Cideb Regulates Diet-Induced Obesity, Liver Steatosis, and Insulin Sensitivity by Controlling Lipogenesis and Fatty Acid Oxidation

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OBJECTIVE—Our previous study suggests that Cidea, a member of Cide family proteins that share sequence homology with the DNA fragmentation factor and are expressed at high levels in brown adipose tissue, plays an important role in the development of obesity. Cideb, another member of Cide family protein, is highly expressed in the liver. We would like to understand the physiological role of Cideb in the regulation of energy expenditure and lipid metabolism.

RESEARCH DESIGN AND METHODS—We generated *Cideb*-null mice by homolog recombination and then fed both wild-type and *Cideb*-null mice with high-fat diet (58% fat). We then characterized the animals' adiposity index, food intake, whole-body metabolic rate, liver morphology, rate of fatty acid synthesis and oxidation, insulin sensitivity, and gene expression profile.

RESULTS—*Cideb*-null mice had lower levels of plasma triglycerides and free fatty acids and were resistant to high-fat diet–induced obesity and live steatosis. In addition, *Cideb* mutant mice displayed significantly increased insulin sensitivity and enhanced rate of whole-body metabolism and hepatic fatty acid oxidation. More importantly, *Cideb*-null mice showed decreased lipogenesis and reduced expression levels of acetyl-CoA carboxylase, fatty acid synthase, and stearol-CoA desaturase. We further demonstrated that expression levels of sterol response element binding protein 1c was significantly decreased in *Cideb*-deficient mice.

CONCLUSIONS—Our data demonstrate that Cideb is a novel important regulator in lipid metabolism in the liver. Cideb may represent a new therapeutic target for the treatment of obesity, diabetes, and liver steatosis. ***Diabetes*** **56:2523–2532, 2007**

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ACC, acetyl-CoA carboxylase; BAT, brown adipose tissue; CPT, carnitine-palmitoyl transferase; FAS, fatty acid synthase; G6P, glucose-6-phosphatase; GTT, glucose tolerance test; IRS, insulin receptor substrate; ITT, insulin tolerance test; NEFA, nonesterified fatty acid; PGC, peroxisome proliferator–activated receptor γ coactivator; PPAR, peroxisome proliferator–activated receptor; SCD1, stearol-CoA desaturase 1; SREBP1c, sterol response element binding protein 1c; TAG, triacylglycerol; WAT, white adipose tissue.

Obesity represents an excessive amount of body fat and is a result of imbalance between energy intake and expenditure. It affects a large population in the world and is a major risk for many metabolic diseases, such as hypertension, stroke, liver steatosis, and even cancer. In particular, obesity has been closely associated with insulin resistance and the development of type 2 diabetes. Liver plays a central role in energy homeostasis because it is the main organ for lipid de novo synthesis, lipid uptake and secretion, fatty acid oxidation, and the production of ketone bodies (1). It is also an important organ for glucose synthesis (gluconeogenesis) and storage (glycogen synthesis). Fatty acid oxidation in the liver begins with the formation of fatty acyl-CoA, which is subsequently transported into the mitochondria by the carnitine-palmitoyl transferase (CPT) (CPT1/CPT2) shuttle-system (2). CPT1 activity is inhibited by malonyl-CoA, the product of acetyl-CoA carboxylase (ACC)-2 in mitochondria (3). Hepatic mitochondrial β-oxidation of fatty acids provides energy, especially during fasting conditions. The regulation of lipid metabolism in liver is controlled by several classes of transcription factors, such as peroxisome proliferator–activated receptors (PPARs) (4) and sterol response element binding proteins (SREBPs) (5). SREBP1c is crucial for triacylglycerol (TAG) synthesis by regulating the expression of several downstream target genes, such as ACC, fatty acid synthase (FAS), and stearol-CoA desaturase 1 (SCD1) (5,6).

Changes of lipid homeostasis in the liver by overexpression or genetic mutation of a variety of factors often result in metabolic defects, such as obesity, diabetes, and liver steatosis. For example, $ACC2^{-/-}$ mice have a higher rate of fatty acid oxidation (7) and are resistant to high-fat diet–induced obesity and diabetes (3). Mice with *SCD1* deficiency, an enzyme catalyzing the desatuation process of palmitic acid to unsaturated fatty acids, have increased fatty acid oxidation, reduced body adiposity, and increased insulin sensitivity and are resistant to diet-induced obesity and liver steatosis (8,9). Targeted overexpression of a constitutively active nuclear form of SREBP-1c in the liver resulted in the activation of an array of downstream target genes and fatty liver formation (10).

Cide proteins, including Cidea, Cideb, and Fsp27 (Cidec), share homology with the DNA fragmentation factor DFF40/45 at the NH_2-terminal region (11). Our previous data revealed that *Cidea* is expressed at high levels in brown adipose tissues (BAT) and that *Cidea*-null mice exhibit higher energy expenditure and enhanced lipolysis in BAT and are resistant to high-fat diet–induced obesity

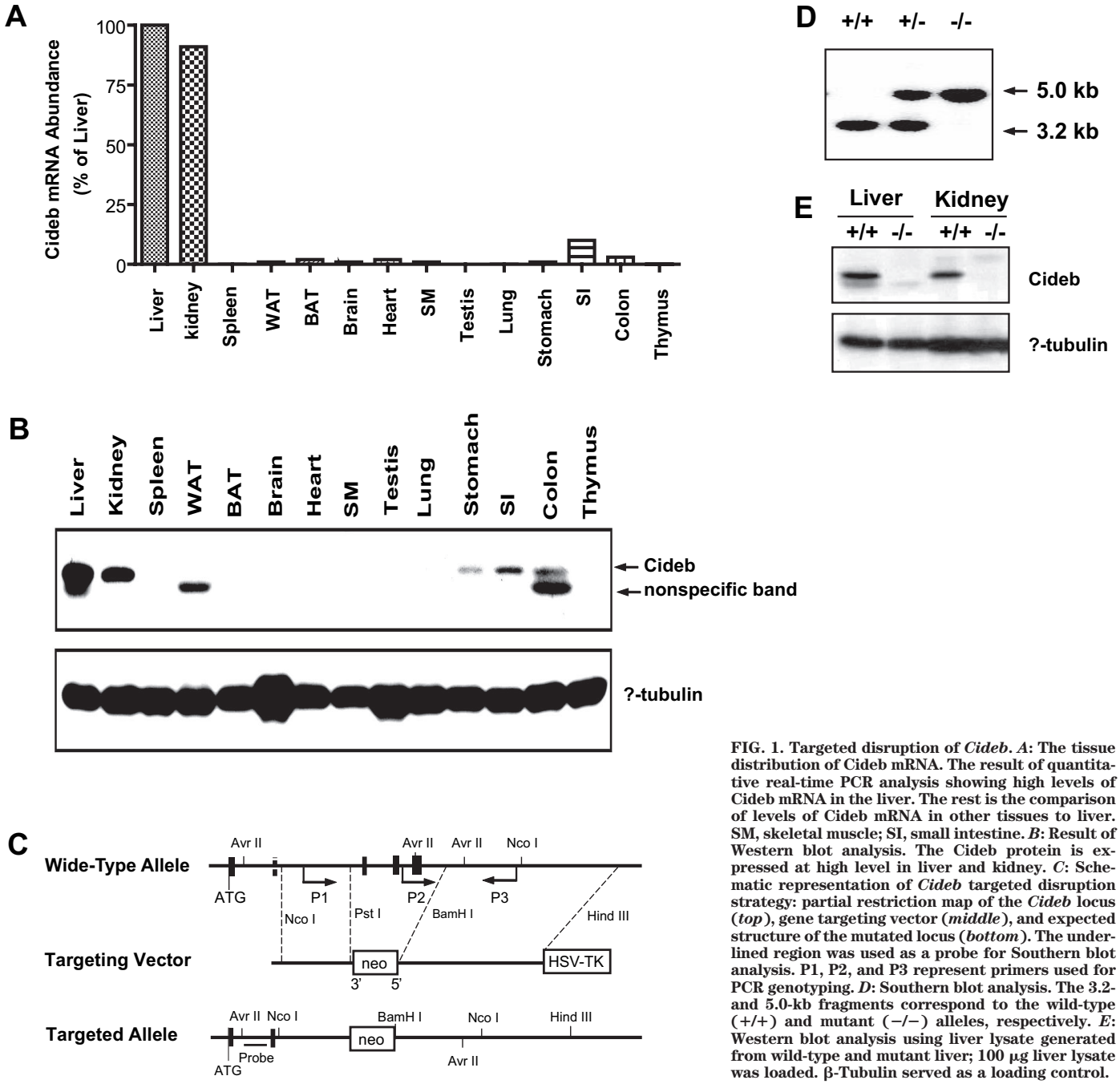

FIG. 1. Targeted disruption of *Cideb*. *A*: The tissue distribution of Cideb mRNA. The result of quantitative real-time PCR analysis showing high levels of Cideb mRNA in the liver. The rest is the comparison of levels of Cideb mRNA in other tissues to liver. SM, skeletal muscle; SI, small intestine. *B*: Result of Western blot analysis. The Cideb protein is expressed at high level in liver and kidney. *C*: Schematic representation of *Cideb* targeted disruption strategy: partial restriction map of the *Cideb* locus (*top*), gene targeting vector (*middle*), and expected structure of the mutated locus (*bottom*). The underlined region was used as a probe for Southern blot analysis. P1, P2, and P3 represent primers used for PCR genotyping. *D*: Southern blot analysis. The 3.2- and 5.0-kb fragments correspond to the wild-type (+/+) and mutant (−/−) alleles, respectively. *E*: Western blot analysis using liver lysate generated from wild-type and mutant liver; 100 μg liver lysate was loaded. β-Tubulin served as a loading control.

and diabetes (12). Recently, Cidea was shown to mediate human obesity by regulating human adipocyte lipolysis (13), and a V115F polymorphism in human was found to be associated with obesity in certain population (14). *Cideb* mRNAs were detected in various other tissues with high expression levels in the liver previously (11); however, its biological role is unclear. When overexpressed in heterologous cells, Cideb proteins can form homo-dimmer and induce caspase-independent cell death (15). In the present study, we generated *Cideb* knockout mice and showed that mice with *Cideb* deficiency exhibited increased energy expenditure and improved insulin sensitivity and are resistant to high-fat diet–induced obesity, hyperlipidemia, or liver steatosis. Our data reveal a novel pathway in controlling lipogenesis and fatty acid oxidation in the liver by Cideb.

RESEARCH DESIGN AND METHODS

Generation of *Cideb*-null mice and maintaining animals. Procedures for the isolation of genomic clones, generation of *Cideb*-deficient mice, and routine maintenance of mouse strain were essentially the same as previously described (12). Animals were fed either normal chow diet (5053, PicoLab Rodent Diet 20) or high-fat diet (D12331, 58% of kilocalories from fat; Research Diets). During a high-fat diet treatment, 3-week-old mice were subjected to high-fat diet feeding for 19 weeks. For fasting and refeeding experiments, mice in the fasting group were fasted for 24 h and in the refeeding group were fasted for 24 h and then allowed access to food and water for 12 h before analysis (16). All mouse research activities were reviewed and approved by the animal research committee of the Hong Kong University of Science and Technology and Tsinghua University.

Southern blot, genotyping by PCR analysis, RNA extraction, and real-time quantitative RT-PCR analysis. Genomic DNA was isolated from mouse tails and subjected to Southern blot analysis as previously described (17). The DNA fragment between the *Avr*II and *Nco*I sites (Fig. 1*C*) of Cideb genomic DNA were used as a probe. PCR analysis was used as a routine

TABLE 1
Metabolic parameters of wild-type and *Cideb*-null mice

Parameter	Normal diet				*P*	High-fat diet				*P*
	Wild type	*n*	*Cideb* null	*n*	value*	Wild type	*n*	*Cideb* null	*n*	value*
TAG (mmol/l) (fed)	0.55 ± 0.04	8	0.40 ± 0.03	7	0.21	0.82 ± 0.15	8	0.48 ± 0.06	8	0.0307†
NEFA (mEq/l) (fed)	1.02 ± 0.15	8	0.60 ± 0.09	7	0.03†	0.97 ± 0.06	8	0.74 ± 0.11	8	0.09
Ketone body (mmol/l) (fed)	0.10 ± 0.01	8	0.10 ± 0.02	7	0.96	0.17 ± 0.05	8	0.18 ± 0.03	8	0.80
Leptin (ng/ml) (fed)	3.50 ± 0.21	8	0.60 ± 0.14	7	0.014†	7.76 ± 0.88	8	2.80 ± 0.54	8	0.0003‡
Glucose (mmol/l) (fed)	9.41 ± 0.46	16	7.56 ± 0.20	15	0.0011§	11.04 ± 0.63	16	8.72 ± 0.29	16	0.0018§
Glucose (mmol/l) (fasted)	7.33 ± 0.26	16	6.78 ± 0.25	15	0.15	8.28 ± 0.27	16	7.39 ± 0.34	16	0.08
Adiponectin (μg/ml) (fed)	9.94 ± 0.64	8	11.72 ± 0.51	7	0.06	6.62 ± 0.61	8	8.53 ± 0.54	8	0.0466†
Resistin (ng/ml) (fed)	5.92 ± 0.74	8	6.17 ± 0.66	7	0.81	9.80 ± 1.87	8	8.59 ± 0.92	8	0.58
Body wt (g)	36.79 ± 1.58	8	33.45 ± 0.54	7	0.08	43.83 ± 1.19	8	37.05 ± 1.32	8	0.0019§
Food intake (g/day)	4.41 ± 0.046	8	5.00 ± 0.066	8	0.0206†	ND		ND		
Liver (g)	1.62 ± 0.09	8	1.51 ± 0.04	7	0.30	1.85 ± 0.08	8	1.66 ± 0.05	8	0.05
Kidney (g)	0.42 ± 0.02	8	0.43 ± 0.02	7	0.60	0.45 ± 0.01	8	0.47 ± 0.02	8	0.24
Spleen (g)	0.10 ± 0.003	8	0.07 ± 0.003	7	0.0002‡	0.113 ± 0.008	8	0.108 ± 0.008	8	0.64
Heart (g)	0.17 ± 0.002	8	0.16 ± 0.007	7	0.12	0.17 ± 0.0057	8	0.17 ± 0.005	8	0.38
Inguinal fat (g)	0.77 ± 0.14	8	0.39 ± 0.02	7	0.03†	2.13 ± 0.11	8	1.08 ± 0.17	8	<0.0001‡
Gonadal fat (g)	1.06 ± 0.19	8	0.46 ± 0.03	7	0.013†	2.47 ± 0.16	8	1.23 ± 0.17	8	<0.0001‡
Retroperitoneal fat (g)	0.57 ± 0.11	8	0.15 ± 0.01	7	0.0037§	1.12 ± 0.13	8	0.47 ± 0.07	8	<0.0001‡
Mesenteric fat (g)	0.65 ± 0.12	8	0.27 ± 0.03	7	0.0124†	1.15 ± 0.06	8	0.45 ± 0.05	8	<0.0001‡
Subcutaneous fat (g)	0.47 ± 0.09	8	0.22 ± 0.02	7	0.0275†	1.48 ± 0.12	8	0.61 ± 0.08	8	<0.0001‡

*P values are the results of nonpaired Student's t test. †$P < 0.05$. ‡$P < 0.001$. §$P < 0.01$. ND, not determined.

method for genotyping. Total RNA was isolated using the TRIzol reagent (Invitrogen, Carlsbad, CA). The first-strand cDNA were synthesized from 1 μg total RNA with oligo-$(dT)_{20}$ primers using Superscript III RT kit (Invitrogen) according to the manufacturer's protocol. Real-time PCRs were performed with ABI SYBR GREEN PCR Master Mix in the MX3000P real-time PCR system (Stratagene) according to the manufacturer's instruction. The results were normalized to β-actin level in each sample. Primer sequences are available on request.

Whole-body oxygen consumption, food intake, glucose/insulin tolerance test, adiposity index, and blood chemistry. Whole-body oxygen consumption, respiration exchange ratio, daily food intake, glucose and insulin tolerance tests (GTT and ITT, respectively), and adiposity index were performed as previously described (12). For blood chemistry, we measured serum levels of triglycerides (TAG), nonesterified fatty acids (NEFAs), insulin, leptin, and glucose as previously described (12). We used a commercial kit to determine serum levels of ketone bodies (Wako Chemical), adiponectin, and resistin (Linco Research).

Western blot analysis, ACC activity, and insulin infusion. Rabbit polyclonal antibody against mouse Cideb was generated by injecting His-tagged truncated Cideb protein (amino acids 1–176) to rabbit as previously described (18). Western blot analysis was performed essentially the same as previously described (12). Liver nuclear and membrane extracts were prepared as previously described (19). Detailed information of antibodies used for Western blot analysis is shown in an online appendix, which is available at http://dx.doi.org/10.2337/db07–0040. Western blot signal was quantified by AlphaEase software (Alpha Innotech, San Leandro, CA). ACC activity was determined using the [^{14}C]bicarbonate fixation assay (20). Insulin infusion experiment was performed using mice that were fasted for 4 h through portal vein for 3 min as previously described (21).

Statistical analysis. All data are presented as means ± SE. Differences between groups were assessed by a two-tailed, nonpaired, or paired Student's t test using the Graphpad Prism statistics software (Graphpad Software).

RESULTS

***Cideb* mRNA is highly enriched in the liver.** To characterize the precise tissue distribution pattern of Cideb, we isolated RNA from various tissues of wild-type mice and performed semiquantitative PCR analysis. We observed that Cideb is highly expressed in the liver and to a lesser extent in the kidney (Fig. 1*A*). Lower expression levels of Cideb (at least 50-fold lower compared with that in liver) were also observed in small intestine and colon. No Cideb mRNA was detected in BAT, white adipose tissue (WAT), or skeletal muscle. Western blot analysis using antibody raised against mouse Cideb further confirmed that Cideb protein is present at high levels in the liver and at moderate levels in the kidney (Fig. 1*B*).

Generation of *Cideb*-deficient mice. To elucidate the physiological role of Cideb, we isolated *Cideb* genomic DNA and generated *Cideb*-null mice by homologous recombination. The gene-targeting strategy for *Cideb* knockout is shown in Fig. 1*C*, in that the three COOH-terminal exons were replaced with the neomycin-resistant gene as a positive selection marker (Fig. 1*C*). A 1.6-kb fragment (*Nco*I-*Pst*I) at the 5′-end of the genomic locus was used as the short arm and a 7.2-kb fragment (*Bam*HI-*Hin*dIII) of the 3′ end of the genomic locus as the long arm. Southern blot analysis with probe corresponding to *Avr*II and *Nco*I fragment revealed a 5.0-kb fragment in Cideb mutant mice instead of 3.2-kb fragment in the wild-type mice, confirming that the Cideb locus had been disrupted as planned (Fig. 1*D*). As shown in Fig. 1*E*, no Cideb protein was detected in the *Cideb*-null mice. Routine genotyping of *Cideb*-null mice was carried by PCR analysis (RESEARCH DESIGN AND METHODS). These data suggest that *Cideb* gene was successfully disrupted, and a functionally null mutation of *Cideb* was obtained.

***Cideb*-deficient mice have lean phenotype.** Because our previous study on *Cidea*-null mice demonstrated that it plays a crucial role in energy homeostasis, we investigated a potential role of Cideb in regulating body weight and adiposity index. When fed with a normal chow diet, *Cideb*-null mice have a body weight similar to that of wild-type mice (Table 1). However, after 19 weeks of high-fat diet feeding, the body weight of *Cideb*-null mice was significantly lower than that of wild-type mice (Fig. 2*A*; $P < 0.001$). Under high-fat diet feeding conditions, *Cideb*-null mice attained an average body weight of 37.05 g compared with 43.83 g for wild-type mice (Table 1; $P < 0.01$). The weight of various tissues, including liver, kidney, and heart, were similar between *Cideb*-null and

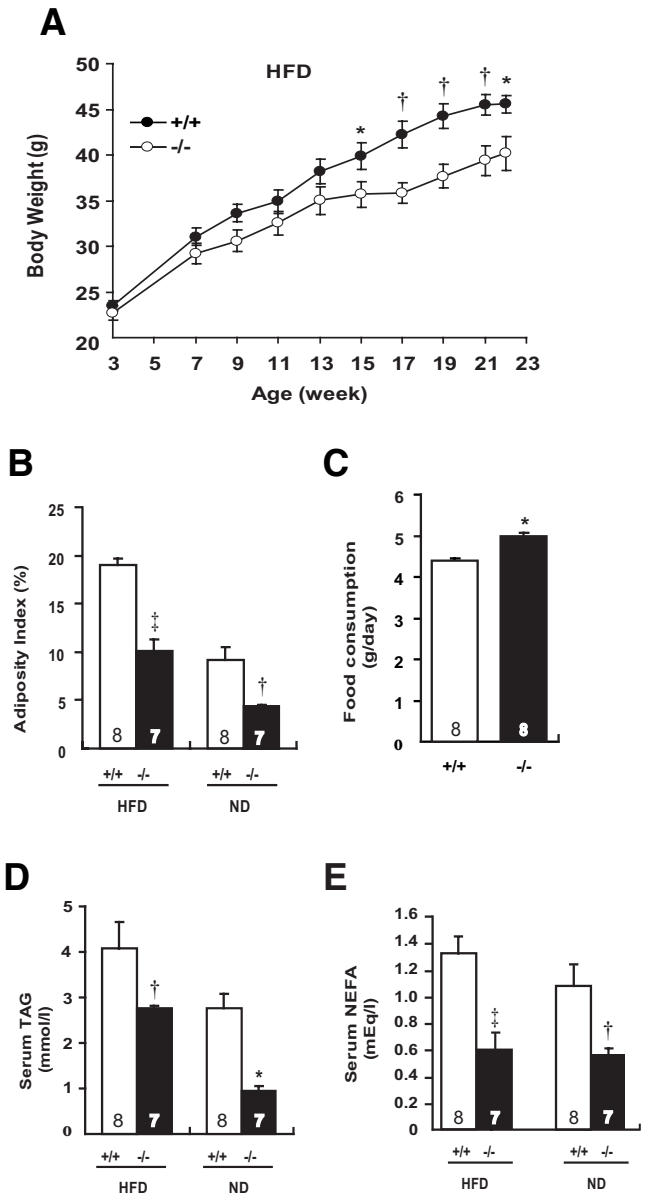

FIG. 2. *Cideb*-null mice have a lean phenotype. *A*: Body weight of male wild-type (+/+) ($n = 13$) and *Cideb*-null (−/−) mice ($n = 12$) fed with a high-fat diet. *$P < 0.05$, †$P < 0.01$. *B*: Adiposity index of mice fed with either a normal diet (ND) or a high-fat diet for 19 weeks. †$P < 0.01$, ‡$P < 0.001$. *C*: Food intake of 8-week-old wild-type and *Cideb*-null male mice. *$P < 0.05$. *D* and *E*: Levels of plasma TAG and NEFA for wild-type and *Cideb*-null male mice under fasting conditions. Number of mice examined in each group is indicated in the respective bars. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$.

wild-type mice (Table 1). Surprisingly, the weight of spleen of *Cideb*-null mice was significantly lower than that of wild-type mice when fed with normal diet (Table 1). No obvious morphological difference was observed between BAT of wild-type and Cideb mutant mice. WAT of Cideb mutant mice is more compact, and adipocytes were slightly smaller than those of wild type mice (Supplementary Fig. A, which is available in an online appendix at http://dx.doi.org/10.2337/db07-0040).

We then examined the adiposity index of mice fed with a normal diet. We found that the adiposity index in *Cideb*-null mice was 52% lower (4.44 compared with 9.22% in wild-type mice; Fig. 2*B*, $P < 0.01$). After high-fat diet feeding, wild-type mice became obese with an adiposity index of 19.01%, whereas the adiposity index of *Cideb*-null mice is 10.10% (Fig. 2*B*), representing a 46% reduction in total body fat ($P < 0.001$). Consistent with the decreased adiposity index, the weight of fat pads from different anatomic locations in Cideb-deficient mice was significantly reduced under both normal and high-fat diet feeding conditions (Table 1). In accordance with the presence of a lower amount of WAT, *Cideb*-null mice had significantly lower levels of serum leptin under both normal diet (3.5 ± 0.21 vs. 0.6 ± 0.14 ng/ml; Table 1; $P < 0.05$) and high-fat diet (7.76 ± 0.88 vs. 2.80 ± 0.54 ng/ml; Table 1; $P < 0.001$) feeding conditions. Consequently, food intake, which is regulated by plasma levels of leptin, was 10% higher in *Cideb*-null mice than in wild-type mice (Fig. 2*C*; 5.00 ± 0.066 vs. 4.41 ± 0.046 g/day; Table 1; $P < 0.05$). Furthermore, levels of adiponectin that are inversely correlated with adiposity index were increased in *Cideb*-deficient mice (Table 1), and no difference in the levels of resistin was observed between wild-type and *Cideb*-null mice. When animals were fed with a high-fat diet, levels of plasma TAG were lower in *Cideb*-null mice under both feeding (Table 1; $P < 0.05$) and fasting (Fig. 2*D*; $P < 0.01$) conditions. When fed with a normal diet, levels of plasma TAG were lower in *Cideb*-null mice (Fig. 2*D*; $P < 0.05$) under fasting conditions but were not different from wild-type mice under normal feeding conditions (Table 1). When fasted overnight, levels of plasma NEFAs were significantly lower in *Cideb*-null mice under both high-fat diet and normal diet feeding conditions (Fig. 2*E*). The reduced adiposity was not due to the reduced adipogenesis in *Cideb*-null mice because the expression levels of several adipogenic markers, such as PPARγ, adipocyte fatty acid–binding protein 2, CEBP (CCAAT enhancer binding protein)/α, CEBP/β, SREBP-1a, SREBP-1c, and SREBP-2, were similar in BAT and WAT of wild-type and Cideb mutant mice (Supplementary Figs. B and C). Our data clearly suggest that *Cideb*$^{-/-}$ mice have a lean phenotype and are resistant to diet-induced obesity.

***Cideb*-deficient mice are resistant to diet-induced liver steatosis.** Because abnormal lipid metabolism in the liver could lead to accumulation of TAG and fatty liver formation, we next measured the amount of TAG in the liver of *Cideb*-null mice and examined the liver morphology by hemotoxylin and eosin or oil red O staining. Liver TAG contents of *Cideb*-null mice were significantly lower than those of wild-type mice after feeding with a high-fat diet (Fig. 3*A*; $P < 0.01$). No difference in the amount of liver TAG was observed between wild-type and mutant mice when they were fed with normal diet (Fig. 3*B*). Morphologically, the liver of wild-type mice contained abundant lipid droplets after high-fat diet feeding (Fig. 3*C* and *E*). In contrast, livers of *Cideb*-null mice contained significantly fewer lipid droplets (Fig. 3*D* and *F*). Electron microscopy analysis further confirmed that hepatocytes of wild-type mice contained much larger lipid droplets compared with those of *Cideb*-null mice (Fig. 3*G* and *H*). The sizes of hepatic glycogen stores, as determined by periodic acid Schiff staining, were similar between *Cideb*-null and wild-type mice (data not shown). No gross morphological difference was observed in kidney, small intestine, pancreata, WAT, skeletal muscle, or heart of wild-type and Cideb mutant mice (data not shown). These data suggest that *Cideb*-null mice were resistant to high-fat diet–induced liver steatosis.

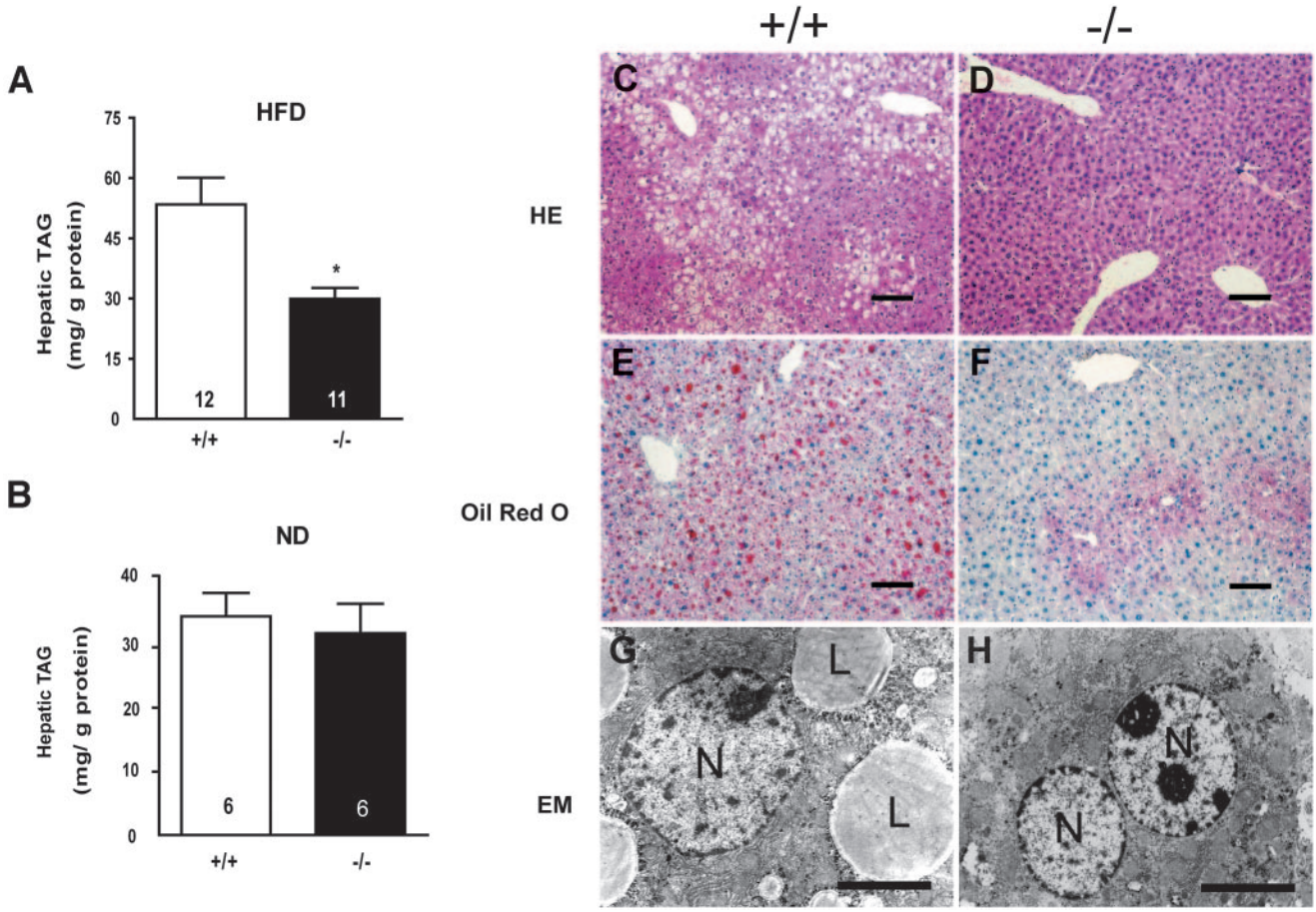

FIG. 3. *Cideb*-deficient mice are resistant to diet-induced fatty liver. *A* and *B*: Liver TAG contents from wild-type and *Cideb*-null mice after animals were fed with high-fat diet (HFD; *A*) or normal diet (ND; *B*) for a period of 19 weeks. Number of mice examined in each group is indicated in the respective bars. Each bar represents the mean ± SE values. *$P < 0.01$. *C–F*: Images of liver sections stained with hemotoxylin and eosin (HE) (*C* and *D*) or oil red O (*E* and *F*) from wild-type and *Cideb*-null mice after 19 weeks of high-fat diet treatment. The empty vacuoles in the hemotoxylin and eosin staining and the red color droplets in oil red O staining represent the lipid droplets in the liver. Scale bars = 100 μm. *G* and *H*: Transmission electron micrograph of liver sections from wild-type and *Cideb*-null mice after 19 weeks of high-fat diet treatment. N, nucleus; L, lipid droplets. Scale bars = 4 μm.

***Cideb*-deficient mice have increased fatty acid oxidation and reduced expression levels of ACC2.** We then evaluated the possibility that the anti-obesity effect of *Cideb*-null mice was due to increased energy expenditure by measuring the whole-body metabolic rate by indirect calorimetry. The *Cideb*-null mice exhibited a 10% higher rate of oxygen consumption than their wild-type littermates when fed with either a high-fat diet or a normal diet per lean body mass (Fig. 4*A*; $P < 0.05$). The derived respiratory exchange ratio (Vco_{2max}/Vo_2) was slightly lower in *Cidea*-null mice but without statistical significance (data not shown). Levels of serum ketone bodies (β-hydroxybutyrate), byproducts of fatty acid oxidation in the liver, were significantly higher in *Cideb*-null mice after 16 h of fasting in both normal and high-fat diet conditions (Fig. 4*B*; $P < 0.01$), suggesting an increased fatty acid oxidation in those mice.

To further evaluate the role of Cideb in regulating fatty acid oxidation, we examined the rate of fatty acid oxidation in isolated hepatocytes using [^{3}H]palmitic acid as a substrate. The rate of palmitic acid oxidization in isolated hepatocytes of *Cideb*-null mice was 30% higher than that of the wild-type mice at each time point tested. These data suggest that the hepatocytes of *Cideb*-null mice have increased rates of fatty acid oxidation (Fig. 4*C*). The increased fatty acid oxidation in hepatocytes was not due to increased mitochondrial activity or an upregulation of key enzymes involved in the fatty acid oxidation pathway, since the expression levels of several key proteins, such as AMP-activated protein kinase α, CPT1, CPT2, PPARδ, PPARγ coactivator (PGC)1, and Cox IV, that are involved in the regulation of fatty acid oxidation are similar between wild-type and *Cideb*-null mice (Fig. 4*D*). In addition, the increased fatty acid oxidation was not caused by an increased rate of fatty acid uptake because both wild-type and *Cideb*-null mice have similar rates of fatty acid uptake (Supplementary Fig. D). It is also important to note that fatty acid oxidation and mitochondrial activity was not increased in BAT, heart, and skeletal muscle (Supplementary Fig. E).

To further explore the mechanism underlying the increased fatty acid oxidation rate in Cideb mutant mice, we analyzed the expression levels of ACC2 in the liver tissue of wild-type and *Cideb*-null mice using quantitative real-time PCR analysis. The mRNA levels of ACC2 were 80 and 60% lower in *Cideb*-null mice under normal feeding or re-fed conditions, respectively (Fig. 4*E*). In an agreement with decreased ACC2 mRNA levels, the amount of ACC2 proteins was lower in *Cideb* mutant mice (Fig. 4*F*). These data suggest that expression levels of ACC2, a negative regulator of CPT1 activity and fatty acid oxidation, were significantly reduced in *Cideb*-null mice, providing a mo-

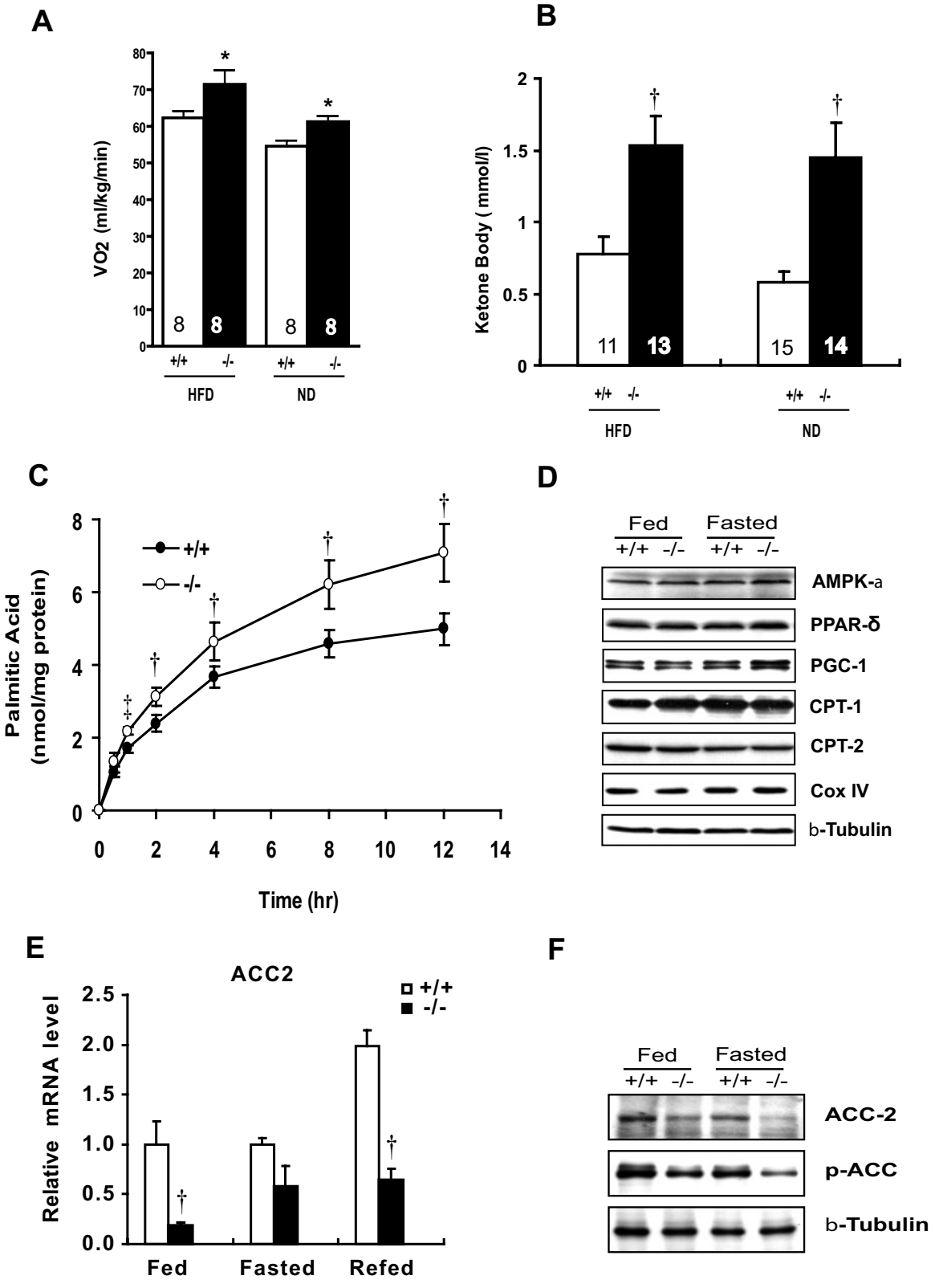

FIG. 4. Increased energy expenditure in *Cideb*-null mice. *A*: Whole-body oxygen consumption rate, represented by mean ± SE of Vo_2. *$P < 0.05$. *B*: Levels of plasma ketone body of wild-type (+/+) and *Cideb*-null (−/−) mice under fasting conditions. Number of mice examined in each group is indicated in the respective bars. †$P < 0.01$. *C*: Fatty acid oxidation in isolated hepatocytes from 3-month-old wild-type and *Cide*-null male mice ($n = 5$). †$P < 0.01$, ‡$P < 0.001$. *D*: Representative Western blot analysis of AMPKα, PPARδ, PGC1, CPT-2, and Cox IV protein levels in livers from 22-week-old wild-type and *Cideb*-null male mice ($n = 3$ each) under fed state or after overnight fasting state. *E*: Relative mRNA expression levels of ACC2 in livers of 22-week-old wild-type and *Cideb*-null mice ($n = 5$ each) in randomly fed state or after fasting with or without refeeding state by quantitative real-time PCR analysis. †$P < 0.01$. The data are shown as mean ± SE. *F*: Representative Western blot analysis of ACC2 and Phospho-ACC (p-ACC) protein levels in livers from 22-week-old wild-type (+/+) and *Cideb*-null (−/−) male mice ($n = 3$ each) under fed state or after overnight fasting, as stated.

lecular explanation of increased fatty acid oxidation in *Cideb*-null mice.

***Cideb*-deficient mice have increased insulin sensitivity.** The fall in plasma TAG and NEFAs in *Cideb*-null mice and their resistance to high-fat diet–induced obesity raised the possibility that their insulin sensitivity might also be increased. To test this, we measured levels of plasma insulin and glucose in both wild-type and *Cideb*-null mice. Levels of plasma glucose in *Cideb*-null mice under regular feeding conditions were significantly lower than those in wild-type mice (Fig. 5*A*). Surprisingly, we observed an 80% reduction in the levels of plasma insulin in *Cideb*-null mice when fed with high-fat diet and a 50% reduction when fed with normal diet (Fig. 5*B*). No difference in insulin levels was observed when animals were fasted for 16 h. Reduced insulin levels did not result from impaired β-cell function in *Cideb* mutant mice, since the stimulated plasma insulin levels in response to acute glucose loading were similar (Supplementary Fig. F). We then measured the rate of glucose disposal and insulin sensitivity in *Cideb*-null mice by GTT and ITT. *Cideb*-null mice had significantly lower levels of blood glucose after administration of an exogenous load of glucose (Fig. 5*C*; $P < 0.01$), suggesting an enhanced glucose disposal. *Cideb*-null mice showed drastically reduced levels of blood glucose when injected with excessive amounts of insulin compared with those of wild-type mice (ITT; Fig. 5*D*; $P < 0.001$). Reduced insulin levels, improved glucose disposal, and lower blood glucose levels in ITT experiments suggest that *Cideb*-null mice have enhanced insulin sensitivity.

To gain mechanistic insight into the enhanced insulin sensitivity in *Cideb*-null mice, we measured levels of tyrosine and serine phosphorylation of insulin receptor substrate (IRS)1/IRS2, the principal mediators of insulin signaling pathway in the liver. Levels of IRS-1 tyrosine phosphorylation were significantly increased in the liver of *Cideb* mutant mice after insulin stimulation (Fig. 5*E*). No difference in IRS2 phosphorylation was observed in *Cideb*-null mice (data not shown). Consistent with the increased IRS-1 tyrosine phosphorylation, the amount of phosphorylated AKT on residue Ser473, a major target of the insulin signaling pathway, was also significantly increased in *Cideb*-null mice after insulin stimulation (Fig. 5*F*). However, levels of IRS-1 tyrosine phosphorylation and AKT phosphorylation in BAT, WAT, and skeletal muscle are similar between wild-type and Cideb mutant mice (data not shown). We then measured the mRNA levels of gluconeogenic enzymes glucose-6-phosphatase (G6P) and PEPCK and glycolysis enzyme glucokinase in the apparently hypoinsulinemic *Cideb*-null mice. We observed no significant differences in mRNA levels of G6P, glucokinase, and PEPCK (Supplementary Fig. G). Furthermore, the rate of glucose production in the liver between wild-type and mutant mice is similar (data not shown). These data suggest that insulin sensitivity in the liver was improved in *Cideb*-null mice. The improved liver insulin sensitivity in *Cideb*-null mice is not dependent on adiposity, as *Cideb*-null mice with 1-month high-fat diet feeding already show improved insulin sensitivity but no difference in adiposity (data not shown).

Decreased fatty acid synthesis in *Cideb*-null mice. To further study the effect of Cideb on lipid metabolism, we measured the in vivo fatty acid synthesis rate in wild-type and *Cideb*-null mice using ^{3}H-labeled water. *Cideb*-null mice showed an ~50% reduction in fatty acid synthesis (Fig. 6*A*; $P < 0.05$) compared with that of wild-type mice. In the absence of citrate (an activator of ACC), ACC activity was low and showed no difference between wild-type and mutant mice (Fig. 6*B*). In the presence of 20 mmol/l citrate, ACC activities in both wild-type and *Cideb* mutant were significantly stimulated, and the stimulated ACC activity was 40% lower in *Cideb*-null mice compared with that in wild-type mice (Fig. 6*B*; $P < 0.001$).

To elucidate the molecular basis of reduced fatty acid synthesis in *Cideb*-null mice, we measured the mRNA levels of ACC1, FAS, and SCD1 using quantitative real-time PCR analysis. The mRNA levels of ACC1, FAS, and SCD1 were significantly lower in *Cideb*-null mice compared with those of wild-type mice under both fed and re-fed conditions (Fig. 6*C*). Because fatty acid synthesis in the liver is regulated by several classes of transcription factors, such as SREBP1a/1c, PPARα, Chrebp, and PGC1α, we measured their mRNA levels in both wild-type and *Cideb* mutant mice. The mRNA levels of SREBP1c were fivefold lower in *Cideb*-null mice than in wild-type mice (Fig. 6*D*; $P < 0.01$). The mRNA levels of SREBP1a, PPARα, Chrebp, and PGC1α were similar between wild-type and *Cideb*-null mice. Consistent with decreased mRNA levels, SREBP1c precursor in the endoplasmic reticulum or its mature nuclear form was also significantly decreased (Fig. 6*E* and *F*). Our data thus suggest that Cideb plays an important and specific role in regulating fatty acid synthesis by specifically controlling the expression of transcription factor SREBP1c and its downstream target genes. The effect of reduced lipogenesis may be due to the alteration of β-oxidation because these two pathways are linked at the level of Malonyl-CoA concentration.

DISCUSSION

Using knockout mice as a model system, we demonstrate here that Cideb, a protein expressed at high levels in the liver, plays an important role in regulating fatty acid synthesis, oxidation, and insulin sensitivity. *Cideb*-null mice exhibited a typical lean phenotype based on the following observations. First, mice deficient in *Cideb* have significantly lower body weight when fed with a high-fat diet and reduced adiposity index under both normal and high-fat diet feeding conditions. Second, levels of plasma TAG and NEFA in *Cideb*-null mice are significantly lower than those in wild-type mice. Third, the amount of high-fat diet–induced accumulation of TAG in the liver is dramatically reduced in *Cideb*-null mice. Besides its role in regulating adiposity, Cideb also plays an important role in modulating insulin sensitivity. Although *Cideb*-null mice had significantly lower levels of plasma insulin, those animals showed increased glucose disposal rate and improved insulin sensitivity by ITT. Furthermore, levels of tyrosine phosphorylation of IRS-1 and phosphorylated-AKT in liver were increased in *Cideb*-null mice. Levels of adiponectin, which enhances liver insulin sensitivity and inversely correlates with adiposity, were higher in *Cideb*-null mice. Therefore, *Cideb*-null mice have improved insulin sensitivity in the liver. The lean and insulin-sensitive phenotypes of *Cideb*-null mice are similar to those observed in *Cidea*-deficient mice, its close homolog expressed at high levels in BAT (12), pointing to a general role for Cide proteins in the homeostatic regulation of energy metabolism and insulin sensitivity. However, the increased insulin sensitivity in *Cidea*-null mice is likely contributed by peripheral tissues, such as BAT and WAT.

The anti-obesity effect of *Cideb* deficiency is in part due

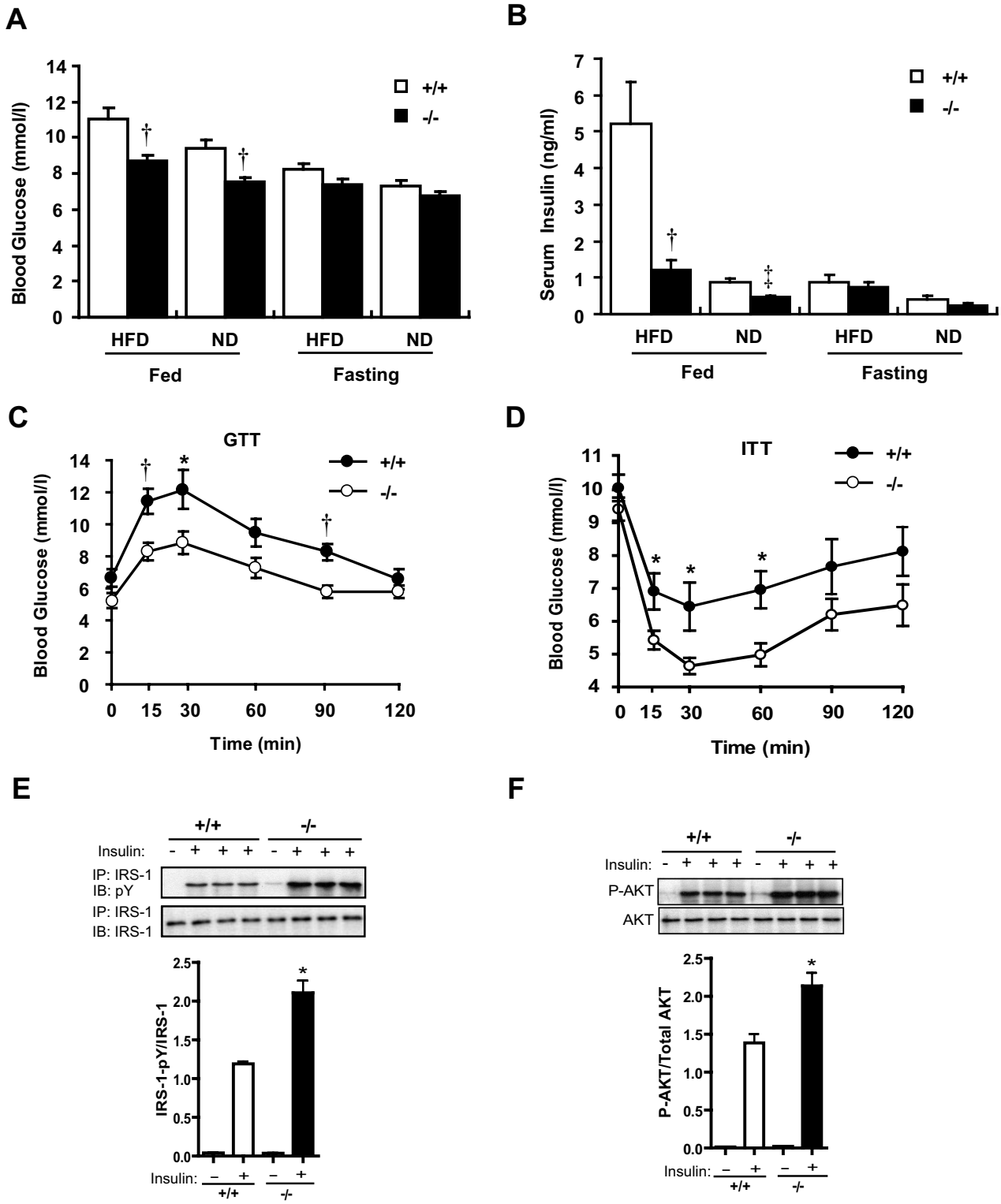

FIG. 5. Increased insulin sensitivity in the liver of *Cideb*-null mice. *A*: Blood glucose levels of wild-type (+/+) (n = 8) and *Cideb*-null (−/−) (n = 7) male mice fed with either a normal or a high-fat diet under fed or overnight fasting state. †P < 0.01. *B*: Serum insulin levels of wild-type and *Cideb*-null (n = 13 each) male mice fed with either a normal or a high-fat diet under fed or overnight fasting state. †P < 0.01, ‡P < 0.001. *C*: GTT on 10-week-old wild-type and *Cideb*-null male mice. *P < 0.05, †P < 0.01. *D*: ITT on 10-week-old wild-type and *Cideb*-null mice. *P < 0.05. n = 8 each. *E*: Western blot analysis of insulin-stimulated IRS-1 tyrosine phosphorylation (pY) and total IRS-1 levels in liver extract of wild-type (+/+) and *Cideb*-null (−/−) mice. Liver tissues were removed after insulin infusion (1 unit/kg) through portal vein for 3 min. One milligram of total liver proteins was used for immunoprecipitation (IP) with anti–IRS-1 antibody and subsequently immunoblotted (IB) with antibodies against phospho-tyrosine (pY) of IRS-1 and IRS-1. Quantification of ratio of insulin-induced IRS-1 phosphorylation to total IRS-1 levels in wild-type (+/+) and *Cideb*-null (−/−) mice is displayed (*bottom*). *F*: Western blot analysis of phospho-AKT (P-AKT) at Ser473 and total AKT levels in livers wild-type (+/+) and *Cideb*-null (−/−) male mice after insulin infusion. *Bottom* displays the quantification of ratio of insulin-induced AKT Ser473 phosphorylation to total AKT levels in wild-type (+/+) and *Cideb*-null (−/−) mice. *P < 0.05. Data are means ± SE.

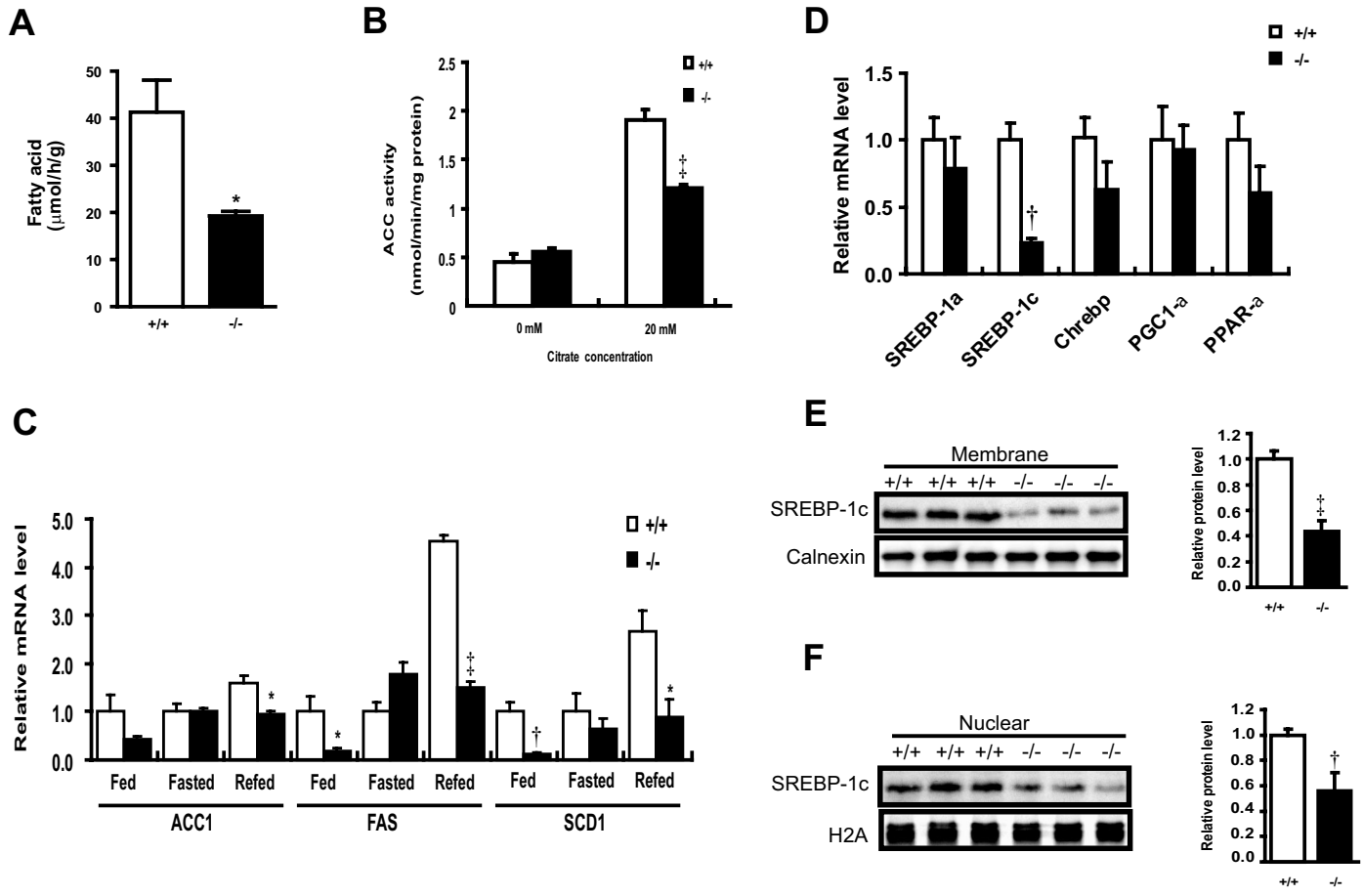

FIG. 6. Decreased lipogenesis in *Cideb*-null mice. *A*: In vivo fatty acid synthesis rate in liver was determined in 3-month-old wild-type (+/+) and *Cideb*-null (−/−) mice under normal diet conditions ($n = 3$). Each mouse was injected intraperitoneally with ^{3}H-labeled water (50 mCi in 0.1 ml isotonic saline) after fasting for 2 h. *$P < 0.05$. *B*: ACC activity in liver of wild-type and *Cideb*-null male mice ($n = 3$) in the absence (*left*) or presence (*right*) of 20 mmol/l citrate. ‡$P < 0.001$. *C*: Relative mRNA levels of ACC1, FAS, and SCD1 in livers of 22-week-old wild-type and *Cideb*-null mice ($n = 5$ each) in randomly fed state or after fasting with or without refeeding state. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$. Data are means ± SE. *D*: Relative mRNA levels of SREBP-1a, SREBP-1c, Chrebp, PGC1α, and PPARα in livers of 22-week-old wild-type (+/+) and *Cideb*-null (−/−) mice ($n = 5$ each) in randomly fed state. *E* and *F*: Western blot analysis of SREBP-1c in membrane (*E*) and nuclear (*F*) extracts from livers of wild type (+/+) and *Cideb*-null (−/−) mice ($n = 3$ each). The *right panels* represent quantitative analysis of SREBP-1c protein level in liver membrane and nuclear extract of *Cideb*-null (−/−) mice compared with wild-type (+/+) mice. †$P < 0.01$, ‡$P < 0.001$. Data are means ± SE. Calnexin and histone 2A (H2A) are loading controls for membrane and nuclear extracts, respectively.

to increased energy expenditure including whole-body metabolism and fatty acid oxidation. We showed that ACC2 expression is significantly lower in *Cideb*-null mice. It is well known that ACC2 plays a crucial role in fatty acid oxidation by converting acetyl-CoA into malonyl-CoA that in turn inhibits CPT1 activity and fatty acid transportation into mitochondria and hence lower oxidation rates. Therefore, reduced ACC2 levels could at least in part account for the increased fatty acid oxidation and energy expenditure in *Cideb*-null mice. Although we cannot completely eliminate the contribution of other tissues such WAT and skeletal muscle in the increased energy expenditure in *Cideb*-null mice, no difference in gross morphology, rate of fatty acid oxidation, mitochondrial activity, or expression levels of enzymes regulating fatty acid oxidation was observed in these tissues between wild-type and *Cideb*-null mice.

The lean phenotype could also be due to the reduced lipogenesis because we observed significantly reduced in vivo fatty acid synthesis and decreased ACC activity in the liver of *Cideb*-null mice. Cideb regulates lipogenesis at the level of transcription because the expression levels of SREBP1c and its downstream target genes ACC, FAS, and SCD1 are downregulated. Therefore, the lean phenotype of *Cideb* deficiency might be a combined effect of increased energy expenditure and decreased lipogenesis in the liver of *Cideb*-null mice, which results in the increased consumption of dietary fat and lower levels of lipid secretion. However, at this point, it is uncertain as to how these liver effects can lead to such a profound influence on adiposity. Further work will be needed to clarify whether other mechanisms could also be involved. Although the precise molecular basis of Cideb underlying the regulation of gene expression of SREBP1c and its downstream targets remains to be resolved, our data reveal a novel pathway of regulating lipid metabolism in the liver that is controlled by Cideb and provide a promising therapeutic target for various metabolic disorders, such as obesity, diabetes, and fatty liver.

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REFERENCES

1. Leonhardt M, Langhans W: Fatty acid oxidation and control of food intake. *Physiol Behav* 83:645–651, 2004
2. Bonnefont JP, Djouadi F, Prip-Buus C, Gobin S, Munnich A, Bastin J: Carnitine palmitoyltransferases 1 and 2: biochemical, molecular and medical aspects. *Mol Aspects Med* 25:495–520, 2004
3. Abu-Elheiga L, Oh W, Kordari P, Wakil SJ: Acetyl-CoA carboxylase 2 mutant mice are protected against obesity and diabetes induced by high-fat/high-carbohydrate diets. *Proc Natl Acad Sci U S A* 100:10207–10212, 2003
4. Evans RM, Barish GD, Wang YX: PPARs and the complex journey to obesity. *Nat Med* 10:355–361, 2004
5. Horton JD, Goldstein JL, Brown MS: SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 109:1125–1131, 2002
6. Oh SY, Park SK, Kim JW, Ahn YH, Park SW, Kim KS: Acetyl-CoA carboxylase beta gene is regulated by sterol regulatory element-binding protein-1 in liver. *J Biol Chem* 278:28410–28417, 2003
7. Abu-Elheiga L, Matzuk MM, Abo-Hashema KA, Wakil SJ: Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2. *Science* 291:2613–2616, 2001
8. Dobrzyn P, Dobrzyn A, Miyazaki M, Cohen P, Asilmaz E, Hardie DG, Friedman JM, Ntambi JM: Stearoyl-CoA desaturase 1 deficiency increases fatty acid oxidation by activating AMP-activated protein kinase in liver. *Proc Natl Acad Sci U S A* 101:6409–6414, 2004
9. Ntambi JM, Miyazaki M, Stoehr JP, Lan H, Kendziorski CM, Yandell BS, Song Y, Cohen P, Friedman JM, Attie AD: Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. *Proc Natl Acad Sci U S A* 99:11482–11486, 2002
10. Shimano H, Horton JD, Shimomura I, Hammer RE, Brown MS, Goldstein JL: Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. *J Clin Invest* 99:846–854, 1997
11. Inohara N, Koseki T, Chen S, Wu X, Nunez G: CIDE, a novel family of cell death activators with homology to the 45 kDa subunit of the DNA fragmentation factor. *EMBO J* 17:2526–2533, 1998
12. Zhou Z, Yon Toh S, Chen Z, Guo K, Ng CP, Ponniah S, Lin SC, Hong W, Li P: Cidea-deficient mice have lean phenotype and are resistant to obesity. *Nat Genet* 35:49–56, 2003
13. Nordstrom EA, Ryden M, Backlund EC, Dahlman I, Kaaman M, Blomqvist L, Cannon B, Nedergaard J, Arner P: A human-specific role of cell death–inducing DFFA (DNA fragmentation factor-α)-like effector A (CIDEA) in adipocyte lipolysis and obesity. *Diabetes* 54:1726–1734, 2005
14. Dahlman I, Kaaman M, Jiao H, Kere J, Laakso M, Arner P: The *CIDEA* gene V115F polymorphism is associated with obesity in Swedish subjects. *Diabetes* 54:3032–3034, 2005
15. Chen Z, Guo K, Toh SY, Zhou Z, Li P: Mitochondria localization and dimerization are required for CIDE-B to induce apoptosis. *J Biol Chem* 275:22619–22622, 2000
16. Matsumoto M, Ogawa W, Akimoto K, Inoue H, Miyake K, Furukawa K, Hayashi Y, Iguchi H, Matsuki Y, Hiramatsu R, Shimano H, Yamada N, Ohno S, Kasuga M, Noda T: PKClambda in liver mediates insulin-induced SREBP-1c expression and determines both hepatic lipid content and overall insulin sensitivity. *J Clin Invest* 112:935–944, 2003
17. Sambrook J, Fritsch EF, Maniatis T: *Molecular Cloning: A Laboratory Manual.* Plainview, NY, Cold Spring Harbor Laboratory Press, 1989
18. Brock DA, Hatton RD, Giurgiutiu DV, Scott B, Jang W, Ammann R, Gomer RH: CF45-1, a secreted protein which participates in Dictyostelium group size regulation. *Eukaryot Cell* 2:788–797, 2003
19. Sheng Z, Otani H, Brown MS, Goldstein JL: Independent regulation of sterol regulatory element-binding proteins 1 and 2 in hamster liver. *Proc Natl Acad Sci U S A* 92:935–938, 1995
20. Majerus PW, Jacobs R, Smith MB, Morris HP: The regulation of fatty acid biosynthesis in rat hepatomas. *J Biol Chem* 243:3588–3595, 1968
21. Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH, Hotamisligil GS: Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 306:457–461, 2004