Nonalcoholic fatty liver disease (NAFLD) is associated with all features of the metabolic syndrome. Although deposition of excess triglycerides within liver cells, a hallmark of NAFLD, is associated with a loss of insulin sensitivity, it is not clear which cellular abnormality arises first. We have explored this in mice overexpressing the lipogenic transcription factor ChREBP (1). On a standard diet, mice overexpressing ChREBP showed normal insulin levels and improved insulin signaling and glucose tolerance compared with controls, despite having greater hepatic steatosis. Finally, ChREBP expression in liver biopsies from patients with nonalcoholic steatohepatitis was increased when steatosis was greater than 50% and decreased in the presence of severe insulin resistance. Together, these results demonstrate that increased ChREBP can dissociate hepatic steatosis from insulin resistance, with beneficial effects on both glucose and lipid metabolism.

**Introduction**

Nonalcoholic fatty liver disease (NAFLD) is gaining increasing recognition as a component of the epidemic of obesity worldwide. NAFLD is the most common cause of liver dysfunction and affects close to 20 million of patients in the USA (1). The spectrum of NAFLD ranges from simple fatty liver (hepatic steatosis), with benign prognosis, to a potentially progressive form, nonalcoholic steatohepatitis (NASH), which may lead to liver fibrosis and cirrhosis, resulting in increased morbidity and mortality. All features of the metabolic syndrome, including obesity, type 2 diabetes, arterial hypertension, and hyperlipidemia (in the form of elevated triglyceride [TG] levels), are associated with NAFLD/NASH (2). Despite being potentially severe, little is known about the natural history or prognostic significance of NAFLD.

Excessive accumulation of TGs in hepatocytes is the hallmark of NAFLD (3, 4). Despite the existing correlation between fatty liver and insulin resistance, it remains unclear whether insulin resistance causes the excessive accumulation of TGs in liver or whether the increase in TG itself or of metabolic intermediates may play a causal role in the development of hepatic or systemic insulin resistance. Studies have favored the concept that the accumulation of intra-hepatic lipids precedes the state of insulin resistance, while others have shown that hepatic TGs themselves are not toxic and may in fact protect the liver from lipotoxicity by buffering the accumulation of deleterious fatty acids (5–8). Such findings suggest that hepatic steatosis is not necessarily associated with insulin resistance (9–11). In agreement with this concept, an identified population of obese humans can stay free of insulin resistance and are metabolically healthy despite morbid obesity (12). These findings suggest that not all lipids are detrimental for insulin sensitivity and that specific lipid species, when present in the proper location and time, may trigger signals that modulate adaptation to stress (13, 14).

The sources of fat contributing to fatty liver include peripheral TGs stored in white adipose tissue that flow to the liver by way of plasma nonesterified fatty acids (NEFAs), dietary fatty acids, and TGs produced de novo through hepatic lipogenesis. After the esterification step, TGs can either be stored as lipid droplets within hepatocytes or secreted into the blood as VLDL, but, depending on the nutritional status, they can also be hydrolyzed to allow the channeling of fatty acids toward the β-oxidation pathway (15). Using a multiple-stable-isotope approach, Dorneley et al. (16) estimated that, of the TG content accounted for
in the livers of patients with NAFLD, 60% arose from NEFA, a little over 10% came from the diet, and close to 30% came from de novo lipogenesis, underlying the importance of de novo TG synthesis in the etiology of NAFLD. Pharmacologic or genetic manipulation of key lipogenic enzymes in mice can have profound metabolic consequences (reviewed in ref. 15). However, whether endogenously synthesized lipids per se affect insulin sensitivity is not clear.

Over recent years, studies reported that the liver transcription factor carbohydrate responsive element–binding protein (ChREBP) is required for the induction of the entire lipogenic and esterification program. C57BL/6J mice were injected intravenously with a single dose of 5 × 10⁹ pfu of GFP or ChREBP adenovirus at day 1. Four weeks later, mice were sacrificed and analyses were performed. (A) Ser196 phosphorylation level of ChREBP in livers of overnight fasted GFP and ChREBP mice. A representative Western blot is shown (n = 10–12 group). (B) Quantification of the ratio of Ser196 ChREBP phosphorylation compared with total ChREBP protein content is shown. ***P < 0.001 ChREBP versus GFP mice. (C) Nuclear ChREBP and SREBP-1c protein content in nuclear extracts from fed GFP versus ChREBP mice. Lamin A/C antibody was used as a loading control. A representative Western blot is shown (n = 10–12/group). mSREBP-1c, mature SREBP-1c. (D) Total ACACA, FASN, and SCD1 protein content in liver lysates from fed GFP and ChREBP mice. β-Actin antibody was used as a loading control. A representative Western blot is shown (n = 10–12/group). (E) qRT-PCR analysis of ChREBP, SREBP-1c, LXR, Pparg, Gck, Pklr, Acaca, Fasn, Scd1, Elovl6, and GPAT in livers of GFP versus ChREBP mice. Results are the mean ± SEM (n = 10–12/group). *P < 0.05, **P < 0.01 ChREBP versus GFP mice.
Figure 2

Overexpression of ChREBP leads to modification in hepatic lipid composition. All analyses were carried out in fed GFP and ChREBP mice. Results are the mean ± SEM (n = 10–12/group, unless specified). (A) Oil Red O staining of liver sections (original magnification, ×40). (B) Liver TGs, DAG, and ceramide concentrations. (C) Liver G6P and glycogen content. (D) PKCε content in cytosol and plasma membrane (PM) from GFP and ChREBP mice. Insulin receptor antibody (IRβ) was used as a control of plasma membrane preparation purity, and β-actin antibody was used as a loading control. A representative Western blot is shown (n = 6–10/group). (E) Phosphorylation levels of NF-κB on Ser536 and total NF-κB p65 protein concentrations in livers of noninjected (NI), GFP, and ChREBP mice. A representative Western blot is shown (n = 6/group). Quantification of NF-κB p65 Ser536/NF-κB p65 is shown (n = 6/group). (F) Fatty acid composition and palmitoleate (C16:1n-7) and oleate (C18:1n-9) concentrations. PUFAs, polyunsaturated fatty acids. (G) Schematic representation of the enzymatic steps involved in fatty acid synthesis from glucose in liver. The enzymes controlled by ChREBP are indicated: L-PK, ACACA, FASN, SCD1, Elovl6, and GPAT. A black arrow shows the change in fatty acid composition caused by ChREBP overexpression. *P < 0.05 ChREBP versus GFP.
In this study, we determined the metabolic impact of enhancing liver lipogenic rates via ChREBP on the outcome of hepatic steatosis and/or insulin resistance. We showed that while stimulating the entire lipogenic/esterification program, the adenosinergic-mediated overexpression of ChREBP in mouse liver does not lead to insulin resistance. The dissociation between ChREBP-mediated steatosis and hepatic insulin resistance was even more apparent under high-fat diet (HFD) feeding. Lipidomic analysis revealed that ChREBP-mediated hepatic steatosis was not deleterious and that beneficial lipid species (monounsaturated fatty acids [MUFAs]) were preferentially enriched. Lastly, we measured the expression of ChREBP in livers of a small cohort of patients with histologically proven NASH. We compared the hepatic levels of ChREBP expression, liver steatosis, and insulin resistance in humans.

**Results**

**Overexpression of ChREBP in liver leads to the induction of the entire lipogenic and esterification program.** To gain insights into the role of ChREBP in lipogenesis and insulin resistance, an adenovirus that expresses a dephosphorylated isoform of ChREBP (DP) was generated (19). Although we recently reported the importance of ChREBP acetylation and O-GlcNAcylation in response to glucose (20, 21), we also described the relevance of modulating the phosphorylation of Ser196 residue for ChREBP localization and/or insulin resistance. We showed that while stimulating liver lipogenic rates via ChREBP on the outcome of hepatic steatosis and/or insulin resistance, an adenovirus that expresses a dephosphorylated isoform of ChREBP (DP) was generated (19). Although we recently reported the importance of ChREBP acetylation and O-GlcNAcylation in response to glucose (20, 21), we also described the relevance of modulating the phosphorylation of Ser196 residue for ChREBP localization and/or insulin resistance. We showed that while stimulating liver lipogenic rates via ChREBP on the outcome of hepatic steatosis and/or insulin resistance, an adenovirus that expresses a dephosphorylated isoform of ChREBP (DP) was generated (19). Although we recently reported the importance of ChREBP acetylation and O-GlcNAcylation in response to glucose (20, 21), we also described the relevance of modulating the phosphorylation of Ser196 residue for ChREBP localization and/or insulin resistance. We showed that while stimulating liver lipogenic rates via ChREBP on the outcome of hepatic steatosis and/or insulin resistance, an adenovirus that expresses a dephosphorylated isoform of ChREBP (DP) was generated (19). Although we recently reported the importance of ChREBP acetylation and O-GlcNAcylation in response to glucose (20, 21), we also described the relevance of modulating the phosphorylation of Ser196 residue for ChREBP localization and/or insulin resistance. We showed that while stimulating liver lipogenic rates via ChREBP on the outcome of hepatic steatosis and/or insulin resistance, an adenovirus that expresses a dephosphorylated isoform of ChREBP (DP) was generated (19). Although we recently reported the importance of ChREBP acetylation and O-GlcNAcylation in response to glucose (20, 21), we also described the relevance of modulating the phosphorylation of Ser196 residue for ChREBP localization and/or insulin resistance. We showed that while stimulating liver lipogenic rates via ChREBP on the outcome of hepatic steatosis and/or insulin resistance, an adenovirus that expresses a dephosphorylated isoform of ChREBP (DP) was generated (19). Although we recently reported the importance of ChREBP acetylation and O-GlcNAcylation in response to glucose (20, 21), we also described the relevance of modulating the phosphorylation of Ser196 residue for ChREBP localization and/or insulin resistance. We showed that while stimulating liver lipogenic rates via ChREBP on the outcome of hepatic steatosis and/or insulin resistance, an adenovirus that expresses a dephosphorylated isoform of ChREBP (DP) was generated (19). Although we recently reported the importance of ChREBP acetylation and O-GlcNAcylation in response to glucose (20, 21), we also described the relevance of modulating the phosphorylation of Ser196 residue for ChREBP localization and/or insulin resistance. We showed that while stimulating liver lipogenic rates via ChREBP on the outcome of hepatic steatosis and/or insulin resistance, an adenovirus that expresses a dephosphorylated isoform of ChREBP (DP) was generated (19). Although we recently reported the importance of ChREBP acetylation and O-GlcNAcylation in response to glucose (20, 21), we also described the relevance of modulating the

**Table 1**

<table>
<thead>
<tr>
<th>Mice</th>
<th>GFP</th>
<th>CD</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>23.78 ± 0.54</td>
<td>24.56 ± 1.16</td>
<td>29.7 ± 1.4</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>1.40 ± 0.07</td>
<td>1.83 ± 0.07^a</td>
<td>2.41 ± 0.12^a</td>
</tr>
<tr>
<td>White adipose weight (epididymal) (g)</td>
<td>0.38 ± 0.03</td>
<td>0.16 ± 0.02^a</td>
<td>0.46 ± 0.05^a</td>
</tr>
<tr>
<td>Glucose (fed [mM])</td>
<td>8.30 ± 0.29</td>
<td>7.22 ± 0.39</td>
<td>9.9 ± 0.8</td>
</tr>
<tr>
<td>Glucose (fasting [mM])</td>
<td>5.19 ± 0.21</td>
<td>4.52 ± 0.25^a</td>
<td>4.95 ± 0.22^a</td>
</tr>
<tr>
<td>Serum insulin (fasting [ng/ml])</td>
<td>0.39 ± 0.10</td>
<td>0.27 ± 0.04^a</td>
<td>0.77 ± 0.54^a</td>
</tr>
<tr>
<td>Serum TGs (mmol/l)</td>
<td>0.56 ± 0.20</td>
<td>0.54 ± 0.46</td>
<td>0.84 ± 0.08</td>
</tr>
<tr>
<td>NEFAs (mg/dl)</td>
<td>0.79 ± 0.26</td>
<td>0.68 ± 0.05</td>
<td>0.88 ± 0.08^a</td>
</tr>
<tr>
<td>Δ Hydroxybutyrate (nM)</td>
<td>0.22 ± 0.03</td>
<td>0.18 ± 0.04</td>
<td>0.24 ± 0.03^a</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>280 ± 18</td>
<td>149 ± 12^a</td>
<td>283 ± 21</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>598 ± 20</td>
<td>526 ± 18</td>
<td>803 ± 27</td>
</tr>
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</table>

All measurements were carried out with 14-week-old mice (n > 6). All data are presented as mean ± SEM. ^aP < 0.05.
concentrations of either saturated fatty acids (SFAs) or polyunsaturated fatty acids (Figure 2F). It should be noted that a specific increase in palmitoleate (C16:1, n-7) and oleate (C18:1, n-9) concentrations was observed (Figure 2F), in agreement with the significant effect of ChREBP overexpression on SCD1 expression (Figure 1D and Figure 2G).

ChREBP-induced steatosis is not associated with insulin resistance. To determine whether the development of hepatic steatosis affected whole glucose homeostasis in ChREBP mice, glucose, insulin, and pyruvate tolerance tests were performed (Figure 3A). While no statistical difference was reached for glucose or insulin tolerance between GFP and ChREBP mice, a difference was observed for pyruvate tolerance (Figure 3A). While plasma glucose rose significantly for both GFP and ChREBP mice after the injection of pyruvate, pyruvate conversion into glucose was lower in ChREBP mice compared with that in GFP mice throughout the time course of the test (Figure 3A). This difference, which reflects lower rates of gluconeogenesis, was in agreement with lower fasting blood glucose concentrations measured in ChREBP mice (Table 1) and with the significant decrease in expression of hepatic PCK1, the rate-limiting enzyme of gluconeogenesis whose expression is inhibited by insulin (Figure 3C and D). The expression of Ppargc1a, which coactivates a number of transcription factors, such as hepatic nuclear factor-4α and forkhead box O1 (Foxo1), was also significantly decreased in livers of ChREBP mice. Foxo1 mRNA levels were also decreased by 50% (Figure 3D).
Modification in liver gene expression was correlated with a 3-fold increase in protein kinase B/Akt Ser473 phosphorylation under fasting conditions (Figure 3B). This increase in Akt Ser473 phosphorylation occurred independently of an increase in insulin concentrations in fasted ChREBP mice, suggesting enhanced insulin sensitivity in these mice (Table 1). In contrast, when a supraphysiological dose of insulin was injected into GFP and ChREBP mice, no apparent modification of the insulin-mediated phosphorylation of Akt (Ser473) or of GSK3β (Ser9) was observed in livers of ChREBP mice compared with that in controls (Supplemental Figure 2B).

Since conditions associated with high rates of lipogenesis are associated with a shift in cellular metabolism from fatty acid β-oxidation to TG esterification, we examined mRNA levels of Cpt1a, the rate-limiting enzyme of mitochondrial β-oxidation, and Ppara, a nuclear receptor that promotes β-oxidation. The expression of Cpt1a and Ppara was significantly decreased in livers of fasted ChREBP mice compared with that of GFP controls, suggesting decreased mitochondrial β-oxidation rates in these mice (Figure 3D). We also measured the expression of fibroblast growth factor 21 (FGF21), a key metabolic regulator whose expression is regulated via PPAR pathways during states requiring increased fatty acid oxidation (33). Fgf21 mRNA levels were 3-fold increased in livers of ChREBP mice, despite decreased Ppara gene expression (Figure 3D). Our results suggest that the transcriptional control exerted by ChREBP on FGF21 (34) may be dominant over that of PPAR and that increased FGF21 expression did not lead to increased mitochondrial β-oxidation rates under our experimental conditions. Altogether, our results suggest that ChREBP-mediated hepatic steatosis is dissociated from insulin resistance in mice.

Figure 4
ChREBP overexpression protects against PALM-induced insulin resistance in primary cultured hepatocytes. Twenty-four hours after plating, mouse hepatocytes were infected with 3 pfu of GFP or ChREBP adenovirus before being incubated for 24 hours with 0.48 mM albumin-bound PALM or 0.48 mM albumin-bound OLE/PALM, as described in the Methods section. An insulin (1 nM) time course was performed for 1, 2, 3, or 5 minutes. (A) Western blot analysis of insulin-mediated Akt phosphorylation on Ser473 and Thr308. Total Akt, ChREBP, SCD1, GFP, non-cleaved caspase-3, and cleaved caspase-3 protein content was determined under similar culture conditions. Representative Western blots are shown (n = 6–10 independent cultures). (B). Quantification of the ratio of Ser473 Akt phosphorylation compared with total Akt protein content is shown. *P < 0.05 GFP PALM versus GFP BSA mice for equivalent time point (n = 6–10 independent cultures). (C). The Δ9-desaturation index was calculated as described in the Methods section. *P < 0.05 versus GFP PALM.
ChREBP overexpression protects against palmitate-induced insulin resistance in primary cultured hepatocytes. Next, we determined whether the increase in hepatic MUFA concentrations measured in livers of ChREBP mice may account for the lack of lipotoxicity and insulin resistance. To address this issue, we tested whether a selective ChREBP-mediated increase in MUFA concentrations (such as oleate) could protect against SFA-mediated insulin resistance in primary hepatocytes. Mouse hepatocytes were first infected with either 3 pfu/cell of GFP or ChREBP adenovirus for 24 hours and then incubated in the presence of 0.48 mM palmitate (PALM, C16:0) for another 24 hours. Hepatocytes infected with GFP (3 pfu/cell) and incubated with a mix of oleate/PALM (OLE/PALM) for 24 hours were used as controls (Figure 4). After adenoviral treatment, insulin (1 nM) was added for times ranging from 0 to 5 minutes. The insulin-mediated phosphorylation of Akt on Ser473 was significantly reduced in the presence of PALM (at time points 3 and 5 minutes) (Figure 4A). The PALM-mediated decreased Akt phosphorylation was not due to cell apoptosis, since no significant increase in cleaved caspase-3 was detected under all the tested culture conditions (Figure 4A). The weak cleaved caspase-3 band was likely due to the adenoviral infection, since it was equivalent regardless of the culture conditions (Figure 4A). Interestingly, when ChREBP was overexpressed, the deleterious effect of PALM on insulin-mediated phosphorylation of Akt on Ser473 and on Thr308 was prevented (Figure 4A) and was associated with a marked induction of SCD1 (the Δ9-desaturase), the enzyme responsible for the conversion of SFA to MUFA (35) (Figure 4A). As a result, a significant increase in Δ9-desaturation index, reflecting the MUFA/SFA ratio, was observed under these experimental conditions (Figure 4C). In agreement with this hypothesis, a similar protective effect was observed when hepatocytes were incubated in the presence of a combination of OLE/PALM (Figure 4, B and C).

To better define the contribution of SCD1 to the ChREBP-mediated protective effects on Akt phosphorylation, we pharmacologically inhibited SCD1 activity (SCD1inhb), as described previously (36). First, within the same set of experiments, we confirmed both
The negative effect of PALM incubation on Akt Ser473 phosphorylation (Supplemental Figure 3, A and B) as well as the protective effect of ChREBP on Akt Ser473 and Thr308 phosphorylation in the presence of PALM (Figure 5, A and B). Performed in parallel, these results are comparable but are presented in 2 separate panels. When SCD1 activity was pharmacologically inhibited in the presence of PALM (Figure 5A), the protective effect of ChREBP on insulin signaling (i.e., Akt phosphorylation on Ser473 and Thr308) was significantly reduced (Figure 5B) and was correlated with a significant decrease in Δ9-desaturation index (Figure 5C). The Δ9-desaturation index was only significantly affected in presence of both ChREBP and PALM (Figure 5C), the only condition also associated with significant modification of the phosphorylated Akt/Akt ratio (Figure 5B). It should be noted here that ChREBP conditions alone (i.e., without PALM) did not increase the MUFA/SFA ratio, since hepatocytes were cultured under low glucose concentrations (5 mM), experimental conditions not sufficient to turn on lipogenic rates (17). Altogether, our results show that ChREBP overexpression reverses PALM-induced insulin resistance by increasing the MUFA/SFA ratio in vitro.

To determine whether ChREBP overexpression in vitro led to metabolic changes downstream of Akt phosphorylation, basal and insulin-mediated inhibition of glucose production were measured in primary hepatocytes overexpressing ChREBP with or without the SCD1 inhibitor (Supplemental Figure 4). First, we observed that basal glucose production was increased by 20% upon PALM incubation. While insulin (100 nM) failed to decrease glucose production under PALM conditions, the presence of ChREBP enabled insulin to significantly decrease glucose production, despite the presence of PALM in the culture medium. This effect was lost when the SCD1 inhibitor was added (Supplemental Figure 4). This set of experiments confirms that ChREBP favors the effect of insulin on a key metabolic pathway such as glucose production in vitro.

**Overexpression of SCD1 recapitulates the beneficial effect of ChREBP on Akt phosphorylation in vitro.** Acyl-CoA desaturases, such as SCD1 (37), introduce a double bond at a specific position on the acyl chain of fatty acids, thereby influencing several structural, metabolic, and signaling properties of the fatty acid or of the fatty acid containing lipids. Among desaturases, SCD1 is particularly critical to convert SFAs (mainly PALM C16:0 and stearate C18:0) into MUFAs (palmitoleate C16:1n-7 and oleate C18:1n-9) in the liver (38). To better define the role of SCD1 in the beneficial effect of ChREBP on insulin signaling in liver cells, we performed experiments in which SCD1 was either overexpressed or knocked down using adenoviral strategies in primary cultured hepatocytes (Figure 6). We observed that SCD1 overexpression restored Akt phosphorylation on both Ser473 (Supplemental Figure 5) and Thr308 to control levels (Figure 6B) in the presence of PALM. In fact, SCD1 and ChREBP overexpression had similar beneficial effects on rescuing Akt phosphorylation (Figure 6, A and B) in the presence of PALM. It should be noted, however, that the levels of SCD1 reached were quite different depending on whether SCD1 or ChREBP was overexpressed (Figure 6A).
this difference, ChREBP efficiently rescued Akt phosphorylation to levels similar to those of SCD1 and control conditions (Figure 6, A and B). Importantly, we observed that the beneficial effect of ChREBP was significantly attenuated when SCD1 was knocked down (Figure 6, A and B). Recent studies have revealed that chronic SFA exposure activates unfolded protein response (UPR) in cultured cells (39, 40). UPR induces transcription of a set of genes whose protein products increase the capacity for protein folding and ER-associated degradation and induces apoptosis when the ER function is severely impaired (41, 42). Of these genes, chop is often used as an UPR marker. As previously described (40), chop expression was increased in cells incubated with PALM (Figure 6C). Interestingly, the PALM-mediated induction of chop was prevented when SCD1 or ChREBP was overexpressed (Figure 6B). These results suggest that ChREBP and/or SCD1 prevented chop induction by increasing the MUFA/SFA ratio (as shown in Figure 5). Surprisingly, no significant reinduction of chop expression was observed in cells overexpressing ChREBP and incubated with PALM (Figure 6C), suggesting that ChREBP alone was sufficient to overcome the effect of PALM on chop expression.

Altogether, these experiments confirm that SCD1 overexpression can recapitulate the protective effect of ChREBP on Akt phosphorylation but also suggest that ChREBP overexpression may also exert its beneficial effects through additional mechanisms.

Exacerbated hepatic steatosis in HFD-fed ChREBP mice. Having determined that ChREBP-mediated liver steatosis was dissociated from insulin resistance under standard chow diet (CD) conditions, we wished to address whether ChREBP overexpression could reverse some of the metabolic alterations induced by HFD feeding. To address this issue, C57BL/6J mice were maintained on a HFD (60% calories coming from fat) for 10 weeks. Six weeks after the beginning of the diet, mice were injected with 1 single dose of $5 \times 10^9$ pfu of GFP or ChREBP adenovirus and maintained on HFD for another 4 weeks. Mice injected with a similar dose of GFP and maintained for 10 weeks on standard CD were used as controls. ChREBP overexpression markedly induced glycolytic ($Pklr$) and lipogenic gene expression ($SREBP-1c$, $Acaca$, $Fasn$, $Scd1$, $Elovl6$) in livers of HFD-fed ChREBP mice (Figure 7A). No difference in the expression of $Cd36$, involved in fatty acid uptake, was observed in livers of HFD-fed ChREBP mice compared with that in HFD-fed GFP mice (Figure 7B). In contrast, a significant decrease in $Cpt1a$ gene expression (Figure 7B) and in β-hydroxybutyrate concentrations (Table 1) was also observed, likely reflecting a decrease in β-oxidation rates in HFD-fed ChREBP mice. As a consequence, lipid droplet accumulation, as revealed by Oil red O staining on liver sections (Figure 8A), and hepatic TG concentrations were further increased (a 2.5-fold increase) (Figure 8C) in HFD-fed ChREBP mice compared with those in HFD-fed GFP mice. Interestingly, while their liver weight was increased (+40%), a marked decrease in white adipose tissue mass (–60%) (epididymal fat depot) was observed for HFD-fed ChREBP mice (Figure 8B), a reduction that was correlated with decreased NEFA concentrations (–40%) (Table 1). Despite exacerbated steatosis, no further increase in the expression of $Tnfa$, chop (Figure 8D), and of known
markers of inflammation (Ccl4, Mrc2, and Mgl1) (ref. 43 and data not shown) was observed in livers of HFD-fed ChREBP mice compared with HFD-fed GFP mice. Interestingly, a significant decrease in Il6 expression was measured (Figure 8D). Altogether, our results suggest that the ChREBP-mediated hepatic steatosis did not aggravate the inflammation status. Importantly, lipidomic analysis revealed that MUFA content was significantly increased in livers of HFD-fed ChREBP mice compared with that in HFD-fed GFP mice, as evidenced by a significant higher desaturation index (Figure 8E). Notably, oleate (C18:1 n-9) enrichment in livers of HFD-fed ChREBP mice occurred at the expense of PALM (C16:0), the percentage of which was significantly decreased (Figure 8F).

**Figure 8**
Exacerbated steatosis in HFD-fed ChREBP mice. Analyses were carried out in HFD-fed GFP and HFD-fed ChREBP mice, as described in the Methods section. GFP mice maintained on a standard CD for a similar number of weeks were used as controls. Mice were sacrificed in the fasted state. (A) Liver macroscopy and Oil Red O staining of liver sections (original magnification, ×40). (B) Liver and adipose tissue weight (epididymal) (expressed as g/g of body weight [BW]). (C) Liver TGs and diacylglycerol concentrations. (D) qRT-PCR analysis of Tnfa, Il6, and chop. (E) Analysis of the Δ9-desaturation index (MUFA/SFA ratio). (F) Percentage of cellular fatty acid content. Nine lipid species (as indicated) were analyzed and used to assess the quality of the steatosis generated. Results are the mean ± SEM (n = 6–8/group). *P < 0.05 HFD-fed GFP versus CD-fed GFP mice; **P < 0.01 HFD-fed ChREBP versus HFD-fed GFP mice; †P < 0.05, ††P < 0.01 HFD versus CD.
the phosphorylation of Akt on Ser473 and Thr308 was reduced by about 50% in livers of HFD-fed GFP mice compared with that in CD-fed GFP mice (Figure 9B). Interestingly, Akt phosphorylation (Ser473 and Thr308) was not reduced in livers of HFD-fed ChREBP mice compared with that in HFD-fed GFP mice and was as high as in the control group (Figure 9A and B). Accordingly, the phosphorylation on Ser/Thr residues of several Akt substrates (ranging from 55 to 250 kDa) was enhanced in HFD-fed ChREBP mice compared with that in HFD-fed GFP mice (Figure 9C). In addition, GSK3β phosphorylation on Ser9 was comparable in livers of HFD-fed ChREBP and CD-fed GFP mice (Figure 9A). Sustained phosphorylation in these key effectors of insulin signaling (namely Akt, Akt substrates, GSK3β) occurred despite higher hepatic TG and DAG concentrations (Figure 8C). Fasting blood glucose concentrations were lower in HFD-fed ChREBP mice compared with those in HFD-fed GFP mice (–30%) (Table 1) and were correlated with a significant decrease in Pck1 (–60%) and Ppargc1a gene expression (Figure 9E), and CRT2 protein content (Figure 9A). Insulin regulates hepatic gluconeogenesis through the FoxO1-PPARGC1A interaction (44) and by promoting the phosphorylation and ubiquitin-dependent degradation of CRT2 (45). The fact that the expression of several key components of the gluconeogenic pathway (namely PCK1, PPARGC1A, CRT2) was decreased in livers of HFD-fed ChREBP mice compared with that in HFD-fed GFP mice is in favor of a rescued insulin-signaling pathway. However, it should be noted that G6pc gene expression, a known target gene of ChREBP (46), was 2-fold increased upon ChREBP overexpression (Figure 9E). Nevertheless, glucose tolerance was improved in HFD-fed ChREBP mice, and hyperinsulinemia was markedly decreased (Figure 10, A and B). As index of glycolytic flux in liver, hepatic pyruvate concentrations were measured (Figure 9D). Consistent with the transcriptional control exerted by ChREBP on L-PK expression (Figure 7A), a significant increase in hepatic pyruvate concentrations was observed (Figure 9D), supporting the idea that glucose flux is preferentially oriented toward glycolysis/lipogenesis in HFD-fed ChREBP mice.

Because Fgf21 gene expression is under the transcriptional control of ChREBP (34) and because FGF21 has beneficial effects on plasma glucose and lipid profiles in diabetic rodents (47), mRNA and cir-

![Figure 9](http://www.jci.org) Improved Akt phosphorylation in livers of HFD-fed ChREBP mice. All analyses were carried out in HFD-fed GFP and ChREBP mice, as described in the Methods section. GFP mice maintained on a standard CD for a similar number of weeks were used as controls. Mice were sacrificed in the fasted state. (A) Ser473, Thr308 Akt phosphorylation, and Ser9 GSK3β phosphorylation in livers of overnight fasted CD-fed GFP, HFD-fed GFP, and HFD-fed ChREBP mice. Total protein content of Akt, GSK3β ChREBP, SCD1, CRT2, and GFP are shown. Representative Western blots are shown. β-Actin was used as a loading control. Samples were run on the same gel, but lanes were not contiguous. (B) Quantification of the ratios of Ser473 and Thr308 Akt phosphorylation compared with total Akt protein content are shown. (C) Phosphorylation of Akt substrates (ranging from 55 to 250 kDa). A representative Western blot is shown. Samples were run on the same gel, but lanes were not contiguous. (D) Liver pyruvate concentrations. (E) qRT-PCR analysis of genes involved in the gluconeogenic pathway (G6pc, Pck1, Ppargc1a). Results are the mean ± SEM (n = 8–10/group). *P < 0.05 HFD-fed GFP versus CD-fed GFP mice; **P < 0.01, ***P < 0.001 HFD-fed ChREBP versus HFD-fed GFP mice.
Finally, significant correlations were found between the HOMA index (4.8) and the values of steatosis (50%) and the presence of either hepatocyte ballooning or intralobular hepatocyte necrosis. Using the median percentage of steatosis was 55% ± 21%, and, between the HOMA index value of less than 4.8 (0.18 ± 0.06 relative expression [SEM]; \( P < 0.01 \)) and the degree of steatosis and inversely to insulin resistance in patients with NASH. In order to translate the findings obtained in mice to human pathology, 25 patients with histologically proven NASH were studied (48). Individual characteristics of patients are listed in Table 2. Mean BMI was 31 ± 5.5 kg/m², and most patients had features of the metabolic syndrome. Mean homeostasis model assessment (HOMA) was 5.6 ± 2.6. Mean percentage of steatosis was 55% ± 21%, and, among the 25 patients, 28% had severe fibrosis (F3, F4). Only 16% had no fibrosis. The diagnosis of NASH was defined as steatosis of more than 20% of the liver and the presence of either hepatic ballooning or intralobular hepatocyte necrosis. Using the median values of steatosis (50%) and the HOMA index (4.8), CHREBP mRNA levels were 2-fold higher in patients with steatosis of more than 50% compared with those in patients with steatosis of less than 50% (0.33 ± 0.03 relative expression [SEM]; \( P < 0.05 \)) (Figure 11A). Conversely, CHREBP expression was decreased by 50% in patients with a HOMA index value of more than 4.8 compared with patients with a HOMA index value of less than 4.8 (0.18 ± 0.06 relative expression [SEM] vs. 0.35 ± 0.13 relative expression [SEM]; \( P = 0.04 \)) (Figure 11B). Finally, significant correlations were found between the HOMA index (\( r^2 = 0.28; \ P = 0.03 \)) or the degree of liver steatosis (\( r^2 = 0.27; \ P = 0.02 \)) (Figure 11D) and CHREBP mRNA levels in livers of these patients.

**Discussion**

NAFLD is an increasing health concern now considered as a component of the metabolic syndrome (1). Excessive accumulation of TGs in hepatocytes is the hallmark of NAFLD. Dysregulated lipogenesis has been shown to contribute to the pathogenesis of hepatic steatosis in both humans (16, 49) and rodents (50). However, the contribution of the glucose-sensitive lipogenic transcription factor CHREBP to the pathophysiology of human steatosis and/or insulin resistance has not been clearly addressed. In addition, despite the widely accepted association between hepatic steatosis and insulin resistance, it remains unclear whether a causal relationship always exists. Our study reports that CHREBP overexpression in mouse liver leads to a significant increase in hepatic TG concentrations and hepatic steatosis development. Interestingly, CHREBP overexpression in livers of HFD mice, while inducing a larger hepatic lipid overload, was protective against liver insulin resistance and was correlated with beneficial lipid species accumulation (i.e., oleate, C18:1 n-9, MUFA) and decreased percentage of PALM (C16:0). To our knowledge, our work is the first to report that hepatic CHREBP expression varies positively according to the degree of steatosis and inversely to insulin resistance in a cohort of patients with NASH.

CHREBP is a basic helix-loop-helix/leucine zipper transcription factor, playing a critical role in hepatic fatty acid synthesis (25–27). To further address the importance of CHREBP in the control of de novo lipogenesis and its relationship to insulin resistance development, we chose to overexpress a dephosphorylated isoform of CHREBP in mouse liver. Although it was previously reported that mutations of Ser196 and/or Thr666 may not result in a constitutively active form of CHREBP (51), we report here that CHREBP target genes were efficiently induced under both substrate abundance (standard CD and HFD) and fasting conditions. Indeed, CHREBP overexpression induced, the entire lipogenic/esterification program in liver independently of an increase in SREBP-1c activity and/or LXRs expression. Aside from previously known targets of CHREBP, such as L-PK, ACACA, FASN, G1AT, G6PC, and FGF21 (19, 34, 52), our results reveal that SCD1 and Elovl6 are key CHREBP target genes. Indeed, a selective enrichment in their lipid products (i.e., MUFA) (Figure 2G) was observed upon CHREBP overexpression in liver under both standard and HFDs. Interestingly, both SCD1 and Elovl6 were identified among 700 other direct target genes of CHREBP in a genome-wide scale analysis performed in HepG2 cells using ChIP sequencing (53).

Altogether, our results strongly support the hypothesis that CHREBP favors TG accumulation and may in fact protect liver from lipotoxicity by buffering the accumulation of detrimental fatty acids. In mammalian cells, fatty acids are required for the synthesis of phospholipid components of the membrane and energy storage as TGs. However, over accumulation of fatty acids can be considered toxic/deleterious (14, 54). To prevent lipotoxicity, cells can enhance incorporation of fatty acids into TGs that are then stored into lipid droplets (55). Under HFD feeding conditions (in terms of energy, 60% coming from fat), enhancing circulating levels of Fgf21 were measured (Figure 7B and Figure 10C). CHREBP overexpression led to a significant increase in Fgf21 mRNA concentrations and circulating levels, raising the possibility that enhanced circulating FGF21 levels may have also contributed to the observed improvement in glucose tolerance (Figure 10A).

**Improved glucose tolerance and insulin resistance in HFD-fed ChREBP mice.** All analyses were carried out in HFD-fed GFP and ChREBP mice, as described in the Methods section. GFP mice maintained on a standard CD for a similar number of weeks were used as controls. (A) Glucose tolerance tests (1 g/kg) were performed in GFP and ChREBP mice. (B) Fasting insulin concentrations. (C) Fasting FGF21 concentrations. Results are the mean ± SEM (\( n = 8–10 \)/group). *P < 0.05 HFD-fed GFP versus CD-fed GFP mice; **P < 0.01 HFD-fed ChREBP versus HFD-fed GFP mice.
endogenous generation of fatty acids via ChREBP overexpression had clear beneficial effects on both hepatic insulin signaling and glucose metabolism. Genes involved in lipogenesis and TG levels were further enhanced (+125%), and a notable switch in cellular fatty acid percentage was observed compared with HFD-fed GFP conditions. ChREBP overexpression induced hepatic steatosis with a greater accumulation of oleate than PALM (45% and 15%, respectively), compared with GFP HFD steatosis, in which TG synthesis and storage in lipid droplets. Lastly, the relevance of desaturase activity (namely SCD1, which introduces a cis double bond in the Δ9 position of saturated 16- and 18-carbon fatty acids) substrates; ref. 35) in fatty acid partitioning has recently been recognized as important as shown in experiments in which knockdown of unsaturates, by decreasing the MUFA/SFA ratio, causes ER stress and apoptosis. Of note, the expression of Dgat1, Elovl6, and Scd1 was significantly increased upon ChREBP overexpression in liver under HFD conditions (Figure 7A).

In fact, our results suggest that the lipogenic SCD1 enzyme was instrumental in the beneficial effects mediated by ChREBP overexpression. By stimulating SCD1 expression in liver, ChREBP led to the subsequent rise in oleate (C18:1n-9) concentrations under CD and HFD feeding. Experiments performed in primary hepatocytes revealed that ChREBP or SCD1 overexpression and/or oleate incubation prevented the deleterious effect of PALM on Akt phosphorylation (Ser473 and Thr308) and on chop induction. Our in vitro experiments support studies showing that lipotoxicity and ER stress are almost exclusively attributed to SFA (6, 39) and that they can be prevented by oleate and/or palmitoleate incubation (61, 62). ChREBP overexpression, through its positive control of SCD1, allowed for the conversion of PALM in the culture medium into specific lipids that are beneficial for insulin sensitivity and for the prevention of ER stress (i.e., chop induction). These results reinforce the concept that TG synthesis is crucial to prevent harmful effects on organelles and/or cell signaling. In agreement with this hypothesis, when SCD1 was inhibited pharmacologically or through a shRNA strategy, the protective effect of ChREBP on insulin signaling was significantly attenuated. Surprisingly, while it has been reported that SCD1 knockdown causes ER stress by causing SFA accumulation (39), no significant reinduction of chop was observed when SCD1 was silenced in the context of ChREBP/PALM conditions, suggesting that ChREBP overexpression alone could be sufficient to overcome the deleterious effect of PALM on chop induction. The contribution of SCD1 to hepatic steatosis, insulin resistance, and/or inflammation is rather complex. On one hand, inactivation of SCD1 in liver prevents fatty liver, insulin resistance, and obesity induced by high carbohydrate feeding (63) but, on the other hand, worsens diabetes in ob/ob mice (64). Furthermore, SCD1 deficiency aggravates the inflammatory state under conditions of methionine choline-deficient diet and in response to dextran sulfate sodium-induced acute colitis, causing hepatic overaccumulation of SFA that triggers hepatocellular apoptosis, liver damage, and NASH (65). Interestingly, oleic acid feeding and in vivo SCD1-rescued expression alleviates the dextran sulfate sodium–induced phenotype, suggesting that SCD1 and its related lipid species may serve as potential targets for intervention and/or treatment of inflammatory diseases (66). Therefore, consumption of an oleic-enriched diet or selective modulation of the lipogenic pathway via ChREBP and/or SCD1 could represent an interesting strategy to ameliorate and/or buffer the toxic effect of SFA by diverting them to safe forms of lipids. In agreement with this concept, a recent study reports that consumption of an oleic-enriched diet improves insulin sensitivity and decreases inflammation in muscles from aged rats (67).

Altogether, our study reveals that HFD-fed ChREBP mice are metabolically healthier than their controls, showing reduced fasting blood glucose, improved glucose tolerance, and reduced hyperinsulinemia, despite a higher grade of hepatic steatosis. It should be noted that ChREBP-mediated steatosis was dissociated from insulin resistance, despite elevated concentrations of DAG (a 2-fold increase under CD but only a 20% under HFD), an important lipid intermediate previously implicated in hepatic insulin resistance assessment using the HOMA index as follows: fasting insulin (mU/l) × fasting plasma glucose (mmol/l)/22.5 (90).

### Table 2
Clinical and biochemical characteristics of patients with NASH evaluated in this study

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>55 ± 11</td>
</tr>
<tr>
<td>Sex ratio (male/female)</td>
<td>17/8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31 ± 5.5</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>107 ± 11</td>
</tr>
<tr>
<td>Diabetes (n)</td>
<td>7</td>
</tr>
<tr>
<td>High blood pressure (n)</td>
<td>11</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>77 ± 40</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>52 ± 29</td>
</tr>
<tr>
<td>γGT (IU/l)</td>
<td>100 ± 11</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>6.6 ± 1.2</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.6 ± 0.36</td>
</tr>
<tr>
<td>TGs (mmol/l)</td>
<td>2.2 ± 1.4</td>
</tr>
<tr>
<td>Insulin (IU/ml)</td>
<td>20.1 ± 8.2</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.9 ± 1.8</td>
</tr>
<tr>
<td>HOMA</td>
<td>5.6 ± 2.6</td>
</tr>
<tr>
<td>Steatosis (%)</td>
<td>55 ± 21</td>
</tr>
<tr>
<td>NAS</td>
<td>4.4 ± 1.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th>Fibrosis stage</th>
<th>F0 (%)</th>
<th>F1 (%)</th>
<th>F2 (%)</th>
<th>F3 (%)</th>
<th>F4 (%)</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>16</td>
<td>8</td>
<td>48</td>
<td>24</td>
<td>4</td>
</tr>
</tbody>
</table>

After a 12-hour overnight fast, venous blood samples were drawn to determine levels of blood glucose and ALT concentrations. Insulin resistance was assessed using the HOMA index as follows: fasting insulin (mU/l) × fasting plasma glucose (mmol/l)/22.5 (90).
resistance in both rodents (31, 32) and humans (8). Of note, our results are consistent with other mouse models in which increased DAG concentrations were not associated with insulin resistance. In these studies, overexpression of DGAT2 in liver (9) or blockade of VLDL secretion mediated by MTP inactivation (68) resulted in a clear dissociation between hepatic steatosis and insulin resistance, despite a significant increase in hepatic DAG and ceramide concentrations. This discrepancy might be explained if different pools of DAG and ceramide exist within the hepatocytes, as previously suggested in muscle cells (69), with only certain pools being able to regulate inhibitors of insulin signaling (70). Another important feature of ChREBP overexpression is that amplified fat storage occurred in liver but was reduced in white adipose depot (under both standard diet and HFD). As a result, serum NEFAs, which predominantly arise from adipose tissue lipolysis at the fasting state, were reduced in HFD-fed ChREBP mice. Altogether, our current results contrast with previous studies reporting that ChREBP deficiency improves hepatic steatosis and insulin resistance in leptin-deficient ob/ob mice (25, 27). As mentioned in the Introduction, paradoxical metabolic/physiologic consequences of ChREBP deficiency have been previously reported, since deleterious or beneficial phenotypes can be observed depending whether ChREBP deficiency is generated under a context of lipid overload. We show here that ChREBP overexpression dissociated hepatic steatosis from insulin resistance by modifying the MUFA/SFA balance. Our results also suggest that enhanced glycolytic rates mediated by ChREBP overexpression (at the level of L-PK, evidenced by increased hepatic pyruvate concentrations) also contributed to the improvement of glucose tolerance in HFD-fed ChREBP mice (Figure 9D). Indeed, it has been previously reported that enhancing glycolytic rates through GCK in obese mice had beneficial influences on overall whole-body energy balance, including glucose tolerance (71). The fact that ChREBP global deficiency leads to decreased glycolytic rates (as evidenced by a lower pyruvate/phosphoenolpyruvate ratio) as well as major alterations in energy substrate utilization also supports this hypothesis. Lastly, the human homolog of ChREBP, WBSCR14 (also known as MondO), is located on a chromosomal region deleted in the Williams-Beuren syndrome, a syndrome characterized in 75% of the cases by severe glucose intolerance (72). Altogether, our results suggest that ChREBP could act as a dual regulator of glycolysis and gluconeogenesis, which have a reciprocal relationship in response to glucose. Indeed, under both standard diets and HFDs, key components of gluconeogenesis (namely PCK1, PPARG1A, FOXO1, CRTC2) were decreased upon ChREBP overexpression. Interestingly, we observed a substantial decrease in the expression of the deacetylase SIRT1 in livers of HFD-fed ChREBP mice compared with that in HFD-fed GFP mice (data not shown), confirming the ChREBP-mediated inhibition of SIRT1 (73). In the liver, SIRT1 controls gluconeogenic activity by modulating the acetylation
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Figure 12

Antilipotoxic effect of ChREBP overexpression in mouse liver. Upon ChREBP overexpression in livers of mice fed a HFD, glycolysis and lipogenesis are stimulated, leading to the development of hepatic steatosis. ChREBP overexpression by stimulating SCD1 activity leads to a modification in lipid composition (namely a change in the mono-unsaturated [MUFA] to saturated [SFA] ratio) and dissociates hepatic steatosis from insulin resistance. Increased phosphorylation of Akt (Ser473 and Thr308) and of downstream substrates was measured in HFD-fed ChREBP mice, and glucose tolerance was improved. Our results provide insights into the causal relationship between hepatic steatosis and insulin resistance and demonstrate that increasing the lipogenic pathway may protect against insulin resistance by raising beneficial lipid species. ChREBP provides original insight on the complex pathogenesis of NAFLD.

Methods

Animals and diets. Seven- to eight-week-old male C57BL/6J mice were purchased from Charles River and adapted to the environment for 2 weeks before study. All mice were housed in colony cages with a 12-hour-light/12-hour-dark cycle in a temperature-controlled environment (the dark cycle going from 3:00 am until 3:00 pm). Mice had free access to water and standard CD (in terms of energy, 65% from carbohydrate, 11% from fat, and 24% from protein). For HFD studies, C57BL/6J mice (Charles River) were maintained on a HFD (EF R/M acc. D12492, Ssniff Spezialdiaten GmbH, modified upon personal request) for a total of 10 weeks, starting at 3 weeks of age. Modified HFD consisted of crude nutrients (fat 35% [SFA], 60% [MUFA], 60% [PUFA], 60% [SFA], 21% from carbohydrate, and 19% from protein). For HFD studies, C57BL/6J mice (Charles River) were maintained on a HFD (EF R/M acc. D12492, Ssniff Spezialdiaten GmbH, modified upon personal request) for a total of 10 weeks, starting at 3 weeks of age. Modified HFD consisted of crude nutrients (fat 35% [SFA], 60% [MUFA], 60% [PUFA], 60% [SFA], 21% from carbohydrate, and 19% from protein). For HFD studies, C57BL/6J mice (Charles River) were maintained on a HFD (EF R/M acc. D12492, Ssniff Spezialdiaten GmbH, modified upon personal request) for a total of 10 weeks, starting at 3 weeks of age. Modified HFD consisted of crude nutrients (fat 35% [SFA], 60% [MUFA], 60% [PUFA], 60% [SFA], 21% from carbohydrate, and 19% from protein).
sacrificed between 9:00 and 10:00 am (during dark cycle). For “fasting” conditions, mice were fasted overnight (12 hours).

Generation of the CHREBP adenovirus construct. The full-length wild-type ChREBP-z isoform mutated on Ser196 and Thr666 (19) was subcloned into the shuttle vector pAd Track-CMV. Recombinant adeno viral constructs ChREBP and GFP recombinant adenoviruses were produced in HEK293T cells and purified on cesium chloride gradients before use by the Laboratoire de Thérapie Génique.

Injection of the CHREBP adenovirus and in vivo insulin stimulation. Male mice were anesthetized with isoflurane before injection through the penis vein with 5 × 10⁹ pfu of either GFP or ChREBP in a final volume of 200 μl of sterile NaCl 9%. Experiments and analysis were performed 4 weeks after the adenoviral injection (a single dose). For insulin signaling experiments, mice were fasted overnight and then injected with NaCl 9% or 1 unit of regular human insulin/kg (Actrapid Penfill, NovoNordisk) via the portal vein. Three minutes after injection of the insulin bolus, livers were removed and snap frozen in liquid nitrogen.

Glucose, insulin, and pyruvate tolerance tests. Glucose tolerance tests were performed 4 weeks after adenoviral injections by glucose gavage (1 g glucose/kg body weight) after an overnight fast. Insulin tolerance tests were performed by intraperitoneal injection of human regular insulin (0.75 unit insulin/kg body weight; Actrapid Penfill, NovoNordisk) 5 minutes after food removal. Pyruvate tolerance tests were performed by intraperitoneal injection of pyruvate (monosodium salt, Boehringer Mannheim) (2 g/kg body weight) after an overnight fast. Blood glucose was determined using the One-Touch AccuChek Glucometer (Roche).

Analytical procedures. Serum concentrations of TG, NEFA, AST, and ALT were determined using an automated Monarch device (Laboratoire de Biochimie, Faculté de Médecine Bichat, Paris, France). Serum insulin concentrations were determined using a rat insulin ELISA Assay Kit (Crystal Chem) using a mouse insulin standard. FGF21 concentrations were measured using the Rat/Mouse FGF21-26K (GeneCust) kit for 24 hours. After the adenovectors period, insulin/kg body weight; Actrapid Penfill, NovoNordisk) for 24 hours. After the adenovectors period, serum albumin (Sigma-Aldrich). After 24 hours in these different conditions, mice were at 315°C and 345°C, respectively.

To measure ceramide and sphingomyelin, total lipids corresponding to 2 mg of tissue were extracted according to the method of Bligh and Dyer in chloroform/methanol/water (2:5:2.5:2.1, v/v/v) in the presence of the internal standards ceramide NC15 (2 μg, prepared according to ref. 86). The dried lipid extract was submitted to a mild alkaline treatment in methanolic NaOH 0.6 N (1 ml) and then to silylation in 50 μl BSTFA (1%TMSCl)/acetonitrile (1.1, v/v) overnight at room temperature. Sample (5 μl) was directly analyzed by gas-liquid chromatography (4890 Hewlett Packard system, using a RESTEK RTX-50 fused silica capillary columns, 30-m × 0.32-mm i.d., 0.1-μm film thickness). Oven temperature was programmed from 195°C to 310°C (12 minutes) at a rate of 3.5°C per minute, and the carrier gas was hydrogen (7.25 psi). The injector and the detector were at 310°C and 340°C, respectively.

To measure total hepatic fatty acid methyl ester (FAME) molecular species, lipids corresponding to an equivalent of 1 mg of liver were extracted in the presence of glyceryl triheptadecanoate (0.5 μg) as an internal standard. The lipid extract was transmethylated with 1 ml of BF3 in methanol (14% solution; Sigma-Aldrich) and 1 ml of hexane for 60 minutes at 100°C and evaporated to dryness, and the FAMES were extracted with hexane/water (2:1). The organic phase was evaporated to dryness and dissolved in 50 μl ethyl acetate. A sample (1 μl) of total FAME was analyzed by gas-liquid chromatography (Clarus 600 Perkin Elmer system, with FAMEs RESTEK fused silica capillary columns, 30-m × 0.32-mm i.d., 0.25-μm film thickness). Oven temperature was programmed from 110°C to 220°C at a rate of 2°C per minute, and the carrier gas was hydrogen (7.25 psi). The injector and the detector were at 225°C and 245°C, respectively.

To measure hepatocyte fatty acid composition, cells were washed twice with ice-cold PBS and scraped into PBS. After centrifugation, the cell pellet was resuspended in PBS and sonicated. Cellular lipids were extracted with hexane/isopropanol (3:2, v/v) prior to transmethylation with 1 ml of BF3 in methanol (14% solution; Sigma-Aldrich) and 1 ml of hexane for 60 minutes at 100°C and evaporated to dryness, and the FAMES were then extracted with hexane and analyzed by gas-liquid chromatography. To assess the effect of SCD1 inhibitor, we estimated the Δ9-desaturation index as the abundance of SCD1 products (oleic and palmitoleic acids) relative to both SCD1 products and substrates (palmitic and stearic acids).

Isolation of total mRNA and analysis by qRT-PCR. Total cellular RNA was extracted using the RNeasy Kit (Qiagen), and 500 ng of RNA were reverse transcribed. qRT-PCR analysis was performed with a LightCycler instrument (Roche Applied Science) and SYBR green detection of amplified products. Primers for G3PDH, Pikh, Acaca, Fasn, Scd1, GPAT, Pck1, ChREBP, SREBP-1c (17, 27), LXR (23), Pparγ (87), and Fgf21 (33) were previously described. Other primers were as follows: Elov60 (sense 5′-AAATGGACCTGTGAGCAAGCATCAGT-3′; antisense 5′-GTACCAGTGCAGGAAGATCAGT-3′); Dgat1...
genes was achieved by the ΔΔCt method (88).

...forward and reverse primers. The sequences of primers are as follows:

- Sense 5′-GCCC-3′;
- Antisense 5′-TTCCGCTTGACCAGAGATCG-3′;
- Sense 5′-TTTTGACCAGATGCGAGACA-3′;
- Antisense 5′-AAGGAATAAGTGGGAACCCAGATCA-3′;
- Sense 5′-TTCTTGAGCTCGACTCTT-3′; and
- Antisense 5′-TGGGAGTAGACAAGGTACAACC-3′.

...from 200 ng RNA was added to 50 μl PCR buffer with 20 pmoles of each forward and reverse primers. The sequences of primers are as follows:

- CHREBP sense 5′-TGGTACTCCAGAGATATGAGA; and
- Antisense 5′-TTGGTACTCCAGAGATATGAGA; and
- Antisense 5′-GAGAGTTAAAGGAA; and
- Antisense 5′-GAGAGACAG). The relative quantification for a given gene was corrected as the percentage of hepatocytes involved within a lobule (0%–100%, steatosis score) and by using a 3+4 classification modified from Kleiner et al. (91). The score is defined as the sum of the scores for steatosis (0, <5%; 1, 5%–33%; 2, 33%–66%; 3, >66%), lobular inflammation (0, none; 1, <2 foci/×200 magnification field; 2, >2–4 foci/×200 magnification field; 3, >4 foci/×200 magnification field), and ballooning (0, none; 1, few; 2, many). This study was approved by the Ethics Committee of the Pitie-Salpetriere Hospital.

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