

Effects of Diet and Genetic Background on Sterol Regulatory Element–Binding Protein-1c, Stearoyl-CoA Desaturase 1, and the Development of the Metabolic Syndrome

Sudha B. Biddinger,^{1,2} Katrine Almind,¹ Makoto Miyazaki,³ Efi Kokkotou,¹ James M. Ntambi,^{3,4} and C. Ronald Kahn¹

Both environmental and genetic factors play important roles in the development of the metabolic syndrome. To elucidate how these factors interact under normal conditions, C57Bl/6 (B6) and 129S6/SvEvTac (129) mice were placed on a low-fat or high-fat diet. Over 18 weeks, the 129 strain developed features of the metabolic syndrome, notably obesity, hyperinsulinemia, and glucose intolerance only on the high-fat diet; the B6 strain on the other hand developed these features on both diets. High-fat feeding of both strains led to decreased serum triglycerides, hepatic steatosis, and hypercholesterolemia; however, B6 mice developed worse steatosis and a larger increase in LDL cholesterol. Both B6 background and high-fat feeding increased sterol regulatory element–binding protein-1c (SREBP-1c), a key regulator of lipogenic gene transcription, and its downstream targets. Stearoyl-CoA desaturase 1 (SCD1), an enzyme that regulates monounsaturated fatty acid (MUFA) synthesis, was also increased at the mRNA and enzyme activity levels by both high-fat feeding and B6 background. Furthermore, lipid analysis revealed increased hepatic triglycerides and MUFAs in B6 and high-fat–fed mice. Thus, dietary fat and genetic background act through SREBP-1c and SCD1 to affect hepatic lipid metabolism contributing to the development of the metabolic syndrome. *Diabetes* 54:1314–1323, 2005

From the ¹Research Division, Joslin Diabetes Center, and Department of Medicine, Harvard Medical School, Boston, Massachusetts; the ²Division of Endocrinology, Children's Hospital, Boston, Massachusetts; the ³Department of Biochemistry, University of Wisconsin, Madison, Wisconsin; and the ⁴Department of Nutritional Sciences, University of Wisconsin, Madison, Wisconsin.

Address correspondence and reprint requests to C. Ronald Kahn, Research Division, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215. E-mail: c.ronald.kahn@joslin.harvard.edu.

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FPLC, fast-performance liquid chromatography; HFD, high-fat diet; LFD, low-fat diet; MUFA, monounsaturated fatty acid; PGC, peroxisome proliferator–activated receptor- γ coactivator; SCD, stearoyl-CoA desaturase; SOCS, suppressor of cytokine signaling; SREBP, sterol regulatory element–binding protein.

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The metabolic syndrome is a constellation of findings, including central obesity, insulin resistance, dyslipidemia, hypertension, and hepatic steatosis, which predispose to diabetes, cardiovascular disease, and cancer. As the prevalence of diabetes, obesity, and the metabolic syndrome reach staggering proportions, much attention has been focused on their etiologies and the relationship among them (1,2). Although both genetic and environmental factors clearly play a role, exactly how these factors interact to produce the metabolic syndrome and its various components remains unclear.

In most humans, insulin resistance appears to be polygenic and heterogeneous (3). Thus, there are multiple genes that potentially contribute to the phenotype, and the development of disease in any individual may involve only a specific subset of these genes that varies from population to population. High-risk populations predisposed to the development of obesity and insulin resistance, such as Pima Indians and Mexican Americans, are thought to be enriched for clusters of genes acting together to produce the metabolic syndrome in the context of an appropriate environmental trigger, such as the high-fat western diet.

Dysregulation of hepatic lipid metabolism may play a central role in the pathogenesis of the metabolic syndrome. McGarry (7) has proposed that increased synthesis of lipids by the liver produces insulin resistance in other tissues, such as muscle. Increased storage of lipids in the liver results in fatty changes that are now known to be a feature of the metabolic syndrome (8). These changes form a spectrum of pathology, labeled nonalcoholic fatty liver disease, ranging from simple benign steatosis to nonalcoholic steatohepatitis, which can progress to cirrhosis and liver failure (9). It is thought that nonalcoholic fatty liver disease may now be the most common cause of cryptogenic cirrhosis in this country (10). Patients with the metabolic syndrome also typically have increased triglycerides and decreased HDL (11). Dyslipidemia is closely tied to the cardiovascular morbidities associated with the metabolic syndrome and may be attributed, at least in part, to the aberrant handling of lipids by the liver (12).

To understand how genes interact with dietary fat to

produce the changes in lipid metabolism that occur in the metabolic syndrome, we used two strains of mice, representing differences in susceptibility to the development of insulin resistance. C57Bl/6 (B6) mice have previously been shown to develop diabetes when subjected to genetically induced insulin resistance due to a double heterozygous deletion of one insulin receptor allele and one insulin receptor substrate-1 allele (13,14). 129Sv (129) mice on the other hand are protected from diabetes when carrying the same insulin receptor/insulin receptor substrate-1 double heterozygous defect. In the present study, B6 and 129 mice were placed on two extremes of diet: a low-fat diet (LFD; 14% calories from fat) and a high-fat diet (HFD; 55% calories from fat). We have compared the effects of genetic and dietary factors not only on glucose, but also on serum and hepatic lipid profiles and hepatic lipogenic gene expression, to better understand how these factors alter lipid metabolism and to identify the key elements controlling the progression of the metabolic syndrome.

RESEARCH DESIGN AND METHODS

Six-week-old male C57Bl/6 and 129S6/SvEvTac (Taconic) mice were placed on a low-fat high-carbohydrate (NIH#31; Taconic) or high-fat low-carbohydrate diet (TD93075; Harlan Teklad). The LFD derives 14% calories from fat, 25% calories from protein, and 61% calories from carbohydrates and was found to contain 1.5% saturated fatty acids, 2.7% monounsaturated fatty acids (MUFAs), and 0.6% polyunsaturated fatty acids by weight. The HFD derives 55% calories from fat, 21% calories protein, and 24% calories from carbohydrates and was found to contain 4.2% saturated fatty acids, 5.0% MUFAs, and 11.2% polyunsaturated fatty acids. The LFD and HFD had 15.4 and 7.1 mg cholesterol per 100 g, respectively. Arachidonic acid was undetectable in both diets. The mice were maintained on a 12-h light-dark cycle; unless otherwise indicated, serum samples were taken and mice were killed between 9:00 and 11:00 A.M., in the nonfasted state at ~6 months of age. Insulin levels were measured in plasma samples of random fed mice using the Crystal Chem ELISA kit and mouse insulin standards. Three independent cohorts were used to perform these experiments.

Serum lipid analysis. Equal volumes of serum from three to four mice fasted for 6 h (beginning in the morning) were pooled. Cholesterol and triglycerides were measured using Sigma kits 352 and 339, adapted for microtiter plates. Additionally, the serum was subjected to fast-performance liquid chromatography (FPLC) as previously described (15), and cholesterol was measured in the eluted fractions. Serum lipid analysis was performed by the Lipid, Lipoprotein and Atherosclerosis Core of the Vanderbilt Mouse Metabolic Phenotyping Centers.

Immunohistochemistry. Livers from the dead animals were frozen in liquid nitrogen, embedded in an optimal temperature cutting compound, and cut into 6- μ m sections. Hematoxylin/eosin and Oil-Red-O staining was performed using standard techniques.

Hepatic lipid analysis. Hepatic lipid analysis was performed by the Lipid, Lipoprotein and Atherosclerosis Core of the Vanderbilt Mouse Metabolic Phenotyping Centers. Lipids were extracted, filtered, and recovered in the chloroform phase. Individual lipid classes were separated by thin-layer chromatography using Silica Gel 60 A plates and visualized by rhodamine 6G. Phospholipids, triglycerides, and cholesterol esters were scraped, methylated, and analyzed by gas chromatography (16,17).

Oligonucleotide microarrays. Total RNA (25 μ g) was pooled from two to three animals to make cRNA as described previously (18). cRNA (15 μ g) was hybridized on Affymetrix murine chips U74Av.2, with four chips representing each group. Data were analyzed using MAS v5, with each chip being normalized to an average intensity of 1,500.

Real-time PCR. Total RNA was extracted and purified using the RNeasy kit (Qiagen) and used to direct cDNA synthesis using the RT for PCR kit (Clontech). RT-PCR was performed using SYBR green master mix (ABI), 5% of the cDNA synthesis reaction, and 300 nmol/l of the relevant primers. Sterol regulatory element-binding protein (SREBP)-1c and SREBP-1a primers were isoform specific and have been previously described (19). Other primers were as follows: suppressor of cytokine signaling (SOCS)-3, 5'-CCTCGGGGACCATAGGAG-3' and 5'-AACTTGCTGTGGGTGACCAT-3'; SREBP-2, 5'-GCGTTCTGGAGACCATGGA-3' and 5'-ACAAAGTTGCTCTGAAAACAAATCA-3'; peroxisome proliferator-activated receptor- γ coactivator (PGC)-1 α , 5'-GTCAACAGCAAAGCCACA-3' and 5'-TCTGGGGTCAGAGGAAGAGAG-3'; and PGC-1 β , 5'-CCCTGT

CCGTGAGGAACG-3' and 5'-ATCCATGGCTTCGTACTTGC-3'. The primers were found to amplify linearly. Because common housekeeping genes such as TATA-binding protein and the ribosomal protein 36B4 varied between strains, expression was normalized to the input RNA and calculated as a function of 2^{-Ct} .

Immunoblotting of SREBP-1. Nuclear protein extracts of mouse liver were prepared as described by Sheng et al. (20). For each condition, equal portions of two to three mouse livers were pooled to produce nuclear extracts; these experiments were done in duplicate or triplicate. Immunoblotting was performed per the Amersham ECL detection system kit protocol, except that the washing solutions were supplemented with 0.1% SDS (wt/vol), 1% (vol/vol) Nonidet P-40, and 0.5% (wt/vol) sodium deoxycholate. Antibodies against mouse SREBP-1 have been previously described (21).

Stearoyl-CoA desaturase enzymatic activity. Conversion of [1- 14 C]stearoyl-CoA to [1- 14 C]oleate was used to measure stearoyl-CoA desaturase (SCD) enzyme activity from microsomes prepared from individual liver extracts as previously described (22).

Immunoblotting of SOCS-3. Approximately 100 mg frozen liver from 16-week-old male mice, fed an HFD for 6 weeks, was homogenized in 25 mmol/l Tris 7.4, 2 mmol/l Na_2VO_4 , 10 mmol/l NaF, 10 mmol/l $\text{Na}_4\text{P}_2\text{O}_7$, 1 mmol/l EGTA, 1 mmol/l EDTA, 1% NP40, and one protease inhibitor tablet (Complete Protease Inhibitor tablets; Roche) in 50 ml and subjected to ultracentrifugation at 50,000 rpm for 45 min in a TLA100.2 rotor. Protein concentration was determined using a Bradford Assay (Bio-Rad). Protein (75 μ g) was subjected to SDS-PAGE, and immunoblotting was performed using a Roche Chemiluminescence Kit with antibodies against SOCS-3 (Santa Cruz).

Statistical analysis. Statistically significant effects of diet and strain were identified using a two-way ANOVA model with interaction. Normality of the data was assessed by the histogram and normal probability plot of residuals of the ANOVA model. Data exhibiting significant departure from normality were transformed in logarithmic scale and refit. In each ANOVA model, the significance of the interaction term was assessed first, and, if the interaction was not statistically significant ($P < 0.05$), the significance of main effects was assessed. The analyses were carried out using the GLM procedure in SAS version 8.2. Unless otherwise indicated, data are presented as the mean \pm SE.

RESULTS

Metabolic response to diet. Six-week-old male 129 and B6 mice were placed either on an LFD or HFD. After 18 weeks, 129 mice weighed 27% more (39 vs. 31 g) on the HFD compared with the LFD, whereas B6 mice weighed 11% more (46 vs. 41 g); however, B6 mice weighed more than 129 mice on either diet (Table 1). High-fat feeding increased liver weights dramatically in B6 (over 75%) but not 129 mice. Glucose homeostasis was impaired in 129 mice by high-fat feeding, with an increase in blood glucose from 92 mg/dl (129 LFD) to 190 mg/dl (129 HFD) during a glucose tolerance test. High-fat feeding of 129 mice also led to a 2.8-fold increase in insulin levels and a 9.4-fold increase in leptin levels. B6 mice on the other hand had no significant changes in either glucose, insulin, or leptin in response to high-fat feeding. Glucose tolerance test and longitudinal weight curves have been described previously (50).

However, B6 mice, in contrast to 129 mice, developed features of the metabolic syndrome even on the LFD. B6 LFD mice had body and liver weights similar to those found in 129 HFD mice. B6 mice on either diet also had higher blood glucose levels while fasting (~115 mg/dl in B6 mice vs. 62 mg/dl in 129 HFD mice) and during a glucose tolerance test (240 mg/dl in B6 mice vs. 190 mg/dl in 129 HFD mice), thus manifesting more severe glucose intolerance than even high-fat-fed 129 mice. Serum insulin was 19-fold higher and serum leptin was 14-fold higher in the B6 strain compared with the 129 strain on the LFD. Thus, high-fat feeding induces obesity and hepatomegaly in both 129 and B6 mice. The 129 mice also developed glucose intolerance, mild hyperinsulinemia, and hyperleptinemia on the HFD. B6 mice, on the other hand, were

TABLE 1
Phenotyping of mice

	129 LFD	129 HFD	B6 LFD	B6 HFD	Significance
Weight (g)					
0 weeks	24.8 ± 1.0	24.6 ± 1.7	23.5 ± 1.7	22.8 ± 1.6	*
18 weeks	30.9 ± 2.5	39.2 ± 4.3	41.5 ± 4.1	46.4 ± 3.2	**, ††
Liver weight (mg)/body weight (g)	32 ± 5	34 ± 2	39 ± 4	69 ± 15	**, ††, ‡‡
Serum glucose (mg/dl)					
Fasting	52 ± 10.6	62 ± 7.6	115 ± 23	117 ± 17	**
Glucose tolerance test	92 ± 8.7	190 ± 24	240 ± 59	239 ± 77	**, †, ‡
Insulin (ng/ml)	1.3 ± 0.6	3.6 ± 1.2	24.8 ± 35	32.7 ± 35	**, †
Leptin (ng/ml)	5.0 ± 1.5	47 ± 17	72 ± 23	82 ± 21	**, ††, ‡‡

Data are means ± SD for four to eight mice. Male mice were placed on an LFD or HFD for 18 weeks, after which weight, liver weight, glucose, insulin, and leptin were measured. Blood glucose was measured after an overnight fast, both before (fasting) and 2 h after a 2-g/kg bolus of glucose i.p. (glucose tolerance test). Significance is indicated in the last column, where **P* ≤ 0.02 for strain effect, †*P* ≤ 0.02 for diet effect, and ‡*P* ≤ 0.02 for their interaction. Double symbols indicate *P* < 0.002.

heavier, more glucose intolerant, hyperinsulinemic, and hyperleptinemic on either diet.

High-fat feeding of both 129 and B6 mice led to significant increases in serum cholesterol (Fig. 1A). FPLC analysis revealed that the excess cholesterol was associated

with the HDL fractions in both strains but that B6 mice also had an increase in LDL cholesterol. Even on the LFD, B6 mice had higher levels of LDL cholesterol than 129 mice, although their total serum cholesterol was slightly lower.

Compared with 129 mice, B6 mice had lower triglycer-

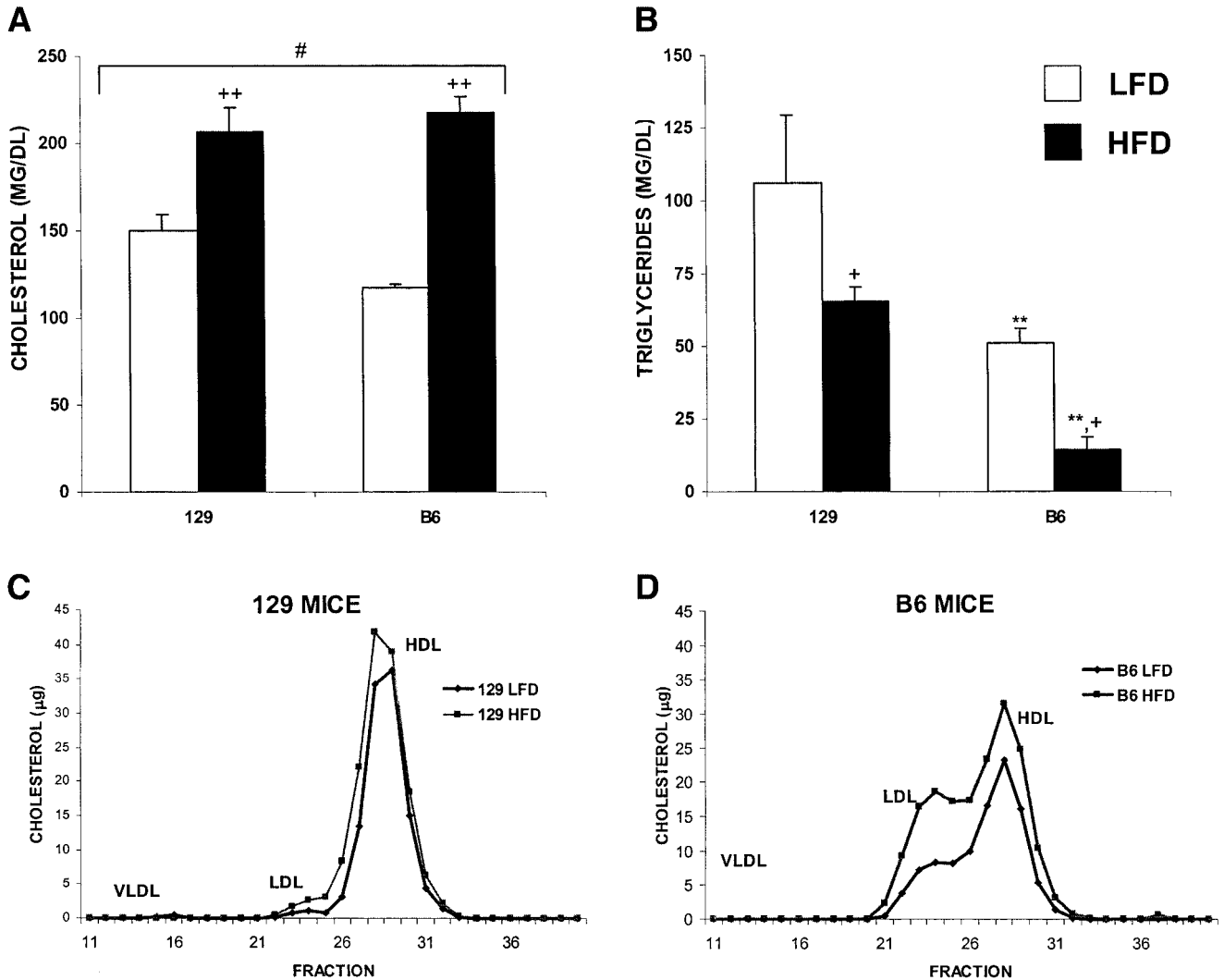


FIG. 1. High-fat feeding leads to hypercholesterolemia but decreased serum triglycerides. Serum from 129 and B6 mice on an HFD or LFD for 16 weeks was taken after a 6-h fast. Cholesterol (A) and triglyceride (B) content was measured; serum was also subjected to FPLC, and the cholesterol content of the eluted fractions was measured (C and D) (*n* = 3–4). **P* < 0.05 for strain effect; +*P* < 0.05 for diet effect; #*P* < 0.05 for their interaction. Double symbols indicate *P* < 0.005.

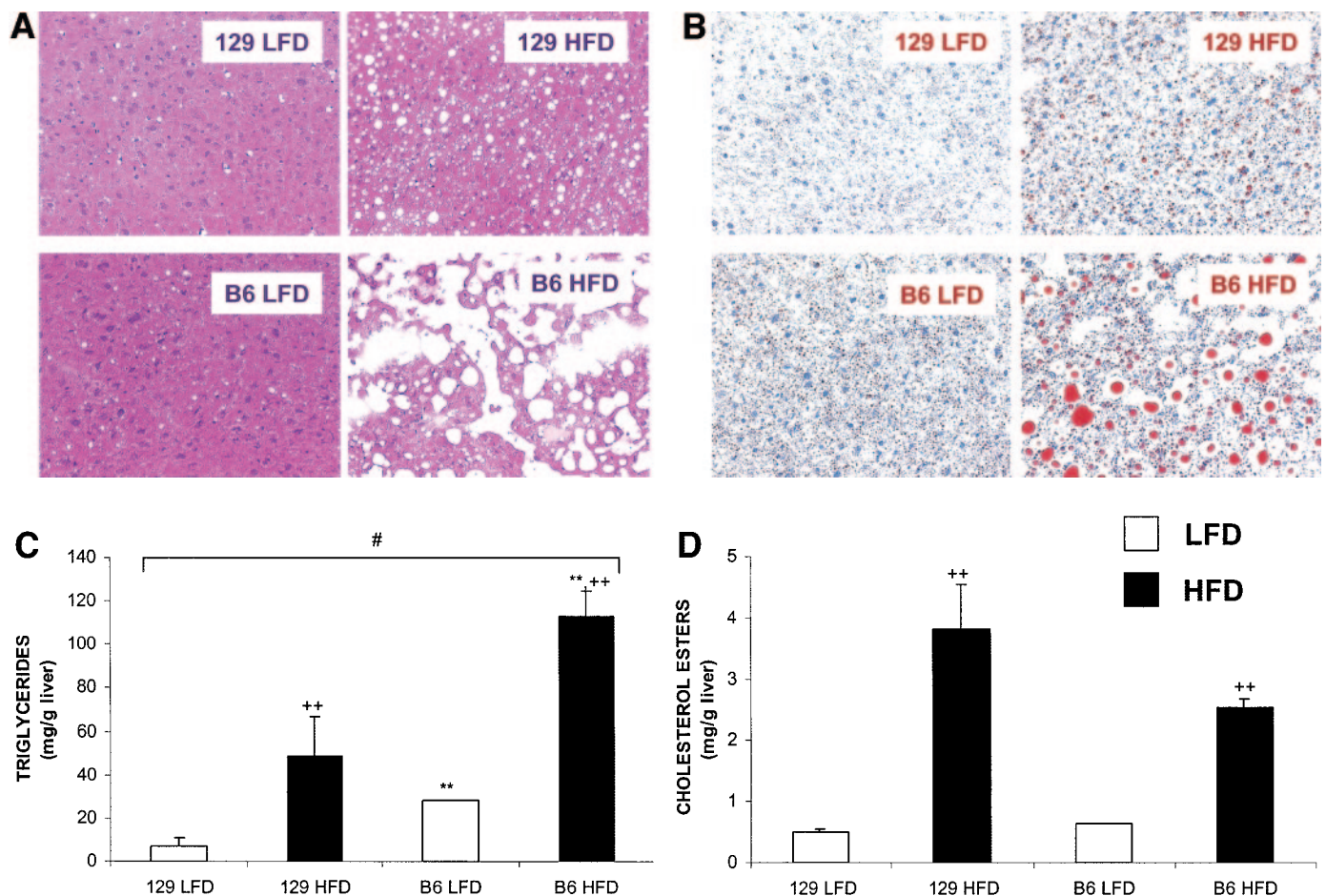


FIG. 2. High-fat feeding leads to steatosis. Livers were stained with hematoxylin and eosin (A) or Oil-Red-O (B). Representative sections are shown (original magnification $\times 200$). Alternatively, hepatic lipids were extracted and triglyceride (C) and cholesterol ester (D) content was measured. * $P \leq 0.01$ for strain effect; + $P \leq 0.01$ for diet effect; # $P \leq 0.01$ for their interaction. Double symbols indicate $P \leq 0.0001$.

ide levels on both diets, although the difference was not significant on the LFD (Fig. 1B). This is consistent with the increased triglyceride clearance that has been previously reported in B6 mice and thought to be due to increased serum lipase and liver CD36/fatty acid translocase (23,24). High-fat feeding lowered triglyceride levels in both strains, although the result was more pronounced in B6 mice. Circulating levels of free fatty acids were similar in B6 and 129 mice on either diet (data not shown).

Hepatic steatosis. It is now recognized that hepatic steatosis is an important component of the metabolic syndrome. Hematoxylin and eosin staining showed normal liver architecture in both 129 and B6 mice fed an LFD (Fig. 2A). On an HFD, 129 mice showed microvesicular fat accumulation, consistent with a mild degree of hepatic steatosis. B6 mice on the other hand showed more profound steatosis with macrovesicular fat accumulation. These changes were confirmed with Oil-Red-O staining (Fig. 2B). Thus, the presence of steatosis appeared to depend on diet, whereas genetic factors were important in determining the degree of steatosis.

Triglycerides and cholesterol esters are the major storage forms of lipids in liver. Hepatic triglyceride and cholesterol ester content were increased sevenfold in 129 mice and fourfold in B6 mice by high-fat feeding (Fig. 2C and D), consistent with the accumulation of lipid associ-

ated with high-fat feeding observed histologically. However, genetic background also had an impact on triglyceride accumulation because B6 mice accumulated approximately threefold more triglyceride than 129 mice on the same diet.

Hepatic lipid profiling. To further analyze the changes in hepatic lipid accumulation, the distribution of fatty acids in each of the major lipid fractions was performed using gas chromatography, yielding several insights (Table 1 of online appendix [available at <http://diabetes.diabetesjournals.org>]). First, in the phospholipid fraction, high-fat feeding led to a decrease in the relative contributions of several polyunsaturated fatty acids such as 18:2, 22:5, and 22:6, but not 20:4. Arachidonic acid (20:4) is a precursor of the prostaglandins and leukotrienes, which are potent mediators of inflammation (25). The relative abundance of arachidonic acid in the phospholipid fraction was increased 40% in both 129 and B6 mice by the HFD.

Another intriguing finding was the relative increase in MUFAs by both high-fat feeding and B6 background. This is depicted in Fig. 3 as the ratio of the monounsaturated (16:1 + 18:1) fatty acids to the saturated fatty acids (16:0 + 18:0). This ratio was increased by both high-fat feeding and B6 genetic background in the triglyceride and phospholipid fractions, although there was no significant change in the cholesterol ester fraction.

Lipogenic gene transcription. To identify the molecular

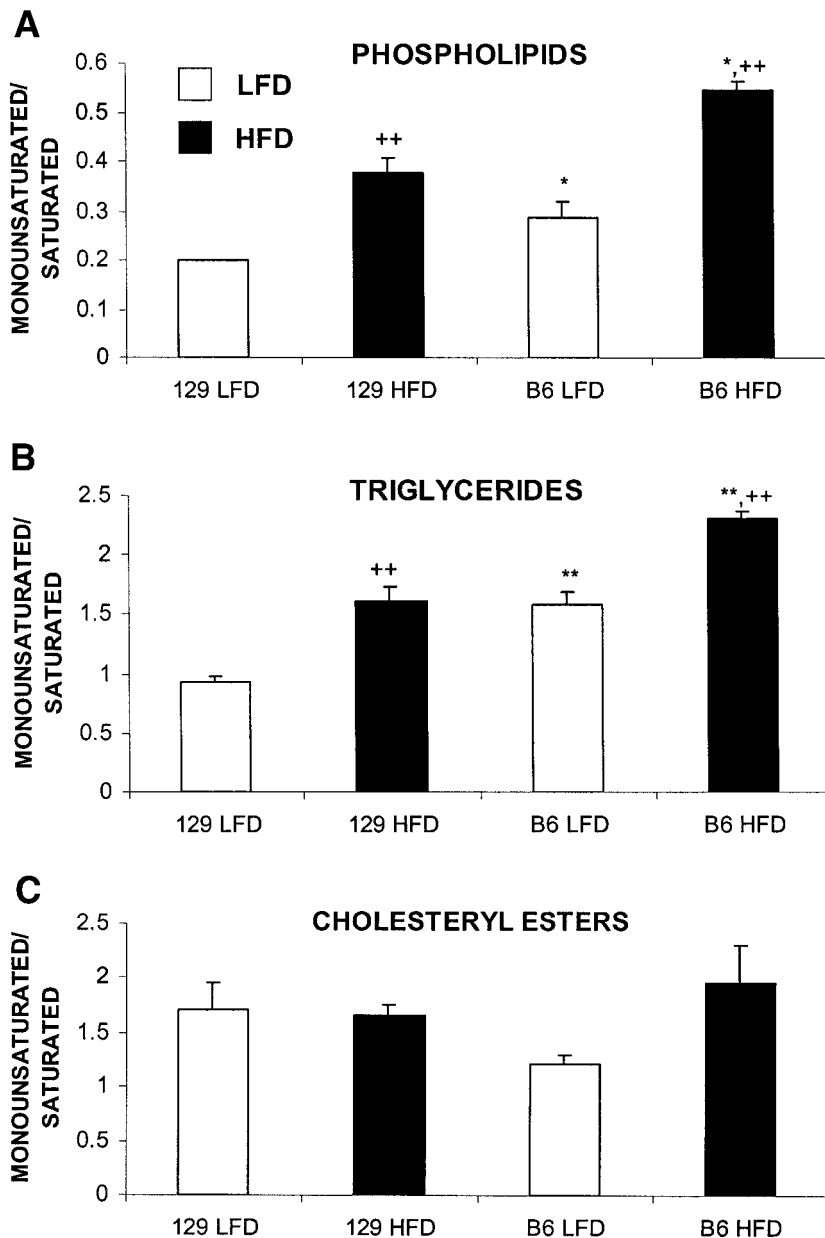


FIG. 3. B6 background and high-fat feeding are associated with an increased ratio of monounsaturated to saturated fatty acids. Hepatic lipids were extracted and analyzed by gas chromatography. The ratio of monounsaturated (16:1 + 18:1) to saturated (16:0 + 18:0) fatty acids in the major lipid fractions was calculated for each mouse ($n = 4$). * $P = 0.002$ for strain effect; + $P = 0.002$ for diet effect. Double symbols indicate $P < 0.0001$.

defects underlying the differences in the lipid profiles of these animals, we performed oligonucleotide microarray analysis. We found that both B6 genetic background and diet increased lipogenic gene expression (Fig. 4). The enzymes of fatty acid synthesis, fatty acid synthase, malic enzyme, and ATP citrate lyase were increased by the B6 background. Δ -5 fatty acid desaturase was increased by high-fat feeding. Long-chain fatty acyl elongase and glycerol 3-phosphate acyltransferase, which is involved in triglyceride synthesis, were increased by both B6 background and high-fat feeding. The B6 genetic background also increased lipogenic gene expression on a chow diet (Table 2 of online appendix).

Alterations in SREBP-1c associated with genetic background and diet. The SREBPs are a family of three nuclear transcription factors encoded by two genes (26). SREBP-1a and SREBP-1c are derived from the same gene, and both appear to regulate lipogenic gene transcription. SREBP-1c, however, is the dominant isoform in liver.

SREBP-1a and SREBP-2 regulate the genes of cholesterol synthesis (26). Using quantitative real-time PCR, we found that both diet and B6 background increased SREBP-1c mRNA (Fig. 5A). SREBP-1a did not change significantly between the four groups (Fig. 5C), whereas SREBP-2 was increased by high-fat feeding (Fig. 5D).

SREBP-1c is subject to complex regulation at the transcriptional and posttranslational levels (26). Its transcript encodes a membrane-bound precursor, which must be proteolytically cleaved in the Golgi to release a soluble fragment that can translocate into the nucleus to activate transcription. Western blots of the nuclear extracts showed that, relative to 129 LFD mice, 129 HFD mice and B6 mice on either diet had increased SREBP-1 (Fig. 5B). Thus, SREBP-1 nuclear protein levels paralleled SREBP-1c mRNA.

Changes in SCD1 with genetic background and diet. The increase in monounsaturated fatty acid content with diet and genetic background seen in Fig. 3 suggests that there might also be changes in SCD1. Stearoyl-CoA desatu-

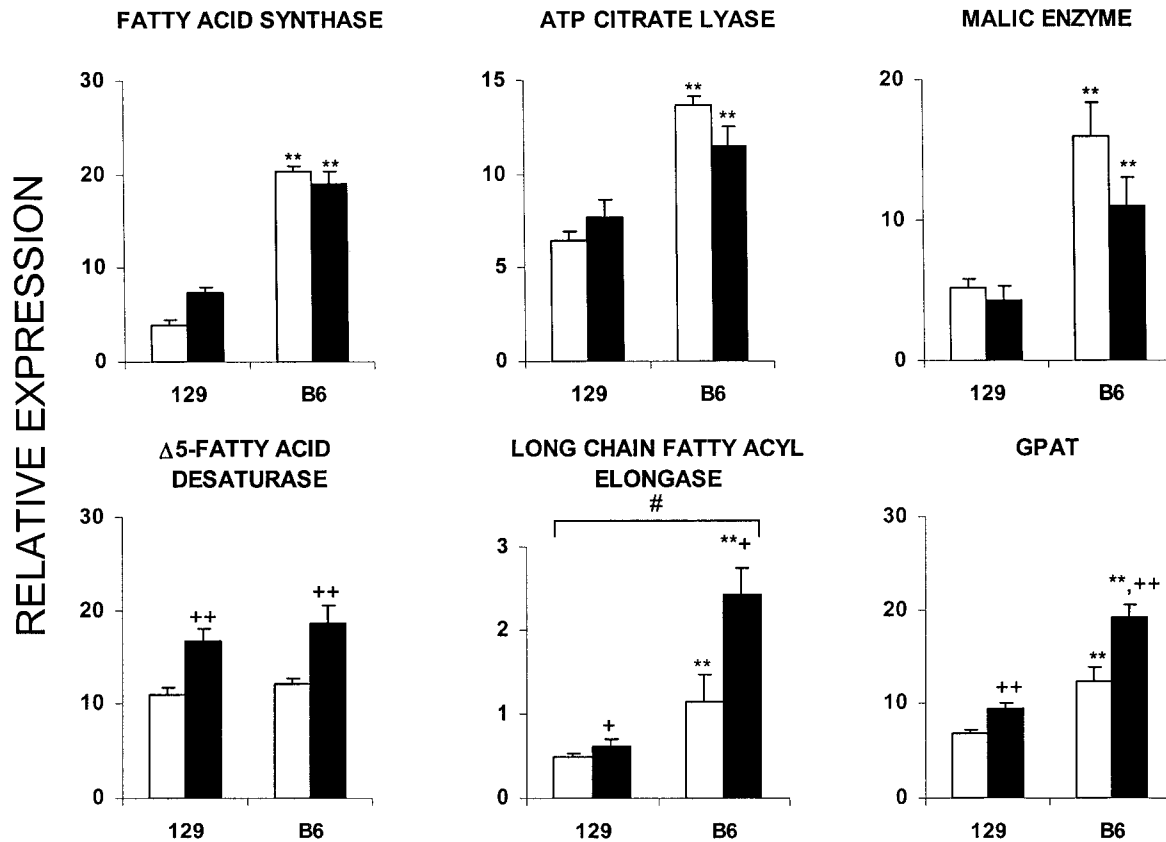


FIG. 4. B6 background and HFD are associated with increased lipogenic gene expression. Microarray analysis was used to determine the changes in hepatic lipogenic gene expression ($n = 4$ Mu74Av2 chips per group, with each chip representing one to two mice). * $P < 0.05$ for strain effect; + $P < 0.05$ for diet effect; # $P < 0.05$ for their interaction. Double symbols indicate $P < 0.005$. □, LFD; ■, HFD. GPAT, glycerol-3- PO_4 acyltransferase.

rases are the rate-determining enzymes in the synthesis of MUFAs, introducing a double bond at the Δ -9 position converting palmitate (16:0) to palmitoleate (16:1) and stearate (18:0) to oleate (18:1) (11). There are four known isoforms of SCD in mice, with SCD1 being the dominant form in liver. Because SCD1 is a target of SREBP-1c, one might expect changes in SCD1 expression (27). As shown in Fig. 6A, SCD1 mRNA was increased 3-fold by high-fat feeding of 129 mice and 1.5-fold by high-fat feeding of B6 mice. There was also a dramatic difference between strains, with B6 LFD mice having three to four times more SCD1 transcript than 129 LFD mice.

To determine if these changes in SCD1 mRNA were reflected in SCD activity, we prepared microsomes from the livers of these animals and measured the production of oleate from stearate (Fig. 6B). We found that there was a progressive increase in SCD activity from 0.2 to 0.4 $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein by high-fat feeding in 129 mice and from 1.1 to 1.6 $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ by high-fat feeding in B6 mice. Thus, high-fat feeding and B6 background exert additive effects on SCD1 activity.

Other candidate genes. PGC-1 α and PGC-1 β are transcriptional coactivators that are important in directing energy metabolism. They coordinate the liver's response to fasting by activating numerous processes including gluconeogenesis, mitochondrial biogenesis, and fatty acid oxidation (28,29). Using real-time PCR, we found that high-fat feeding increases PGC-1 α by approximately two-fold in the B6 strain (Fig. 7A). However, high-fat feeding

had no effect on PGC-1 α mRNA in the 129 strain. There were no significant effects of diet or strain on PGC-1 β (Fig. 7B).

Insulin and leptin resistance are known to increase SOCS-1 and SOCS-3 (30,31). Moreover, we have previously shown that increases in SOCS proteins can induce SREBP-1c transcription (32). Real-time PCR and Western blotting revealed that SOCS-3, like PGC-1 α , was increased by high-fat feeding in B6 mice, but lower in B6 LFD mice compared with 129 LFD mice, despite the fact that B6 mice are more insulin resistant (Fig. 7C and D). No differences were observed in SOCS-1 mRNA (data not shown).

DISCUSSION

Dysregulation of lipid metabolism is a central feature of the metabolic syndrome and is intimately associated with the development of hepatic steatosis, dyslipidemia, and insulin resistance. The pathogenesis of the metabolic syndrome is poorly understood but clearly involves both genetic and environmental factors. In this study, we have taken advantage of the natural genetic variation between two "normal" laboratory mouse strains: the B6 strain, which is prone to the development of obesity and insulin resistance, and the 129 strain, which is not. Subjecting these strains to LFDs and HFDs produces a spectrum of pathology, ranging from the normal 129 LFD mice to the severely impaired B6 HFD mice, providing an opportunity to observe the effects of genetic strain and diet on the metabolic syndrome.

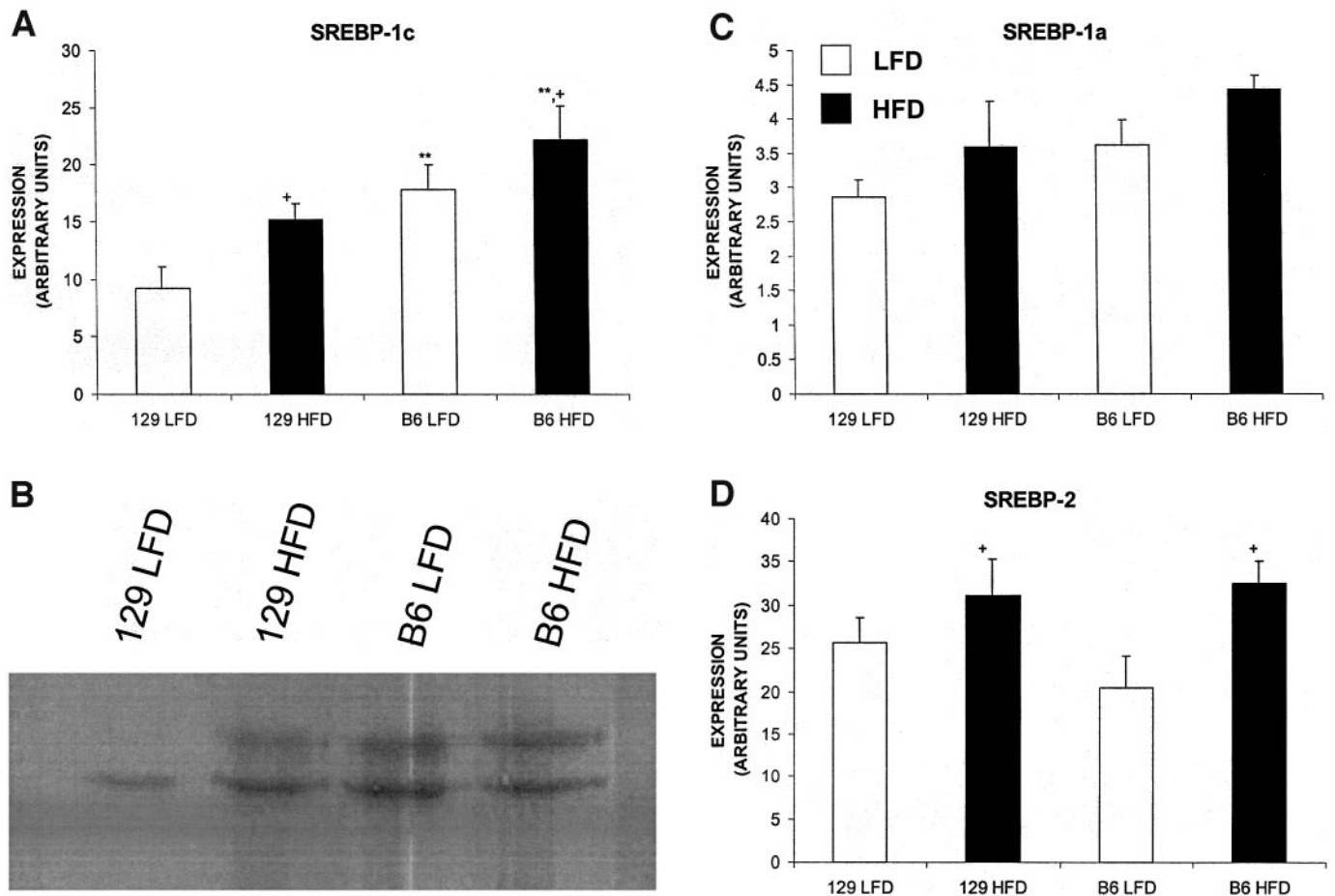


FIG. 5. The effects of genetic background and high-fat feeding on the SREBPs. Quantitative real-time PCR ($n = 4-8$) was performed using primers specific for the SREBP-1c (A), SREBP-1a (C), or SREBP-2 (D). $*P \leq 0.02$ for strain effect; $+P \leq 0.02$ for diet effect. Double symbols indicate $P < 0.002$. No significant differences were found between SREBP-1a levels. **B:** Nuclear SREBP-1 is also increased by high-fat feeding or B6 background. Hepatic nuclear extracts were prepared from the mice described in Table 1, and 30 μg was subjected to immunoblotting with an antibody that recognizes both SREBP-1 isoforms.

In certain respects, both 129 and B6 strains respond similarly to high-fat feeding. On the HFD, both gain more weight and develop hypercholesterolemia, a reduction in serum triglycerides, and an increase in hepatic lipid accumulation. They both accrue triglycerides and phospholipids enriched in monounsaturated fatty acids. We also find that both strains of mice have a relative increase in phospholipid-associated arachidonate (20:4) when exposed to an HFD. Because arachidonate is the precursor for prostaglandins and leukotrienes, which are potent mediators of inflammation, it is tempting to speculate that its increased abundance contributes to the proinflammatory state associated with the metabolic syndrome (33,34). Finally, both strains respond to high-fat feeding with an increase in glycerol-3- PO_4 acyltransferase and long-chain fatty acyl elongase, a finding also noted in other strains of mice (35).

Despite similarities in their response to high-fat feeding, dramatic differences exist between the two strains. B6 mice are more obese, glucose intolerant, hyperinsulinemic, and hyperleptinemic than 129 mice on either diet. Whereas both strains develop hypercholesterolemia in response to high-fat feeding, the excess in serum cholesterol is associated with HDL particles in 129 HFD mice, but both LDL and HDL particles in B6 HFD mice. B6 mice also

have a higher content of liver triglycerides and an increased abundance of MUFAs in the triglyceride fraction. The expression of fatty acid synthase, malic enzyme, and citrate lyase was also higher in B6 mice compared with 129 mice.

These changes in lipogenic gene expression and MUFA content found with transcriptional and lipid profiling suggested that there might be changes in SREBP-1c, a transcription factor capable of activating transcription of all the enzymes required for MUFA synthesis, and SCD1, which catalyzes the rate-determining step in the production of MUFAs. Indeed, both SREBP-1c mRNA and nuclear protein and SCD1 mRNA and activity are increased by high-fat feeding and the B6 genetic background. Our findings are somewhat in contrast to those of Kakuma et al. (38) that Sprague-Dawley rats fed an HFD for 6 weeks have an 80% suppression of SCD1 transcript. Whether this represents a difference in species or the duration of high-fat feeding is unknown, but clearly the effect of prolonged high-fat feeding in our study is to increase both mRNA and activity of this enzyme, and this is consistent with the increased MUFA content of the triglyceride and phospholipid fractions.

The importance of SREBP-1c in regulating lipid metabolism is illustrated by transgenic mice expressing a trun-

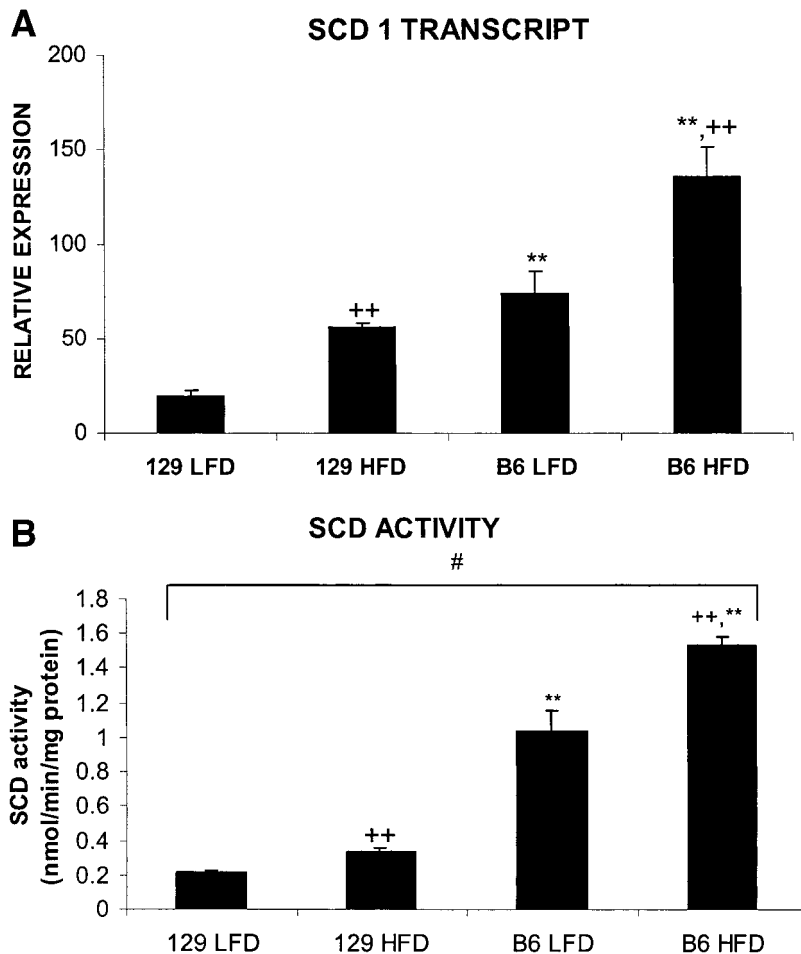


FIG. 6. SCD transcript and activity is increased by HFD and B6 genetic background. Expression of the SCD1 transcript (A) was quantitated using oligonucleotide microarrays, whereas SCD activity (B) was measured as the production of oleate from stearate by liver microsomes prepared from the mice described in Table 1. $n = 4$ chips or 4 mice in Fig. 6A and B, respectively. * $P \leq 0.02$ for strain effect; + $P \leq 0.02$ for diet effect; # $P \leq 0.02$ for their interaction. Double symbols indicate $P < 0.002$.

cated constitutively active form of SREBP-1c. These mice have increased transcription of all the enzymes needed for the synthesis of unsaturated fatty acids (27), increased hepatic lipid synthesis, particularly MUFAs, and hepatic steatosis (39). Additionally, SREBP-1c transgenic mice, like B6 mice, have decreased serum triglycerides; this is thought to be due to the fourfold induction of lipoprotein lipase transcription by SREBP-1c resulting in increased triglyceride clearance (39). Conversely, *ob/ob* mice with a superimposed knockout of SREBP-1 do not develop the massive steatosis characteristic of *ob/ob* mice. Thus, SREBP-1 appears to be necessary and sufficient for the development of steatosis.

SCD1 activation also appears to be required for the development of hepatic steatosis, since *ob/ob* mice with a knockout of SCD1 do not develop steatosis (22,40). Interestingly, *ob/ob* mice with a knockout of SCD1 also have decreased obesity and increased energy expenditure compared with *ob/ob* mice (22). Similarly, SCD1 knockout mice without leptin deficiency exhibit increased insulin sensitivity, energy expenditure, and fatty acid oxidation and are resistant to diet-induced obesity compared with wild-type controls (41). These data are consistent with a global role for SCD1 in regulating energy metabolism.

The dramatic increases in SREBP-1c and SCD1 observed in leptin-deficient *ob/ob* mice and lipoatrophic mice (22,43,44) are thought to mediate, at least in part, the hepatic steatosis and insulin resistance found in these models. Our data suggest that even more modest changes

in SREBP-1c and SCD1 resulting from differences in genetic background or diet may produce similar effects, and account for some of the phenotypic differences between the B6 and other strains. For example, B6 mice, with relatively high levels of SREBP-1c and SCD1 have been shown in this study and others to have a genetic predisposition toward steatosis and low serum triglycerides. Thus, the A-ZIP/F-1 mutation causes lipoatrophy and severe hyperinsulinemia on both B6 and FVB/N backgrounds (24). However, lipoatrophic B6 mice have more marked steatosis and lower serum triglycerides than lipoatrophic FVB/N mice. Similarly, the *ob/ob* mutation also leads to worsened steatosis but decreased serum triglycerides on the B6 background compared with the FVB/N background (23). Conversely, 129 mice, with relatively low levels of SCD1, are somewhat similar to the SCD1 knockout mouse. They have previously been shown to gain less weight than B6 mice while eating more and being less active (50). The metabolic rate of 129 mice, measured by O_2 consumption, is 6 and 14% higher than that of B6 mice on the LFD and HFD, respectively (50).

The mechanism by which SREBP-1c and SCD1 are activated by the B6 genetic background is not clear. Neither SREBP-1c nor SCD1 is near the quantitative trait loci thus far associated with insulin resistance in these strains. Because insulin is a known positive regulator of both genes, it is possible that changes in SREBP-1c and SCD1 are secondary to high serum insulin levels. In fact, B6 mice have fivefold higher insulin levels than 129 mice.

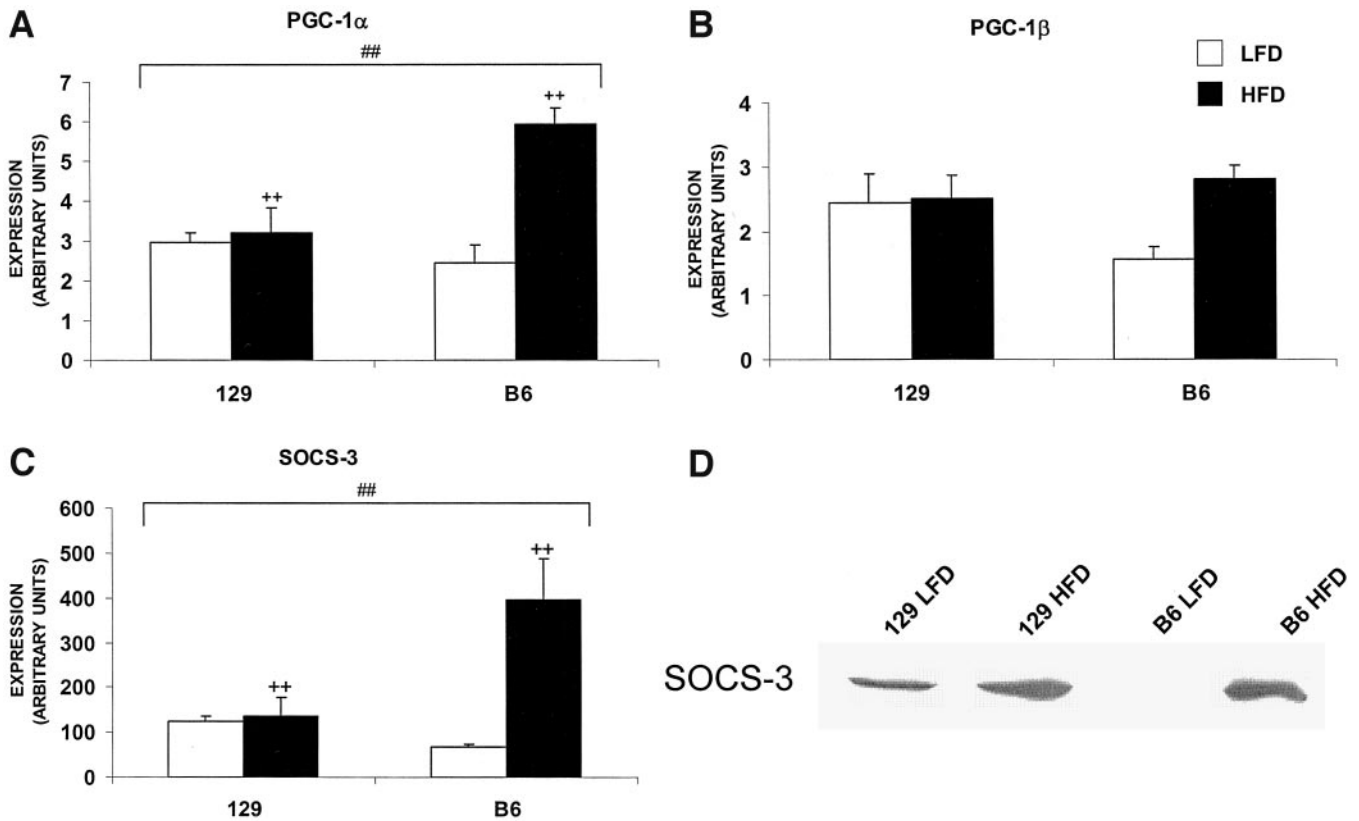


FIG. 7. *A–C*: PGC-1 α and SOCS-3 are increased in B6 mice by HFD. Quantitative RT-PCR for PGC-1 α (*A*), PGC-1 β (*B*), and SOCS-3 (*C*) was performed ($n = 4–8$). $+P \leq 0.02$ for diet effect; $\#P \leq 0.02$ for the interaction of strain and diet. Double symbols indicate $P < 0.002$. *D*: Liver homogenate (75 μ g) from 16-week-old mice fed an HFD for 6 weeks was subjected to immunoblotting with antibodies against SOCS-3.

This may be due to differences in insulin secretion by the islets, since other studies have shown that B6 mice have increased insulin secretion in vivo in response to glucose and arginine compared with 129 mice (45). Furthermore, islets isolated from B6 mice have increased insulin content and sensitivity to glucose compared with those isolated from 129 mice (45).

In summary, we have characterized a model of the metabolic syndrome in which genetic heterogeneity and high-fat feeding interact to produce obesity, insulin resistance, hepatic steatosis, and hypercholesterolemia. Using a combination of transcriptional and lipid profiling, we have identified two key regulators that, at least in part, appear to mediate these changes: SREBP-1c and SCD1. SREBP-1c can be activated by either diet or strain, whereas there is a synergistic effect of diet and strain on SCD1. We propose that these genes are on a common final pathway in the progression of the metabolic syndrome and represent important targets for therapeutic intervention.

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