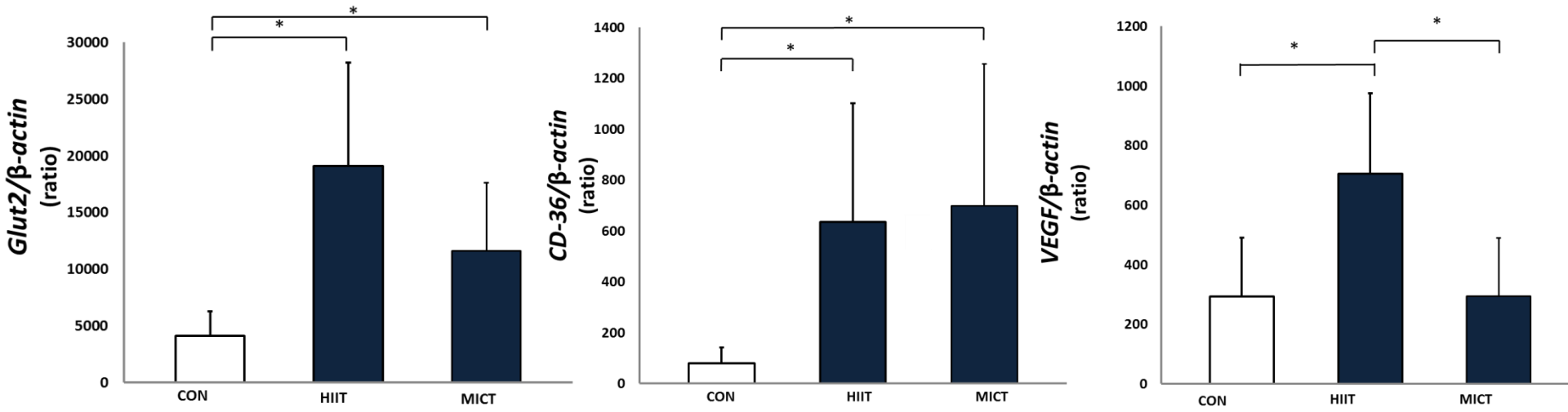

HIIT
MICT
Duodenum
Jejunum
Colon
Deltoid
QF
Glucose uptake (µmol/min/100g)
Free fatty acid uptake (µmol/min/100g)
Training p = 0.27
Time p = 0.99
Time*training p = 0.44
Training p = 0.77
Time p = 0.86
Time*training p = 0.08
Training p = 0.44
Time p = 0.04
Time*training p = 0.02
Training p = 0.50
Time p = 0.18
Time*training p = 0.30
Training p = 0.40
Time p < 0.001
Time*training p = 0.27
Training p = 0.54
Time p = 0.01
Time*training p = 0.28
Training p = 0.14
Time p = 0.13
Time*training p = 0.76
Training p = 0.70
Time p = 0.10
Time*training p = 0.08
Training p = 0.99
Time p = 0.65
Time*training p = 0.20
Training p = 0.65
Time p = 0.66
Time*training p = 0.39
Baseline
2 weeks

Figure 3


r = 0.46
p = 0.03
VO2 (ml/kg/min)
Jejunal glucose uptake (µmol/min/100ml)
r = - 0.45
p = 0.05
Visceral fat (kg)
Jejunal glucose uptake (µmol/min/100ml)
r = 0.17
p = 0.63
Whole body glucose uptake (µmol/kg*min)
Colonic glucose uptake (µmol/min/100ml)

a)


Glut2/β-actin (ratio)
30000
25000
20000
15000
10000
5000
0
CON
HIIT
MICT
*
*
CD-36/β-actin (ratio)
1400
1200
1000
800
600
400
200
0
CON
HIIT
MICT
*
*
VEGF/β-actin (ratio)
1200
1000
800
600
400
200
0
CON
HIIT
MICT
*
*

b)

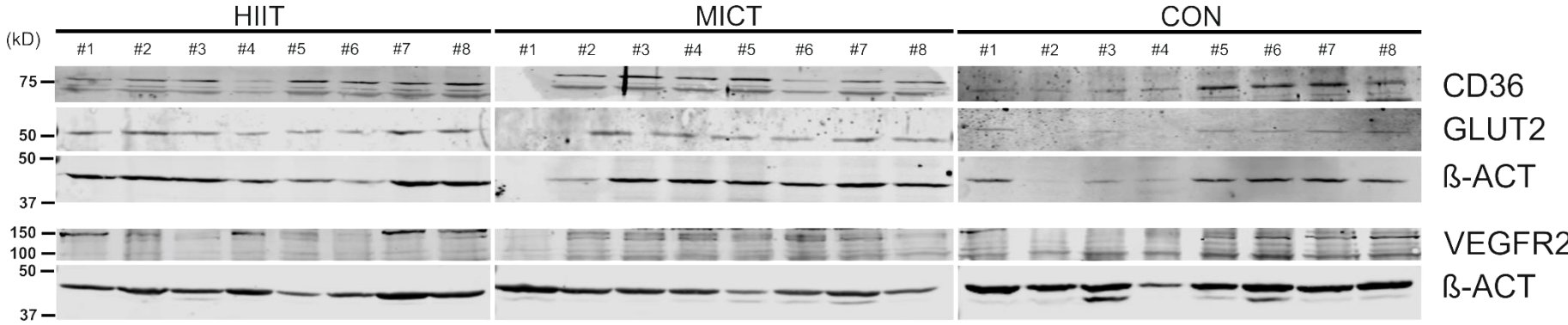

HIIT
MICT
CON
(kD)
#1 #2 #3 #4 #5 #6 #7 #8
75
50
50
37
150
100
50
37
CD36
GLUT2
ß-ACT
VEGFR2
ß-ACT

Table 1: Subject characteristics at baseline and after the exercise intervention

Parameter	HIIT n=13			MICT n=13			P value	
	Pre	Post	Δ %	Pre	Post	Δ %	Time	Time x group interaction
Anthropometrics								
BMI (kg/m^2)	25.9 [24.5, 27.3]	25.7 [24.3, 27]	-1	26.4 [25.0, 27,7]	26.4 [25.0, 27.7]	0	0.14	0.19
Whole body fat (%)	22.2 [19.8, 24.6]	21.2 [18.8, 23.6]	-5	22.9 [20.5; 25.3]	22.1 [19.7, 24.5]	-3	**<0.0001**	0.56
Subcutaneous fat mass (kg)	4.03 [3.3, 4.8]	3.93 [3.2, 4.7]	-2	4.44 [3.7, 5.2]	4.38 [3.6, 5.1]	-1	**0.04**	0.54
Visceral fat mass (kg)	2.91 [2.1, 3.8]	2.80 [1.9, 3.7]	-4	2.66 [1.7, 3.5]	2.59 [1.8, 3.4]	-3	**0.046**	0.73
VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{-1}$)	34.7 [32.4, 37.1]	36.7 [34.3, 39.1]	6	33.7 [31.3, 36]	34.7 [32.4, 37.1]	3	**0.001**	0.27
Glucose Profile								
$Glucose_{fasting}$ ($mmol \cdot l^{-1}$)	5.5 [5.3, 5.7]	5.4 [5.2, 5.6]	-1	5.7 [5.5, 5.9]	5.6 [5.4, 5,8]	-1	0.43	0.77
$Glucose_{clamp}$ ($mmol \cdot l^{-1}$)	5.0 [4.7, 5.3]	4.9 [4.6, 5.2]	-3	4.9 [4.5, 5.2]	5.0 [4.7, 5.3]	3	0.96	0.20
$Insulin_{fasting}$ ($mU \cdot l^{-1}$) †	5.2 [3.8, 7.2]	4.8 [3.4, 6.6]	-8	5.8 [4.1, 8.1]	6.0 [4.3, 8.5]	4	0.80	0.46
$Insulin_{clamp}$ ($mU \cdot l^{-1}$)	75.3 [66.8, 83.9]	73.8 [65.1, 82.6]	-2	75.4 [66.5, 84.3]	79.4 [70.3, 88.6]	5	0.64	0.31
HbA_{1c} (mmol/mol)	36.5 [34.3, 38.6]	35.2 [33.0, 37.4]	-4	37.4 [35.3, 39.5]	34.3 [32.1, 36.5]	-8	**<0.001**	0.11

M-value ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	38.2 [30.1, 46.4]	42.8 [34.5, 51.0]	12	31.9 [23.1, 40.7]	34.2 [25.4, 43.1]	7	**0.03**	0.45
Lipid Profile								
$FFA_{fasting}$ ($mmol \cdot l^{-1}$)	0.61 [0.50, 0.71]	0.59 [0.48, 0.70]	-3	0.78 [0.67, 0.89]	0.67 [0.54, 0.79]	-15	0.052	0.14
FFA_{clamp} ($mmol \cdot l^{-1}$)	0.06 [0.05, 0.08]	0.06 [0.05, 0.08]	0	0.08 [0.06, 0.10]	0.07 [0.05, 0.09]	-14	0.41	0.43
Cholesterol ($mmol \cdot l^{-1}$)	5.3 [4.8, 5.7]	4.6[4.1, 5.0]	-14	4.7 [4.3, 5.2]	4.4 [3.9, 4.9]	-7	**<0.001**	0.06
HDL ($mmol \cdot l^{-1}$) †	1.4 [1.2, 1.6]	1.2 [1.1, 1.4]	-10	1.4 [1.2, 1.5]	1.3 [1.1, 1.5]	-5	**<0.001**	0.28
LDL ($mmol \cdot l^{-1}$)	3.4 [3.0, 3.8]	2.8 [2.4, 3.3]	-16	2.9 [2.5, 2.3]	2.7 [2.3, 3.1]	-6	**<0.001**	**0.03**
HDL Ox	28.7 [26.3, 31.1]	29.4 [27.0, 31.9]	3	27.4 [24.9, 30.0]	27.6 [25.1, 30.1]	1	0.58	0.74
LDL Ox	30.3 [26.0, 34.5]	31.9 [27.6, 36.1]	5	28.0 [23.6, 32.4]	28.4 [24.0, 32.9]	2	0.26	0.50
Triglycerides ($mmol \cdot l^{-1}$)	1.02 [0.85, 1.19]	0.97 [0.79, 1.15]	-5	0.96 [0.78, 1.13]	0.80 [0.62, 0.98]	-16	0.07	0.37

All values are model based means [SE]. BMI, body mass index; AUC, area under the curve; HbA1c, glycosylated hemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein; HDL Ox , oxidized high density lipoprotein; LDL Ox, oxidized low density lipoprotein; MICT, moderate intensity continuous training; HIIT, high intensity interval training. † Log transformation was done to achieve normal distribution. The p-value for time indicates the change in the whole study group. The p-value for time x group interaction indicates if the change in the parameter was different between the HIIT and MICT training modes.

Table 2: Animal characteristics at baseline and after the exercise intervention

Table 2:Animal characteristics at baseline and the changes induced after the exercise intervention

Parameter	CON n=8			HIIT n=8			MICT n=8			P value	
	Pre	Post	Δ %	Pre	Post	Δ %	Pre	Post	Δ %	Time	Time x group interaction
Anthropometrics											
Weight (g)	282 [269, 294]	351 [338, 364]*	25	297 [285, 309]	346 [331, 360]*	16	281 [269, 293]	350 [337, 364]*	25	**<.0001**	**0.002**
Fat free mass (%)	239 [229, 248]	282 [271, 294]	18	253 [244, 263]	296 [285, 307]	17	248 [238, 257]	291 [279, 302]	17	**<.0001**	0.99
Fat mass (g)†	36.8 [33.6, 40.4]	47.2 [42.2, 52.7]*	28	38.4 [35.0, 42.1]	40.5 [36.3, 45.2]	6	35.9 [32.7, 39.4]	40.4 [36.2, 45.1]*	13	**<.0001**	**<0.001**
Fat (%)	11.9 [11.0, 12.9]	12.7 [11.6;13.8]*	6	11.7 [10.8, 12.7]	10.7 [9.7, 11.8]*	-8	11.4 [10.4, 12.3]	10.8 [9.7, 11.9]*	-5	0.09	**<.001**
VO_2max (ml/min/kg)	69.0 [65.1, 72.9]	68.9 [65.0, 72.8]	0	70.1 [66.2, 74.0]	72.9 [69.0, 76.8]*	4	71.2 [67.3, 75.1]	72.8 [68.9, 76.7]	2	**0.01**	**0.05**
OGTT											
Glucose 0 (mmol·l^{-1})	5.0 [4.6, 5.4]	4.9 [4.5, 5.3]	-2	5.1 [4.7, 5.5]	4.9 [4.5, 5.4]	-3	4.9 [4.5, 5.3]	4.7 [4.2, 5.1]	-5	0.31	0.93
Glucose 120 (mmol·l^{-1})	5.5 [5.0, 6.1]	5.3 [4.9, 5.8]	-3	4.8 [4.3, 5.4]	5.2 [4.8, 5.6]	8	5.3 [4.7, 5.8]	4.9 [4.4, 5.3]	-8	0.73	0.23
Glucose AUC (min*mmol·l^{-1})	840 [779, 900]	813 [767, 859]	-3	806 [745, 866]	786 [728, 844]	-2	774 [713,834]	742 [693, 791]	-4	0.18	0.97

All values are mean [95 % confidence intervals]. AUC, Area under the curve; CON, control group no exercise; MICT, moderate intensity continuous training; HIIT, high intensity interval training. †Log transformation was done to achieve normal distribution. The p-value for time indicates the change in the whole study group. The p-value for time x group interaction indicates if the change in the parameter was different between the CON, HIIT and MICT training modes and * pre vs post p value < 0.05.