Nonobese, insulin-deficient Ins2ΔAkita mice develop type 2 diabetes phenotypes including insulin resistance and cardiac remodeling

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Nonobese, insulin-deficient Ins2ΔAkita mice develop type 2 diabetes phenotypes including insulin resistance and cardiac remodeling. Am J Physiol Endocrinol Metab 293: E1687–E1696, 2007. First published October 2, 2007; doi:10.1152/ajpendo.00256.2007.—Although insulin resistance has been traditionally associated with type 2 diabetes, recent evidence in humans and animal models indicates that insulin resistance may also develop in type 1 diabetes. A point mutation of insulin 2 gene in Ins2ΔAkita mice leads to pancreatic β-cell apoptosis and hyperglycemia, and these mice are commonly used to investigate type 1 diabetes and complications. Since insulin resistance plays an important role in diabetic complications, we performed hyperinsulinemic-euglycemic clamps in awake Ins2ΔAkita and wild-type mice to measure insulin action and glucose metabolism in vivo. Nonobese Ins2ΔAkita mice developed insulin resistance, as indicated by an ~80% reduction in glucose infusion rate during clamps. Insulin resistance was due to ~50% decreases in glucose uptake in skeletal muscle and brown adipose tissue as well as hepatic insulin action. Skeletal muscle insulin resistance was associated with a 40% reduction in total GLUT4 and a threefold increase in PKCε levels in Ins2ΔAkita mice. Chronic phloridzin treatment lowered systemic glucose levels and normalized muscle insulin action, GLUT4 and PKCε levels in Ins2ΔAkita mice, indicating that hyperglycemia plays a role in insulin resistance. Echocardiography showed significant cardiac remodeling with ventricular hypertrophy that was ameliorated following chronic phloridzin treatment in Ins2ΔAkita mice. Overall, we report for the first time that nonobese, insulin-deficient Ins2ΔAkita mice develop type 2 diabetes phenotypes including peripheral and hepatic insulin resistance and cardiac remodeling. Our findings provide important insights into the pathogenesis of metabolic abnormalities and complications affecting type 1 diabetes and lean type 2 diabetes subjects.

The prevalence of diabetes is increasing at an alarming rate, and despite decades of investigative effort the mechanisms by which diabetes and its complications develop remain elusive (41, 42). Insulin resistance is a major characteristic of type 2 diabetes and plays a pivotal role in the pathogenesis of the disease (26, 35). Insulin resistance has also been shown to contribute to multiorgan affecting complications of type 2 diabetes (4, 50). Recent evidence in humans and animal models suggest that insulin resistance also develops in type 1 diabetes (9). In this regard, nonobese diabetic (NOD) mice have been used as a classical model of type 1 diabetes for more than 30 years (39), but recent reports from Chaparro and colleagues (5, 7) indicate that NOD mice also develop insulin resistance. These findings suggest that insulin resistance may be a common etiology of type 1 and type 2 diabetes and their associated complications (6, 15, 40).

The Ins2ΔAkita mouse, a C57BL/6 mutant mouse, spontaneously develops diabetes with significant early loss of pancreatic β-cell mass resulting from a conformation-altering missense mutation (Cys96Tyr) in the insulin 2 gene that disrupts a disulfide bond between A and B chains of insulin (47, 48). Intracellular accumulation of misfolded proinsulin promotes ER stress in β-cells that leads to apoptosis via induction of ER-stress-associated apoptosis factor Chop (29). Hence, Ins2ΔAkita mice develop progressive hyperglycemia secondary to hypoinsulinemia in the absence of insulitis. As a mouse model of type 1 diabetes with early-onset hyperglycemia, recent studies have used Ins2ΔAkita mice to examine diabetic complications including retinopathy, nephropathy and neuropathy (2, 10, 44). Barber et al. (2) demonstrated that Ins2ΔAkita mice showed increased retinal vascular permeability and apoptosis, resembling diabetic retinopathy. Others found elevated urinary albumin excretion in Ins2ΔAkita mice and suggested the use of these mice to investigate diabetic nephropathy (10). Ins2ΔAkita mice further showed enhanced expression of αβ-crystallin in oligodendrocytes, suggesting a role for hyperglycemia or insulin deficiency in diabetic neuropathy (44). Although Ins2ΔAkita mice are increasingly used to study complications associated with type 1 diabetes, there is little to no information on the metabolic phenotypes of these mice. This is an important gap, as altered glucose and lipid metabolism, as well as insulin resistance, may play a role in the etiology of diabetic complications affecting retina, kidneys, heart, and central nervous system (25). Thus, we performed hyperinsulinemic-euglycemic clamps in awake Ins2ΔAkita mice and wild-type (C57BL/6) mice to determine insulin action and glucose metabolism in individual organs. We report for the first time that nonobese, insulin-deficient Ins2ΔAkita mice develop insulin resistance in skeletal muscle, liver, and brown adipose tissue that is partly due to chronic hyperglycemia. Ins2ΔAkita mice also develop significant complications.
cardiac remodeling including ventricular hypertrophy. Chronic phlorizin (PHZ) treatment normalizes muscle insulin action and ameliorates ventricular hypertrophy in Ins2Akita mice, indicating a role of hyperglycemia in the metabolic abnormalities of Ins2Akita mice.

**EXPERIMENTAL PROCEDURES**

**Animals, ¹H-MRS body composition, and metabolic cages.** Male Ins2Akita mice and wild-type littersmates (C57BL/6 background) at 8–13 wk of age were housed under controlled temperature and lighting with free access to food and water. Whole body fat and lean mass were noninvasively measured in awake Ins2Akita and wild-type mice at 8 and 13 wk of age (n = 7 for each group) using ¹H-MRS (Echo Medical Systems, Houston, TX). In an additional group of mice (n = 4 for each group), 3-day measurements of food/water intake, energy expenditure, respiratory exchange ratio, and physical activity were performed using metabolic cages (TSE Systems, Bad Homburg, Germany). All procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee.

**Hyperinsulinemic-euglycemic clamps.** At 4–5 days before clamp experiments, mice were anesthetized, and an indwelling catheter was inserted into the right internal jugular vein (16). On the day of clamp experiment, a 3-way connector was attached to the catheter to intra-venously deliver solutions (e.g., glucose, insulin). Mice were placed in a rat-size restrainer (to minimize stress during experiments in awake experiment, a 3-way connector was attached to the catheter to intra-venously deliver solutions (e.g., glucose, insulin). Mice were placed in a rat-size restrainer (to minimize stress during experiments in awake state) and were tail restrained using a tape to obtain blood samples from the tail vessels.

Following an overnight fast (~15 h), Ins2Akita mice remained severely hyperglycemic (~30 mM), and PHZ (inhibitor of renal glucose reabsorption) was infused at 100 μg·kg⁻¹·min⁻¹ for 90 min prior to the clamps to lower basal glucose levels. A 2-h hyperinsulinemic-euglycemic clamp was conducted in awake Ins2Akita and wild-type mice at ~13 wk of age (n = 8–11) with a primed (150 μl/kg body wt), continuous infusion of human regular insulin (Humulin, Eli Lilly, Indianapolis, IN) at a rate of 2.5 μl/kg·min⁻¹ to raise plasma insulin within a physiological range (16). 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. Basal and insulin-stimulated whole body glucose turnover were estimated with a continuous infusion of [³-³H]glucose (PerkinElmer, Boston, MA) for 2 h prior to the clamps (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-O-[¹-¹¹C]glucose (2-¹¹C)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, [¹H]O₂, 2-¹¹C)DG concentrations, and/or insulin concentrations. At the end of the clamps, mice were euthanized, and tissues were taken for biochemical and molecular analysis (16).

**Biochemical assays.** Glucose concentrations during clamps were analyzed using 10 μl of plasma by a glucose oxidase method on a Beckman Glucose Analyzer 2 (Beckman, Fullerton, CA). Plasma insulin concentrations were measured by ELISA using kits from Alpco Diagnostics (Salem, NH). Plasma triglyceride and FFA concentrations were measured using Sigma diagnostic kits (Sigma Diagnostics, St. Louis, MO) and spectrophotometry. Plasma concentrations of [³-³H]glucose, 2-¹¹C)DG, and [¹H]O₂ were determined following deproteinization of plasma samples, as previously described (16). 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 2-¹¹C)DG-6-P content, tissue samples were homogenized, and the supernatants were subjected to an ion exchange column to separate 2-¹¹C)DG-6-P from 2-¹¹C)DG.

Tissue-specific triglyceride concentrations were determined by digesting tissue samples in chloroform-methanol (30).

**Calculations.** Rates of basal hepatic glucose production (HGP) and insulin-stimulated whole body glucose turnover were determined as the ratio of the [³H]glucose infusion rate to the specific activity of plasma glucose at the end of basal period and during the final 30 min of clamp, respectively (16). Insulin-stimulated rate of HGP during clamp was determined by subtracting the glucose infusion rate from whole body glucose turnover. Whole body glycolysis and glycogen plus lipid synthesis from glucose were calculated as previously described (16). Insulin-stimulated glucose uptake in individual tissues (i.e., skeletal muscle, white and brown adipose tissue, heart) was assessed by determining the tissue content of 2-¹¹C)DG-6-P and plasma 2-¹¹C)DG profile.

**Western blot for skeletal muscle GLUT4 and PKC expression.** To measure total GLUT4, PKCθ, and PKCe expression in skeletal muscle, 50 μg of gastrocnemius tissues was ground in a mortar with liquid N₂. 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 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 Chemical, Rockford, IL). One hundred micrometers 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 into 10% gel for SDS-PAGE. Protein loading was controlled with GAPDH. Proteins resolved by SDS-PAGE were transferred to a

Fig. 1. Body composition analysis using ¹H-MRS in Ins2Akita and wild-type mice at 8 and 13 wk of age. A: whole body fat mass. B: whole body lean mass. Values are means ± SE for 7 experiments. *P < 0.05 vs. wild-type mice.
nitrocellulose membrane (Bio-Rad, Hercules, CA). The membrane
was blocked by gently shaking with 5% nonfat milk in TBS-T for 1 h
at room temperature and incubated with polyclonal antibodies against
GLUT4 (Chemicon International, Temecula, CA), PKCθ, and PKCe
(Santa Cruz Biotechnology, Santa Cruz, CA) in 1% nonfat milk in
TBS-T overnight at 4°C. Detection of immunoreactive bands was
achieved using ECL, and quantification was performed using a GS-
800 densitometer (Bio-Rad).

Cardiac function and structure. In an additional group of Ins2Akita
and wild-type mice (n = 5 for each group), cardiac function and
structure were examined using two-dimensional directed M-mode
echocardiography with a Sequoia C256 Echocardiography System
equipped with a 7.5-MHz transducer in mice lightly anesthetized with
ketamine. Once anesthetized, mice were placed on a heating pad and
covered with surgical towels to limit heat loss and maintain core body
temperature. For all mice, 3–5 beats were recorded with the transducer
in the same position. The end diastolic and end systolic, interventric-
ular septum, and posterior wall dimensions were obtained using the
leading edge method (23). Ventricular fractional shortening was
calculated as EDD (end diastolic distance) / ESD (end systolic
distance) divided by EDD. Parameters were measured using electronic
calipers, and mean calculations were obtained from three or more
consecutive cardiac cycles.

Chronic treatment of PHZ. To determine the chronic effects of
normalizing glucose levels in Ins2Akita mice, PHZ (0.4 g/kg body wt)
was administered subcutaneously twice daily for 10 days or 6 wk in
Ins2Akita mice (n = 6–7 for each group), and insulin clamp and
echocardiography were performed at the end of treatment. Wild-
type mice were treated with vehicle injection and served as controls (n = 6).

Statistical analysis. Data are expressed as means ± SE. The
significance of the difference in mean values of Ins2Akita mice vs.
wild-type mice or PHZ-treated Ins2Akita mice vs. wild-type mice was
evaluated using Student’s t-test. The statistical significance was at the
P < 0.05 level.

RESULTS

Basal metabolic parameters. Ins2Akita mice showed smaller
body weights than wild-type mice (20.7 ± 0.2 vs. 23.2 ± 0.6 g
in wild-type mice, P < 0.05). Body composition analysis using
1H-MRS showed that whole body fat mass was significantly
reduced in Ins2Akita mice at 8 and 13 wk of age compared with
age-matched wild-type mice (Fig. 1A). In contrast, whole body
lean mass was not altered in Ins2Akita mice (Fig. 1B). Metabolic
cages were used to noninvasively measure energy balance in
Ins2Akita and wild-type mice (n = 4 for each group). Daily food
intake was increased twofold in Ins2Akita mice compared with
wild-type mice (Fig. 2A), and this is likely due to reduced
adiposity (and possibly leptin levels) in Ins2 Akita mice.
Ins2Akita mice were less active (Fig. 2B) but showed increased
energy expenditure during the 24-h cycle (Fig. 2C). Both rates
of O2 consumption and CO2 production were increased by
40% in Ins2Akita mice (Fig. 2D). Increased energy expendi-

Fig. 2. Three-day analysis of energy balance using metabolic cages in Ins2Akita and wild-type mice at 13 wk of age. A: food intake during a 24-h cycle. Averaged
daily food intake (inset). B: physical activity during a 24-h cycle. C: whole body energy expenditure during a 24-h cycle. D: averaged rates of VO2 and VCO2.
Values are means ± SE for 4 mice of each group. *P < 0.05 vs. wild-type mice.
Ins2Akita mice (33). Consistent with this notion, respiratory exchange ratio was significantly reduced in Ins2Akita mice (0.64 ± 0.01 vs. 0.69 ± 0.01 in wild-type mice), reflecting increased lipid oxidation.

Ins2Akita mice remained hyperglycemic (~30 mM) following the overnight fast, and PHZ was infused for 90 min to lower plasma glucose levels to ~10 mM before the clamps (Fig. 3A). Basal (overnight-fasted) plasma insulin levels tended to decrease in Ins2Akita mice (P = 0.07), whereas fed insulin levels were significantly reduced in Ins2Akita mice (Fig. 3B). Basal plasma triglyceride concentrations were reduced by 40% in Ins2Akita mice (Fig. 3C), and plasma fatty acids levels showed a tendency to be reduced in these mice (P = 0.12; Fig. 3D), likely due to enhanced whole body lipid oxidation. Insulin clamp caused a 50% reduction in plasma triglyceride levels in wild-type mice but failed to affect plasma triglyceride levels in Ins2Akita mice (Fig. 3C).

Whole body and skeletal muscle insulin action during clamps. To measure insulin action and glucose metabolism in vivo, 2-h hyperinsulinemic-euglycemic clamps were conducted in awake male Ins2Akita mice and wild-type littermates at 13 wk of age. During the clamps, plasma insulin concentration was raised to ~300 pM (Fig. 3B), whereas the plasma glucose concentration was maintained at ~7 mM by a variable infusion of glucose in both groups of mice (Fig. 3A). Ins2Akita mice were severely insulin resistant, as indicated by ~80% decreases in glucose infusion rates required to maintain euglycemia during the last 30 min of clamps (steady state) compared with wild-type mice (Fig. 4A). Insulin resistance was partly attributed to an ~40% reduction in insulin-stimulated whole body glucose turnover in Ins2Akita mice (Fig. 4B). Insulin-stimulated whole body glycolysis and glycogen synthesis were similarly reduced by 40–50% in Ins2Akita mice (Fig. 4C), which were due to lower glucose turnover in peripheral organs of Ins2Akita mice.

Skeletal muscle accounts for the majority (~70%) of whole body glucose disposal during insulin-stimulated state (13), and muscle glucose uptake was reduced by ~30% in Ins2Akita mice (Fig. 5A). Skeletal muscle insulin resistance was associated with a 40% reduction in total GLUT4 levels (Fig. 5B) and a threefold increase in PKCε expression in Ins2Akita mice (292 ± 18% vs. 100 ± 26% in wild-type mice). In contrast, total and membrane-associated PKCθ expression (Fig. 5B) and IRS-2-associated insulin signaling were not significantly altered in Ins2Akita mice (data not shown). Intramuscular triglyceride levels were reduced by almost 90% in Ins2Akita mice, which was consistent with significant decreases in circulating triglyceride levels and whole body fat mass in Ins2Akita mice (Fig. 5C). However, it is likely that changes in intracellular lipid metabolism secondary to hypoinsulinemia further contributed to markedly reduced intramuscular lipid levels in Ins2Akita mice.

Glucose and lipid metabolism in adipose tissue and liver. Insulin-stimulated glucose uptake in white adipose tissue was increased more than threefold in Ins2Akita mice (Fig. 6A). In contrast, insulin-stimulated glucose uptake in brown adipose tissue was significantly reduced in Ins2Akita mice (Fig. 6A). Thus, there was a reciprocal change in adipose tissue insulin action in Ins2Akita mice, and insulin resistance developed selectively in brown adipose tissue. This is consistent with other insulin-resistant models (e.g., high-fat diet model) in which glucose metabolism is similarly altered in skeletal muscle and brown adipose tissue (31).

Basal HGP was significantly increased in Ins2Akita mice, and this increase contributes to overnight-fasted hyperglycemia in these mice (Fig. 6B). The insulin clamp caused a 70% suppression of basal HGP in wild-type mice (Fig. 6B). HGP remained significantly elevated during the insulin clamp in Ins2Akita mice, resulting in markedly reduced hepatic insulin action in Ins2Akita mice (~30% suppression of basal HGP; Fig. 6C). Similar to skeletal muscle, intrahepatic triglyceride levels...
were significantly reduced in Ins2Akita mice (Fig. 6D). Taken together, these data demonstrate impaired insulin action on glucose metabolism in a nonobese, insulin-deficient animal, findings that are similar to those observed in models of type 2 diabetes. In contrast, lower lipid levels in skeletal muscle and liver were opposite to those commonly found in obese type 2 diabetic models but consistent with those found in lean type 2 diabetic subjects who also develop insulin resistance (14, 45).

Cardiac function and structure. To determine the effects of chronic hyperglycemia and insulin resistance on cardiac function, echocardiography was performed in lightly anesthetized Ins2Akita and wild-type mice. Representative echocardiograms of Ins2Akita and wild-type mice showing clear differences in cardiac parameters (EDD and ESD) are presented in Fig. 7A (wild-type mouse) and 7B (Ins2Akita mouse). Interventricular septum thickness and left ventricular posterior wall thickness were significantly increased in Ins2Akita mice, suggesting ventricular hypertrophy (Fig. 7C). In this regard, ventricular hypertrophy is a cardiovascular event commonly associated with diabetes (8). Ventricular fractional shortening and ejection fraction as markers of cardiac function were also altered in Ins2Akita mice (data not shown), which resemble our previous observations in obese, diabetic mice (31). Despite significant changes in cardiac structure and function, insulin-stimulated glucose uptake in heart (1,096 ± 104 vs. 1,134 ± 69 nmol·g⁻¹·min⁻¹ in Ins2Akita mice) and intramyocardial tri-

![Fig. 4. Insulin-stimulated whole body glucose metabolism in awake Ins2Akita and wild-type mice at 13 wk of age. A: steady state glucose infusion rate, obtained from averaged rates of 90–120 min of hyperinsulinemic-euglycemic clamps. B: insulin-stimulated whole body glucose turnover. C: insulin-stimulated whole body glycolysis and glycojen plus lipid synthesis. Values are means ± SE for 8–11 experiments. *P < 0.05 vs. wild-type mice.](image)

![Fig. 5. Skeletal muscle glucose and lipid metabolism in Ins2Akita and wild-type mice at 13 wk of age. A: insulin-stimulated glucose uptake in skeletal muscle (gastrocnemius). B: total GLUT4 and PKC0 levels in skeletal muscle (gastrocnemius). C: intramuscular triglyceride concentrations (quadriceps). Values are means ± SE for 5–11 experiments. *P < 0.05 vs. wild-type mice.](image)
glyceride levels (2.0 ± 0.3 vs. 1.9 ± 0.1 μmol/g in Ins2Akita mice) were not significantly altered in Ins2Akita mice.

Effects of chronic PHZ treatment. The role of hyperglycemia per se on insulin resistance was examined by treating Ins2Akita mice with PHZ to chronically lower circulating glucose levels. Plasma glucose levels were reduced from 25 mM to 10 mM after 2 days of PHZ treatment and remained thereafter in Ins2Akita mice (Fig. 8A). Chronic PHZ treatment for 10 days or 6 wk completely normalized peripheral insulin action in Ins2Akita mice, and insulin-stimulated whole body glucose turnover and skeletal muscle glucose uptake were comparable between PHZ-treated Ins2Akita mice and wild-type mice (Fig. 8, B and C). In contrast, PHZ treatment failed to normalize hepatic insulin action in Ins2Akita mice, which remained insulin...
resistant (Fig. 8D). Improved muscle glucose metabolism following chronic PHZ treatment was associated with increased GLUT4 expression and reduced PKCε levels in skeletal muscle of PHZ-treated Ins2Akita mice compared with untreated Ins2Akita mice (Fig. 9). Additionally, chronic PHZ treatment normalized cardiac function \((67 \pm 2 \text{ vs. } 63 \pm 2\% \text{ ventricular fractional shortening in PHZ-treated Ins2Akita mice, } P > 0.05)\) and cardiac structure (interventricular septum and left ventricular posterior wall thickness) in Ins2Akita mice (Fig. 7D).

**DISCUSSION**

Mutation of the insulin gene in pancreas of Ins2Akita mice results in hypoinsulinemia and hyperglycemia, as observed in type 1 diabetes. Although Ins2Akita mice are not obese and have lower lipid levels in circulation and tissues, these mice develop severe insulin resistance in multiple organs including skeletal muscle, brown adipose tissue, and liver. Insulin resistance in Ins2Akita mice is associated with reduced GLUT4 expression and increased PKCε levels in skeletal muscle. Ins2Akita mice also develop blunted cardiac function and ventricular hypertrophy. Chronic PHZ treatment lowers systemic glucose levels and improves insulin action selectively in skeletal muscle, whereas liver remains insulin resistant. PHZ-mediated recovery of muscle insulin action is associated with normalized levels of GLUT4 and PKCε in skeletal muscle of PHZ-treated Ins2Akita mice. Thus, nonobese, insulin-deficient Ins2Akita mice develop type 2 diabetes phenotypes including insulin resistance that may contribute to complications affecting other organs.

Insulin resistance is a major characteristic of obese and type 2 diabetic subjects and is largely due to intracellular accumulation of lipid and downregulation of insulin signaling in skeletal muscle (3, 18). The latter event may be due to lipid-mediated activation of PKCε, as observed in rodents, which increases serine phosphorylation of IRS-1 and causes insulin resistance in skeletal muscle (3). Insulin resistance also develops in subjects with type 1 diabetes and lean type 2 diabetic subjects, but the underlying mechanism remains unresolved (11, 14, 32, 45). Marked increases in intramyocellular lipid content are observed in type 1 diabetic subjects who develop insulin resistance in skeletal muscle (32). Defects in insulin-mediated suppression of lipolysis contribute to peripheral insulin resistance in adolescents with poorly controlled type 1 diabetes (11). Increased plasma levels of acylation stimulating protein and complement C3 are also associated with insulin resistance in lean type 2 diabetic subjects (45). In the present study, Ins2Akita mice developed insulin resistance in skeletal muscle in the absence of elevated intramuscular or circulating lipid levels, which were in fact reduced in Ins2Akita mice. Ins2Akita mice also developed insulin resistance in liver associated with reduced intrahepatic lipid levels. Thus, insulin resistance in Ins2Akita mice is unlikely due to lipid-mediated factors, and this is consistent with unaltered PKCε expression in skeletal muscle of Ins2Akita mice.

Insulin resistance in Ins2Akita mice involves reduced muscle expression of GLUT4, the major insulin-sensitive glucose transporter in skeletal muscle (13), and mice with muscle-specific deletion of GLUT4 developed severe insulin resistance in skeletal muscle (51). Studies using pancreatectomy or streptozotocin (STZ)-induced hyperglycemia found insulin resistance in skeletal muscle that was primarily due to reduced total muscle GLUT4 protein content (36, 49), consistent with our observation in Ins2Akita mice. Although the underlying mechanism is unclear, hyperglycemia and activation of PKCε, which is increased threefold in Ins2Akita mice, may play a role.
in the downregulation of GLUT4 in skeletal muscle. Chronic hyperglycemia ("glucose toxicity") promotes insulin resistance in skeletal muscle (4, 46), and increased PKCe levels were associated with insulin resistance in chronically glucose-infused rats (19). However, in the study of Laybutt et al. (19), hyperglycemia-induced insulin resistance and activation of PKCe were associated with intramuscular accumulation of long-chain acyl-CoAs. In this regard, it is important to note that their study was performed in normal Wistar rats, and chronic PHZ treatment ameliorated muscle insulin resistance in STZ-treated rats (28, 37). Alternatively, the hexosamine biosynthesis pathway (HBP) and elevated production of UDP-hexosamines may be involved in hyperglycemia-induced insulin resistance (24). Although recent findings have argued against the role of this pathway in hyperglycemia-induced insulin resistance in type 2 diabetes (34), the role of HBP in Ins2Akita mice and type 1 diabetes remains unknown. Taken together, our findings indicate that alterations in GLUT4 and PKCe secondary to β-cell pathogenesis and hyperglycemia may contribute to insulin resistance in type 1 diabetic and lean type 2 diabetic subjects.

In addition to peripheral organs, Ins2Akita mice developed insulin resistance in liver that was associated with reduced intrahepatic lipid levels, suggesting lipid-independent factors as the cause of insulin resistance. We (17) have previously shown that mice with muscle-specific deletion of GLUT4 developed insulin resistance in skeletal muscle and liver and that hepatic insulin resistance was due to glucose toxicity in these mice. Chronic PHZ treatment normalized systemic glucose levels and hepatic insulin action in GLUT4-null mice (17). Thus, chronic hyperglycemia may also contribute to hepatic insulin resistance in Ins2Akita mice (27). Interestingly, chronic PHZ treatment failed to ameliorate hepatic insulin resistance in Ins2Akita mice. It is unclear why hepatic insulin action was differently affected by PHZ treatment in GLUT4-null mice and Ins2Akita mice.

In addition to dramatic changes in glucose metabolism, Ins2Akita mice also showed alterations in lipid metabolism. Reduced lipid levels in skeletal muscle and liver may be due to multiple factors, including lower circulating lipid levels and alterations in intracellular lipid metabolism secondary to hyperinsulinemia in Ins2Akita mice. In this regard, Perseghin et al. (33) found reduced intrahepatic fat content and increased lipid oxidation in type 1 diabetic patients, and these results are consistent with enhanced whole body lipid oxidation in Ins2Akita mice. Other studies have shown that insulin regulates fatty acid uptake and lipid metabolism in peripheral organs (43), and the combined effects of insulin deficiency and insulin resistance may contribute to dramatic reductions in tissue lipid content in Ins2Akita mice. However, it remains unclear why intramyocellular lipids are elevated in type 1 diabetic subjects but reduced in Ins2Akita mice. One possibility may involve species difference in muscle lipid metabolism. Also, untreated diabetes with extreme hyperglycemia does not reproduce treated type 1 diabetes in humans. Moreover, insulin-mediated inhibition of adipocyte lipolysis may be blunted in Ins2Akita mice, since plasma lipid levels were not significantly altered between basal and insulin clamp state. This observation is consistent with previous findings indicating impaired suppression of lipolysis in poorly controlled type 1 diabetic subjects (11). Despite blunted insulin action on lipolysis, insulin-stimulated glucose uptake in white adipose tissue was increased in Ins2Akita mice. This may be a compensatory mechanism to partition more glucose into lipogenesis in adipose tissue of Ins2Akita mice.

Cardiac dysfunction and ventricular hypertrophy represent major complications associated with diabetes, and cardiovascular disease is the most common cause of mortality in diabetic subjects (41). Although the mechanism by which diabetes leads to heart disease is unknown, perturbations in cardiac metabolism and insulin resistance may precede and be causally associated with functional and pathological changes in the diabetic heart (22). Our recent study found that diet-induced...
insulin resistance was associated with ventricular hypertrophy and reduced ventricular fractional shortening in high-fat-fed C57BL/6 mice (31). Additionally, hyperglycemia and insulin resistance were associated with left ventricular hypertrophy in diabetic patients (12), and lowering systemic glucose levels with an α-glucosidase inhibitor attenuated heart failure in mice (21). In the present study, hyperglycemic and insulin-resistant Ins2Akita mice developed extensive cardiac remodeling that resulted in ventricular hypertrophy and alterations in ventricular dimension, ventricular fractional shortening, and ejection fraction. Our findings are consistent with the cardiovascular remodeling observed in insulin-resistant diabetic subjects (20). However, most diabetic patients exhibiting cardiac remodeling are hypertensive, a common comorbidity of diabetes that may contribute to diabetic heart disease (38). In contrast, Ins2Akita mice are not hypertensive (10) and showed normal insulin sensitivity in myocardium. Additionally, chronic PHZ treatment ameliorated defects in cardiac function and structure in Ins2Akita mice, suggesting the potential role of hyperglycemia in cardiac remodeling in these mice. Thus, our results indicate that chronic hyperglycemia and systemic insulin resistance may contribute to cardiac remodeling in Ins2Akita mice.

Overall, nonobese, insulin-deficient Ins2Akita mice, a model of type 1 diabetes, develop metabolic abnormalities associated with obesity and type 2 diabetes, including insulin resistance in skeletal muscle, adipose tissue, and liver. In contrast to obese and type 2 diabetic subjects, systemic insulin resistance in Ins2Akita mice develops without intracellular lipid accumulation and is mostly due to chronic hyperglycemia. Hyperglycemia and insulin resistance may also contribute to ventricular hypertrophy in Ins2Akita mice, since chronic PHZ treatment ameliorated metabolic and cardiac abnormalities in these mice. In this regard, NOD mice, a classical model of type 1 diabetes, were also found to express type 2 diabetes phenotypes, including insulin resistance (5). Additionally, lean type 2 diabetic subjects are reported to develop insulin resistance without intracellular lipid accumulation. Thus, our results provide important insights into the pathogenesis of metabolic abnormalities in type 1 diabetes and lean type 2 diabetes subjects. Also, our findings indicate that insulin-resistant Ins2Akita mice may be an ideal model to investigate the common etiology of complications associated with type 1 and type 2 diabetes.

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