Interaction between Altered Insulin and Lipid Metabolism in CEACAM1-inactive Transgenic Mice*

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Inactivation of CEACAM1 in L-SACC1 mice by a dominant-negative transgene in liver impairs insulin clearance and increases serum free fatty acid (FFA) levels, resulting in insulin resistance. The contribution of elevated FFAs in the pathogenesis of insulin resistance is herein investigated. Treatment of L-SACC1 female mice with carnitine restored plasma FFA content. Concomitantly, it normalized insulin levels without directly regulating receptor-mediated insulin internalization and prevented glucose tolerance in these mice. Similarly, treatment with nicotinic acid, a lipolysis inhibitor, restored insulin-stimulated receptor uptake in L-SACC1 mice. Taken together, these data suggest that chronic elevation in plasma FFAs levels contributes to the regulation of insulin metabolism and action in L-SACC1 mice.

Insulin action is mediated by its binding to and activation of the insulin receptor tyrosine kinase to phosphorylate itself and other substrates (1). CEACAM1, an insulin receptor substrate in liver, but not in muscle or adipose tissue, regulates insulin action by promoting its receptor-mediated uptake and degradation in a phosphorylation-dependent manner (2–4). Overexpressing the dominant negative, phosphorylation-defective S503A CEACAM1 mutant in liver impaired insulin clearance and produced hyperinsulinemia in L-SACC1 transgenic mice (5). Hyperinsulinemia caused insulin resistance in the L-SACC1 male mice at 2 months of age, the earliest age examined. These mice also developed altered fat metabolism with increased visceral adiposity, increased fasting plasma free fatty acids (FFAs), and triacylglycerols (TG) and increased hepatic TG content (5).

The L-SACC1 mouse highlights the notion that intra-abdominal visceral adiposity and elevated plasma FFAs are commonly associated with impaired insulin clearance (6–11). It also emphasizes the important role of a CEACAM1-dependent insulin signaling downstream of the insulin receptor to directly regulate insulin clearance and sensitivity in liver and further regulate insulin action in extrahepatic tissues. Thus far, models of hepatic insulin resistance, including the LIRKO mouse with liver-specific insulin receptor ablation, which developed impaired insulin clearance, have primarily demonstrated that insulin signaling in hepatocytes is required to mediate insulin sensitivity in liver and extrahepatic tissues (12, 13). It is interesting, however, that the extent of impairment of fat metabolism is disproportionate to the extent of insulin resistance. For instance, LIRKO and other mice with primary hepatic insulin resistance did not develop elevated FFAs despite higher insulin levels than L-SACC1 transgenics (12, 14). This suggests that additional mechanisms may underlie insulin resistance in these models.

The association of hepatic insulin resistance with elevation in FFAs levels and increased visceral adiposity in L-SACC1 mice provides a potential mechanism to explain this apparent discrepancy. Chronic hyperinsulinemia caused by impaired insulin clearance in L-SACC1 mice may lead to increased hepatic triglyceride content and output (15, 16), thus contributing to hepatic insulin resistance that is brought about by the transgene. With normal pancreatic β-cell function in L-SACC1 mice, elevation in plasma triglycerides may, in turn, promote insulin secretion and prolongation of visceral adipose tissue (17). This eventually increases plasma FFAs output even in the absence of lipolysis (18).

Exogenous plasma FFAs are preferentially removed by re-esterification in liver and by oxidation in muscle, heart, liver, and other tissues (19). When the uptake of FFAs is exceedingly high, it may interfere with glucose uptake. It may also elevate the level of long chain fatty acyl-CoA, reducing the inhibition of carnitine palmitoyltransferase 1 by malonyl-CoA (20). This leads to increased transport of long chain fatty acyl-CoAs to the mitochondrial matrix to undergo β-oxidation (21). Conditions that partition the β-oxidation product, acetyl-CoA, to the citric cycle interfere with glucose metabolism and promote insulin resistance (22). The tight correlation between high fasting plasma FFAs and insulin resistance has been supported by the observation that fasting plasma FFAs are commonly elevated in obese and insulin-resistant individuals (23).

Given the regulatory role of plasma FFAs in insulin sensitivity, we investigated the role of FFAs in the pathogenesis of insulin resistance in L-SACC1 mice. To this end, we treated...
L-SACC1 females, which, unlike their male counterparts, did not develop hyperglycemia until 8 months of age, following increased visceral adiposity and impaired insulin clearance, with carnitine to normalize FFAs levels (24). We report that normalization of FFAs levels by carnitine in 6-month-old L-SACC1 mice restored insulin levels and prevented hyperglycemia. By decreasing lipolysis, nicotinic acid also restored receptor-mediated insulin uptake. Because carnitine does not modulate insulin internalization directly, these data suggest that increased visceral adiposity contributes to the pathogenesis of insulin resistance in L-SACC1 females.

EXPERIMENTAL PROCEDURES

Animal Maintenance and Treatment—Animals were kept in a 12-h dark/light cycle and fed standard chow ad libitum. All procedures were approved by the relevant Institutional Animal Care and Utilization Committees at the Medical College of Ohio and Veterans Affairs Medical Center, San Francisco. When carnitine was used, 6-month-old wild type (WT) and L-SACC1 mice were treated at 1600 h on 1 or 2 weeks with a daily intraperitoneal injection of saline (vehicle-treated) or 0.2–1.5 g/kg body weight of L-carnitine, 0.4 g/ml saline (L-carnitine; Inner Salt Solution [ISS]). The 6-month-old mice were treated with two daily intraperitoneal injections of 200 ml/kg body weight of nicotinic acid (Sigma) or saline for 2 weeks (25).

Phenotypic Analysis—Following an overnight fast (with food being removed at 1700 h on the day prior to the experiment), mice were anesthetized with sodium pentobarbital (30 mg/g body weight) between 1100 and 1200 h. Whole venous blood was drawn from the retro-orbital sinususes to measure fasting glucose levels using a glucometer (Accu-chek; Roche Applied Science), plasma insulin, C-peptide, and leptin levels by radioimmunoassays (Linco Research), plasma FFAs using the NEFA C kit (Wako), triglycerides using the Infinity Triglycerides reagent (Sigma), and cholesterol using the Infinity Cholesterol reagent (Sigma). For liver and kidney functions, serum ALT (Sigma) and blood urea nitrogen (Infinity BUN reagent; Sigma) were measured, respectively. Visceral adipose tissues were weighed, and visceral adiposity was expressed as a percentage of total body weight. Liver and muscle triacylglycerols were determined as described previously (5).

Serum β-Hydroxybutyrate—Following an overnight fast, mice were anesthetized, and blood was drawn to determine serum 3-hydroxybutyric acid levels by gas chromatography/mass spectroscopy following ethyl acetate/diethyl ether extraction of plasma deproteinized with 7% perchloric acid (36).

Serum Carnitine Levels—Free carnitine and acylcarnitines were quantified in serum from fasted mice by tandem mass spectrometry (27) that were in turn analyzed by electrospray tandem mass spectrometry of the precursor ions of carnitine that were normalized to a known amount of internal standard (26).

Dried extract was derivatized with butanolic HCl to form butyl esters (36). Following an overnight fast (with food being removed at 1700 h on the day prior to the experiment), mice were anesthetized fasted mice, as described previously (5).

Acetyl-CoA Carboxylase 1 Phosphorylation—The liver was removed from fasted 6-month-old mice and homogenized in buffer containing 20 mM Tris, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, and protease inhibitors (32). The homogenate was centrifuged at 12,500 × g for 30 min at 4 °C, and fatty acid synthase activity was assayed in the postmitochondrial supernatant (33).

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Acetyl-CoA Carboxylase 1 Phosphorylation—The liver was removed from fasted 6-month-old mice and homogenized in buffer containing 20 mM Tris, pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, and protease inhibitors (32). The equal amounts of protein were immunoprecipitated with agarose-streptavidin, resolved by 6–15% gradient SDS-PAGE, and immunoblotted with an α-phospho-acyl-CoA carboxylase 1 (pACC1) antibody (Upstate Biotechnology, Inc., Lake Placid, NY) to detect phosphorylated ACC1 followed by blotting with horseradish peroxidase-streptavidin to determine the total amount of ACC1 in the immunopellet. The pACC1/ACC1 ratio was used as a measure of ACC1 activation (35).

mRNA Levels—Liver mRNA was purified using the MicroPoly(A) Pure kit (Ambion), analyzed by Northern blot, and probed with cDNA for phosphorylase kinase, glucose-6-phosphatase, and glucokinase mRNA levels were normalized with β-actin.

Statistics—Data were analyzed with Statview software (Abacus Concepts) using one-factor analysis of variance analysis. p values less than 0.05 were considered to be statistically significant.

RESULTS

Abnormal Metabolism in L-SACC1 Female Transgenic—Similar to L-SACC1 males (5), L-SACC1 females developed a 2–5-fold increase in plasma insulin levels by 2 months of age (Table I). They maintained normal glucose uptake in soleus muscle at submaximal insulin concentrations (200 microunits/ml) (Fig. 1A) and glucose transporter-4 mRNA levels (Fig. 1B). Glucose levels decreased to a similar extent in L-SACC1 and WT mice following insulin injection (Fig. 1C). This suggests that peripheral tissues in L-SACC1 mice, especially skeletal muscle, maintained intrinsic insulin sensitivity to glucose uptake. However, in control mice, glucose levels returned to normal within 3 h, whereas in L-SACC1 mice, they remained suppressed (Fig. 1C), consistent with the observation that L-SACC1 removed injected insulin less efficiently than WT mice.

Intraperitoneal glucose tolerance tests indicated that L-SACC1 females became increasingly glucose-intolerant with age (Fig. 2A). In view of the fact that muscle glucose uptake is normal, glucose intolerance is likely to arise from hepatic in-
Role for Plasma FFAs in Hyperglycemia

Phenotype characterization of age-matched 2-, 4-, and 6-month-old WT and L-SACC1 mice was performed as described under “Experimental Procedures.” 10–12 mice per category were tested. Values are expressed as mean ± S.E.

### Table 1

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<tr>
<th>Metabolic parameters in L-SACC1 female mice</th>
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<tr>
<td>Visceral fat/body weight (%)</td>
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<tr>
<td>Fed glucose (mg/dl)</td>
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<tr>
<td>Fasting glucose</td>
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<td>Plasma insulin (pm)</td>
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<td>Plasma FFA (pm)</td>
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<td>Plasma TG (mg/dl)</td>
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### Footnotes

* p < 0.05 L-SACC1 versus WT control mice.

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**Fig. 1. Muscle insulin sensitivity.** Soleus muscle was removed from 8-month-old WT and L-SACC1 mice (5–7 each), and 2-deoxy-D-glucose (2-DG) uptake was measured in the absence (basal, filled bars) or presence of insulin (open bars) (A). Values are mean ± S.E.; †, p < 0.05 versus basal. B, mRNA was purified from the gastrocnemius muscle of these mice and probed with glucose transporter 4 and β-actin cDNA to be normalized. C, insulin tolerance measured in 6-month-old vehicle-treated WT (open squares), vehicle-treated L-SACC1 (open triangles), and carnitine-treated L-SACC1 (filled triangles) mice (seven of each). Mice were injected with insulin (0.125 units/kg), and blood was drawn for glucose determination at times from 0 to 180 min. Values are mean ± S.E.; †, p < 0.05 versus WT; ‡, p < 0.05 versus vehicle-treated.

**Fig. 2. Hepatic insulin resistance in L-SACC1 females.** A, glucose tolerance test in 2- and 8-month-old WT and L-SACC1 mice (10 of each). Following injection with glucose (2 g/kg), blood was drawn to determine glucose levels. Values are mean ± S.E. B, insulin receptor number in primary hepatocytes of 6-month-old mice (four of each). Assays were performed in triplicate, and values are mean ± S.E. C, mRNA from livers of 6- and 8-month-old WT and L-SACC1 mice (five or six of each) were probed with phosphoenolpyruvate carboxykinase, glucose-6-phosphatase, glucokinase, and β-actin.

4.1 mg/dl versus 112.2 ± 4.1 in WT), consistent with normal β-cell insulin secretory function in L-SACC1 mice (5).

**Altered Hepatic FFA Metabolism in L-SACC1 Females—**Like their male counterparts (5), L-SACC1 females exhibited increased body weight (not shown) and visceral adiposity at all ages examined (p < 0.05) (Fig. 3A). However, plasma leptin levels were normal (Fig. 3B, p > 0.05). This suggests that the food intake in L-SACC1 was normal and that visceral obesity is not attributed to changes in food intake. L-SACC1 mice also showed elevated fasting plasma FFAs and TG starting at 2 months of age (Table I). Hepatic acetyl- and malonyl-CoA levels were normal (Table II). Together with normal ACC1 activity in fasted L-SACC1 mice (pACC/ACC = 0.9–1.1 versus 1.1–1.3 in WT mice), this suggests that fatty acid synthesis is normal in L-SACC1 females under fasting conditions. Under nonfasting conditions, fatty acid synthase activity in liver extracts of 2-month-old L-SACC1 females was normal (174.3 ± 6.6 cpm/μg versus 177.7 ± 6.5 in WT mice); so was hepatic de novo fatty acid (FA) synthesis measured in vivo in 7-month-old L-SACC1 females (Table III). Similarly, fatty acid synthesis in whole
body, skeletal muscle, heart, and small intestine of nonfasted L-SACC1 females was normal (Table III). Hepatic TG content was elevated in L-SACC1 females (Table II). Elevation in Glc-6-P levels (0.043 ± 0.006 μmol/g versus 0.015 ± 0.003 in WT; p < 0.05) suggests that this is in part due to increased esterification and TG synthesis in L-SACC1 liver. Because fatty acid synthesis is normal, FA substrates of TG synthesis are likely to derive from adipose tissue.

Serum acyl- and acylcarnitine/total carnitine ratios were high (Table II). Because carnitine esters that are released from the liver equilibrate with plasma more rapidly than those released from muscle and brain (36, 37), this suggests increased FA uptake into L-SACC1 hepatic mitochondria. This is supported by the observation that hepatic malonyl-CoA levels were not significantly elevated (Table II) to reduce FA uptake into the mitochondria. Moreover, the slight (-3-fold) but significant elevation in plasma β-hydroxybutyrate levels in L-SACC1 mice (Table II) suggests that FA mitochondrial oxidation is not impaired in the L-SACC1 mouse liver and that elevation of acylcarnitine was not due to its accumulation in the mitochondria.

Effects of L-carnitine on Fat and Glucose Metabolism in 6-Month-old L-SACC1 Female Mice—To address the role of elevated plasma FFAs in the pathogenesis of insulin resistance, we investigated the effect of lowering serum FFAs levels on metabolism in L-SACC1 females. Treatment with low dose carnitine (0.2 g/kg body weight) did not reduce plasma FFAs (not shown). Treatment with 1.5 g/kg for 1 week decreased plasma FFA content (0.43 ± 0.03 mm versus 0.61 ± 0.07 in vehicle-treated mice; p < 0.05) but did not restore it (0.43 ± 0.03 mm in carnitine-treated L-SACC1 versus 0.27 ± 0.03 in vehicle-treated WT mice; p < 0.05). Prolonged treatment (2 weeks) normalized it completely (Table II). The higher dose of carnitine did not impair liver or kidney function (data not shown), in agreement with a reported lack of carnitine toxicity (38). Although carnitine reduced visceral adipose mass substantially in L-SACC1 mice, it remained higher than normal (Table II). This occurred without a significant effect on body weight gain (increase in body weight of 1.75 ± 0.53 g in carnitine-treated versus 1.68 ± 0.23 in vehicle-treated L-SACC1 mice and 1.17 ± 0.53 g in carnitine-treated versus 1.63 ± 0.27 in vehicle-treated WT mice). Additionally, carnitine lowered plasma TG content in L-SACC1 mice but failed to normalize it (Table II). Consistently, hepatic TG content remained elevated (Table II). High hepatic TG levels may in part derive from elevated de novo fatty acid synthesis by carnitine as measured by [14C]acetate incorporation in primary hepatocytes (80.4 ± 16.4 cpm/μg in carnitine-treated versus 45.0 ± 6.0 in vehicle-treated L-SACC1; p < 0.05). It can also result from increased FFA uptake and esterification in the liver of carnitine-treated mice. Nonetheless, carnitine did not appear to significantly alter hepatic FA oxidation in L-SACC1 mice, as suggested by the absence of an effect of carnitine on plasma acetyl- and acylcarnitine/carnitine and β-hydroxybutyrate levels (Table II). Consistently, carnitine did not negatively affect glucose metabolism. Instead, it restored glucose tolerance in L-SACC1 mice (Fig. 4).

In control WT mice, carnitine reduced serum FFAs levels without significantly affecting visceral adipose mass (Table II). It significantly elevated acetyl-CoA/CoA ratio in liver (Table II), suggesting increased FA mitochondrial uptake. Normal ketogenesis, as indicated by unaltered levels of β-hydroxybutyrate in carnitine-treated WT mice (Table II), rules out routing of the oxidative products to ketogenesis in liver. However, carnitine markedly reduced hepatic acetyl-CoA levels (Table II), suggesting reduced glucose oxidation in carnitine-treated WT mice. In support of this hypothesis, WT mice became glucose-intolerant upon carnitine treatment (Fig. 4).

Concomitantly, carnitine increased ACC1 activity in WT mouse livers, as indicated by reduced ACC1 phosphorylation (pACC/ACC = 0.4–0.5 in carnitine-treated versus 1.1–1.3 in vehicle-treated mice; p < 0.05) and increased hepatic malonyl-CoA levels (Table II). This suggests that carnitine increased fatty acid synthesis in WT mice, which in turn could underlie increased hepatic TG content in these mice (Table II). Despite increased hepatic TG content, plasma TG levels remained normal in carnitine-treated WT mice (Table II). Taken together, these observations suggest that carnitine reduces plasma FFAs levels in WT mice mainly by increasing their oxidation in liver.

Carnitine Restores Insulin Metabolism in L-SACC1 Females—Carnitine treatment normalized insulin levels in L-SACC1 female mice (Table II). This was due to restoration of insulin clearance, as indicated by normal C-peptide/insulin molar ratio (Table II). The lack of a significant effect on C-peptide levels (Table II) suggests that carnitine did not affect insulin secretion. Consistent with the improvement of insulin clearance, carnitine-treated L-SACC1 mice displayed normal glucose levels 3 h after insulin injection as opposed to vehicle-treated L-SACC1 mice, in which glucose levels remained suppressed (Fig. 1C).

Lack of a Direct Effect of Carnitine on Insulin Clearance—To examine whether carnitine directly regulates receptor-mediated insulin uptake, primary hepatocytes were treated with or without carnitine for 48 h followed by measuring [125I]insulin internalization at 37 °C. As Fig. 5 shows, 4-month-old L-SACC1 females exhibited a marked decrease in receptor-mediated insulin uptake that was not modified by carnitine. The data suggest that carnitine does not regulate insulin clearance directly. This hypothesis is supported by the observation that carnitine treatment did not modify insulin clearance in WT mice, as indicated by the comparable C-peptide/insulin ratio in carnitine- and vehicle-treated WT mice (Table II).

Effect of Plasma FFAs on Insulin Clearance—The effect of carnitine on visceral adiposity and plasma FFAs levels in L-SACC1 mice may be secondary to its effect on oxidation in different tissues. To assess whether carnitine restored insulin metabolism in L-SACC1 mice mainly by reducing the supply of FFAs, we treated mice with nicotinic acid, a direct inhibitor of lipolysis, and investigated its effect on receptor-mediated insulin endocytosis. To this end, we measured the loss of biotin-labeled surface membrane α-subunit of the insulin receptor (IRα) in primary hepatocytes before (-) and after (+) insulin treatment (Fig. 6). Immunoprecipitation (Ip) with IRα antibody...
TABLE II
Effects of L-carnitine on lipid and insulin metabolism of 6-month-old L-SACC1 females

Vehicle- or carnitine-treated 6-month-old WT and L-SACC1 female mice (5–9 mice/group) were fasted overnight, and the whole venous blood was drawn to determine serum β-hydroxybutyrate carnitine, FFA, TG, insulin, and C-peptide levels. Liver and gastrocnemius muscle were removed and frozen in liquid nitrogen to measure CoA metabolites and TG. Visceral adipose tissues were collected, weighed, and expressed as a percentage of total body weight. Values are expressed as mean ± S.E.

<table>
<thead>
<tr>
<th>Lipid metabolism</th>
<th>WT vehicle</th>
<th>WT carnitine</th>
<th>L-SACC1 vehicle</th>
<th>L-SACC1 carnitine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visceral fat/body weight (%)</td>
<td>0.46 ± 0.11</td>
<td>0.33 ± 0.03</td>
<td>4.00 ± 0.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.62 ± 0.28&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma FFA (mmol/liter)</td>
<td>0.58 ± 0.04</td>
<td>0.43 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.14 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.63 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma TG (mg/dl)</td>
<td>35.9 ± 2.83</td>
<td>32.4 ± 2.55</td>
<td>60.6 ± 5.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.0 ± 5.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver TG (μg/mg protein)</td>
<td>40.2 ± 4.20</td>
<td>104. ± 22.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95.8 ± 12.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>74.6 ± 13.0&lt;sup&gt;c&lt;/sup&gt;</td>
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TABLE III

In vivo fatty acid synthesis (μmol of 1<sup>4</sup>H<sub>1</sub>O incorporation/kg)

The experiment was performed on 7-month-old WT and L-SACC1 females (5–8 each). Values are expressed as mean ± S.E.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>WT</th>
<th>L-SACC1</th>
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<tbody>
<tr>
<td>Whole body</td>
<td>56.7 ± 8.10</td>
<td>43.9 ± 3.10</td>
</tr>
<tr>
<td>Liver</td>
<td>31.5 ± 3.06</td>
<td>41.6 ± 5.88</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>1.95 ± 0.19</td>
<td>1.61 ± 0.22</td>
</tr>
<tr>
<td>Heart</td>
<td>5.39 ± 0.63</td>
<td>5.23 ± 0.37</td>
</tr>
<tr>
<td>Small intestine</td>
<td>10.6 ± 2.06</td>
<td>10.0 ± 0.47</td>
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<sup>a</sup> p < 0.05 versus vehicle-treated WT.

<sup>b</sup> p < 0.05 carnitine versus vehicle-treated.

<sup>c</sup> p < 0.05 carnitine-treated L-SACC1 versus vehicle-treated WT.

FIG. 4. Carnitine restores glucose tolerance in L-SACC1 females. Glucose tolerance was determined in 6-month-old WT (squares) and L-SACC1 (triangles) mice (6–8 of each) following treatment with vehicle (open symbols) or carnitine (filled symbols). Values are mean ± S.E. * p < 0.05 versus WT; †, p < 0.05 versus vehicle-treated.

followed by immunoblotting (Ib) with streptavidin revealed that the IR<sub>a</sub> content on the surface was higher in L-SACC1 than WT in the absence of insulin (Fig. 6A, lanes 5 and 7 versus lanes 1 and 2). Because the level of the insulin receptor in L-SACC1 hepatocytes was identical to that of WT (Fig. 6B, lanes 5 and 7 versus lanes 1 and 2), accumulation of IR<sub>a</sub> on the surface of L-SACC1 hepatocytes was not due to increased synthesis and assembly. Consistent with impaired receptor-mediated insulin endocytosis in L-SACC1 mice, insulin treatment led to a complete loss of the surface membrane content of IR<sub>a</sub> in WT but not in L-SACC1 mice (Fig. 6, lane 4 versus lane 3 in WT as compared with lane 8 versus lane 7 in L-SACC1). Treatment with nicotinic acid (+ NA), restored insulin-induced endocytosis of the receptor, as assessed by the complete loss of biotin-labeled IR<sub>a</sub> in primary hepatocytes of treated L-SACC1 mice in response to insulin (Fig. 6, lane 6 versus lane 5).
DISCUSSION

We have shown previously that overexpressing a dominant-negative phosphorylation-defective Ceacam1 transgene in mouse liver inhibits CEACAM1-mediated insulin clearance and results in hyperinsulinemia (5). The latter caused insulin resistance by inducing insulin receptor down-regulation and desensitization of the insulin signaling pathway and was associated with altered fat metabolism (5). We now report that L-SACC1 females develop hyperinsulinemia and increased visceral adiposity, elevated plasma FFAs, and triglycerides before they develop hyperglycemia and marked glucose intolerance. This progressive phenotypic pattern supports the well-known protective effect of female gender against the development of the metabolic syndrome and of insulin-resistant diabetes in the C57BL/6J background (36).

Multiple studies have employed environmentally induced visceral adiposity in rodents to investigate the role of elevated plasma FFAs in the pathogenesis of insulin resistance. However, the potential underlying effect of impaired insulin clearance in these studies has been vastly ignored. In the L-SACC1 mice with impaired insulin clearance, the mechanism of the development of visceral adiposity and elevation of plasma FFAs is not completely delineated. Normal food intake suggests that it could arise from increased de novo tissue lipogenesis in proportion to increased insulin levels, as has been reported in hyperinsulinemic-euglycemic normal rats (39). Because CEACAM1 levels are insignificant in muscle and adipose tissue, it is conceivable that the deranged phenotype of L-SACC1 mice begins with altered metabolism in the liver. Thus, we propose that hyperinsulinemia initially elevates lipogenesis in the L-SACC1 liver, which in turn, leads to increased VLDL-TG output. With the insulin secretory function of pancreatic β-cells in L-SACC1 mice being intact (5), it is conceivable that increased TG levels cause an increase in insulin secretion, which in turn, leads to proliferation of adipocytes and increases visceral adiposity.

In these studies, we took advantage of the sequential progression of metabolic abnormalities in L-SACC1 females and treated them at 6 months of age with carnitine to normalize their serum FFAs and investigate whether this prevents altered glucose metabolism. Although further examination of metabolic fluxes are needed to confirm our observation, the current data suggest that, similar to its effect on obese Zucker (40) and Wistar rats (41), carnitine normalized plasma FFA levels in L-SACC1 mice mainly by reducing its supply. That carnitine may have increased oxidation of FFAs in several sites, including muscle, is possible. However, improved glucose tolerance in carnitine-treated L-SACC1 mice suggests that carnitine did not significantly increase FFAs oxidation to a significant level that would have interfered with glucose oxidation (22), as it did in WT mice that became glucose-intolerant when treated with carnitine. Nonetheless, decreased plasma FFA levels by carnitine restored insulin metabolism and prevented alteration in glucose metabolism. Because carnitine does not directly affect insulin uptake and degradation in hepatocytes, normalization of insulin levels and glucose tolerance by carnitine via its lowering effect on FFA availability in L-SACC1 mice supports the hypothesis that increased visceral adiposity and FFA output contribute to deranged insulin metabolism and action in L-SACC1 mice. Restoration of receptor-mediated insulin endocytosis by nicotinic acid supports this hypothesis.

Because restoration of plasma FFA levels by carnitine and nicotinic acid reverses insulin endocytosis and metabolism in L-SACC1 mice bearing inactivation of CEACAM1 in liver, it is possible that plasma FFAs are implicated in a CEACAM1-independent regulatory pathway of insulin metabolism and action. More studies are needed to further address this issue. Nonetheless, the data are in agreement with the notion that high plasma FFAs levels impair glucose utilization (42) and prevent insulin suppression of glucose production in uncomplicated obesity (43). Moreover, these observations are supported by epidemiological studies in Caucasians (44) and Pima Indians (45) that revealed that elevated plasma FFAs levels are independent indicators of the deterioration of glucose tolerance.

Although hepatic and plasma TG levels were substantially reduced in L-SACC1 mice by carnitine, they remained high. This indicates that L-SACC1 mice retain altered hepatic lipid metabolism in the presence of inactive CEACAM1. Thus, it is possible that with CEACAM1 being inactive, hepatic production and secretion of TG remain elevated, perpetuating a vicious cycle of increased hepatic TG synthesis and adipocyte proliferation (17). Sustained high levels of hepatic and plasma TG despite normalization of insulin levels by carnitine suggests that CEACAM1 regulates lipid metabolism in liver. Whether CEACAM1 modulates lipid metabolism independently of its effect on insulin clearance is not clear at the moment and requires further study. Nonetheless, the data suggest that altered FFA metabolism is an important regulator of insulin action. The data also highlight the complex interaction between fatty acids metabolism, insulin clearance, and hepatic insulin sensitivity, which can act independently or in concert to cause the metabolic syndrome.

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