Original Article

CD36-Facilitated Fatty Acid Uptake Inhibits Leptin Production and Signaling in Adipose Tissue

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Leptin plays an important role in regulating energy expenditure in response to food intake, but nutrient regulation of leptin is incompletely understood. In this study using in vivo and in vitro approaches, we examined the role of fatty acid uptake in modulating leptin expression and production. Leptin levels are doubled in the CD36-null mouse, which has impaired cellular fatty acid uptake despite a 40% decrease in fat mass. The CD36-null mouse is protected from diet-induced weight gain but not from that consequent to leptin deficiency. Leptin secretion in the CD36-null mouse is strongly responsive to glucose intake, whereas a blunted response is observed in the wild-type mouse. This indicates that leptin regulation integrates opposing influences from glucose and fatty acid and loss of fatty acid inhibition allows unsuppressed stimulation by glucose/insulin. Fatty acid inhibition of basal and insulin-stimulated leptin release is linked to CD36-facilitated fatty acid flux, which is important for fatty acid activation of peroxisome proliferator–activated receptor γ and likely contributes to the nutrient sensing function of adipocytes. Fatty acid uptake also may modulate adipocyte leptin signaling. The ratio of phosphorylated to unphosphorylated signal transducer and activator of transcription 3, an index of leptin activity, is increased in CD36-null fat tissue disproportionately to leptin levels. In addition, expression of leptin-sensitive fatty acid oxidative enzymes is enhanced. Targeting adipocyte CD36 may offer a way to uncouple leptin production and adiposity. *Diabetes* 56: 1872–1880, 2007

Adipose tissue plays a central role in nutrient sensing and energy homeostasis (1). It stores surplus calories and secretes peptide hormones or adipokines that regulate food intake and energy use. One of the most studied adipokines is leptin (2), a 16-kDa peptide released in response to food ingestion that serves to prevent obesity by limiting further intake (1–3) and enhancing fatty acid catabolism in muscle, liver, and adipose tissues (4,5). Absence of leptin in both rodents and humans results in severe obesity (6), but its effectiveness in preventing obesity in humans is limited by the development of leptin resistance (7). Centrally, resistance has been shown to involve defects in leptin transport to the hypothalamus and impaired intracellular leptin signaling (8). Peripherally, the expanding adipocytes gain the ability to resist leptin action to increase fatty acid oxidation (9).

The adipocyte function in integrating nutrient sensing and adipokine secretion plays an essential role in adiposity and maintenance of body weight (1,4). Fatty acids are abundant dietary nutrients and major component of adipose tissue stores; as such, elucidating their role in leptin production is important. High glucose and insulin (10,11) with feeding promote adipose tissue expansion and chronically stimulate leptin levels. By comparison, fatty acid regulation of leptin production is poorly understood. Fatty acid mobilization during fasting promotes adipocyte shrinkage, whereas uptake of fatty acid from hydrolysis of triglycerides in postprandial lipoproteins promotes cell enlargement. Thus fatty acids are handled by adipocytes as fasting and feeding currency, and fatty acid regulation of leptin is predicted to distinguish fatty acid uptake versus release to tailor effects to the nutritional status. In vitro, fatty acids inhibit leptin release (12,13), but this could not be documented in vivo (14,15). We speculated that the $CD36^{-/-}$ mouse would provide a unique model to explore the contribution of fatty acid uptake to leptin regulation in vivo. This mouse lacks a plasma membrane protein that facilitates cellular fatty acid uptake, and abnormalities of fatty acid metabolism are notable phenotypical traits in this mouse. $CD36^{-/-}$ adipocytes exhibit impaired lipid accumulation (16–18), but the resulting effects on leptin regulation are unknown. Our results using fatty acid administration in vivo and in vitro document a regulatory role of CD36-facilitated fatty acid uptake on adipocyte leptin expression, production, and signaling.

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ACO, acyl-CoA oxidase; CPT1, carnitine palmitoyl transferase 1; ΔCt, threshold crossing of each sample after normalization; PPARγ, peroxisome proliferator–activated receptor γ; *p*-STAT3, signal transducer and activator of transcription 3 phosphorylation; STAT3, signal transducer and activator of transcription 3; UCP2,uncoupling protein 2.

RESEARCH DESIGN AND METHODS

Animals and diets. CD36-null ($CD36^{-/-}$) and wild-type mice on the C57BL6 background (17,18) were housed in a facility with a 12-h light cycle and fed ad libitum chow (5001; Purina, St. Louis, MO) or high-fat (F3282; Bio-Serv, Frenchtown, NJ) diets. Mice (5 weeks) were fed the high-fat diet for 15 weeks to induce obesity with body weight measured weekly.

Energy balance. Studies were performed ($n = 6$ per group) in metabolic chambers (Metabowl; Jencons Scientific), as described previously (19). Energy intake was obtained by measuring food consumption multiplied by the diet caloric content (2.98 kcal/g). Metabolic rate was determined by indirect calorimetry with an Oxymat/Ultramat 6 analyser (Siemens) that monitors O_2 and CO_2 concentrations using data acquisition hardware (Analogic) and software (Labtech). The respiratory quotient equals Vco_2/Vo_2. To measure activity, mice were housed for 24 h individually at 18°C and a 12-h light cycle. Cages were placed into frames with infrared photocells interfaced with a computer (San Diego Instruments). Body composition was by dual energy X-ray analysis (GE Lunar, Madison, WI).

Intragastric glucose and fat loads on blood leptin. Fasted (16 h) $CD36^{-/-}$ and wild-type mice were given via an intragastric tube 500 ml saline or saline with glucose (7.46 mg/g) or olive oil emulsion (3.5 mg/g) (20). Blood and organs were collected 2.5 h later. Plasma lipids and glucose were tested enzymatically (16), and insulin was tested by RIA (Linco, St. Louis, MO).

Adipocyte sizing. Epididymal fat was formalin-fixed, ethanol-dehydrated, transitioned into xylene, and paraffin-embedded. Sections (3 mm) were stained with hematoxylin-eosin. Sections were viewed at ×10 magnification with a Nikon Eclipse TE2000-U microscope in epiflourescence using an EXFO-Xcite-TM120 PC Illumination System and a Photometrics Cool SNAPcf monochrome camera (Roper Scientific, Tuscon, AZ). Morphometery was by MetaMorph v6.2r6 (Universal Imaging Corp) with 0.45 μm/pixel. Mean cell size was calculated using Excel (Microsoft, Redmond, WA).

Adipose tissue culture and leptin measurement. Epididymal fat from six to eight wild-type and $CD36^{-/-}$ mice was minced, and 500-mg samples were placed in 2 ml serum-free M199 under 95/5 O_2/CO_2 with daily medium replenishment (21). Leptin production was the sum of leptin in medium and tissue. Tissue (500 mg) was homogenized (Teflon pestle, clearance B) in 2 ml cold buffer (10 mmol/l Tris, 1 mmol/l EDTA, and 250 mmol/l sucrose, pH 7.4) with 3.5 μg/ml aprotinin, 2.5 μg/ml leupeptin, and 1 mmol/l phenylmethylsulfonyl fluoride. After centrifugation (14,000 rpm, 15 min, 4°C) infranatants and media were assayed for leptin by ELISA (Linco). Adiponectin was assayed as described previously (22) or by ELISA (Linco).

Fatty acid incorporation into lipids was as previously described (17). Fatty acid oxidation was measured from the CO_2 produced from [^{14}C]oleic acid (23) using isolated adipocytes washed four times in buffer with 1% albumin.

Western blots. Tissues were homogenized (50 mmol/l Tris, pH 7.5, 150 mmol/l NaCl, 1% Triton X-100, 1% sodium deoxycholic acid, 1 mmol/l phenylmethylsulfonyl fluoride, 50 mmol/l sodium fluoride, 1 mmol/l sodium orthovanadate, 50 μg/ml aprotinin, and 50 μg/ml leupeptin). After centrifugation (15 min at 4°C), proteins in supernatants separated by SDS-PAGE were transferred to nylon-enhanced nitrocellulose membranes that were blocked in 0.1% Tween/PBS with 5% fat-free milk (1 h) before incubation with antibodies for peroxisome proliferator–activated receptor γ (PPARγ) (Upstate, Lake Placid, NY) (1:1,000, 2 h, room temperature), signal transducer and activator of transcription 3 (STAT3), or *p*-STAT3 (Cell Signaling, Danvers, MA) (1:1,000, overnight, 4°C). After three washings with 0.1% Tween/PBS, the membranes were re-blocked and incubated with peroxidase-labeled secondary antibody (1 h, 1:10,000). Immunodetection was by ECL (Amersham, Arlington, IL), and band intensity was determined by densitometry scanning (Umax) with analysis by ImageJ (National Institutes of Health, Bethesda, MD).

Quantitative PCR. Isolated RNA (Trizol; Invitrogen) was washed in 75% ethanol, dried, and resuspended in UltraPure Water (Gibco). Amplification was using Superscript III Platinum SYBR Green (Invitrogen) on a SmartCycler (Cephied, Sunnyvale, CA). Cyclophilin, bActin, or 18S was used to normalize expression of tested genes. The threshold crossing of each sample after normalization (ΔCt) was used to calculate levels of each mRNA relative to controls using $2^{-\Delta Ct}$. Primers used are listed in the online appendix (available at http://dx.doi.org/10.2337/db06–1699).

Statistical methods. Data are shown as means ± SE and tested for statistically significant differences using *t* tests and $P < 0.05$. Differences in growth curves between groups were compared by ANOVA followed by the Tukey-Kramer test when appropriate.

RESULTS

Plasma leptin in wild-type and $CD36^{-/-}$ mice. The $CD36^{-/-}$ mouse exhibits a major defect in fatty acid uptake by adipocytes (17,18), providing a unique model for examining the physiological role of fatty acid uptake on leptin secretion. If adipocyte fatty acid uptake regulates leptin, one might predict that the $CD36^{-/-}$ mouse, with high fatty acid levels but impaired fatty acid uptake and metabolism, would lack this regulatory effect. We compared blood leptin levels in CD36-null and wild-type mice. As shown in Fig. 1, plasma leptin was twofold higher in $CD36^{-/-}$ mice compared with age- and sex-matched wild-type mice (Fig. 1*A*). Leptin levels in females were 40% higher than in males, but similar effects of genotype on leptin were observed regardless of sex (data not shown), therefore, only male mice were used for the rest of the studies. The hyperleptenemia in $CD36^{-/-}$ mice was associated with increased leptin mRNA in adipose tissue (Fig. 1*B*), suggesting that it was due to enhanced transcription of the ob gene. To determine specificity of the effect of CD36 deficiency on leptin, we measured levels of the adiponectin (Fig. 1*C* and *D*). Blood levels of adiponectin and its adipose tissue expression were significantly lower in $CD36^{-/-}$ compared with wild-type mice. Thus CD36 deficiency affected adiponectin in the opposite direction to leptin.

The high leptin levels in $CD36^{-/-}$ mice did not reflect increased adiposity. Epididymal fat pads of $CD36^{-/-}$ mice (Fig. 1*E*) weighed less than those of wild-type mice, and mean adipose cell size was similar for tissues from both genotypes (Fig. 1*F*).

Energy balance of $CD36^{-/-}$ mice. To determine whether the increased leptin levels in $CD36^{-/-}$ mice were effective in limiting energy intake and fat deposition, food intake, growth curves, fat versus lean body mass, bone density, metabolic rate, and respiratory quotient were examined. $CD36^{-/-}$ mice gained less body weight than wild-type mice (Fig. 2*A*) with differences becoming significant ($P < 0.01$) for the period of 12–16 weeks. Lean body mass was similar, but total body fat was 38% lower in $CD36^{-/-}$ mice (Fig. 2*B*), in line with the lower fat pad weight. Bone mass (Fig. 2*B*, *right*) was significantly decreased (12%) in $CD36^{-/-}$ mice, which may be consistent with reports documenting leptin inhibition of bone formation (24). Food intake (Fig. 2*C*), determined during the 24-h calorimetry, was 20% lower for $CD36^{-/-}$ mice compared with age-matched controls. $CD36^{-/-}$ mice had a similar metabolic rate to that of wild-type mice when the metabolic rate is related to unit body weight or lean body mass (Fig. 2*D*). Without this adjustment, metabolic rate of $CD36^{-/-}$ mice was 15% lower than that of wild-type mice ($P < 0.05$). The respiratory quotient was similar for both genotypes (Fig. 2*E*).

The lack of change in the respiratory quotient (Fig. 2*E*) suggested that fuel oxidation was similar for both genotypes. $CD36^{-/-}$ mice exhibit decreased fatty acid utilization in peripheral tissues (16,17). In contrast, hepatic fatty acid uptake is slightly increased (17,25). We examined circulating ketone bodies in wild-type and $CD36^{-/-}$ mice as an index of hepatic fatty acid oxidation. Plasma β-hydroxybutyrate levels (mg/dl) were increased (12.3 ± 1.0 in $CD36^{-/-}$ versus 7.8 ± 1.1 in wild-type, $P < 0.05$, $n = 9$), suggesting enhanced hepatic fatty acid oxidation. The reductions in energy intake and fat mass and the increase in blood ketone bodies in $CD36^{-/-}$ mice were consistent with known actions of leptin to reduce energy intake (3) and to increase hepatic fatty acid oxidation (26).

We also compared the effects of fat feeding on body weight and leptin in wild-type versus $CD36^{-/-}$ mice. As shown (Fig. 3*A*), $CD36^{-/-}$ mice gained significantly less

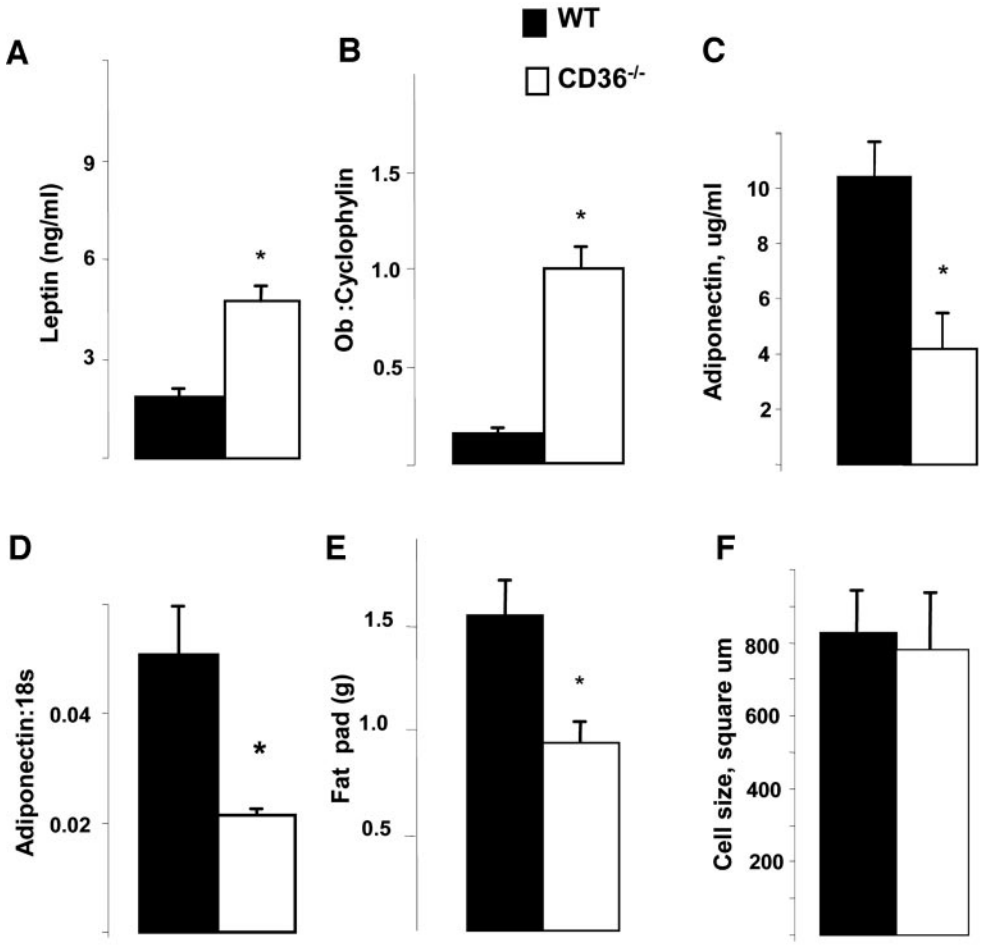

FIG. 1. Blood levels and adipose tissue expression of leptin and adiponectin in wild-type (WT) and CD36-null ($CD36^{-/-}$) mice. Blood was obtained from age- (12–16 weeks) and sex-matched wild-type or CD36-null mice (n = 12/group) in the fed state. Differences between groups were evaluated for significance using the t test. *$P < 0.01$.

weight than wild-type mice. Fat pad mass at the end of the diet period was 80% larger in wild-type compared with $CD36^{-/-}$ mice (Fig. 3*B*). Leptin levels in $CD36^{-/-}$ mice were twice those of wild-type mice, and ob gene expression in $CD36^{-/-}$ adipose tissue was three times higher (Fig. 3*C* and *D*, respectively). These data indicated that $CD36^{-/-}$ mice were protected from diet-induced obesity. To determine whether CD36 deletion would protect from obesity consequent to leptin deficiency, we generated a mouse double null for CD36 and leptin by crossing $CD36^{-/-}$ and *ob/ob* mice. The $CD36^{-/-}$-*ob/ob* mouse (Fig. 3*E*) was obese, indicating that blocking fatty acid uptake does not reverse obesity of the *ob/ob* mouse and supporting the contribution of leptin to the lean phenotype of CD36 deficiency.

Mechanism for the increased leptin levels. Levels of insulin and glucose, which chronically induce leptin (10), were similar in fed wild-type and $CD36^{-/-}$ mice (data not shown). Fasted $CD36^{-/-}$ mice were hypoinsulinemic and hypoglycemic (data not shown) compared with wild-type mice, consistent with previous findings (16,17). However, leptin levels of fasted $CD36^{-/-}$ mice were still double those of fasted wild-type mice (data not shown).

The acute response of leptin secretion to nutrient ingestion was examined next by monitoring leptin after intragastric and equicaloric doses of glucose or olive oil. As expected (Fig. 4), the glucose load was cleared faster in $CD36^{-/-}$ mice (Fig. 4*A*), whereas no significant effects were observed on blood fatty acid levels (Fig. 4*A*, *inset*), which were significantly higher in the $CD36^{-/-}$ group. Glucose increased plasma leptin in wild-type mice by 21% at 2 h, (from 3.9 to 4.7 ng/ml, $P < 0.05$). At 4 h, leptin levels did not differ from basal or pre-glucose levels. The response of leptin to intragastric glucose was strongly enhanced in $CD36^{-/-}$ mice. Levels rose from 5.7 to 8.5 ng/ml at 2 h and up to 9.5 at 4 h after gavage (by 49 and 67%, respectively, $P < 0.01$) (Fig. 4*B*). Gavage with olive oil (Fig. 4*C* and *D*) produced little effect on blood leptin in wild-type or $CD36^{-/-}$ mice. Glucose levels were unaltered in both groups, but blood fatty acid increased in $CD36^{-/-}$ mice (Fig. 4*C*), consistent with delayed fatty acid clearance. Together, the above data suggest that insulin and glucose are potent stimulators of leptin production, as previously reported (10). However, the stimulatory effect is antagonized by CD36-facilitated fatty acid uptake in vivo. CD36 deletion allows uninhibited rise in leptin levels in response to glucose and insulin.

CD36 recognizes a variety of ligands in addition to fatty acid (rev. in 27). To further establish the link between CD36-facilitated fatty acid uptake and leptin inhibition, we examined adipose tissue from wild-type and $CD36^{-/-}$ mice ex vivo in the absence of CD36 ligands other than fatty acid. $CD36^{-/-}$, compared with wild-type adipose tissue, exhibited a 60% defect in fatty acid uptake and incorpora-

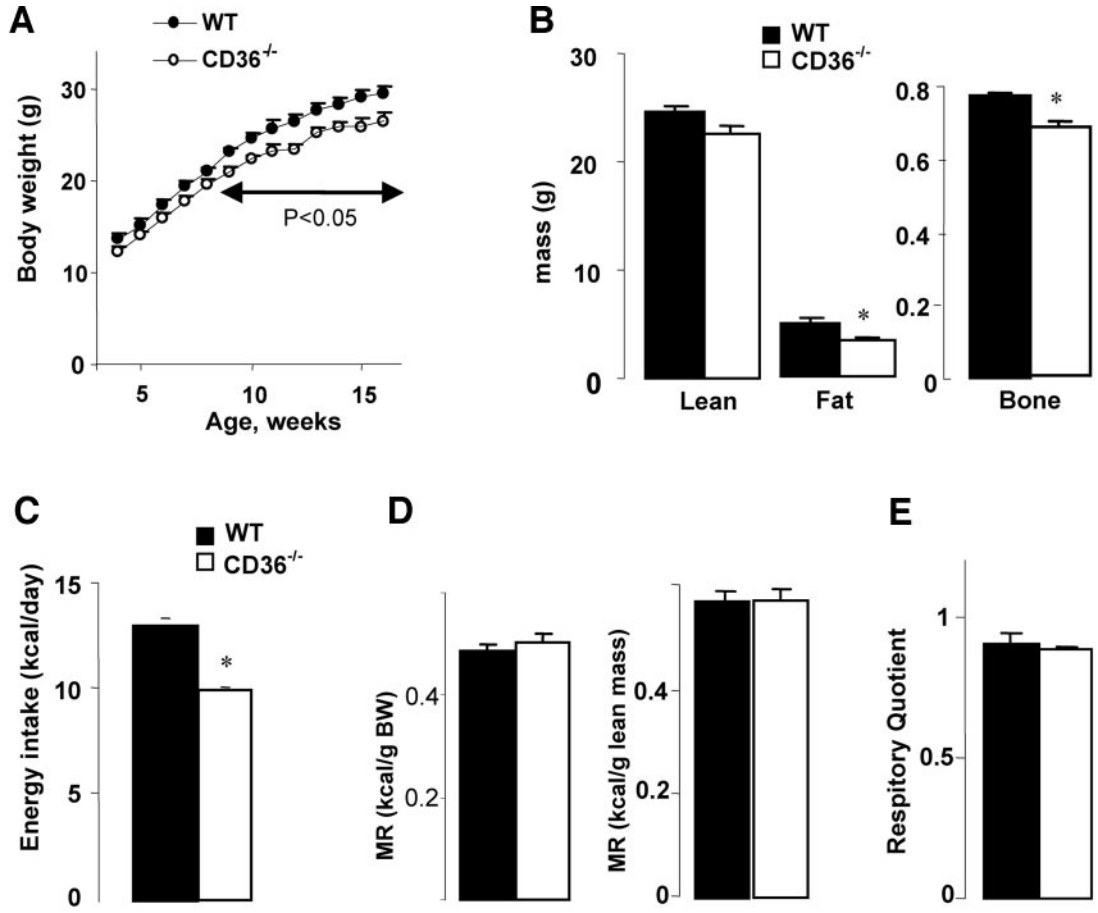

FIG. 2. The energy balance of wild-type and CD36-null mice. *A*: Body weight. *B*: Body composition showing lean mass, fat mass, and bone density. *C*: Energy intake. *D*: Metabolic rate expressed per gram body weight or per gram lean mass. *E*: Respiratory quotient. Measurements were obtained as detailed in RESEARCH DESIGN AND METHODS. *$P < 0.05$, $n = 6–8$.

tion into triglycerides (data not shown) as previously reported (17,18). Leptin secretion was twofold higher in tissue from CD36$^{-/-}$ compared with wild-type mice (Fig. 5*A*). Addition of either palmitic or oleic acids (0.075 mmol/l, 0.3 mmol/l BSA, unbound fatty acid 1.6 and 1.8 nmol/l for palmitate and oleate, respectively) significantly reduced basal leptin production by wild-type tissue but had no effect on CD36$^{-/-}$ tissue (Fig. 5*B*). Addition of insulin (Fig. 5*C*) stimulated leptin production by about threefold in cultures from wild-type or CD36$^{-/-}$ mice (compare *y* axes in Fig. 5*B* and *C*). Importantly, the insulin effect was inhibited in wild-type tissue when fatty acids were added (~60%), whereas no inhibition occurred in CD36$^{-/-}$ tissue. These data indicate that CD36–fatty acid uptake antagonizes the stimulatory effect of glucose and insulin on leptin production. Fatty acid inhibition is lost in CD36 deficiency where insulin plus glucose can fully activate leptin release. Thus fatty acid uptake by CD36-independent mechanisms, which accounts for ~40% of uptake, is ineffective in suppressing leptin. The in vivo rise in blood leptin in CD36$^{-/-}$ mice given glucose (Fig. 4*B*) would reflect uninhibited glucose/insulin stimulation as a result of impaired fatty acid sensing by adipocytes.

PPARγ activity in CD36$^{-/-}$ adipose tissue. PPARs are activated by fatty acid or their derivatives and are essential to the nutrient-sensing function of adipose tissue, which includes leptin secretion. Thiazolidinediones, which activate PPARγ, inhibit leptin release (13,28,29), and mice heterozygotes for PPARγ deficiency exhibit high leptin (30). We examined whether the high leptin of CD36$^{-/-}$ mice could reflect a defect in fatty acid activation of adipose tissue PPARγ. We tested levels and activity of adipose tissue PPARγ in mice after intragastric administration of saline or olive oil. Levels of PPARγ protein at 2.5 h after saline gavage were reduced in CD36$^{-/-}$ adipose tissue (Fig. 6, *top*). The effect of intragastric oil administration on expression of the PPARγ targets INSIG-1 (31) and aP2 (32) is shown in Fig. 6, *bottom*. Increases were observed in wild-type tissue but not in CD36$^{-/-}$ tissue. Expression of ap2 in the experiment shown in Fig. 6 trended lower without reaching statistical significance. However, basal ap2 expression measured in two experiments was significantly decreased by 45% ($n = 5$, $P = 0.03$). The data suggest impaired basal levels, basal activity, and fatty acid responsiveness of PPARγ in CD36$^{-/-}$ tissue. This helps explain the reduced adipose tissue expression and blood levels of adiponectin (Fig. 1) in CD36$^{-/-}$ mice because adiponectin is induced by PPARγ (33).

Leptin sensitivity of CD36$^{-/-}$ adipose tissue. The energy balance data suggested leptin sensitivity was intact in CD36$^{-/-}$ mice despite the hyperleptinemia. To examine leptin sensitivity of adipose tissue, we compared expression of fatty acid–oxidative genes previously shown responsive to leptin (9) in tissues from wild-type and CD36$^{-/-}$ mice (Fig. 7). Expression of carnitine palmitoyl transferase 1 (CPT1), uncoupling protein 2 (UCP2), and acyl-CoA oxidase (ACO) was significantly increased in

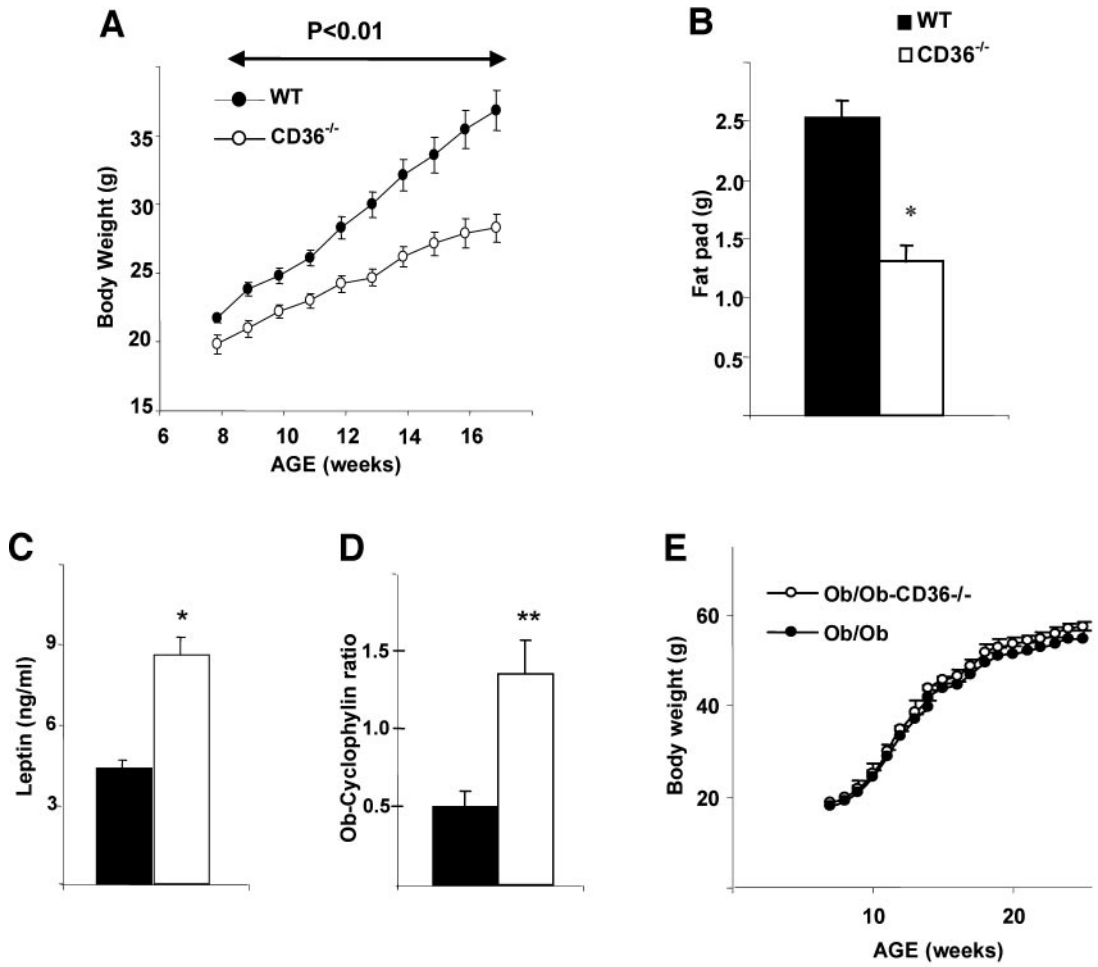

FIG. 3. High-fat feeding and generation of a CD36-deficient mouse on the *ob/ob* background. Wild-type and CD36$^{-/-}$ mice (n = 10 per group) were fed a diet high in saturated fat beginning at 8 weeks. *A*: Body weight. *B*: Fat pad weight. *C*: Plasma leptin. *D*: Leptin expression in epididymal fat tissue. *P < 0.01, **P < 0.001. *E*: Mice double null for CD36 and leptin were generated by crossing the CD36-null mouse with the *ob/ob* mouse. Growth curves are shown for the double-null mouse compared with the *ob/ob* mouse on a chow diet (n = 12 per genotype).

CD36$^{-/-}$ adipose tissue. Consistent with this, oleate oxidation in adipocytes isolated from CD36$^{-/-}$ compared with wild-type mice was enhanced (0.02 vs. 0.01 nmol · 10,000 cells^{-1} · h^{-1}, respectively; $P < 0.01$). We also examined leptin signaling. Leptin stimulates several intracellular signaling pathways. The pathway involving STAT3 is central to metabolic effects of leptin, and its dysfunction results in obesity and leptin resistance (34,35). STAT3 phosphorylation (*p*-STAT3), a sensitive indicator of leptin activity (4,34,35) was ~10-fold higher in CD36$^{-/-}$ compared with wild-type tissue, whereas total STAT3 content was similar (Fig. 8). The higher *p*-STAT3–to–STAT3 ratio reflected the ambient hyperleptinemia (2.2 vs. 4 ng/ml for wild type and CD36$^{-/-}$, respectively) and indicated that leptin sensitivity was intact. The fact that the 10-fold increase in *p*-STAT3 to STAT3 was out of proportion with the 1.8-fold increase in leptin levels suggested enhanced leptin signaling.

DISCUSSION

The present study examined the role of fatty acid uptake in leptin regulation using both in vivo and in vitro approaches. The following novel findings were obtained related to nutrient regulation of leptin production: First, uptake of dietary fatty acid via CD36 inhibits leptin production by adipocytes blunting stimulation by glucose and insulin. CD36 deletion allows uninhibited stimulation of leptin by glucose and insulin, which uncouples the normal association between adipose tissue mass and leptin level. Second, the effect of CD36-facilitated fatty acid uptake on leptin production appears linked to low PPARγ levels and activity in CD36$^{-/-}$ adipose tissue. Because fatty acid uptake still occurs in CD36$^{-/-}$ by CD36-independent mechanisms, the CD36–fatty acid pathway may modulate PPARγ levels and supply the fatty acid–derived ligand for PPARγ activation. Third, leptin sensitivity is intact and possibly enhanced in CD36 deficiency despite the hyperleptinemia. CD36$^{-/-}$ adipocytes exhibited upregulated *p*-STAT3 and fatty acid oxidative enzymes responsive to leptin. These data indicate that CD36-facilitated fatty acid uptake modulates leptin production in vivo and may contribute to leptin resistance of adipose tissue.

Dietary fatty acid regulation of leptin production. Glucose and insulin stimulate leptin production in vitro (10,11). In vivo, intragastric glucose had modest and transient effects on leptin levels in wild-type mice versus strong and sustained upregulation in CD36$^{-/-}$ mice. This indicates that glucose and insulin are potent stimulators of leptin secretion in vivo but their effect is blunted by fatty

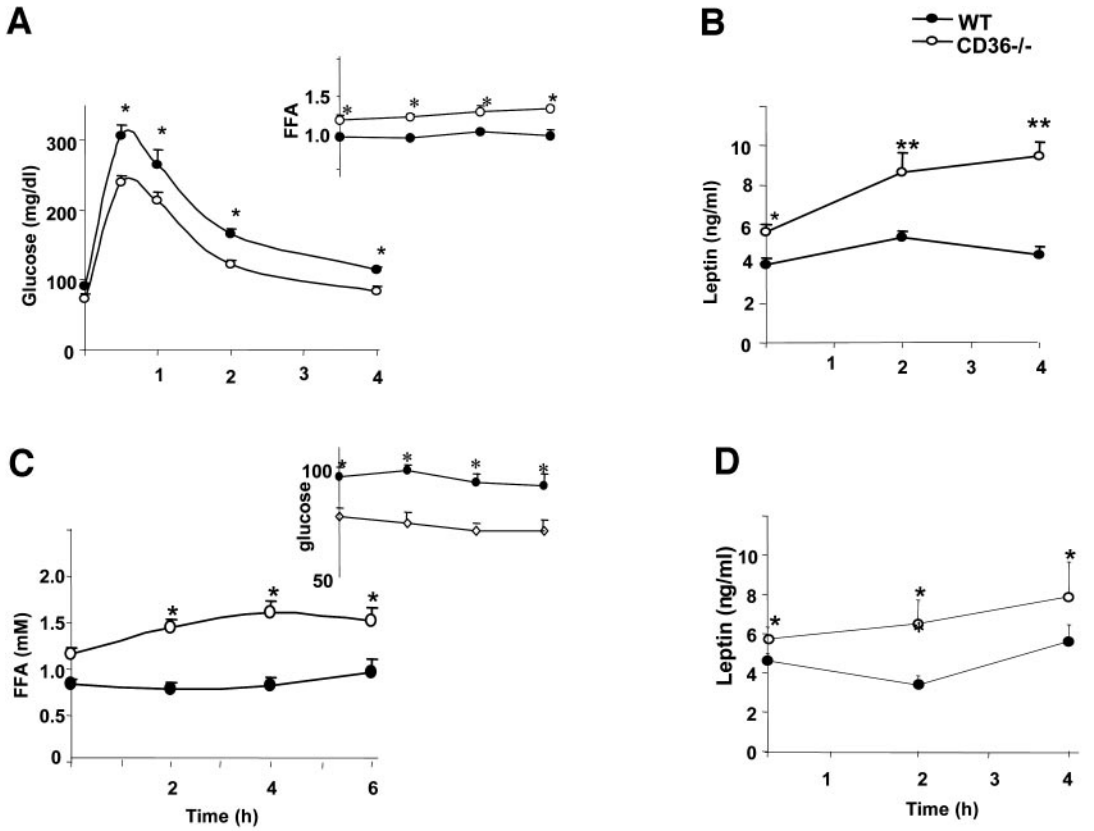

FIG. 4. Leptin response to intragastric administration of glucose or olive oil. Male wild-type and CD36$^{-/-}$ mice (n = 6 per group) were fasted (16 h) and then given via an intragastric tube either glucose or olive oil equivalent to 28.4 cal/g body mass. Blood was collected at the times indicated for measuring circulating leptin, glucose, and fatty acids. *A*: Glucose levels after glucose gavage; *inset*: fatty acid levels at 0, 2, 4, and 6 h. *B*: Leptin levels after glucose gavage. *C*: Fatty acid levels after gavage with olive oil; *inset*: glucose levels at 0, 2, 4, and 6 h. *D*: Leptin levels after oil gavage. Statistically significant differences between wild-type and CD36$^{-/-}$ mice are indicated by *P < 0.05, **P < 001.

acid. As further confirmed in vitro, absence of fatty acid influx via CD36 is required for full stimulation of leptin production by insulin and glucose.

Fatty acid inhibition, like glucose stimulation of leptin production, likely contributes to the nutrient sensing function of adipose tissue. Fatty acid inhibition was specific to CD36-facilitated fatty acid uptake because the CD36-independent component (40%) was ineffective in suppressing leptin or inducing adiponectin. The effect of CD36-mediated fatty acid uptake on leptin may in part be exerted via modulating insulin signaling and/or glucose metabolism. It could be argued that CD36 deficiency enhances insulin sensitivity of adipocytes, which would stimulate leptin production. Although such an effect could contribute to chronic leptin regulation, it would not explain why adiponectin expression is decreased because this usually correlates with insulin resistance. Previously, disruption of the adipocyte insulin-responsive glucose transporter (36) did not alter circulating leptin, but the response of leptin to nutrient intake and in particular to fatty acid was not tested. As a result, additional work is needed to fully understand how the metabolism of glucose and fatty acid might interact in regulating leptin production. Although we believe that the data in this study, when taken together, strongly suggest an important role for the transcription factor PPARγ in mediating inhibitory effects of CD36-facilitated fatty acid uptake on leptin levels, they do not rule out contribution of additional mechanisms such as changes in insulin signaling or glucose metabolism. PPARγ is a major fatty acid sensor that, when activated, inhibits release of leptin (14,29,37) and induces that of adiponectin (33). CD36 deletion resulted in lower PPARγ protein levels with reduced basal expression of PPARγ targets: ap2 by 45%, adiponectin by 60% (n = 5, P = 0.008), and lipoprotein lipase by 50% (n = 4, P < 0.04). In addition, there was blunted PPARγ activation by fat administration in CD36-null mice (Fig. 6). Possibly, activity of the fatty acid–CD36-PPARγ pathway with subsequent suppression of leptin and induction of adiponectin would promote clearance of nutrients from the circulation. Less leptin favors fatty acid storage in adipose tissue, whereas more adiponectin promotes their oxidation in muscle and liver (33).

Fatty acids on peripheral leptin sensitivity. Leptin sensitivity was intact in CD36$^{-/-}$ mice despite the hyperleptinemia as shown by the reduced food intake, the decreased bone density, the enhanced hepatic fatty acid oxidation, and the induction of leptin-responsive fatty acid–catabolizing enzymes in adipose tissue. In addition at the level of adipose tissue, the increase in leptin signaling estimated from the p-STAT–to–STAT ratio was disproportional to the increased in vivo leptin levels, also supporting enhanced sensitivity. The data suggest that the fatty acid–CD36 pathway may be relevant to the development of leptin resistance of adipose tissue.

The effects of leptin on peripheral tissues are less well characterized than its central effects, and this is especially true of adipose tissue of which little information is available. Leptin does not appear to regulate glucose uptake by adipocytes (38), but there is evidence that it reduces fatty

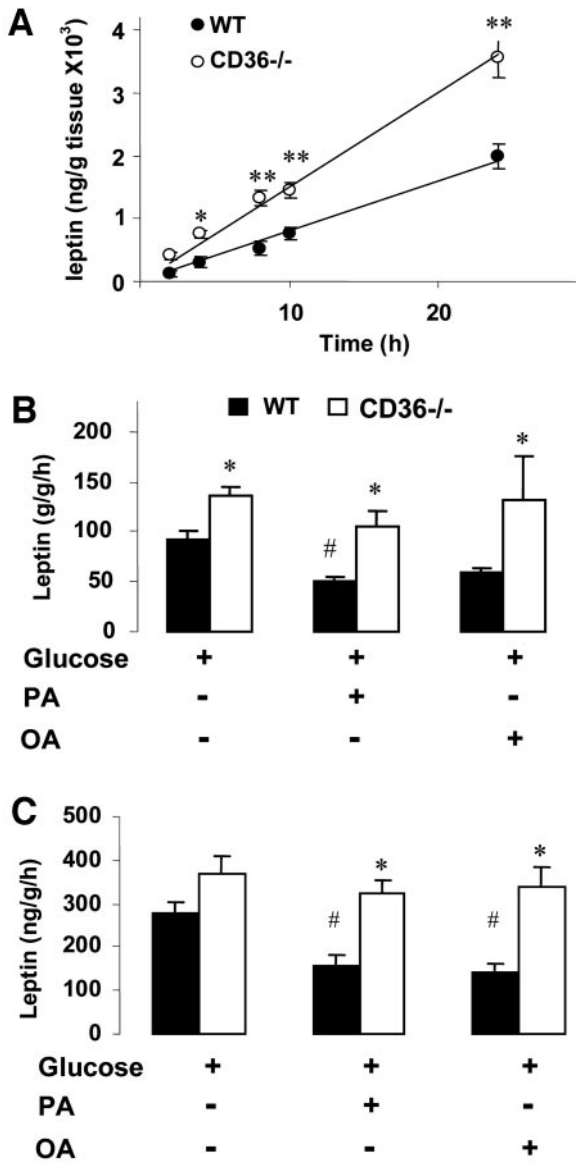

FIG. 5. Leptin production by cultured adipose tissue. *A*: Time-dependent secretion of leptin from adipose tissue of wild-type or CD36-null mice, $n = 6$ mice per group. Regulation of leptin production by palmitate and oleate alone (*B*) and palmitate and oleate (0.075 mmol/l, 0.3 mmol/l BSA) in combination with 100 nmol/l insulin (*C*). Adipose tissue from wild-type and CD36-null mice ($n = 3$–9) were preincubated for 24 h in medium containing either palmitate (PA) or oleate (OA) complexed with BSA (0.25:1) alone or in presence of 100 nmol/l insulin. Leptin production was measured after a 3-h incubation. Data are means shown with their SE and are from two experiments done in triplicates. *Wild type versus CD36$^{-/-}$, #fatty acid versus no fatty acid, $P < 0.05$.

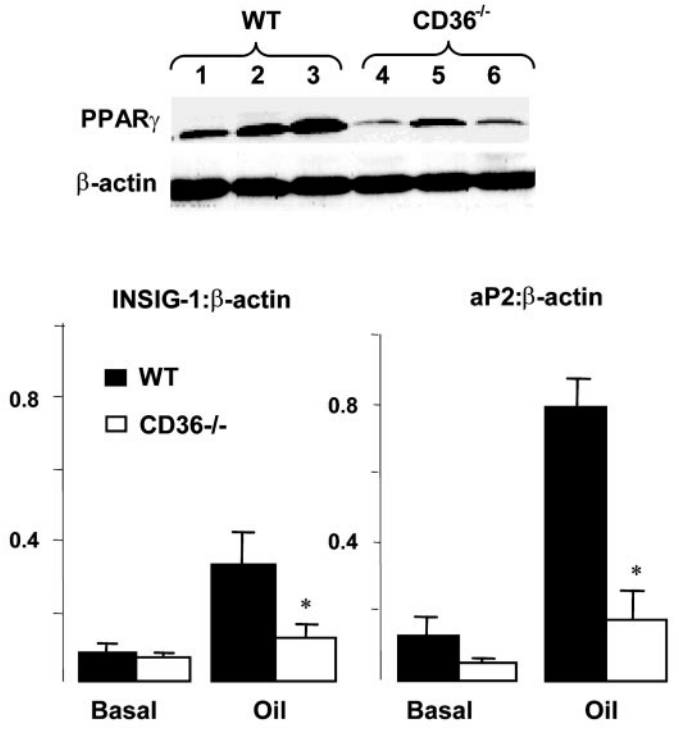

FIG. 6. PPARγ levels and activity in adipose tissue in response to intragastric administration of olive oil. Fasted wild-type and CD36$^{-/-}$ mice received an intragastric bolus of olive oil, and adipose tissue was harvested 2.5 h later. PPARγ and -β actin protein levels were detected using the respective antibodies as described in RESEARCH DESIGN AND METHODS. Expression of the PPARγ target genes aP2 and insulin-induced gene-1 (INSIG-1) in adipose tissue was tested using RT-PCR. Statistical differences between CD36$^{-/-}$ and wild type are indicated with an asterisk. *$P < 0.05$. The data are representative of two experiments with three mice per group per experiment. The decrease in basal ap2 in CD36$^{-/-}$ tissue missed statistical significance for the experiment shown. However, basal aP2 expression determined in separate experiments was down by 45%; $n = 5$, $P = 0.03$.

acid uptake by these cells (39) or that it antagonizes the effect of insulin to stimulate it (38). Interestingly, it has been reported that adipose tissue of *ob/ob* mice has severalfold higher CD36 expression that is normalized by leptin administration (39). The tissue also exhibits enhanced rates of fatty acid uptake that drop early after leptin injection, preceding reductions in food intake (39).

In contrast to its actions to decrease adipocyte fatty acid uptake and CD36, leptin upregulates both in muscle cells (40). This suggests that regulation of fatty acid uptake may mediate some of the metabolic effects of leptin to inhibit fatty acid storage and increase fatty acid catabolism.

It is well established that central resistance to leptin signaling plays a major role in the etiology of obesity (3,41,42) but resistance at the level of muscle and adipose tissue may also be important (4,9,43). Adipocyte-specific downregulation of the leptin receptor in mice results in obesity in the absence of effects on food intake (43). Our findings suggest that suppressing adipocyte CD36 could improve leptin regulation in obesity where a selective increase in fatty acid uptake by adipocytes in rodents and humans (44) has been reported.

Relevance to humans. CD36 deficiency is relatively high (1–3%) in some subpopulations such as Asians and Africans (45). Humans deficient in CD36 exhibit abnormalities of fatty acid utilization that are similar to those identified in rodents and it was proposed that the deficiency may be related to the phenotypic expression of the metabolic syndrome (46). Common polymorphisms in the CD36 gene in Caucasians have been linked to lipid abnormalities (47) and to diabetes incidence (48). None of the studies addressed leptin levels or sensitivity. However, there is indirect evidence to suggest that what we describe in the CD36-null mouse may be relevant to the human case. CD36 levels are upregulated in adipose tissue of obese subjects (49). Also a common polymorphism in the upstream promoter of the CD36 gene was associated with a higher incidence of diabetes type 2 that was most pronounced in subjects with a high BMI (48), suggesting unfavorable interaction of the polymorphism with BMI as a modifier of diabetes risk. Finally, the CD36-PPARγ-adiponectin link that we propose may help explain the

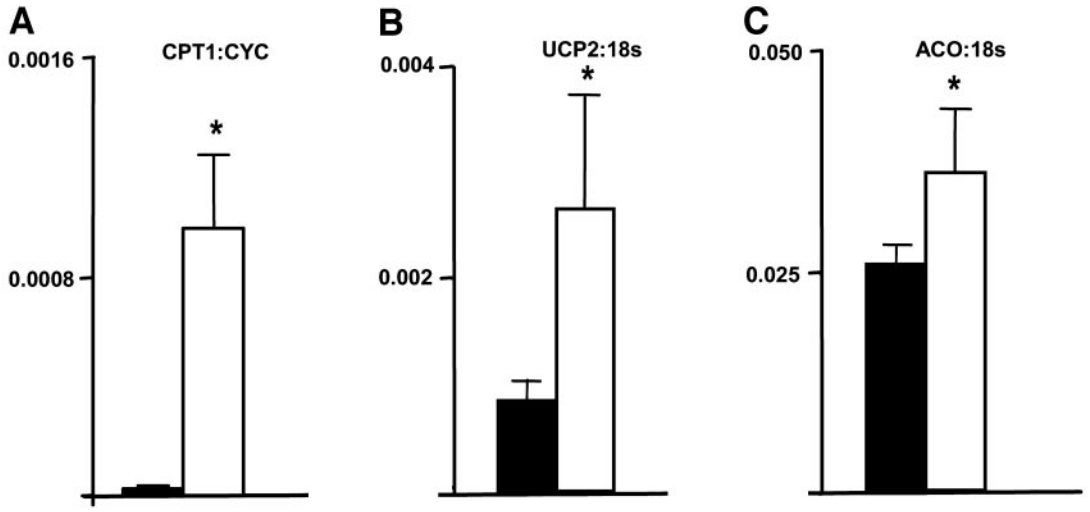

FIG. 7. Expression of oxidative genes in adipose tissue from wild-type and CD36$^{-/-}$ mice. Expression of the genes for CPT1 (*A*), UCP2 (*B*), and ACO (*C*) was measured using real-time PCR as described in RESEARCH DESIGN AND METHODS. Data are presented as means ± SE. Statistical significance was performed using Student's *t* test, and significant differences are indicated with an asterisk. *$P < 0.05$.

correlation previously noted between low adiponectin levels and a CD36 promoter variant in a French population (50).

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REFERENCES

1. Trayhurn P: Endocrine and signalling role of adipose tissue: new perspectives on fat. *Acta Physiol Scand* 184:285–293, 2005

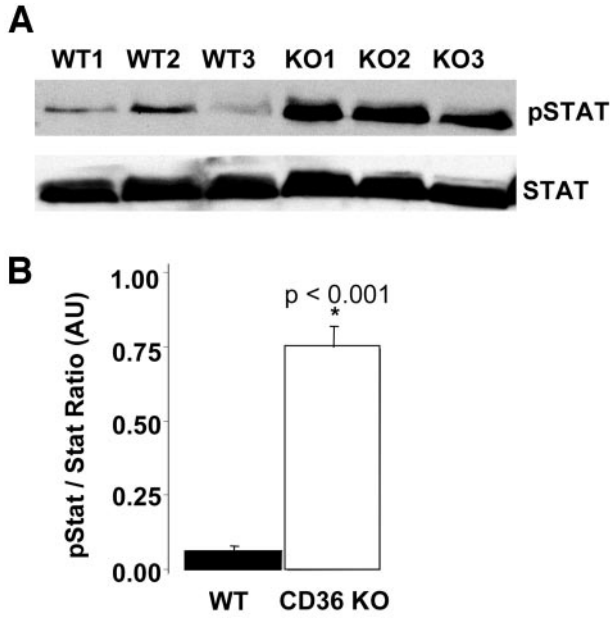

FIG. 8. Leptin signaling in wild-type and CD36$^{-/-}$ adipose tissue. *A*: Epididymal fat pads from wild-type and CD36-null mice were removed and analyzed for total and phosphorylated STAT3 as described in RESEARCH DESIGN AND METHODS. *B*: Densitometry scan showing level of *p*-STAT3 normalized to that of total STAT3. Data are representative of two experiments.

2. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425–432, 1994
3. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM: Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269:543–546, 1995
4. Bjorbaek C, Kahn BB: Leptin signaling in the central nervous system and the periphery. *Recent Prog Horm Res* 59:305–331, 2004
5. Shimabukuro M, Koyama K, Chen G, Wang MY, Trieu F, Lee Y, Newgard CB, Unger RH: Direct antidiabetic effect of leptin through triglyceride depletion of tissues. *Proc Natl Acad Sci U S A* 94:4637–4641, 1997
6. Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA, Cheetham CH, Earley AR, Barnett AH, Prins JB, O'Rahilly S: Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 387:903–908, 1997
7. Cohen P, Friedman JM: Leptin and the control of metabolism: role for stearoyl-CoA desaturase-1 (SCD-1). *J Nutr* 134:2455S–2463S, 2004
8. El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, Flier JS: Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *J Clin Invest* 105:1827–1832, 2000
9. Wang MY, Orci L, Ravazzola M, Unger RH: Fat storage in adipocytes requires inactivation of leptin's paracrine activity: implications for treatment of human obesity. *Proc Natl Acad Sci U S A* 102:18011–18016, 2005
10. Fried SK, Ricci MR, Russell CD, Laferrere B: Regulation of leptin production in humans. *J Nutr* 130:3127S–3131S, 2000
11. Mueller WM, Gregoire FM, Stanhope KL, Mobbs CV, Mizuno TM, Warden CH, Stern JS, Havel PJ: Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. *Endocrinology* 139:551–558, 1998
12. Cammisotto PG, Gelinas Y, Deshaies Y, Bukowiecki LJ: Regulation of leptin secretion from white adipocytes by free fatty acids. *Am J Physiol Endocrinol Metab* 285:E521–E526, 2003
13. Shintani M, Nishimura H, Yonemitsu S, Masuzaki H, Ogawa Y, Hosoda K, Inoue G, Yoshimasa Y, Nakao K: Downregulation of leptin by free fatty acids in rat adipocytes: effects of triacsin C, palmitate, and 2-bromopalmitate. *Metabolism* 49:326–330, 2000
14. Peino R, Fernandez Alvarez J, Penalva A, Considine RV, Rodriguez-Segade S, Rodriguez-Garcia J, Cordido F, Casanueva FF, Dieguez C: Acute changes in free-fatty acids (FFA) do not alter serum leptin levels. *J Endocrinol Invest* 21:526–530, 1998
15. Stumvoll M, Fritsche A, Tschritter O, Lehmann R, Wahl HG, Renn W, Haring H: Leptin levels in humans are acutely suppressed by isoproterenol despite acipimox-induced inhibition of lipolysis, but not by free fatty acids. *Metabolism* 49:335–339, 2000
16. Hajri T, Han XX, Bonen A, Abumrad NA: Defective fatty acid uptake modulates insulin responsiveness and metabolic responses to diet in CD36-null mice. *J Clin Invest* 109:1381–1389, 2002
17. Coburn CT, Knapp FF Jr, Febbraio M, Beets AL, Silverstein RL, Abumrad NA: Defective uptake and utilization of long chain fatty acids in muscle and adipose tissues of CD36 knockout mice. *J Biol Chem* 275:32523–32529, 2000
18. Febbraio M, Abumrad NA, Hajjar DP, Sharma K, Cheng W, Pearce SF,

Silverstein RL: A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism. *J Biol Chem* 274:19055–19062, 1999
19. Jensen DR, Gayles EC, Ammon S, Phillips R, Eckel RH: A self-correcting indirect calorimeter system for the measurement of energy balance in small animals. *J Appl Physiol* 90:912–918, 2001
20. Drover VA, Abumrad NA: CD36-dependent fatty acid uptake regulates expression of peroxisome proliferator activated receptors. *Biochem Soc Trans* 33:311–315, 2005
21. Fried SK, Moustaid-Moussa N: Culture of adipose tissue and isolated adipocytes. In *Adipose Tissue Protocols*. Ailhaud G, Ed. Totowa, NJ, Humana Press, 2001, p. 197–212
22. Fisher FF, Trujillo ME, Hanif W, Barnett AH, McTernan PG, Scherer PE, Kumar S: Serum high molecular weight complex of adiponectin correlates better with glucose tolerance than total serum adiponectin in Indo-Asian males. *Diabetologia* 48:1084–1087, 2005
23. Bastie CC, Hajri T, Drover VA, Grimaldi PA, Abumrad NA: CD36 in myocytes channels fatty acids to a lipase-accessible triglyceride pool that is related to cell lipid and insulin responsiveness. *Diabetes* 53:2209–2216, 2004
24. Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL: Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. *Endocrinology* 140:1630–1638, 1999
25. Goudriaan JR, Dahlmans VE, Teusink B, Ouwens DM, Febbraio M, Maassen JA, Romijn JA, Havekes LM, Voshol PJ: CD36 deficiency increases insulin sensitivity in muscle, but induces insulin resistance in the liver in mice. *J Lipid Res* 44:2270–2277, 2003
26. Lee Y, Wang MY, Kakuma T, Wang ZW, Babcock E, McCorkle K, Higa M, Zhou YT, Unger RH: Liporegulation in diet-induced obesity: the antisteatotic role of hyperleptinemia. *J Biol Chem* 276:5629–5635, 2001
27. Febbraio M, Guy E, Coburn C, Knapp FF Jr, Beets AL, Abumrad NA, Silverstein RL: The impact of overexpression and deficiency of fatty acid translocase (FAT)/CD36. *Mol Cell Biochem* 239:193–197, 2002
28. De Vos P, Lefebvre AM, Miller SG, Guerre-Millo M, Wong K, Saladin R, Hamann LG, Staels B, Briggs MR, Auwerx J: Thiazolidinediones repress ob gene expression in rodents via activation of peroxisome proliferator-activated receptor gamma. *J Clin Invest* 98:1004–1009, 1996
29. Kallen CB, Lazar MA: Antidiabetic thiazolidinediones inhibit leptin (ob) gene expression in 3T3–L1 adipocytes. *Proc Natl Acad Sci U S A* 93:5793–5796, 1996
30. Yamauchi T, Kamon J, Waki H, Murakami K, Motojima K, Komeda K, Ide T, Kubota N, Terauchi Y, Tobe K, Miki H, Tsuchida A, Akanuma Y, Nagai R, Kimura S, Kadowaki T: The mechanisms by which both heterozygous peroxisome proliferator-activated receptor gamma (PPARgamma) deficiency and PPARgamma agonist improve insulin resistance. *J Biol Chem* 276:41245–41254, 2001
31. Kast-Woelbern HR, Dana SL, Cesario RM, Sun L, de Grandpre LY, Brooks ME, Osburn DL, Reifel-Miller A, Klausing K, Leibowitz MD: Rosiglitazone induction of Insig-1 in white adipose tissue reveals a novel interplay of peroxisome proliferator-activated receptor gamma and sterol regulatory element-binding protein in the regulation of adipogenesis. *J Biol Chem* 279:23908–23915, 2004
32. Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM: mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev* 8:1224–1234, 1994
33. Kadowaki T, Yamauchi T: Adiponectin and adiponectin receptors. *Endocr Rev* 26:439–451, 2005
34. Bates SH, Stearns WH, Dundon TA, Schubert M, Tso AW, Wang Y, Banks AS, Lavery HJ, Haq AK, Maratos-Flier E, Neel BG, Schwartz MW, Myers MG Jr: STAT3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature* 421:856–859, 2003
35. Buettner C, Pocai A, Muse ED, Etgen AM, Myers MG Jr, Rossetti L: Critical role of STAT3 in leptin's metabolic actions. *Cell Metab* 4:49–60, 2006
36. Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, Minnemann T, Shulman GI, Kahn BB: Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature* 409:729–733, 2001
37. Hollenberg AN, Susulic VS, Madura JP, Zhang B, Moller DE, Tontonoz P, Sarraf P, Spiegelman BM, Lowell BB: Functional antagonism between CCAAT/Enhancer binding protein-alpha and peroxisome proliferator-activated receptor-gamma on the leptin promoter. *J Biol Chem* 272:5283–5290, 1997
38. Ho M, Foxall S, Higginbottom M, Donofrio DM, Liao J, Richardson PJ, Maneuf YP: Leptin-mediated inhibition of the insulin-stimulated increase in fatty acid uptake in differentiated 3T3–L1 adipocytes. *Metabolism* 55:8–12, 2006
39. Fan X, Bradbury MW, Berk PD: Leptin and insulin modulate nutrient partitioning and weight loss in ob/ob mice through regulation of long-chain fatty acid uptake by adipocytes. *J Nutr* 133:2707–2715, 2003
40. Palanivel R, Eguchi M, Shuralyova I, Coe I, Sweeney G: Distinct effects of short- and long-term leptin treatment on glucose and fatty acid uptake and metabolism in HL-1 cardiomyocytes. *Metabolism* 55:1067–1075, 2006
41. Lam TK, Pocai A, Gutierrez-Juarez R, Obici S, Bryan J, Aguilar-Bryan L, Schwartz GJ, Rossetti L: Hypothalamic sensing of circulating fatty acids is required for glucose homeostasis. *Nat Med* 11:320–327, 2005
42. Munzberg H, Flier JS, Bjorbaek C: Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. *Endocrinology* 145:4880–4889, 2004
43. Huan JN, Li J, Han Y, Chen K, Wu N, Zhao AZ: Adipocyte-selective reduction of the leptin receptors induced by antisense RNA leads to increased adiposity, dyslipidemia, and insulin resistance. *J Biol Chem* 278:45638–45650, 2003
44. Petrescu O, Fan X, Gentileschi P, Hossain S, Bradbury M, Gagner M, Berk PD: Long-chain fatty acid uptake is upregulated in omental adipocytes from patients undergoing bariatric surgery for obesity. *Int J Obes (Lond)* 29:196–203, 2005
45. Aitman TJ, Cooper LD, Norsworthy PJ, Wahid FN, Gray JK, Curtis BR, McKeigue PM, Kwiatkowski D, Greenwood BM, Snow RW, Hill AV, Scott J: Malaria susceptibility and CD36 mutation. *Nature* 405:1015–1016, 2000
46. Yamashita S, Hirano KI, Kuwasako T, Janabi M, Toyama Y, Ishigami M, Sakai N: Physiological and pathological roles of a multi-ligand receptor CD36 in atherogenesis: insights from CD36-deficient patients. *Mol Cell Biochem* 299:19–22, 2007
47. Ma X, Bacci S, Mlynarski W, Gottardo L, Soccio T, Menzaghi C, Iori E, Lager RA, Shroff AR, Gervino EV, Nesto RW, Johnstone MT, Abumrad NA, Avogaro A, Trischitta V, Doria A: A common haplotype at the CD36 locus is associated with high free fatty acid levels and increased cardiovascular risk in Caucasians. *Hum Mol Genet* 13:2197–2205, 2004
48. Corpeleijn E, van der Kallen CJ, Kruijshoop M, Magagnin MG, de Bruin TW, Feskens EJ, Saris WH, Blaak EE: Direct association of a promoter polymorphism in the CD36/FAT fatty acid transporter gene with type 2 diabetes mellitus and insulin resistance. *Diabet Med* 23:907–911, 2006
49. Bonen A, Tandon NN, Glatz JF, Luiken JJ, Heigenhauser GJ: The fatty acid transporter FAT/CD36 is upregulated in subcutaneous and visceral adipose tissues in human obesity and type 2 diabetes. *Int J Obes (Lond)* 30:877–883, 2006
50. Lepretre F, Linton KJ, Lacquemant C, Vatin V, Samson C, Dina C, Chikri M, Ali S, Scherer P, Seron K, Vasseur F, Aitman T, Froguel P: Genetic study of the CD36 gene in a French diabetic population. *Diabete Metab* 30:459–463, 2004