Diabetic myopathy differs between \(\text{Ins2}^{\text{Akita}+/—}\) and streptozotocin-induced Type 1 diabetic models

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Submitted 3 December 2008; accepted in final form 20 February 2009

Krause MP, Riddell MC, Gordon CS, Imam SA, Cafarelli E, Hawke TJ. Diabetic myopathy differs between \(\text{Ins2}^{\text{Akita}+/—}\) and streptozotocin-induced Type 1 diabetic models. J Appl Physiol 106: 1650–1659, 2009. First published February 26, 2009; doi:10.1152/japplphysiol.91565.2008.—Mechanistic studies examining the effects of Type 1 diabetes mellitus (T1DM) on skeletal muscle have largely relied on streptozotocin-induced diabetic (STZ) rodents. Unfortunately, characterization of diabetic myopathy in this model is confounded by the effects of streptozotocin on skeletal muscle independent of the diabetic phenotype. Here we define adolescent diabetic myopathy in a novel, genetic model of T1DM, \(\text{Ins2}^{\text{Akita}+/—}\) mice, and contrast these findings with STZ mice. Eight weeks of diabetes resulted in significantly reduced gastrocnemius-plantaris-soleus mass (control: 0.16 ± 0.005 g; \(\text{Ins2}^{\text{Akita}+/—}\): 0.12 ± 0.003 g; STZ: 0.12 ± 0.01 g) and II/B/D fiber area in \(\text{Ins2}^{\text{Akita}+/—}\) (1.294 ± 94 μm²) and STZ (1.768 ± 163 μm²) compared with control (2.241 ± 144 μm²). Conversely, STZ type I fibers (1.535 ± 165 μm²) were significantly larger than \(\text{Ins2}^{\text{Akita}+/—}\) (915 ± 76 μm²) but not control (1.152 ± 86 μm²). Intramyocellular lipid increased in STZ (122.9 ± 3.6% of control) but not \(\text{Ins2}^{\text{Akita}+/—}\) likely resultant from depressed citrate synthase (control: 6.2 ± 1.2 μmol·s⁻¹·mg⁻¹; \(\text{Ins2}^{\text{Akita}+/—}\): 5.2 ± 0.8 μmol·s⁻¹·mg⁻¹; STZ: 2.8 ± 0.5 μmol·s⁻¹·mg⁻¹) and 3-β-hydroxyacyl coenzyme-A dehydrogenase (control: 4.2 ± 0.6 mmol·s⁻¹·mg⁻¹; \(\text{Ins2}^{\text{Akita}+/—}\): 5.0 ± 0.6 mmol·s⁻¹·mg⁻¹; STZ: 2.7 ± 0.6 mmol·s⁻¹·mg⁻¹) enzyme activity in STZ muscle. In situ muscle stimulation revealed lower absolute peak tetanic force in \(\text{Ins2}^{\text{Akita}+/—}\) (70.2 ± 8.2% of control) while STZ exhibited an insignificant decrease (87.6 ± 7.9% of control). Corrected for muscle mass, no force loss was observed in \(\text{Ins2}^{\text{Akita}+/—}\), while STZ was significantly elevated vs. control and \(\text{Ins2}^{\text{Akita}+/—}\). These results demonstrate that atrophy and specific fiber-type loss in \(\text{Ins2}^{\text{Akita}+/—}\) muscle did not affect contractile properties (relative to muscle mass). Furthermore, we demonstrate distinctive contractile, metabolic, and phenotypic properties in STZ vs. \(\text{Ins2}^{\text{Akita}+/—}\) diabetic muscle despite similarity in hyperglycemia/hypoinsulinemia, raising concerns of our current state of knowledge regarding the effects of T1DM on skeletal muscle.

insulin-dependent diabetes mellitus; muscle lipids; fiber-type shift; muscle stimulation; capillary-to-fiber ratio; twitch force

THE CAPACITY of skeletal muscle for growth, adaptation, and regeneration is truly remarkable. Nowhere is this ability more evident than during childhood/adolescence where increases in the overall size (hypertrophy) and strength of the muscle are the result of increased protein synthesis and fusion of satellite cell progeny (termed myoblasts) to existing muscle fibers (29). Naturally, this adaptability of muscle is not limited solely to hypertrophic stimuli. Atrophic stimuli, such as casting, weightlessness, and various pathological situations, result in a decrease in muscle mass, strength, and satellite cell number (21, 46, 56). It is important to note that, in contrast to adult muscle, atrophic stimuli placed on adolescent muscle results in a rapid and irreversible remodeling process involving a decrease in satellite cell content, impaired proliferative capacity, and myonuclear accretion (21, 46, 56).

One of the most common clinical conditions in which the capacity of muscle growth and performance may be dramatically altered is Type 1 diabetes mellitus (T1DM). Unfortunately, T1DM diagnosis often occurs in childhood or early adolescence, when management of glycemic control is often suboptimal (28). Although the effects of T1DM on human adolescent muscle phenotype and function have yet to be fully elucidated, the end result is an overall reduction in muscle mass and physical performance variables referred to as diabetic myopathy (1, 49). Studies of adult T1DM muscle performance indicate a reduction in peak strength (5) but improved fatigue resistance (2). To date, there are limited studies examining the effect of T1DM on skeletal muscle phenotype in adolescent or adult humans. Interestingly, of the studies conducted, it has been shown that type 1 diabetic muscle exhibits an increase in glycolytic metabolism (20) and a shift in fiber type toward more glycolytic or fast-twitch muscle fibers (26), which is contrary to what is typically observed in animal studies: a shift toward more oxidative fiber-type composition (7, 35). A shift in fiber-type composition is suggestive of possible alterations to substrate metabolism, although human studies measuring intramyocellular lipid (IMCL) have demonstrated no change (10) or, similar to animal studies (17, 35), an increased IMCL content (48).

Much of our current knowledge regarding diabetic myopathy is the result of studies performed using adult streptozotocin-induced diabetic rodents. Streptozotocin is a glucosamine-nitrosourea compound taken up by pancreatic β-cells, causing DNA damage followed by cell death, rendering the animal insulin deficient (9, 23, 32). The basic indexes of skeletal muscle phenotype and function, such as fiber-type composition, fiber size, IMCL, capillary-to-fiber ratio, and contractile parameters, have all been demonstrated to be altered in the streptozotocin-induced diabetic model (6–8, 17, 19, 35, 52). However, we have recently demonstrated that streptozotocin, independent of hyperglycemia/hypoinsulinemia, impairs body weight gain and attenuates muscle fiber growth in vivo, likely as a result of a reactive oxygen-species (ROS)-mediated G2/M phase cell cycle arrest in myoblasts (31). Thus it remains unclear if changes observed in streptozotocin-treated skeletal muscle (that were previously attributed to T1DM) were in fact...
the result of a direct action of streptozotocin. Clearly, a new non-streptozotocin model of diabetes is urgently needed to study the potentially deleterious complications of hyperglycemia/hypoinsulinemia to skeletal muscle.

Heterozygotic Ins2Akita mice (Ins2Akita+/−) become spontaneously diabetic due to a mutation in one allele of the insulin-2 gene, resulting in reduced levels of circulating insulin beginning during mouse adolescence (3–4 wk old) (38, 41, 57). With concerns raised regarding the use of streptozotocin for the study of diabetic skeletal muscle (31), we investigated the Ins2Akita+/− mouse as an alternative, nonpharmacological model for the study of diabetic myopathy. We hypothesized that although both streptozotocin-induced (STZ) and Ins2Akita+/− diabetic models exhibit characteristic hyperglycemia and hypoinsulinemia, there would be a number of functional and phenotypic variables that would be different between them. Consistent with this hypothesis, our findings indicate that STZ diabetic mice, compared with Ins2Akita+/− mice, display 1) a more drastic attenuation in weight gain, 2) elevated plasma nonesterified fatty acid (NEFA) levels, 3) elevated IMCL levels, 4) reduced muscle lipid and oxidative enzyme activity, 5) a paradoxical maintenance of absolute peak muscle contractile force, and 6) altered muscle twitch rate and relaxation times.

Not only is skeletal muscle responsible for our physical capacities for movement, it is also the largest organ for glucose disposal. Thus understanding the effects of T1DM on skeletal muscle in growth and development is of paramount importance if we are to properly and accurately develop therapeutic strategies to combat this devastating disease. Taken together, our results raise concerns regarding the current state of knowledge on the effects of T1DM on skeletal muscle, which is largely based on STZ-induced diabetic muscle, and suggest that nonpharmacological models should be used to confirm and validate previous findings.

METHODS

Animal characteristics. Age-matched male Ins2Akita+/− and C57Bl/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Ins2Akita+/− mice became hyperglycemic (>15 mM) over a period of 2–3 days at 4 wk of age. Correspondingly, at 4 wk of age, C57Bl/6 mice were randomly assigned into streptozotocin-treated (STZ) or control groups. The STZ group were administered a single intraperitoneal injection of 120 mg/kg streptozotocin (Sigma-Aldrich, Oakville, Canada) dissolved in sterile saline and became diabetic within 2–3 days or were excluded from the study. In total, 10 control, 10 Ins2Akita+/−, and 9 STZ mice were studied for an 8-wk period. The animal room was maintained at 21°C, 50% humidity, and a 12:12-h light-dark cycle, and all mice had access to standard diet and water ad libitum. Mice had no access to running wheels; however, enrichment was provided in the form of nesting material and cardboard tubing. Although not specifically measured, it appeared that Ins2Akita+/− and STZ mice were less active than control mice, particularly in the final weeks of the experimental period, consistent with that observed by others (30). Blood glucose was monitored daily (OneTouch Ultra glucometer; maximum 35 mmol/L; Johnson and Johnson) in the diabetic groups until glycemic values peaked and stabilized; thereafter blood glucose was measured less frequently. Body mass was measured weekly with animals in the fed state (0900) and once in the fasted state just before in situ muscle measurements (see below). At 4 wk of diabetes, fasted plasma samples were obtained to determine glucose and NEFA concentrations. At 5.5 wk of diabetes, food and water consumption were determined over a 24-h period. All experiments were approved by the York University Animal Care Committee in accordance with Canadian Council for Animal Care guidelines.

Muscle stimulation. Following the 8-wk experimental period, mice were fasted overnight and sedated with a ketamine-xylazine intraperitoneal injection (ketamine: 150 mg/kg; xylazine: 10 mg/kg) before surgery. Supplemental doses were unnecessary as the mice were unresponsive to pinch reflex tests for the duration of the protocol. The surgical procedure and muscle stimulation protocol were performed as described previously with minor modifications (37). Optimal voltage was determined by generating single-twitch contractions at increasing voltages until no increase in single-twitch force production was observed. Optimal muscle length was determined in a similar manner. Specifically, muscle length was manipulated and single-twitch force production was observed. The length and voltage at which a single twitch produced the greatest force were used throughout the entire stimulation protocol. The average voltage used was 30 V. The pulse duration was set to 0.1 ms for all twitch and tetanic contractions. The direct muscle stimulation protocol consisted of a force-frequency curve to determine peak tetanic force followed by a 2-min low-frequency (2 Hz) stimulation period to determine fatigue resistance. Twitch contractions were performed before and after the stimulation protocol. The force-frequency curve was determined by stimulating the muscle with trains 1 s in duration separated by 9 s of rest with the pulse frequency beginning at 20 Hz and increased by 10 Hz per train until no further increase in force was observed. The greatest force achieved was considered the absolute peak tetanic force. Direct muscle stimulation was chosen over sciatic nerve stimulation so as to directly assess the functional capacity of the muscle and remove potential confounding effects of impaired nerve/neuromuscular junction function (2–4, 24). Twitch amplitude, rise time, and half-relaxation time were determined before and after the stimulation protocol. Rise time was defined as the time elapsed from the base to the peak of a single twitch. Half-relaxation time was defined as the time elapsed from the peak of a single twitch to the point of the twitch amplitude returning halfway to baseline. All muscle function data were collected through an AD Instruments Bridge Amp and Powerlab 4/30 and analyzed with Chart5 PowerLab software.

Tissue collection and blood analyses. Following the muscle stimulation protocol, the animal was euthanized via decapitation, and tissues were immediately collected and either snap-frozen in liquid nitrogen or mounted on cork using mounting medium and quick-frozen in liquid nitrogen-cooled isopentane. Blood samples were collected and centrifuged at 16,000 g for 2 min to separate plasma. Plasma samples were analyzed for NEFA (HR Series NEFA-HR2 kit; Wako Diagnostics, Richmond, VA), glucose (YSI-2300 Stat-Plus glucose/lactate analyzer, Interscience, Toronto, Canada), and insulin concentrations (90080 Ultra Sensitive Mouse Insulin ELISA Kit, Crystal Chem).

Enzyme activity assays. 3-β-Hydroxacyl coenzyme-A dehydrogenase (β-HAD) and citrate synthase (CS) activity were determined as described previously (13) on soleus and white gastrocnemius muscle. Sample activities were normalized via Bradford-determined protein concentration (11).

Western blot analysis. Homogenates of soleus and white gastrocnemius muscles were prepared as described previously (45). Equal amounts of each protein sample (11) were loaded, resolved by SDS-PAGE, transferred to PVDF membranes, and probed using antibodies for fatty acid translocase (FAT/Cd36 (Santa Cruz, CA), cytochrome c [courtesy of Dr. D. A. Hood, York University (54)], and glyceroldehyde-3-phosphate dehydrogenase (GAPDH; AbCam). Primary antibodies were detected using the species-appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies and signals visualized on film using chemiluminescent reagent (Amersham). Target band optical intensity was quantified using Scion Image software (Scion) and presented as arbitrary units (AU).

Histochemical analyses. Metachromatic fiber-type staining was performed on gastrocnemius-plantaris cryosections (10 μm) using a
modified Ogilvie and Feeback protocol (37, 47). The red gastrocnemius, white gastrocnemius, and plantaris regions were identified on each section and a representative image of each muscle region was acquired for analysis. Approximately 200 fibers were counted per microscope image per animal to determine fiber-type composition. Fiber area was assessed on ~25 fibers/image for each fiber type using Scion Image (n = 9 in each group).

IMCL content was determined by Oil-Red-O staining of tibialis anterior sections (36). Optical intensity of IMCL droplet staining was assessed in the red region of the tibialis anterior as previously described (37) with Scion Image (~38 fibers/image were quantified; control n = 9, Ins2Akita+/−/ n = 7, STZ n = 9).

Muscle capillary-to-fiber ratio was assessed by the lead ATPase stain as previously described (37, 50) using the same muscle regions examined for fiber type and fiber area. An average of 377 capillaries and 196 fibers were manually counted in each microscope image and expressed as a capillary:fiber ratio (control n = 7, Ins2Akita+/−/ n = 8, STZ n = 8). Edge effect was not accounted for in this analysis; however, images were captured at low magnification. Consequently, the number of fibers and capillaries in the interior of the image greatly outnumber those on the edge, minimizing the edge effect.

All images were acquired with a Nikon Eclipse 90i microscope and Q-Imaging MicroPublisher 3.3 RTV camera with Q-Capture software.

Data analyses. All statistical analyses were performed with GraphPad Prism 5 software. Differences between control, Ins2Akita+/−/ and STZ groups were determined by one-way (area under fatigue curve, body and tissue masses, body growth rate, fasting plasma glucose, and NEFA concentrations, food and water consumption, white gastrocnemius fiber area, Western blot optical intensities, enzyme activities) or two-way (capillary-to-fiber ratio, fiber-type composition, red gastrocnemius and plantaris fiber areas, 2-min fatigability curve, all pre- vs. postfatigue contraction measures) ANOVA followed by Bonferroni post hoc tests. P values < 0.05 were considered significant. All data presented are means ± SE.

RESULTS

Animal characteristics. Assessment of fed body mass through-out the experiment revealed that control mice gained an average of 0.86 ± 0.07 g/wk, demonstrating that they were in the adolescent phase of development (Fig. 1A). Ins2Akita+/−/ mouse fed body mass did not significantly differ from control until the seventh week of diabetes, while STZ mouse body mass became significantly different from both control and Ins2Akita+/−/ mice at 3 wk of diabetes (Fig. 1A). Assessment of fasted body mass following the 8-wk experimental period revealed no difference between STZ and Ins2Akita+/−/ mice, while both weighed significantly less than control (Fig. 1B). Surprisingly, only Ins2Akita+/−/ mice exhibited hyperglycemia (Fig. 1C), while polydipsia was markedly elevated in both diabetic groups (Fig. 1D).

Both diabetic models exhibited significantly lower absolute muscle, heart, and epididymal fat mass compared with control mice (Fig. 1, E–G). In addition to the severe reduction in epididymal fat mass, other fat depots (subcutaneous and perirenal) were virtually undetectable in both diabetic models (data not shown). When tissue masses were expressed relative to body mass, a relative fat mass loss was also observed (Fig. 1H). Both diabetic models exhibited a significant decrease in relative muscle mass, indicating a specific loss of skeletal muscle mass (i.e., diabetic myopathy). STZ mice have a significantly lower relative heart mass compared with Ins2Akita+/−/ mice although the cause of this is unknown.

Ins2Akita+/−/ and STZ mice had similar fed blood glucose profiles throughout the 8-wk diabetic period although STZ mice were slower to reach peak blood glucose values (Fig. 2A). STZ and Ins2Akita+/−/ mice had similar plasma glucose values following an overnight fast (Fig. 2B). Fasting plasma insulin was not statistically different between any group (control: 0.214 ± 0.022 ng/ml; Ins2Akita+/−/ 0.173 ± 0.019 ng/ml; STZ: 0.175 ± 0.022 ng/ml), consistent with previous diabetes studies using STZ or Ins2Akita+/−/ mice in the fasted state (15, 16, 30, 57).

Muscle phenotype. In red gastrocnemius, a significant loss of type IIB/D fibers was observed in Ins2Akita+/−/ compared with control mice, while no significant fiber type loss was observed in STZ mice (Fig. 3A). Determination of fiber area in this muscle revealed a significantly lower type IIB/D fiber size in both diabetic models compared with control, while Ins2Akita+/−/ also displayed smaller type IIA fibers compared with control (Fig. 3B). Notably, STZ muscle exhibited significantly larger type I fibers compared with Ins2Akita+/−/ muscle. In the plantaris, a muscle generally devoid of type I fibers, no significant fiber-type percentage shift occurred (control: 0.7 ± 0.7% type I, 38.7 ± 3.3% type II, 60.6 ± 3.3% type IIB/D; Ins2Akita+/−/ 0.2 ± 0.1% type I, 40.0 ± 1.4% type IIA, 59.8 ± 1.4% type IIB/D; STZ: 5.4 ± 1.9% type I, 33.8 ± 3.1% type IIA, 60.8 ± 3.4% type IIB/D). However, a significant reduction in type IIB/D fiber area in both diabetic models vs. control was observed. STZ also exhibited a reduced type IIA fiber area in the plantaris muscle (control: 1.256 ± 0.022 μm² type IIA, 2.177 ± 0.143 μm² IIB/D; Ins2Akita+/−/ 1.003 ± 0.085 μm² type IIA, 1.551 ± 0.138 μm² type IIB/D; STZ: 83.4 ± 0.59 μm² type IIA, 1.320 ± 0.07 μm² type IIB/D). Examination of white gastrocnemius fiber area also demonstrated smaller IIB/D fibers in STZ and Ins2Akita+/−/ compared with control (control: 2.456 ± 0.120 μm²; Ins2Akita+/−/ 1.698 ± 0.09 μm²; STZ: 1.836 ± 0.115 μm²). Taken together, these findings suggest that diabetic skeletal muscles shift away from a glycolytic phenotype.

As alterations in fiber-type composition may be indicative of changes to other fiber characteristics, such as lipid storage, we determined IMCL content of individual tibialis anterior muscle fibers. The optical intensity of Oil-Red-O was significantly elevated in STZ muscle fibers compared with both control and Ins2Akita+/−/ (Fig. 4A). Given this striking change in lipid storage, it was prudent to examine measures of lipid uptake and utilization. Fasting NEFA concentrations were found to be significantly elevated in STZ mice compared with both control and Ins2Akita+/−/ mice (Fig. 4B). FAT/CD36, a protein involved in fatty acid transport into the muscle fiber (14, 34), was significantly elevated in the oxidative soleus of both diabetic models (Fig. 4D) but not the largely glycolytic white gastrocnemius (data not shown). While no change in cytochrome c expression in the soleus of either diabetic model was observed (control: 1.00 ± 0.07 AU; Ins2Akita+/−/ 0.80 ± 0.12 AU; STZ: 0.83 ± 0.14 AU), β-HAD activity was depressed in STZ soleus compared with Ins2Akita+/−/ (Fig. 4E) and CS activity was depressed in STZ soleus compared with control (Fig. 4F). A decrease in muscle capillary-to-fiber ratio has been previously demonstrated in STZ muscle (52); therefore, we were interested to determine if this change occurred in Ins2Akita+/−/ mice. A significant reduction in capillary-to-fiber ratio in both diabetic models compared with control mice was detected in
the plantaris and red gastrocnemius muscles, but not in white gastrocnemius (Fig. 5A).

Muscle function. To ascertain if the phenotypic and metabolic changes occurring in T1DM muscle resulted in functional impairment, we examined several contractile parameters of the gastrocnemius-plantaris-soleus muscle group. At each stimulation frequency tested, \textit{Ins2Akita/H11001/H11002} mice consistently displayed reduced tetanic contractile force compared with other groups (Fig. 6A; significant main effect of mouse model). Examination of absolute peak tetanic force revealed a significant \(~29\%\) loss of force in \textit{Ins2Akita/H11001/H11002} mice compared with control mice before fatigue (Fig. 6C). When expressed relative to muscle mass, there was no significant reduction in force, suggesting that the cause of reduced absolute force was the decrease in muscle mass (Fig. 6D). In contrast to \textit{Ins2Akita/H11001/H11002} muscle, STZ mice exhibit the greatest relative peak force (Fig. 6D; significant main effect of mouse model). No differences in fatigue rates were detected between groups during the low-frequency fatigue protocol (Fig. 6B).
To gain insight into muscle twitch characteristics and Ca\(^{2+}\) handling capacity of each mouse model, we measured twitch rise time and half-relaxation time. Analysis of twitch rise time revealed a significant difference between STZ and Ins2Akita/+ mice both before and following muscle fatigue (Fig. 6G). Half-relaxation time was also found to be elevated in STZ mice following the fatigue protocol compared with both control and Ins2Akita/+ mice (Fig. 6H).

**DISCUSSION**

The goal of the present study was to gain a more comprehensive understanding of diabetic myopathy by studying skeletal muscle from two different rodent models of T1DM: the commonly utilized, pharmacologically induced STZ mouse and the Ins2Akita/+ mouse (57), a genetic model of T1DM. While both models exhibited extreme hyperglycemia throughout the 8-wk experimental period and both developed some myopathic conditions, we have identified several novel dissimilarities between these models that require consideration given the divergent modes in which diabetes is initiated. Of particular note, we found that compared with muscle from Ins2Akita/+ mice, the muscles from STZ mice have 1) an increase in IMCL content likely the result of a twofold increase in circulating NEFA and reduced CS and \(\beta\)-HAD activity, 2) elevated type I fiber area compared with Ins2Akita/+ muscle, 3) a paradoxical increase in relative muscle twitch and tetanic force and, following fatigue, a reduced rate of relaxation. These changes do not appear to be the result of differences in hyperglycemia/hypoinsulinemia as both groups had similar exposure to glucose throughout the last 6 wk of diabetes, and fasted insulin levels at the time of harvest were not different between models. Rather, we believe that the pharmacological agent streptoz-

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Fig. 2. Alterations to blood glucose and feeding habits in the diabetic state. A: blood glucose values peaked in Ins2Akita/+ mice before STZ mice (‡significant difference between STZ and Ins2Akita/+ groups), although both diabetic models became significantly hyperglycemic within 3 days (*significant difference between control and both diabetic groups). B: following an overnight fast, Ins2Akita/+ and STZ mice had similar plasma glucose values.

![Figure 2](http://www.jap.org)

Fig. 3. Differences in fiber-type composition and fiber area are found between diabetic models. A: examination of fiber type percentage of red gastrocnemius revealed a significant interaction between fiber type and mouse model \((P = 0.0015)\). A significant decrease (*) in the percentage of type IIB/D fibers in Ins2Akita/+ mice compared with control (Con) mice was detected, while STZ mice did not differ from either group. B: examination of red gastrocnemius fiber area revealed that type I fibers were significantly larger in STZ mice compared with Ins2Akita/+ mice, while type IIA fibers were significantly smaller in Ins2Akita/+ mice compared with control mice. Ins2Akita/+ mice also had significantly smaller type IIB/D fibers compared with both STZ and control mice, although STZ IIB/D fibers were also significantly smaller than those of control (*significant difference between control group; †significant difference compared with Ins2Akita/+ group). C: representative images of metachromatic fiber type stains of control, Ins2Akita/+ and STZ mouse red gastrocnemius. Fiber types are indicated (I = type I, A = type IIA, B = type IIB/D). Note the visibly larger type I fibers in the STZ muscle. Bar represents 100 μm. *Significant difference compared with control group. †Significant difference compared with Ins2Akita/+ group.

![Figure 3](http://www.jap.org)

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1654 MYOPATHY DIFFERS BETWEEN AKITA AND STZ-INDUCED DIABETIC MICE

**J Appl Physiol • VOL 106 • MAY 2009 • www.jap.org**
tocin has other effects on muscle that are independent of the

As direct effects of streptozotocin on muscle both at the
molecular and cellular level have previously been demon-
strated (31), we propose that interpretation of previous diabetic
myopathy research employing STZ-induced diabetic models

![Fig. 4. Intramyocellular lipid (IMCL) accumulation and elevated plasma nonesterified fatty acids (NEFA) occurs in STZ but not Ins2Akita−/− mice. A: mean optical intensity of IMCL stained by oil-red O expressed relative to control mean. STZ mice exhibit elevated IMCL optical intensity compared with both control and Ins2Akita−/− mice. B: STZ mice exhibited a significant increase of NEFA measured in the blood plasma compared with both control and Ins2Akita−/− mice. C: representative images of an oil red O stain of control, Ins2Akita−/−, and STZ mouse tibialis anterior muscles. Bar represents 50 μm. D: Western blot analysis of fatty acid transportase (FAT/CD36) showed that both diabetic groups had significantly increased FAT/CD36 protein compared with control. Activity of the enzyme 3-β-hydroxyacyl coenzyme-A dehydrogenase (β-HAD; E) was depressed in STZ compared with Ins2Akita−/− mice, while citrate synthase (CS) activity (F) was lower in STZ compared with control mice. *Significant difference compared with control group. †Significant difference compared with Ins2Akita−/− group.](http://jap.physiology.org/)

should be made cautiously. The present study used a streptozotocin dose lower than what is commonly used to induce T1DM in mice [120 vs. ~200 mg/kg (24, 25, 39, 55)], suggesting that, even at lower doses, toxicity may still be a concern. Although rats typically do not require doses as high as mice, the changes to muscle phenotype observed in our STZ
mice are congruent with those seen previously in STZ rats (7, 8, 17, 52).

IMCL levels are a result of the balance between lipid availability (and uptake) and metabolism. Here we demonstrate that IMCL content in STZ muscle was elevated compared with \( \text{Ins2}^{\text{Akita}+/-} \) and control muscle (Fig. 4, A and C) and propose this is the result of elevated NEFA levels (Fig. 4B), increased FAT/CD36 protein expression (Fig. 4D), and depressed CS and \( \beta \)-HAD activity (Fig. 4, E and F). Hyperglycemia and hypoinsulinemia have been demonstrated to induce FAT/CD36 expression (18, 42), resulting in elevated transport of fatty acids across muscle membranes (42) and thereby increasing IMCL levels. While an increase in FAT/CD36 was observed in \( \text{Ins2}^{\text{Akita}+/-} \) muscle, no increase in IMCL was observed, possibly due to sustained activity of muscle enzymes involved in lipid metabolism (Fig. 4, E and F). Similar to the present findings, Hong et al. (30) demonstrate normal plasma NEFA levels, as well as lower plasma triglyceride and IMCL levels, in \( \text{Ins2}^{\text{Akita}+/-} \) at 13 wk of age. It remains possible that IMCL and plasma NEFA levels were elevated in \( \text{Ins2}^{\text{Akita}+/-} \) mice at some point during the 8-wk experimental period but had normalized by the time of tissue collection because of the muscle’s high reliance on this fuel as a source of ATP. Human studies have demonstrated either increased (48) or unchanged (10) IMCL levels.

The present study did not assess hepatic lipid content or lipid transport proteins such as FAT/CD36 in the liver of \( \text{Ins2}^{\text{Akita}+/-} \) mice, although Hong et al. (30) demonstrate low levels of liver triglycerides in \( \text{Ins2}^{\text{Akita}+/-} \) mice. Similar to the present study, plasma NEFA was not different from control mice, although plasma triglycerides were lower in \( \text{Ins2}^{\text{Akita}+/-} \) mice (30). Using STZ-diabetic rats, Luiken et al. (42) demonstrate low palmitate transport across hepatic membranes, despite increased FAT/CD36 expression. Whether a similar limitation in transport or increased metabolism of lipids is responsible for decreased liver triglycerides in the \( \text{Ins2}^{\text{Akita}+/-} \) liver is unknown.

Differences in fiber-type composition and fiber area were also detected in diabetic muscle. \( \text{Ins2}^{\text{Akita}+/-} \) red gastrocnemius displayed a loss of fast-glycolytic (type IIB/D) fiber number and an atrophy of all fast (type IIA and IIB/D) fibers, while STZ red gastrocnemius displayed atrophied type IIB/D fibers and hypertrophy of slow oxidative (type I) fibers compared with \( \text{Ins2}^{\text{Akita}+/-} \) (Fig. 3, A and B). Although the term atrophy has been applied to the reduced fiber area observed in these adolescent diabetic models, it cannot be ruled out that the reduced fiber area is the result of attenuated fiber growth. The findings in our STZ mice are consistent with observations in STZ rodents (7, 35). The cause of the increase in type I fiber area is currently unknown, although abundant availability of lipid may have promoted the increased expression of type I myosin. Support for this comes from a recent study demonstrating increased type I myosin expression in obese mice during the early stages of metabolic syndrome (22). It is important to note that human studies demonstrate increased glycolytic fiber-type composition (26) and increased glycolytic metabolism (20), in opposition to the findings of the present study and others (7, 35). This could be reflective of several factors, such as age, disease duration, physical activity levels, diet, and likely most importantly, insulin treatment.

Our findings of a decreased capillary-to-fiber ratio, although surprising given the reduction in glycolytic fiber number and fast fiber atrophy, are consistent with previous results (52). One would hypothesize that capillary-to-fiber ratio is influenced by fiber area, and therefore the reduction in fiber area was accompanied by a proportionate decrease in capillary-to-fiber ratio. \( \text{Ins2}^{\text{Akita}+/-} \) and control mice both became significantly heavier than STZ mice by 3 wk of diabetes (Fig. 1A). \( \text{Ins2}^{\text{Akita}+/-} \) fed body mass did not significantly differ from control mice until 7 wk of diabetes, while the STZ mice remained behind in growth throughout the experiment. Essentially, STZ mice failed to grow during adolescence, a period characterized by rapid growth. Previous studies have demonstrated that streptozotocin caused 1) an immediate loss of body and muscle mass despite aggressive insulin therapy and maintenance of euglycemia (31), 2) \( G_2 \) cell cycle arrest of muscle progenitor cells (31), and 3) DNA alkylation (9, 12). Thus it is likely that these direct effects of streptozotocin on skeletal muscle contributed to the 6-wk delay in growth in STZ mice. Conversely, \( \text{Ins2}^{\text{Akita}+/-} \) mice did not differ from control mice until 7 wk of diabetes. The \( \text{Ins2}^{\text{Akita}+/-} \) mice also exhibited hyperphagia, which may have helped maintain body mass for the first 6 wk of diabetes. This is clinically relevant, as it demonstrates that attenuated weight gain may not become apparent until several weeks following disease onset, although accelerated food consumption may be a recognizable early
symptom of the disease. Early recognition of T1DM is critical, given that early intervention and aggressive insulin treatment can significantly reduce the severity of T1DM complications and normalize growth (33). Importantly, it remains to be elucidated whether early intervention attenuates physical disability later in life. As skeletal muscle is the primary organ of glucose disposal and an individual’s physical capacities are tightly coupled to their muscle mass, understanding the basic mechanisms underlying diabetic myopathy has tremendous importance to the development of appropriate and successful long-term therapeutic strategies to improve life expectancy and quality of life and reduce overall healthcare costs.
Given the notable changes in muscle phenotype, we hypothesized that muscle strength would be reduced following 8 wk of diabetes. Indeed, a significant decrease in absolute force was observed in \textit{Ins2Akita} mice (Fig. 6, A and C), with this magnitude of force loss consistent with previous human studies (2, 5). In contrast, however, muscles from STZ mice demonstrated similar absolute peak force to the control mice despite marked muscle atrophy (Fig. 6, A and C). In human studies, strength or torque measurements cannot be easily corrected for the mass of the muscle tested; however, in the present study, when force is corrected for muscle mass, \textit{Ins2Akita} did not exhibit a force deficit, while STZ mice displayed increased relative force (Fig. 6D). Previous studies employing STZ diabetic rodents have demonstrated mixed findings with respect to force production. Consistent with human studies (2, 5), absolute force is usually found to decrease (24, 40, 51, 55). However, studies presenting force data relative to muscle mass reveal decreases (40, 44, 53), increases (27, 43, 55), or no change (40, 44, 51, 53). The basis for these discrepancies is not clearly understood, although it may involve the variability in STZ dosage, duration of untreated diabetes, the muscle group or fiber types studied, or the broad range of muscle stimulation protocols employed. A previous study that approximates the design of the present study demonstrated absolute force loss in \textit{Ins2Akita} and STZ skeletal muscle compared with nondiabetic controls (55). However, similar to the present study, when force was expressed relative to muscle mass, STZ mice exhibited greater contractile force compared with nondiabetic controls.

The basis for this improved contractile performance in STZ-treated diabetic mice is surprising given the shift away from fast-glycolytic fiber type composition. A possible explanation for the increased relative force in STZ muscle may be that calcium release/uptake dynamics and/or sensitivity have been increased (27, 43, 44). Our examination of muscle calcium handling characteristics revealed that STZ mice exhibited increased twitch force, a trend for longer rise time, and longer half-relaxation time in the postfatigue period (Fig. 6, E–H), consistent with previous studies of STZ muscle twitch characteristics (43, 44). Furthermore, STZ muscle was previously demonstrated to exhibit hyperactive Ca$^{2+}$ kinetics and sarcoplasmic reticulum (SR) Ca$^{2+}$-ATPase activity (27), and augmented contractile force following caffeine exposure (40), which may be an effect of SR or plasma membrane instability (27). In the present study these alterations to muscle twitch characteristics were observed in the STZ mice but not the \textit{Ins2Akita} mice, indicating that STZ may be directly altering Ca$^{2+}$ handling, irrespective of the diabetic muscle phenotype. The present study has comprehensively characterized the phenotype and functionality of skeletal muscle from two different models of T1DM. Our focus for future diabetic myopathy research is now on the \textit{Ins2Akita} mouse, which exhibits a fiber type shift away from fast-glycolytic fibers and an increased ability for lipid transport into the muscle although no dyslipidemia following 8 wk of overt diabetes. Despite marked muscle atrophy, contractile properties were not altered when expressed relative to muscle mass, suggesting that metabolic changes within muscle fibers precede detectable impairments in contractile properties. Little is currently known about human muscle during the early stages of T1DM before diagnosis and insulin therapy; thus it is critical that contributions to this field of research are physiologically relevant and not a result of pharmaceutical side effects of the diabetes-inducing agent as has been reported (31). As \textit{Ins2Akita} mice become diabetic due to a spontaneous mutation of the Insulin-2 gene, glycemia and insulinemia have been the only factors identified as altered (55, 57). While most of what is understood about muscle phenotype and function in early stage T1DM comes from studies employing STZ-induced diabetic rodents, the present study has illustrated several deviations in muscle phenotype, metabolism, and function from the \textit{Ins2Akita} mouse. Given the known toxic effects of STZ on skeletal muscle (31), interpretation of results from previous reports using STZ-induced diabetic models should be made cautiously, and future animal studies should consider alternative models of T1DM.

REFERENCES


