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Effects of exercise and dietary protein sources on adiposity and insulin sensitivity in obese mice☆,☆☆

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Abstract

Low-fat diets and exercise are generally assumed to ameliorate obesity-related metabolic dysfunctions, but the importance of exercise *vs.* dietary changes is debated. Male C57BL/6J mice were fed a high-fat/high-sucrose (HF/HS) diet to induce obesity and then either maintained on the HF/HS or shifted to low-fat (LF) diets containing either salmon or entrecote. For each diet, half of the animals exercised voluntarily for 8 weeks. We determined body composition, glucose tolerance, insulin sensitivity and hepatic triacylglycerol levels. The microbiota composition in cecal and fecal samples was analyzed using 16S ribosomal RNA gene amplicon sequencing. Voluntary exercise improved insulin sensitivity but did not improve glucose tolerance. Voluntary exercise did not reduce adiposity in mice maintained on an HF/HS diet but enhanced LF-induced reduction in adiposity. Hepatic triacylglycerol levels were reduced by voluntary exercise in LF- but not HF/HS-fed mice. Voluntary exercise induced shifts in the cecal and fecal microbiota composition and functional potential in mice fed LF or HF/HS diets. Whereas voluntary exercise improved insulin sensitivity, a switch to an LF diet was the most important factor related to body weight and fat mass reduction.

Keywords: Mice; Obesity; Exercise; Low-fat diet; Dietary protein source; Gut microbiota

1. Introduction

Nonpharmacological interventions to reduce body weight and improve metabolic dysfunction have been conducted extensively in mice using low-fat (LF) diets [1], calorie restriction [2] and exercise [3,4]. However, the importance of exercise as a remedy to ameliorate obesity related metabolic dysfunctions *vs.* dietary changes is debated. Changes of macronutrient composition, % energy from fat, carbohydrates and protein in the diet have been demonstrated to profoundly affect the development of obesity and insulin resistance in several studies. In rodents, the amount of dietary fat content rather than the obese state appears to be a key determinant for dysregulated glucose homeostasis [5–7]. However, increasing the protein:carbohydrate ratio in high-fat diets prevents development of high-fat-diet-induced obesity and insulin resistance [8–14]. Moreover, a number of studies have demonstrated that the protein source modulates the potential of high-protein diets to attenuate obesity development [15] and the obesogenic potential of Western diets [16–19]. However, it is still unclear to what extent the (anti)obesogenic potential of different protein sources relates to differences in the amino acid composition or if other factors are of importance, as protein sources also differ with respect to the type of endogenous fat, micronutrients and undesirables, such as pollutants and medical residues [20]. The composition of the gut microbiota has been established as a potential therapeutic target for treatment of obesity and other metabolic disorders [21–25]. Voluntary exercise has been demonstrated to induce a shift in the composition of the gut microbiota and to prevent high-fat (HF) diet-induced weight gain and glucose intolerance in casein-based diets [26]. The protein source can modulate the composition of the gut microbiota [20]; however, to what extent intake of LF diets containing different protein sources with different amino acid and fatty acid composition combined with voluntary exercise affects the composition of the gut microbiota in the obese state has not been addressed.

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Thus, the aim of the present study was to evaluate how voluntary exercise and LF diets based on different protein sources affected weight loss and metabolic parameters in obese C57BL/6J mice at thermoneutral condition. Further, we aimed to investigate how diets and exercise modulated the composition and functional potential of the gut microbiota. Obesity was induced by high-fat/high-sucrose (HF/HS) feeding followed by a shift to LF diets containing salmon or entrecote with or without possibilities for voluntary exercise. The experimental LF diets were supplemented with an equal amount of casein and balanced with fat to reach isocaloric levels, but the diets differed in amino acid and fatty acid composition. LF diets containing entrecote or salmon induced the same loss in body and fat mass. However, the LF diet containing salmon had a more pronounced effect on hepatic triacylglycerol (TAG) levels and insulin sensitivity compared to mice given an LF diet containing entrecote. Both LF diets and exercise modulated the composition and functional potential of the gut microbiota in a complex manner.

2. Materials and methods

2.1. Diets

A regular casein-based LF diet (S8672-E050) from Ssniff Spezialdiäten GmbH (Soest, Germany) was used for 1-week acclimatization. We prepared three experimental diets based on the macro- and micronutrient composition of LF and HF/HS diets for rodents produced by Ssniff (S8672-E050 and S8672-E056). The protein sources were selected to represent three different types of protein: entrecote as a representative of red meat, salmon as a representative of fatty fish and casein as a representative of dairy protein. Endogenous fat from the protein sources were quantified, but not extracted, in order to avoid chemical modification of the added entrecote or salmon filet. Due to the high fat content of salmon and entrecote, the LF diets were prepared by 50% casein and 50% protein from either salmon (Marine Harvest, Bergen, Norway) or entrecote (H. Brakstad Eftf. AS, Bergen, Norway). The experimental HF/HS diet was prepared using one-third casein, one-third protein from salmon and one-third protein from entrecote. Both salmon and entrecote were added to the experimental diets after heat treatment (core temperature of 75°C), freeze-drying (to 95%–97% dry matter) and homogenization. Endogenous fat and nitrogen contents in both the salmon and entrecote filet were quantified, and corn oil was added to the experimental LF diet containing entrecote to achieve isocaloric experimental diets with the same level of fat. The compositions of the experimental diets and energy content are shown in Table 1. Amino acid composition (Supplementary Table 1) and fatty acid composition (Supplementary Table 2) in the diets were determined as described previously [16].

2.2. Ethical statements and animal housing

The animal experiment was performed in accordance with the approval given by the Norwegian Animal Research Authority (FOTS id.nr 5358). We did not observe any adverse effects of the experimental diets during the experiment.

We obtained 70 male C57BL/6J (BomTac) mice from Taconic (Ejby, Denmark). To quantify energy intake and apparent fat and nitrogen digestibility, all mice were housed individually. To avoid thermal stress, all mice were housed at thermoneutrality (30°C± 1°C) [27] and with a standard 12/12-h light/dark cycle. After 1 week acclimatization, all mice were fed the obesogenic HF/HS diet for 8 weeks. Thereafter, 60 representative animals were divided into 6 equal experimental groups based on body weight and body composition and subjected to an 8-week intervention. Twenty mice continued on the HF/HS diet, while the remaining 40 mice were given LF diets *ad libitum* based on a 1:1 mixture of casein and salmon or entrecote protein. Ten mice within each of the three experimental diet groups were allowed to exercise voluntarily (Supplementary Fig. 1A). Body mass was measured weekly. Fat mass, lean mass and free water were determined in conscious mice by noninvasive scanning (Bruker Minispec LF50 Body Composition Analyzer mq7.5, Bruker Optik GmbH, Germany) before the intervention and after 7 weeks on the experimental diets. After 8 weeks, the animals were anesthetized using isoflurane (Isoba-vet, Schering-Plow, Denmark) and sacrificed by cardiac puncture. Liver and adipose tissue depots (eWAT and iWAT) were dissected out, weighted and snap-frozen in liquid nitrogen. All tissues and feces samples were stored at −80°C until further analyses.

2.3. Voluntary exercise

The mice were allowed to exercise using low-profile wireless running wheels. We quantified voluntary exercise level by measuring the average running distance per week using standard setup and settings (ENV-044, Low Profile Wireless Running Wheel for Mice) as described by the manufacturer (Med Associates, Inc.). No restrictions on distance or period (night/day) were made.

Table 1
Ingredient list and analyses of nitrogen, fat and total energy content of the experimental diets

Diet	HF/HS	LF salmon	LF entrecote
Ingredients (g/kg)			
Entrecote	111.1	-	145.9
Salmon	138.6	178.2	–
Casein	70.0	103.6	103.6
L-Cystine	3	3	3
Starch (normal)	9.5	–	–
Sucrose (powdered sugar)	410.5	91.8	91.8
Cellulose	50	50	50
Dextrin	10	524	524
Corn oil	150	2.10	33.9
t-Butylhydroquinone	0.02	0.02	0.02
Mineral mix (AIN93G)	35	35	35
Vitamin mix (AIN93VX)	10	10	10
Choline Bitartrate	2.5	2.5	2.5
Sum	1000	1000	1000
Content analysis (g/100 g)			
Fat	24.9	8.4	8.4
Protein	21.8	17.3	17.3
Energy (kJ/g)	23.1	18.4	18.3

Ingredient list of the experimental diets. Total fat content was determined gravimetrically after organic extraction, and total protein content was calculated based on nitrogen content measured in the experimental diets. Values obtained were based on duplicate measurement of each experimental diet.

2.4. Energy intake, feed efficiency and apparent nitrogen and fat digestibility

The mice were fed three times per week and given approximately 3 g of feed per day. The total energy intake was calculated based on the amount of feed eaten and energy content in the experimental diets. Feed efficiency was calculated based on body weight gain and energy intake during the 8-week intervention. Apparent fat and nitrogen digestibility were estimated based on measurements of fat and nitrogen concentrations in feces collected during 1 week (the fifth week of the intervention) in the experiment, and apparent digestibility was calculated using the formula: $\frac{(\text{amount of fat or nitrogen eaten} - \text{amount of fat or nitrogen excreted})}{\text{amount of fat or nitrogen eaten}} \times 100$. The fatty acid composition in feces was determined by the method described earlier [28,29].

2.5. Hematoxylin and eosin (H&E) staining of tissue samples

Tissue samples of eWAT, iWAT and liver were fixated, dehydrated and embedded in paraffin. Five-micrometer-thick sections were cut throughout the tissue and stained with H&E as previously described [30]. One representative micrograph from each group is presented, and tissue from five mice per group was quantified. The five animals chosen in each group were selected based on body weight and reflected the mean within each of the groups.

2.6. TAG quantification in liver tissue

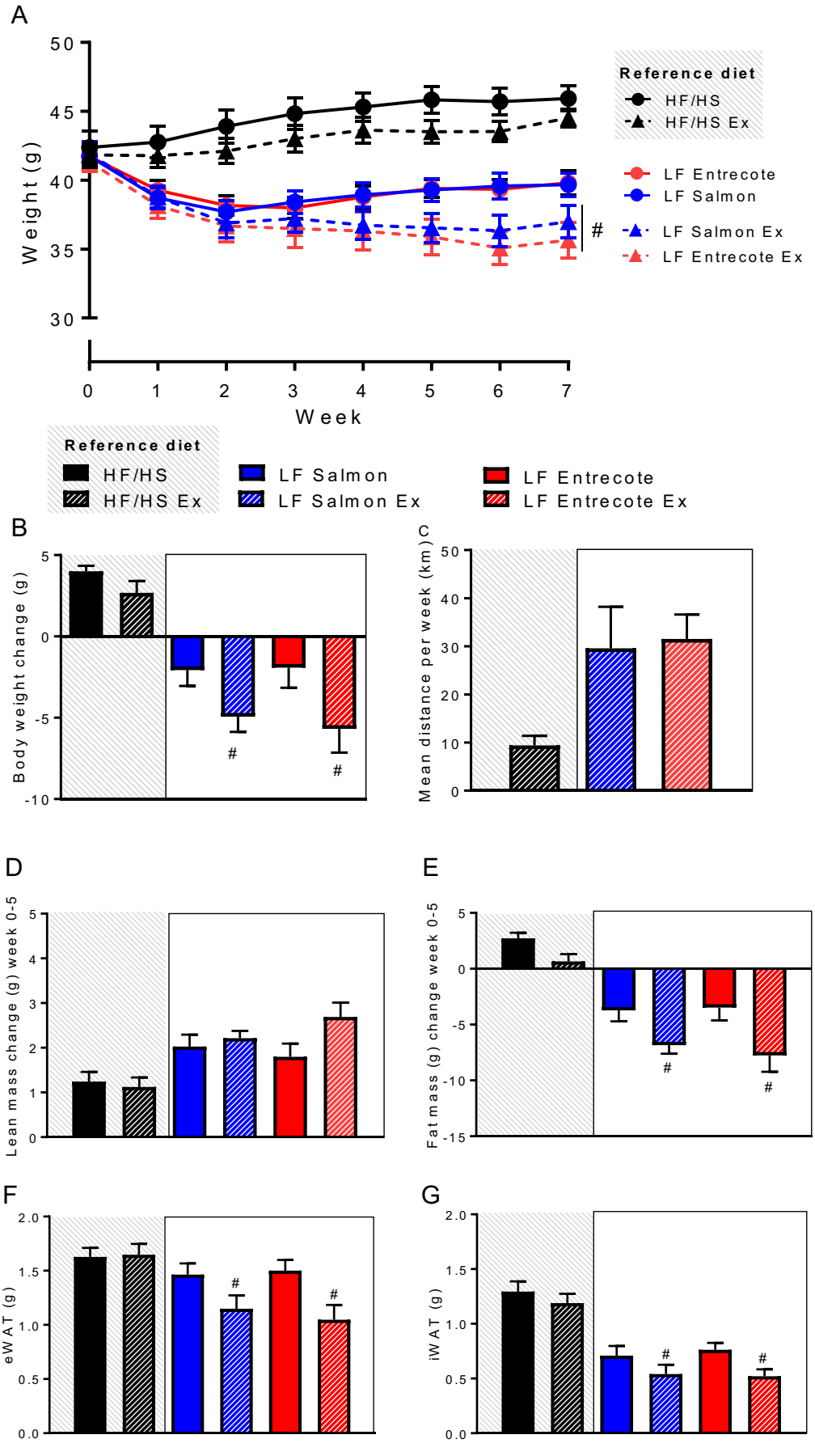
TAG measurements were performed with free glycerol reagent (Sigma-Aldrich, F6428) and triglyceride reagent (Sigma-Aldrich, T2449) on liver tissue from termination. Fifty to hundred milligrams of tissue was homogenized in isopropanol and centrifuged at 5000 rcf for 5 min. One microliter of tissue extract was diluted in 150 μl free glycerol reagent followed by 37.5 μl triglyceride reagent. All samples were measured at 450-nm absorbance after 1-h incubation at 37°C.

2.7. Glucose (GTT) and insulin tolerance test (ITT)

A GTT was performed during the sixth week of the intervention period in 6-h feed-deprived animals using 3 mg glucose/g lean mass given by oral gavage. Blood glucose was measured with a glucometer (Ascensia Contour, Bayer) 15, 30, 60 and 120 min after glucose administration. Blood samples were collected at baseline and 15 and 120 min after glucose administration, and insulin was quantified with EIA-3439 as described in the manufacturer's instructions (DRG Diagnostics GmbH). An ITT was performed during the seventh week of the intervention period. Animals were transferred to a clean cage 2 h prior to ITT. The test was performed at 10 a.m. by an intraperitoneal injection of 1 U insulin/kg lean mass. Blood glucose levels were measured prior injection and 15, 30, 45 and 60 min after insulin injection.

2.8. 16S ribosomal rDNA amplicon sequencing and bioinformatics

Microbiota composition was analyzed using fresh spot fecal samples collected prior to start of the intervention and during the seventh week of the intervention period


A
Weight (g)
Week
Reference diet
HF/HS
HF/HS Ex
LF Entrecote
LF Salmon
LF Salmon Ex
LF Entrecote Ex
#
B
Body weight change (g)
C
Mean distance per week (km)
D
Lean mass change (g) week 0-5
E
Fat mass (g) change week 0-5
F
eWAT (g)
G
iWAT (g)

(Supplementary Fig. 1A). At the end of the experiment, samples of cecum content were collected, snap-frozen and stored at −80°C until bacterial DNA extraction (NucleoSpin soil kit, Macherey-Nagel) was performed. Polymerase-chain-reaction-based library formation and sequencing were performed as previously described [19]. Alpha diversity was estimated using unfiltered data and phyloseq package [31].

2.9. Statistical analyses

All results are expressed as mean±S.E.M., and all data were analyzed for normal distribution and homogeneity of variance before applicable statistic was performed. A two-way analysis of variance (ANOVA) was performed on experimental data, only including mice given LF diets, using exercise and protein source as two independent variables. A significant effect caused by exercise is marked with #, and an effect of the dietary protein source is denoted by different letters (a, b) ($P<.05$). In case of an interaction effect between exercise and protein source, a *post hoc* Fisher least significant difference test was performed. A regular *t* test was performed on the animals who received the reference diet (HF/HS) with and without voluntary exercise. Significant differences are marked with *. Gene expression data were log transformed before statistical analysis was performed, and expression was normalized according to the sedentary mice fed the HF/HS diet. Adonis statistical methods were applied to all PCoA-based figures. Figures and statistical analyses were performed using GraphPad Prism v6 (GraphPad Software Inc.).

3. Results

3.1. Voluntary exercise enhances LF-induced reduction in obesity but does not reduce adiposity in mice maintained on an HF/HS diet

Obesity was induced by feeding all mice an HF/HS diet (Table 1) *ad libitum* for 8 weeks (Supplementary Fig. 1A and B). This was followed by an 8-week intervention where the obese mice were given isocaloric LF diets containing salmon or entrecote, while a group of mice was maintained on the HF/HS (Table 1, Supplementary Table 1 and Supplementary Table 2). For each diet, half of the animals was allowed to exercise (Supplementary Fig. 1A). As expected, due to a shift to diets with lower energy content, both LF diets led to reduced body weight independent of exercise (Fig. 1A). We observed no difference in body mass change between mice fed the salmon- or entrecote-containing LF diets (Fig. 1A and B). Voluntary exercise enhanced LF-induced weight loss but did not affect weight in mice maintained on the experimental HF/HS diet (Fig. 1B). The mean running distance was significantly higher for mice fed LF diets (LF salmon Ex and LF entrecote Ex) than for mice fed an HF/HS diet (HF/HS Ex) (Fig. 1C). This effect was evident from week 2 in the intervention period.

Body composition was determined after 5 weeks on the experimental diets, prior to the glucose and insulin tolerance tests. Despite a reduction in body mass, mice fed LF diets exhibited increased lean body mass (change from week 0 to 5) compared to mice fed an HF/HS diet (Fig. 1D). When comparing fat mass in mice allowed to exercise, all mice given LF diets exhibited a reduction in total fat mass compared to HF/HS-fed mice. The LF-induced reduction in fat mass was overall increased by exercise, whereas no reduction in fat mass was seen by exercise in mice fed HF/HS (Fig. 1E). Similarly, eWAT and iWAT masses in mice allowed to exercise and fed LF diets were lower than eWAT masses in sedentary LF-fed mice (Fig. 1F–G). No differences in body weights, fat masses or lean masses were observed comparing mice fed LF salmon or LF entrecote.

3.2. A switch to LF diets reduces energy intake and modulates nitrogen and fat absorption

We quantified the energy intake throughout the intervention period, and as expected, the cumulative energy intake during the study was significantly lower in mice fed an LF diet than in mice maintained on an HF/HS-diet (Fig. 2A). However, no significant compensatory increase in cumulative or mean energy intake per week was observed with voluntary exercise in mice fed LF or HF/HS diets (Fig. 2A and B). All mice fed LF diets (LF salmon ± Ex and LF entrecote ± Ex) had a negative feed efficiency (g BW/Mcal intake), which was further accentuated by voluntary exercise. By contrast, feed efficiency was similar in sedentary and exercised mice fed the HF/HS diet (Fig. 2C).

We quantified apparent nitrogen and fat digestibility after 5 weeks on the experimental diets (Supplementary Fig. 1C). Nitrogen digestibility was not affected by exercise in either LF- or HF/HS-fed mice (Fig. 2D). However, nitrogen digestibility was lower in all mice fed LF diets compared to HF/HS-fed mice (Fig. 2D). Mice fed the LF diet containing entrecote had lower fat digestibility compared to mice given LF diet containing salmon, but the apparent fat digestibility was unaffected by exercise in any of the experimental groups (Fig. 2E).

To investigate possible factors accounting for the reduced fat digestibility in mice fed the entrecote-containing diet, we determined the fecal levels of saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs). These analyses revealed higher excretion levels of total SFAs, MUFAs and PUFAs (Fig. 3A), including 16:0 and 18:0 SFAs, 18:1n-9 and 18:1n-11 MUFAs and 18:2n-6 and 20:4n-6 PUFAs (Supplementary Fig. 2), in feces of LF entrecote-fed mice compared to LF salmon-fed mice. No difference in excretion of fatty acids was observed by exercise in any of the experimental groups (Fig. 3 and Supplementary Fig. 2).

3.3. Voluntary exercise and an LF diet containing salmon reduce hepatic TAG levels

To evaluate the effect of LF diets and exercise on hepatic TAG levels in obese mice, liver sections of samples obtained after 8-week intervention were evaluated. Examination of H&E-stained sections revealed the presence of lipid droplets in mice fed HF/HS ± Ex and in livers from sedentary mice fed LF entrecote, but not in mice fed LF salmon ± Ex or in mice fed LF entrecote combined with voluntary exercise (Fig. 4A). In accordance with examination of the H&E-stained liver sections, hepatic TAG levels were reduced in mice fed an LF salmon diet compared to LF entrecote (Fig. 4B). Reduction in hepatic TAG was observed when exercise was combined with LF diets, whereas exercise did not reduce hepatic TAG in mice fed an HF/HS diet (Fig. 4B). Liver weight was not affected by the dietary protein source or exercise (Fig. 4C), but hepatic mRNA expression of *Col1a1*, a marker of liver steatosis, revealed a reduction in exercised mice given the HF/HS diet or LF diets (Fig. 4D). Increased liver TAG may be caused by reduced beta-oxidation and/or increased fatty acid synthesis. The expression level of *Acox* in liver indicated a significantly higher beta-oxidation in mice fed LF diet containing salmon compared to entrecote. In addition, the hepatic expression of *Scd1* and *Fasn* tended to be higher in mice fed LF entrecote, indicating lower fatty acid synthesis in mice fed an LF salmon diet (Fig. 4E–G).

Fig. 1. Body weight development, body weight change, average distance run per week, lean mass and fat mass after the 7 week intervention period in mice fed LF and HF/HS diets with or without voluntary exercise. (A) Body weight development in mice given an HF/HS diet or LF diets containing salmon or entrecote in combination with voluntary exercise. (B) Body weight change, weight at termination of the study at week 7 minus weight prior to the intervention (*P* value comparing HF/HS *vs.* HF/HS Ex: .377). (C) Average distance run (km per week) in experimental groups with voluntary exercise. (D) Changes in lean mass after 5-week intervention. (E) Changes in fat mass after 5 week intervention (*P* value comparing HF/HS *vs.* HF/HS Ex: .139). Lean mass and fat mass were determined by MRI scanning prior to the glucose and insulin tolerance test. (F–G) Weight of epididymal white adipose tissue (eWAT) and inguinal white adipose tissue (iWAT) at termination of the study. All results are presented as mean±S.E.M. and tested for normality and homogeneity of variances ($n=9–10$). Data on average distance run were analyzed using a regular *t* test. The rest of the data were analyzed using two-way ANOVA test, only including mice given LF diets, using exercise and protein source as two independent variables. $P<.05$ was considered significant, an effect caused by exercise is marked with #, and an effect of the dietary protein source is denoted by different letters (a, b).

3.4. LF-diet-induced improvement in insulin sensitivity, but not glucose tolerance, is augmented by voluntary exercise

Blood glucose levels in mice given the experimental diets for 6 weeks were measured in animals feed-deprived for 6-h prior to the GTT. A lower blood glucose level was observed in feed-deprived mice given LF diets with either salmon or entrecote, but blood glucose levels did not decrease further by voluntary exercise (Fig. 5A). Similarly, blood glucose levels at all time points and area under the curve during the glucose challenge revealed that voluntary exercise did not augment the LF-diet-induced improvement of glucose tolerance. Neither did voluntary exercise improve glucose tolerance in HF/HS-fed mice (Fig. 5B and C). Plasma insulin levels were quantified in feed-deprived animals and 15 and 60 min after a glucose injection to evaluate glucose-stimulated insulin secretion in first and second phase. Lower plasma insulin levels 60 min after a glucose injection were observed in all mice fed the LF diets compared to mice fed the HF/HS diet. No significant reduction in plasma insulin levels was observed in response to exercise in mice fed HF/HS or LF diets (Fig. 5D–F).

Insulin sensitivity was evaluated after 6 week intervention. Mice fed LF diets, compared to HF/HS-fed mice, exhibited an improved insulin response evaluated by delta blood glucose 15-0 (Fig. 5G). A tendency towards improved initial insulin response was observed in mice given HF/HS combined with exercise compared to sedentary mice fed HF/HS (Fig. 5G). Further, a significant improvement in area over the curve was observed with exercise in both HF/HS- and LF-fed mice (Fig. 5H–I).

3.5. Dietary fat level strongly modulates the gut microbiota composition in obese mice

Whereas the gut microbiota has been shown to be strongly affected by the amount and type of dietary fat and protein [7,11,19,20,32–34], less is known about how a shift from an HF/HS diet to LF diets with different protein sources affects the gut microbiota in already obese mice.

We collected spot feces from each randomized group of the obese mice prior to and after 7 weeks of intervention. In addition, we collected cecum content after 8 weeks at termination. Bacterial DNA was isolated and analyzed using 16S rRNA V4 gene amplicon sequencing. PCoA analyses of unweighted and weighted UniFrac distances showed no significant separation of the randomized groups prior to the intervention, but as expected, clear diet-driven separations were observed by week 7 (Supplementary Fig. 3A–D). Similar to the analysis of the spot feces samples, analyses of the cecum samples collected at termination revealed a strong diet-driven separation (Supplementary Fig. 3E–F). Interestingly, PCoA using weighted UniFrac distances indicated that the separation in the gut microbiota between mice fed the HF/HS and the LF diets in both spot feces and cecum samples was most pronounced for the entrecote-containing LF diet (Supplementary Fig. 3D and F). Alpha diversity in neither fecal nor cecum samples was significantly different between the experimental groups (Supplementary Fig. 4A–B). We observed as expected differences in the relative abundances of taxa at the phylum and family levels comparing spot feces and cecum sample, but in addition, we also observed consistent changes in responses to the diets (Fig. 6A–D). At the phylum level, the most conspicuous general changes included an increase in the abundance of Verrucomicrobia and a decrease in the abundance of Proteobacteria in mice fed the LF diets. At the family level, *Erysipelotrichaceae* dominated, and we observed a trend towards higher relative abundances of *Verrucomicrobiaceae* in response to voluntary exercise. Reduced abundances of *Rikenellaceae*, *Desulfovibrionaceae* and *Clostridiaceae* were observed in response to LF feeding in samples collected from cecum (Fig. 7). The high level of *Erysipelotrichaceae* may reflect HF/HS feeding at thermoneutral condition as observed previously [35]. An increased abundance of *Clostridiaceae* is a feature of HF feeding [32], and accordingly, we observed a decrease in the abundance of *Clostridiaceae* when mice were shifted to the LF diets (Fig. 7).

3.6. The protein source and voluntary exercise induce changes in the composition and functional potential of the gut microbiota

To further dissect the effect of diet composition and training, we focused our analyses on the microbiota of the cecum. The fat content of the diet was as expected a strong driver determining the bacterial composition (Supplementary Fig. 5A–B). Hence, the effect of exercise was analyzed in HF/HS- and LF-fed mice separately. Voluntary exercise did not lead to significant changes in the overall composition of the gut microbiota in LF-fed mice (Supplementary Fig. 5C–D). Still, using LefSe [36] to calculate differences in bacterial composition in LF-fed mice, we observed an increase in the relative abundances of the Verrucomicrobia phylum and the *Dorea* genus in mice allowed to exercise (Supplementary Fig. 5E-F). Further analyses of mice maintained on the HF/HS diet revealed no significant changes in the bacterial composition by voluntary training using weighted UniFrac distances, whereas PCoA using unweighted UniFrac distances revealed two statistically distinct groups (not shown). Accordingly, using LefSe to search for functional differences, we found a number of KEGG modules that were enriched in either sedentary or exercised mice. Specifically, we noted that bacterial genes involved in biosynthesis of secondary bile acids and secretory systems in the gut microbiota were enriched in the nonexercised mice, whereas genes involved in sugar transport were enriched in the mice allowed to exercise (Supplementary Fig. 5G).

We next performed comparisons between the gut microbiota in sedentary mice fed the two different salmon- or entrecote-containing LF diets. We observed no differences in the composition of the cecal microbiota using PCoA analyses of unweighted and weighted UniFrac distances (Supplementary Fig. 6A–B) but noted a differential enrichment of a limited number of KEGG modules (Supplementary Fig. 6C). Comparing salmon- or entrecote-fed mice allowed to exercise, we noted a significant separation on PCoA between the cecal microbiota of mice fed the two diets using weighted UniFrac distances and a trend using unweighted UniFrac distances (Supplementary Fig. 7 A–B). In this case, MetaSeq analyses also identified a number of taxa that differed significantly in abundances, with *Desulfovibrionales, Burkholderiales* and *Verrucomicroiales* being more abundant in the gut microbiota of mice fed salmon, whereas the abundance of *Caccae* was higher in mice fed entrecote (Supplementary Table 3). Comparison of KEGG modules revealed a large number of modules that were differentially enriched in exercised mice fed salmon or entrecote (Supplementary Fig. 7C). In the salmon-fed mice, we observed enrichment in modules involved in amino acid transport, whereas in the entrecote-fed mice, we observed a broader enrichment involving numerous pathways with no clear pattern (Supplementary Fig. 7C).

Although PCoA analyses revealed no overall effect of exercise on LF-fed mice (Supplementary Fig. 5C–D), we used MetSEq-based analyses and LefSe to examine if exercise had an effect on either salmon- or entrecote-fed mice. These analyses revealed a significant decrease in the abundance of the *Caccae* genus and a differential enrichment of modules involved in lysine degradation and branched-chain amino acid transport in mice allowed to exercise in response to exercise in salmon-fed mice (Supplementary Fig. 8A). In the sedentary mice, we observed an enrichment of modules involved in lysine, GABA and pyrimidine biosynthesis (Supplementary Fig. 8A). In the entrecote-fed mice, an increase in the abundance of *Akkermansia* and *Dorea* and enrichment of a module involved in dermatan sulfate

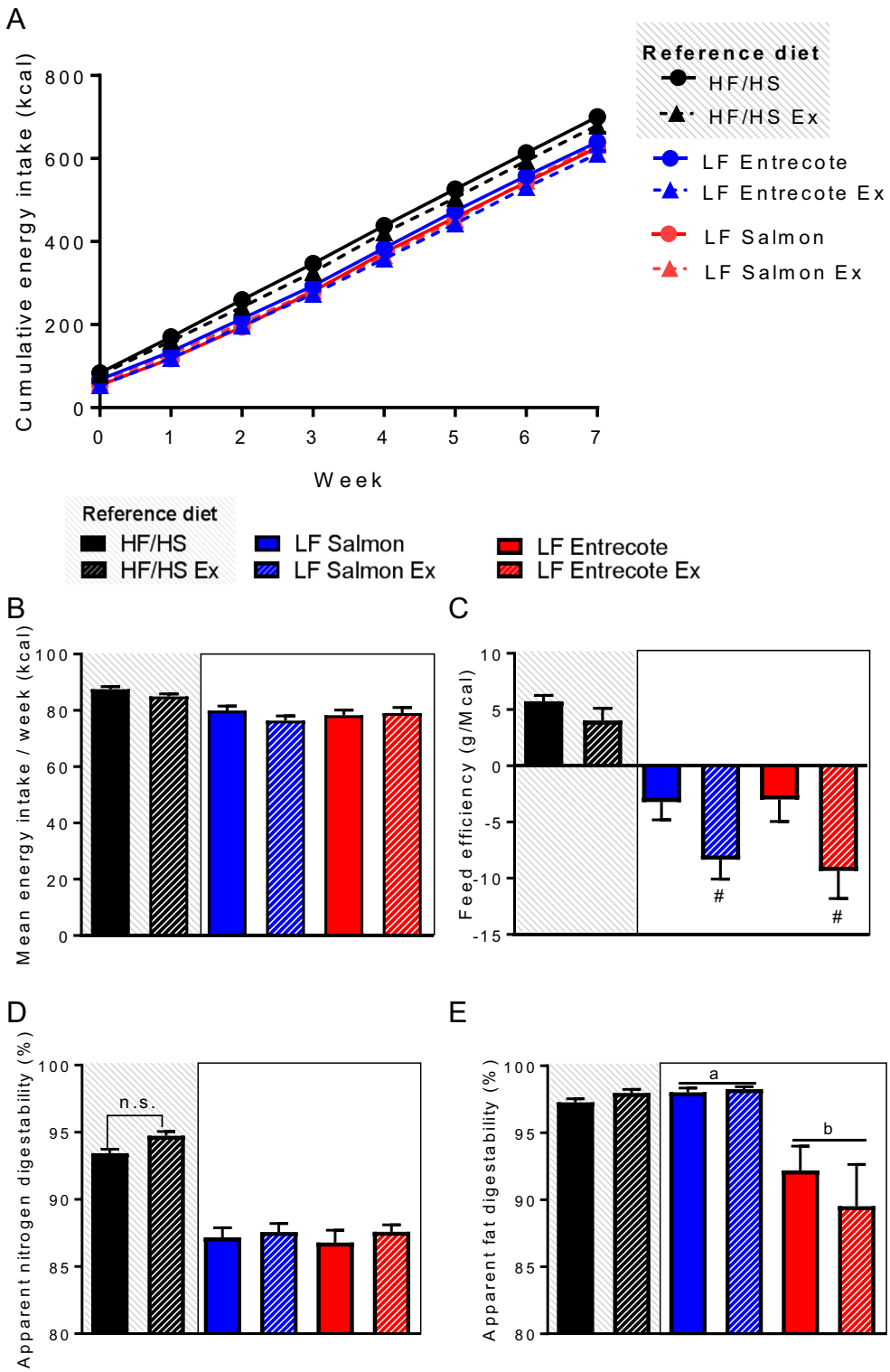


Fig. 2. Energy intake, feed efficiency and apparent nitrogen and fat digestibility during the intervention period. (A) Cumulative energy intake during the experimental period (*P* value comparing HF/HS *vs.* LF salmon: <.001, LF salmon Ex: <.001, LF entrecote: <.001, LF entrecote Ex: <.001). (B) Average energy intake per week in the experimental period. (C) Calculated energy efficiency during the intervention period (*P* value comparing HF/HS *vs.* HF/HS Ex: .291). (D) Calculated apparent digestibility of nitrogen for 7 days. (E) Calculated apparent digestibility of nitrogen for 7 days. Results were based on intake and excretion of nitrogen and fat in week 5 of the experiment. All results are presented as mean±S.E.M. and tested for normality and of homogeneity of variances (n=9–10). All data were analyzed using two-way ANOVA test, only including mice given LF diets, using exercise and protein source as two independent variables. P<.05 was considered significant, an effect caused by exercise is marked with #, and an effect of the dietary protein source is denoted by different letters (a, b).

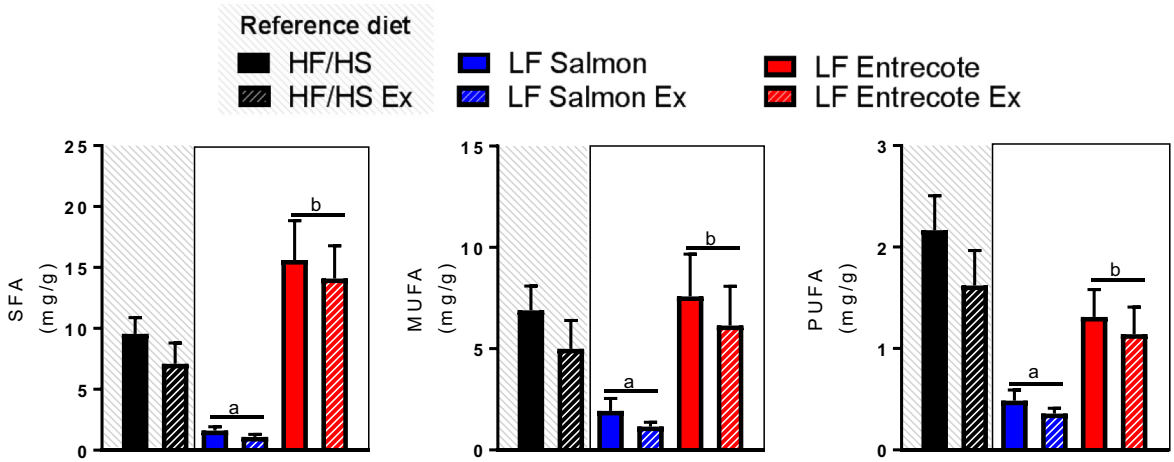


Fig. 3. Excretion of SFA, MUFA and PUFA in feces. (A) Quantitative levels of SFA, MUFA and PUFA fatty acids in spot feces from mice given the experimental diets. (B) Excretion of 16:0 and 18:0, 18:1n-9 and 18:1n-11 fatty acids in feces samples collected (n=8–10). All results are presented as mean±S.E.M. and tested for normality and homogeneity of variances. Data were analyzed using two-way ANOVA test, only including mice given LF diets, using exercise and protein source as two independent variables. P<.05 was considered significant, an effect caused by exercise is marked with #, and an effect of the dietary protein source is denoted by different letters (a, b).

degradation were observed in response to exercise (Supplementary Fig. 8B).

4. Discussion

Animal studies have shown that LF diets and voluntary or forced exercise attenuate adiposity development in mice [37,38]. However, less is known about the ability of exercise to enhance LF-diet-induced reduction in body weight and fat mass in already obese animals. Further, to what extent intake of LF diets containing different protein sources combined with voluntary exercise affects the composition of the gut microbiota in the obese state has not been addressed.

A switch to an LF diet, and thereby reduced energy intake, was the most important factor related to body weight and fat mass reduction. We observed that voluntary exercise enhanced LF-diet-induced reduction in adiposity. However, in contrast to a previous study that reported reduced obesity by forced exercise in HF-fed mice [39], we observed no significant effect on obesity by voluntary exercise in HF/HS-fed mice. The lack of a reduction in body weight and fat mass in mice on an HF/HS diet in response to exercise was not caused by a compensatory increase in energy intake as reported in other studies [37,39]. However, we observed that mice fed an HF/HS diet only ran about half the distance per week compared to mice fed the LF diets, which at least in part could explain the lack of reduction in body weight and fat mass in the HF/HS fed mice. It has been reported that exposure to obesogenic diets reduces the level of the D2-type dopamine receptor which lowers the level of physical activity [40,41], and such a change might contribute to the reduced activity of the HF/HS-fed mice. Since body weight and fat mass are important factors determining the extent of voluntary exercise, it is also likely that the rapid decrease in body weight 1 to 2 weeks after the switch to LF diets further contributed to the increased activity level in LF fed mice.

In HF/HS-fed mice, voluntary exercise improved insulin sensitivity without changing glucose tolerance, feed-deprived blood glucose and glucose-stimulated insulin secretion. In contrast to several studies [42–44], we failed to observe dramatic effects of voluntary exercise on the gut microbiota composition in mice maintained on the HF/HS diet. This may be related to the reduced running activity and the obese state of the mice prior to the intervention. Another reason for these differences could be the rapid weight loss observed in our study. Weight loss has been reported to elicit changes in the gut microbiota [45], and such weight-loss-dependent changes may have masked the exercise-induced changes. Detailed analyses revealed that bacterial genes involved in biosynthesis of secondary bile acids were enriched only in sedentary mice maintained on the HF/HS diet, whereas genes involved in sugar transport were enriched in the mice allowed to exercise. Especially, the observed differences in abundance of genes involved in secondary bile acid metabolism would be predicted to influence metabolism and might thereby also contribute to the lack of weight loss in mice maintained on the HF/HS diet and allowed to exercise [46,47]. We observed that a shift from an HF/HS diet to LF diets for 6 weeks improved glucose tolerance in accordance with earlier reports [39]. The improvement in glucose tolerance was independent of the dietary protein source and voluntary exercise.

The shift from the HF/HS diet to the LF diet, irrespective of exercise, led to pronounced changes in the composition and functional potential of the gut microbiota, where we at the phylum level observed an increase in the abundance of Verrucomicrobia and a decrease in the abundance of Proteobacteria. The changes in the abundance of Verrucomicrobia fit the well-characterized beneficial effect of *Akkermansia* on metabolic health, whereas the changes in Proteobacteria are more complex, with increased abundance of Proteobacteria being associated with metabolic health [33,35]. At the family level, we further noted the decrease in abundances of *Rikenellaceae*, *Desulfovibrionaceae* and *Clostridiaceae* in response to LF feeding. High-fat feeding has been associated with an increased abundance of *Clostridiaceae* [32], and thus, the present study clearly demonstrates the fat-responsive changes in the abundance of this family. The reduced abundance of *Rikenellaceae* in response to the LF diet shift is of interest since it has been reported to increase in mice in response to high-fat feeding [48]. A higher abundance of *Rikenellaceae* in mice fed entrecote compared to salmon indicates a less favorable response. However, an increased abundance of *Rikenellaceae* has also been associated with improved immune responses and gastrointestinal health in mice [49]. The decreased abundance of *Desulfovibrionaceae* in response to the LF diet shift is difficult to reconcile with the finding that exchanging fish oil with saturated fat is reported to decrease the abundance of *Desulfovibrio* [33], but content of omega-3 in the HF/HS diet may in part explain this change in abundance.

By analyzing the effect of exercise independently of feed type, we observed that exercise also increased the relative abundance of the

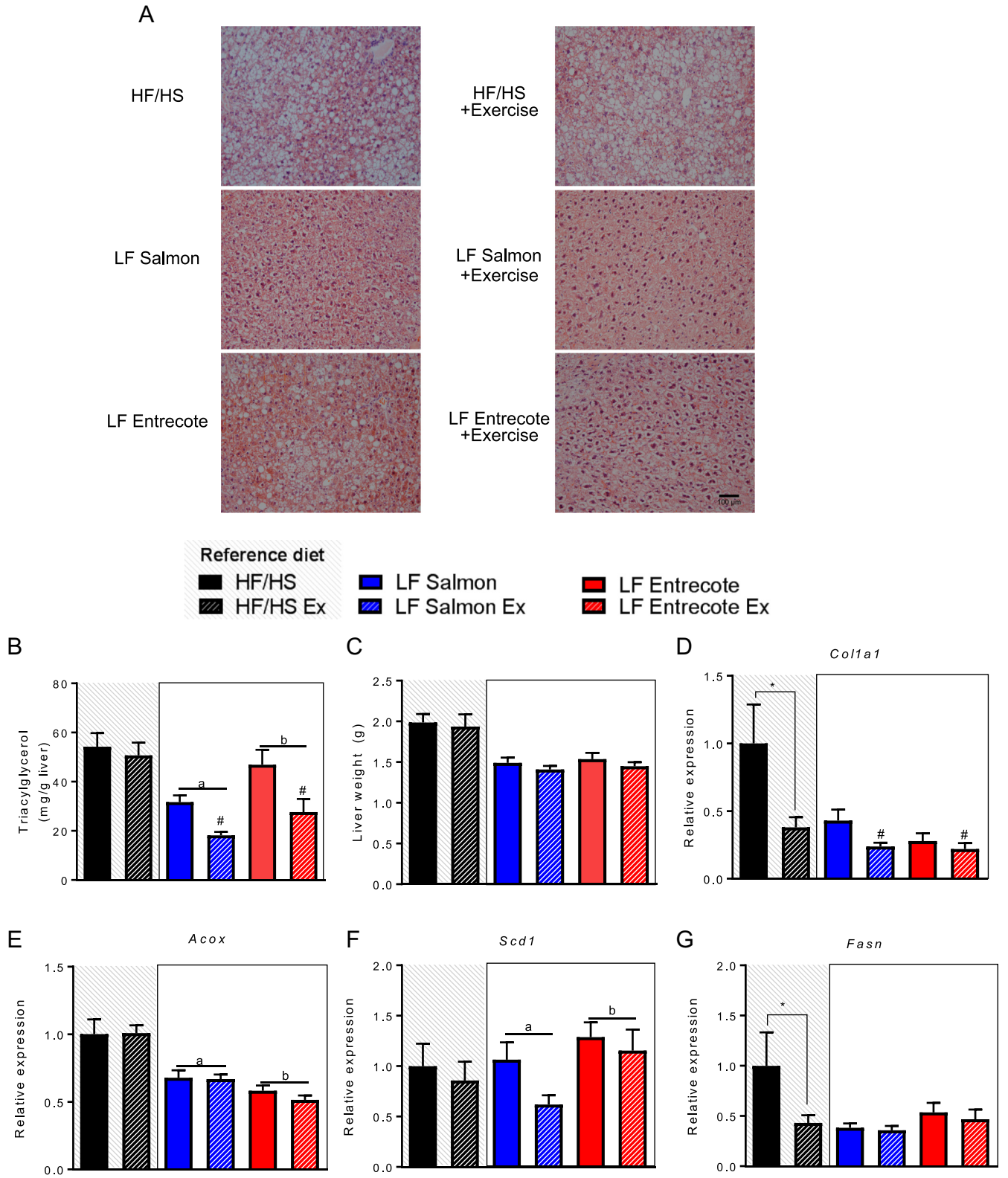


Fig. 4. Micrograph of hepatic tissue, liver weight and liver gene expression after the 7 week intervention period in mice fed LF and HF/HS diets with or without voluntary exercise. (A) Representative micrographs of liver tissues from each treatment. (B) Liver TAG concentration quantified in mice at termination of the study. (C) Liver weight at termination of the study. (D) Relative gene expression of *Col1a1* (*P* value comparing HF/HS *vs.* HF/HS Ex: .048). (E) *Acox*, (F) *Scd1* and (G) *Fasn* (*P* value comparing HF/HS *vs.* HF/HS Ex: .008) in hepatic tissue. Gene expression is normalized to expression of *Tbp*, and relative expression is related to gene expression in mice fed the HF/HS diet (expression=1) (n=8–10). All results are presented as mean±S.E.M. and tested for normality and homogeneity of variances. Data were analyzed using two-way ANOVA test, only including mice given LF diets, using exercise and protein source as two independent variables. $P<.05$ was considered significant. An effect caused by exercise is marked with #, and an effect of the dietary protein source is denoted by different letters (a, b).

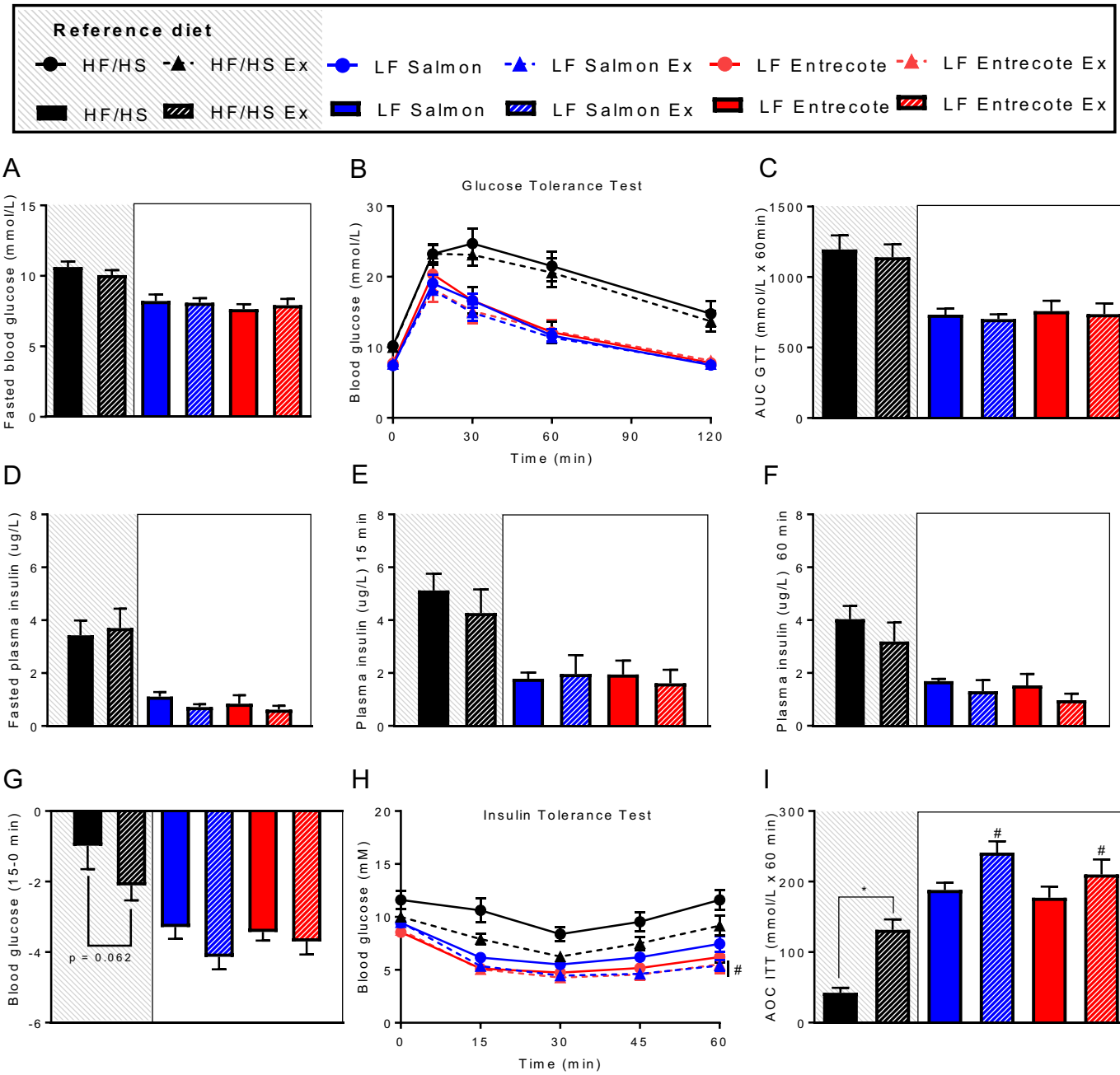


Fig. 5. Fasted blood glucose, GTT, plasma insulin levels and ITT. (A) Feed-deprived (6 h) blood glucose measured in animals after 6 week intervention (*P* value comparing HF/HS *vs.* HF/HS Ex: .238). (B) GTT performed in feed-deprived animals (6 h) and blood glucose measurements 15, 30, 60 and 120 min after injection. The injection was given based on lean mass (3 mg/g lean mass). (C) Area under the curve is calculated from GTT in Fig. 5B. (D) Plasma insulin levels measured in samples collected during the GTT. Plasma levels in feed-deprived mice at time point 0 (prior to the glucose injection), (E) 15 min (F) and 60 min after injection. (G) Delta blood glucose calculations of the change in blood glucose from start and 15 min after insulin injection. (H) ITT with blood glucose levels measured 15, 30, 45 and 60 min after insulin injection. (I) Area over the curve calculated from the ITT (*P* value comparing HF/HS *vs.* HF/HS Ex: <.001). Results are presented as mean±S.E.M.. and tested for normality and homogeneity of variances (n=8–10). The data were analyzed using two-way ANOVA test, only including mice given LF diets, using exercise and protein source as two independent variables. P<.05 was considered significant, an effect caused by exercise is marked with #, and an effect of the dietary protein source is denoted by different letters (a, b).

Verrucomicrobia phylum and the *Dorea* genus. The importance of Verrucomicrobia for metabolic health and gut homeostasis is well documented. The increased abundance of *Dorea* is surprising and difficult to explain, as the abundance of *Dorea* is enriched in the gut microbiota of HF-fed mice [32] and obese Han Chinese [50]. Finally, a systematic analysis of changes in the gut microbiota following bariatric surgery and weight loss reported on a decreased abundance of *Dorea* [51].

The type of protein did not influence neither obesity or glucose homeostasis. However, sedentary mice fed the salmon-containing LF diet exhibited a reduction in liver TAG accumulation, whereas exercise was necessary to reduce liver TAG levels in mice fed an LF entrecote-containing diet. This observation is in agreement with previous studies demonstrating that intake of omega-3 fatty acids and fish proteins is able to diminish liver lipid accumulation in diet-induced obesity [52,53]. Since the salmon filets used in the feed

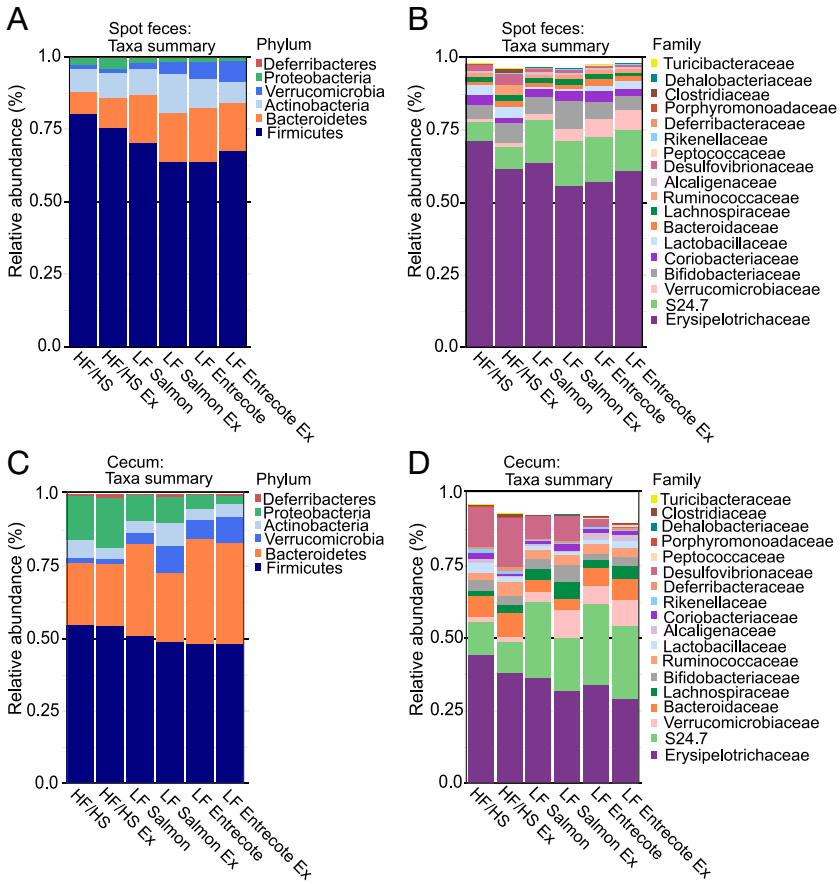


Fig. 6. Taxa summary for spot feces and cecal microbiota at phyla and family level. (A–B) Taxa summary at phylum and family level in spot feces collected after 6 week intervention (n=8–10). (C–D) Taxa summary at phylum and family level in cecum samples collected at termination of the study (n=9–10). All values are expressed as relative abundance (%).

contain a significant amount of omega-3 fatty acids, we cannot determine if the reduction in liver lipids should be attributed to omega-3 fatty acids or certain amino acids in the salmon filet or a combination of both. We also noted significant differences elicited in response to exercise on the cecal microbiota dependent on the type of LF diet. We observed that *Desulfovibrionales*, *Burkholderiales* and *Verrucomicrobiales* were more abundant in mice fed salmon, whereas the abundance of *Caccae* was higher in mice fed entrecote. Considering the reported beneficial effects of *Desulfovibrionales* and *Verrucomicroiales* on metabolism, this finding may suggest an additional beneficial effect of LF diets containing salmon. The ability of different protein sources to elicit metabolic changes may apart from differences in amino acid and fatty acid composition also reflect different content of micronutrients, glycosylation patterns, possible medical residues or environmental contaminants, all factors known to influence on the gut microbiota [20].

The mechanisms by which exercise and diet cause changes in the gut microbiota are far from being fully understood but may well involve changes in stool transit time, which will differentially affect the abundance of bacteria dependent on the rate of multiplication [54,55]. Regardless of the mechanism, our results demonstrate that a combination of particularly reduced dietary fat content and exercise is needed in order to elicit metabolically beneficial changes as well as reduction in adiposity and that different protein sources will affect the process. While, undoubtedly, a reciprocal interaction between host and gut bacteria also is involved, the details of this interaction remain to be elucidated.

5. Conclusion

This study demonstrates that voluntary exercise enhances LF-diet-induced reduction in body weight, fat mass and insulin sensitivity in obese mice. Still, by evaluating the effects of diet and exercise, we show that a dietary change to an LF diet is the most important single factor related to a reduction in body weight and improvement in glucose homeostasis. Whereas the type of protein did not influence either weight loss or improvement of glucose homeostasis, mice fed salmon had lower hepatic TAG levels than entrecote-fed mice. Intake of LF diets containing salmon or entrecote leads to protein-dependent shifts in the gut microbiota composition.

Author contributions

The authors have declared no conflict of interest.

All authors of this manuscript have directly participated in the execution and/or analysis of the study and approved the manuscript.

E.F., L.S.M. and L.M. conceived and designed the experiments.

E.F., L.S.M., D.O.L., H.A., J.B.H., P.K., R.H. and B.L. performed the experiments.

E.F., L.S.M., K.K. and L.M. wrote the paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jnutbio.2019.01.003.

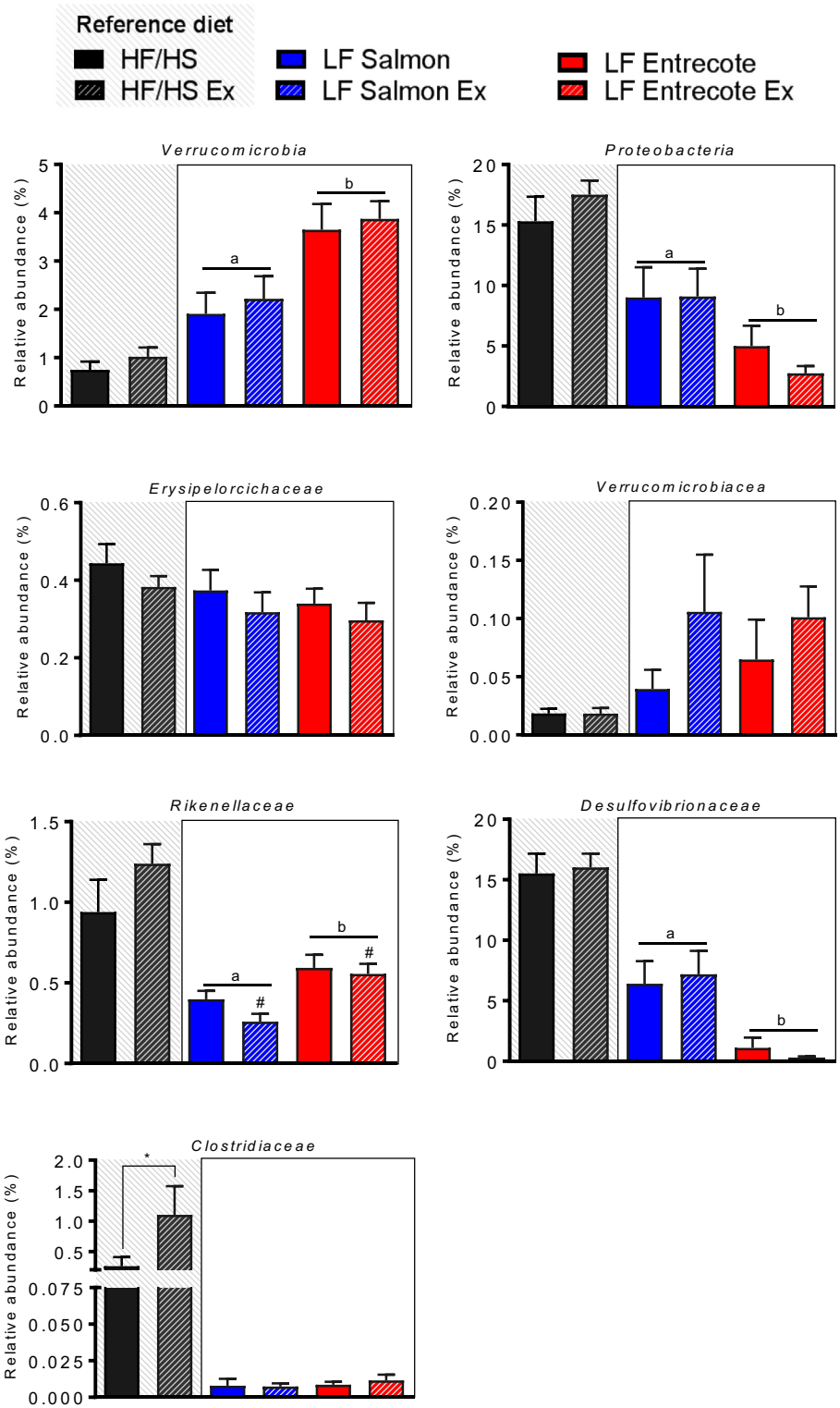


Fig. 7. Relative abundance of selected bacteria in cecum samples at phyla, family and genus level. Bar charts of the relative abundance of selected bacteria in cecum (n=8–10). All results are presented as mean±S.E.M. and tested for normality and homogeneity of variances. The data were analyzed using two-way ANOVA test, only including mice given LF diets, using exercise and protein source as two independent variables. P<.05 was considered significant, an effect caused by exercise is marked with #, and an effect of the dietary protein source is denoted by different letters (a, b).

References

[1] Hsieh C-H, Rau C-S, Wu S-C, Yang JC-S, Wu Y-C, Lu T-H, et al. Weight-reduction through a low-fat diet causes differential expression of circulating microRNAs in obese C57BL/6 mice. BMC Genomics 2015;16:699.

[2] Gao X, Yan D, Zhao Y, Tao H, Zhou Y. Moderate calorie restriction to achieve normal weight reverses β-cell dysfunction in diet-induced obese mice: involvement of autophagy. Nutr Metab 2015;12:34.

[3] Mercken EM, Carboneau BA, Krzysik-Walker SM, de Cabo R. Of mice and men: the benefits of caloric restriction, exercise, and mimetics. Ageing Res Rev 2012;11:390–8.

[4] Bradley RL, Jeon JY, Liu F-F, Maratos-Flier E. Voluntary exercise improves insulin sensitivity and adipose tissue inflammation in diet-induced obese mice. Am J Physiol Endocrinol Metab 2008;295:E586–94.
[5] Harris RB, Kor H. Insulin insensitivity is rapidly reversed in rats by reducing dietary fat from 40 to 30% of energy. J Nutr 1992;122:1811–22.
[6] Fjære E, Aune UL, Røen K, Keenan AH, Ma T, Borkowski K, et al. Indomethacin treatment prevents high fat diet-induced obesity and insulin resistance but not glucose intolerance in C57BL/6J mice. J Biol Chem 2014;289:16032–45.
[7] Jensen BAH, Nielsen TS, Fritzen AM, Holm JB, Fjære E, Serup AK, et al. Dietary fat drives whole-body insulin resistance and promotes intestinal inflammation independent of body weight gain. Metabolism 2016;65:1706–19.
[8] Madsen L, Pedersen LM, Liaset B, Ma T, Petersen RK, van den Berg S, et al. cAMP-dependent signaling regulates the adipogenic effect of n-6 polyunsaturated fatty acids. J Biol Chem 2008;283:7196–205.
[9] Ma T, Liaset B, Hao Q, Petersen RK, Fjære E, Ngo HT, et al. Sucrose counteracts the anti-inflammatory effect of fish oil in adipose tissue and increases obesity development in mice. PLoS One 2011;6:e21647.
[10] Hao Q, Lillefosse HH, Fjaere E, Myrmel LS, Midtbø LK, Jarlsby RH, et al. High-glycemic index carbohydrates abrogate the antiobesity effect of fish oil in mice. Am J Physiol Endocrinol Metab 2012;302:E1097–112.
[11] Kiilerich P, Myrmel LS, Fjære E, Hao Q, Hugenholtz F, Sonne SB, et al. Effect of a long-term high-protein diet on survival, obesity development, and gut microbiota in mice. Am J Physiol Endocrinol Metab 2016;310:E886–99.
[12] Freudenberg A, Petzke KJ, Klaus S. Comparison of high-protein diets and leucine supplementation in the prevention of metabolic syndrome and related disorders in mice. J Nutr Biochem 2012;23:1524–30.
[13] Freudenberg A, Petzke KJ, Klaus S. Dietary L-leucine and L-alanine supplementation have similar acute effects in the prevention of high-fat diet-induced obesity. Amino Acids 2013;44:519–28.
[14] Morens C, Keijzer M, de Vries K, Scheurink A, van Dijk G. Effects of high-fat diets with different carbohydrate-to-protein ratios on energy homeostasis in rats with impaired brain melanocortin receptor activity. Am J Physiol Regul Integr Comp Physiol 2005;289:R156–63.
[15] Liisberg U, Myrmel LS, Fjære E, Rønnevik AK, Bjelland S, Fauske KR, et al. The protein source determines the potential of high protein diets to attenuate obesity development in C57BL/6J mice. Adipocyte 2016;5:196–211.
[16] Tastesen HS, Keenan AH, Madsen L, Kristiansen K, Liaset B. Scallop protein with endogenous high taurine and glycine content prevents high-fat, high-sucrose-induced obesity and improves plasma lipid profile in male C57BL/6J mice. Amino Acids 2014;46:1659–71.
[17] Tastesen HS, Rønnevik AK, Borkowski K, Madsen L, Kristiansen K, Liaset B. A mixture of cod and scallop protein reduces adiposity and improves glucose tolerance in high-fat fed male C57BL/6J mice. PLoS One 2014;9:e112859.
[18] Liisberg U, Fauske KR, Kuda O, Fjære E, Myrmel LS, Norberg N, et al. Intake of a Western diet containing cod instead of pork alters fatty acid composition in tissue phospholipids and attenuates obesity and hepatic lipid accumulation in mice. J Nutr Biochem 2016;33:119–27.
[19] Holm JB, Rønnevik A, Tastesen HS, Fjære E, Fauske KR, Liisberg U, et al. Diet-induced obesity, energy metabolism and gut microbiota in C57BL/6J mice fed Western diets based on lean seafood or lean meat mixtures. J Nutr Biochem 2016;31:127–36.
[20] Madsen L, Myrmel LS, Fjære E, Liaset B, Kristiansen K. Links between dietary protein sources, the gut microbiota, and obesity. Front Physiol 2017;8:1047.
[21] Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. Cell 2012;148:1258–70.
[22] Gilbert JA, Quinn RA, Debelius J, Xu ZZ, Morton J, Garg N, et al. Microbiome-wide association studies link dynamic microbial consortia to disease. Nature 2016;535:94–103.
[23] Khan MT, Nieuwdorp M, Bäckhed F. Microbial modulation of insulin sensitivity. Cell Metab 2014;20:753–60.
[24] Sonnenburg JL, Bäckhed F. Diet-microbiota interactions as moderators of human metabolism. Nature 2016;535:56–64.
[25] Ussar S, Griffin NW, Bezy O, Fujisaka S, Vienberg S, Softic S, et al. Interactions between gut microbiota, host genetics and diet modulate the predisposition to obesity and metabolic syndrome. Cell Metab 2015;22:516–30.
[26] Evans CC, LePard KJ, Kwak JW, Stancukas MC, Laskowski S, Dougherty J, et al. Exercise prevents weight gain and alters the gut microbiota in a mouse model of high fat diet-induced obesity. PLoS One 2014;9:e92193.
[27] Fischer AW, Cannon B, Nedergaard J. Optimal housing temperatures for mice to mimic the thermal environment of humans: an experimental study. Mol Metab 2018;7:161–70.
[28] Lie O, Lambertsen G. Fatty acid composition of glycerophospholipids in seven tissues of cod (Gadus morhua), determined by combined high-performance liquid chromatography and gas chromatography. J Chromatogr 1991;565:119–29.
[29] Torstensen B, Froyland L, Ornsrud R, Lie O. Tailoring of a cardioprotective muscle fatty acid composition of Atlantic salmon (Salmo salar) fed vegetable oils. Food Chem 2004;87:567–80.
[30] Dankel SN, Degerud EM, Borkowski K, Fjære E, Midtbø LK, Haugen C, et al. Weight cycling promotes fat gain and altered clock gene expression in adipose tissue in C57BL/6J mice. Am J Physiol Endocrinol Metab 2014;306:E210–24.
[31] McMurdie PJ, Holmes S. Phyloseq: a bioconductor package for handling and analysis of high-throughput phylogenetic sequence data. Pac Symp Biocomput 2012:235–46.
[32] Xiao L, Sonne SB, Feng Q, Chen N, Xia Z, Li X, et al. High-fat feeding rather than obesity drives taxonomical and functional changes in the gut microbiota in mice. Microbiome 2017;5:43.
[33] Caesar R, Tremaroli V, Kovatcheva-Datchary P, Cani PD, Bäckhed F. Crosstalk between gut microbiota and dietary lipids aggravates WAT inflammation through TLR signaling. Cell Metab 2015;22:658–68.
[34] Zhu Y, Lin X, Zhao F, Shi X, Li H, Li Y, et al. Meat, dairy and plant proteins alter bacterial composition of rat gut bacteria. Sci Rep 2015;5:15220.
[35] Ziętak M, Kovatcheva-Datchary P, Markiewicz LH, Ståhlman M, Kozak LP, Bäckhed F. Altered microbiota contributes to reduced diet-induced obesity upon cold exposure. Cell Metab 2016;23:1216–23.
[36] Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol 2011;12:R60.
[37] Gollisch KSC, Brandauer J, Jessen N, Toyoda T, Nayer A, Hirshman MF, et al. Effects of exercise training on subcutaneous and visceral adipose tissue in normal- and high-fat diet-fed rats. Am J Physiol Endocrinol Metab 2009;297:E495–504.
[38] Linden MA, Pincu Y, Martin SA, Woods JA, Baynard T. Moderate exercise training provides modest protection against adipose tissue inflammatory gene expression in response to high-fat feeding. Physiol Rep 2014;2. https://doi.org/10.14814/phy2.12071.
[39] Vieira VJ, Valentine RJ, Wilund KR, Antao N, Baynard T, Woods JA. Effects of exercise and low-fat diet on adipose tissue inflammation and metabolic complications in obese mice. Am J Physiol Endocrinol Metab 2009;296:E1164–71.
[40] Kravitz AV, O'Neal TJ, Friend DM. Do dopaminergic impairments underlie physical inactivity in people with obesity? Front Hum Neurosci 2016;10:514.
[41] Beeler JA, Faust RP, Turkson S, Ye H, Zhuang X. Low dopamine D2 receptor increases vulnerability to obesity via reduced physical activity, not increased appetitive motivation. Biol Psychiatry 2016;79:887–97.
[42] Lambert JE, Myslicki JP, Bomhof MR, Belke DD, Shearer J, Reimer RA. Exercise training modifies gut microbiota in normal and diabetic mice. Appl Physiol Nutr Metab 2015;40:749–52.
[43] Cerdá B, Pérez M, Pérez-Santiago JD, Tornero-Aguilera JF, González-Soltero R, Larrosa M. Gut microbiota modification: another piece in the puzzle of the benefits of physical exercise in health? Front Physiol 2016;7:51.
[44] Lamoureux EV, Grandy SA, Langille MGI. Moderate exercise has limited but distinguishable effects on the mouse microbiome. mSystems 2017;2:e00006–17.
[45] Dao MC, Everard A, Clément K, Cani PD. Losing weight for a better health: role for the gut microbiota. Clin Nutr Exp 2016;6:39–58.
[46] Gu Y, Wang X, Li J, Zhang Y, Zhong H, Liu R, et al. Analyses of gut microbiota and plasma bile acids enable stratification of patients for antidiabetic treatment. Nat Commun 2017;8:1785.
[47] Sayin SI, Wahlström A, Felin J, Jäntti S, Marschall H-U, Bamberg K, et al. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. Cell Metab 2013;17:225–35.
[48] Daniel H, Gholami AM, Berry D, Desmarchelier C, Hahne H, Loh G, et al. High-fat diet alters gut microbiota physiology in mice. ISME J 2013;8:295–308.
[49] Ma G, Kimatu BM, Zhao L, Yang W, Pei F, Hu Q. In vivo fermentation of a Pleurotus eryngii polysaccharide and its effects on fecal microbiota composition and immune response. Food Funct 2017;8:1810–21.
[50] Liu R, Hong J, Xu X, Feng Q, Zhang D, Gu Y, et al. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. Nat Med 2017;23:859–68.
[51] Guo Y, Liu C-Q, Shan C-X, Chen Y, Li H-H, Huang Z-P, et al. Gut microbiota after Roux-en-Y gastric bypass and sleeve gastrectomy in a diabetic rat model: increased diversity and associations of discriminant genera with metabolic changes. Diabetes Metab Res Rev 2016;33:e2857.
[52] Midtbø LK, Ibrahim MM, Myrmel LS, Aune UL, Alvheim AR, Liland NS, et al. Intake of farmed Atlantic salmon fed soybean oil increases insulin resistance and hepatic lipid accumulation in mice. PLoS One 2013;8:e53094.
[53] Midtbø LK, Borkowska AG, Bernhard A, Rønnevik AK, Lock E-J, Fitzgerald ML, et al. Intake of farmed Atlantic salmon fed soybean oil increases hepatic levels of arachidonic acid-derived oxylipins and ceramides in mice. J Nutr Biochem 2015;26:585–95.
[54] Gao R, Gao Z, Huang L, Qin H. Gut microbiota and colorectal cancer. Eur J Clin Microbiol Infect Dis 2017;36:757–69.
[55] Oettle GJ. Effect of moderate exercise on bowel habit. Gut 1991;32:941–4.