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ABSTRACT

Introduction: Intestinal metabolism and microbiota profiles are impaired in obesity and insulin resistance. Moreover, dysbiotic gut microbiota has been suggested to promote systemic low-grade inflammation and insulin resistance through the release of endotoxins particularly lipopolysaccharides. We have previously shown that exercise training improves intestinal metabolism in healthy men. To understand whether changes in intestinal metabolism interact with gut microbiota and its release of inflammatory markers, we studied the effects of sprint interval (SIT) and moderate intensity continuous training (MICT) on intestinal metabolism and microbiota in insulin resistance.

Methods: Twenty-six, sedentary subjects (prediabetic n=9, T2D n=17; age 49[SD 4] years; BMI 30.5[SD 3]) were randomized into SIT or MICT. Intestinal insulin-stimulated glucose uptake (GU) and fatty acid uptake (FAU) from circulation were measured using PET. Gut microbiota composition was analysed by 16S rRNA gene sequencing and serum inflammatory markers with multiplex assays and enzyme-linked immunoassay kit.

Results: VO_{2peak} improved only after SIT ($p=0.01$). Both training modes reduced systematic and intestinal inflammatory markers (TNF α, LBP) (time $p<0.05$). Training modified microbiota profile by increasing Bacteroidetes phylum (time $p=0.03$) and decreasing Firmicutes/Bacteroidetes ratio (time $p=0.04$). Moreover, there was a decrease in *Clostridium* genus (time $p=0.04$) and *Blautia* (time $p=0.051$). Only MICT decreased jejunal FAU ($p=0.02$). Training had no significant effect on intestinal GU. Colonic GU associated positively with

Bacteroidetes and inversely with Firmicutes phylum, ratio Firmicutes/Bacteroidetes and *Blautia* genus.

Conclusion: Intestinal substrate uptake associates with gut microbiota composition and activity and whole-body insulin sensitivity. Exercise training improves gut microbiota profiles and reduces endotoxemia.

Keywords: Exercise training, gut microbiota, metabolic endotoxemia, intestinal glucose uptake, intestinal free fatty acid uptake, sprint interval training.

INTRODUCTION

Gut microbiota has been recognized to play a key role in human health and well-being. Gut microbial profiles have been shown to differ in healthy subjects compared to subjects with obesity, metabolic syndrome and inflammatory bowel disease (IBD) (1). It is suggested that the impairments in gut microbiota composition induce metabolic endotoxemia through the release of endotoxins, particularly lipopolysaccharide (LPS), which promote systemic low-grade inflammation and insulin resistance (2).

The effect of exercise training on gut microbiota remains elusive. It has been suggested that there is a link between physical fitness and health-associated gut microbial parameters such as taxonomic diversity (3) and richness (4). Previously, Mika et al. have suggested that regular physical activity promotes the psychological and metabolic health through the development of diverse microbiota in childhood and adolescence (5). Exercise training has been shown to effect the gut microbiota first, through its effects on autonomic nervous system (vagal tone) which is also known as “brain-gut axis” (6) and second by its impact on improvement of immune function (7). It has been suggested that alterations in the vagal nerve influence the gut microbiota through their control of inflammatory alterations and that exercise training improves this vagal tone by improving gut microbiota composition (8). Additionally, inflammation in the intestinal mucosa associated with the IBD has been shown to alter gut microbiota (9). Training has been demonstrated to be an effective treatment for multiple inflammatory conditions including IBD (10). Even though there is lot of speculation about the beneficial effects of exercise training on gut microbiota (4), studies in humans are scarce.

Recently we reported in healthy middle-age sedentary men, that short term moderate-intensity continuous training (MICT) improves intestinal insulin-stimulated glucose uptake (GU) (from circulation into intestine) and fasting free fatty acid uptake (FAU) more efficiently compared to sprint interval training (SIT) (11). We also demonstrated a positive correlation between intestinal insulin GU and whole-body GU (i.e. insulin sensitivity) (11). However, it is unclear whether the changes in the intestinal metabolism are associated with gut microbiota composition and activity and whether this interaction further reflects whole-body metabolism.

In the present study, we investigated the effects of short-term training on intestinal insulin-stimulated GU, fasting FAU, gut microbiota composition and metabolic endotoxemia (lipopolysaccharide binding protein, LBP) in prediabetic and T2D subjects. Based on our previous study we hypothesized, that training responses are more rapidly detectable in intestinal metabolism and gut microbiota after MICT than SIT training.

MATERIALS AND METHODS

Study design

This subproject is part of a bigger 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). All studies were performed at Turku PET Centre, University of Turku, Turku University hospital, Åbo Akademi University (Turku, Finland) and Paavo Nurmi Centre (Turku, Finland). The various studies performed before and after the exercise intervention are illustrated in (Figure 1a and 1b). Participants were also asked to abstain from any caffeinated and alcoholic drinks, avoid strenuous exercise and stop all oral hypoglycaemic

medication 48 hours prior to these studies. The study was approved by the ethics 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 informed consent was obtained before any measurements were performed.

Subjects

Twenty-six sedentary middle-aged insulin resistant subjects (Prediabetic n=9, T2D n=17; males/females 16/10) were randomized either into SIT or MICT group. The randomization was done by permuted blocks of 1:1 ratio. The subjects (aged 40-55 years, VO_{2peak} < 40 ml•kg^{-1}•min^{-1}) had no previous background of exercise training. All 26 subjects met the criteria of defective glucose tolerance set by American Diabetes Association criteria (12) and had an HbA1c less than 7.5 mmol/l. Out of the 26 subjects, 17 (male n = 11, female n = 6) met the criteria of T2DM (median duration for T2D 4 years) and 9 had prediabetes, having either impaired fasting glucose and/or impaired glucose tolerance (12). Out of the 17 T2DM subjects 13 were treated with oral hypoglycaemic medication (11 metformin; 5 DPP-IV and 1 sulfonylurea) while the rest 4 were diagnosed at the screening and were not taking any medication for T2DM. All diabetes medication was discontinued before any studies were performed. The inclusion and exclusion criteria have been explained previously in detail (13).

Three of the subjects failed to complete the study during the intervention one due to training induced migraine and two due to personal reasons. The fecal samples were available from 18 out of the 26 subjects and therefore the results presented here are from these 18 subjects.

Exercise interventions

In both training interventions participants exercised six times (3/week) over two weeks. All sessions were performed under supervision. The training protocol is described in detailed previously (14). During the screening phase participants were familiarized with SIT (2 x 30s bouts). Each SIT session consisted 30s exercise bouts (4-6) of all out cycling efforts (Wingate protocol, load 10% of fat-free mass in kg, Monark Ergomedic 828E, Monark, Vansbro, Sweden) with 4 min of recovery in between the exercise bouts. MICT training involved 40-60 min of moderate intensity (60% of VO2peak intensity) cycling (Tunturi E85, Tunturi Fitness, Almere, Netherlands). Both training interventions were progressive; with number of exercise bouts increasing from 4 to 5 and finally to 6 in the SIT group while in the MICT group the training duration was increased from 40 min to 50 min and then to 60 min after every second training session.

Primary outcomes

PET scans

The PET imaging method has been described in detail previously (11). Briefly, intestinal FAU and GU [^{18}F]FTHA PET and [^{18}F]FDG PET imagining were performed on two different days using 14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoic acid (FTHA) and 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) radiotracers. The [^{18}F]FTHA PET was done under fasting conditions while the [^{18}F]FDG PET was done under euglycemic hyperinsulinemic clamp. The PET raw data was corrected for attenuation, dead time and decay. The data was reconstructed with 3D-OSEM method and was analysed with Carimas software (version 2.9, www.turkupetcentre.fi/carimas). Regions of interest (ROIs) were drawn manually on sections of intestine (duodenum, jejunum

and colon) as explained previously (11). From these tubular ROIs tissue time activity curves were obtained. Fractional uptake rate was calculated from the plasma and tissue time activity curves using graphical analysis (15). The regional (duodenal, jejunal and colonic) FAU and GU was calculated by multiplying the regional fractional uptake rate with plasma free fatty acid or glucose concentration, respectively, during the scan.

Fecal DNA extraction and specific qPCR

Stool samples were collected before and after the exercise intervention and kept frozen at −80 °C until processed for analysis. Total DNA was isolated from the fecal samples using the MasterPure Complete DNA & RNA Purification Kit (Epicentre) according to the manufacturer's instructions with some modifications as described previously (16).

PCR primers targeted to *Bacteroides* group, *Bifidobacterium* group and also, Enterobacteriaceae family were used as previously described (17). Specific qPCR was performed in LightCycler® 480 Real-Time PCR System (Roche®) by use of SYBR® Green PCR Master Mix (Roche®). The fluorescent products were detected in the last step of each amplification cycle. A melting curve analysis was made at the end of the PCR to distinguish the un-targeted PCR product. The bacterial concentration in each sample was calculated comparing the Ct values obtained from standard curves.

16S rRNA gene sequencing analysis

Total DNA concentrations were measured using a Qubit® 2.0 Fluorometer (Life Technology, Carlsbad, CA, USA) and diluted to 5 ng/μL. 16S rDNA gene (V3-V4 region) was

amplified by PCR using Illumina adapter overhang nucleotide sequences following Illumina protocols. After 16S rDNA gene amplification, the mutiplex step was performed using Nextera XT Index Kit (Illumina, San Diego, CA, USA). PCR product was checked with a Bioanalyzer DNA 1000 chip (Agilent Technologies, Santa Clara, CA, USA) and libraries were sequenced using a 2x300pb paired-end run (MiSeq Reagent kit v3) on a MiSeq-Illumina platform (FISABIO sequencing service, Valencia, Spain) according to manufacturer's instructions (Illumina).

Data processing was performed using the QIIME pipeline (version 1.9.0) (18). The sequences were clustered into OTUs at 97% similarity and checked for chimeras. Representative sequences were obtained for each microbial phylotype and taxonomy was assigned using Greengenes GG 13.8 database. Sequences that could not be classified to domain level, or were classified as Cyanobacteria and Chloroplasts, were removed from the dataset as they likely represent ingested plant material.

Biomarkers in plasma and stool LBP, CRP and TNF α measurements:

Concentrations of interleukins IL-1B, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12 P70, IL-13, Interferon gamma (IFN-g), Tumor Necrosis Factor (TNF-alpha), and also, C-reactive Protein (CRP) were measured by multiplex bead assay analysis (12-plex, Luminex® Performance Assay Multiplex Kit; Procarta® Immunoassay Affymetrix, Santa Clara US) according to the manufacturer's instructions. Plasma samples were analyzed with LUMINEX® 200™ using the Luminex xPonent software (Luminex, USA). Concentrations of some of the interleukins were under the detection limit and were therefore not included in the final analysis. Human

Lipopolysaccharide-binding protein (LBP) was determined by ELISA Kit (FineTest® Ref.- EH1560, Wuhan Fine Biotech Co., Ltd.) in both, plasma and fecal samples. Levels of human calprotectine (FineTest® Ref.- EH4140, Wuhan Fine Biotech Co., Ltd.) and zonulin (MyBiosource Ref. MBS749365-96) in fecal samples were also analysed by ELISA.

Secondary outcomes

Other measurements

Euglycemic hyperinsulinemic clamp technique was performed to calculate the whole-body insulin sensitivity (M-value) (19) . A cycle ergometer (Ergoline 800 s; VIASYS Healthcare, Germany) was used to determine the VO_{2peak} as previously described (14) . Bioimpedence monitor (Inbody 720, Megaelectonics Ltd., Kuopio, Finland) was used to measure the body composition. Abdominal subcutaneous and visceral fat masses were measured using magnetic resonance imaging (MRI) and SliceOmatic software version 4.3 (http://www.tomovision.com/products/sliceomatic.html) as previously described (11).

Statistics

The sample size for the whole study (NCT01344928) was based on the primary outcome skeletal muscle glucose uptake (quadriceps femoris). To achieve > 90% power of detecting a 20 % unit change in insulin-stimulated GU in quardriceps femoris a total of 20 prediabetic/T2D subjects (SIT = 10 and MICT = 10) were required. To accommodate for the drops outs and technical problems during the study, an extra six subjects were recruited as described previously (20). No sample size calculations were done specifically on the outcomes measures of the current study. Descriptive statistics shown in the tables are presented by model-based means and [95 %

confidence intervals, CI] while the figures are based on model-based means and (95% confidence intervals). Association between anthropometrics, glucose profile, lipid profile and training groups, time points, training*time, interaction were performed with hierarchical linear mixed models, using the compound symmetry covariance structure for repeated measurements. The medication status (taking oral hypoglycaemic medication/not taking oral hypoglycaemic medication) and gender were used as additional factors for all the analyses. Subjects with one value, but another missing (drop outs, technical problems) are included in this model and therefore model-based mean (SAS least square means) values are reported for all the parameters. Transformations (logarithmic or square root) were done to (whole body fat percentage, subcutaneous and visceral fat volume, glucose fasting, insulin fasting, FFA clamp, ratio Firmicutes/Bacteroidetes, and *Clostridium* genus) variables to achieve the normal distribution assumption. Multivariate redundancy analysis (RDA) plots were created with Calypso Multivariate tool, an online platform for mining, visualizing and comparing multiple microbial community composition data (cgenome.net/calypso). In addition, Pearson correlation coefficient was calculated. 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

Aerobic capacity (VO_{2peak}) improved only after SIT and not after MICT training (Time*training = 0.03). However, both training modes reduced whole-body fat percentage and the abdominal visceral fat mass (both time p = 0.04) and improved glycosylated haemoglobin (HBA_1c) (time p = 0.003) (Table 1).

Both training modes significantly reduced systemic inflammatory marker TNF α (time p = 0.03), tended to reduce CRP (time p = 0.08) and intestinal inflammatory marker LBP (time p = 0.02) (Fig 2 a-c). There were no changes in other plasma cytokines and intestinal inflammatory markers (Calprotectin and Zonulin) measured from fecal samples (Supplementary figure 1, Supplemental Digital Content 1, Changes in the inflammatory markers, http://links.lww.com/MSS/B712). LBP correlated positively with HBA_1c (r = 0.54; p = 0.02) (Supplementary figure 2, Supplemental Digital Content 2, correlations between different parameters, http://links.lww.com/MSS/B713).

Gut microbiota composition was influenced by exercise training. Both training modes decreased the ratio of Firmicutes/Bacteroidetes (time p = 0.04), mainly due to the increase in the relative abundance of Bacteroidetes phyla (time p = 0.03) (Fig 3a, 3b) while no change was found in the Firmicutes levels. At genus level, both training modes decreased the abundance of *Blautia* spp. (time p = 0.051) and *Clostridium* spp. (time p = 0.04) (Fig 3c, d). While *Lachnospira* genus was present in higher abundance after SIT compare to baseline (p = 0.025) and significant higher abundance of *Veillonella* genus (and also, *Veillonella dispar*) was observed after MICT compared to baseline (p = 0.036) and compared to SIT (p = 0.055). Interestingly, the abundance of *Faecalibacterium genus (F. prausnitzii)* was increased after MICT (p = 0.057) while no change after SIT was found (Supplementary figure 3, Supplemental Digital Content 3, changes in relative abundance of *Feacalibacterium* and *Akkermansia, http://links.lww.com/MSS/B714*). There were no differences in microbiome richness and diversity between the training modes (by Chao1, Shannon, observed otus and PD whole tree)

(Supplementary figure 4, Supplemental Digital Content 4, effects of exercise on microbial diversity, http://links.lww.com/MSS/B715). While at OTUs level, MICT training increased significantly ($p = 0.035$) the relative abundance of *Veillonella dispar* (OTU 4034) whereas no change was seen after SIT. In addition, multivariate redundancy analysis (RDA) test showed significant differences in the gut microbiota according to the training response (SIT vs. MICT) (Supplementary figure 5, Supplemental Digital Content 5, multivariate redundancy analysis, http://links.lww.com/MSS/B716).

There was no change in insulin-stimulated intestinal GU in either training group (Fig 4a). While MICT reduced the fasting FAU in the jejunum no changes were observed either in duodenum or colon (Fig 4b) or after SIT. Intestinal FAU correlated negatively with whole-body insulin sensitivity ($r = -0.81$; $p = 0.049$) after MICT training only (Supplementary figure 2, Supplemental Digital Content 2, http://links.lww.com/MSS/B713). Interestingly, at baseline insulin-stimulated colonic GU associated inversely with the abundance of Firmicutes ($r = -0.59$; $p = 0.03$), Firmicutes/Bacteroidetes ratio ($r = -0.62$; $p = 0.024$) and *Blautia* ($r = -0.57$; $p = 0.049$) and positively with the abundance of Bacteroidetes ($r = 0.71$; $p = 0.007$) (Fig 5 a-d). In addition, lower abundance of *Blautia* genus was associated with better whole-body insulin sensitivity (M-value) ($r = -0.53$; $p = 0.04$) (Supplementary figure 2, Supplemental Digital Content 2, correlations between different parameters, http://links.lww.com/MSS/B713).

DISCUSSION

The present study shows for the first time that exercise training reduces intestinal inflammation and modulates gut microbiota profiles in insulin resistant subjects. Both training

modes reduced endotoxemia by decreasing the intestinal inflammatory marker (LBP). Training also decreased the ratio of Firmicutes/Bacteroidetes (obesity) (21), *Clostridium* genus (immune response) (1) and *Blautia* genus (inflammation) (22), and increased Bacteroidetes (protection against obesity) (23). Only MICT decreased jejunal FAU while no training response was observed in intestinal insulin sensitivity.

Gut microbiota has been suggested to induce whole-body systematic low-grade inflammation through the release of inflammatory products (LPS, TNF α) (22;24). Healthy gut microbiota has been reported to release small amount of LPS in the blood, which is essential for the maintenance and development of the host immune system. But when LPS is released in massive amounts, it is associated with pathophysiological reactions in various organs, in adipose tissue (induces inflammation and insulin resistance), in liver (damages hepatocytes leading to progression from simple fatty liver to steatohepatitis), in endothelium (contributes to plagues formation and rupture) and in some cases irreversible shock (2). Due to the limitations in measuring LPS in biologic fluids (25), endogenous protein LBP has been used as an alternative clinical marker for measuring endotoxemia and immune response (26;27). LBP binds to LPS and enhances the binding of LPS to "Cluster of Differentiation 14" (CD14) (28). The CD14-LPS complex is transduced into the cell nucleus and it initiates a cascade of inflammatory cytokines (29). Elevated LBP levels have been associated with obesity, T2D and metabolic syndrome (30). In our study both SIT and MICT significantly reduced LBP after two weeks of training. This significant reduction in the LBP levels can be due to changes in gut microbiota. Cani et al. showed that high fat diet significantly reduces the number of *Bifidobacterium*, *Eubacterium rectale-Blautia coccoides* and *Bacteroides* genus (24). This reduction leads to an increase in the

Gram-negative to Gram-positive ratio leading to an increase in the levels of LPS in blood (24). Compared to the Cani et al. data after the high fat diet, our findings indicate the opposite. We found a reduction in Firmicutes/Bacteroidetes ratio, mainly due to the increase in the relative abundance of Bacteroidetes and a decrease in the abundance of *Blautia* spp. and *Clostridium* spp. at genus level. These modulations can possibly explain the observed improvement in the LBP level in our study as the improvement in the relative abundance of Bacteroidetes can lead to a reduction in Gram-negative to Gram-positive ratio leading to a decrease in the LPS levels. Moreover, the improvement in Bacteroidetes levels can also lead to an improvement in the intestinal inflammation as Bacteroidetes induces regulatory T cells to produce IL-10 (anti-inflammatory cytokine) inside the gut (31). Furthermore, in the present study *Bacteroidetes* at the species level correlated negatively with plasma inflammatory makers LBP, TNF α and CRP levels (data not shown) highlighting the importance of Bacteroidetes in intestinal inflammation.

Exercise training also reduced the relative abundance of *Clostridium* and tended to reduce the *Blautia* genus. *Clostridium* bacteria has been suggested to play an important role in whole-body immune responses (22). *Blautia* genus has been shown to be one of the most abundant genus in pre diabetes and type 2 diabetes (T2D) compared to healthy subjects (32) and has been suggested to increase the release of pro inflammatory cytokines (TNFα, cytokines) (22). Interestingly, in the present study *Blautia* did decrease ($p = 0.051$) and we also observed a significant reduction in the TNFα after two weeks of training (Fig 2a). This reduction is important as TNFα plays a critical role in the inflammatory processes such as inflammatory bowel disease (33). Thus, our data suggests that exercise training reduces the inflammation in the

gut and whole-body and thereby another manner through which training reduces the risk of acquiring various diseases.

One of the most interesting results in our study is the reduction of Firmicutes/Bacteroidetes ratio. The ratio has been shown to have a significant relevance in the normal/healthy human gut microbiome (23). In obesity the Firmicutes/Bacteroidetes ratio is elevated (34;35), it is reversible after dietary intervention and correlates with body weight loss (23). One of the reasons how Firmicutes contribute to obesity is the speculation that they are able to harvest more energy from food (34;36). In our study, two weeks of training reduced the ratio between Firmicutes/Bacteroidetes mainly through the significant increase in the relative abundance of Bacteroidetes at the phylum level (Fig 3b). In addition, to measuring the Bacteroidetes relative abundance we also measured its levels in the feces using qPCR. Both exercise modes also tended to increase the overall Bacteroidetes levels measured with qPCR ($p = 0.07$). The increase in Bacteroidetes is significant as it plays an essential role in the metabolic conversions of complex sugar polymers and degradation of proteins (37). Additionally, in obesity and irritable bowel syndrome (IBS) there is reduced relative abundance of Bacteroidetes (23;38).

In addition, to quantifying bacteria's at the phylum and genus level we also performed multivariate redundancy analysis (RDA). RDA identifies significant associations between microbial communities according to their composition at baseline and after the intervention (SIT and MICT). The RDA analysis showed that the training adaptation was different between SIT and MICT at the OTU level (Supplementary figure 3, Supplemental Digital Content 3, changes

in relative abundance of *Feacalibacterium* and *Akkermansia, http://links.lww.com/MSS/B714*). OTU's are a cluster of microorganisms which share similar sequences and represent more phylogenetically similar organisms. MICT increased Veillonella OTU significantly compared to baseline, while no OTU's were different after the SIT intervention. However, when analysed further we did not find any differences between SIT and MICT at the genus and phylum level. This observation needs to be further studied with longer intervention duration and higher number of study subjects.

Previous cross-sectional studies in humans have shown that physically active subjects have higher microbial abundance *Akkermansia muciniphila, Faecalibacterium prausnitzi,* and *Roseburia* and higher microbial diversity compared to sedentary subjects (4;39). Many training intervention studies in animals have suggested that exercise indeed has a positive effect on the gut microbiome composition (increased Bacteroidetes and reduced Firmicutes phylum, as well as increased Actinobacteria phylum towards *Bifidobacterium* genus) (40;41). Moreover, intervention studies done with humans have shown an increase in the abundance of the six of the major bacterial phyla/genera (42), an increase in *Akkermansia*, *Coriobacteriaceae*, and *Succinivibrionaceae*, a decrease in Proteobacteria phylum (43;44), and positive impact on the butyrate producing bacteria (45;46). Exercise training has been suggested to modify the gut microbiota through its anti-inflammatory effects (7), mainly via the release of cytokines and peptides (also known as "myokines") by the contracting skeletal muscles (36). Another way through which exercise modifies gut microbiota is through the digestive physiology. Moderate intensity exercise has been shown to accelerate the digestive transit time (34), this is important as it has been suggested that with advancing age the gut microbiome composition alters due to

changes in the digestive time compared to younger adults (37). In our study the digestive transit might have changed after both SIT and MICT but unfortunately, we did not measure it in this study. However, this hypothesis warrants further studies.

Our data shows that already short-term MICT decreases jejunal FAU in insulin resistant subjects. This finding is consistent with our previous data regarding the training responses in healthy subjects (11). And also in line with recent study by Koffert et al. (47) showing increased jejunal FAU in morbidly obese compared to normal weight healthy subjects. One of the probable explanations for the change in intestinal FAU is the change in the plasma FFA supply to the intestine. In morbidly obese subjects circulating FFA levels are increased and in contrast training decreases FFA levels by reducing lipolysis (reduction in visceral mass and increase in insulin sensitivity). In the present study plasma FFA level decreased after MICT by -11%, but the decrease did not reach statistical significance.

Opposite to our previous data in healthy humans (11) training did not improve insulin-stimulated GU in insulin-resistant subjects in the present study. It might be that two weeks training period is too short to induce adaptations in insulin resistant intestine.

In the present study colonic FAU correlated negatively with whole-body insulin sensitivity ($r = -0.81$; $p = 0.049$) in the MICT group. Additionally, Firmicutes/Bacteroidetes ratio (increased in obesity) and Firmicutes correlated negatively and Bacteroidetes (reduced in obesity) positively with the insulin-stimulated colonic GU (Fig 5 a,b and d). These results highlight the importance of intestinal substrate uptake on the whole-body and that the changes

especially in the glucose uptake might have a positive effect on the gut microbiota as well. In this study colonic GU correlated negatively with *Blautia* genus (Fig 5 c) which releases pro inflammatory markers (TNFα). As we know exercise reduces the risk of IBD (10), the reduction in *Blautia* genus can be one of the mechanism through which exercise reduces the risk of acquiring IBD. Moreover, as we know that the risk of acquiring type 2 diabetes, cardiovascular disease and certain cancers is directly proportional to the degree of obesity (48), exercise by altering the Firmicutes/Bacteroidetes ratio can reduce the comorbidities associated with obesity.

Limitations:

Our study contains some limitations. First, we did not have dietary control in our study, but subjects were asked to maintain their dietary habit throughout the study period. However, the possible effects of diet on the intestinal metabolism cannot be ruled out. Furthermore, there were more males than females in the study (21;22) and differences in medication between the subjects. Previous studies have shown contradictory findings regarding the effects of gender on microbiota (21;22), we found no differences in the microbiota composition between the sexes in our study at baseline. Moreover metformin has been shown to have a therapeutic impact on gut microbiota by increasing the relative abundance of *Butyrivibrio, Bifidobacterium bifidum*, and *Megasphaera* (production of short chain fatty acids) and *Akkermansia muciniphila* (that use mucin, a complex glycosylated protein, as a carbon and nitrogen source) which plays an important role in the maintenance of intestinal mucosa (49;50). Metformin was taken into account as a covariate in the statistics and were found to have no effect on the results. Additionally, diabetic status (i.e. prediabetic and T2D) was also taken into account as a covariate in the statistics and it showed to have no effect on the results. The study assessments were done 48 to 96 hours post training. As

the training intervention was short, it is possible that due to the detraining effect some training adaptations were not detectable anymore 72- or 96-hours post training. Also, it is highly likely that with longer training intervention period more changes would have been detected.

In conclusion, this study suggests that short-term exercise training improves the gut microbiota at the phylum and genus levels. Both training modes induced changes mostly in the major bacteria inhabiting the intestine and reduced the whole-body systemic and gut microbiota specific endotoxemia markers. RDA analysis indicated differences in the gut microbiota after SIT and MICT, observation to be further studied. Moreover, baseline correlations between the microbiota, intestinal substrate uptake and whole-body insulin sensitivity suggest another possible mechanism by which exercise can alter the gut and whole-body metabolism.

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Contribution statement

KKK and JCH designed the study; JJE, KAV, KKK and JCH collected data. KKM analyzed PET images; MCC and SE performed fecal data analysis; KKM, MCC and EL analyzed data; KKM, MCC, PN and JCH interpreted data; KKM and MCC wrote the manuscript and prepared the figures. All authors critically reviewed the manuscript and approved the final version. JCH is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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FIGURE CAPTIONS

Figure 1. a) Consort flow diagram showing the total number of subjects recruited and analysed. T2D, type 2 diabetes; SIT, sprint interval training; MICT, moderate-intensity continuous training. b) Study design: Subjects were studied on three separate days before and after the exercise intervention. OGTT, oral glucose tolerance test; VO_{2peak}, aerobic capacity; PET, positron emission tomography; FTHA, [^{18}F]-labelled 6-thia-heptadecanoic acid; PET-FDG, [^{18}F]-fluorodeoxyglucose.

Figure 2. Impact of exercise intervention on specific inflammatory markers. a) TNF α, Tumor necrosis factor alpha; b) CRP, C-reactive protein and c) LBP, lipopolysaccharide binding protein. All values are expressed as model-based means and bars are confidence intervals [95 % CI]. (§) Log transformation was performed to achieve normal distribution. * p value for time interaction (i.e. the groups (SIT + MICT) behaved similarly for the change in the parameter with a significant difference between them.

Figure 3. Impact of exercise intervention on the gut microbiota composition. a) ratio (Firmicutes/Bacteroidetes), b) Bacteroidetes, and the genus c) *Blautia* and d) *Clostridium*. All values are expressed as model-based means and bars are confidence intervals [95 % CI]. (§) Log transformation was performed to achieve normal distribution. * p value for time interaction (i.e. the groups (SIT + MICT) behaved similarly for the change in the parameter with a significant difference between them.

Figure 4. a) Insulin stimulated glucose uptake and b) fasting free fatty acid uptake in different parts of intestine before and after two weeks of either sprint interval training (SIT) and moderate intensity continuous training (MICT). All values are expressed as model-based means and bars are confidence intervals [95 % CI]. (§) Log transformation was performed to achieve normal distribution. ** p-value for time*training interaction (i.e. the groups behaved differently for the change in the parameter with a significant difference between them). SIT, sprint interval training; MICT, moderate intensity continuous training.

Figure 5. Correlation between insulin-stimulated colonic glucose uptake and a) Firmicutes b) ratio (Firmicutes/Bacteroidetes), c) *Blautia* and d) Bacteroides phylum in pooled analysis of both SIT and MICT subjects at baseline. (◆) sprint interval training (SIT) and (●) moderate intensity continuous training (MICT). (§) Log transformation was performed to achieve normal distribution.

Supplemental figures

Supplementary figure 1: Changes in the inflammatory markers. a) IL1b, Interleukin 1 beta; b) IL2, Interleukin 2; c) IL10, Interleukin 10; d) Calprotectin and e) Zonulin. All values are expressed as model-based means and bars are confidence intervals [95 % CI]. (§) Log transformation was performed to achieve normal distribution.

Supplementary figure 2: Correlation a) LBP (lipopolysaccharide binding protein) and HbA1c (glycosylated hemoglobin) in pooled analysis of both SIT and MICT subjects at baseline, b) between jejunal free fatty acid uptake and whole-body insulin sensitivity in MICT group after exercise, c) Blautia genus and whole-body insulin sensitivity in pooled analysis of both SIT and MICT subjects at baseline. LBP, (◆) sprint interval training (SIT) and (●) moderate intensity continuous training (MICT).

Supplementary figure 3: Changes in the relative abundance of *Faecalibacterium prausnitzi* and *Akkermansia muciniphila*. All values are expressed as model-based means and bars are confidence intervals [95 % CI].

Supplementary figure 4: Changes in microbiome richness and diversity. a) Chao1; b) Shannon; c) observed otus and d) PD whole tree). All values are expressed as model-based means and bars are confidence intervals [95 % CI]

Supplementary figure 5: Multivariate Redundancy analysis (RDA) plot to identify significant associations between microbial communities according to baseline (pre-intervention) and after two different interventions (SIT and MICT). SIT, sprint interval training and MICT, moderate intensity continuous training.

Figure 1 (a)

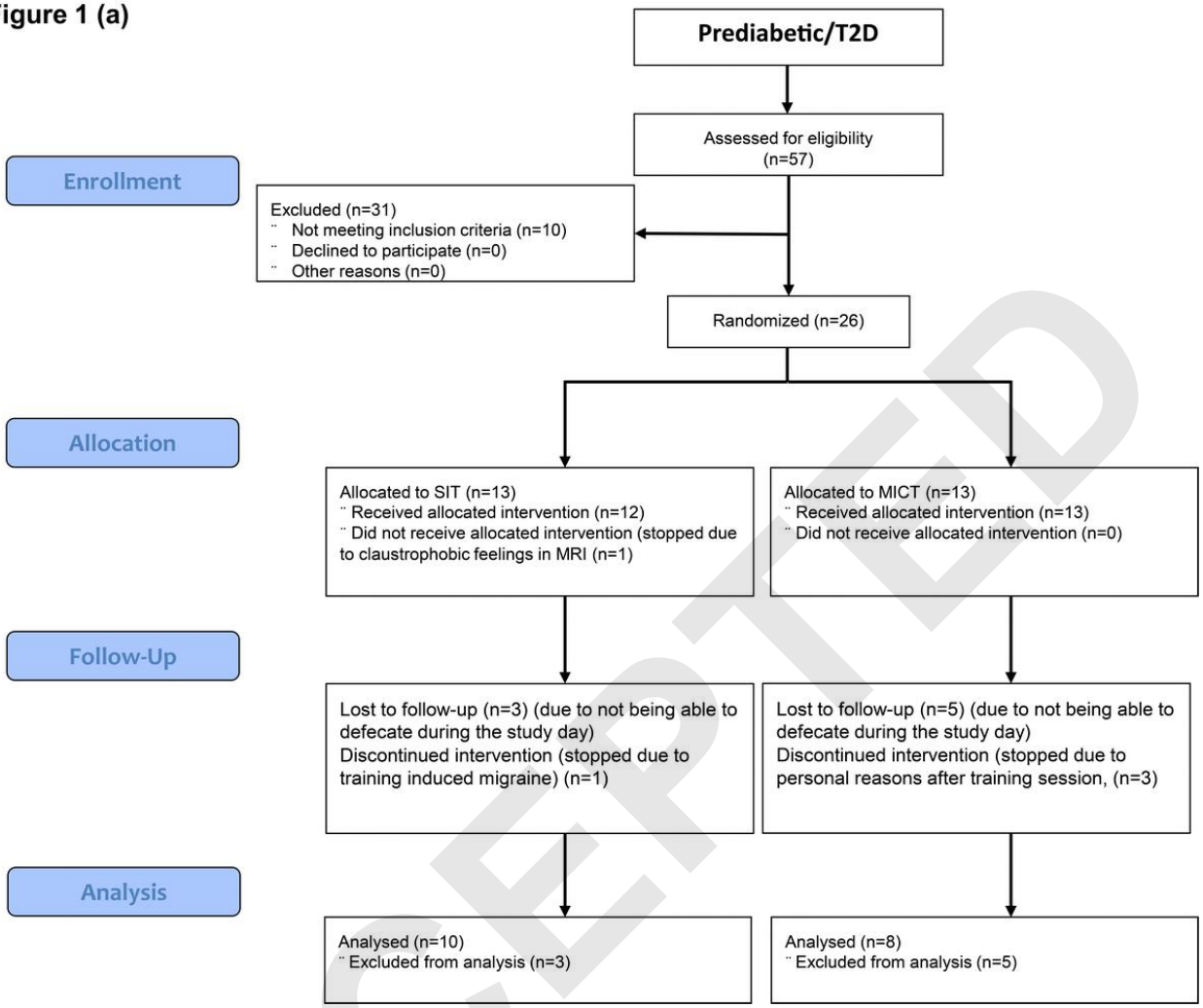


Figure 1 (b)

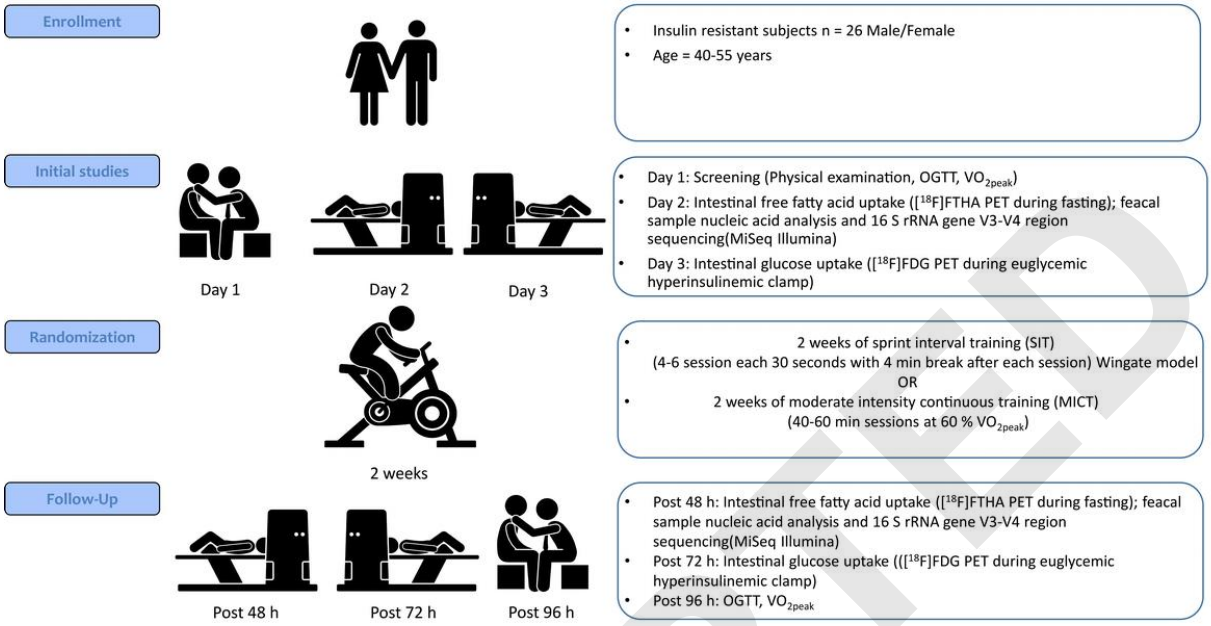


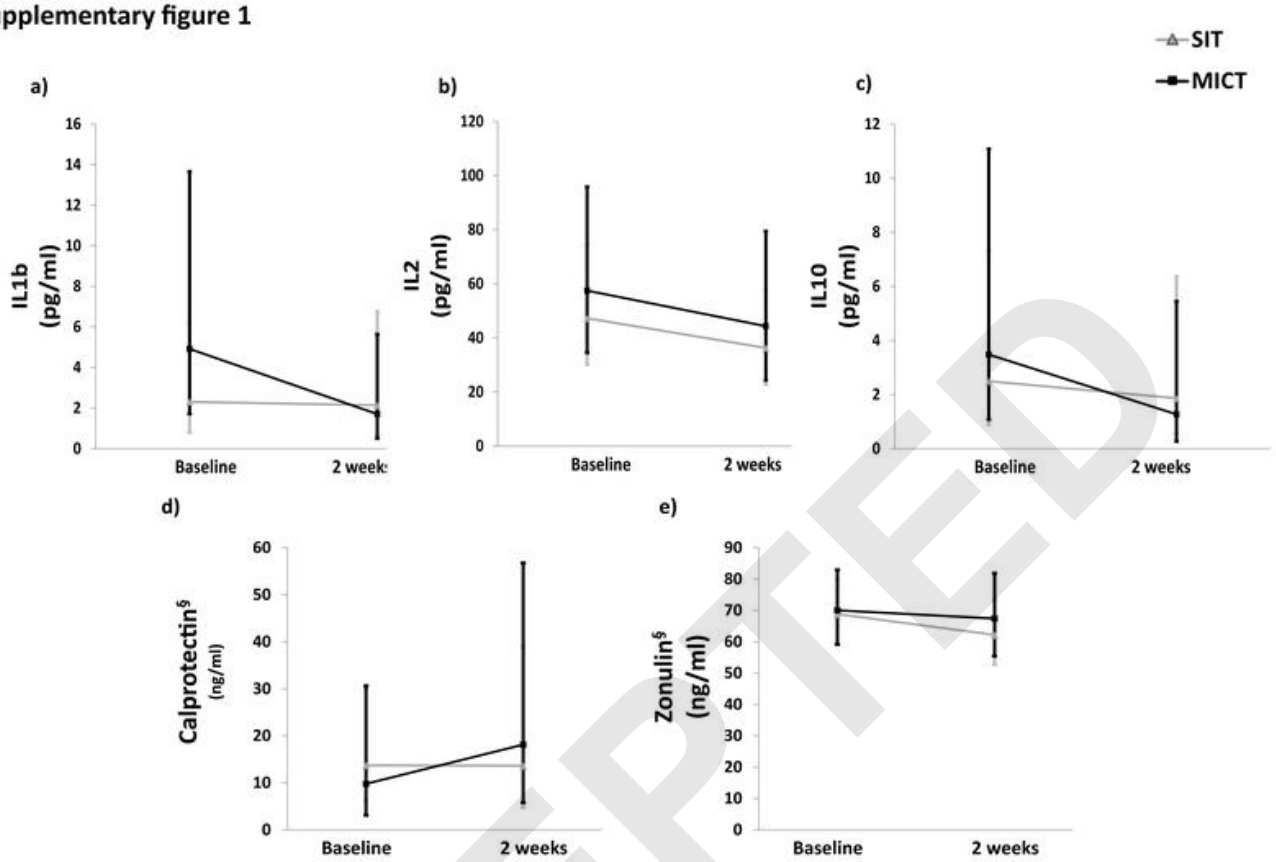
Table 1: Subject characteristics at baseline and the changes induced after the exercise intervention.

	SIT Pre	SIT Post	MICT Pre	MICT Post	Training
N	10		8		
men/women, n	7/3		6/2		
n Prediabetic/T2D	9/1		6/2		
SIT/MICT	10		8		
Glucose lowering medication					
Metformin	6		2		
DPP-4 inhibitors (sitagliptin)	4				
Sulfonylurea (glimepiride)	1				
Anthropometrics					
Weight (kg)	87.9 [80.7,95.1]	87.4 [80.2, 94.6]	91.7 [83.6, 99.7]	91.5 [83.5, 99.5]	0.45
BMI (kg/m^2)	29.3 [27.5, 31.0]	29.1 [27.3; 30.8]	30.7 [28.9,32.6]	30.7 [28.8, 32.6]	0.22
Whole body fat[§] (%)	32.6 [27.8, 38.3]	31.6 [26.9, 37.1]	30.5 [25.8, 36.2]	29.7 [25.1, 35.2]	0.57
Subcutaneous fat mass[§] (kg)	6.6 [5.2, 8.5]	6.5 [5.1, 8.4]	5.9 [4.5, 7.6]	5.8 [4.5, 7.6]	0.50
Visceral fat mass[§] (kg)	2.81 [1.95;4.05]	2.76 [1.92, 3.98]	3.99 [2.71, 5.88]	3.70 [2.51, 5.46]	0.22
VO_{2peak} (ml/kg/min)	27.1 [23.7, 30.5]	28.7 [25.2, 32.1]	28.6 [25.0, 32.3]	28.2 [24.6, 31.9]	0.82

Glucose Profile					
$Glucose_{fasting}$ [§] (mmol/L)	7.0 [6.5 7.6]	7.1 [6.5, 7.7]	6.2 [5.7, 6.7]	6.2 [5.7, 6.7]	0.03
$Glucose_{clamp}$ (mmol/L)	4.7 [4.6, 4.9]	4.9 [4.7, 5.1]	4.9 [4.7, 5.1]	5.0 [4.8, 5.2]	0.18
$Insulin_{fasting}$ [§] (mU/L)	11.9 [7.2, 19.6]	10.7 [6.5, 17.8]	8.9 [5.4, 14.8]	9.7 [5.8, 16.1]	0.56
$Insulin_{clamp}$ (mU/L)	85.4 [76.1, 94.7]	87.0 [77.1, 97.0]	85.8 [76.5, 95.1]	85.0 [75.0, 94.9]	0.88
Whole body insulin sensitivity (M-value)(µmol/min/kg)	22.4 [14.1, 30.8]	26.8 [18.2, 35.4]	21.9 [13.5, 30.3]	21.9 [13.3, 30.4]	0.62
HbA_{1c} (mmol/mol)	39.8 [36.2, 43.3]	37.5 [33.9, 41.1]	38.6 [34.6, 42.6]	37.0 [33.0, 41.1]	0.75
Lipid Profile					
$FFA_{fasting}$ (mmol/L)	0.73 [0.60, 0.80]	0.74 [0.60, 0.87]	0.81 [0.67, 0.95]	0.72 [0.57, 0.87]	0.70
FFA_{clamp} [§] (mmol/L)	0.06 [0.04, 0.10]	0.06 [0.04, 0.08]	0.08 [0.05, 0.11]	0.07 [0.04, 0.10]	0.56

All values are model based means [95 % confidence intervals]. SIT, Sprint interval training; MICT, moderate intensity continuous training; BMI, body mass index; VO2peak, aerobic capacity; HbA1c, glycosylated hemoglobin. [§] Log transformation was performed to achieve normal distribution. The p-value for time indicates the change between pre- and post-measurements in the whole study group. The p-value for training*time interaction indicates if the change in the parameter was different between the SIT and MICT training modes.

Supplementary figure 1



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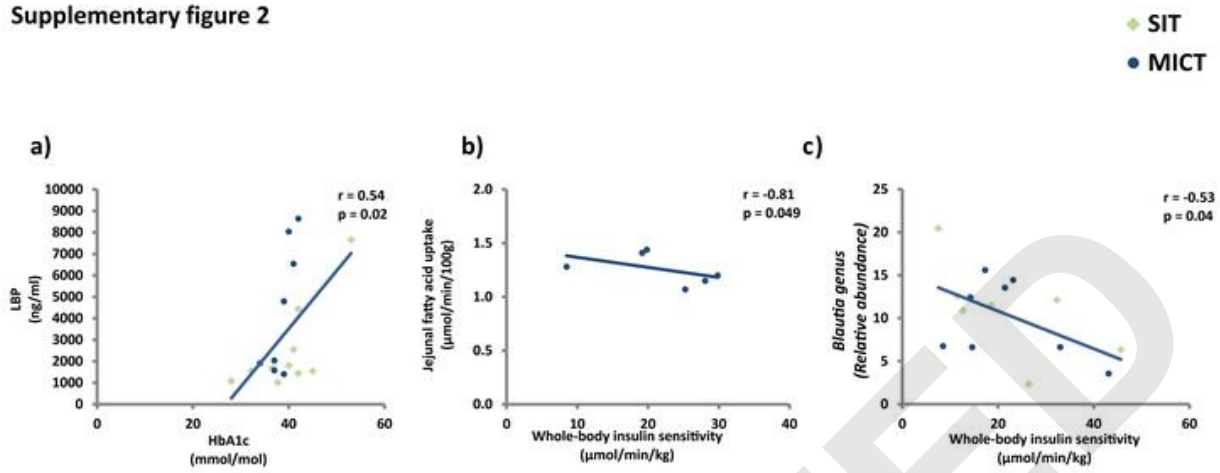

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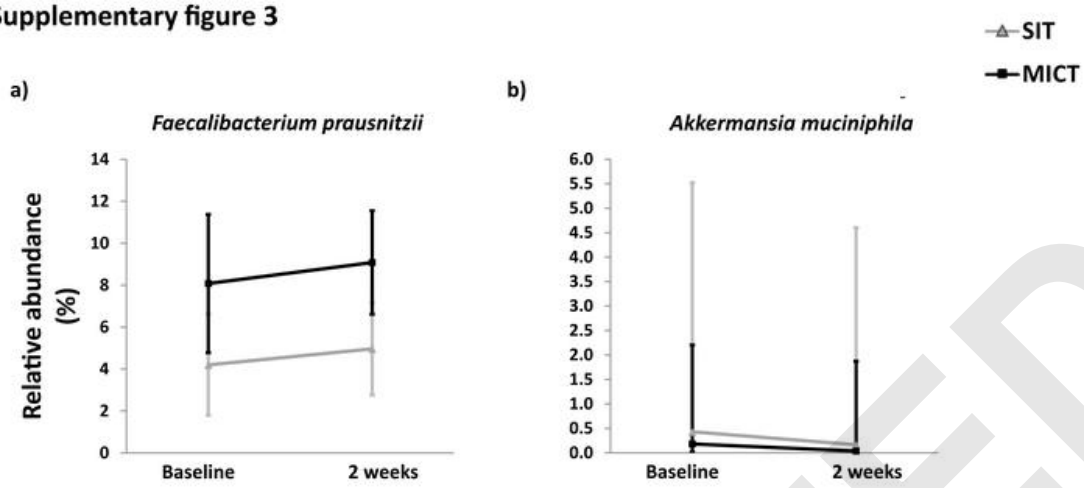
Supplementary figure 3



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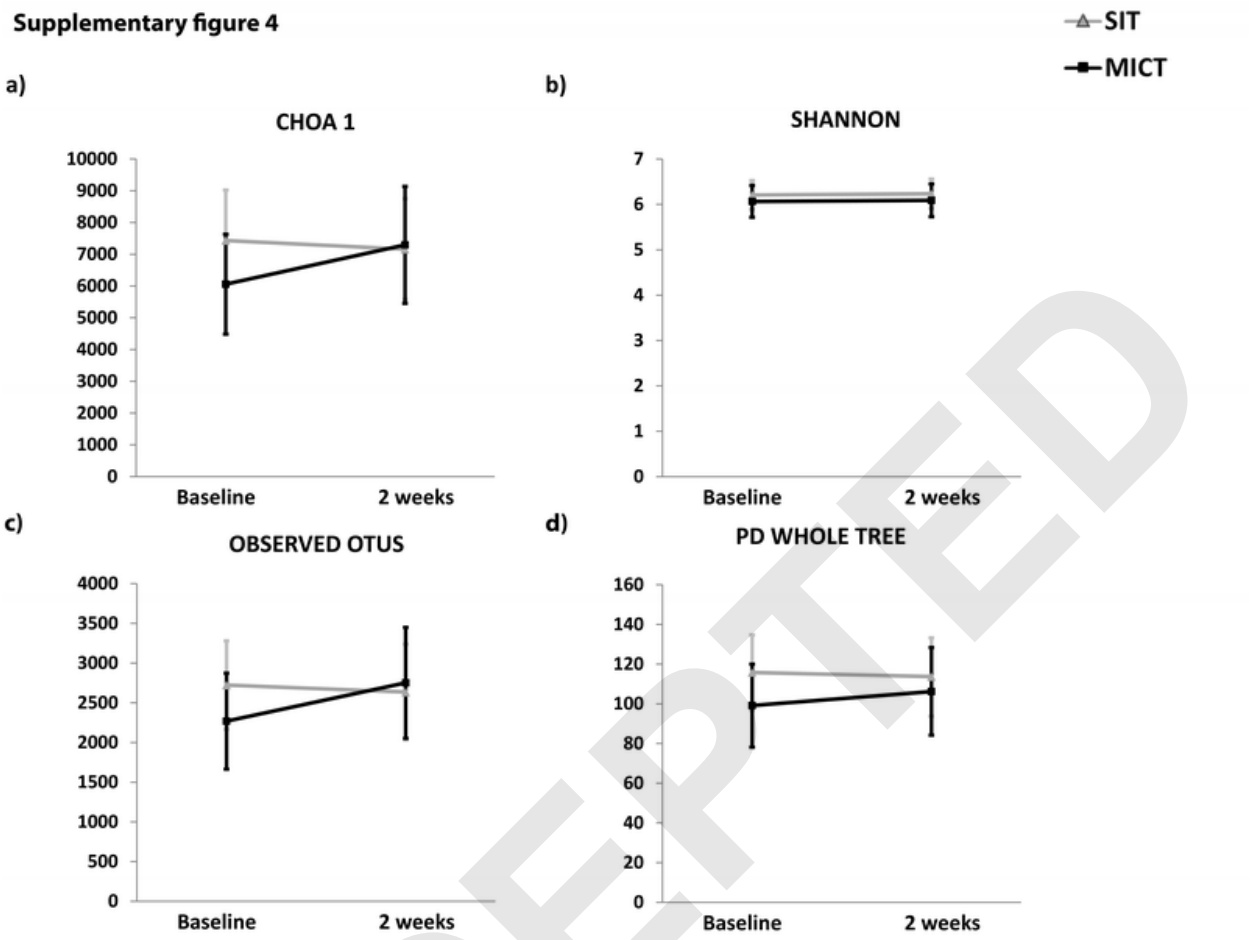

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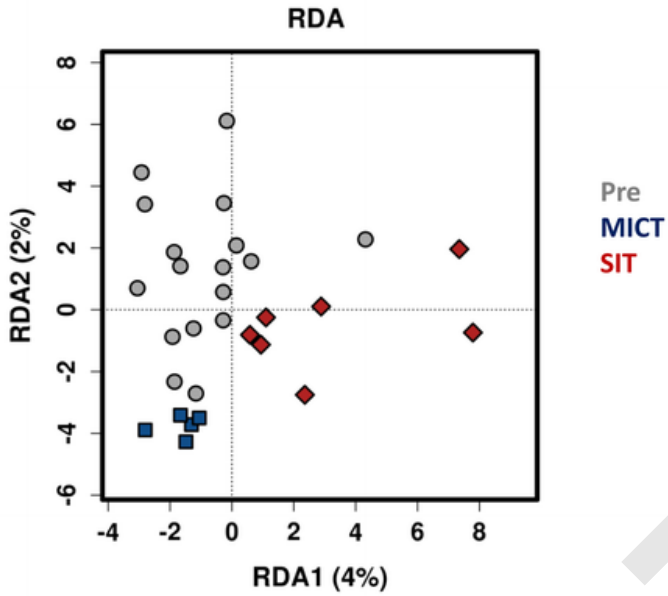
Supplementary figure 5



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