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Lactobacillus plantarum HAC01 regulates gut microbiota and adipose tissue accumulation in a diet-induced obesity murine model

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Abstract The functional features of *Lactobacillus plantarum* HAC01 (HAC01), isolated from fermented Korean kimchi, were studied with regard to the fat mass, immunometabolic biomarkers and dysbiosis in a diet-induced obesity (DIO) murine model. *L. rhamnosus* GG (LGG) served as reference strain and a PBS-treated group as control. The administration of *L. plantarum* HAC01 resulted in reduction of the mesenteric adipose depot, the conjunctive tissue closely associated with the gastrointestinal tract, where lipid oxidative gene expression was upregulated compared to the control group. Metagenome analysis of intestinal microbiota showed that both strains HAC01 and LGG influenced specific bacterial families such as the *Lachnospiraceae* and *Ruminococcaceae* rather than the phyla *Firmicutes* and *Bacteroidetes* as a whole. The relative abundance of the *Lachnospiraceae* (phylum *Firmicutes*) was significantly higher in both LAB-treated groups than in the control. Comparing the impact of the two *Lactobacillus* strains on microbial composition in the gut also suggests strain-specific effects. The study emphasises the need for deeper studies into functional specificity of a probiotic organism at the strain level. Alleviation of obesity-associated dysbiosis by modulation of the gut microbiota appears to be associated with "indicator" bacterial taxa such as the family *Lachnospiraceae*. This may provide further insight into mechanisms basic to the mode of probiotic action against obesity and associated dysbiosis.

Keywords *Lactobacillus plantarum* · *Lactobacillus rhamnosus* GG · Mesenteric adipose tissue · Diet-induced obesity · Dysbiosis · Gut microbiota

Introduction

Lactic acid bacteria (LAB) are characterised as Gram-positive, non-spore forming, facultatively aerobic bacteria, producing lactic acid by fermentation (Holzapfel and Wood 2014). Although genera such as *Streptococcus* also harbour pathogenic species, the LAB are generally recognised as safe. They represent the major functional microorganisms in most fermented foods, while a wide array of strains, in particular of *Lactobacillus* spp., are commonly applied as probiotics. Putative probiotic LAB strains have been reported to be effective against obesity and obesity-related metabolic syndromes as well as gastrointestinal disorders such as irritable bowel syndrome and immune disorders (Ji et al. 2012; Kwon et al. 2008; Masood et al. 2011; Parvez et al. 2006; Zeng et al. 2008). There also have been several reports that LAB administration ameliorated chronic low-grade inflammation associated with pathological aspects of obesity or metabolic syndrome (Li et al. 2003; Miyoshi et al. 2014). The universal role

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of LAB in the gastrointestinal tract (GIT) is still considered ambiguous. This may be related to the huge biodiversity within this large bacterial group but may also be due to open questions on the specific mode of action in complex ecosystems. Key issues relate to their functional role and underlying mechanisms within the autochthonous and overwhelming complexity of competing gut microbiota (Holzapfel and Wood 2014). An example is the beneficial impact of some LAB strains on host immunometabolic disorders, which, for lack of a comprehensive mechanistic explanation, is not yet fully understood (Hemarajata and Versalovic 2012).

Increasing interest and intensified research on intestinal microbiota have now moved the focus on the importance of the formerly "forgotten organ of the host" (O'Hara and Shanahan 2006), with approximately 1000 bacterial species encoding estimated 5 million genes (D'Argenio and Salvatore 2015). Concerted research efforts such as the Human Microbiome Project Consortium (Consortium HMP 2012) have contributed decisively to this new awareness and to the opening of new research ventures. Various studies have revealed discordant features of gut microbiota when comparing healthy and unhealthy populations. This provides a cornerstone for restoration of a healthy condition by modulating the gut microbiome. Obesity, in particular, is one of the most widely studied areas relating this condition to the role of gut microbiota, their impact on host metabolism and the microbial biotransformation of external energy sources (Backhed et al. 2004; Kimura et al. 2013; Sanz et al. 2013). Convincing evidence has shown that gut microbiota is regulated by the complex interaction of environmental factors and host genetics, and thereby play a key role in the development of obesity, insulin resistance and immunological disorders of the host (Caricilli et al. 2011; Spor et al. 2011).

Several strategies, including probiotics, prebiotics and bacterial therapy, have been suggested to maintain a healthy gut microbial environment (Lozupone et al. 2012). Prompted by increasing evidence on the interaction of gut microbiota with host health, efforts to recover or maintain a healthy microbiota by application of beneficial bacteria (probiotics) appear highly promising as an approach to overcome abnormal host conditions. Such attempts, involving probiotics, may aim at modulating the meta-performance of microbiota to overcome various host immunometabolic disorders including obesity. Efficacy of a probiotic, as reflected by alleviation of host metabolic disorders and modulation of GIT microbiota, was investigated in several in vivo studies (Ji et al. 2012; Kadooka et al. 2010; Kim et al. 2016; Kim et al. 2013; Miyoshi et al. 2014; Ritze et al. 2014; Wang et al. 2015). These include reports on the positive effects of the administration of LAB and probiotic strains on diet-induced obese (DIO) mice accompanied by the modulation of faecal microbiota (Wang et al. 2015). This study also emphasised strain-specific impacts on host metabolic syndrome-associated parameters. We have previously reported on the microbial modulation in a murine model receiving a normal diet (Ji et al. 2012). Weight and epididymal fat mass were reduced after administering probiotics, concomitantly with a reduction of the *Clostridium* cluster XIVab group and the F/B (*Firmicutes/Bacteroides*) ratio in the small intestine but not in faecal samples. These results strongly suggest that consumption of probiotics can influence host health not only by direct microbe/host interaction but also indirectly by modulation of the GIT microbiota.

In this study, we selected *L. plantarum* HAC01 isolated from Korean white (*baek*) kimchi (Park et al. 2016) that we administered to a high-fat diet (HFD)-induced obesity murine model. A major objective was to investigate the in vivo potential of *Lactobacillus plantarum* strain HAC01 as a putative probiotic and as a beneficial modulator of gut microbiota for amelioration of metabolic syndrome, including obesity.

Materials and methods

Bacterial strains and culture conditions

L. plantarum HAC01 was isolated from Korean white (*baek*) kimchi and has shown antiobesity effects in an HFD-induced obesity murine model (Park et al. 2016). The strain has been deposited in Korean Collection for Type Cultures (KCTC; WDCM597) under the number KCTC 12647BP. *L. rhamnosus* GG (LGG) (ATCC 53103) served as reference for this study. The strains were grown in MRS broth (Difco Laboratories INC., Franklin Lakes, NJ, USA) and prepared daily for feeding during the intervention period.

Animal experiments

Four-week-old C57BL/6J male mice supplied by Koatec (Gyeonggi, Korea) were housed separately in sterilised cages at 23 ± 1 °C and 55 ± 10% humidity, in a 12-h light/dark cycle and were provided filtered water and 60 kcal% fat rodent diet no. D12492 (Research Diet, USA) ad libitum for 12 weeks. Mice were acclimated for 3 weeks and 1×10^8 CFU viable cells of *L. plantarum* HAC01 and *L. rhamnosus* GG were orally administered for 8 more weeks once a day ($N = 7$ per group). The microorganisms were suspended in 20 μL of PBS for oral administration while the control groups only received 20 μL of PBS. Each LAB strain was grown for 8 h in MRS medium at 37 °C and washed twice with PBS after harvesting

at 16,000×*g* for 5 min. The weight of each animal and its feed consumption was measured once a week. At the end of the experimental period, the animals were sacrificed by cervical dislocation. Blood serum samples were taken by centrifugation of the whole blood specimen at 2500×*g* for 20 min. Adipose tissue and serum samples were frozen and stored at −80 °C without repeated freeze-and-thaw steps. Several stool pellets from each mouse were collected within 6 h after change of bedding, submerged in RNAlater® Solution (Ambion, USA) and stored as described in the manual.

Blood analysis

Fifty microlitres of blood serum sample of each mouse was diluted into 450 μL of PBS, and glucose, triglycerides and cholesterol levels were measured using an automated biochemical analyser BS-200 (Mindray, China) at Pohang Technopark (South Korea). The concentration of leptin and adiponectin in the serum was determined by enzyme-linked immunosorbent assay (ELISA) using an ELISA Kit, pink-ONE (Koma, Korea) as described in the manual.

Transcriptional analysis of mouse tissues

Extraction of mRNA from tissues was conducted according to the specific protocol of Reliaprep RNA tissue miniprep system (Promega, USA). Briefly, each organ sample was homogenised by a hand-held homogeniser (IKA, Germany) in a lysis buffer and centrifuged. The supernatant was mixed with isopropanol and passed through a column provided with the kit. After several washing steps and DNase treatment, the purity and concentration of the eluted complementary DNA (cDNA) were measured by SPECTROstar (BMG LABTECH, Germany). Two to three micrograms of complementary DNA was prepared using the GoScript™ Reverse Transcription System (Promega, USA) through a Verity 96-well thermal cycler (ABI Research, USA) and 5 min of incubation with oligo-dT primer at 70 °C. Quantitative real-time PCR was performed by SYBR Premix Ex Taq™ II (Takara, Japan) with 20 ng of cDNA for each reaction using Step-One Plus real-time PCR System (Applied Biosystems, USA). Specific primers used for the analysis are described in Table S1.

Microbiota analysis of faecal samples

Bacterial RNA from faecal samples of each group was used for metagenome analysis of the microbiota. Faeces frozen with liquid nitrogen was first pulverised in a mortar and pestle while thawing of samples was carefully avoided. Bacterial RNA in the pulverised faecal sample was extracted using ReliaPrep RNA Miniprep System (Promega, USA) as described before, following cell lysis using a mini-beadbeater (Biospec, USA) with 0.3 g of 0.1-mm zirconia/silica beads (Biospec, USA). Two micrograms of RNA was converted into complementary DNA with random primers following the same protocol explained above.

Gut microbiota analysis based on faecal samples, using NGS

Metagenomic analysis was performed with complementary DNA obtained from bacterial RNA in mice faecal samples using V1-V3 (forward, 8f: 5′-AGAGTTTGATCMTGGCTCAG-3′; reverse 518r: 5′-ATTACCGCGGCTGCTGG-3′) variable region of the 16S rRNA gene. Forward primers were tagged with 10-bp unique barcode labels at the 5′ end along with the adaptor sequence (5′-CCATCTCATCCCTGCGTGTCTCCGAC TCAG-3′) to allow multiple samples to be included in a single 454 GS FLX Titanium sequencing plate as previously described (Carroll et al. 2012). 16S rRNA PCR products were quantified, pooled and purified for the sequencing reaction. 454 GS FLX Titanium sequencing was performed on a 454 Life Sciences Genome Sequencer FLX machine (Roche, Florence, SC). Since the pattern of sequence has an irregular flow order (flow pattern B), we used FlowClus (Gaspar and Thomas 2015) to filter and denoise it. The reads truncated prior to the reverse primer, the first N in the sequence and a window of 50 quality scores whose average is below 25.0 were filtered as minimum 200 bp to a maximum of 600 bp. Denoised sequences were analysed via MacQIIME 1.9.1 pipeline with its default analyse options after removal of chimera identified by Usearch6.1. Sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity, and taxonomic assignment was performed with Greengenes 16S rRNA reference database. Alpha- and beta-diversity were also computed and visualised within MacQIIME1.9.1. The sequenced data have been deposited at the NCBI Sequence Read Archive database (SRP081181) under BioProject PRJNA338338.

Statistical analysis

Data are shown as mean and SD. ANOVA was used for comparing data with post hoc analysis such as Dunnett's test and Scheffe's test after Levene's test of variance homogeneity. Statistical analyses were performed using the IBM SPSS Statistics version 20 (IBM, USA). Significance was accepted at $P < 0.05$.

Results

Antiobesity effect of *L. plantarum* HAC01 on DIO mice

The initial body weight of HF-PBS, HF-HAC and HF-GG was 28.36 ± 1.72, 28.32 ± 1.43 and 28.25 ± 2.23 g

(mean ± SD; $P > 0.7$), respectively. After 4 weeks, both lactobacilli-treated groups showed lower body weight gain compared to the control group (HF-PBS). Significant weight reduction was detected for the HF-HAC group compared to HF-PBS, while the reduction of the HF-GG group was not statically significant due to the high variation, starting from the first week (Fig. 1a). Finally, the body weight at the end of the experiment (8 weeks) was markedly lower by 10% in the HF-HAC group than in the control group (Table 1), although overall feed consumption was not significantly different for all groups (Fig. 1b). In addition to differences in total body weight gains, the administration of *Lactobacillus* strains resulted in a reduction in mesenteric fat mass, located around the intestine, but it was significant only for the *L. plantarum* HAC01 group as compared to the control (HF-PBS) (Fig. 1c and Table 1). The values of liver and epididymal fat mass were also noticeably reduced in the LAB-fed groups compared to the control (Table 1). A decrease in blood glucose and cholesterol levels resulted from the administration of *L. plantarum* strain HAC01. Moreover, levels of other indicators such as the glucose/lipid metabolism-associated biomarkers, the blood glucose, total cholesterol (TC) and triacylglycerol (TG) levels were all alleviated in the HF-LP group (Table 1), while the concentrations of circulating blood leptin and adiponectin either significantly decreased or increased, respectively, indicating amelioration of the metabolic dysfunction induced by an HFD (Table 1).

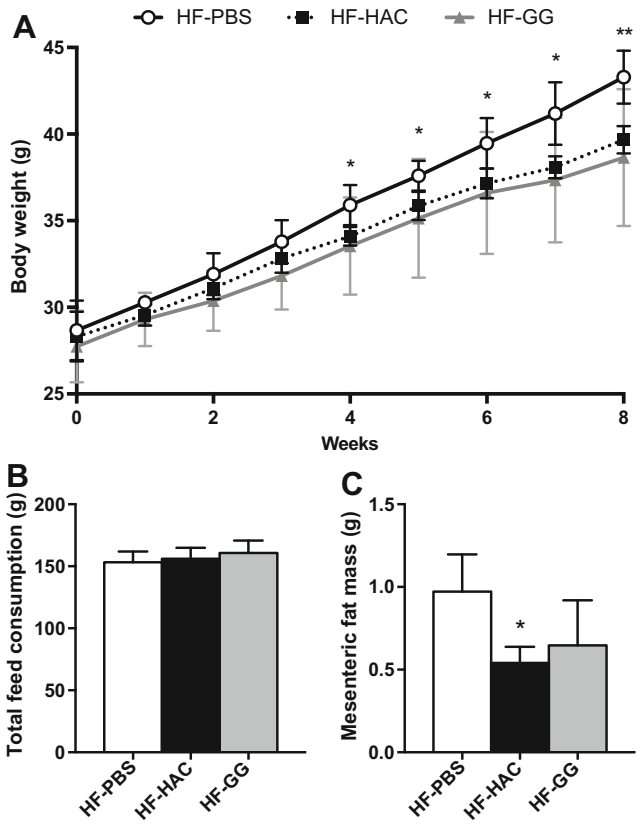

Fig. 1 The effect of LAB administration on the weight of DIO mice receiving a high-fat (HF) diet over 8 weeks. Whole body weight (**a**) and total feed consumption (**b**) during an administration period and mesenteric fat mass (**c**) were measured after the experiment was finished ($N = 6$). HF-PBS: control group treated with sterile PBS; HF-HAC: group receiving *L. plantarum* HAC01; HF-GG: group receiving *L. rhamnosus* GG. Data of each group are shown as mean ± SD and were analysed with one-way ANOVA with Dunnett's test compared HF-PBS group. Significant differences between HF-HAC and HF-PBS groups are presented as *asterisks*. The HF-GG group did not show significant differences from any point. *$p < 0.05$; **$p < 0.01$

Expression of lipid metabolism-associated biomarkers in mesenteric adipose tissue

Expression levels of mRNA involving fatty acid oxidation in adipose tissue such as acyl-coenzyme A oxidase (ACOX), carnitine palmitoyltransferase1 (CPT1), peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α) and peroxisome proliferator-activated receptor alpha (PPAR-α) were significantly upregulated in the mesenteric adipose tissue (MAT) in the HF-HAC group compared to the control group (Fig. 2a). LGG administration to DIO mice also increased mRNA expression of PGC-1α and PPAR-α; however, it did not induce noticeable changes in the expression of enzymes for beta-oxidation such as ACOX and CPT1. Moreover, the mRNA expression of lipogenic genes such as fatty acid synthase (FAS) and stearoyl-CoA desaturase (SCD-1) showed slight but not significant modulation in the *Lactobacillus* treatment groups compared to the control (Fig. 2b). As the leptin and adiponectin concentrations in the blood noticeably changed, the respective mRNA expression levels were also analysed, with the results closely correlating with the data obtained from the serum (Fig. 2c). These results suggest a lower expression level of enzymes associated with the de novo synthesis and storage of fatty acids in the adipose tissue, while oxidation of fatty acids was increased by the administration of *L. plantarum* HAC01 in the DIO mice model. In addition, LAB administration improved regulation of the inflammatory status in obese mice and resulted in lowering of lipid metabolism as reflected by changes in the level of adiponectin and leptin in the LAB-fed groups. On the other hand, changes in mRNA expression levels, especially for FAS and SCD1, were not reflected in a similar way in the epididymal adipose tissue (EAT) of the HF-LP group and with less reduction in weight as compared to the control group (Figure S1).

Modulation of the gut microbial population following LAB administration

The administration of *L. plantarum* strain HAC01 resulted in a strong reduction, in both weight gain and mesenteric fat mass, as well as other obesity-related indicators. LGG administration also showed noticeable impacts on these parameters yet

Table 1 The individual effects of two lactobacilli on weight and other obesity-related indicators in the blood of DIO mice receiving HF diet

Parameters	HF-PBS	HF-HAC	HF-GG
Body weight (g)			
Initial	28.36 ± 1.72	28.32 ± 1.43	28.25 ± 2.23
End point	43.28 ± 1.53	39.67 ± 0.80**	39.75 ± 4.46
Weight gain	14.62 ± 3.04	11.35 ± 1.22	11.50 ± 3.23
Liver	1.28 ± 0.34	1.08 ± 0.08	1.19 ± 0.20
EAT	2.29 ± 0.17	2.09 ± 0.22	2.05 ± 0.55
MAT	0.97 ± 0.23	0.54 ± 0.10*	0.69 ± 0.32
Blood analysis			
Glucose (mg/dL)	237.50 ± 9.57	187.50 ± 23.63*	200.00 ± 49.67
TG (mg/dL)	105.00 ± 12.91	87.50 ± 17.08	107.50 ± 29.86
TC (mg/dL)	202.50 ± 23.63	162.50 ± 15.00	165.00 ± 34.16
Adiponectin (μg/mL)	5.27 ± 0.80	7.08 ± 0.67*	6.63 ± 0.51
Leptin (ng/mL)	71.18 ± 21.60	35.87 ± 12.12*	48.49 ± 34.47

The weight of the whole body, liver and adipose tissue was measured after 8 weeks from the initial point ($N = 6$). Indicators in the blood were measured either by a blood analyser ($N = 4$) or ELISA method for detection of adiponectin and leptin concentration ($N = 6$). Data were shown as mean ± SD and analysed with one-way ANOVA with Dunnett's test compared to HF-PBS group

EAT epididymal adipose tissue, *MAT* mesenteric adipose tissue, *TG* triacylglyceride, *TC* total cholesterol, *HF-PBS* control group treated with sterile PBS, *HF-HAC* group receiving *L. plantarum* strain HAC01, *HF-GG* group receiving *L. rhamnosus* strain GG

*$p < 0.05$; **$p < 0.01$

not significant due to a high variation within the group. These results opened the question as to whether these strains had a modulatory effect on the microbial composition in the intestinal tract. To assess changes in the active microbial population in the gut, we used reverse-transcribed bacterial 16S rRNA information from faecal samples of each group and sequenced it up to 550 bp based on the V1-V3 region of the bacterial 16S rRNA, using the 454 GS FLX+ system. There was significant discrimination between the treatment groups in beta-diversity using principal coordinate analysis (PCoA) with both unweighted and weighted UniFrac distances (Fig. 3a, b) while alpha-diversity did not show clear distinction among the

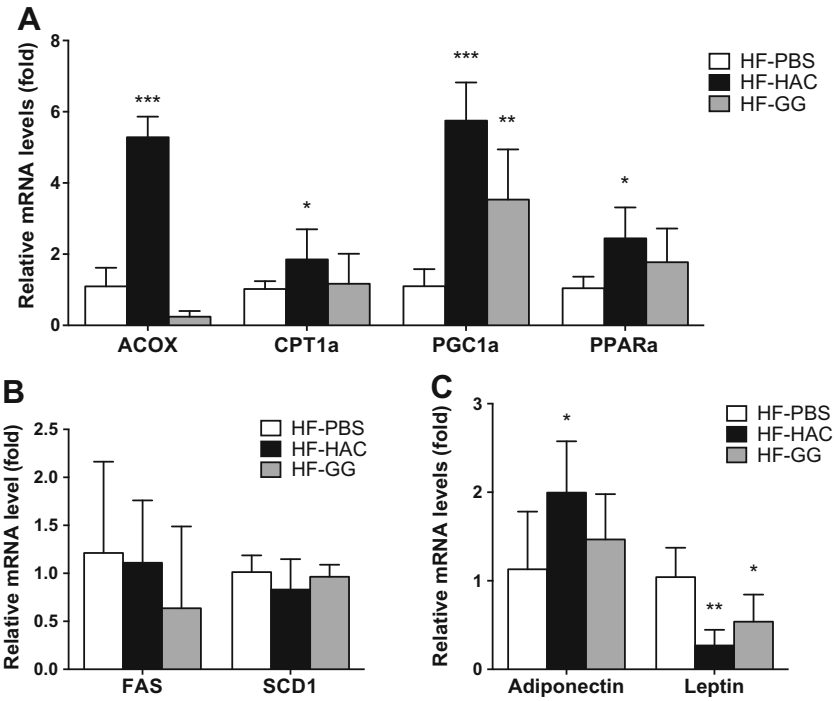

Fig. 2 Change in mRNA expression in the mesenteric adipose tissue of DIO mice ($N = 5$–6) receiving HF diet over 8 weeks. Relative amounts of mRNA expression of biomarkers associated with fat oxidation (**a**), lipogenesis (**b**), and adipokines (**c**) in the adipose tissue are shown. Expression levels were measured by qRT-PCR and normalised by GAPDH. HF-PBS: control group treated with sterile PBS; HF-HAC: group receiving *L. plantarum* HAC01; HF-GG: group receiving *L. rhamnosus* GG. Data are shown as mean ± SD and were compared by one-way ANOVA with Dunnett's test compared to the HF-PBS group. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

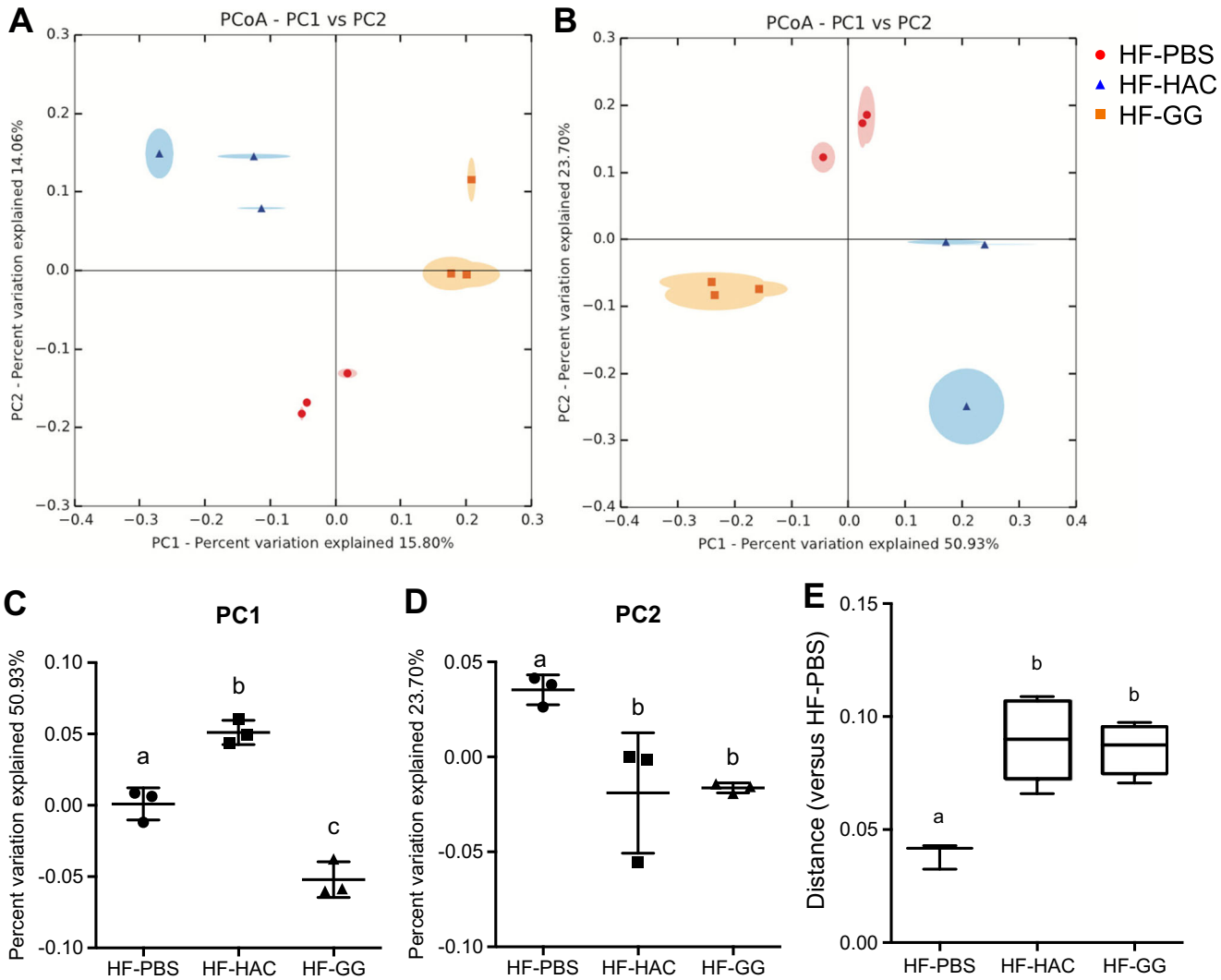

Fig. 3 Beta-diversity of faecal microbiota in each group of DIO mice receiving HF diet over 8 weeks ($N = 3$). Principal coordinates analysis (PCoA) plots were generated from unweighted (**a**) and weighted (**b**) UniFrac distance metrics. The score of each PC1 (**c**) and PC2 (**d**) from weighted UniFrac distance matrix is shown individually and the distance of samples between HF-PBS and each group (**e**) was also computed. HF-PBS: control group treated with sterile PBS; HF-LP: group receiving *L. plantarum* HAC01; HF-LG: group receiving *L. rhamnosus* GG. Data were analysed by Macqiime ver. 1.9.1. and shown as mean ± SD and were compared by one-way ANOVA with Scheffe's test, and statistical differences ($p < 0.05$) were represented by different *letters* above each bar

different treatment groups (Figure S2). Especially, PC1 of beta-diversity showed significantly distinct microbial composition of all three groups, while the administration of the two LAB strains induced microbiota modulation of the control group in a similar fashion according to PC2 (Fig. 3a–d). The distance of samples within HF-PBS also significantly differed from the control group and each LAB-treated group, indicating a modulation of intestinal microbiota in DIO mice by LAB administration (Fig. 3e).

LAB administration resulted in diverging changes in several bacterial taxa, both on family and genus level (Fig. 4). The relative abundance of the family *Lachnospiraceae* (phylum *Firmicutes*) was significantly higher in both LAB-treated groups than in the control (PBS) group (8.27 ± 0.13% for HF-PBS, 10.64 ± 0.53% for HF-HAC and 9.81 ± 0.40% for HF-GG of total reads per sample; p values were 0.001 and 0.008 compared to HF-PBS, respectively). In contrast, the low abundance of the family *Deferribacteraceae* in the LAB-administrated groups (0.25 ± 0.03% for HF-HAC and 0.50 ± 0.10% for HF-GG, compared to 0.85 ± 0.10% for HF-PBS; p values were less than 0.001 and 0.007 compared to HF-PBS, respectively) was probably due to a comparatively significant reduction in numbers of the genus *Mucispirillum* (Fig. 4a, b). Some modulatory effects of the two LAB strains were however quite different. The proportion of the families *Bacteroidaceae* and *Rikenellaceae* (both phylum *Bacteroidetes*) was significantly lower in HF-HAC (4.95 ± 0.41 and 2.20 ± 0.11%) compared to HF-PBS (6.25 ± 0.07 and 2.62 ± 0.09%, $p < 0.05$) but without a significant change in the HF-GG group (5.73 ± 1.27 and 3.05 ± 0.71%) (Fig. 4a). At the genus level, these two families

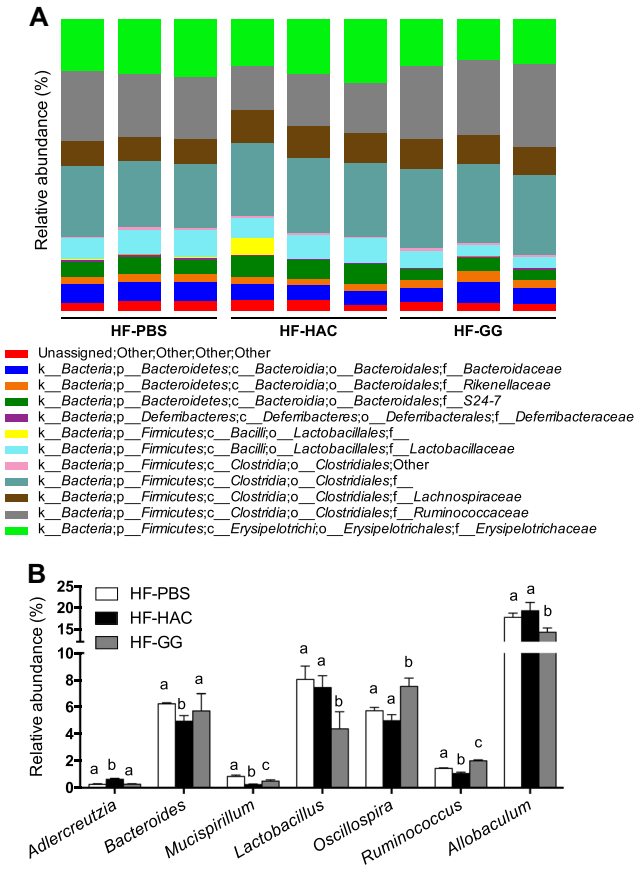

Fig. 4 Composition of microbiota from stool samples. Relative abundance of assigned OTUs on family level (**a**) was obtained by trimming OTUs having less than 0.5%. More detailed information on the relative ratio of the abundance for several OTUs is given at genus level (**b**), normalised with values of relative abundance (%) of the HF-PBS group (*dashed line*), also shown as mean ± SD. Reads were assigned by using uclust taxonomy classifier against Greengenes reference sequences through Macqiime 1.9.1. HF-PBS: control group treated with sterile PBS; HF-LP: group receiving *L. plantarum* HAC01; HF-LG: group receiving *L. rhamnosus* GG. Data were compared by one-way ANOVA with Dunnett's test compared to HF-PBS group. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

(e.g., comparing abundance of the genus *Bacteroides*) were also significantly lower in HF-HAC compared to other groups (Fig. 4b). However, the identity of the major assigned genus in the family *Rikenellaceae* was not defined by the database (data not shown). Another *Bacteroidetes* family, S24-7, was more abundant in HF-HAC (6.71 ± 0.34%) and much less so in HF-GG (3.89 ± 0.50%) than in HF-PBS (5.27 ± 0.65%; p values 0.038 and 0.045, respectively) (Fig. 4a). On the other hand, the ratio of the *Firmicutes* family *Ruminococcaceae* was decreased in HF-HAC (16.26 ± 1.34%, p value = 0.011) and increased in HF-GG (26.10 ± 1.64%, p value = 0.034), compared to HF-PBS (21.80 ± 1.46%) (Fig. 4a). The relative proportions of the genera *Ruminococcus* and *Oscillospira* (both family *Ruminococcaceae*) were also smaller in HF-HAC and higher in HF-GG (Fig. 4b). The relative abundance of the genus *Allobaculum* (family *Erysipelotrichaceae*) was significantly smaller in HF-GG than in other groups (Fig. 4b). Interestingly, administration of the respective *Lactobacillus* strains, particularly of LGG, resulted in relative decreases in the family *Lactobacillaceae* (8.04 ± 1.00% HF-PBS, 7.52 ± 0.94% HF-HAC and 4.39 ± 1.26% HF-GG; p = 0.846 and 0.017 compared to HF-PBS, respectively) (Fig. 4). The relative abundance of the genus *Lactobacillus* was also reduced in both LAB-treated groups and, in fact, was statistically significant in the HF-GG group (Fig. 4). There was no significant change in the relative proportion of *Firmicutes* and *Bacteroidetes*, the phyla typical of the mouse gut microbiota (80.79 ± 0.84% HF-PBS, 81.17 ± 1.23% HF-HAC and 83.21 ± 2.26% HF-GG according to the abundance for phylum *Firmicutes*; 14.45 ± 0.61% HF-PBS, 14.07 ± 0.63% HF-HAC and 12.90 ± 2.41% HF-GG according to the abundance for phylum *Bacteroidetes*; $p > 0.1$).

Discussion

We investigated the functionality of LAB strains isolated from fermented Korean white kimchi in a DIO murine model with regard to amelioration of host metabolic symptoms and modulation of GIT microbiota. Supplementation with *L. plantarum* strain HAC01 has resulted in a reduction of adipose tissue accumulation in the DIO murine model and the modulation of specific groups of gut microbiota. We have also monitored changes in gene expression associated with lipid metabolism in mesenteric adipose tissue of DIO mice. Adipose tissue is one of the major storage sites for triglycerides, and, considering its role as endocrine organ in energy homeostasis, it has a direct impact on the development of metabolic disorders such as cardiovascular disease (CVD) and type II diabetes mellitus (T2DM) (Cusi 2010; Maghbooli and Hossein-nezhad 2015; Richardson et al. 2013; Sethi and Vidal-Puig 2007; Xu 2013). Both MAT and EAT are categorised as visceral adipose depot and considered to be primary derivatives of developing host immunometabolic dysfunctions. Yet, contrasting responses were measured when two different LAB strains were administered. *L. plantarum* HAC01 induced a significant upregulation of fat oxidation-related (ACOX) gene expression in MAT, while LGG treatment resulted in downregulation of oxidative gene expression compared to the control group (HF-PBS) (Fig. 2a). Administration of probiotics may promote the lowering of MAT mass along with the reduction of serum glucose and TG levels as well as alleviation of circulating leptin/adiponectin ratio in DIO animals. These data show agreements with former studies including human clinical trials (Oda et al. 2008). HAC01 treatment, however, did not significantly affect EAT mass and expression of the fatty acid oxidative genes (Table 1; Figure S1). The contradicting results for MAT and EAT may implicate LAB-induced modulation of the intestinal microbiota, thereby affecting the MAT, the conjunctive tissue to the gastrointestinal tract.

Our approach for the analysis of gut microbiota was to investigate the active population using microbial ribosomal RNA (rRNA) instead of genomic DNA (gDNA) (Gosalbes et al. 2011; Perez-Cobas et al. 2013). We also used 454 titanium pyrosequencing to analyse the change in major microbial groups in the intestinal tract. The major advantage of 454 pyrosequencing over Illumina is the reading of longer sequences thereby ensuring more reliable results on taxa assignment in spite of higher costs. Some previous reports have shown the change in microbial composition triggered by treatment with a single *Lactobacillus* strain to be on the level of the less abundant taxa level rather than in the overall structure (Kwok et al. 2015; Roos et al. 2013). Thus, by analysing longer and more reads with a smaller number of samples from each group, we expected more detailed and accurate analysis of changes on the taxon level.

Ravussin et al. (2012) found that the relative abundance of the genera *Mucispirillum* and *Bacteroides* appeared at a lower level in a calorie-restricted group of DIO mice than in the ad libitum DIO group; they also showed the positive correlation of *Mucispirillum* abundance with the circulating leptin level. The abundance of these two genera, belonging to the families *Deferribacteriaceae* and *Bacteroidaceae*, respectively, was also lower in the HF-HAC than in the control group, concomitantly with a reduction in blood leptin concentration. The higher relative abundance of the genus *Allobaculum* (family *Erysipelotrichaceae*) in HF-HAC, although not statistically significant, appeared to be negatively correlated with leptin concentration, in line with the report by Ravussin et al. (2012). Another report suggested the enrichment of *Allobaculum*, one of the short-chain fatty acid-producing genera, to correlate with improved gut permeability and alleviation of blood glucose and insulin concentrations by berberine treatment in HFD-fed rats (Zhang et al. 2012). Our current study confirms our formerly reported decrease in the relative abundance in the murine gut of the genus *Lactobacillus* resulting from LAB consumption (Ji et al. 2012). This, however, appears to contradict the results reported by Kwok et al. (2015) showing a significant increase in abundance of the genus *Lactobacillus* by consumption of *L. plantarum* in a human clinical trial. This may be explained by expected differences in results between using either genomic DNA or ribosomal RNA, the latter of which indicate active microbial population groups. It may also be noted that oral treatment with *Bacteroides fragilis* has not resulted in colonisation of the *B. fragilis* strain but rather in a modulation of the relative proportion of specific groups of bacteria in a mouse model (Hsiao et al. 2013), thereby also resulting in correction of gut permeability and alleviation of intestinal disorders. These results indicate that the administration of specific bacteria may result not only in the increase of a particular population and/or its activity in the gut but also in the modulation of gut microbiota composition without colonisation. Modes of interaction of a modulated microbial population and mechanisms by which metabolic pathways in the adipose tissue around the intestine are affected still need further investigation. However, several possible mechanisms may be linked to changes of a few genera in the active microbial population. For example, elevated levels of SCFA can stimulate respective receptors such as GPR43 in the adipose tissue, previously demonstrated to influence inhibition of fat accumulation in this tissue (Kimura et al. 2013).

In conclusion, our data suggest that administration of the single LAB strain *L. plantarum* HAC01, previously shown to survive simulated conditions of the upper intestinal tract at high levels (Park et al. 2016), alleviated negative symptoms of obesity while modulating the active gut microbial composition in a DIO mouse model. Based on these results, we propose a possible explanation of dynamic processes resulting from LAB administration, leading to modulation of the intestinal microbiota and thereby influencing the regulation of gene expression related to lipid metabolism in the MAT. This interactive cascade may lead to a reduction in fat mass and alleviation of host metabolism in DIO mice. Future investigations should be directed to understanding the metabolic role and features of the modulated host gut microbiota. Monitoring (strain-) specific responses in vivo, following the administration of a single bacterial strain, may enable a deeper understanding of probiotic mechanisms of action based on indirect host microbiota modulation.

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Conflict of interest The authors declare that they have no conflict of interest.

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References

Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI (2004) The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 101(44):15718–15723. doi:10.1073/pnas.0407076101

Caricilli AM, Picardi PK, de Abreu LL, Ueno M, Prada PO, Ropelle ER, Hirabara SM, Castoldi Â, Vieira P, Camara NO (2011) Gut microbiota is a key modulator of insulin resistance in TLR 2 knockout mice. PLoS Biol 9(12):e1001212

Carroll IM, Ringel-Kulka T, Siddle JP, Ringel Y (2012) Alterations in composition and diversity of the intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. Neurogastroenterol & Motility 24(6):521–e248

Consortium HMP (2012) Structure, function and diversity of the healthy human microbiome. Nature 486(7402):207–214

Cusi K (2010) The role of adipose tissue and lipotoxicity in the pathogenesis of type 2 diabetes. Curr Diabetes Rep 10(4):306–315

D'Argenio V, Salvatore F (2015) The role of the gut microbiome in the healthy adult status. Clin Chim Acta 451:97–102

Gaspar JM, Thomas WK (2015) FlowClus: efficiently filtering and denoising pyrosequenced amplicons. BMC Bioinformatics 16:105. doi:10.1186/s12859-015-0532-1

Gosalbes MJ, Durban A, Pignatelli M, Abellan JJ, Jimenez-Hernandez N, Perez-Cobas AE, Latorre A, Moya A (2011) Metatranscriptomic approach to analyze the functional human gut microbiota. PLoS One 6(3):e17447. doi:10.1371/journal.pone.0017447

Hemarajata P, Versalovic J (2012) Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. Therap Adv Gastroenterol 6(1):39–51. doi:10.1177/1756283X12459294

Holzapfel WH, Wood BJ (2014) Lactic acid bacteria: biodiversity and taxonomy. John Wiley & Sons, Chichester

Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, Codelli JA, Chow J, Reisman SE, Petrosino JF (2013) Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 155(7):1451–1463

Ji YS, Kim HN, Park HJ, Lee JE, Yeo SY, Yang JS, Park SY, Yoon HS, Cho GS, Franz CM, Bomba A, Shin HK, Holzapfel WH (2012) Modulation of the murine microbiome with a concomitant anti-obesity effect by *Lactobacillus rhamnosus* GG and *Lactobacillus sakei* NR28. Benef Microbes 3(1):13–22. doi:10.3920/BM2011.0046

Kadooka Y, Sato M, Imaizumi K, Ogawa A, Ikuyama K, Akai Y, Okano M, Kagoshima M, Tsuchida T (2010) Regulation of abdominal adiposity by probiotics (*Lactobacillus gasseri* SBT2055) in adults with obese tendencies in a randomized controlled trial. Eur J Clin Nutr 64(6):636–643. doi:10.1038/ejcn.2010.19

Kim B, Park K-Y, Ji Y, Park S, Holzapfel W, Hyun C-K (2016) Protective effects of *Lactobacillus rhamnosus* GG against dyslipidemia in high-fat diet-induced obese mice. Biochem Biophys Res Comm 473(2):530–536

Kim S-W, Park K-Y, Kim B, Kim E, Hyun C-K (2013) *Lactobacillus rhamnosus* GG improves insulin sensitivity and reduces adiposity in high-fat diet-fed mice through enhancement of adiponectin production. Biochem Biophys Res Comm 431(2):258–263

Kimura I, Ozawa K, Inoue D, Imamura T, Kimura K, Maeda T, Terasawa K, Kashihara D, Hirano K, Tani T, Takahashi T, Miyauchi S, Shioi G, Inoue H, Tsujimoto G (2013) The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. Nat Commun 4:1829. doi:10.1038/ncomms2852

Kwok L, Guo Z, Zhang J, Wang L, Qiao J, Hou Q, Zheng Y, Zhang H (2015) The impact of oral consumption of *Lactobacillus plantarum* P-8 on faecal bacteria revealed by pyrosequencing. Benef Microbes 6(4):405–413

Kwon H-K, So J-S, Lee C-G, Sahoo A, Yi H-J, Park J-N, S-y L, Hwang K-C, Jun C-D, Chun J-S (2008) Foxp3 induces IL-4 gene silencing by affecting nuclear translocation of NFκB and chromatin structure. Mol Immunol 45(11):3205–3212

Li Z, Yang S, Lin H, Huang J, Watkins PA, Moser AB, DeSimone C, Xy S, Diehl AM (2003) Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. Hepatol 37(2):343–350

Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R (2012) Diversity, stability and resilience of the human gut microbiota. Nature 489(7415):220–230

Maghbooli Z, Hossein-nezhad A (2015) Transcriptome and molecular endocrinology aspects of epicardial adipose tissue in cardiovascular diseases: a systematic review and meta-analysis of observational studies. Biomed Res Int 2015:926567 12 pages, http://dx.doi.org/10.1155/2015/926567

Masood MI, Qadir MI, Shirazi JH, Khan IU (2011) Beneficial effects of lactic acid bacteria on human beings. Crit Rev Microbiol 37(1):91–98

Miyoshi M, Ogawa A, Higurashi S, Kadooka Y (2014) Anti-obesity effect of *Lactobacillus gasseri* SBT2055 accompanied by inhibition of pro-inflammatory gene expression in the visceral adipose tissue in diet-induced obese mice. Europ J Nutr 53(2):599–606

O'Hara AM, Shanahan F (2006) The gut flora as a forgotten organ. EMBO Rep 7(7):688–693. doi:10.1038/sj.embor.7400731

Oda N, Imamura S, Fujita T, Uchida Y, Inagaki K, Kakizawa H, Hayakawa N, Suzuki A, Takeda J, Horikawa Y (2008) The ratio of leptin to adiponectin can be used as an index of insulin resistance. Metabolism 57(2):268–273

Park S, Ji Y, Park H, Lee K, Park H, Beck BR, Shin H, Holzapfel WH (2016) Evaluation of functional properties of lactobacilli isolated from Korean white kimchi. Food Control 69:5–12. doi:10.1016/j.foodcont.2016.04.037

Parvez S, Malik K, Ah Kang S, Kim HY (2006) Probiotics and their fermented food products are beneficial for health. J Appl Microbiol 100(6):1171–1185

Perez-Cobas AE, Gosalbes MJ, Friedrichs A, Knecht H, Artacho A, Eismann K, Otto W, Rojo D, Bargiela R, von Bergen M, Neulinger SC, Daumer C, Heinsen FA, Latorre A, Barbas C, Seifert J, dos Santos VM, Ott SJ, Ferrer M, Moya A (2013) Gut microbiota disturbance during antibiotic therapy: a multi-omic approach. Gut 62(11):1591–1601. doi:10.1136/gutjnl-2012-303184

Ravussin Y, Koren O, Spor A, LeDuc C, Gutman R, Stombaugh J, Knight R, Ley RE, Leibel RL (2012) Responses of gut microbiota to diet composition and weight loss in lean and obese mice. Obesity (Silver Spring) 20(4):738–747. doi:10.1038/oby.2011.111

Richardson VR, Smith KA, Carter AM (2013) Adipose tissue inflammation: feeding the development of type 2 diabetes mellitus. Immunobiol 218(12):1497–1504

Ritze Y, Bárdos G, Claus A, Ehrmann V, Bergheim I, Schwiertz A, Bischoff SC (2014) *Lactobacillus rhamnosus* GG protects against non-alcoholic fatty liver disease in mice. PLoS One 9(1):e80169

Roos S, Dicksved J, Tarasco V, Locatelli E, Ricceri F, Grandin U, Savino F (2013) 454 pyrosequencing analysis on faecal samples from a randomized DBPC trial of colicky infants treated with *Lactobacillus reuteri* DSM 17938. PLoS One 8(2):e56710

Sanz Y, Rastmanesh R, Agostonic C (2013) Understanding the role of gut microbes and probiotics in obesity: how far are we? Pharmacol Res 69(1):144–155

Sethi JK, Vidal-Puig AJ (2007) Thematic review series: adipocyte biology. Adipose tissue function and plasticity orchestrate nutritional adaptation. J Lipid Res 48(6):1253–1262

Spor A, Koren O, Ley R (2011) Unravelling the effects of the environment and host genotype on the gut microbiome. Nature Rev Microbiol 9(4):279–290

Wang J, Tang H, Zhang C, Zhao Y, Derrien M, Rocher E, van- Hylckama Vlieg JE, Strissel K, Zhao L, Obin M, Shen J (2015) Modulation of gut microbiota during probiotic-mediated attenuation of metabolic syndrome in high fat diet-fed mice. ISME J 9(1):1–15. doi:10.1038/ismej.2014.99

Xu H (2013) Obesity and metabolic inflammation. Drug Discovery Today: Disease Mechanisms 10(1):e21–e25

Zeng J, Li YQ, Zuo XL, Zhen YB, Yang J, Liu CH (2008) Clinical trial: effect of active lactic acid bacteria on mucosal barrier function in patients with diarrhoea-predominant irritable bowel syndrome. Alim Pharmacol Therap 28(8):994–1002

Zhang C, Zhang M, Pang X, Zhao Y, Wang L, Zhao L (2012) Structural resilience of the gut microbiota in adult mice under high-fat dietary perturbations. ISME J 6(10):1848–1857. doi:10.1038/ismej.2012.27