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Diet-induced alterations in intestinal and extrahepatic lipid metabolism in liver fatty acid binding protein knockout mice

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Abstract

Liver fatty acid binding protein (L-FABP) is highly expressed in both enterocytes and hepatocytes and binds multiple ligands, including saturated (SFA), unsaturated fatty acids (PUFA), and cholesterol. *L-fabp*^{-/-} mice were protected against obesity and hepatic steatosis on a high saturated fat (SF), high cholesterol “Western” diet and manifested a similar phenotype when fed with a high SF, low cholesterol diet. There were no significant differences in fecal fat content or food consumption between the genotypes, and fatty acid (FA) oxidation was reduced, rather than increased, in SF-fed *L-fabp*^{-/-} mice as evidenced by decreased heat production and serum ketones. In contrast to mice fed with a SF diet, *L-fabp*^{-/-} mice fed with a high PUFA diet were not protected against obesity and hepatic steatosis. These observations together suggest that *L-fabp*^{-/-} mice exhibit a specific defect in the metabolism of SFA, possibly reflecting altered kinetics of FA utilization. In support of this possibility, microarray analysis of muscle from Western diet-fed mice revealed alterations in genes regulating glucose uptake and FA synthesis. In addition, intestinal cholesterol absorption was decreased in *L-fabp*^{-/-} mice. On the other hand, and in striking contrast to other reports, female *L-fabp*^{-/-} mice fed with low fat, high cholesterol diets gained slightly less weight than control mice, with minor reductions in hepatic triglyceride content. Together these data indicate a role for L-FABP in intestinal trafficking of both SFA and cholesterol.

Keywords

Diet-induced obesity; Hepatic triglyceride; Steatosis; Nutrient-gene interactions

Introduction

The fatty acid binding proteins (FABP) are a family of highly abundant, cytosolic lipid-binding proteins with a defined tissue-specific expression pattern (for review see [1]). Distinct isoforms are present in tissues involved in fatty acid metabolism and/or trafficking, including muscle, skin, intestine, liver, and adipose tissues. *FABP* family members are genetically and structurally conserved with 10 anti-parallel β -sheets that form a lipid-binding pocket, followed by a helix turn helix loop that guards the hydrophobic pore and promotes protein-protein interactions [2–5]. The reason for multiple FABP isoforms with tissue-specific distribution is not completely understood, though it is postulated that amino acid variability in the helix turn helix region may direct cell-type-specific protein-protein interactions [5].

Liver FABP (L-FABP, FABP1) is expressed abundantly in the mammalian liver and small intestine, with lower levels of expression in the kidney and colon [6]. L-FABP is similar in structure to the other family members, yet it has a more flexible lipid-binding pocket that allows the binding of two fatty acid (FA) molecules [4,7]. Moreover, L-FABP has broad fatty acid binding specificity with high affinity for long chain, polyunsaturated FAs [7,8], but the larger binding site of L-FABP also allows it to bind to other hydrophobic ligands, including cholesterol, acyl-CoA, bile acid, and phytanic acid [9–14]. These findings suggest that L-FABP might be reasonably predicted to participate in the regulation of intracellular trafficking and delivery of a wide range of lipid molecules, many of which are important regulators of energy balance and lipid homeostasis throughout the body.

Several lines of evidence indicate that L-FABP is required for efficient FA trafficking and metabolism and facilitates triglyceride (TG) synthesis in both enterocytes and hepatocytes. In vitro studies have demonstrated increased fatty acid uptake and trafficking in cells with upregulated expression of L-FABP [15–17]. Complementing these findings, in vivo studies in mice with germline disruption of *L-fabp* are beginning to clarify the physiological role of FABP in mammalian hepatic and intestinal FA trafficking [18–21]. *L-fabp*^{−/−} mice are fertile with no overt phenotype in mice fed with a chow diet [18,22], although an obesity phenotype has been reported in *L-fabp*^{−/−} mice fed with a low fat, phytol-free semi-synthetic diet [19]. Following a 48 h fast, *L-fabp*^{−/−} mice exhibit decreased hepatic TG accumulation, reduced VLDL production, and decreased ketogenesis compared to C57BL/6 mice [18]. FA availability is unchanged (serum FFA levels are similar in fasted wild-type (WT) and *L-fabp*^{−/−} mice), but hepatocytes isolated from fasted *L-fabp*^{−/−} mice exhibit reduced incorporation of radiolabeled FA into cellular TG and phospholipids compared to WT hepatocytes [18]. Collectively, these findings are consistent with reduced net FA uptake and metabolic channeling into triglyceride, both for storage and for export as VLDL.

Dietary studies have begun to elucidate the role of L-FABP in intestinal FA uptake and processing. *L-fabp*^{−/−} mice fed with a high saturated fat, high cholesterol Western diet are protected against diet-induced obesity and exhibit decreased steatosis compared to C57BL/6 control mice [20]. Energy balance studies determined that this phenotype is not due to malabsorption of dietary fat or increased oxidation of fatty acid. Using a radiolabeled lipid gavage, it was shown that the kinetics of intestinal FA uptake and TG secretion are altered in enterocytes of *L-fabp*^{−/−} mice, a finding similar to that observed in isolated hepatocytes [18,20]. It is not clear, however, whether or not this difference in intestinal FA trafficking is

directly responsible for the protection against high fat diet-induced weight gain and whether alterations in FA trafficking lead to secondary alterations in feeding behavior or satiety signaling. Furthermore, the possibility also exists that compensatory changes in extrahepatic metabolism—the result of altered substrate delivery—might underlie the protection against diet-induced obesity and hepatic steatosis.

Adding further complexity to these questions, our findings pointing to the protection against diet-induced obesity and hepatic steatosis in *L-fabp*^{-/-} mice are in conflict with an independent study showing that *L-fabp*^{-/-} mice display increased obesity when fed with a high (1.25%) cholesterol (low fat) diet [19]. Because the “Western” diet contains both elevated cholesterol (0.2%) and high saturated FA, we initiated studies to independently examine the role of cholesterol and fat in the resistance to diet-induced obesity in *L-fabp*^{-/-} mice [21]. Our findings, using a range of different dietary regimens, demonstrate both an extrahepatic and an intestinal phenotype in *L-fabp*^{-/-} mice, which likely reflect altered trafficking of both saturated FA and cholesterol.

Materials and methods

Materials

Serum β -hydroxybutyrate was determined using a commercially available kit (Wako Chemicals, USA, Richmond VA). Serum lactate was monitored enzymatically using a Roche COBAS MIRA chemistry analyzer by the Diabetes Research and Training Center Immunoassay Core (Washington University, St Louis, MO).

Animals

C57BL/6J congenic *L-fabp*^{-/-} mice were generated previously in our laboratory [18,20]. Age-matched C57BL/6J mice (Jackson Laboratory) were used as controls. Female mice were used for all experiments. Mice were housed in a full barrier facility with a 12-h light/dark cycle and were maintained on standard chow (PicoLab Rodent Diet 20, 4.5% fat (0.8% saturated fat), 0.015% cholesterol) with free access to food and water, unless otherwise noted. All animal protocols were approved by the Washington University Animal Studies Committee and conformed to the criteria outlined in the National Institutes of Health “Guide for the Care and Use of Laboratory Animals”.

Dietary studies

Details of respective diets are shown in Table 1 and [21]. The 2% cholesterol, chow diet was obtained from MP Biomedicals (#904691; Solon, Ohio). The 1.25% cholesterol, semi-synthetic diet was the same formulation as that used by Martin et al. [19] (D01091702; Research Diets Inc., New Brunswick, NJ). The high SF diet (#960242) and the high polyunsaturated fat (PUFA) diet (#960244) were obtained from MP Biomedicals (Solon, Ohio). Mice were started on the cholesterol-containing diets at ~8 weeks of age and maintained on the diet for 10–12 weeks. Mice were started on the SF and PUFA diets at ~12 weeks of age. Mice were weighed weekly during all dietary studies. Weekly weight gain was calculated from weight gained after 10–12 weeks on the diet, divided by the number of weeks on the diet. Data are expressed as weight gain to control for differences in starting weight, since mice were started on the diets at different ages. At the conclusion of the dietary studies, mice were sacrificed following a 4 h fast. Body, liver, and gonadal fat pad weights were measured. Serum and tissues were flash frozen in liquid nitrogen and stored at -80°C for later analyses.

Food consumption and fecal fat determinations were performed 8–12 weeks after the mice were placed on the respective diets as described [20]. Food intake was measured and feces

were collected individually for at least 3 days for each animal. Fecal fat content was determined gravimetrically as described [23]. Lipid mass was normalized to food consumption and dietary fat content to determine percent fat absorption.

Gene expression

RNA was isolated from frozen muscle tissue using TRIzol (Invitrogen Life Technologies) as directed by the manufacturer. RNA from four animals per genotype was pooled and hybridized to Agilent Technologies Mouse Whole Genome Microarray (G4122A).

Cholesterol absorption

Cholesterol absorption was measured by a fecal dual-isotope ratio method as described [23,24]. Mice were gavaged with 150 μ l corn oil containing 1 μ Ci [14 C]-cholesterol (Perkin Elmer) and 2 μ Ci [3 H]-Sitostanol (American Radiolabeled Chemicals, Inc), then housed individually in metabolic cages. Feces were collected for 48 h and extracted with chloroform/methanol. The ratio of [14 C] to [3 H] in the feces was determined and corrected for the ratio in the dosing mixture, and percent cholesterol absorption was calculated as described [24].

Results

Susceptibility of C57BL/6 and *L-fabp*^{-/-} mice to diet-induced obesity

C57BL/6 congenic *L-fabp*^{-/-} mice and age-matched C57BL/6 control mice were fed with a chow diet until 8–12 weeks of age and then switched to a lipid-enriched diet. Details of the individual diets are shown in Table 1. The average weekly weight gain after 10–12 weeks on each diet is shown in Fig. 1. *L-fabp*^{-/-} mice fed with either a 2% cholesterol, chow-based diet or a 1.25% cholesterol, phytol-free semi-synthetic diet (identical to the diet used by Martin et al. [19]) exhibited decreased weekly weight gain compared to C57BL/6 mice. These findings are in direct conflict with previous studies in which female *L-fabp*^{-/-} mice fed with the 1.25% cholesterol diet showed a 3-fold increase in body weight after 5 weeks, with a marked increase in adipose tissue [19]. The reasons for this discrepancy are unclear since we attempted to control for differences in diet, gender, and genetic background in these experiments.

L-fabp^{-/-} mice fed with a high SF, low cholesterol diet exhibit markedly reduced weekly weight gain compared to SF-fed C57BL/6 animals (Fig. 1), with absolute values and differences between the genotypes recapitulating the phenotype observed with mice that were fed with the Western diet (Fig. 1), which contains >60% SFA. In contrast, no difference in weekly weight gain was observed between *L-fabp*^{-/-} and C57BL/6 mice fed with a high PUFA diet. This is particularly surprising in light of the high affinity of L-FABP for PUFA but may reflect differences in the way saturated and polyunsaturated FA are metabolized, both in the lumen of the small intestine and within the enterocyte [25]. This possibility will require further experimental examinations. Interestingly, the average weekly weight gain of *L-fabp*^{-/-} mice fed with the PUFA diet is not significantly different from the average weekly weight gain of C57BL/6 mice fed with either the Western diet or SF diet (Fig. 1), indicating that *L-fabp*^{-/-} mice are capable of marked weight gain when fed with high fat diets enriched in distinct species of FA.

Differences in food intake or energy utilization do not account for protection against diet-induced obesity and steatosis in *L-fabp*^{-/-} mice fed with a high fat diet

The decreased weekly weight gain of *L-fabp*^{-/-} mice fed with the Western and the SF diet is not due to differences in food consumption or fat absorption. As shown in Table 2, there is no difference in the percentage of dietary fat absorbed by either genotype in Western, SF, or

PUFA-fed mice, as determined by fecal fat mass. Moreover, food consumption is not significantly different between WT and *L-fabp*^{-/-} mice fed with any of the diets, though there is a trend toward decreased food consumption in *L-fabp*^{-/-} mice (Table 2). These differences are minimized, however, when food consumption is normalized to body weight (Table 2).

We explored an alternative possibility, namely that increased energy utilization might account for the protection against obesity and hepatic steatosis in *L-fabp*^{-/-} mice fed with Western or SF diets. However, this was not the case. As shown in Table 2, the average heat production was not significantly different between the genotypes of mice fed with a Western diet. In contrast, average heat production was significantly lower in SF-fed *L-fabp*^{-/-} mice compared to SF-fed C57BL/6 mice, suggesting decreased oxidation of fatty acids. Moreover, levels of serum β -hydroxybutyrate, a ketone body, were reduced in *L-fabp*^{-/-} mice fed with Western [20] and SF diets (C57BL/6, 403 $\pm$ 51; *L-fabp*^{-/-}, 196 $\pm$ 55; $P = 0.018$), due either to decreased availability of FA for ketogenesis or increased reliance on ketones as an energy source. *L-fabp*^{-/-} mice fed with a Western diet exhibit elevated serum lactate [20], consistent with increased use of glucose as an energy source. In addition, intestinal lipid absorption was virtually quantitative in animals of both genotypes fed with different diets (ranging from approximately 97 to 99%, Table 2), suggesting that differences in fat absorption were not a factor in producing the observed phenotype. Taken together, these data indicate that the protection against diet-induced obesity and steatosis in SF and Western fed *L-fabp*^{-/-} mice is not due to increased oxidation or defective absorption of fatty acid. Rather, the data suggest that there is decreased availability of dietary FA in *L-fabp*^{-/-} mice.

Altered gene expression in soleus muscle of Western diet-fed *L-fabp*^{-/-} mice

In order to begin exploring the possibility that alterations in substrate delivery to extrahepatic tissues might contribute to the protection against diet-induced obesity, we undertook microarray analysis on mRNA obtained from the soleus muscle of control and *L-fabp*^{-/-} mice fed with a Western diet. Consistent with increased reliance on glucose as an energy source [20], expression of several genes involved in glucose uptake and metabolism (Glut1, Pdk4, and Pepck) was increased in the muscle of *L-fabp*^{-/-} mice (Table 3). Expression of FA synthase was upregulated in *L-fabp*^{-/-} mice (Table 3, [20]), while genes involved in FAO oxidation were either unchanged (Cpt1b) or only moderately increased (Cpt1a, MCAD, LCAD). Interestingly, *L-fabp*^{-/-} mice displayed increased expression of both genes involved in cellular proliferation (Jun, Fos) and genes induced by glucocorticoids and muscle wasting [26–28] (CAAT/enhancer binding protein beta and delta). Taken together, these data suggest that there is compensatory upregulation of genes involved in glucose uptake and FA synthesis but that FAO is not upregulated in the skeletal muscle of *L-fabp*^{-/-} mice fed with a Western diet. In addition, the findings suggest the intriguing possibility that the expression of fasting- and wasting-associated genes is increased.

Altered cholesterol absorption in chow-fed *L-fabp*^{-/-} mice

Because average weekly weight gain is slightly reduced in *L-fabp*^{-/-} mice fed with cholesterol-enriched diets (Fig. 1), we asked whether intestinal cholesterol absorption was altered in *L-fabp*^{-/-} mice fed with either a chow or 1.25% cholesterol semi-synthetic diet. Cholesterol absorption was assessed using an extensively validated, dual-isotope balance method and shown to be significantly reduced in female *L-fabp*^{-/-} mice fed with a chow diet compared to control mice (Fig. 2). Similar findings were obtained using chow-fed male *L-fabp*^{-/-} animals (data not shown). In mice fed with 1.25% cholesterol diet, overall cholesterol absorption is reduced, but there was no difference between the genotypes (Fig.

2). These findings again emphasize that there are subtle distinctions in intestinal lipid trafficking in *L-fabp*^{-/-} mice.

Discussion

The data summarized in this manuscript suggest that the protection against diet-induced obesity and steatosis observed in *L-fabp*^{-/-} mice fed with a 0.2% cholesterol, high SF “Western” diet is due primarily to the altered metabolism of saturated FA, rather than the altered utilization of cholesterol. *L-fabp*^{-/-} mice fed with cholesterol-supplemented diets gained slightly less weight than control mice and displayed a slight reduction in hepatic TG content (Fig. 1, [21]). In contrast to previous studies [19], cholesterol-fed *L-fabp*^{-/-} mice did not develop an obesity phenotype and the absolute weight gain over the course of the experiment was minimal. Taken together, these data suggest that the elevated cholesterol present in the Western diet (0.2%) does not reverse the protection against high SF-induced obesity in *L-fabp*^{-/-} mice. Our findings suggest, if anything, that cholesterol supplementation might contribute to the protective phenotype observed with SF feeding.

In comparison to mice fed with high-cholesterol diets, the difference in average weekly weight gain between C57BL/6 and *L-fabp*^{-/-} mice fed with either the Western or SF diet were dramatic (Fig. 1). *L-fabp*^{-/-} mice gained ~60% less weight per week than control mice on both diets, with dramatically reduced hepatic TG content [21]. Reduced serum ketones in *L-fabp*^{-/-} mice suggest either decreased ketogenesis or increased utilization of ketones as an energy source. Interestingly, the protection against hepatic steatosis correlates with the protection against diet-induced obesity [21]. Hepatic TG content was reduced in *L-fabp*^{-/-} mice fed with either the Western or SF diets [20,21]. In contrast, PUFA-fed *L-fabp*^{-/-} mice develop similar degrees of hepatic steatosis as PUFA-fed C57BL/6 mice, with no difference in weight gain (Fig. 1, [21]). These data suggest that the absence of L-FABP does not prevent the development of hepatic steatosis per se. Rather, the findings suggest that the protection against hepatic steatosis in *L-fabp*^{-/-} mice may reflect differences in the trafficking and/or availability of dietary lipid, rather than an inability of the liver to accumulate TG.

The mechanism[s] by which *L-fabp*^{-/-} mice are protected against high fat diet-induced obesity are still incompletely resolved. We observed no difference in dietary fat absorption, and either no difference or reduced FA oxidation in *L-fabp*^{-/-} mice, as monitored by indirect calorimetry (Table 2). Moreover, expression of genes related to FA oxidation is not markedly increased in the liver, muscle, and adipose tissue of *L-fabp*^{-/-} mice fed with the Western diet (Table 3, [20], and data not shown). Food consumption was marginally decreased in *L-fabp*^{-/-} mice consuming either the Western or SF diet, though the differences are not significant (Table 2). On the other hand, energy balance calculations suggest that even a minimal decrease in consumption of these high caloric diets could potentially be sufficient to produce a difference in weight gain similar to that observed. For example, a 0.8 g/week decrease in food consumption in mice fed with the SF diet could explain a 0.39 g/week difference in weight gain (0.8 g food × 4.4 kcal/g SF diet = 3.52 kcal ÷ 9 kcal/g fat = 0.39 g fat differential). Thus, although the difference in food consumption between the genotypes is not statistically significant, the trend toward decreased food consumption in *L-fabp*^{-/-} mice could be sufficient to explain their reduced weight gain and protection against hepatic steatosis.

It is tempting to speculate on several possible explanations for the trend toward reduced food consumption in *L-fabp*^{-/-} mice. It is possible that an altered rate of FA trafficking through *L-fabp*^{-/-} enterocytes may result in more rapid or stronger induction of a satiety signal. In support of this possibility, recent studies have shown that L-FABP is involved in

the budding of pre-chylomicron transport vesicles from the endoplasmic reticulum of the enterocyte [29], which is consistent with delayed appearance of dietary TG in serum of *L-fabp*^{-/-} mice [20]. In addition, preliminary data suggests that expression of genes involved in FA uptake and metabolism may be decreased in enterocytes of *L-fabp*^{-/-} mice fed with SF diet, compared to expression in SF-fed C57BL/6 mice (data not shown). Finally, a possibility remains that *L-fabp*^{-/-} mice might exhibit a decreased oral preference for dietary fat and thus consume less. Against this possibility, however, is that the average food consumption was similar in *L-fabp*^{-/-} mice fed with PUFA and SF diets, despite disparate average weight gain.

It is unclear why the phenotype of *L-fabp*^{-/-} mice fed with cholesterol-supplemented diet described in these studies is diametrically opposite that reported previously, despite the use of apparently similar methodology [19,21]. It is possible that subtle differences in genetic background, construct generation, or generation number of the mice used for these studies might influence the phenotype, but no clear explanation is yet at hand. The mice used in our study were backcrossed to a C57BL/6 background using a speed congenic approach in which mice containing minimal amounts of non-C57 genomic DNA, particularly surrounding the *fabp1* locus, were identified and selected for further breeding. This is of particular importance because different mouse strains display significant differences in cholesterol absorption, due to variations in specific genetic loci [30,31]. Genes within 5 cM on either side of the FABP1 locus on chromosome 6 include *Abcg2*, neuropeptide Y, and aquaporin 1, as well as QTLs associated with differences in body weight (obq13, bw18, bwq2) and epididymal fat weight (efw) [32–35].

One of the most striking findings of this study was that *L-fabp*^{-/-} mice fed with a high PUFA diet exhibit similar average weekly weight gain and hepatic steatosis as PUFA-fed C57BL/6 mice (Fig. 1, [21]). Moreover, the weekly weight gain of PUFA-fed *L-fabp*^{-/-} mice was not significantly different from C57BL/6 mice fed with the SF or Western diet, indicating that *L-fabp*^{-/-} mice are, in fact, susceptible to diet-induced obesity when fed with certain fatty acids. Based on the high binding affinity of L-FABP for long chain, polyunsaturated FA [7,8], these findings are somewhat surprising. It is possible, however, that differences in susceptibility of *L-fabp*^{-/-} mice to PUFA versus SF diet-induced weight gain may reflect fundamental differences in the mechanism of intestinal hydrolysis, absorption, and re-esterification of PUFA compared to SFA, rather than the relative affinity of each fatty acid for L-FABP in vitro. For example, compared to oleic acid, a higher percentage of dietary linoleic acid (18:2) is incorporated into phospholipid secreted from enterocytes, with decreased incorporation into TG [25]. Along these lines, it should be emphasized that the fatty acids in the PUFA and SF diets differ not only in their degree of saturation but also in their chain length with the SF diet containing primarily medium chain (C12) fatty acids (Table 1). Accordingly, it might be informative in future studies to examine the role of L-FABP in the absorption of dietary FAs of similar chain length but with different degrees of saturation. In addition, our observations point to a subtle defect in cholesterol absorption in chow-fed *L-fabp*^{-/-} mice. This observation raises the question of whether decreased cholesterol absorption in chow-fed *L-fabp*^{-/-} mice reflects altered expression of cholesterol transporters in the intestinal mucosa of these mice. This and other issues regarding the kinetics of lipid delivery and trafficking will be the focus of future reports.

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Fig. 1.

Average weekly weight gain of C57BL/6 and *L-fabp*^{-/-} mice on high-fat and high-cholesterol diets. Mice were started on the high cholesterol diets at 8 weeks of age, and on the high-fat diets at 10–12 weeks of age. Mice were weighed weekly and maintained on diet for 10–20 weeks. Weekly weight gain for each mouse was calculated from weight gain after 10–12 weeks on diet divided by number of weeks on diet. Data are expressed as average $\pm$ SE, with *n* of 7–18 mice per group. Black bars C57BL/6 mice; gray bars *L-fabp*^{-/-} mice. Asterisks indicate *P* < 0.05 versus C57BL/6 mice fed with the same diet

Fig. 2.

Intestinal cholesterol absorption in chow and cholesterol-fed mice. Cholesterol absorption was examined in the same group of female mice ($n = 4-5$) fed with either a chow diet (studied at 8 weeks, immediately prior to start of cholesterol diet feeding) or after 8 weeks on the 1.25% cholesterol semi-synthetic diet. Black bars: C57BL/6 mice; gray bars: *L-fabp*^{-/-} mice. Asterisks indicate $P < 0.05$ versus C57BL/6 mice fed with the same diet

Table 1

Fat and cholesterol content of chow, cholesterol, and high fat diets

Diet	Kcal/g	Cholesterol (%)	Fat (%)	Fat source	Primary fatty acid
Chow	3.4	0.015	~4.5	Corn Oil	~50% (18:2)
2% Cholesterol	3.4	2.0	~4.5	Corn Oil	~50% (18:2)
1.25% Cholesterol	3.9	1.25	5.0	Corn Oil	~50% (18:2)
Western	4.5	0.2	21	Milkfat	~25% (18:1), ~25% (16:0)
High SF	4.4	0.015	20	Coconut Oil	~50% (12:0)
High PUFA	4.4	0.015	20	Safflower Oil	~75% (18:2)

Percentages of fat and cholesterol in each diet are calculated in grams of fat or cholesterol per gram diet $\times 100$ (w/w). Fat content of chow and the 2% cholesterol, chow-based diet are approximate since these are not defined diets

Table 2Fat absorption, food consumption, and heat production in C57BL/6 and *L-fabp*^{-/-} mice fed with high fat diets

	Fat absorption (% absorbed)	Food consumption (g/day)	Food consumed/g body weight (g/ day/g body)	Heat production (kcal/h)
Western				
C57BL/6	98.7 ± 0.1	2.94 ± 0.13	0.117 ± 0.005	0.433 ± 0.028
<i>L-fabp</i> ^{-/-}	98.7 ± 0.1	2.86 ± 0.09	0.118 ± 0.005	0.419 ± 0.018
Sat Fat				
C57BL/6	98.7 ± 0.1	2.73 ± 0.09	0.121 ± 0.004	0.415 ± 0.006
<i>L-fabp</i> ^{-/-}	98.6 ± 0.1	2.50 ± 0.09	0.117 ± 0.005	0.352 ± 0.007*
PUFA				
C57BL/6	97.4 ± 0.2	2.66 ± 0.14	0.075 ± 0.011	nd
<i>L-fabp</i> ^{-/-}	96.9 ± 0.2	2.56 ± 0.14	0.077 ± 0.009	nd

All studies were performed after mice were on the respective diets for at least 8 weeks. Fat absorption was calculated based on fecal fat mass and food consumption as described. Food consumption was normalized to body weight of each mouse at the start of food consumption study. Heat production was calculated from oxygen consumption and carbon dioxide production. *Nd* not determined

* $P < 0.05$ versus C57BL/6 mice fed with the same diet

Table 3Altered gene expression in soleus muscle of Western diet-fed *L-fabp*^{-/-} mice

Gene name	Common name	Accession number	Ratio
Fasn	Fatty acid synthase	NM_007988	2.121
Angptl4	FIAF, fasting induced adipose factor	NM_020581	2.078
Lpl	Lipoprotein lipase	NM_008509	2.060
Cpt1a	Carnitine palmitoyltransferase, liver	NM_013495	1.592
Cpt1b	Carnitine palmitoyltransferase, muscle	NM_009948	0.968
Acadm	MCAD	NM_007382	1.426
Acadl	LCAD	NM_007381	1.311
Cyp3a16		NM_007820	0.260
Slc2a1	Glut1	NM_011400	2.337
Pdk4	Pyruvate dehydrogenase kinase 4	NM_013743	2.463
Pck1	Pepck	NM_011044	1.462
Crem	cAMP responsive element modulator	NM_013498	2.284
Cebpd	C/EBP delta	NM_007679	2.714
Cebpb	C/EBP beta	NM_009883	2.036
Fos		NM_010234	2.245
Jun		NM_010591	2.294
Pln	Phospholamban	NM_023129	2.311
Sln	Sarcolipin	NM_025540	3.063
Retnla	Resistin like alpha	NM_020509	1.471

RNA was isolated from the soleus muscle of female C57BL/6 and *L-fabp*^{-/-} mice maintained on Western diet for 18 weeks and pooled (*n* = 4 animals/genotype). Results are expressed as a ratio of expression in *L-fabp*^{-/-} versus C57BL/6 mice. Ratios less than 1 indicate decreased expression in *L-fabp*^{-/-} animals. *MCAD* medium chain acyl-CoA dehydrogenase; *LCAD* long chain acyl-CoA dehydrogenase; *C/EBP* CAAT/enhancer binding protein; *Pepck* phosphoenolpyruvate carboxykinase