

# Interaction between dietary lipids and gut microbiota regulates hepatic cholesterol metabolism<sup>1</sup>

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**Abstract** The gut microbiota influences many aspects of host metabolism. We have previously shown that the presence of a gut microbiota remodels lipid composition. Here we investigated how interaction between gut microbiota and dietary lipids regulates lipid composition in the liver and plasma, and gene expression in the liver. Germ-free and conventionally raised mice were fed a lard or fish oil diet for 11 weeks. We performed lipidomics analysis of the liver and serum and microarray analysis of the liver. As expected, most of the variation in the lipidomics dataset was induced by the diet, and abundance of most lipid classes differed between mice fed lard and fish oil. However, the gut microbiota also affected lipid composition. The gut microbiota increased hepatic levels of cholesterol and cholesteryl esters in mice fed lard, but not in mice fed fish oil. Serum levels of cholesterol and cholesteryl esters were not affected by the gut microbiota. Genes encoding enzymes involved in cholesterol biosynthesis were downregulated by the gut microbiota in mice fed lard and were expressed at a low level in mice fed fish oil independent of microbial status. **In summary, we show that gut microbiota-induced regulation of hepatic cholesterol metabolism is dependent on dietary lipid composition.**—Caesar, R., H. Nygren, M. Orešič, and F. Bäckhed. **Interaction between dietary lipids and gut microbiota regulates hepatic cholesterol metabolism.** *J. Lipid Res.* 2016. 57: 474–481.

**Supplementary key words** cholesterol/biosynthesis • fish oil • gene expression • lipidomics • liver • microarrays • germ-free

The composition of dietary fat is known to affect metabolic health. Consumption of saturated lipids is associated with metabolic perturbations such as obesity, hyperglycemia,

dyslipidemia, increased hepatic lipogenesis, and liver steatosis (1). In contrast, consumption of polyunsaturated fatty acids is associated with a lean and metabolically healthy phenotype (2–4). Emerging evidence suggests that some of the effects of dietary lipids on host metabolism may be mediated by modifications of the gut microbiota composition, which result in altered metabolic properties of the gut microbiota (5–8) and/or by alterations in gut integrity, which affect the extent of leakage of microbially derived metabolites into the circulation (6).

Metabolites produced by the gut microbiota may be utilized as a source of energy by the host or may act as signaling molecules that influence host metabolism (8). These metabolites can have a local effect in the intestine, but may also be transferred from the gut into the circulation, thereby affecting peripheral tissues (8). Indeed, germ-free (GF) mice have been shown to have improved glucose sensitivity and to be protected against obesity and dyslipidemia (9–12), exemplifying the systemic importance of the microbiota. Because of its proximity to the gastrointestinal tract, the liver is strongly affected by the microbiota, and bacterial status has been shown to affect both hepatic lipid biosynthesis (9) and lipid degradation (13). Several mechanisms linking bacterial metabolic processes with liver metabolism have been described (14); for example, microbial processing of dietary fibers, bile acids, and choline produces metabolites that regulate the activity of hepatic transcription factors and, hence, metabolic processes.

Abbreviations: CONV-R, conventionally raised; FDR, false discovery rate; GF, germ-free; GO, gene ontology; PBST, PBS Tween-20; PCA, principle component analysis; SREBP2, sterol regulatory element-binding protein 2.

<sup>1</sup>The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (Caesar et al., 2016) and are accessible through GEO Series accession number GSE73195 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE73195>).

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Here, we aimed to determine how the gut microbiota affects hepatic lipid metabolism during the metabolic challenge of a high-fat diet and how interaction between dietary lipids and the gut microbiota influences lipid composition and regulation of metabolic pathways.

## MATERIALS AND METHODS

### Mice

C57Bl/6 mice were maintained under standard specific-pathogen free or GF conditions, as described previously (10). All mice were males, age matched, and 12–14 weeks of age. Mice were fed irradiated isocaloric diets (45% kcal fat) of identical composition except for the source of fat, which was either menhaden fish oil (Research Diets, D05122102) or lard (Research Diets, D10011202) for 11 weeks (7). Fatty acid composition of the diets is displayed in supplementary Table 1. The mice were fasted for 4 h before they were euthanized. Blood samples and liver samples were harvested at the end of the experiment. All experiments were performed with protocols approved by the University of Gothenburg Animal Studies Committee.

### Lipidomics analysis

Lipids were analyzed by ultra-performance LC coupled with TOFMS using a Waters Q-TOF Premier (Waters, Milford, MA) with a methodology described earlier (15). Briefly, the samples were extracted with a chloroform:methanol mixture after addition of internal standards containing lysophosphatidylcholine (17:0), phosphatidylcholine (17:0/17:0), phosphatidylethanolamines (17:0/17:0), ceramide (d18:1/17:0) (Avanti Polar Lipids, Alabaster, AL), and triglyceride (17:0/17:0/17:0) (Larodan Fine Chemicals, Malmö, Sweden). Finally, an additional external standard solution composed of three stable isotope-labeled reference compounds [lysophosphatidylcholine (16:1-D<sub>3</sub>), phosphatidylcholine (16:1/16:1-D<sub>6</sub>), and triglyceride (16:0/16:0/16:0-<sup>13</sup>C<sub>3</sub>)] was added. The data were processed using MZmine 2 (16), which included alignment of peaks, peak integration, isotopic grouping, normalization, and peak identification. Lipids were identified using an in-house lipid species database and based on their MS/MS spectra. The data were normalized using the internal standards representative of each class of lipid present in the samples, as previously described (15). Sphingomyelins were normalized to the phosphatidylcholine standard.

The serum cholesterol concentrations were obtained by a metabolomics method based on 2D GC-MS. Briefly, each serum sample (30  $\mu$ l) was spiked with 10  $\mu$ l of internal standard solution (valine-d<sub>8</sub>, c = 37 mg/l; heptadecanoic acid, c = 187 mg/l; and succinic-d<sub>4</sub> acid, c = 63 mg/l), extracted with 400  $\mu$ l of methanol, centrifuged, and evaporated to dryness. Cholesterol was converted into its methoxime and TMS derivative(s) by two-step derivatization using methoxime and n-methyl-n-trimethylsilyl trifluoroacetamide reagents, and a retention index standard mixture (n-alkanes) in hexane was added. The analysis was performed as previously described (17) using a Leco Pegasus 4D GC $\times$ GC-TOFMS instrument (Leco Corp., St. Joseph, MI) equipped with a cryogenic modulator and with an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) as the GC part of the instrument. The raw data processing was done using ChromaTOF version 4.22 (Leco Corp.) software. Finally, alignment and normalization of the data were done using an in-house developed program, Guineu (17), and concentrations of cholesterol were calculated using a calibration curve. Hepatic free cholesterol was extracted

using the Folch method (18) and quantified using straight-phase HPLC coupled to evaporative light scattering detection (19).

### Microarray expression analysis

RNA was isolated from the left hepatic lobe using RNeasy lipid tissue mini kit (Qiagen, Hilden, Germany). cDNA synthesis, labeling, hybridization to GeneChip<sup>®</sup> ST arrays (GeneChip<sup>®</sup> Mouse Gene 1.0 ST array), washing, and scanning were performed as previously described (7). The raw data were normalized using the robust multi-array average method (20). Differential expression of microarray data was evaluated by Student's *t*-test followed by correction for false discovery rate (FDR). Analysis of interactions, using colonization status and diet as independent variables, was performed by two-way analysis of interaction followed by correction for FDR. Analysis of enrichment of regulated genes within functional categories [gene ontology (GO) categories] (21) was performed using the software DAVID (<http://david.abcc.ncifcrf.gov/>) (22). The results of the enrichment calculations were filtered for GO categories that were significantly enriched ( $P < 0.05$ ) after Bonferroni correction. Redundancy within lists of GO terms was reduced by the Revigo software (<http://revigo.irb.hr/>) (23) with a similarity score set to 0.5. Principle component analysis (PCA) was performed in MultiExperiment Viewer. Microarray data have been uploaded to the Gene Expression Omnibus (GEO) database with accession number GSE73195.<sup>2</sup>

### Immunoblot

Protein extracts (20  $\mu$ g) were loaded onto 10% NuPAGE Bis-Tris gels (Invitrogen, Carlsbad, CA) and blotted to Hybond P polyvinylidene difluoride membranes (Invitrogen). After 1 h blocking in PBS Tween-20 (PBST) supplemented with 5% fat-free milk powder, the membranes were incubated overnight with primary antibodies [sterol regulatory element-binding protein 2 (SREBP2) (ab30682; Abcam, Cambridge, UK) or actin (1615; Santa Cruz Biotechnology, Santa Cruz, CA)] diluted 1:1,000 in blocking buffer. The membranes were subsequently washed in PBST, incubated for 1 h with secondary antibodies [donkey anti-rabbit IgG (NA934V; GE Healthcare, Little Chalfont, UK), or donkey anti-goat IgG (Sc2056; Santa Cruz Biotechnology)] diluted 1:2,000 in blocking buffer, and finally washed in PBST. Proteins were detected with LumiGLO blotting detection kit (Cell Signaling, Danvers, MA).

### Statistical analysis

Data are represented as mean  $\pm$  SEM. Statistical comparison of two groups was performed by Student's *t*-test. Analysis of datasets with 2  $\times$  2 factorial design (comparing bacterial status and diet) was performed by two-way ANOVA with Tukey's multiple comparison test. Statistical analysis was performed in GraphPad Prism 6 unless otherwise stated.

## RESULTS

### Interaction between dietary lipids and gut microbiota regulates the levels of cholesteryl esters in the liver

We have previously shown that the gut microbiota affects liver and serum lipid composition in mice fed a chow diet (24) and that interaction between gut microbiota and dietary lipids influences adiposity, adipose tissue inflammation, and systemic glucose metabolism (7). Here we performed lipidomics analysis of liver and serum from conventionally raised (CONV-R) and GF mice fed a lard or fish oil diet for 11 weeks to study how interaction between

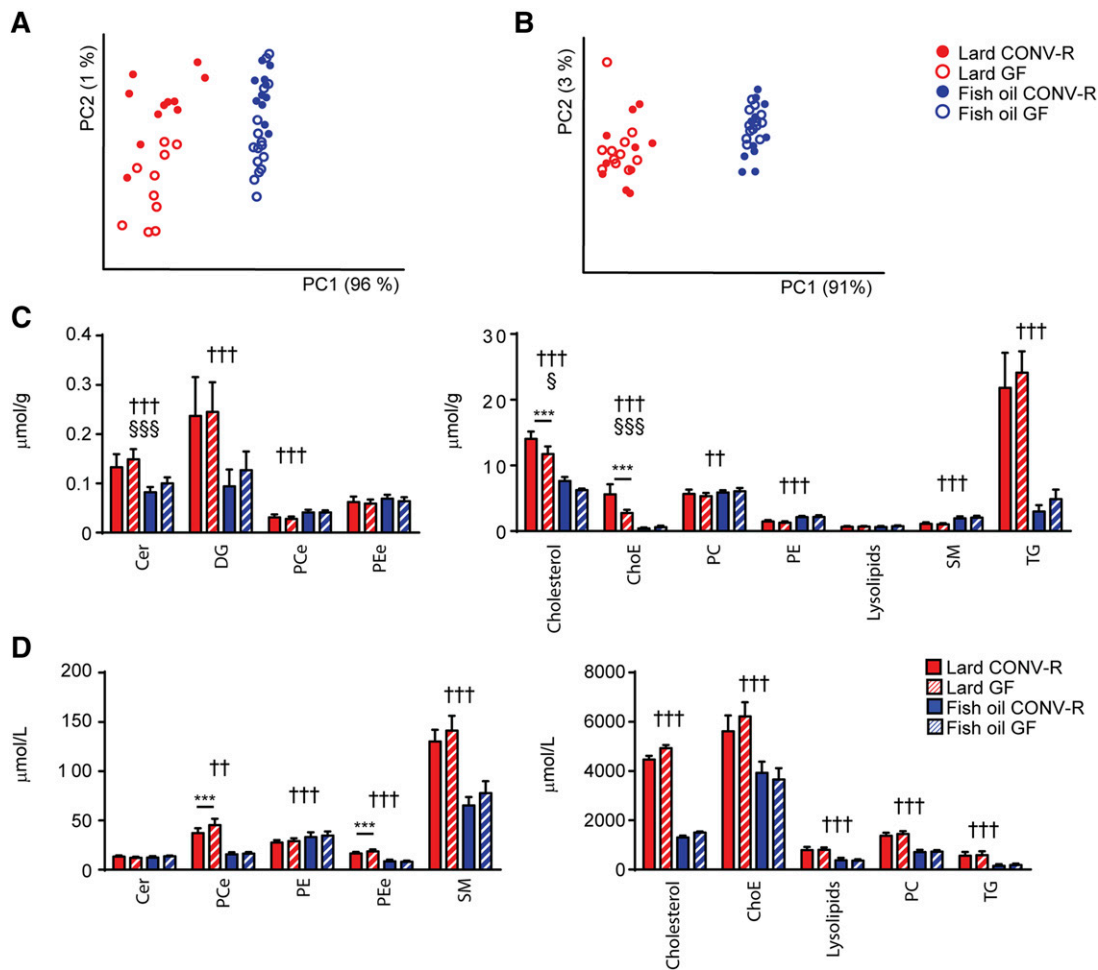
the gut microbiota and dietary lipids affects hepatic and serum lipid composition.

GF mice gained approximately 30% less weight than CONV-R mice (data not shown). To investigate the influence of gut microbiota on lipid metabolism independent of differences in body weight, we used GF mice that were slightly heavier than their CONV-R counterparts at the beginning of the feeding period. At the end of the experiment the CONV-R and GF mice had matching body weights (supplementary Fig. 1A). Diet consumption was similar between CONV-R and GF mice, while mice fed lard consumed more food than mice fed fish oil (supplementary Fig. 1B). PCA of lipidomics data showed that most of the variation in the dataset was induced by diet. Samples separated by diet in the first dimension, which accounted for 96% of the variation among liver samples (Fig. 1A) and for 91% of the variation among serum samples (Fig. 1B). Liver samples from mice fed lard separated on microbial

status in the second dimension (Fig. 1A), which represented approximately 1% of the total variation in the dataset. Serum samples did not separate on microbial status in either of the dietary groups (Fig. 1B).

Dietary lipids affected the abundance of the majority of lipid classes both in liver and in serum (Fig. 1C, D). However, the gut microbiota only induced increased hepatic levels of cholesterol and cholesteryl esters (Fig. 1C) and decreased serum levels of ether phosphatidylcholines and ether phosphatidylethanolamines in mice fed lard, but not in mice fed fish oil (Fig. 1D).

Despite the relatively small contribution by the microbiota to the overall variation in the lipidomics dataset, many individual lipid species in the liver and in serum were regulated by the gut microbiota (supplementary Tables 1, 2). To investigate whether the effect of the gut microbiota on individual lipid species in the liver and serum was similar, we plotted regulation induced by the gut microbiota in the



**Fig. 1.** Lipidomics analysis of liver and serum from CONV-R and GF mice fed lard or fish oil for 11 weeks. PCA of lipidomics data from liver (A) and serum (B) samples from CONV-R and GF mice fed lard or fish oil. Liver samples:  $n = 11$  (CONV-R lard); 10 (GF lard); 11 (CONVR fish oil); 18 (GF fish oil). Serum samples:  $n = 11$  (CONV-R lard); 10 (GF lard); 10 (CONV-R fish oil); 15 (GF fish oil). Abundance of lipid classes in the liver (C) and in serum (D) of CONV-R and GF mice fed lard or fish oil. Liver cholesterol:  $n = 9$  mice per group; other liver lipids:  $n = 11$  (CONV-R lard); 10 (GF lard); 11 (CONVR fish oil); 18 (GF fish oil); all serum lipids:  $n = 11$  (CONV-R lard); 10 (GF lard); 10 (CONVR fish oil); 15 (GF fish oil). Cer, ceramide; DG, diglyceride; PCe, ether phosphatidylcholines; PEe, ether phosphatidylethanolamine; ChoE, cholesteryl ester; PC, phosphatidylcholines; PE, phosphatidylethanolamine. Mean values  $\pm$  SEM are plotted. Variation induced by the diet: †† $P < 0.01$ , ††† $P < 0.001$ . Variation induced by the gut microbiota: † $P < 0.05$ , †† $P < 0.01$ , ††† $P < 0.001$ . Post hoc multiple comparison analysis: †††† $P < 0.0001$ , ††††† $P < 0.00001$ , †††††† $P < 0.000001$ .



liver against regulation induced by the gut microbiota in serum and observed a positive linear relationship in both dietary groups (Fig. 2A, B). An exception to this trend was observed for cholesteryl ester species, which were upregulated by the gut microbiota in the liver, but not in serum (except for a small increase in 18:1) of mice fed lard (Fig. 2A, C, D), but were not influenced by the gut microbiota in either the liver or serum of mice fed fish oil (Fig. 2B–D).

### Interaction between dietary lipids and gut microbiota regulates hepatic cholesterol biosynthesis

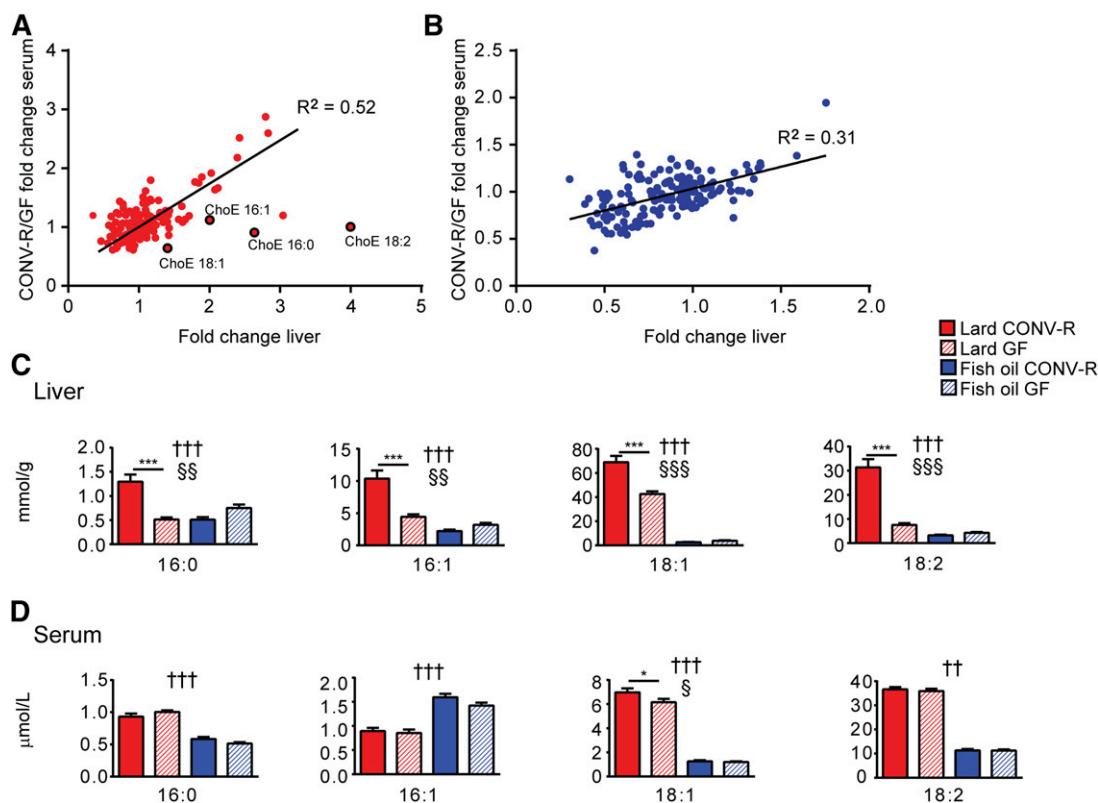
To study how the gut microbiota and dietary lipids regulate liver lipid metabolism, we performed microarray analysis of liver samples from CONV-R and GF mice fed lard or fish oil for 11 weeks.

PCA of hepatic gene expression data revealed that mice separated on diet in the first dimension and on microbial status in the second dimension (Fig. 3A). To compare how the gut microbiota affects hepatic gene expression in mice fed lard or fish oil, we plotted differences in hepatic gene expression level that were significant between CONV-R and GF mice, with values for mice fed fish oil on the y axis and values for mice fed lard on the x axis (Fig. 3B). We found a linear relationship between microbiota-induced gene regulation in liver from mice fed lard or fish oil (Fig. 3B),

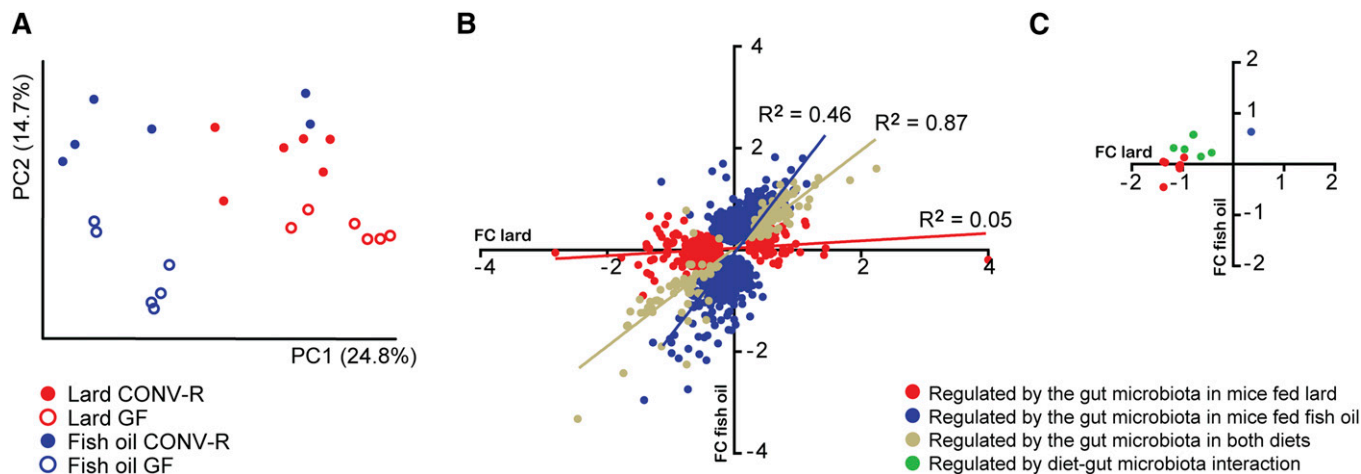
indicating that many genes are regulated by the gut microbiota independently of dietary lipids.

GO analysis showed that the microbiota induced hepatic expression of genes involved in the immune response in mice fed fish oil (Table 1). Most of these genes also showed a tendency toward being upregulated in mice fed lard, but did not reach significance in these mice (data not shown). Gene expressions of the macrophage markers *Cd68*, *Emr1* (encoding F4/80), and *Clec4f* were upregulated by the gut microbiota on both diets (supplementary Fig. 2A–C) indicating that the microbiota induces infiltration of macrophages to the liver. However, the expression of genes encoding interleukins was not affected by either dietary lipids or microbial status (data not shown). Genes in functional categories associated with cholesterol biosynthesis were downregulated by the gut microbiota in mice fed lard (Table 1, Fig. 3C). Two-way ANOVA also showed that genes regulated by the interaction between dietary lipids and the gut microbiota were highly enriched in processes associated with cholesterol biosynthesis (Table 1, Fig. 3C).

We found that the gut microbiota reduced the hepatic expression of many genes encoding enzymes in: 1) the mevalonate pathway, including the rate-limiting enzyme HMG-CoA reductase encoded by *Hmgcr* (Fig. 4A, B);



**Fig. 2.** Cholesteryl esters in the liver are increased by the gut microbiota in mice fed lard for 11 weeks. Microbial regulation of liver and serum lipid species in mice fed lard (A) and fish oil (B). Fold changes for lipid species significantly regulated by the microbiota in serum and liver are plotted on the y axis and x axis, respectively. ChoE, cholesteryl ester. Abundance of cholesteryl ester species in the liver (C) and in serum (D) from CONV-R and GF mice fed lard or fish oil. Liver samples: n = 11 (CONV-R lard); 10 (GF lard); 11 (CONV-R fish oil); 18 (GF fish oil). Serum samples: n = 11 (CONV-R lard); 10 (GF lard); 10 (CONV-R fish oil); 15 (GF fish oil). Mean values  $\pm$  SEM are plotted. Variation induced by the diet:  $^{\dagger\dagger}P < 0.01$ ,  $^{\dagger\dagger\dagger}P < 0.001$ . Variation induced by the gut microbiota:  $^{\S}P < 0.05$ ,  $^{\S\S}P < 0.01$ ,  $^{\S\S\S}P < 0.001$ . Post hoc multiple comparison analysis:  $^*P < 0.05$ ,  $^{***}P < 0.001$ .



**Fig. 3.** Gene expression in the liver of CONV-R and GF mice fed lard or fish oil for 11 weeks. A: PCA of global hepatic gene expression in CONV-R and GF mice fed lard or fish oil ( $n = 6$  mice per group). B: Microbial regulation of hepatic genes in mice fed lard (x axis) or fish oil (y axis). Fold changes (FC) for genes significantly regulated ( $P < 0.05$ , corrected for FDR) are displayed ( $n = 6$  mice per group). C: Microbial regulation of hepatic genes within GO category GO:016126 (sterol biosynthesis) in mice fed lard (x axis) or fish oil (y axis). Fold changes for genes significantly regulated ( $P < 0.05$ , corrected for FDR) are displayed ( $n = 6$  mice per group).

and 2) the steroid biosynthesis pathway (Fig. 4A, C) in mice fed lard. The expression levels of these genes were low in mice fed fish oil independent of microbial status (Fig. 4B, C).

To study the mechanisms underlying the regulation of cholesterol biosynthesis by gut microbiota and dietary lipids, we analyzed gene expression and protein cleavage of sterol regulatory element-binding protein 2 (SREBP2), a key regulator of cholesterol homeostasis. Gene expression of *Srebfp2* (encoding SREBP2) was increased in mice fed lard compared with mice fed fish oil (Fig. 4D). The bioactive cleaved form of SREBP2 was higher in mice fed fish oil than in mice fed lard, and there was a trend toward a reduction by the microbiota in mice fed lard (Fig. 4E, F).

The rate-limiting enzyme of bile acid synthesis, CYP7A1, which uses cholesterol as substrate, was downregulated by

the gut microbiota both in mice fed lard and in mice fed fish oil (Fig. 4G).

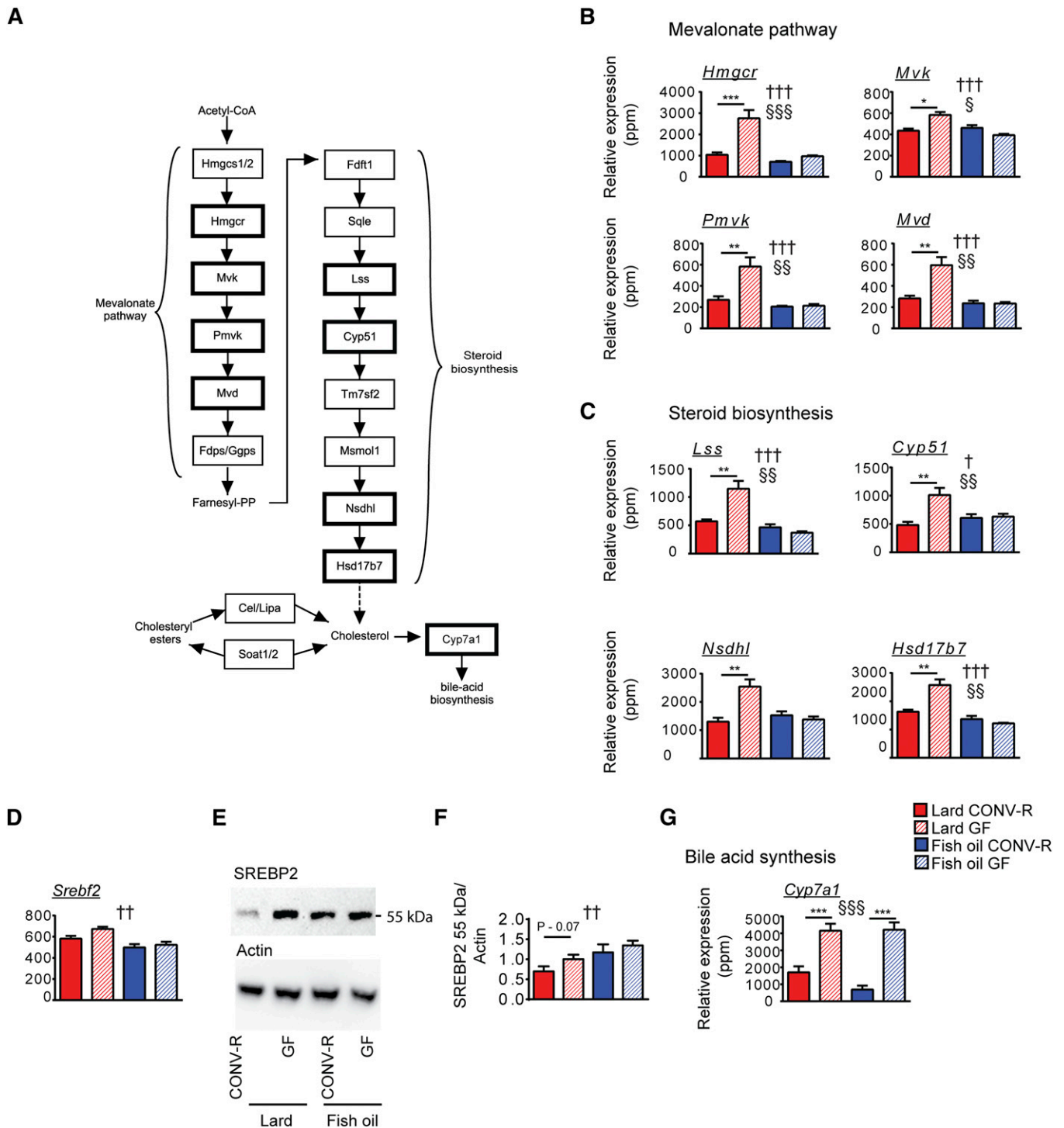
Taken together, we found that genes involved with cholesterol biosynthesis were downregulated: 1) by the gut microbiota in mice fed lard; and 2) by fish oil independent of microbial status.

## DISCUSSION

In the present study, we demonstrate that the interaction between gut microbiota and dietary lipids regulates cholesterol metabolism in the liver. The gut microbiota increased hepatic levels of cholesterol and cholesteryl esters and decreased the expression of genes involved in cholesterol biosynthesis in mice fed lard, but not in mice fed fish oil.

TABLE 1. Gene categories enriched in genes regulated by the gut microbiota during lard and fish oil diet

GO	Term	Fold Enrichment	FDR (Adjusted $P$ value)
Enriched in genes induced by the gut microbiota in mice fed lard			
—			
Enriched in genes reduced by the gut microbiota in mice fed lard			
16126	Sterol biosynthetic process	30.4	1.31E-09
6695	Cholesterol biosynthetic process	36.0	1.67E-09
8202	Steroid metabolic process	8.7	3.36E-08
16125	Sterol metabolic process	14.0	3.17E-08
8203	Cholesterol metabolic process	14.2	1.42E-07
6694	Steroid biosynthetic process	14.0	1.38E-07
8610	Lipid biosynthetic process	4.9	5.25E-05
7155	Cell adhesion	3.4	1.36E-04
22610	Biological adhesion	3.4	1.24E-04
8299	Isoprenoid biosynthetic process	18.8	1.40E-02
6720	Isoprenoid metabolic process	10.1	2.98E-02
Enriched in genes induced by the gut microbiota in mice fed fish oil			
6955	Immune response	3.1	5.79E-03
Enriched in genes reduced by the gut microbiota in mice fed fish oil			
—			
Enriched in genes regulated by interaction between dietary lipids and gut microbiota			
6694	Steroid biosynthetic process	15.0	1.10E-04
8202	Steroid metabolic process	7.3	3.45E-03
16126	Sterol biosynthetic process	19.7	2.99E-02
30198	Extracellular matrix organization	8.2	4.15E-02



**Fig. 4.** Expression of genes involved in cholesterol biosynthesis is decreased by the gut microbiota in mice fed lard. **A:** Schematic overview of de novo cholesterol biosynthesis pathways, including the mevalonate pathway and steroid biosynthesis, and synthesis of cholesteryl esters. Genes reported in **(B)** and **(C)** are indicated by bold borders. **B:** Genes in the mevalonate pathway significantly regulated by the gut microbiota in mice fed lard ( $n = 6$  mice per group). **C:** Genes in the steroid biosynthesis pathway significantly regulated by the gut microbiota in mice fed lard ( $n = 6$  mice per group). **D:** Expression of *Srebf2* encoding the transcription factor SREBP2 ( $n = 6$  mice per group). **E:** Representative immunoblot showing the 55 kDa fragment of SREBP2 and actin. **F:** Quantification of SREBP2 determined by the ratio between SREBP2 and actin ( $n = 9$  mice per group). **G:** Expression of *Cyp7a1* ( $n = 6$  mice per group). Mean values  $\pm$  SEM are plotted. Variation induced by the diet:  $^{\dagger}P < 0.05$ ,  $^{\ddagger}P < 0.01$ ,  $^{\ddagger\ddagger}P < 0.001$ . Variation induced by the gut microbiota:  $^{\S}P < 0.05$ ,  $^{\S\S}P < 0.01$ ,  $^{\S\S\S}P < 0.001$ . Post hoc multiple comparison analysis:  $^*P < 0.05$ ,  $^{**}P < 0.01$ ,  $^{***}P < 0.001$ .

Fatty acids are absorbed from the intestine and utilized as building-blocks in the biosynthesis of lipids. In accordance with previous reports (25), we found that dietary

lipids strongly affected the abundance and composition of lipids in the liver and in serum. We have previously shown that the presence of a gut microbiota in mice fed a chow


diet regulates lipid composition in serum and liver and decreases abundance of hepatic cholesteryl esters (24). The present study demonstrates that the gut microbiota also influences lipid composition in mice on a high-fat diet. The gut microbiota regulated the abundance of many lipid species, but had a modest effect on the abundance of lipid classes with the exception of cholesterol and cholesteryl esters, which were upregulated by the gut microbiota in the liver of mice fed lard. The genes encoding sterol *O*-acyltransferase (*Soat1/2*), carboxyl ester lipase (*Cel*), and lipase A (*Lipa*), which convert cholesterol into cholesteryl esters, were not regulated by the gut microbiota (data not shown). The increased levels of cholesteryl esters in CONV-R mice fed lard may instead result from the increased availability of hepatic free cholesterol.

Depending on dietary composition, GF mice may be protected against obesity and associated conditions such as increased levels of triglycerides in the liver (9, 12). However, this is not always the case and Fleissner et al. (26) have shown that CONV-R and GF mice differ in adiposity and hepatic triglyceride levels on a Western diet, but not on a high-fat diet. Here we show that weight-matched GF and CONV-R mice on a high-fat diet have similar levels of lipids in the liver.

In accordance with Rabot et al. (12), we found that genes involved in cholesterol biosynthesis were downregulated by the gut microbiota in liver from mice fed lard. Interestingly, we also found that expression of genes involved in cholesterol biosynthesis was low in mice fed fish oil, independent of microbial status. Cholesterol biosynthesis is controlled by negative feedback through inhibition of SREBP2 processing. We found that the increased levels of hepatic cholesterol induced by the gut microbiota and by a lard diet were inversely related to the levels of activated SREBP2. Microbiota-induced inhibition of SREBP2 was paralleled by decreased expression of genes involved in cholesterol biosynthesis in mice on a lard diet. Surprisingly, however, mice fed fish oil had low expression of genes encoding cholesterol biosynthesis enzymes despite high levels of activated SREBP2. Saturated lipids have been shown to induce cholesterol biosynthesis (27), while lipids rich in polyunsaturated fatty acids have been shown to reduce cholesterol biosynthesis in the liver (28). Moreover, *in vitro* experiments have previously demonstrated that the polyunsaturated fatty acid, DHA, activates SREBP2 without increasing cholesterol biosynthesis (29, 30), suggesting that polyunsaturated fatty acids regulate cholesterol biosynthesis through alternative mechanisms.

Cholesterol is the precursor for bile acids, and hence bile acid production is a potential sink for the hepatic cholesterol pool. In accordance with previous reports on mice fed a chow diet (31), we found that expression of CYP7A1, the rate-limiting enzyme in the bile acid biosynthesis pathway, was downregulated by the gut microbiota in mice fed lard and in mice fed fish oil. The decreased levels of hepatic cholesterol in GF mice may therefore be related to increased bile acid production associated with suppression of the FXR-mediated negative feedback loop due to increased levels of the FXR antagonist,  $\beta$ -muricholic acid

(31). Previous investigators have observed increased expression of genes encoding the membrane transporters ABCG5 and ABCG8 in the liver of GF mice (12) and suggested that these proteins may mediate increased cholesterol secretion and decreased hepatic cholesterol levels in GF mice. However, we did not find any differences in the expression of *Abcg5* or *Abcg8* between GF and CONV-R mice (data not shown). The dissimilarity in regulation of ABCG5 and ABCG8 may be related to differences in experimental design between the studies. In the present study, diets with 45% energy from fat were used and CONV-R and GF mice were matched for body weight, while in the previous study diet, 60% energy from fat was used and GF mice were leaner than their CONV-R counterparts (12).

In summary, we show that interaction between the gut microbiota and dietary lipids regulates hepatic cholesterol biosynthesis and that the influence of gut microbiota on hepatic cholesterol metabolism is greater in mice fed a lard diet compared with those on a fish oil diet. 

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