Streptozotocin induces G2 arrest in skeletal muscle myoblasts and impairs muscle growth in vivo

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Johnston AP, Campbell JE, Found JG, Riddell MC, Hawke TJ. Streptozotocin induces G2 arrest in skeletal muscle myoblasts and impairs muscle growth in vivo. Am J Physiol Cell Physiol 292: C1033–C1040, 2007. First published November 8, 2006; doi:10.1152/ajpcell.00338.2006.—Streptozotocin (STZ) is used extensively to induce pancreatic β-cell death and ultimately diabetes mellitus in animal models. However, the direct effects of STZ on muscle are largely unknown. To delineate the effects of STZ from the effects of hypoinsulinemia/hyperglycemia, we injected young rats with 1) saline (control), 2) STZ (120 mg/kg) or 3) STZ and insulin (STZ-INS; to maintain euglycemia). STZ rats demonstrated significantly elevated blood glucose throughout the 48-h protocol, while control and STZ-INS rats were euglycemic. Body mass increased in control (13 ± 4 g), decreased by 19 ± 2 g in STZ and remained unchanged in STZ-INS rats (−0.3 ± 2 g). Cross-sectional areas of gastrocnemius muscle fibers were smaller in STZ vs. control (1,480 ± 149 vs. 1,870 ± 40 μm², respectively; P < 0.05) and insulin treatment did not rescue this defect (STZ-INS: 1,476 ± 143 μm²).

Western blot analysis revealed a detectable increase in ubiquitinated proteins in the STZ skeletal muscle cells compared with control and STZ-INS. To further define the effects of STZ on skeletal muscle, independent of hyperglycemia, myoblasts were exposed to varying doses of STZ (0.25–3.0 mg/ml) in vitro. Both acute and chronic exposures of STZ significantly impaired proliferative capacity in a dose-dependent manner. Within STZ-treated myoblasts, increased reactive oxygen species was associated with significant G2/M phase cell-cycle arrest. Taken together, our findings show that the effects of STZ are not β-cell specific and reveal that STZ should not be used for studies examining diabetic myopathy.

satellite cell; diabetes; diabetic model; type 1 diabetes mellitus; cell cycle; proliferation; hypertrophy

type 1, or insulin-dependent, diabetes mellitus (T1DM) is typified by an autoimmune mediated destruction of the pancreatic β-cells. Over time, T1DM progresses to a significant decrease, or even complete abolishment, of pancreatic β-cell mass, rendering the patient in a state of hypoinsulinemia and hyperglycemia (15). A host of complications are associated with T1DM, including retinopathy, nephropathy, neuropathy, and myopathy (1, 7, 8). Much of our present knowledge concerning the pathogenesis and treatment of T1DM is due to the study of animal models of the disease. Within animal models, diabetes can be induced by surgical or pharmacological means, as well as the use of spontaneously diabetic animals such as the nonobese diabetic mouse (31). It is believed that many of the pharmacological agents used to induce diabetes, such as streptozotocin (STZ), selectively destroy the pancreatic β-cell (12, 16). The use of pharmacological agents to induce diabetes allows the investigator to 1) study a reproducible type of diabetes in a variety of species, 2) use smaller animal models for economy of care, and 3) define the disease progression as many of the end-organ effects of diabetes occur with these models.

Administration of STZ, a nitrosourea derivative, induces T1DM with a single high-dose (22, 29) or multiple low-dose treatments (31), whereas type 2 diabetes can be induced with a single high-dose treatment of STZ on the day of birth (29). Within pancreatic β-cells, STZ has been shown to act as a powerful alkylating agent, directly methylating DNA (3), causing DNA strand breaks (37) and inducing apoptosis and necrosis (32). It has recently been demonstrated that rats treated with STZ to induce T1DM possess a compromised ability for normal skeletal muscle growth (1). The authors proposed the impaired skeletal muscle growth in the young rats was the result of the STZ-induced hypoinsulinemia and/or hyperglycemia. However, while the effects of STZ are believed to be pancreatic β-cell specific, STZ administration has been shown to adversely affect the renal and hepatic tissues (11, 28).

Therefore, the purpose of the present study was to investigate the direct effects of STZ on skeletal muscle. Here, we demonstrate that administration of STZ to young rats results in an impaired skeletal muscle fiber growth that is independent of the diabetic phenotype; despite a restoration of protein ubiquitination to control levels. We further demonstrate that both chronic and acute administration of STZ in an in vitro culture model of skeletal muscle growth result in a dose-dependent decrease in proliferative capacity due to a G2/M phase arrest within the muscle myoblast cell cycle. Taken together, these data suggest that the impairment in skeletal muscle fiber growth is not due to a T1DM-induced increase in protein degradation, but a direct effect of STZ on skeletal muscle myoblasts. In summary, our findings reveal that the effects of STZ are not solely specific to the pancreatic β-cells, but implicate direct effects on skeletal muscle. These findings further suggest that caution is warranted when utilizing and evaluating studies that have used the STZ model to study the etiology and mechanisms underlying diabetic myopathy.

EXPERIMENTAL METHODS AND MATERIALS

In vivo analysis. Male Sprague-Dawley rats (Charles River, Québec, Canada; 46–49 days of age and weighing 178.0 ± 2.1 g at beginning of the experimental protocol) were individually housed in clear cages in temperature and humidity-controlled rooms. The animals were fed rat chow (Ralston...
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Purina, St. Louis, MO) and water ad libitum and were acclimatized to a 12-h light cycle (lights on between 0700 and 1900 h) for a period of 1 wk before experimental manipulation. All experiments were approved by the Animal Care Committee at York University and were conducted in accordance with guidelines set forth by the Canadian Council for Animal Care.

Three groups of rats were used: 1) normal controls, 2) streptozotocin-treated (STZ; Sigma-Aldrich), and 3) streptozotocin-insulin-treated (STZ-INS) with \( n = 3 \) to 4 per group. A single intravenous injection of STZ (120 mg/kg in sterile saline) was given to the STZ and STZ-INS rats while control animals received a saline injection under similar conditions. Body weight and food intake were measured daily (0900 h) and blood glucose concentration was measured using a tailnick and glucometer (One Touch Ultra, Lifescan) approximately every 3 h during the treatment period. In an effort to dissociate the direct effects of STZ from the effects of STZ-induced hyperglycemia/hypoinsulinemia, blood glucose levels in the STZ-INS group were maintained euglycemic (\( \leq 11 \) mM) by intraperitoneal injections of insulin (Humulin-N and Humalog; 100 U/ml each; Lilly). The quantity of insulin administered varied depending on the antecedent blood glucose measurement and injections were given on an “as needed” basis. The STZ-INS rats received 4–5 injections of insulin per day with a total of 14.3 ± 1.3 IU insulin administered over the 48-h experimental period. To maintain consistency and remove the variable of stress associated with insulin injections, control and STZ animals were handled frequently, in addition to having repeated tail nicks for blood glucose measurements. Insulin therapy in the STZ-INS rats began 6 h following STZ administration. Blood glucose measurements were taken regularly (approximately every 3 h) with the data presented in Fig. 1A being the average of the two blood glucose measurements taken during each 6-h period. Forty-eight hours following the initial injection of saline or STZ, the rats were euthanized by decapitation. The right gastrocnemius/soleus complex from each leg was removed and placed in cryoprotectant (10% sucrose solution) for 3 h before being embedded in OCT mounting media for tissue sectioning while the left gastrocnemius/soleus complex was snap frozen in liquid nitrogen and stored at −80°C until used for Western blot analysis. Blood samples were collected at the time of harvest for analysis of serum creatine kinase using a CK/MB immunoassay kit (Biocheck, Foster City, CA) and serum insulin using an ELISA kit (Crystal Chem) and quantified using a plate reader (Trinity Biotech). Serum corticosterone levels were measured at euthanization using an ELISA kit (Assay Designs, Ann Arbor, MI).

Western blot analysis. The left gastrocnemius muscle was homogenized in buffer containing 20 mM Tris-HCl, pH 8, 137 mM NaCl, 10% glycerol, 1% Nonidet P-40, 2 mM EDTA, and a protease inhibitor cocktail (Pierce, Rockford, IL). Protein concentrations were determined on all samples through the Bradford protein assay (Bio-Rad, Hercules, CA). Equal amounts of total protein were then resolved on SDS-PAGE gels and subsequently transferred to PVDF membranes and incubated with Ponceau S (Sigma-Aldrich) to demonstrate successful transfer and equal loading as previously described (21). Membranes were blocked with 5% nonfat milk before being probed with anti-ubiquitin (Abcam; rabbit polyclonal; 1:500) for 1 h at room temperature. Following primary incubation, blots were then washed 5 × 10 min in TBST and incubated with secondary antibody for 1 h at room temperature (anti-rabbit conjugated with horseradish peroxidase; 1:10,000). Signals were visualized using Supersignal chemiluminescent reagent (Pierce) as per supplier instructions. Equal loading control was demonstrated by stripping the membrane and reprobing for GAPDH (Abcam; mouse monoclonal; 1:5,000).

Fiber cross-sectional area. Cryosections of the right gastrocnemius muscle (10 μm thick) were stained with hematoxylin and eosin to demonstrate fiber architecture. Images were taken using a Nikon E80i upright microscope using brightfield optics. Fiber area (\( n = 3 \) for each experimental group with >50 fibers analyzed per muscle) was calculated using AlphaEaseFC software (Alpha Innotech, San Leandro, CA).

Cell culture. Proliferating C2C12 myoblasts, a mouse skeletal muscle satellite cell-derived cell line, were maintained at 37°C in Dulbecco’s modified Eagle’s medium (10% fetal bovine serum, 1% penicillin/streptomycin).

In vitro STZ treatment. To assess the effect of STZ on cell proliferation, C2C12 myoblasts were exposed to three different experimental protocols: 1) continuous STZ exposure, 2) acute, single STZ exposure, or 3) acute, multiple low-dose STZ exposure.

STZ was dissolved in warmed Dulbecco’s modified Eagle’s medium at the desired concentration just before addition to
myoblasts. First, for continuous exposure, 200,000 cells were plated in 35 mm wells \((n = 3\) for each condition) and allowed to adhere and proliferate. In the STZ-administered cells, STZ was dissolved in growth medium at concentrations of 0.25, 1.0, 2.0, and 3.0 mg/ml. STZ concentrations utilized were consistent with concentrations that have been utilized previously for in vitro studies \((2, 4, 9)\). Control cells remained untreated. After 1 day, cells were counted and replated at a density of 200,000 cells. Medium was changed daily with STZ administered cells continually exposed to growth media supplemented with the appropriate dosage of STZ. This protocol was followed for 3 consecutive days for the 1.0, 2.0, and 3.0 mg/ml STZ treated cells and for 5 consecutive days for the 0.25 mg/ml STZ treated cells. Second, for single exposure, 200,000 myoblasts were plated into 35 mm wells and allowed to adhere and proliferate for 1 day. Cells were then counted and replated at a cell density of 200,000. The first day served as the control period for each experimental well. Cells were then exposed to a single treatment of growth medium supplemented with STZ at a concentration of 3.0 mg/ml for 6 h \((n = 3\) for each condition). Following 6 h of STZ treatment, cells were rinsed thoroughly with phosphate-buffered saline (PBS) to remove any excess STZ and fresh growth medium (without streptozotocin) was applied. Cells were grown in growth media and their proliferative capacity was assessed through cell counting for 3 days following the end of STZ treatment. Following each cell counting, 200,000 cells were replated to maintain consistency in cell density between time points. Third, multiple low-dose myoblasts \((200,000)\) were plated into 35 mm wells and allowed to adhere and proliferate for 1 day. Cells were counted and replated at a cell density of 200,000. The first day served as the control period for each well. Cells were then exposed to a single treatment of STZ \((0.25 \text{ mg/ml})\) for 1 h, then washed with PBS and fresh growth medium (STZ free) was applied. This procedure was repeated for 5 consecutive days.

To determine whether pretreatment with an antioxidant could rescue the decrease in cell proliferation, four experimental groups were utilized \((n = 3\) for each): 1) control, 2) STZ treated \((0.25 \text{ mg/ml})\), 3) vitamin E-treated \((50 \mu \text{g/ml})\), and 4) STZ \((0.25 \text{ mg/ml}) + \text{ vitamin E (50 } \mu \text{g/ml})\). Cells were plated \((200,000)\) cells into 35 mm wells and allowed to adhere before the beginning of the experiment. Cells receiving vitamin E treatment were given a 16-h pretreatment before the beginning of the experiment. Streptozotocin exposure lasted for 1 h and upon completion cells were washed with PBS and fresh growth medium was added. Cell number was assessed 1 day following STZ exposure.

**Cell cycle analysis.** Proliferating myoblasts from untreated (control) and 6-h STZ-treated cells \((0.5 \text{ mg/ml and 1.5 mg/ml})\) were harvested 1 day after STZ treatment, fixed with 70% ethanol, permeated with 0.1% \((\text{vol/vol})\) Triton X-100 and incubated with a propidium iodide staining solution \((1.8 \text{ mg/ml RNase A, 50 } \mu \text{g/ml propidium iodide})\) for >3 h to label DNA \((21)\). With the use of a FACSCalibur flow cytometer \((BD Biosciences)\), 25,000 live event cells \((n = 3\) for each group) were acquired on the basis of DNA content using Cellquest \((BD Biosciences)\) and the percentage of cells in each phase of the cell cycle was quantified using ModFit LT for Mac version 3.0 software.

**Reactive oxygen species analysis.** To determine whether STZ induced an increase in reactive oxygen species (ROS), myoblasts were plated on gelatin-coated coverslips and allowed to proliferate overnight. The following day, cells were exposed to one of the following protocols: 1) control-no dichlorofluorescein (DCF; Molecular Probes), 2) STZ-no DCF, 3) control with DCF, and 4) STZ \((4 \text{ h of 3.0 mg/ml})\) with DCF. Cells were incubated with 90 μM DCF (in ethanol) or an equal volume of ethanol (control-no DCF and STZ-no DCF) for 1 h to assess the degree of ROS production in the presence and absence of STZ. In the final 15 min of DCF incubation, 4′,6-diamidino-2-phenylindole \((10 \mu \text{M})\) was added to the media to label all nuclei. Live cells were imaged under a Nikon E80i upright fluorescent microscope after several rinses and placement onto microscope slides with Vectashield mounting media \((

**RESULTS**

**STZ attenuates muscle growth independent of hyperglycemia and hypoinsulinemia.** To dissociate the direct effects of STZ on skeletal muscle from the effects of STZ-induced hyperglycemia/hyperinsulinemia \((i.e., \text{diabetes})\), blood glucose levels in the STZ-INS group were stabilized by intraperitoneal injections of insulin. As seen in Fig. 1A, STZ treatment resulted in a significant increase in blood glucose concentration and insulin treatment was successful in maintaining STZ-INS rats in a glycemic range that was not different from control. Serum insulin measurements illustrate that STZ resulted in a near abolishment of insulin secretion \((\text{Fig. 1B})\). Importantly, the insulin injections given to the STZ-INS group resulted in serum insulin levels that were similar to controls thereby demonstrating that these rats were euinsulinemic, at least at the time of death. Serum corticosterone levels, although variable among STZ treated animals, were elevated \((P < 0.05)\) in both STZ \((459 \pm 381 \text{ ng/ml})\) and STZ-INS \((769 \pm 405 \text{ mg/ml})\) rats compared with controls \((18 \pm 2 \text{ ng/ml})\). As STZ animals were not given routine saline injections \((\text{as a control for insulin administration})\), we must consider the possibility that the stress associated with serial intraperitoneal injections was, at least in part, responsible for the elevations in glucocorticoids in the STZ group. In fact, the observation that glucocorticoid levels in the STZ-INS group are almost double that measured in STZ suggests that the stress of streptozotocin and serial intraperitoneal injections \((\text{of insulin})\) may be additive.

Administration of STZ to growing rats resulted in a significant decrease in body mass within a 48-h period that was attenuated, but not returned to control values, with insulin treatment \((\text{Fig. 2A})\). The food intake over the 48-h experimental period was significantly higher in control rats \((36.9 \pm 1.3 \text{ g/48 h})\) when compared with STZ \((23.6 \pm 1.5 \text{ g/48 h})\) and STZ-INS rats \((24.2 \pm 2.1 \text{ g/48 h})\). Fiber area was significantly decreased 48 h following STZ treatment compared with control muscles \((\text{Fig. 2B})\). The maintenance of blood glucose levels
with insulin treatment (STZ-INS) did not rescue the decreased muscle fiber area induced by STZ treatment (Fig. 2B) suggesting that it was the STZ and not the diabetic state that was responsible for the decrease in fiber cross-sectional area. The decrease in fiber area with STZ was not the result of overt necrosis as fiber architecture was not visibly different between groups (as assessed on hematoxylin- and eosin-stained sections) and serum creatine kinase (CK), a measure of muscle damage, was not detectable in any of the groups at the time of death.

Increased proteolysis through the ubiquitin-proteasome pathway has been demonstrated previously in STZ treated animals and is proposed to be responsible for the loss of muscle protein with acute diabetes (26). To ascertain whether this was occurring in our study, Western blot analysis of muscle homogenates from each of the three experimental groups was undertaken. In agreement with previous investigations (26), we observed that STZ-treated animals displayed an increase in the quantity of proteins targeted for degradation (ubiquitinated proteins) when compared with saline-injected controls or STZ-INS rats (Fig. 2C). Furthermore, analysis of serum CK/MB, as well as hematoxylin and eosin staining of cryo-sectioned skeletal muscle, revealed that muscle fibers were not undergoing overt muscle necrosis as no difference was observed between any of the experimental groups. These observations suggest that factors other than necrosis and protein degradation may be involved in the impaired growth-associated hypertrophy of skeletal muscle fibers caused by STZ treatment. To investigate this in more detail, we undertook in vitro studies to determine if STZ could directly affect myoblast activity.

**STZ treatment impairs myoblast proliferation in vitro.** Continuous exposure of skeletal muscle myoblasts to STZ significantly impaired their proliferative capacity in a dose-dependent manner (Fig. 3). A significant decrease in cell number compared with controls was observed after only 1 day when myoblasts were exposed to doses of STZ between 0.25 mg/ml and 3.0 mg/ml (Fig. 3). Exposure of myoblasts to 0.25 mg/ml STZ for 5 consecutive days resulted in a 70.5 ± 3.6% decrease in cell number relative to control values. Three consecutive days of higher STZ doses resulted in an even greater decrease in proliferative capacity with 1.0 mg/ml STZ decreasing cell number by 89.4 ± 3.6% compared with control, while 2.0 and 3.0 mg/ml STZ decreased cell number by 116 ± 3.7% and 131 ± 2.9% compared with control, respectively.

To assess whether a single, acute exposure of STZ could affect myoblast function for an extended period of time, myoblasts were treated with 3.0 mg/ml of STZ dissolved in growth media for 6 h and cell number was assessed up to 3 days post treatment. Six hours of STZ treatment resulted in a significant decrease in myoblast number by 89.9 ± 3.3% one day following removal of STZ, and this inhibition persisted throughout the experimental period such that by 3 days following STZ removal, proliferation was completely abolished and there was an actual net loss of cells (7.1%; P ≤ 0.05) from what was originally plated.

We were also interested to determine whether repeated short-duration, low-concentration exposure to STZ could affect myoblast proliferative capacity. Myoblasts were exposed to 0.25 mg/ml of STZ for 1 h daily consecutively for 5 days. It was observed that 1 h of STZ exposure significantly decreased myoblast number by 52.3 ± 24.8% 24 h after treatment (vs. controls) and this suppression of myo-

![Fig. 2. STZ treatment induces weight loss, decreased skeletal muscle cross-sectional area, and increased protein ubiquitination in vivo. A: body weight (g) change 48 h post-STZ treatment in control, STZ, and STZ-INS rats. There was a significant decrease in body weight with STZ that was only partly attenuated with insulin therapy. *Significantly different from control. †P ≤ 0.05 vs. control and STZ. B: muscle cross-sectional area (μm²) in control, STZ, and STZ-INS rats. STZ treatment significantly decreased fiber cross-sectional area and this was not recovered with the restoration of euglycemia in STZ-INS rats. *Significantly different from control. ‡Significantly different from control. C: representative Western blot of muscle homogenates from control, STZ, and STZ-INS rats incubated with anti-ubiquitin antibody. The bands (indicated by arrows) denote various muscle proteins that have been ubiquinated and therefore targeted for proteolysis by the 26S proteosome. There was a noticeable increase in the intensity of the bands in the STZ muscle that was returned to control levels in the STZ-INS muscle.](http://ajpcell.physiology.org/)

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Blast proliferation was evident throughout, with STZ significantly decreasing cell number by 73.3 ± 15.8% by the completion of the experiment.

**STZ-treated myoblasts display a G<sub>2</sub>/M phase cell cycle arrest.** To determine the mechanism for the reduction in cell proliferative capacity with STZ treatment, control and STZ-treated myoblasts were sorted on the basis of DNA content using flow cytometric analysis. Exposure to STZ induced a G<sub>2</sub>/M phase cell cycle arrest in myoblasts at all concentrations tested (Fig. 4). When compared with controls, cells treated with 0.5 mg/ml STZ had a significantly higher propensity to arrest in the G<sub>2</sub>/M phase of the cell cycle (controls: 15.3% vs. STZ: 21.3%). This G<sub>2</sub>/M arrest was more pronounced with administration of a higher dose of STZ (1.5 mg/ml) with almost 20% more cells (vs. controls) arrested in the G<sub>2</sub>/M phase.

**STZ increases ROS in skeletal muscle myoblasts.** STZ has been demonstrated to increase reactive oxygen species (ROS) within many cell types (6, 9). In the present study, myoblasts treated with 3.0 mg/ml STZ for 4 h displayed visibly higher levels of ROS compared with that observed within untreated myoblast cells (Fig. 5). Pretreatment of myoblasts with 50 μg/ml vitamin E for 16 h before STZ exposure (0.25 mg/ml) did not prevent the STZ-induced decrease in proliferative capacity (41.8 ± 7.3% and 51.0 ± 5.3% for STZ and STZ-INS vs. control, respectively).

![Fig. 3. STZ treatment impairs myoblast proliferation in a dose-dependent fashion. The % change in cell number vs. control in C2C12 myoblasts treated with 0.25 to 3.0 mg/ml STZ for 24 h. Control cell number was normalized to 100%. Therefore, any value <100% represents a decrease in proliferative capacity relative to untreated, control cells. All STZ concentrations resulted in significant decreases in proliferation relative to control. Furthermore, significant differences were noted between STZ concentrations as indicated. The 0.25 mg/ml concentration was continued for 5 days with a significant decrease noted each of the 5 days. The 1.0, 2.0, and 3.0 mg/ml concentrations were continued for 3 days with significant decreases in proliferative capacity noted each day. *P < 0.05, lowest STZ concentration vs. all other concentrations.](image)

![Fig. 4. STZ arrests myoblasts in the G<sub>2</sub>/M phase of the cell cycle. Representative FACS profiles of untreated proliferating C2C12 myoblasts (A), C2C12 myoblasts treated with 0.5 mg/ml of STZ for 6 h followed by 1 day of proliferation (B), and C2C12 myoblasts treated with 1.5 mg/ml of STZ for 6 h followed by 1 day of proliferation (C). D: table of means ± SE of percentage of cells in G<sub>1</sub>, G<sub>2</sub>/M and S phases of the cell cycle. *P ≤ 0.5, significance compared with control. †P ≤ 0.5, significance compared with control and 0.5 mg/ml STZ.](image)
FIG. 5. STZ-treated myoblasts display increased reactive oxygen species (ROS) production. A: representative image of control (untreated) C2C12 myoblasts incubated with 4′,6-diamidino-2-phenylindole (DAPI; blue) to label all nuclei and dichlorofluorescein (green) to label ROS. B: representative image of STZ-treated (3.0 mg/ml; 4 h) C2C12 myoblasts incubated with DAPI and dichlorofluorescein. Note the higher incidence of green fluorescence with STZ treatment, indicating an increased ROS production (arrows).

DISCUSSION

The injection of STZ to induce pancreatic β-cell death causing severe hyperglycemia/hypoinsulinemia is one of the most common animal models to study T1DM and its related complications including diabetic myopathy. In the present study, we have delineated the direct effects of STZ on skeletal muscle from the effects associated with the STZ-induced T1DM phenotype. Our findings demonstrate that STZ directly results in a decreased body mass and reduced muscle fiber cross-sectional area that cannot be explained by overt necrosis or increased ubiquitin-mediated muscle proteolysis. Furthermore, at all doses utilized, STZ negatively affected myoblast proliferative capacity in vitro, even when exposure was limited to just 1 h. The impaired proliferation in response to STZ was the result of a G2/M phase cell cycle arrest that was associated with an increased in ROS production.

The perception that the genotoxic effects of STZ are confined solely to the β-cell requires reevaluation. In 1974, Bhuyan and colleagues (5) demonstrated that a variety of tissues, including skeletal muscle, have detectable STZ levels following in vivo injection. Subsequent studies demonstrated that STZ can negatively affect hepatic and renal tissue, causing DNA strand breaks (28), unscheduled DNA synthesis (36) and tumorigenesis (22). Furthermore, STZ treatment has been linked to chromosome abnormalities (24) and sister chromatid exchanges (35) within various human cell types. Taken together with our observations in skeletal muscle, it appears that STZ treatment in rodents has widespread deleterious effects in a variety of tissues, including pancreas, skeletal muscle, liver, and kidney, likely through a common mechanism of DNA damage and cell cycle arrest.

Although the mechanism(s) behind the potent effects of STZ on pancreatic β-cells and other tissue types has yet to be fully elucidated, there is a general agreement that STZ treatment results in DNA alkylation and fragmentation through increases in free radicals, ROS, and H2O2 production (2, 3, 34). Consistent with the proposed mechanism of action of STZ in pancreatic β-cells, we demonstrate a significant G2/M arrest and detectable increase in ROS activity in myoblasts treated with STZ. Since the repair of DNA damage primarily occurs during the G2 phase as the cell prepares to enter the mitotic (M) phase, we would propose that STZ treatment of myoblasts elicits considerable DNA damage which arrests the cell within the G2 phase of the cell cycle. This is in agreement with the in vitro treatment of L1210 (lymphocytic leukemia) cells where doses as low as 0.25 mg/ml of STZ resulted in a significant G2/M phase cell cycle arrest and reduced M phase entry (4).

Of particular interest are the findings of Blasiak et al. (6) who observed that STZ administration (0.01–100 μmol/l) induced DNA damage in both normal human lymphocytes and HeLa cancer cells in a dose-dependent manner. However, these cell types were able to repair their respective DNA damage within 30 and 60 min, respectively. Although we did not measure DNA damage specifically in the present study, we did observe that myoblast proliferation was significantly impaired 24 h after a 1-h treatment of STZ (0.25 mg/ml or 940 μmol/l) and a significant cell cycle arrest was observed 24 h following treatment with 0.5 mg/ml (1,886 μmol/l) STZ. Although the concentration of STZ used in the present study was higher than that used by Blasiak et al. (6), the fact that cell cycle progression was still impaired 24 h after STZ removal would suggest that skeletal muscle myoblasts are unable to repair STZ-induced DNA damage in a similar time frame to lymphocytes. Clearly, future studies should be undertaken to investigate the time course of DNA repair in myoblasts following STZ exposure.

Studies investigating diabetic skeletal muscle have observed an increase in protein breakdown and overall loss of skeletal muscle mass (19, 26, 33). In particular, it has been hypothesized that the diabetic phenotype results in elevated muscle proteolysis through an ATP (ubiquitin)-mediated pathway (27, 33). Consistent with these previous reports, we observed that STZ impaired normal weight gain and increased muscle protein ubiquitination. However, despite the restoration of normal ubiquitin-mediated proteolytic pathway activity with insulin therapy in the present study, a significant decrease in muscle fiber area was still observed with STZ-INS administration. The apparent decrease in muscle fiber area in both STZ and STZ-INS animals was not due to overt muscle necrosis as analysis of serum CK/MB as well as hematoxylin and eosin staining of cryo-sectioned skeletal muscle revealed that muscle fibers were normal and not structurally different between groups.

While a reduced protein synthesis with STZ treatment could explain the decrease in fiber area, previous reports (18) indicate that protein synthesis rates are restored when insulin levels are reestablished in STZ-treated rats. It may be that a reduced food intake and/or an increase in glucocorticoids in the STZ and STZ-INS rats could explain, at least in part, the apparent decrease in muscle fiber area.
attenuation in body weight gain and skeletal muscle growth. Indeed, STZ administration appears to influence food intake in rats such that administration of 65 mg/kg STZ results in hyperphagia, whereas administration of 125 mg/kg resulted in a marked fall in food consumption (17). Moreover, STZ administration to rats increases glucocorticoid levels, which are restored after four days of insulin therapy (13). In line with these previous observations, we observed that food intake was suppressed and circulating glucocorticoids elevated in both STZ and STZ-INS animals compared with controls. Since insulin therapy was started immediately after STZ induction of diabetes in our study, we believe that it may be the STZ, per se, rather than the diabetes that impairs food intake and elevates stress hormone levels in these animals. As such, we cannot rule out the possibility that that the reduction in food intake and the elevation in glucocorticoids contribute to the impaired muscle growth and body weight gain in these animals. On the basis of our hypothesis that STZ directly influences muscle growth by causing G2 arrest, or indirectly by lowering food intake and elevating glucocorticoids, we feel that a reexamination of previous studies using STZ to study diabetic myopathy is necessary.

Aragno and colleagues (1) induced the T1DM phenotype in 3-wk-old rats with STZ (50 mg/kg iv injection). Similar to the current study, these researchers observed an impaired muscle growth and also noted a decreased myogenic marker expression in STZ-treated rats. These researchers hypothesized that their findings were due to an increase in oxidative stress resulting from hyperglycemia, as antioxidant treatment resulted in a partial restoration of muscle growth. It is, however, conceivable that their findings may be explained by a direct effect of STZ on skeletal muscle, as restoration of the T1DM phenotype with insulin therapy in the present study did not rescue the STZ-induced decrease in muscle fiber area. Furthermore, our finding of elevated ROS in STZ-treated myoblasts (independent of hyperglycemia) suggests that the oxidative stress observed by Aragno and colleagues (1) may be a direct effect of STZ, rather than an effect of STZ-induced hyperglycemia.

While a partial restoration of muscle growth with antioxidant treatment has been observed in vivo (1), our administration of vitamin E to STZ-treated myoblasts in vitro had no effect. The disparity between findings may be due to many factors including: in vivo vs. in vitro methodologies, the dose of vitamin E that was utilized, vitamin E can impair in vitro cell proliferation at high doses (10), or finally, the localization of vitamin E to the mitochondrial and endoplasmic reticulum fractions (25), where it may not have been sufficiently bioavailable to scavenge the high levels of ROS produced by STZ exposure in vitro.

The observations demonstrate that even short-term STZ exposure impaired skeletal muscle myoblast proliferation in the present study is particularly novel. As the ability for skeletal muscle growth and regeneration is a function of the population of resident satellite cells and their progeny, i.e., myoblasts (20), affecting their proliferative capacity will have a profound effect on overall muscle mass, particularly if STZ is provided in the early stages of growth, as in this study and others (1). This is clearly illustrated by the work of Collins and colleagues (14) who demonstrated that as few as seven transplanted satellite cells were able to give rise to >100 new muscle fibers containing thousands of myonuclei, as well as repopulating the satellite cell population.

The findings of the present study have significant implications regarding the use of STZ for the study of T1DM; in particular, our results demonstrate that the toxic effects of STZ are not limited to the pancreatic β-cell and suggest a reevaluation of this experimental model for the study of diabetic myopathy.

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