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Liver Fatty Acid-Binding Protein Gene-Ablated Female Mice Exhibit Increased Age-Dependent Obesity[1–3]

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Abstract

Previous work conducted in our laboratory suggested a role for liver fatty acid-binding protein (L-FABP) in obesity that develops in aging female L-FABP gene-ablated (−/−) mice. To examine this possibility in more detail, cohorts of wild-type (+/+) and L-FABP (−/−) female mice were fed a standard, low-fat, nonpurified rodent diet for up to 18 mo. Various obesity-related parameters were examined, including body weight and fat and lean tissue mass. Obesity in (−/−) mice was associated with increased expression of nuclear receptors that induce PPARα (e.g. hepatocyte nuclear factor 1α, genotype effect) and of PPARα-regulated proteins involved in uptake of free (lipoprotein lipase and fatty acid transport protein, genotype, and/or age effect) and esterified (scavenger receptor class B type 1, genotype effect) long-chain fatty acids (LCFA). Hepatic total lipid and neutral lipid levels were not affected by age or genotype, consistent with absence of gross and histologic steatosis. There was increased mRNA expression of liver proteins involved in LCFA oxidation [mitochondrial 3-oxoacyl-CoA thiolase (genotype effect) and butyryl-CoA dehydrogenase (genotype and/or age effect)], increased expression of LCFA esterification enzymes [glycerol-3-phosphate acyltransferase (age x genotype effect) and acyl-CoA:cholesterol acyltransferase-2 (genotype and/or age effect)], and increased expression of proteins involved in intracellular transfer and secretion of esterified LCFA [liver microsomal triacylglycerol transfer protein (genotype effect), serum apolipoprotein (apo) B (genotype or age effect), and liver apoB (age and age x genotype effect)]. The data support a working model in which obesity development in these mice results from shifts toward reduced energy expenditure and/or more efficient energy uptake in the gut. J. Nutr. 138: 1859–1865, 2008.

Introduction

Liver fatty acid-binding protein (L-FABP),[6] a small, 14-kDa protein, is present at a high level (3–5% of soluble protein) in tissues active in long-chain fatty acid (LCFA) uptake and metabolism, i.e. liver and intestine (review in 1). Although its physiological function is not completely clear, studies in vitro and with transfected cells indicate that L-FABP has both direct and indirect roles in LCFA uptake and metabolism.

Earlier in vitro studies of L-FABP function focused on structure, ligand binding characteristics, and L-FABP activity in LCFA uptake, diffusion, esterification, and oxidation, suggesting involvement (2–10) in both anabolic and catabolic LCFA pathways (review in 1). These putative functions were recently confirmed with cultured primary hepatocytes and livers from L-FABP null mice (11–16), suggesting that L-FABP may be important in regulating lipid metabolism and affecting the obese state in mice.

L-FABP also indirectly influences cellular LCFA metabolism by transporting LCFA to the nucleus for interaction with PPARα, a nuclear receptor that regulates transcription of multiple enzymes involved in LCFA uptake, transport, esterification, and oxidation (review in 17–19). Both L-FABP (review in 1,2) and PPARα (20–22) exhibit acyl chain-dependent high affinity (i.e. nmol/L K_d) for LCFA and LCFA-CoA, both of which alter conformation of these proteins. Studies in vitro and with purified nuclei (23) as well as with intact living cells (24–26) show L-FABP enhances LCFA and LCFA-CoA distribution to nuclei, directly binds PPARα, and induces PPARα transcription of

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[3] Supplemental Table 1 is available with the online posting of this paper at jn.nutrition.org.

[6] Abbreviations used: ACAT-2, acyl-CoA:cholesterol acyltransferase-2; apo, apolipoprotein; CPT I, carnitine palmitoyl transferase I; FABP, fatty acid-binding protein; FAT/CD36, fatty acid translocase; FATP-4, fatty acid transport protein-4; FTM, fat tissue mass; GPAT, glycerol-3-phosphate acyltransferase; GST, glutathione *S*-transferase; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; HNF, hepatocyte nuclear factor; 3α-HSD, 3α-hydroxysteroid dehydrogenase; LCFA, long-chain fatty acid; L-FABP, liver FABP; LPL, lipoprotein lipase; LTM, lean tissue mass; MTP, microsomal triacylglyerol transfer protein; SCP-2, sterol carrier protein-2; SCP-x, sterol carrier protein-x/3-ketoacyl-CoA thiolase; SR-B1, scavenger receptor class B type 1; TTM, total tissue mass; (+/+), liver fatty acid binding protein wild-type mice; (−/−), liver fatty acid binding protein gene-ablated mice.

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LCFA oxidative enzymes. Consequently, L-FABP may regulate its own expression via PPARα (review in 27).

Previous studies in our laboratory (12) demonstrated that under conditions of stable energy intake (standard nonpurified diet), L-FABP (−/−) mice exhibit similar, but less severe, age-dependent obesity without morphologically evident hepatic steatosis as observed in PPARα null mice (28,29). To further explore the mechanistic basis of obesity in older female L-FABP (−/−) mice, the current study was undertaken to determine the age dependence of hepatic lipid distribution, serum lipid distribution, and expression of select proteins involved in LCFA metabolism. These studies provided new insights into the mechanistic basis of hepatic lipid metabolism and obesity in L-FABP gene-ablated female mice and support a working model in which the age-dependent obesity that develops in the (−/−) female mice is a result of physiological shifts in these mice toward reduced energy expenditure and/or more efficient intestinal energy uptake.

Materials and Methods

Materials. Protease inhibitor cocktail (catalog no. P8340) was from Sigma-Aldrich. Protein was quantified by Protein Assay Dye reagent (Bio-Rad Laboratories). Rabbit polyclonal antisera directed against recombinant rat L-FABP, mouse acyl-CoA-binding protein, and mouse sterol carrier protein-2 (SCP-2) were produced as described in (30). A rabbit polyclonal antibody directed against mouse SCP-x, which recognizes all SCP-x/SCP-2 gene products (58-kDa SCP-x, 46-kDa thiolase, 15-kDa pro-SCP-2, and 13.2-kDa SCP-2), was made as in (31). Rabbit polyclonal anti-human antibodies against PPARα, sterol regulatory element binding protein-1, caveolin-1, goat polyclonal anti-human antibodies against fatty acid transport protein-4 (FATP-4), fatty acid translocase (FAT/CD36), carnitine palmitoyltransferase I (CPT I), lipoprotein lipase (LPL), apolipoprotein (apo) B, microsomal triacylglycerol transfer protein (MTP), mitochondrial 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) synthase, hepatocyte nuclear factor-1α (HNF-1α), and HNF-4α, and goat polyclonal anti-mouse antibodies against LDL receptor and apoAI were from Santa Cruz Biotechnology. Rabbit polyclonal anti-human acyl-CoA:cholesterol acyltransferase-2 (ACAT-2) was from Cayman Chemical. Rabbit polyclonal anti-mouse scavenger receptor class B type 1 (SR-B1) was from Novus Biologicals. Rabbit anti-human HMG-CoA reductase was from Upstate Cell Signaling Solutions. Rabbit polyclonal anti-mouse glutathione *S*-transferase (GST) and anti-*Pseudomonas* 3α-hydroxysteroid dehydrogenase (3α-HSD) were from US Biological. Rabbit polyclonal anti-glycerol-3-phosphate acyltransferase (GPAT) was a generous gift of Dr. Rosalind Coleman (Department of Nutrition, University of North Carolina, Chapel Hill, NC). Alkaline phosphatase-conjugated goat anti-rabbit IgG and rabbit anti-goat IgG were from Sigma-Aldrich. All other reagents and solvents used were of the highest grade available and were cell-culture tested.

Mouse studies. C57BL/6 mice were obtained from the National Cancer Institute (Frederick Cancer Research and Development Center, Frederick, MD). L-FABP gene-ablated (−/−) C57BL/6 mice were generated by targeted disruption of the L-FABP gene through homologous recombination (12) and bred to generation 6. The Animal Care and Use Committee of Texas A&M University approved all animal protocols. Mice were housed individually in ventilated microisolator cages in a temperature-controlled (25°C) facility on a constant 12-h-light/-dark cycle and were allowed to consume food and water ad libitum. All mice were fed a commercial, standard low-fat (5% of energy from fat) pelleted nonpurified diet (**Supplemental Table 1**; Harlan Teklad Rodent Diet 8604). The current aging study was initiated using 30 age-matched (2 mo old) (−/−) female mice. Thirty age-matched (2 mo old) (+/+) C57BL/6 female cohorts were used as controls. Every 2 d, each mouse was weighed and the amount of food consumed by each mouse was measured (14). At age 3 mo, 15 (+/+) and 15 (−/−) mice were deprived of food overnight (12 h) and anesthetized (ketamine, 100 mg/kg; xylazine, 10 mg/kg). Blood was collected by cardiac puncture and processed to serum for storage at −80°C and subsequent lipid and protein analysis. After killing the mice, livers were removed and weighed prior to further processing (14). At the end of the study (age 18 mo), the remaining (+/+) and (−/−) mice (n = 12–13/genotype) were food-deprived overnight (12 h) and killed as described above. Blood and liver were collected and processed as described above. At the time of analysis, liver (0.1–0.2 g) was minced and homogenized (motor-driven Teflon pestle) on ice in 0.4–0.5 mL of PBS (pH 7.4) with protease inhibitor cocktail.

Whole-body phenotype analysis. We analyzed whole-body phenotype longitudinally throughout the current study in mice at age 2, 3, 6, 9, and 18 mo by dual-energy X-ray absorptiometry utilizing a Lunar PIXImus densitometer to determine fat tissue mass (FTM) and bone-free lean tissue mass (LTM) according to a previously published procedure (31). Prior to PIXImus analysis, the mouse was anesthetized by an intraperitoneal injection of a mixture of ketamine and xylazine (0.01 mL/g body weight; 10 g/L ketamine and 1 g/L xylazine in 0.9% saline solution). Following the procedure, the mouse was injected with yohimbine (0.11 μg/g body weight) to facilitate recovery. The mouse was injected with warm saline solution for rehydration, kept warm during recovery with heat pads to minimize heat loss, and the mouse was checked every 30 min until recovery was complete. Determination of body composition was performed by exposing the entire mouse, minus the head region, to sequential beams of low- and high-energy X-rays with an image taken of the X-rays impacting a luminescent panel. Separation of bone mass from soft tissue mass was accomplished by measuring the ratios of signal attenuation at the different energy levels. We further separated soft tissue mass into lean and FTM to provide accurate values of body composition. Instrument calibration was performed utilizing a phantom mouse of known bone mineral density and FTM, followed by correlation to chemical extraction techniques.

Lipid analysis. Lipid, protein, and RNA data were generated from age-matched cohorts of female mice at age 3 and 18 mo. Lipids were extracted from liver homogenates (5 mg protein) and analyzed as described (12,16). We quantified serum lipids and apolipoproteins using commercially available kits: total cholesterol, Wako no. 276–64909; free cholesterol, Wako no. 274–47109; nonesterified fatty acid, Wako no. 994–75409; triacylglycerol, Wako no. 998–40391/no. 994–40491; phospholipid, Wako no. 990–54009; apoA1, Wako no. 991–27201; and apoB, Wako no. 993–27401 (Wako Diagnostics). Serum cholesteryl ester was determined by subtracting free cholesterol from total cholesterol.

Western blotting analysis. Protein expression levels in liver homogenates were measured by Western blotting of equivalent amounts of protein loaded in each lane (15,16), quantified by densitometry (14,31), and expressed in arbitrary units relative to that in 3-mo-old (+/+) mice defined as 1.0. For each Western blot, we used the following procedure to control for potential blot-to-blot variability. Aliquots of each 3-mo-old (+/+) liver homogenate containing equivalent amounts of total protein were pooled. The pooled sample was gently mixed, divided into 50-μL aliquots, and stored at −80°C for subsequent SDS-PAGE/Western blot analysis. An aliquot of the pooled 3-mo-old (+/+) homogenate that contained an amount of total protein equivalent to that of the individual liver homogenate samples to be examined by SDS-PAGE and subsequent Western blotting was removed and loaded onto each gel. After color development and densitometry, the integrated density value of the pooled 3-mo old (+/+) sample was defined as 1.0; the integrated density values of the individual liver homogenate samples on the blot of interest were normalized to this value.

Mouse liver mRNA quantification. Mouse liver mRNA levels were quantified as previously described (32). The amount of mRNA detected by RT-PCR was expressed in arbitrary units, with that present in the samples from (+/+) mice defined as 1.0.

Statistics. The current investigation used cohorts of female mice in which all (−/−) mice were of the n = 6 generation. We used repeated-

measures ANOVA to analyze body weight, food intake, and fat and LTM. Tukey's post hoc test was conducted when the interaction was significant. Data from the separate groups of 3- and 18-mo-old mice were analyzed by 2-way ANOVA (genotype × age). Tukey's post hoc test was used to compare age within genotype or genotype within age when the interaction was significant (GraphPad Prism version 3.02). Data are expressed as means ± SEM. Differences with $P < 0.05$ were considered significant. Graphical analysis was accomplished using SigmaPlot 2002 for Windows version 8.02 (SPSS).

Results

Mouse body weight. The initial body weight of 2-mo-old L-FABP (−/−) mice did not differ from that of L-FABP wild-type cohorts (**Fig. 1***A*). By 9 mo of age, weight differences were significant ($P < 0.05$) and L-FABP (−/−) mice were 4 g heavier than the wild-type cohorts by 18 mo (Fig. 1*A*). There were no obvious differences in physical activity by subjective observation and food consumption did not differ between (+/+) and (−/−) mice at any age examined (Fig. 1*B*).

Body FTM did not differ between 2-mo-old (−/−) and (+/+) mice (Fig. 1*C*). At 2 mo, (+/+) mice had 13.1 ± 0.6% of total tissue mass (TTM) as FTM, which did not differ from (−/−) mice at that time that had 14.3 ± 0.7% of TTM as FTM. In contrast, at 18 mo, FTM in (−/−) mice was 58% greater than in (+/+) mice (Fig. 1*C*). The amount of body weight gained as LTM did not differ significantly throughout the study as a result of genotype (Fig. 1*D*). As a result, the amount of FTM as a percentage of TTM increased to 31.5 ± 0.9% in (−/−) mice by the end of the aging study (18 mo), which was higher ($P < 0.05$) than the amount of FTM as a percentage of TTM measured in (+/+) mice (22.2 ± 0.7%).

Hepatic lipid concentrations. L-FABP gene ablation significantly increased the concentration of hepatic cholesterol, cholesteryl ester, and phospholipid in female mice (**Table 1**). For primarily neutral storage lipids, hepatic triacylglycerol declined whereas cholesteryl ester increased in old mice (Table 1). L-FABP gene ablation did not further alter triacylglycerol concentration; however, L-FABP gene ablation resulted in an increased cholesteryl ester concentration in both young and old mice. Finally, LCFA increased with genotype or age in mice (Table 1).

Serum lipid concentrations. Serum cholesterol, cholesteryl ester, phospholipid, and triacylglycerol were not altered in 18-mo-old (+/+) mice compared with 3-mo-old (+/+) mice; however, the serum concentration of nonesterified fatty acids was lower in the older (+/+) than in the younger (+/+) mice (**Table 2**). L-FABP gene ablation alone resulted in a significant increase in serum triacylglycerol levels in 3-mo-old mice (Table 2). Serum cholesterol concentration was increased in 18 mo (−/−) mice (genotype × age); however, serum cholesteryl ester was decreased in these mice (Table 2). In summary, the greater obesity observed in older (−/−) mice was reflected in elevated serum total lipids, especially lipid classes typically enriched in VLDL (triacylglycerol, cholesterol).

Effects of L-FABP gene ablation and aging on proteins important in LCFA delivery to hepatocytes as esterified lipids in lipoproteins. Serum apoB (high in VLDL, LDL) levels increased in 3-mo-old (−/−) mice and in 18-mo-old (+/+) mice (**Table 3**). The hepatic apoB concentration was unaltered by L-FABP gene ablation in younger mice but was significantly increased in older (+/+) and (−/−) mice (Table 3). Serum and liver levels of apoA1 in these mice were unaffected by L-FABP gene ablation and/or aging (not shown). The hepatic SR-B1 was upregulated in young and old (−/−) mice (Table 3).

Effects of L-FABP gene ablation and aging on proteins that transport LCFA into hepatocytes, release LCFA from lipoproteins for transport into hepatocytes, and transport LCFA within hepatocytes. Albumin-bound LCFA is delivered to plasma membrane LCFA transporters (caveolin-1, FAT/CD36, FATP) for translocation into the cell. Whereas neither FAT/CD36 nor caveolin-1 was increased by L-FABP gene ablation or aging (not shown), FATP increased in (−/−) mice and increased with age regardless of genotype (Table 3). At the surface of hepatic endothelial cells, LPL also releases LCFA from lipoproteins (e.g. VLDL). As with FATP expression, LPL concentration was increased in (−/−) mice and increased with age regardless of genotype (Table 3). With regards to the intracellular LCFA and LCFA-CoA transport proteins, L-FABP gene ablation resulted in the complete absence of L-FABP but without concomitant upregulation of SCP-2 or acyl-CoA-binding protein (not shown).

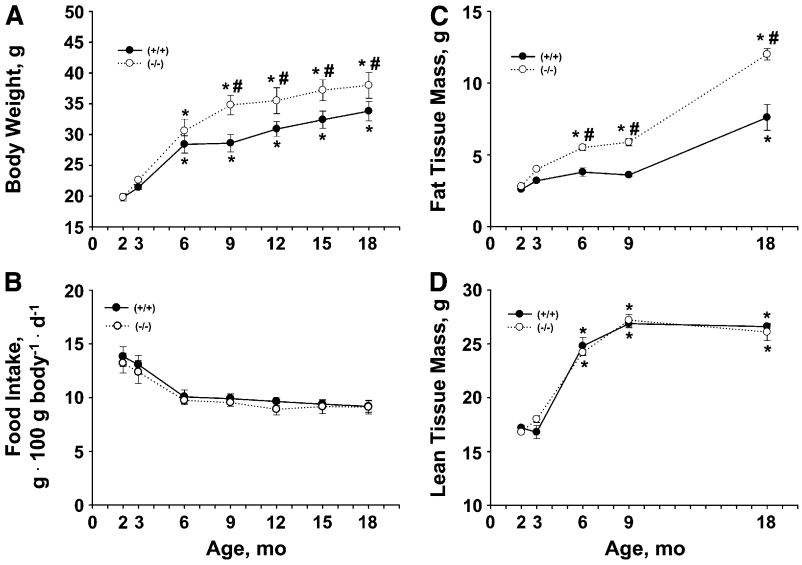

FIGURE 1 Body weight (*A*), food intake (*B*), FTM (*C*), and LTM (*D*) in L-FABP (−/−) vs. (+/+) female mice. Values are means ± SEM, $n = 10$. *Different from 2 mo old for that genotype, $P < 0.01$. #Different from (+/+) at that age, $P < 0.05$.

TABLE 1 Liver lipid concentrations in 3- and 18-mo-old (+/+) and (−/−) mice[1]

	3 mo		18 mo		2-Way ANOVA *P*-value		
Lipid	+/+	−/−	+/+	−/−	Genotype	Age	Genotype × age
			mmol/kg protein				
Cholesterol	8 ± 2	21 ± 2[c]	5 ± 1	14 ± 2[a]	<0.001	0.222	0.048
Cholesteryl ester	3 ± 1	14 ± 2[c]	13 ± 1[c]	19 ± 2[c]	<0.001	<0.001	<0.001
Total cholesterol	12 ± 2	34 ± 4[c]	18 ± 1[a]	32 ± 5[b]	<0.001	0.020	0.001
Phospholipid	75 ± 9	140 ± 10[c]	48 ± 3[a]	64 ± 4	<0.001	0.016	0.309
Nonesterified fatty acid	13 ± 1	17 ± 1[a]	25 ± 3[b]	27 ± 2[c]	0.010	0.001	<0.001
Triacylglycerol	155 ± 1	130 ± 8[b]	40 ± 7[c]	39 ± 5[c]	0.005	<0.001	<0.001

[1] Values are means ± SEM, $n = 12$ (3-mo-old mice) or 10 (18-mo-old mice). [a]Different from 3-mo old (+/+), $P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$.

Effects of L-FABP gene ablation and aging on liver proteins of LCFA anabolic metabolism: esterification and intracellular transport for secretion of esterified LCFA. The activity of GPAT, the rate-limiting enzyme in glyceride (phospholipid, triacylglycerol) synthesis, is age and L-FABP expression dependent. L-FABP gene ablation did not affect liver GPAT concentrations in young or old mice; however, there was a significant decrease in GPAT concentration in 18-mo (+/+) mice (**Table 4**). Aging did not affect the expression of MTP, a protein that transports triglycerides and cholesteryl esters in cells for secretion as VLDL (Table 4). In contrast, L-FABP gene ablation increased liver MTP concentrations in young and old mice (Table 4).

In terms of the acyl-CoA cholesterol acyltransferase enzymes, L-FABP gene ablation or aging had very little effect on the liver concentration of ACAT-1 (not shown). In contrast, ACAT-2 (predominant form of ACAT in liver) levels were age and L-FABP gene ablation dependent (Table 4). L-FABP gene ablation or aging resulted in increased hepatic ACAT-2 levels in mice (Table 4). At least 2 proteins compete with ACAT for cholesterol substrate by enhancing active transport of bile salts and cholesterol within the hepatocyte and across the hepatocyte plasma membranes: GST and 3α-HSD. Neither L-FABP gene ablation nor aging had any effect on GST or 3α-HSD levels in mice (not shown). L-FABP gene ablation and/or aging had no effect on the level of SCP-x, a key enzyme in oxidation of the branched side chain of cholesterol to form bile acids (not shown).

HMG-CoA synthase is involved in de novo synthesis of cholesterol (precursor of cholesteryl ester), but expression of this protein was not affected by age or L-FABP ablation (not shown). Age and/or L-FABP gene ablation also did not alter HMG-CoA reductase levels in females (not shown).

Effects of L-FABP gene ablation and aging on liver proteins important for LCFA catabolic metabolism: mitochondrial and peroxisomal oxidation. CPT I, a rate-limiting enzyme in mitochondrial LCFA oxidation, increased with age or L-FABP gene ablation in mice (Table 4). L-FABP gene ablation resulted in increased hepatic levels of mitochondrial 3-oxoacyl-CoA thiolase mRNA in 3- and 18-mo-old mice (Table 4). Butyryl-CoA dehydrogenase mRNA concentration significantly increased in older mice regardless of genotype (Table 4).

Effects of L-FABP gene ablation and aging on key nuclear receptors of LCFA and cholesterol metabolism. We examined the expression of certain key nuclear receptors involved in regulating transcription of enzymes/proteins participating in LCFA uptake, transport, esterification, and oxidation. Neither L-FABP gene ablation nor aging affected levels of the active 68-kDa nuclear form of sterol regulatory element binding protein-1 involved in cholesterol and fatty acid metabolism (not shown). PPARα, which regulates expression of proteins participating in LCFA transport into the cell, LCFA oxidation, and glucose metabolism, was increased by age regardless of genotype (Table 4). HNF1α and HNF4α regulate expression of multiple proteins in LCFA metabolism, including PPARα. HNF1α expression was increased by L-FABP gene ablation in young mice (Table 4), but levels of HNF4α were not affected in either young or old mice (not shown).

Discussion

L-FABP may regulate liver LCFA metabolism not only directly but also through nuclear transcription mechanisms, including enhanced fatty acid distribution to nuclei (24,25), interaction with PPARα in nuclei (25,26), and regulation of PPARα

TABLE 2 Serum lipid concentrations in 3- and 18-mo old (+/+) and (−/−) mice[1]

	3 mo		18 mo		2-Way ANOVA *P*-value		
Lipid	+/+	−/−	+/+	−/−	Genotype	Age	Genotype × age
			mmol/L				
Cholesterol	0.5 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	1.0 ± 0.1[b]	0.171	1.000	0.002
Cholesteryl ester	1.2 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	0.6 ± 0.1[c]	1.000	0.176	<0.001
Total cholesterol	1.7 ± 0.2	1.9 ± 0.2	1.4 ± 0.1	1.5 ± 0.1	0.487	0.222	0.411
Phospholipid	2.9 ± 0.2	2.7 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	0.487	0.176	0.095
Nonesterified fatty acid	1.6 ± 0.1	1.5 ± 0.1	1.0 ± 0.1[c]	1.0 ± 0.1[c]	0.487	<0.001	<0.001
Triacylglycerol	0.2 ± 0.1	1.7 ± 0.3[c]	0.3 ± 0.1	2.0 ± 0.2[c]	<0.001	0.491	<0.001

[1] Values are means ± SEM, $n = 12$ (3-mo-old mice) or 10 (18-mo-old mice). [a]Different from 3-mo old (+/+), $P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$.

TABLE 3 Serum apoB concentration and relative liver concentrations of key proteins important in hepatocyte LCFA transport in 3- and 18-mo old (+/+) and (−/−) mice[1]

	3 mo		18 mo		2-Way ANOVA *P*-value		
Protein	+/+	−/−	+/+	−/−	Genotype	Age	Genotype × age
Serum ApoB, $\mu mol\ L^{-1}$	2.2 ± 0.2	13.0 ± 0.8[c]	10.0 ± 0.9[c]	7.0 ± 0.8[c]	<0.001	<0.001	<0.001
Liver	*Fold of 3 mo (+/+)*						
ApoB	1.0 ± 0.1	1.0 ± 0.1	1.4 ± 0.1[b]	2.0 ± 0.1[c]	1.000	0.011	<0.001
SR-B1	1.0 ± 0.2	3.2 ± 0.4[c]	1.6 ± 0.5	3.5 ± 0.4[c]	<0.001	0.248	<0.001
FATP	1.0 ± 0.2	2.1 ± 0.2[b]	3.2 ± 0.2[c]	5.0 ± 0.3[c]	0.001	<0.001	<0.001
LPL	1.0 ± 0.1	1.5 ± 0.2[a]	2.2 ± 0.1[c]	4.0 ± 0.3[c]	0.036	<0.001	<0.001

[1] Values are means ± SEM, $n = 12$ (3-mo-old mice) or 10 (18-mo-old mice). [a]Different from 3-mo old (+/+), $P < 0.05$; [b]$P < 0.01$; [c] $P < 0.001$.

transcriptional activity of genes involved in LCFA uptake, oxidation, lipoprotein transport, and glucose metabolism (17,24–26). If, as suggested by in vitro and cultured cell studies, L-FABP contributes significantly to physiological regulation of PPARα, then L-FABP null mice should exhibit phenotypic similarities to PPARα null mice, which show age-dependent obesity without hepatic steatosis but with increased serum triacylglycerols (28,33). L-FABP (−/−) female male mice exhibit age-dependent obesity without hepatic steatosis, in a phenotype similar to, but less severe than, that observed in PPARα null mice (28,33). The data presented herein yielded several mechanistic insights into age-dependent obesity in L-FABP null mice.

First, obesity in older (−/−) mice was associated with select elevation in serum triacylglycerol and cholesterol and serum apoB, whereas serum cholesteryl ester decreased. This pattern of lipid and apo changes was consistent with, but does not prove, increased VLDL (review in 34,35) similar to that observed in older PPARα (−/−) female mice (28), suggesting that changes in L-FABP may regulate PPARα-related pathways.

Second, obesity in older (−/−) mice was associated with selective increases in liver cholesterol, cholesteryl ester, and phospholipid (but not triacylglycerol) as well as apoB. In contrast, older PPARα (−/−) female mice had not only higher liver cholesterol but also higher cholesteryl ester and triacylglycerol (28). These chemical analyses were consistent with lack of morphological evidence of overt steatosis in livers of older (−/−) female mice. Although steatosis was also not morphologically evident in livers of older PPARα (−/−) female mice, chemical analysis indicated that older female PPARα (−/−) mice exhibited increased liver lipid levels compared with older L-FABP (−/−) female mice, consistent with the fact that L-FABP is only one (26) of several proteins (review in 17–19) that activate PPARα transcriptional activity. The absence of overt steatosis in PPARα (−/−) female mice and in L-FABP (−/−) females suggests more efficient packaging and export of lipids from the livers of these mice.

Third, as shown by Western blotting and/or RT-PCR, the age-dependent lipid changes observed prominently in old obese (−/−) female mice were associated with increased expression of select liver proteins LPL and FATP involved in LCFA tissue release/uptake. The increased hepatic level of LPL is especially significant in view of LPL not being synthesized in liver, but rather in adipose and muscle tissue from where it must be transported to endothelial cells in liver as well as adipose, muscle, and other tissues. The finding of increased LPL and FATP in older obese L-FABP (−/−) females indicates increased LCFA hepatic uptake and suggests, but does not prove, an increased level of LPL in adipose tissue that could contribute to the adiposity noted in older obese (−/−) mice. Whether these proteins are elevated in older obese PPARα (−/−) females is not known.

Fourth, obesity in older (−/−) mice was associated with increased LCFA esterification (ACAT-2) and intracellular transfer/secretion of esterified LCFA (MTP, apoB). Interestingly, coordinate repression of MTP (MTP inhibitor 8aR) and L-FABP (gene ablation) in standard nonpurified diet-fed young mice resulted in decreased VLDL secretion without hepatosteatosis (36). However, L-FABP gene ablation alone increased MTP expression in female mice.

The age-dependent obesity in L-FABP (−/−) mice was unique compared with that of other known FABP-targeted mice,

TABLE 4 Relative liver concentrations of key proteins/mRNAs important in hepatocyte LCFA/cholesterol metabolism in 3- and 18-mo old (+/+) and (−/−) mice[1]

	3 mo		18 mo		2-Way ANOVA *P*-value		
Protein/mRNA	+/+	−/−	+/+	−/−	Genotype	Age	Genotype x age
	Fold of 3 mo (+/+)						
Protein GPAT	1.0 ± 0.1	1.1 ± 0.1	0.4 ± 0.1[c]	0.8 ± 0.1	0.487	<0.001	0.176
MTP	1.0 ± 0.1	1.9 ± 0.2[b]	1.0 ± 0.1	1.9 ± 0.2[b]	0.001	1.000	0.001
ACAT-2	1.0 ± 0.1	1.4 ± 0.1[a]	1.7 ± 0.1[c]	2.1 ± 0.1[c]	0.010	<0.001	<0.001
CPT I	1.0 ± 0.1	1.8 ± 0.1[c]	2.0 ± 0.1[c]	2.7 ± 0.2[c]	<0.001	<0.001	<0.001
PPARα	1.0 ± 0.1	1.3 ± 0.2	2.1 ± 0.1[c]	2.3 ± 0.3[c]	0.193	<0.001	<0.001
HNF1α	1.0 ± 0.1	1.3 ± 0.1[a]	0.7 ± 0.1[a]	1.1 ± 0.1	0.045	0.048	0.491
mRNA 3-Oxoacyl-CoA thiolase	1.0 ± 0.1	2.0 ± 0.4[a]	1.2 ± 0.1	2.8 ± 0.4[c]	0.024	0.176	<0.001
Butyryl-CoA dehydrogenase	1.0 ± 0.2	2.1 ± 0.5	2.0 ± 0.2[b]	4.0 ± 0.2[c]	0.053	0.002	<0.001

[1] Values are means ± SEM, $n = 12$ (3-mo-old mice) or 10 (18-mo-old mice). [a]Different from 3-mo old (+/+), $P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$.

including adipocyte FABP (−/−) mice, which exhibited increased body weight gain compared with their wild-type counterparts only when fed a high-fat diet (37); intestinal FABP (−/−) mice, which displayed no differences in body weight gain when fed either a control diet or a high-fat, high-cholesterol diet compared with their wild-type counterparts (38); and epidermal FABP (−/−) mice, which were phenotypically normal (39).

Ultimately, the increased body weight and FTM observed in L-FABP (−/−) female mice must be due to an energy imbalance in these mice. This energy imbalance can arise from increased food intake, change in metabolic rate, and/or differences in feed efficiency. Although (+/+) and (−/−) mice did not differ in food intake or physical activity, these conclusions were based on subjective observations and need to be examined in more detail.

In summary, the net effect of the lipid and protein changes in older obese (−/−) mice was to shift the balance of hepatic LCFA metabolism toward a net increase in LCFA uptake (LPL, FATP), esterification (ACAT-2, GPAT), transport/secretion as VLDL (MTP, apoB), and utilization by adipose tissue for storage. This phenotype shared many, but not all, attributes with those reported for PPARα (−/−) mice, supporting the view that L-FABP and PPARα influence some common pathways that regulate lipid metabolism. The data reported herein support a working model in which loss of L-FABP results in obesity without obvious steatosis. The absence of steatosis could result from more efficient packaging and export of lipids from the liver. Because food consumption was equivalent in wild-type and L-FABP gene-ablated mice, obesity development in the knockout mice must have resulted from shifts toward a reduced energy expenditure and/or more efficient energy uptake in the gut.

Literature Cited

1. McArthur MJ, Atshaves BP, Frolov A, Foxworth WD, Kier AB, Schroeder F. Cellular uptake and intracellular trafficking of long chain fatty acids. J Lipid Res. 1999;40:1371–83.
2. Frolov A, Cho TH, Murphy EJ, Schroeder F. Isoforms of rat liver fatty acid binding protein differ in structure and affinity for fatty acids and fatty acyl CoAs. Biochemistry. 1997;36:6545–55.
3. Murphy EJ, Prows DR, Jefferson JR, Schroeder F. Liver fatty acid binding protein expression in transfected fibroblasts stimulates fatty acid uptake and metabolism. Biochim Biophys Acta. 1996;1301:191–8.
4. Murphy EJ. L-FABP and I-FABP expression increase NBD-stearate uptake and cytoplasmic diffusion in L-cells. Am J Physiol. 1998;275: G244–9.
5. Weisiger RA. Cytosolic fatty acid binding proteins catalyze two distinct steps in intracellular transport of their ligands. Mol Cell Biochem. 2002; 239:35–43.
6. Schroeder F, Jolly CA, Cho TH, Frolov AA. Fatty acid binding protein isoforms: structure and function. Chem Phys Lipids. 1998;92:1–25.
7. Woldegiorgis G, Bremer J, Shrago E. Substrate inhibition of carnitine palmitoyltransferase by palmitoyl-CoA and activation by phospholipids and proteins. Biochim Biophys Acta. 1985;837:135–40.
8. Bhuiyan AKMJ, Pande SV. Carnitine palmitoyltransferase activities: effects of serum albumin, acyl-CoA binding protein and fatty acid binding protein. Mol Cell Biochem. 1994;139:109–16.
9. Atshaves BP, Storey SM, Petrescu AD, Greenberg CC, Lyuksyutova OI, Smith R, Schroeder F. Expression of fatty acid binding proteins inhibits lipid accumulation and alters toxicity in L-cell fibroblasts. Am J Physiol. 2002;283:C688–703.
10. Atshaves BP, Storey S, Huang H, Schroeder F. Liver fatty acid binding protein expression enhances branched-chain fatty acid metabolism. Mol Cell Biochem. 2004;259:115–29.
11. Atshaves BP, McIntosh AL, Lyuksyutova OI, Zipfel WR, Webb WW, Schroeder F. Liver fatty acid binding protein gene ablation inhibits branched-chain fatty acid metabolism in cultured primary hepatocytes. J Biol Chem. 2004;279:30954–65.
12. Martin GG, Danneberg H, Kumar LS, Atshaves BP, Erol E, Bader M, Schroeder F, Binas B. Decreased liver fatty acid binding capacity and altered liver lipid distribution in mice lacking the liver fatty acid binding protein (L-FABP) gene. J Biol Chem. 2003;278:21429–38.
13. Martin GG, Huang H, Atshaves BP, Binas B, Schroeder F. Ablation of the liver fatty acid binding protein gene decreases fatty acyl CoA binding capacity and alters fatty acyl CoA pool distribution in mouse liver. Biochemistry. 2003;42:11520–32.
14. Atshaves BP, McIntosh AL, Payne HR, Mackie J, Kier AB, Schroeder F. Effect of branched-chain fatty acid on lipid dynamics in mice lacking liver fatty acid binding protein gene. Am J Physiol. 2005;288:C543–58.
15. Martin GG, Atshaves BP, McIntosh AL, Mackie JT, Kier AB, Schroeder F. Liver fatty acid binding protein (L-FABP) gene ablation alters liver bile acid metabolism in male mice. Biochem J. 2005;391:549–60.
16. Martin GG, Atshaves BP, McIntosh AL, Mackie JT, Kier AB, Schroeder F. Liver fatty acid binding protein (L-FABP) gene ablation potentiates hepatic cholesterol accumulation in cholesterol-fed female mice. Am J Physiol Gastrointest Liver Physiol. 2006;290:G36–48.
17. Adida A, Spener F. Intracellular lipid binding proteins and nuclear receptors inolved in branched-chain fatty acid signaling. Prostaglandins Leukot Essent Fatty Acids. 2002;67:91–8.
18. Desvergne B, Michalik L, Wahli W. Be fit or be sick: peroxisome proliferator-activated receptors are down the road. Mol Endocrinol. 2004;18:1321–32.
19. Frederiksen KS, Wulf EM, Wassermann K, Sauerberg P, Fleckner J. Identification of hepatic transcriptional changes in insulin-resistant rats treated with peroxisome proliferator activated receptor-alpha agonists. J Mol Endocrinol. 2003;30:317–29.
20. Lin Q, Ruuska SE, Shaw NS, Dong D, Noy N. Ligand selectivity of the peroxisome proliferator-activated receptor α. Biochemistry. 1999;38:185–90.
21. Hostetler HA, Petrescu AD, Kier AB, Schroeder F. Peroxisome proliferator activated receptor alpha (PPARalpha) interacts with high affinity and is conformationally responsive to endogenous ligands. J Biol Chem. 2005;280:18667–82.
22. Hostetler HA, Kier AB, Schroeder F. Very-long-chain and branched-chain fatty acyl CoAs are high affinity ligands for the peroxisome proliferator-activated receptor alpha (PPARalpha). Biochemistry. 2006;45:7669–81.
23. Lawrence JW, Kroll DJ, Eacho PI. Ligand dependent interaction of hepatic fatty acid binding protein with the nucleus. J Lipid Res. 2000;41:1390–401.
24. Huang H, Starodub O, McIntosh A, Kier AB, Schroeder F. Liver fatty acid binding protein targets fatty acids to the nucleus: real-time confocal and multiphoton fluorescence imaging in living cells. J Biol Chem. 2002;277:29139–51.
25. Huang H, Starodub O, McIntosh A, Atshaves BP, Woldegiorgis G, Kier AB, Schroeder F. Liver fatty acid binding protein colocalizes with peroxisome proliferator receptor alpha and enhances ligand distribution to nuclei of living cells. Biochemistry. 2004;43:2484–500.
26. Wolfrum C, Borrmann CM, Borchers T, Spener F. Fatty acids and hypolipidemic drugs regulate PPARalpha and PPARgamma gene expression via L-FABP: a signaling path to the nucleus. Proc Natl Acad Sci USA. 2001;98:2323–8.
27. Escher P, Wahli W. Peroxisome proliferator activated receptors: insights into multiple cellular functions. Mutat Res. 2000;448:121–38.
28. Costet P, Legendre C, More J, Edgar A, Galtier P, Pineau T. PPARalpha deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. J Biol Chem. 1998;273:29577–85.
29. Kersten S. Peroxisome proliferator activated receptors and obesity. Eur J Pharmacol. 2002;440:223–34.
30. Atshaves BP, Petrescu A, Starodub O, Roths J, Kier AB, Schroeder F. Expression and intracellular processing of the 58 kDa sterol carrier protein 2/3-oxoacyl-CoA thiolase in transfected mouse L-cell fibroblasts. J Lipid Res. 1999;40:610–22.
31. Atshaves BP, Payne HR, McIntosh AL, Tichy SE, Russell D, Kier AB, Schroeder F. Sexually dimorphic metabolism of branched chain lipids in C57BL/6J mice. J Lipid Res. 2004;45:812–30.
32. Atshaves BP, McIntosh AL, Landrock D, Payne HR, Mackie J, Maeda N, Ball JM, Kier AB, Schroeder F. Effect of SCP-x gene ablation on branched-chain fatty acid metabolism. Am J Physiol Gastronintest Liver Physiol. 2007;292:G939–51.
33. Kersten S, Seydoux J, Peters JM, Gonzalex FJ, Desvergne B, Wahli W. PPARalpha mediates the adaptive response to fasting. J Clin Invest. 1999;103:1489–98.

34. Fielding PE, Fielding CJ. Dynamics of lipoprotein transport in the circulatory system. In: Vance DE, Vance J, editors. Biochemistry of lipids, lipoproteins, and membranes. New York: Elsevier; 1991. p. 427–59.
35. Fielding PE, Fielding CJ. Dynamics of lipoprotein transport in the human circulatory system. In: Vance DE, Vance J, editors. Biochemistry of lipids, lipoproteins, and membranes. New York: Elsevier; 2002. p. 527–52.
36. Spann NJ, Kang S, Li AC, Chen AZ, Newberry EP, Davidson NO, Hui STY, Davis RA. Coordinate transcriptional repression of liver fatty acid binding protein and microsomal triglyceride transfer protein blocks hepatic VLDL secretion without hepatosteatosis. J Biol Chem. 2006; 281:33066–77.
37. Hotamisligil GS, Johnson RS, Distel RJ, Ellis RF, Papaioannou VE, Spiegelman BM. Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. Science. 1996;274:1377–9.
38. Vassileva G, Huwyler L, Poirer K, Agellon LB, Toth MJ. The intestinal fatty acid binding protein is not essential for dietary fat absorption in mice. FASEB J. 2000;14:2040–6.
39. Owada Y, Suzuki I, Noda T, Kondo H. Analysis on the phenotype of E-FABP gene knockout mice. Mol Cell Biochem. 2002;239:83–6.