Hyperglycemia, maturity-onset obesity, and insulin resistance in NONcNZO10/LtJ males, a new mouse model of type 2 diabetes

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Cho Y-R, Kim H-J, Park S-Y, Ko HJ, Hong E-G, Higashimori T, Zhang Z, Jung DY, Ola MS, LaNoue KF, Leiter EH, Kim JK. Hyperglycemia, maturity-onset obesity, and insulin resistance in NONcNZO10/LtJ males, a new mouse model of type 2 diabetes. Am J Physiol Endocrinol Metab 293: E327–E336, 2007; doi:10.1152/ajpendo.00376.2006.—As a new mouse model of obesity-induced diabetes generated by combining quantitative trait loci from New Zealand Obese (NZO/HILt) and Nonobese Nondiabetic (NON/LtJ) mice, NONcNZO10/LtJ (RCS10) male mice developed type 2 diabetes characterized by maturity onset obesity, hyperglycemia, and insulin resistance. To metabolically profile the progression to diabetes in preobese and obese states, a 2-h hyperinsulinemic euglycemic clamp was performed and organ-specific changes in insulin action were assessed in awake RCS10 and NON/LtJ (control) males at 8 and 13 wk of age. Prior to development of obesity and attendant increases in hepatic lipid content, 8-wk-old RCS10 mice developed insulin resistance in liver and skeletal muscle due to significant decreases in insulin-stimulated glucose uptake and GLUT4 expression in muscle. Transition to an obese and hyperglycemic state by 13 wk of age exacerbated insulin resistance in skeletal muscle, liver, and heart associated with organ-specific increases in lipid content. Thus, this polygenic mouse model of type 2 diabetes, wherein plasma insulin is only modestly elevated and obesity develops with maturity yet insulin action and glucose metabolism in skeletal muscle and liver are reduced at an early prediabetic age, should provide new insights into the etiology of type 2 diabetes.

Glucose metabolism; skeletal muscle; liver; lipids; transgenic mouse

Type 2 diabetes is a progressive disease with insulin resistance being the primary event that leads to a series of metabolic changes including compensatory hyperinsulinemia, dyslipidemia, decompensation of pancreatic β-cells, and hyperglycemia (3, 6, 13, 32, 35). Although the mechanism of insulin resistance remains unclear, obesity and altered lipid metabolism likely play a causative role (1, 25, 31). Adipocytes affect glucose metabolism by releasing fatty acids into circulation, and obesity-associated increase in circulating fatty acids has been shown to cause insulin resistance (2, 3, 47). The mechanism may involve intracellular accumulation of lipid in insulin-sensitive organs (i.e., skeletal muscle, liver, and heart) and lipid-mediated downregulation of insulin signaling and glucose transport system (24, 27, 42, 50, 55). Adipocytes further modulate whole body glucose homeostasis by releasing hormones/cytokines such as resistin, adiponectin, leptin, tumor necrosis factor (TNF)-α, and interleukin (IL)-6 (collectively termed adipokines) that affect glucose metabolism, and obesity is associated with dysregulated production of adipokines (11, 14, 17, 45, 46, 49, 52).

To better understand the relationship between obesity and diabetes, new polygenic mouse models of obesity-induced diabetes ("diabesity") were recently generated by combining independent diabetes risk-conferring quantitative trait loci (QTL) from two unrelated parental strains of New Zealand Obese (NZO/HILt) and nonobese nondiabetic (NON/LtJ) mice (36). Recombinant congenic strains (RCS) were then created by back-crossing the F1 hybrids onto the parental NON/LtJ background, with selection for different subsets of diabesity QTL sorting into each RCS. Among the RCS generated, one, NONcNZO10/LtJ, contains the greatest number of diabesity contributions from both parental backgrounds and develops a maturity-onset obesity and hyperglycemia with 100% of the mice exhibiting type 2 diabetes phenotype by 24 wk of age (28). To determine the metabolic changes underlying the development of diabetes in these mice, we performed a hyperinsulinemic euglycemic clamp to assess the changes in organ-specific glucose metabolism and insulin action in preobese and obese NONcNZO10/LtJ males relative to the parental NON/LtJ strain males.

Experimental Procedures

Animals and Surgery

NONcNZO10/LtJ (JAX no. 4456) will hereafter be designated as RCS10 for brevity. Control NON/LtJ males (JAX no. 2423) and young prediabetic RCS10 males were obtained from two sources at The Jackson Laboratory, a research vivarium (conventional SPF) as well as from colonies of each maintained behind full barrier (a kind donation of JAX Research Systems). Two cohorts of each strain were shipped from Bar Harbor, Maine, to Yale University, one at 3 wk of age and the second at 7 wk of age. Upon arrival, each group was aged in the quarantine facility of NIH-Yale Mouse Metabolic Phenotyping Center for 6 wk prior to the in vivo metabolic studies. Mice were housed under controlled temperature (23°C) and lighting (12 h of light, 0700–1900; 12 h of dark, 1900–0700) with free access to standard mouse chow diet (6% fat by calories; Harlan Teklad, Madison, WI) and water.

At least 4 days before scheduled in vivo experiments, whole body fat and lean mass was measured in awake mice by 1H-MRS (Bruker...
Mini-Spec Analyzer; Echo Medical Systems, Houston, TX). Following the body 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 the 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 awake state) and tail restrained using a tape to obtain blood samples from the tail vessels. All procedures were approved by the Yale University and Penn State University Animal Care and Use Committee.

Hyperinsulinemic Euglycemic Clamps

After an overnight fast (~15 h), a 2-h hyperinsulinemic euglycemic clamp was conducted 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 physiological range (~200 pM) (18). 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 glucose at basal concentrations (~6 mM). 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 the clamps (0.05 μCi/min) and throughout the clamps (0.1 μCi/min), respectively. To estimate insulin-stimulated glucose uptake in individual tissues, 2-deoxy-D-[1-14C]glucose (2-[14C]DG; PerkinElmer Life and Analytical Sciences) 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 the clamps for the measurement of plasma [3H]glucose, [3H]2O, 2-[14C]DG concentrations, and/or insulin concentrations. At the end of the clamps, mice were euthanized, and tissues were taken for biochemical and molecular analysis (18).

Biochemical Assays

Glucose concentrations during clamps were analyzed using 10 μl of plasma by a glucose oxidase method on a Beckman Glucose Analyzer 2 (Fullerton, CA). Plasma insulin concentrations were measured by radioimmunoassay (RIA) using kits from Linco Research (St. Charles, MO). Plasma concentrations of [3-3H]glucose, 2-[14C]DG, and [3H]2O were determined following deproteinization of plasma samples, as previously described (18). The radioactivity in the tissue glycogen was determined by digesting tissue samples in KOH and precipitating glycogen with ethanol. For the determination of tissue 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 (19). Briefly, the lipid layer was separated using H2SO4, and concentrations were determined using a triglyceride assay kit (Sigma Diagnostics, St. Louis, MO) and spectrophotometry.

Western Blot for Muscle Expression of IRS-1, GLUT4, and PKC

To measure IRS-1 expression in skeletal muscle, immunoblotting was performed using powdered gastrocnemius muscle tissue dissolved in lysis buffer (50 mM HEPES, pH 7.4, 137 mM NaCl, 2 mM
EDTA, 10 mM NaF, 1 mM MgCl$_2$, 10 mM sodium pyrophosphate, 2 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 10 mM sodium vanadate, 1% Nonidet P-40, 1 μM microcystin LR, and protease inhibitor cocktail), homogenized by short sonication burst and centrifuged for 15 min at 15,000 g. The supernatant was harvested, and protein concentrations were determined using the Bio-Rad DC protein assay (Hercules, CA). The proteins were dissolved in SDS gel sample buffer and resolved by electrophoresis on 8% polyacrylamide gels. Total protein (50 μg) was loaded per well. After electrophoresis, proteins were transferred to nitrocellulose membrane (Bio-Rad), blocked in 5% nonfat milk in 50 mM Tris–HCl (pH 7.5), 100 mM NaCl, and 0.1% Tween 20 and incubated with IRS-1 antibody (Upstate, 1:1,000 dilution). The membranes were then incubated in goat anti-rabbit secondary antibody conjugated to horseradish peroxidase (1:5,000 dilution; Pierce Chemical, Rockford, IL). Blots were developed in SuperSignal West Pico (Pierce) and exposed to X-ray film. The film was scanned with a GS-800 scanner (Bio-Rad), and the density was quantified by Quantity One software (Bio-Rad). Insulin-stimulated tyrosine phosphorylation of IRS-1 was examined in muscle samples (gastrocnemius) obtained at the end of the insulin clamps and using anti-phosphotyrosine antibodies for IRS-1 (Upstate).

To measure GLUT4 and PKCθ expression in skeletal muscle, 50 mg of quadriceps tissues were ground with a mortar under a continuous flow of liquid N$_2$. Powdered tissues were lysed in 800 μl of ice-cold lysis buffer (25 mM HEPES, 4 mM EDTA, 25 mM benzamidine, pH 7.4) containing protease inhibitor cocktail (1.04 mM E329

Table 1. Metabolic parameters at basal state (overnight fasted) and during a 2-h hyperinsulinemic euglycemic clamp experiment in RCS10 and NON/LtJ mice at ~8 and ~13 wk of age

<table>
<thead>
<tr>
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<th>Basal Period</th>
<th>Clamp Period</th>
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<tr>
<td></td>
<td>n</td>
<td>Body Weight, g</td>
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<tr>
<td>NON/LtJ, 8 wk</td>
<td>8 ± 10</td>
<td>23 ± 0</td>
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<tr>
<td>RCS10, 8 wk</td>
<td>12</td>
<td>23 ± 1</td>
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<tr>
<td>NON/LtJ, 13 wk</td>
<td>6 ± 10</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>RCS10, 13 wk</td>
<td>8</td>
<td>32 ± 1*</td>
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Values are means ± SE. *P < 0.05 vs. NON/LtJ mice of respective age.

Fig. 2. Insulin-stimulated glucose and lipid metabolism in RCS10 and NON/LtJ (control) mice at 8 wk of age. A: insulin-stimulated glucose uptake in skeletal muscle (gastrocnemius). B: intramuscular (quadriceps) triglyceride levels. C: total IRS-1 protein levels in skeletal muscle (gastrocnemius). D: total GLUT4 and PKCθ expression levels in skeletal muscle (quadriceps). Values are means ± SE for 8–12 experiments. *P < 0.05 vs. NON/LtJ mice.
AEBSF, 0.8 μM aprotinin, 20 μM leupeptin, 40 μM bestatin, 15 μM Pepstatin A, 14 μM E-64; Sigma-Aldrich) and 1% Triton X-100 (final concentration). Tissue lysates were sonicated with an ultrasonic processor (Cole Pamer, Vernon Hill, IL) at an amplitude of 40 for 1 min on ice, incubated by periodically vortexing on ice for 1.5 h, and centrifuged for 40 min at 20,000 g. The supernatants were harvested, and protein concentrations were determined using the BCA reagent (Pierce). One hundred micrograms of each protein was mixed with 2× sample loading buffer (125 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 2% β-mercaptoethanol, 0.06% bromophenol blue) and loaded in 10% gel for SDS-PAGE. Proteins resolved by SDS-PAGE were transferred to nitrocellulose membrane (Bio-Rad). The membrane was blocked by gentle shaking with 5% nonfat milk in TBST for 1 h at room temperature and incubated with polyclonal GLUT4 and PKC antibodies (Chemicon International, Temecula, CA) in 1% nonfat milk in TBST overnight at 4°C. Detection of immunoreactive bands was achieved using ECL, and quantification was performed using a GS-800 densitometer (Bio-Rad).

**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 (dpm/min) to the specific activity of plasma glucose (dpm/mol) at the end of basal period and during the final 30 min of clamp, respectively. Insulin-stimulated rate of HGP during clamp was determined by subtracting the glucose infusion rate from whole body glucose uptake. Whole body glycolysis and glycogen plus lipid synthesis from glucose were calculated as previously described (18). Insulin-stimulated glucose uptake in individual tissues was assessed by determining the tissue (e.g., skeletal muscle, heart) content of 2-[14C]DG-6-P and plasma 2-[14C]DG profile, which was fitted with a double exponential or linear curve using MLAB (Civilized Software, Bethesda, MD). Insulin-stimulated glycolysis and glycogen synthesis in skeletal muscle were calculated as previously described (18).

**Hyperglycemic Clamps to Assess In Vivo Pancreatic β-Cell Function**

In an additional group of mice, hyperglycemic clamps were performed in awake RCS10 and NON/LtJ mice at 8 wk (n = 5–6 per group) and 13 wk (n = 6–7 per group) of age. Survival surgery was performed at 4–5 days before clamps to establish indwelling catheter in jugular vein. Following an overnight fast (~15 h), a 2-h hyperglycemic clamp was conducted with a continuous and variable infusion of 20% glucose to maintain hyperglycemia at ~300 mg/dl. Blood samples were taken at 15- to 20-min intervals for the measurement of plasma glucose and insulin levels. Furthermore, metabolic cages (TSE Systems, Bad Homburg, Germany) were used in an additional group of RCS10 and NON/LtJ mice at 8 wk of age (n = 4 for each group) to noninvasively measure food/water intake, energy expenditure, indirect calorimetry, and physical activity.

**Statistical Analysis**

Data are expressed as means ± SE. The significance of the difference in mean values between RCS10 and NON/LtJ mice was calculated as previously described.
evaluated using Student’s t-test. The statistical significance was at the 
\( P < 0.05 \) level.

RESULTS

Preobese, Hyperglycemic 8-wk-old RCS10 Mice

Basal metabolic parameters. Whole body fat and lean mass, as measured by \(^{1}H\)-MRS in awake mice, were similar in RCS10 and NON/LtJ mice at 8 wk of age (Fig. 1A). Despite normal body weight, RCS10 mice showed diabetic phenotypes with significantly elevated basal (overnight fasted) plasma glucose and insulin levels compared with NON/LtJ mice (Table 1). Additionally, metabolic cage analysis indicated no alterations in food intake, energy expenditure, \( V\dot{O}_{2} \) consumption, \( V\dot{CO}_{2} \) production, and total physical activity in RCS10 mice (data not shown).

Whole body glucose metabolism during hyperinsulinemic-euglycemic clamps. To determine the changes in insulin action and glucose metabolism in vivo, a 2-h hyperinsulinemic euglycemic clamp was conducted in awake male RCS10 and NON/LtJ mice at 8 wk of age. During the clamps, plasma insulin concentration was raised to \( \sim 200 \) pM, whereas the plasma glucose concentration was maintained at \( \sim 6 \) mM by a variable infusion of glucose in all groups (Table 1). RCS10 mice were insulin resistant, as indicated by a 50% reduction in glucose infusion rate during clamps, and this is consistent with basal hyperinsulinemia in these mice (Fig. 1B).

\[^{3}H\]glucose turnover data confirmed that insulin-stimulated whole body glucose uptake was significantly reduced in 8-wk-old RCS10 males compared with age-matched NON/LtJ males (Fig. 1B). In RCS10 males, insulin-stimulated whole body glycolysis was decreased by \( \sim 30\% \), but whole body glycogen plus lipid synthesis was unaffected (Fig. 1C).

Skeletal muscle insulin action. Muscle insulin action and glucose metabolism were assessed using radioactive labeled 2-deoxyglucose, which is a nonmetabolizable glucose analog, during clamps. Whole body insulin resistance in RCS10 mice was partly due to a 20% decrease in insulin-stimulated glucose uptake in skeletal muscle (gastrocnemius) (Fig. 2A). Although body weights of 8-wk-old RCS10 males were not significantly different from NON/LtJ male controls, intramuscular triglyceride levels were modestly but significantly elevated compared with age-matched NON/LtJ mice (Fig. 2B). Skeletal muscle expression of IRS-1 was not altered in RCS10 mice (Fig. 2C). Insulin-stimulated tyrosine phosphorylation of IRS-1 was also not altered in RCS10 mice (data not shown). In contrast, RCS10 mice showed more than 60% decrease in total GLUT4 levels in skeletal muscle, which in part accounts for muscle insulin resistance in these mice (Fig. 2D). Total PKC\( \epsilon \) expression in muscle was not altered in RCS10 males compared with NON/LtJ males.

Fig. 4. Body composition and whole body glucose metabolism in RCS10 and NON/LtJ (control) mice at 13 wk of age. A: whole body fat and lean mass as measured by \(^{1}H\)-MRS. B: steady-state glucose infusion rate, obtained from averaged rates of 90–120 min of hyperinsulinemic euglycemic clamps, and insulin-stimulated whole body glucose uptake. C: insulin-stimulated whole body glycolysis and glycogen plus lipid synthesis. Values are means \( \pm \) SE for 6–8 experiments. \(* P < 0.05\) vs. NON/LtJ mice.
Glucose metabolism in liver and heart. Basal HGP was not significantly altered in RCS10 mice at 8 wk of age (Fig. 3A). In contrast, insulin’s ability to suppress HGP was blunted in RCS10 mice, as reflected by significantly elevated HGP during insulin clamp compared with NON/LtJ mice. Hepatic insulin resistance in these RCS10 males was not due to increased intrahepatic triglyceride levels, which did not differ significantly from those in NON/LtJ controls (Fig. 3B). In contrast to liver and gastrocnemius muscle, insulin-stimulated glucose uptake in heart and intramyocardial triglyceride levels were not altered in 8-wk-old preobese RCS10 males (Fig. 3, C and D).

Obese, Hyperglycemic 13-wk-old RCS10 Mice

Basal metabolic parameters. By 13 wk of age, RCS10 mice showed significant increases in body weight, which was mostly due to ~50% increases in whole body fat mass compared with NON/LtJ mice (Fig. 4A). Basal plasma glucose concentrations remained higher in RCS10 mice, but plasma insulin concentrations were not significantly altered in the cohort of 13-wk-old RCS10 males tested (Table 1). Thus, 13-wk-old RCS10 males were relatively obese and hyperglycemic, consistent with previous observations (28).

Whole body glucose metabolism. In the 13-wk-old cohort of RCS10 males, the steady-state glucose infusion rate to maintain euglycemia during insulin clamp was further reduced by 70% compared with age-matched NON/LtJ mice (Fig. 4B). Older RCS10 males remained insulin resistant, as reflected by significant reductions in both insulin-stimulated whole body glucose uptake (Fig. 4B) and ~60% reduction in whole body glycogen plus lipid synthesis (Fig. 4C).

Insulin action in skeletal muscle, liver, and heart. Whole body insulin resistance in 13-wk-old RCS10 mice was partly due to an ~40% decrease in insulin-stimulated glucose uptake in skeletal muscle (Fig. 5A). Intramuscular triglyceride levels increased more than twofold in 13-wk-old RCS10 mice compared with 8-wk-old RCS10 mice (14.4 ± 1.6 vs. 6.4 ± 2.0 μmol/g, P < 0.005) and remained significantly higher than age-matched NON/LtJ mice (7.4 ± 1.7 μmol/g; Fig. 5B). Hepatic insulin resistance was exacerbated, and insulin completely failed to suppress HGP in 13-wk-old RCS10 mice (Fig. 5C). In contrast to the metabolic data in 8-wk-old mice, hepatic insulin resistance in 13-wk-old RCS10 males was associated with an approximately threefold increase in intrahepatic triglyceride levels (Fig. 5D). These results are consistent with previously observed hepatic steatosis in chronically hyperglycemic 24-wk-old RCS10 males (28). Additionally, 13-wk-old RCS10 mice developed cardiac insulin resistance, as reflected by a 50% decrease in insulin-stimulated glucose uptake in heart that was associated with significantly elevated intramyocardial triglyceride levels compared with age-matched NON/LtJ mice (Fig. 6, A and B). Insulin-stimulated glucose uptake in white adipose tissue was also reduced in RCS10 mice (Fig. 6C).

Fig. 5. Glucose and lipid metabolism in skeletal muscle and liver of RCS10 and NON/LtJ (control) mice at 13 wk of age. A: insulin-stimulated glucose uptake in skeletal muscle (gastrocnemius). B: intramuscular (quadriceps) triglyceride levels. C: hepatic glucose production during basal and insulin-stimulated (clamp) states. D: intrahepatic triglyceride levels. Values are means ± SE for 6–8 experiments. *P < 0.05 vs. NON/LtJ mice.
To examine the pancreatic β-cell function in vivo, hyperglycemic clamps were conducted in awake RCS10 mice and NON/LtJ mice at 8 and 13 wk of age. Plasma glucose levels were raised with variable rates of glucose infusion and maintained at ~300 mg/dl in both groups of mice (Fig. 7, A and C). At 8 wk of age, hyperglycemia induced insulin secretion in NON/LtJ mice, as indicated by a rapid rise in plasma insulin levels at 20–30 min of clamps (P < 0.05 at 20 min, P < 0.05 at 30 min vs. basal insulin levels; Fig. 7B). In contrast, plasma insulin levels did not significantly change during hyperglycemic clamps in 8-wk-old RCS10 mice (Fig. 7B). There was no clear pattern of changes in plasma insulin levels during hyperglycemic clamps in 13-wk-old NON/LtJ and RCS10 mice (Fig. 7D).

DISCUSSION

To determine the mechanism by which RCS10 mice develop insulin resistance, organ-specific insulin action and glucose metabolism were assessed during hyperinsulinemic euglycemic clamps in awake RCS10 and NON/LtJ mice. At 8 wk of age, body weight and whole body fat mass were comparable between RCS10 males and NON/LtJ males (controls). RCS10 mice were hyperglycemic and developed whole body insulin resistance that was due to marked decreases in insulin-stimulated glucose uptake in skeletal muscle and insulin’s ability to suppress HGP. Skeletal muscle insulin resistance was partly due to reduced GLUT4 expression. At 13 wk of age, RCS10 mice became relatively obese and exhibited hyperglycemia with only modest changes in plasma insulin levels. The maturity-onset obesity in RCS10 males was accompanied by more severe insulin resistance developing in skeletal muscle, liver, and heart that was associated with significant increases in organ-specific triglyceride levels.

The mechanism by which preobese, 8-wk-old RCS10 males develop skeletal muscle insulin resistance may involve chronic hyperglycemia (i.e., glucose toxicity). Previous studies showed that partial pancreatectomy- and streptozotocin-induced hyperglycemia caused significant decreases in GLUT4 protein levels and insulin-stimulated glucose uptake in rat skeletal muscle (37, 38). Since insulin treatment normalized GLUT4 mRNA and protein levels in pancreatectomized rats (37), hypoinsulinemia and hyperglycemia may downregulate GLUT4 expression to protect the cells from the toxic effects of high glucose. Thus, early bouts of hyperglycemia in 8-wk-old RCS10 males may be responsible for reduced GLUT4 expression and insulin resistance in skeletal muscle. Alternatively, reduced skeletal muscle expression of GLUT4 may be causally associated with hyperglycemia in RCS10 mice.

Despite normal whole body adiposity, intramuscular triglyceride levels were modestly but significantly elevated in 8-wk-old RCS10 mice. This is consistent with a previous study of Laybutt et al. (27), which showed intramuscular lipid accumulation following chronic glucose infusion in rats. Inverse relationship between intramuscular lipid content and insulin sens-
Sensitivity is well documented in studies involving humans and animal models (26, 47). Increasing fatty acid delivery into skeletal muscle by overexpressing lipoprotein lipase caused insulin resistance (19), whereas decreasing fatty acid uptake into skeletal muscle with the deletion of fatty acid translocase (FAT/CD36) or fatty acid transport protein 1 improved insulin sensitivity (10, 21). The mechanism may involve intracellular accumulation of fatty acid-derived metabolites (i.e., fatty acyl-CoAs, diacylglycerol, ceramide), activation of serine kinases, including PKC and IκB kinase-β, and subsequent serine phosphorylation of IRS-1, which downregulates insulin signaling and glucose transport activity (20, 41, 42, 55, 56). In 8-wk-old RCS10 mice, the modest increase in intramuscular triglyceride levels did not affect IRS-1 protein expression or IRS-1 tyrosine phosphorylation. Although we cannot rule out the possible changes in downstream insulin signaling activity, such as phosphatidylinositol 3-kinase and Akt, our findings indicate that IRS-associated insulin signaling does not play a major role in mediating skeletal muscle insulin resistance in RCS10 mice.

Insulin-stimulated whole body glycolysis was significantly reduced, but whole body glycogen plus lipid synthesis was unaltered in 8-wk-old RCS10 mice. Skeletal muscle accounts for the majority of glucose disposal during the insulin-stimulated state (43), and the pattern of changes in whole body glucose metabolic flux may reflect those in skeletal muscle. Consistent with this notion, insulin-stimulated muscle glycolysis was reduced by 25% in the RCS10 mice (322 ± 30 vs. 433 ± 22 nmol·g$^{-1}$·min$^{-1}$ in NON/LtJ mice; $P < 0.05$) whereas insulin-stimulated glycogen synthesis in skeletal muscle, measured by [$^{3}$H]glucose incorporation into muscle glycogen, was unaltered in RCS10 mice (17.1 ± 2.8 vs. 16.7 ± 2.7 nmol·g$^{-1}$·min$^{-1}$ in NON/LtJ mice). Since glucose transport is the rate-determining step of muscle glucose utilization (12), reduced expression of GLUT4 and glucose uptake in skeletal muscle may be responsible for the decreases in whole body and muscle glycolysis in RCS10 mice. Furthermore, insulin-stimulated muscle glycogen synthesis involves activation of IRS-1- and Akt-mediated insulin signaling and subsequent stimulation of glycogen synthase (5, 12, 13). Thus, normal IRS-1 expression in skeletal muscle may promote insulin-stimulated glycogen synthesis in 8-wk-old RCS10 mice.

In addition to muscle insulin resistance, preobese 8-wk-old RCS10 mice developed hepatic insulin resistance. Since intrahepatic triglyceride levels were normal, hepatic insulin resistance may be due to factors other than altered lipid metabolism, which may involve early bouts of hyperglycemia (4, 22, 39, 51). We (22) have previously shown that mice with muscle-specific deletion of GLUT4 developed hepatic insulin resistance, which was secondary to skeletal muscle insulin resistance and chronic hyperglycemia. Additionally, Brihard et al. (4) showed that phloridzin treatment of streptozotocin-diabetic rats normalized glycemia and hepatic glucose metabolism, implicating the causative role of hyperglycemia on hepatic insulin resistance. Alternatively, hepatic insulin resistance may
be due to changes in intracellular levels of lipid-derived metabolites, such as long-chain fatty acyl-CoAs, diacylglycerol, and ceramide, which may not reflect intrahepatic triglyceride levels. We (55) have previously shown that such lipid metabolites, and not triglyceride per se, are the causative factors of insulin resistance.

At 13 wk of age, RCS10 mice became relatively obese, and insulin resistance in skeletal muscle and liver was exacerbated. This is most likely due to the more severe increase in intramuscular triglyceride levels and intrahepatic triglyceride content that was significantly elevated in 13-wk-old RCS10 mice (normal hepatic lipid in 8-wk-old RCS10 mice). Increased circulating levels of fatty acids directed from adipocytes may contribute to elevated lipid contents and insulin resistance in skeletal muscle and liver of RCS10 mice (7). These results are consistent with the causative role of intracellular lipid in insulin resistance (42). Despite severe insulin resistance, basal insulin levels were not significantly altered compared with those of insulin-sensitive NON/LtJ mice. Available data (29) suggest that the early progression to hyperglycemia in RCS10 males is associated with altered insulin secretion dynamics in response to glucose in 13-wk-old RCS10 mice. Our findings from hyperglycemic clamps confirm that pancreatic β-cell function and glucose-induced insulin secretion are blunted in RCS10 mice, and these defects contribute to hyperglycemia in these mice.

Although mitochondrial lipid oxidation is the principal energy source for constitutively energy-demanding heart, glucose metabolism and insulin action play an important role in normal cardiac function (15, 40, 44, 48). Previous studies using isolated perfused-heart preparations, cultured cardiomyocytes, and positron emission tomography uniformly showed blunted glucose metabolism and insulin resistance in diabetic heart (23, 40, 54). Recent studies showed that mice with cardiac-selective overexpression of PPARα developed metabolic and functional phenotypes similar to that of the diabetic heart, including increased lipid oxidation and reduced glucose metabolism (9, 34). Importantly, those studies demonstrated the causative role of increased lipid supply to the myocardium in the pathogenesis of diabetic heart (8). Our findings that insulin-stimulated cardiac glucose uptake was reduced in obese 13-wk-old RCS10 mice are consistent with previous reports of cardiac insulin resistance in obese and diabetic rodent models such as leptin-deficient (ob/ob) mice (23, 30, 40). Additionally, cardiac insulin resistance was associated with a significant increase in intramyocardial triglyceride content in RCS10 mice, which is consistent with the role of myocardial lipid metabolism in insulin resistance (8, 23, 40). In contrast, preobese and hyperglycemic 8-wk-old RCS10 mice maintained normal cardiac insulin action and triglyceride levels. In this regard, chronic hyperglycemia has been implicated in the pathogenesis of diabetic heart with mechanisms involving hyperglycemia-mediated changes in myocardial lipid metabolism, activation of PKC isoforms, and increases in glycosylated proteins such as advanced glycation end products (16, 27, 33, 51, 53). However, our results suggest that, at least in RCS10 mice, chronic but modest hyperglycemia did not affect cardiac glucose metabolism.

Overall, RCS10 mice, as a new mouse model of obesity-associated type 2 diabetes, developed diabetes phenotypes due to insulin resistance in skeletal muscle, liver, and heart. Preobese 8-wk-old RCS10 mice developed insulin resistance in skeletal muscle due to reduced GLUT4 expression, which was associated with chronic hyperglycemia. Hepatic insulin resistance developed in these mice possibly due to factors other than lipid. By 13 wk of age, RCS10 males became relatively obese and developed insulin resistance in skeletal muscle, liver, and heart, which was mostly due to organ-specific increases in lipid content. Thus, our results indicate a contribution of the mild hyperglycemia in insulin-resistant 8-wk-old RCS10 mice, with the development of obesity exacerbating the impairment in glucose homeostasis by 13 wk of age. Our findings that insulin resistance develops via different mechanisms in hyperglycemic and obese states of RCS10 mice provide important insights to understanding nonobese and obese type 2 diabetic subjects. Additionally, RCS10 mice develop age-associated metabolic abnormalities in individual organs that resemble the progressive development of human type 2 diabetes and thus are ideal models to understand and identify therapeutic targets in the treatment of diabetes.

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