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Mechanism of glucose intolerance in mice with dominant negative mutation of CEACAM1

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The prevalence of obesity has reached epidemic proportions in the Westernized countries and is a major factor for the recent dramatic increase in incidence of type 2 diabetes throughout the world (29, 33). Insulin resistance is a major characteristic and requisite event in the development of type 2 diabetes, and a wealth of previous studies (1, 5, 12, 21, 24) has shown that obese humans, as well as genetically or experimentally-induced obese animals, developed insulin resistance in skeletal muscle, adipose tissue, and liver. The mechanism underlying obesity-mediated insulin resistance involves tissue-specific accumulation of fat and fatty acid metabolites and their deleterious effects on insulin signaling and glucose transport activity (2, 20, 34). Additionally, adipocytes produce a host of metabolic hormones and inflammatory cytokines, including resistin, adiponectin, leptin, tumor necrosis factor-α, and interleukin-6, and dysregulated production of these factors has been shown to alter whole body insulin sensitivity (10, 13–15, 37, 40).

The carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is a transmembrane glycoprotein expressed in various cell types, including endothelial and epithelial cells and B and T lymphocytes, and in the liver among insulin-sensitive organs (32, 39). CEACAM1 is a substrate of the insulin receptor tyrosine kinase and a regulator of receptor-mediated endocytosis and degradation of insulin in liver (25, 35). The physiological role of CEACAM1 in the metabolic regulation has been demonstrated using mice with liver-specific overexpression of dominant negative phosphorylation-defective S503A-CEACAM1 mutant (L-SACC1 mice) that developed a significant defect in hepatic insulin clearance and subsequent hyperinsulinemia (30). Interestingly, L-SACC1 mice developed increased visceral adiposity and glucose intolerance (30). Although the circulating leptin levels were normal, L-SACC1 mice exhibited elevated free fatty acid (FFA) levels, and inhibition of lipolysis with nicotinic acid restored hepatic insulin sensitivity (4). To determine the underlying mechanism of altered glucose homeostasis, tissue-specific insulin action and glucose metabolism were examined during a 2-h hyperinsulinemic euglycemic clamp in awake L-SACC1 and wild-type mice.

MATERIALS AND METHODS

Animals and surgery. Male L-SACC1 mice and wild-type littermates were obtained from the animal facility of the Medical University of Ohio at Toledo at 8–10 wk of age (30). Upon arrival, mice were housed in the animal facility of Yale Mouse Metabolic Phenotyping Center for 5–6 wk before the metabolic studies. Mice were housed under controlled temperature (23°C) and lighting [12:12-h light (0700–1900)-dark (1900–0700) cycle] with free access to standard mouse chow diet (6% fat by calories; Harlan Teklad, Madison, WI) and water. At least 4 days before the in vivo experiments, whole body fat and lean mass were measured in awake mice by using 1H-MRS (magnetic resonance spectroscopy, Bruker Mini-Spec Analyzer; Echo Medical Systems, Houston, TX). Following the body.

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composition measurement, mice were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg body wt) and xylazine (10 mg/kg body wt), and an indwelling catheter was inserted in the right internal jugular vein. On the day of clamp experiment, a three-way connector was attached to the catheter to intravenously deliver solutions (e.g., glucose, insulin). Also, mice were placed in a rat-size restrainer (to minimize stress during experiments in the awake state) and tail-restrained using a tape to obtain blood samples from tail vessels. All procedures were approved by the Yale University Animal Care and Use Committee.

Hyperinsulinemic euglycemic clamps to assess insulin action in vivo. Following an overnight fast (~15 h), a 2-h hyperinsulinemic euglycemic clamp was conducted in awake L-SACC1 and wild-type mice (n = 11–12), with a primed (150 mU/kg body wt) and continuous infusion of human regular insulin (Humulin; Eli Lilly, Indianapolis, IN) at a rate of 15 pmol·kg⁻¹·min⁻¹ to raise plasma insulin within a physiological range (~300 pM). Blood samples (20 μl) were collected at 20-min intervals for the immediate measurement of plasma glucose concentration, and 20% glucose was infused at variable rates to maintain euglycemia. Basal and insulin-stimulated whole body glucose turnover was estimated with a continuous infusion of [3-3H]glucose (PerkinElmer Life and Analytical Sciences, Boston, MA) for 2 h prior to (0.05 μCi/min) and throughout the clamps (0.1 μCi/min), respectively. All infusions were performed using the microdialysis pumps (CMA/Microdialysis, North Chelmsford, MA). To estimate insulin-stimulated glucose uptake in individual tissues, 2-deoxy-d-[1-14C]glucose (2-[14C]DG) was administered as a bolus (10 μCi) at 75 min after the start of clamps. Blood samples were taken before, during, and at the end of clamps for the measurement of plasma [3H]glucose, H₂O, 2-[14C]DG, and/or insulin concentrations. At the end of clamps, mice were anesthetized, and tissues were taken for biochemical and molecular analysis (15).

Biochemical assays. Glucose concentrations during clamps were analyzed using 10 μl of plasma by a glucose oxidase method on Beckman Glucose Analyzer 2 (Beckman, Fullerton, CA). Plasma insulin concentrations were measured by radioimmunoassay using kits from Linco Research (St. Charles, MO). Plasma concentrations of [3-3H]glucose, 2-[14C]DG, and H₂O were determined following deproteinization of plasma samples, as previously described (15). The radioactivity of [3H] in tissue glycogen was determined by digesting tissue samples in KOH and precipitating glycogen with ethanol. For the determination of tissue 2-[14C]DG-6-phosphate (2-[14C]DG-6-P) content, tissue samples were homogenized, and the supernatants were subjected to an ion exchange column to separate 2-[14C]DG-6-P from 2-[14C]DG. Tissue-specific triglyceride concentrations were determined by digesting tissue samples in chloroform-methanol (16). Briefly, the lipid layer was separated using H₂SO₄, and concentrations were determined using triglyceride assay kit (Sigma Diagnostics, St Louis, MO) and spectrophotometry.

In additional overnight fasted (to minimize stress during experiments in the awake state) and tail-restrained using a tape to obtain blood samples from tail vessels. All procedures were approved by the Yale University Animal Care and Use Committee.

Calculation. Rates of basal hepatic glucose production (HGP) and insulin-stimulated whole body glucose uptake were determined as the ratio of the [3H]glucose infusion rate (disintegrations/min) to the specific activity of plasma glucose (dpm/μmol) at the end of basal period and during the final 30 min of clamp, respectively (15). Insulin-stimulated rate of HGP during clamp was determined by subtracting the glucose infusion rate from whole body glucose uptake. Whole body glycolysis was calculated from the rate of increase in plasma H₂O concentration, determined by linear regression of the measurements at 80, 90, 100, 110, and 120 min of clamps. Whole body glycolysis plus lipid synthesis from glucose was estimated by subtracting whole body glycolysis from whole body glucose uptake (15). Because 2-deoxyglucose is a glucose analog that is phosphorylated but not further metabolized, insulin-stimulated glucose uptake in individual tissues can be estimated by determining the tissue (i.e., skeletal muscle, adipose tissue) content of 2-[14C]DG-6-P. On the basis of this, glucose uptake in individual tissues was calculated from plasma 2-[14C]DG profile, which was fitted with a double exponential or linear curve by using MLAB (Civilized Software, Bethesda, MD) and tissue 2-[14C]DG-6-P content. Skeletal muscle glycogen synthesis was calculated from 3H incorporation to muscle glycogen, and skeletal muscle glycogenesis was estimated as the difference between muscle glucose uptake and muscle glycogen synthesis (15).

Intracellular levels of glucose 6-phosphate and coenzyme A ester. For the measurement of postprandial intracellular concentrations of glucose 6-phosphate (G-6-P), liver was removed from L-SACC1 and wild-type mice at 3 mo of age and immediately frozen using liquid N₂. One gram of tissue was homogenized, resuspended in 5 ml of 6 N perchloric acid, and centrifuged (3,000 g for 10 min at 4°C). The pH of the supernatant was adjusted to 3.5, placed on ice, and mixed with 0.2 M triethanolamine buffer, 0.2 mM NADP, and 5 mM MgCl₂ before 170 U/ml G-6-P-dehydrogenase was added. Absorbance was measured at 340 nm before and after the addition of enzyme, and G-6-P content was calculated in nmol tissue (4). For the intracellular coenzyme A (CoA) ester levels, 100–200 mg of liver samples were added to an ice-cold mortar and pestle containing 1.5 ml of 6% perchloric acid and homogenized. The homogenate was centrifuged (2,000 g for 10 min at 4°C), 50 μl of 0.32 M dithiothreitol were added to the supernatant, and CoA esters were measured using a modified HPLC procedure (4).

Hepatic glycogen content. Liver samples were obtained at 0800 (postprandial state) or at 1700 (steady state) from age-matched 3-mo-old male L-SACC1 and wild-type mice. Frozen tissue samples (20–30 mg) were digested in 1 N NaOH for 2 h at 65°C. Homogenates were centrifuged at 14,000 rpm, and supernatants were spotted on Whatman filter paper. After papers were washed in 70% ethanol, samples were incubated in 0.2 M sodium acetate-amylglucosidase for 4 h. Afterwards, glucose trinder (Sigma) was added, and samples were incubated at room temperature for 15 min, analyzed at A₅₀₅ nm, and converted to milligrams glycogen per gram of wet tissue weight.

Lipoprotein lipase activity. Following an overnight fast, plasma samples were collected from the age-matched 3-mo-old male L-SACC1 and wild-type mice following intravenous injection of heparin (100 IU/kg body wt) via tail vein. Samples (5 μl) were diluted in water and incubated for 60 min at 25°C shaking water bath in 180 μl of incubation medium [10 μl of substrate emulsion containing 10% intralipid (120 mM triglycerol) into which a trace amount of 14 MBq [3H]triolein was introduced, 10 μl heat-inactivated serum as a source of apolipoprotein C-II, 60 μl of deionized water, and 100 μl of incubation buffer (12% fatty acid free bovine serum albumin, 0.02% standard heparin, 0.2 M NaCl, and 0.3 M Tris-HCl, pH 8.5)]. The reaction was stopped by addition of distilled water (0.5 ml) and 2 ml of isopropanol-heptane-H₂SO₄ (48:48:3:1 vol/vol/vol). Total lipids were extracted and fatty acids separated from triglycerol as follows: after centrifugation (2,000 g for 3 min, 4°C), a sample of the upper phase (800 μl) containing total lipids was transferred to new tubes into which 1 ml alkaline ethanol (ethanol 95%-water-2 M NaOH, 500:475:25 vol/vol/vol) and 3 ml heptane were added. After a second centrifugation, the upper heptane phase containing unhydrolysed triglycerol was discarded. A new extraction was performed with 3 ml of heptane. Finally, an aliquot (800 μl) of the remaining alkaline ethanol phase containing fatty acids was counted. All incubations were performed in triplicate. Lipoprotein lipase (LPL) activity was then calculated in millinits per milliliter plasma (with 1 mU representing 1 nmol of fatty acids released per min) (7).

Western blotting for fatty acid synthase and fatty acid transport protein-1. To determine the expression levels of fatty acid synthase (FAS) and fatty acid transport protein-1 (FATP-1), proteins in frozen tissues were lysed in 0.5% Triton (20), and 75 μg of lysates were analyzed directly by 6 and 10% SDS-PAGE followed by immuno-blotting with polyclonal antibodies against FAS (11) and FATP-1.
Body composition and plasma profiles in L-SACC1 mice. The L-SACC1 mice showed a significant increase in body weight (Table 1) that was attributed to a twofold increase in whole body fat mass, as assessed by $^1$H-MRS (Fig. 1A). In contrast, whole body lean mass did not differ between the groups (Fig. 1A). Following an overnight fast, plasma glucose levels were similar, but plasma insulin levels were significantly elevated in the L-SACC1 mice (Table 1).

Hepatic glucose metabolism during hyperinsulinemic euglycemic clamp. To assess organ-specific insulin action and glucose metabolism in vivo, a 2-h hyperinsulinemic euglycemic clamp was conducted in awake male wild-type and L-SACC1 mice. During the clamps, plasma insulin concentration was raised to ~300 pM in both groups of mice while plasma glucose concentration was maintained at 5–6 mM by a variable infusion of glucose in all groups (Table 1). The glucose infusion rate required to maintain euglycemia during clamps increased rapidly in the wild-type mice and reached a steady state within 90 min. In contrast, the steady state glucose infusion rate was significantly lower in the L-SACC1 mice (236 ± 16 vs. 352 ± 10 μmol·kg$^{-1}$·min$^{-1}$ in the wild-type mice; Fig. 1B). This indicates that whole body glucose metabolism in response to insulin was markedly blunted in the L-SACC1 mice, consistent with previously reported glucose intolerance in these mice (30). Whole body insulin resistance was partly attributed to reduced insulin sensitivity in liver. Although basal HGP was not altered, the rates of HGP during insulin clamp were increased more than threefold in the L-SACC1 mice (49 ± 8 vs. 83 ± 9% in wild-type mice; Fig. 1C). This resulted in blunted insulin-mediated suppression of HGP (hepatic insulin resistance) in the L-SACC1 mice (49 ± 8 vs. 83 ± 9% in wild-type mice; Fig. 1D). Also consistent with elevated HGP, intracellular level of G-6-P in liver during the postprandial state was significantly increased in the L-SACC1 mice (0.34 ± 0.07 vs. 0.19 ± 0.02 μmol/g wet tissue in the wild-type mice; Fig. 2A).

Table 1. Metabolic parameters of the wild-type and L-SACC1 mice at basal and during a 2-h hyperinsulinemic euglycemic clamp experiment

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<th>Basal Period</th>
<th>Clamp Period</th>
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<tr>
<td></td>
<td>Plasma glucose, mM</td>
<td>Plasma insulin, pM</td>
</tr>
<tr>
<td>Wild type</td>
<td>7.3 ± 0.6</td>
<td>31 ± 3</td>
</tr>
<tr>
<td>L-SACC1</td>
<td>7.1 ± 0.4</td>
<td>67 ± 10*</td>
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</tbody>
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Values are means ± SE. L-SACC1, liver-specific overexpression of dominant negative phosphorylation-defective S503A-CEACAM1 mutant. *P < 0.05 vs. wild-type mice by Student’s t-test; †basal plasma insulin levels were measured in a separate group of age-matched mice following an overnight fast.
Insulin-mediated suppression of HGP (hepatic insulin action) involves inhibition of gluconeogenesis and stimulation of net hepatic glucose uptake, where the former pathway involves insulin’s modulation of phosphoenolpyruvate carboxykinase (PEPCK) and the latter pathway mostly involves insulin’s stimulation of hepatic net glycogen synthesis (5). In this regard, we have previously shown that PEPCK levels were increased in the hepatocytes of L-SACC1 mice (30). Additionally, postprandial hepatic glycogen content was reduced by ~70% in the L-SACC1 mice (1.75 ± 0.10 vs. 6.52 ± 1.89 mg/g wet tissue in the wild-type mice; Fig. 2B), suggesting blunted glycogen synthesis.

Hepatic lipid metabolism. In addition to changes in glucose metabolism, hepatic lipid metabolism was altered in the L-SACC1 mice, resulting in a twofold increase in intrahepatic triglyceride content (11.6 ± 1.6 vs. 6.9 ± 1.9 μmol/g in the wild-type mice; Fig. 2C, left). This was associated with significant increases in intracellular levels of long-chain fatty acyl-CoAs (0.225 ± 0.045 vs. 0.095 ± 0.021 μmol/g dry wt in the wild-type mice; Fig. 2C, right) and protein content of FAS, a key enzyme in de novo lipogenesis, in the liver of L-SACC1 mice (Fig. 2D). These results are consistent with our recent report (26) indicating increased FAS activity in the L-SACC1 mice compared with the wild-type mice. Circulating levels of β-hydroxybutyrate were markedly reduced in male L-SACC1 mice (48 ± 7 vs. 482 ± 148 mM in the wild-type mice, P < 0.05), suggesting reduced fatty acid β-oxidation. Furthermore, total and hepatic activity of LPL, a rate-determining enzyme in the hydrolysis of circulating triglyceride (7), was unaltered in the L-SACC1 mice (14.0 ± 4.0 vs. 14.5 ± 3.2 μmol FFA·mL−1·h−1 for total LPL and 4.6 ± 0.5 vs. 5.2 ± 0.5 μmol FFA·mL−1·h−1 for hepatic LPL in the wild-type mice). Taken together, these results suggest that increases in hepatic lipogenesis and triglyceride release may be responsible for elevated levels of hepatic and circulating triglyceride levels, whereas whole body lipolysis may not be significantly altered in the L-SACC1 mice (30).

Whole body and peripheral glucose metabolism. Insulin-stimulated whole body glucose uptake was reduced by ~20% in the L-SACC1 mice (289 ± 7 vs. 356 ± 13 μmol·kg−1·min−1 in the wild-type mice; Fig. 3A), indicating insulin resistance in peripheral tissues. Similarly, insulin-stimulated whole body glucose plus lipid synthesis was reduced by ~30% (107 ± 17 vs. 155 ± 14 μmol·kg−1·min−1 in the wild-type mice), but insulin-stimulated glycolysis was unaltered in the L-SACC1 mice (Fig. 3, B and C). Peripherial insulin resistance was mostly accounted for by an ~40% decrease in insulin-stimulated glucose uptake in the skeletal muscle of L-SACC1 mice (241 ± 28 vs. 382 ± 28 nmol·g−1·min−1 in the wild-type mice; Fig. 4A). Insulin-stimulated glycolysis in skeletal muscle was decreased by ~40% in the L-SACC1 mice (227 ± 28 vs. 370 ± 29 nmol·g−1·min−1 in the wild-type mice), whereas glycogen synthesis was unaltered (Fig. 4B). Reduced muscle glycolysis despite a normal rate of whole body glucose glycolysis in the L-SACC1 mice suggests that organs other than skeletal muscle may have altered glycolysis. Skeletal muscle insulin resistance was associated with a more than twofold increase in intramuscular triglyceride levels in the L-SACC1 mice (10.2 ± 2.7 vs. 4.1 ± 2.0 μmol/g in the wild-type mice; Fig. 4C). The protein level of FATP-1 was unaltered in the skeletal muscle of L-SACC1 mice (Fig. 4D).

Furthermore, insulin-stimulated glucose uptake in white and brown adipose tissues was reduced by 30–50% in the L-SACC1 mice (34 ± 7 vs. 75 ± 13 nmol·g−1·min−1 and 2,044 ± 254 vs. 3,088 ± 294 nmol·g−1·min−1 in white and brown adipose tissue of wild-type mice, respectively; Fig. 5, A and B). In addition to altered glucose metabolism, lipid metabolism in white adipose tissue was enhanced in the L-SACC1 mice, as reflected by significantly elevated expression of FAS and FATP-1 (Fig. 5, C and D).
DISCUSSION

We have previously shown that L-SACC1 caused defects in hepatic insulin clearance, chronic hyperinsulinemia, increased visceral adiposity, and glucose intolerance (30). In this study, we performed a hyperinsulinemic euglycemic clamp with labeled glucose to determine the mechanism by which L-SACC1 mice develop altered glucose homeostasis. Our results indicate that L-SACC1 mice are insulin resistant, owing to defects in hepatic insulin action and insulin-mediated glucose uptake in skeletal muscle and adipose tissue. Insulin resistance in skeletal muscle was associated with increased intramuscular fat content. Insulin resistance in liver and adipose tissue was associated with altered lipid metabolism and elevated expression of genes associated with lipogenesis. Thus our findings indicate that insulin resistance in the L-SACC1 mice is due to altered glucose and lipid metabolism in liver, skeletal muscle, and adipose tissue.

Fig. 3. Insulin-stimulated whole body glucose metabolism in vivo in the L-SACC1 mice and wild-type littermates. A: insulin-stimulated whole body glucose uptake. B: insulin-stimulated whole body glycogen plus lipid synthesis. C: insulin-stimulated whole body glycolysis. Values are means ± SE for 11–12 experiments. *P < 0.05 vs. wild-type mice.

Fig. 4. Glucose metabolism and triglyceride in the skeletal muscle of L-SACC1 mice and wild-type littermates. A: insulin-stimulated glucose uptake in skeletal muscle (gastrocnemius). B: insulin-stimulated glycolysis and glycogen synthesis in skeletal muscle (gastrocnemius). C: intramuscular triglyceride levels (quadriceps). Values are means ± SE for 11–12 experiments. D: skeletal muscle fatty acid transport protein-1 (FATP-1) represented in Western blot and density normalized to actin. Tissue lysates from wild-type and L-SACC1 mice (n = 5) were sequentially probed with α-FATP-1 and α-actin antibodies. *P < 0.05 vs. wild-type mice.
Previous studies have shown that CEACAM1 is required for receptor-mediated insulin endocytosis and that inhibition of its activity impairs hepatic clearance of insulin (3, 6). Recently, we have described a mechanism by which CEACAM1 participates in the transient suppression of hepatic triglyceride synthesis by insulin (26), which arguably counters the lipogenic effect of portal insulin to regulate hepatic triglyceride levels. Our findings of elevated intrahepatic triglyceride and fatty acyl-CoA levels in the L-SACC1 mice further support the regulatory role of CEACAM1 on hepatic lipid metabolism. Additionally, because FAS expression was increased, whereas LPL activity was unaltered in the liver of L-SACC1 mice, increased de novo lipogenesis due to blunted insulin-mediated suppression of proteins associated with lipid metabolism (e.g., FAS) may be responsible for elevated hepatic fat contents (26).

Altered lipid metabolism and intrahepatic accumulation of lipid may be responsible for hepatic insulin resistance in the L-SACC1 mice. Inverse relationship between intracellular fat content and insulin sensitivity has been well documented in studies involving both humans and animal models (23, 22, 38). Increasing fatty acid delivery into liver by overexpressing LPL caused insulin resistance (16), whereas decreasing fatty acid uptake into tissues with the deletion of fatty acid translocase (FAT/CD36) or FATP-1 improved insulin sensitivity (9, 17). The molecular mechanism by which intracellular accumulation of fat causes insulin resistance involves activation of serine kinase cascade, of which PKC and/or IκB kinase-β may play a role, by fatty acid metabolites (i.e., fatty acyl-CoAs, diacylglycerol, ceramide), leading to the serine phosphorylation of insulin receptor substrates (IRS) (8, 18, 28, 41, 42). Subsequent to the serine phosphorylation of IRS, insulin-mediated tyrosine phosphorylation of IRS, activation of phosphatidylinositol 3-kinase and Akt, and GLUT4 translocation are impaired (31). However, because IRS-2 phosphorylation was unaltered in the liver of L-SACC1 mice (30), lipid-mediated alteration of other IRS isoforms or downstream signaling proteins may mediate hepatic insulin resistance in these mice. Alternatively, hyperinsulinemia-mediated downregulation of insulin receptor in liver may play a role in the pathogenesis of hepatic insulin resistance in the L-SACC1 mice (30).

In addition to altered hepatic glucose and lipid metabolism, L-SACC1 mice developed a profound increase in visceral adiposity in association with altered lipid metabolism in adipocytes and chronic hyperinsulinemia (30). Our previous study showed that mice with muscle-specific deletion of insulin receptor (MIRKO) developed insulin resistance and hyperinsulinemia and elevated whole body fat mass (19). Selective insulin resistance in the skeletal muscle caused hyperinsulinemia-mediated redistribution of substrates to adipose tissue, leading to increased adiposity in the MIRKO mice (19). Our findings of elevated expression of FAS and FATP-1 in the adipocytes of L-SACC1 mice further support increased lipid flux and de novo lipogenesis, possibly due to hyperinsulinemia in these mice (36). In this regard, increased circulating levels of fatty acids directed from adipocytes may further contribute to elevated fat contents and insulin resistance in the liver of L-SACC1 mice. In support of this notion, we have recently shown that reduction of visceral obesity and nicotinic acid-mediated inhibition of lipolysis ameliorated hepatic insulin resistance in the L-SACC1 mice (4).

Although the genetic mutation was directed to a liver-specific protein, CEACAM1, it was surprising to find severe insulin resistance in the skeletal muscle of L-SACC1 mice. In this regard, increased visceral adiposity is also likely to mediate blunted glucose metabolism in skeletal muscle. Our finding of a more than twofold increase in intramuscular triglyceride content suggests that lipid flux into skeletal muscle was enhanced in response to increased adiposity in the L-SACC1 mice. Intramuscular accumulation of fat and fatty acid-derived metabolites may in turn affect insulin signaling and glucose...
metabolism (8, 9, 17, 18, 22, 23, 28, 31, 38, 41, 42). Interestingly, our previous in vitro study (30) showed normal insulin-mediated glucose transport in the soleus muscle of 2-mo-old L-SACC1 mice that was associated with unaltered intramuscular triglyceride level in isolated soleus muscle of 8-mo-old L-SACC1 mice. The discrepant findings may be attributed to different experimental setting (in vivo vs. in vitro), fiber type (type 1 fiber-consisting soleus muscle vs. mixed fiber-consisting gastrocnemius muscle), and/or age of mice studied (2 and 8 mo vs. 4–5 mo). Nonetheless, the present results clearly indicate that insulin-stimulated glucose metabolism in vivo is reduced in the L-SACC1 mice, and this defect may be associated with increased intramuscular fat contents. Moreover, insulin-stimulated glucose uptake in white and brown adipose tissue was also reduced in the L-SACC1 mice, and this is consistent with the effects of diet-induced obesity on adipocyte glucose metabolism (27).

We thus propose the following mechanism by which L-SACC1 mice develop whole body insulin resistance. Inactivation of CEACAM1 may cause hepatic insulin resistance by increasing the expression and activity of lipogenic enzymes and lipogenesis in liver. Subsequent accumulation of intrahepatic fat and fatty acid metabolites may downregulate insulin signaling that leads to defects in glucose metabolism. Additionally, inactivation of CEACAM1 may indirectly cause hepatic insulin resistance by blunting hepatic insulin clearance and altering lipid metabolism in adipose tissue in association with chronic hyperinsulinemia. This event, combined with increased adiposity, may further contribute to intrahepatic accumulation of lipid. Altered lipid metabolism and obesity in the L-SACC1 mice may also be responsible for increased intramuscular fat and insulin resistance in skeletal muscle and adipose tissue. Overall, the demonstration that a distinct CEACAM1-dependent signaling pathway modulates insulin clearance without affecting intracellular signaling proposes a mechanism by which hyperinsulinemia may be a cause, and not a consequence, of insulin resistance. Our findings thus indicate the potential role of CEACAM1 and hepatic insulin clearance in the pathogenesis of obesity and insulin resistance.

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