Hepatic steatosis is common in non-insulin-dependent diabetes and can be associated with fibrosis and cirrhosis in a subset of individuals. Increased rates of fatty acid synthesis have been reported in livers from rodent models of diabetes and may contribute to the development of steatosis. Sterol regulatory element-binding proteins (SREBPs) are a family of regulated transcription factors that stimulate lipid synthesis in liver. In the current studies, we measured the content of SREBPs in livers from two mouse models of diabetes, obese ob/ob mice and transgenic aP2-SREBP-1c mice that overexpress nuclear SREBP-1c only in adipose tissue. The aP2-SREBP-1c mice exhibit a syndrome that resembles congenital generalized lipodystrophy in humans. Both lines of mice develop hyperinsulinemia, hyperglycemia, and hepatic steatosis. Nuclear SREBP-1c protein levels were significantly elevated in livers from ob/ob and aP2-SREBP-1c mice compared with wild-type mice. Increased nuclear SREBP-1c protein was associated with elevated mRNA levels for known SREBP target genes involved in fatty acid biosynthesis, which led to significantly higher rates of hepatic fatty acid synthesis in vivo. These studies suggest that increased levels of nuclear SREBP-1c contribute to the elevated rates of hepatic fatty acid synthesis that leads to steatosis in diabetic mice.

Non-insulin-dependent diabetes mellitus is a common disorder that affects approximately 5% of the population. Affected patients are usually obese, and they manifest insulin resistance, hyperinsulinemia, and hyperglycemia. As many as 40% of non-insulin-dependent diabetics develop evidence of hepatic steatosis or “fatty liver,” a condition that leads to hepatic fibrosis and cirrhosis in a subset of individuals (1). Hepatic fatty acid synthesis is increased in rodent models of hyperinsulinemia and likely contributes to the development of fatty livers (2, 3).

Sterol regulatory element-binding proteins (SREBPs) are a family of transcription factors that activate the entire program of cholesterol and fatty acid synthesis in liver (4, 5). SREBPs belong to the basic helix-loop-helix-leucine zipper family of transcription factors (4). Unlike other members of the basic helix-loop-helix-leucine zipper family, SREBPs are synthesized as ~150-amino acid precursors bound to the endoplasmic reticulum and nuclear envelope (4). To be active, the NH2-terminal segment must be released from the membrane by a sequential two-step cleavage process (6, 7). Following the second cleavage (site-2 cleavage), the ~500-amino acid NH2-terminal segment of SREBP is released from the membrane and translocates to the nucleus, where it binds to enhancer regions of target genes to activate transcription.

To date, three SREBP isoforms have been identified and characterized (4, 8). SREBP-1a and -1c are derived from a single gene through the use of alternative transcription start sites that produce alternate forms of exon 1 (9). The third SREBP isoform, SREBP-2, is derived from a separate gene and is ~45% identical to SREBP-1a (10). In most cultured cell lines, the predominant SREBP-1 isoform is SREBP-1a (11). In contrast, most animal tissues, including liver, express SREBP-1c as the predominant SREBP-1 isoform (11). Multiple lines of evidence suggest that SREBP-1 and SREBP-2 have different relative effects on target genes. SREBP-1 is relatively selective in activating genes involved in fatty acid synthesis, while SREBP-2 preferentially activates genes involved in cholesterol biosynthesis (12–15).

To gain insight into the separate roles of each SREBP isoform in vivo, we previously produced and characterized transgenic mice that overexpress the truncated, transcriptionally active nuclear forms of human SREBP-1a, -1c, or -2 in liver (13, 15). Mice that overexpressed SREBP-1c, the predominant SREBP-1 isoform, had a 4-fold increase in the rate of fatty acid synthesis, as measured by the incorporation of [3H]water. Corresponding 2–6-fold increases were measured in mRNA levels for the lipogenic genes ATP citrate lyase, acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), glycerol-3-phosphate acetyltransferase (GPAT), malic enzyme, and glucose-6-phosphate dehydrogenase (Glu-6-PD) (13, 17). No changes were measured in the mRNAs for the cholesterol biosynthetic enzymes 3-hydroxy-3-methylglutaryl-CoA synthase, HMG-CoA reductase, and squalene synthase or in the in vivo rates of cholesterol synthesis (13, 15).

In contrast, mice that overexpressed nuclear SREBP-2 (nSREBP-2) had 28-fold higher rates of cholesterol synthesis in vivo, and corresponding 10–12-fold increases in mRNAs for several enzymes in the cholesterol biosynthetic pathway (15). The SREBP-2 transgenic mice also had 4-fold higher rates of fatty acid biosynthesis in vivo. This demonstrated that at high levels of expression, nSREBP-2 is capable of activating the enzyme cascade required for fatty acid biosynthesis, albeit much less efficiently than for that of cholesterol biosynthesis.

In the current studies, we demonstrate that the amount of nSREBP-1 is increased in fatty livers from two distinct animal
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RNA Analysis—For Northern gel analysis, equal aliquots of total RNA made from each mouse liver were pooled (total, 10 μg), denatured with formaldehyde and formamide, subjected to electrophoresis in a 1% agarose gel, and transferred to Hybond N+ membranes (Amersham Pharmacia Biotech) for hybridization. Hybridization conditions and cDNA probe preparation were carried out as described (15–17). As a loading control, a cDNA probe for mouse β-actin was prepared using reverse transcriptase-PCR and mouse liver poly(A) RNAs as a template as described previously (16). The PCR primers used were as follows: 5’ primer, 5′-CATTGGAACATGACATTCCAGCTTAGAGGACAC-3′; and 3’ primer, 5′-GCATCCTGTCTTCAATGTAGCCAACA-3′. Northern blot filters were exposed to a Fujifilm PhosphorImager, and the resulting bands were quantified using a Bio-Imaging Analyzer with BAS1000 Mac-BAS software (Fujin Medical Systems, Stamford, CT). The fold change for each mRNA was calculated after normalization by the signal generated by β-actin. The RNAse protection assay for SCID1 and SCID2 mRNA transcripts was carried out as described previously (17).

**Tissue Cholesterol and Fatty Acid Composition**—The relative hepatic fatty acid compositions in the indicated lipid fractions were measured as described previously (17).

### RESULTS

Table I shows the phenotypic characteristics of the mice used in the current studies. In experiment A, we studied male ob/ob mice on the C57BL/6J background and age-matched wild-type C57BL/6J male mice as controls. In experiment B, we studied aP2-SREBP-1c mice that overexpress human nSREBP-1c exclusively in adipose tissue and sex-matched littermate wild-type controls. The aP2-SREBP-1c mice were derived from the C57BL/6J and SJL strains. The ob/ob and aP2-SREBP-1c mice had similar elevations in plasma glucose and insulin, indicating they had similar levels of insulin resistance and diabetes. Liver cholesterol was increased 2–3-fold in both diabetic models. Liver triglyceride content was markedly elevated in livers from ob/ob (10-fold) and aP2-SREBP-1c (16-fold) mice compared with wild-type levels. The two models differ in that the ob/ob mice had massive peripheral fat stores and higher plasma free fatty acid concentrations compared with their wild-type controls. Conversely, the aP2-SREBP-1c mice had markedly reduced peripheral fat stores with a slight reduction in plasma free fatty acid levels, compared with their littermate controls.

Fig. 1 shows immunoblots of SREBP-1 and -2 in nuclear extracts (N) and membranes (P) from livers of ob/ob mice, and aP2-SREBP-1c mice.
aP2-SREBP-1c mice, and their respective wild-type controls after a brief 1–2-h fast. The amount of transcriptionally active nSREBP-1 was 3–4-fold increased in livers from ob/ob (lane 2) and aP2-SREBP-1c (lane 6) mice compared with their respective wild-type controls (lanes 1 and 5). Consistent with previous experiments (11, 17, 25), the predominant SREBP-1 mRNA transcript present was SREBP-1c as determined by an RNase protection assay (data not shown). This suggests that the SREBP-1 isoform measured by immunoblot is predominantly the SREBP-1c isoform. In contrast to the increase in nSREBP-1c, the amount of nSREBP-2 showed very little change in livers from either diabetic model (lanes 4 and 8 versus lanes 3 and 7, respectively). Similar results were obtained in one additional independent experiment (data not shown).

To confirm that the changes measured in the mRNAs for the lipogenic enzymes resulted in increased rates of fatty acid synthesis in vivo, we used [3H]water to directly measure newly synthesized cholesterol and fatty acids in livers of wild-type and diabetic mice (Table II). The fatty acid synthetic rates were 6× higher per gram of liver and 23-fold higher per organ in ob/ob mouse. Livers from aP2-SREBP-1c mice had fatty acid synthetic rates that were 2.5- and 5.4-fold increased per gram and organ, respectively. In contrast, livers from ob/ob and aP2-SREBP-1c mice had 6- and 3-fold reductions in the rate of incorporation of [3H]water into digitonin-precipitable sterols per gram of liver, respectively. The lower cholesterol synthetic rates presumably reflect post-transcriptional suppression of HMG-CoA reductase activity owing to the increased level of hepatic cholesterol (26).

A major fatty acid modifying enzyme in liver is the ∆9 desaturase, also known as stearoyl-CoA desaturase (SCD). Two isoforms of SCD (SCD1 and SCD2) are currently known (27). The major substrates for both SCD isoforms are palmitic (16:0) and stearic (18:0) acids, which are converted, respectively, to palmitoleic (16:1) and oleic (18:1) acids, respectively (27). We have previously reported that the mRNA for SCD1 was increased in transgenic mice that overexpress nuclear SREBP-1a, -1c, or -2 in liver (17). The SCD1 isoform is the only SCD isoform mRNA found in wild-type liver (28). However, in livers from SREBP-1a and -2 transgenic mice, the mRNA for SCD2 was also expressed (17).

To determine whether SCD1 and/or SCD2 mRNA was elevated in livers of the two diabetic mouse models, we employed a RNase protection assay to detect the mRNA for each gene (Fig. 3). Lanes 1 and 2 show the protected bands for the SCD1 and SCD2 mRNA in 5 μg of total RNA from epididymal fat pads from wild-type mice. Inasmuch as both SCD1 and SCD2 mRNA transcripts are present in white fat, this mRNA was used as a positive control (28). Compared with their respective wild-type levels, the SCD1 mRNA was 3.5-fold higher in ob/ob mouse liver (lanes 3 and 4) and 2-fold higher in aP2-SREBP-1c mouse liver (lanes 7 and 8).
increased mRNA levels for SCD1 but not SCD2 (12, 13).

This result is consistent with previous observations that nSREBP-1c overexpression in liver and cultured cells results in increased mRNA levels for SCD1 but not SCD2 (12, 13).

To determine whether the increased SCD1 mRNA resulted in increased hepatic monounsaturated fatty acid content, we measured the relative fatty acid compositions of various lipid fractions from livers of the mice described in Table I. Table III shows the relative fatty acid compositions in total lipid extracts as well as the three major lipid classes after fractionation. Livers from ob/ob and aP2-SREBP-1c mice had similar 2–3-fold increases in the percentage of 16:1 and 18:1 in total lipid extracts. Therefore, approximately 50% of the total fatty acids in the livers from the diabetic mice are monounsaturated (primarily oleic acid). These differences are primarily a result of changes in fatty acid composition in the triglyceride and cholesterol ester fractions.

**DISCUSSION**

The purpose of the current studies was severalfold: 1) to determine whether the amounts of nSREBP-1c were altered in fatty livers from obese and lipodystrophic mouse models of non-insulin-dependent diabetes, 2) to correlate the changes in nSREBP-1c content with the relative mRNA levels of known lipogenic SREBP-1c target genes, and 3) to determine whether increased nSREBP-1c expression contributes to the development of fatty liver in both mouse models of diabetes.

Previous studies in fasted and refed mice suggested that nSREBP-1c was regulated in parallel with the amounts of mRNA encoding lipogenic enzymes (29). Fasted mice had markedly reduced levels of hepatic nSREBP-1c and nSREBP-2. Refeeding a high carbohydrate/low fat diet led to a 4-fold “overshoot” in the amount of nSREBP-1c compared with pre-fasted levels, while nSREBP-2 returned only to pre-fasted levels (29). The pattern of regulation of nSREBP-1c closely paralleled the changes in mRNAs for lipogenic genes, whereas the changes in nSREBP-2 protein paralleled the changes in mRNA levels for genes encoding enzymes of cholesterol synthesis (29). These studies suggested that nSREBP-1c contributed to the fasting and refeeding response that has been reported for lipogenic enzymes in liver (30). This response has been previously attributed to the direct effects of ingested glucose or the secondary effect of the elevated insulin that occurs in response to ingested glucose (30).

Inasmuch as lipogenesis is increased in livers from ob/ob mice (3), we hypothesized that increased levels of nSREBP-1c could contribute to this process. Indeed, we found markedly elevated levels of nSREBP-1c protein in livers from ob/ob mice. To address whether this observation is specific to ob/ob mice or whether it represents a more general consequence of hyperinsulinemia/hyperglycemia, we studied a phenotypically different model of insulin-resistant diabetes, the aP2-SREBP-1c transgenic mouse. These mice exhibited similar 3–4-fold elevations in hepatic nSREBP-1c. The increased nSREBP-1c levels in both models were associated with increased mRNAs for multiple lipogenic enzymes and increased rates of fatty acid synthesis and triglyceride accumulation in liver. The predominant fatty acid synthesized was oleic acid, presumably as a consequence of the increase in SCD1 mRNA. Further in vivo studies showed that the increased nSREBP-1c levels in both diabetic models were associated with increased mRNAs for multiple lipogenic enzymes and increased rates of fatty acid synthesis and triglyceride accumulation in liver.

**TABLE II**

| Genotype of mice | Liver weight | Digitonin-precipitable sterols | Fatty acids |
|------------------|-------------|-------------------------------|------------|
|                  | g | µmol/h/g | µmol/h/organ | µmol/h/g | µmol/h/organ |
| Wild-type        | 1.10 ± 0.04 | 1.0 ± 0.14 | 1.2 ± 0.19 | 6.9 ± 0.5 | 7.6 ± 0.7 |
| ob/ob            | 4.06 ± 0.13* | 0.15 ± 0.01* | 0.60 ± 0.04* | 43 ± 2.3* | 175 ± 12* |
| Wild-type        | 1.42 ± 0.07 | 0.73 ± 0.09 | 1.06 ± 0.12 | 11 ± 2.4 | 16 ± 3.2 |
| aP2-SREBP-1c     | 3.01 ± 0.22* | 0.24 ± 0.06* | 0.69 ± 0.14 | 28 ± 4.5* | 87 ± 16* |

*p < 0.05 (Student's t test) between ob/ob or aP2-SREBP-1c mice and their respective wild-type controls.

**FIG. 2.** Changes in the amounts of mRNAs for SCD1 and SCD2 in livers from wild-type (WT), C57BL/6J (ob/ob) (ob), and transgenic aP2-SREBP-1c (Tg) mice, as measured by the RNase protection assay. Total RNA was isolated from epididymal fat of C57BL/6J mice, and 5-µg aliquots were subjected to an RNase protection assay for SCD1 (lane 1) and SCD2 (lane 2) as described under “Experimental Procedures.” White fat contains mRNA transcripts for both SCD1 and SCD2, and was thus used as a positive control. Total RNA isolated from livers of mice described in Table I was pooled, and 5-µg aliquots were hybridized with 32P-labeled cRNA probe for SCD1 (lanes 3 and 4 and lanes 7 and 8) or SCD2 (lanes 5 and 6 and lanes 9 and 10). After RNase digestion, the protected fragments were separated by gel electrophoresis and exposed to film at –80 °C for 6 h.

Nuclear SREBP-2 levels were unchanged in livers from the two diabetic mouse models, and no significant changes were measured in the mRNAs for genes involved in cholesterol homeostasis. These studies support previous cell culture and in vivo studies showing that the isoforms of SREBP-1 preferen-
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Liver samples from individual mice of each genotype were extracted, and the major classes of lipids were separated on silica columns. The lipid fractions were methyl-esterified and quantified by gas-liquid chromatography as described under “Experimental Procedures.” Each value represents the mean from the mice described in Table I. Standard errors of the means were all less than 15% of the mean and are omitted for clarity. Bold values denote a level of statistical significance of \( p < 0.05 \) between wild-type and \( ob/ob \) or \( aP2-SREBP-1c \) mice.

**Table III**

Fatty acid composition of livers from wild-type, \( ob/ob \), and \( aP2-SREBP-1c \) mice

| Genotype of mice | Fatty acid | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:4 |
|------------------|-----------|------|------|------|------|------|------|------|
| **Total fatty acids** |            |      |      |      |      |      |      |      |
| Wild-type        | 21        | 2.1  | 11   | 18   | 21   | 1.1  | 10   |
| \( ob/ob \)      | 22        | 7.1  | 2.9  | 52   | 8.5  | 1.5  | 1.3  |
| Wild-type        | 23        | 1.9  | 12   | 17   | 13   | 0.6  | 16   |
| \( aP2-SREBP-1c \) | 24       | 5.6  | 4.5  | 46   | 7.1  | 1.4  | 4.4  |
| **Cholesteryl esters** |          |      |      |      |      |      |      |      |
| Wild-type        | 39        | 4.6  | 5.3  | 22   | 13   | 2.1  |      |
| \( ob/ob \)      | 23        | 7.1  | 1.5  | 57   | 6.8  | 1.5  | 0.1  |
| Wild-type        | 38        | 3.4  | 5.6  | 36   | 7.7  | 1.5  | 2.6  |
| \( aP2-SREBP-1c \) | 26       | 6.1  | 1.6  | 55   | 6.2  | 1.6  | 0.6  |
| **Triglycerides** |            |      |      |      |      |      |      |      |
| Wild-type        | 22        | 4.1  | 1.9  | 31   | 28   | 2.2  | 1.5  |
| \( ob/ob \)      | 21        | 9.2  | 1.2  | 51   | 10   | 1.9  | 0.5  |
| Wild-type        | 24        | 5.6  | 2.1  | 41   | 16   | 1.5  | 2.1  |
| \( aP2-SREBP-1c \) | 24       | 7.5  | 1.3  | 50   | 9.3  | 1.6  | 1.1  |
| **Phospholipids** |            |      |      |      |      |      |      |      |
| Wild-type        | 29        | 1.0  | 19   | 10   | 16   | 0.2  | 9.8  |
| \( ob/ob \)      | 25        | 2.4  | 19   | 19   | 9.6  | 0.5  | 9.7  |
| Wild-type        | 25        | 1.5  | 12   | 14   | 12   | 0.4  | 17   |
| \( aP2-SREBP-1c \) | 23       | 1.5  | 17   | 16   | 8.8  | 0.4  | 16   |

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