Inhibition of Plasminogen Activator Inhibitor-1 Restores Skeletal Muscle Regeneration in Untreated Type 1 Diabetic Mice

Matthew P. Krause,1,2 Jasmin Moradi,1,2 Aliyah A. Nissar,1,2 Michael C. Riddell,2 and Thomas J. Hawke1,2

OBJECTIVE—Type 1 diabetes leads to impairments in growth, function, and regenerative capacity of skeletal muscle; however, the underlying mechanisms have not been clearly defined.

RESEARCH DESIGN AND METHODS—With the use of Ins2WT/C96Y mice (model of adolescent-onset type 1 diabetes), muscle regeneration was characterized in terms of muscle mass, myofiber size (cross-sectional area), and protein expression. Blood plasma was analyzed for glucose, nonesterified fatty acids, insulin, and plasminogen activator inhibitor-1 (PAI-1). PAI-039, an effective inhibitor of PAI-1, was orally administered to determine if PAI-1 was attenuating muscle regeneration in Ins2WT/C96Y mice.

RESULTS—Ins2WT/C96Y mice exposed to 1 or 8 weeks of untreated type 1 diabetes before chemically induced muscle injury display significant impairments in their regenerative capacity as demonstrated by decreased muscle mass, myofiber cross-sectional area, myogenin, and Myh3 expression. PAI-1, a physiologic inhibitor of the fibrinolytic system and primary contributor to other diabetes complications, was more than twofold increased within 2 weeks of diabetes onset and remained elevated throughout the experimental period. Consistent with increased circulating PAI-1, regenerating muscles of diabetic mice exhibited excessive collagen levels at 5 and 10 days postinjury with concomitant decreases in active urokinase plasminogen activator and matrix metalloproteinase-9. Pharmacologic inhibition of PAI-1 with orally administered PAI-039 rescued the early regenerative impairments in noninsulin-treated Ins2WT/C96Y mice.

CONCLUSIONS—Taken together, these data illustrate that the pharmacologic inhibition of elevated PAI-1 restores the early impairments in skeletal muscle repair observed in type 1 diabetes and suggests that early interventional studies targeting PAI-1 may be warranted to ensure optimal growth and repair in adolescent diabetic skeletal muscle. Diabetes 60:1964–1972, 2011

With type 1 diabetes onset predominantly occurring during youth, a time of critical growth and development, two important issues related to the current study must be considered: 1) atrophic stimuli placed on young, growing muscle results in a rapid and irreversible remodeling process (1–3), and 2) populations with pediatric type 1 diabetes consistently display elevated plasminogen activator inhibitor-1 (PAI-1) levels, irrespective of HbA1c (4). Unfortunately, assessment of skeletal muscle health in type 1 diabetes has not been a consideration in the clinical setting because it is assumed that insulin therapy alone is enough to restore normal muscle health by balancing protein synthesis and degradation. However, several studies have demonstrated that insulin treatment does not restore this balance (5–8), and the information to date indicates that young patients with diabetes score significantly lower on maximal strength tests (9) and that adolescents newly diagnosed with type 1 diabetes experience reduced muscle fiber size and altered muscle morphology (10). Studies using appropriate animal models of adolescent type 1 diabetes also demonstrate significant limitations in muscle growth and contractile function (11–13).

For skeletal muscle tissue to stay healthy, it must continuously be maintained, adapt to changing needs, and be capable of repair in instances of overuse, exercise, or trauma. The repair of skeletal muscle is a complex orchestration of events including degeneration, extracellular matrix (ECM) remodeling, and repair/replacement of damaged muscle fibers (14). This regenerative process must proceed in an orderly and efficient manner if skeletal muscle is to be maintained as a healthy, functioning organ. Although it has been reported that the type 1 diabetes environment may affect muscle regeneration after injury (15–17), it has been proposed, although never demonstrated, that the lack of insulin’s anabolic action is the sole reason for the deficits observed. However, the role of insulin in skeletal muscle repair and regeneration has yet to be established. It is now becoming increasingly evident from studies conducted in various tissues that other factors, such as alterations in circulating PAI-1, may be as important in diabetes complications as hypoinsulinemia/hyperglycemia (18–20). In skeletal muscle, alterations in PAI-1 levels, an inhibitor of the fibrinolytic system, can have profound effects on ECM remodeling and ultimately delay muscle regeneration after injury (21–25).

In the current study, we sought to determine the temporal pattern of regeneration and elucidate the underlying mechanism(s) resulting in deficits in the regenerative capacity of skeletal muscle in adolescent type 1 diabetes using a genetic murine model of the disease, the Ins2WT/C96Y mouse.

RESEARCH DESIGN AND METHODS
Animal care. Male C57BL/6-Ins2Akita/J (hereafter Ins2WT/C96Y) mice and their wild-type (WT) littermates were purchased at 3 weeks of age from Jackson Laboratory (Bar Harbor, ME). Mice (N = 16/group) were studied over a period of 8 to 13 weeks of untreated type 1 diabetes. A separate group of Ins2WT/C96Y and WT mice (N = 3/group) were used for the 1 week of type 1 diabetes.
regeneration study. Ins2<sup>WT/C96Y</sup> mice become spontaneously diabetic at ~4 weeks of age because of a heterozygous mutation in the Ins2 gene (26). Exact onset of diabetes was determined by monitoring blood glucose as previously described (12). The Ins2<sup>WT/C96Y</sup> mice were chosen instead of the commonly used streptozotocin-induced diabetic rodent model because of known growth-arresting effects of streptozotocin on skeletal muscle (27).

The animal room was maintained at 21°C, 50% humidity, and 12 h/12 h light-dark cycle. All mice had access to standard breeder chow and water ad libitum.

Blood glucose and body mass were measured biweekly (fed state: 1200–1400 h) in the 8-week experimental groups. Blood samples were collected at 2, 4, and 6 weeks of diabetes for analysis of metabolites and hormones. All animal experiments were approved by the McMaster and York University Animal Care Committees in accordance with Canadian Council for Animal Care guidelines.

Skeletal muscle injury. Skeletal muscle injury was induced with an intramuscular injection of 10 μL cardiotonic (CTX; Latoxan, France) as previously described (28). Injuries were generated in the left tibialis anterior (TA) and 6 weeks of diabetes for analysis of metabolites and hormones. All animal analyses were performed by the McMaster and York University Animal Care Committees in accordance with Canadian Council for Animal Care guidelines.

Blood analyses. Heparinized blood plasma was analyzed for insulin and total PAI-1 (MADPK-71 K; Millipore, Billerica, MA) at all collection time points. Plasma was also analyzed for nonesterified fatty acids with the use of a colorimetric assay (Wako Diagnostics, Richmond, VA).

Western blot analysis. Snap-frozen quadriceps or TA samples were homogenized, analyzed for protein concentration, electrophoretically separated on acrylamide gels, and transferred to polyvinylidene fluoride membranes as previously described (29). Primary antibodies included Myh3 (Hybridoma Bank FI.652), GAPDH (Abcam 8245, loading control; Cambridge, MA), myogenin (Hybridoma Bank F53), MMP9 (Abcam 38898), and uPA (Abcam 28230). uPA was analyzed for unbound (active) uPA and uPA bound to PAI-1 as a measure of PAI-1 activity in the muscle (30). Active uPA is found at ~48 kDa, and inactive (PAI-1-bound) uPA is found at ~93 kDa. Appropriate horseradish peroxidase-conjugated secondary antibodies were used and visualized with the addition of chemiluminescent reagent (Amersham, Piscataway, NJ). Images were acquired with a Fusion FX7 imager (Vilber Lourmat, Eberhard, Germany) and analyzed with ImageJ.

Histochemical and immunofluorescent analyses. Eight-micron skeletal muscle cross-sections were mounted on glass slides and stained as described below.

Hematoxylin–eosin. Hematoxylin–eosin staining was used to determine the average fiber area of uninjured and injured TA. Images spaced evenly throughout the TA (~1 mm apart) were used for analysis where 25 fibers per image were analyzed for area (75 total fibers per TA). We have previously demonstrated that in this muscle, quantification of this number of fibers provides a reasonable assessment of fiber area.

Picrosirius red. To stain for collagen content, sections were immersed in picrosirius red solution (0.1% w/v Direct Red 80 [Sigma 365548]; St. Louis, MO) in a saturated aqueous solution of picric acid [Sigma P6744] for 1 h. Sections were briefly rinsed in two changes of acidified dH2O (0.5% glacial acetic acid), dehydrated, cleared, and mounted.

Immunofluorescence. Sections were fixed with ice-cold 2% paraformaldehyde, blocked with 10% normal goat serum/1.5% BSA, followed by mouse IgG Block (BMK 2202, Vector Laboratories Inc., Burlingame, CA), and incubated with 1:1 dilution of anti-MYH3 overnight at 4°C. Alexa 488 anti-mouse secondary antibody (A-11001; Invitrogen, Carlsbad, CA) was used for detection, and 4,6-diamidino-2-phenylindole was used to identify nuclei.

RESULTS

Ins2<sup>WT/C96Y</sup> mice spontaneously developed type 1 diabetes (hyperinsulinemia/hyperglycemia) at ~4 weeks of age, which was maintained throughout the study period (Tables 1 and 2) and compared with WT littermates. Relative to WT mice, Ins2<sup>WT/C96Y</sup> mice also displayed decreased body mass gain and developed hyperlipidemia by 6 weeks of untreated diabetes (Tables 1 and 2), consistent with previous findings (33).

Histologic assessment of the uninjured TA served as an index for the effects of type 1 diabetes on skeletal muscle growth. We found that uninjured Ins2<sup>WT/C96Y</sup> muscles displayed no impairment in myofiber cross-sectional area within the first 17 days of type 1 diabetes (Table 1), whereas a significant reduction (12%) in myofiber cross-sectional area accrual occurred by 8 weeks of type 1 diabetes that did not significantly worsen with increasing disease duration (up to 13 weeks, Table 2). This suggests that impaired growth, rather than progressive atrophy, is responsible for the reduced myofiber area observed in Ins2<sup>WT/C96Y</sup>, at least until such time as significant neuropathic complications develop (34).

In response to muscle damage, deficits in muscle health became considerably more apparent. Ins2<sup>WT/C96Y</sup> muscles exposed to the type 1 diabetes environment for 1 week before injury demonstrated a 21% decrement in myofiber cross-sectional area at the 10-day regeneration mark (Fig. 1B). This novel finding suggests that even short-term exposure to type 1 diabetes has profound effects on skeletal muscle’s ability to repair after damage.

The regenerative capacity was also impaired at 8 weeks of disease progression because muscle masses and myofiber cross-sectional areas of regenerating Ins2<sup>WT/C96Y</sup> muscles were significantly less than WT muscles from 10 days of regeneration onward (Fig. 1C–E). Loss of mass and myofiber area was significant even when expressed as a percentage of either the uninjured, contralateral TA or body mass (Supplementary Fig. 1), confirming that the poor regeneration in type 1 diabetes extends beyond the reduced growth rate. These changes in overall mass and myofiber area were preceded by alterations in the protein expression of markers of the regenerative process, myogenin and embryonic myosin heavy chain (Myh3). Myogenin is a myogenic regulatory factor that is expressed during early time points in regeneration and is important for cell-cycle exit of myoblasts and consequent terminal differentiation (35,36). Western blot analysis showed suppressed expression of myogenin in Ins2<sup>WT/C96Y</sup> muscle.
FIG. 1. Type 1 diabetes impairs regeneration of skeletal muscle after CTX injury. Ten days after injury, TA muscle of the 1-week diabetic Ins2\(^{WT/C96Y}\) mice demonstrates a significant loss of (A) mass (t test: \(P = 0.015\); \(N = 3\)) and (B) myofiber cross-sectional area (t test: \(P = 0.014\); \(N = 3\)) compared with WT, indicating impaired regeneration after injury at only 7 days of type 1 diabetes. C: Hematoxylin–eosin staining of TA injured at 8 weeks of type 1 diabetes demonstrates loss of (D) muscle mass (\(N = 16\)) and (E) myofiber area (\(N = 16\)) compared with WT beginning at 10 days postinjury. The uninjured time point in both panels is the contralateral TA to the 5-day post-CTX muscle and is included to illustrate the decrease in muscle mass and myofiber area associated with the impaired growth of skeletal muscle in the type 1 diabetic state. The values for the uninjured time point are not included in the statistical analysis. A main effect of diabetes (main effect: \(P < 0.001\)) is observed in both mass and fiber area with the asterisk (*) denoting specific differences between WT and Ins2\(^{WT/C96Y}\) as defined by post hoc analysis. Note that the type 1 diabetic muscle does not return to WT mass/fiber size at later time points, but continues to lag in regeneration. Because early expression of myogenic proteins is critical to the early stages of regeneration, (F) myogenin (main effect: \(P = 0.062\), interaction: \(P = 0.017\); \(N = 8\)) and (G) embryonic myosin heavy chain (main effect: \(P = 0.134\), interaction: \(P = 0.017\); \(N = 8\)) were decreased at 5 days postinjury. H: myoD (main effect: \(P = 0.062\), interaction: \(P = 0.017\); \(N = 8\)) was not changed at 10 days postinjury.
**TABLE 1**
Characteristics of WT and diabetic mice at 8 weeks of diabetes (−13 weeks old)

| Measure               | Group                  | 2 weeks post-CTX | 4 weeks of diabetes | 6 weeks of diabetes | 8 weeks of diabetes | Diabetes main effect and interaction with time |
|-----------------------|------------------------|------------------|---------------------|---------------------|---------------------|-------------------------------------------------|
| Body mass (g)         | WT                     | 20.1 ± 0.3       | 23.1 ± 0.4          | 24.9 ± 0.4          | 26.2 ± 0.4          | DME = 0.0004                                    |
|                       | Ins2                   | 19.1 ± 0.3       | 21.5 ± 0.3b         | 22.9 ± 0.4b         | 23.9 ± 0.4b         | Int = 0.0035                                    |
| Insulin (pg/mL)       | WT                     | 789 ± 86         | 778 ± 175           | 814 ± 106           | 845 ± 155           | DME < 0.0001                                    |
|                       | Ins2                   | 225 ± 24        | 224 ± 35b           | 222 ± 56b           | 223 ± 58b           | Int = NS                                        |
| Blood glucose (mmol/L)| WT                     | 11.3 ± 0.6       | 9.1 ± 0.3           | 8.2 ± 0.3           | 8.1 ± 0.3           | DME < 0.0001                                    |
|                       | Ins2                   | 29.1 ± 1.0b      | 32.2 ± 0.7b         | 32.4 ± 0.7b         | 33.1 ± 0.5b         | Int < 0.0001                                    |
| Nonesterified fatty   | WT                     | 0.63 ± 0.06      | 0.77 ± 0.08         | 1.10 ± 0.06         | 1.26 ± 0.12         | DME < 0.0001                                    |
| acids (mmol/L)        | Ins2                   | 1.05 ± 0.10      | 0.85 ± 0.07         | 1.14 ± 0.12         | 1.21 ± 0.07         | Int = 0.0003                                    |

Biweekly data for the long-term (8 weeks) type 1 diabetic and WT groups (N = 16). Ins2WT/C96Y mice are labeled Ins2. Two-factor ANOVA was run to determine the main effects of diabetes, time, and interaction; P values for diabetes main effect and interaction are listed next to the respective data. Absence of a significant main effect or interaction is indicated as NS. DME, diabetes main effect; Int, interaction with time. *Significant difference at that time point by Bonferroni post hoc comparison.

compared with WT muscle at 5 days postinjury (Fig. 1F). Myh3, a developmental myosin isoform, is expressed transiently during skeletal muscle regeneration (37) and used as a reference point to assess the process of differentiation (38). Similar to myogenin, the protein expression of Myh3 was reduced in Ins2WT/C96Y muscle at 5 days of regeneration. Both Western blot and immunofluorescent staining of regenerating muscles demonstrated this expression pattern (Fig. 1G–I). Neither group displayed expression of Myh3 at 21 or 35 days after regeneration, suggesting that the impairments in the regenerative process are within the early phases after injury with diabetes development (≤10 days), and after this time, maturation proceeds, albeit delayed.

Skeletal muscle regeneration is a complex process that is heavily dependent, particularly during the early phase, on the optimal functioning of the fibrinolytic system (21–25). We speculated that impairments in type 1 diabetes muscle regeneration may be due, at least in part, to elevated PAI-1 of the MMP associated with ECM remodeling in skeletal muscle (39,40), was significantly elevated at 5 days of regeneration in WT but not Ins2WT/C96Y muscle, with levels between the two groups similar by 10 days postinjury (Fig. 2E).

With PAI-1 elevated within ~2 weeks of type 1 diabetes onset (Fig. 2A), we hypothesized that the defect in regeneration observed in Ins2WT/C96Y mice diabetic for 1 week before CTX injury would also display defective ECM remodeling. As hypothesized, collagen content in regenerating Ins2WT/C96Y mice, diabetic for a total of 17 days, was significantly elevated (Fig. 2F), consistent with a role of PAI-1 in the impaired regeneration.

By having identified that increased PAI-1 levels in Ins2WT/C96Y mice are associated with early impairments in muscle regeneration, we then determined if these deficits could be restored with pharmacologic inhibition of PAI-1, even in the absence of insulin therapy. Twice-daily oral dosing of PAI-039 (tiplaxtinin), a pharmacologic inhibitor of PAI-1 (29), effectively increased the amount of active (free) uPA in the injured muscle of 8-week diabetic Ins2WT/C96Y mice compared with untreated Ins2WT/C96Y mice (Fig. 3A) and increased the ratio of active to inactive uPA (uPA/PAI-1-uPA) (Ins2WT/C96Y: 1.22 ± 0.13 AU vs. Ins2WT/C96Y+PAI-039: 1.74 ± 0.14 AU; P = 0.02). WT mice treated with vehicle alone demonstrated no significant change in active uPA levels (WT: 5978 ± 1362 AU vs. WT + vehicle: 7007 ± 778 AU; P = 0.27) or uPA/PAI-1-uPA (WT: 1.40 ± 0.18 AU vs. WT + vehicle: 1.28 ± 0.09 AU; P = 0.29). The downstream effect of elevated active uPA levels, resultant from PAI-039 treatment, was an increase in active MMP9 (Fig. 3B) and a normalization of collagen content in the regenerating Ins2WT/C96Y muscles to the levels observed in WT mice (Fig. 3C and D). The recovery of the fibrinolytic pathway with PAI-039 in Ins2WT/C96Y mice restored not only normal ECM remodeling but also Myh3 expression to levels similar to WT regenerating muscles (Fig. 3E and F). Injured TA fiber area demonstrated no significant difference between groups (WT + vehicle: 461 ± 29 μm² vs. Ins2WT/C96Y+PAI-039: 396 ± 32 μm²; P = 0.19), whereas TA mass exhibited a small but significant

(Myh3) expression (main effect: P = 0.117, interaction: P = 0.009; N = 8) were determined in quadriceps muscle and demonstrated significantly increased expression at 5 days postinjury in WT but not Ins2WT/C96Y (labeled Ins2, F and G). H: Immunofluorescent staining of injured TA with anti-Myh3 confirms (J) the lack of Myh3 positive fibers in Ins2WT/C96Y compared with WT (main effect: P = 0.013; interaction: P < 0.001; N = 8) at 5 days postinjury. *Differences between groups at specific time points identified by Bonferroni post hoc analysis after 2-way ANOVA (D–G, I). A–I: White bars represent WT, and black bars represent Ins2WT/C96Y. (A high-quality digital representation of this figure is available in the online issue.)

diabetes.diabetesjournals.org

DIABETES, VOL. 60, JULY 2011 1967
PAI-1 IMPAIRS MUSCLE REPAIR IN TYPE 1 DIABETES

DISCUSSION

Our results indicate that the type 1 diabetic environment negatively affects the health of skeletal muscle, as defined by impaired growth and poor regenerative capacity. The deficits in regenerative capacity occur rapidly with exposure to type 1 diabetes (within ~2 weeks) and, as we demonstrated, are consistent with elevated PAI-1 and ineffective ECM remodeling.

Maintaining a healthy muscle mass in the type 1 diabetic population has not typically been addressed in the clinical setting. Unfortunately, many studies demonstrate impairments in skeletal muscle health (e.g., impaired morphology, decreased strength, and metabolic capacity) observed early in patients with type 1 diabetes who are receiving insulin therapy, changes that may precede other diabetes complications (13). The results presented support these previous findings as we demonstrate that repair from muscle damage is significantly blunted in the diabetic state with as little as 7 days of uncontrolled type 1 diabetes before muscle injury. Furthermore, we also demonstrate that PAI-1 is significantly elevated within the first 2 weeks of type 1 diabetes onset and that inhibition of this hormone restores the regenerative capacity of type 1 diabetic mice, irrespective of the hypoinsulinemia. Although skeletal muscle is capable of maintaining basic function in the face of extreme stressors, this does not equate to a healthy muscle mass that is functioning optimally. We and others of extreme stressors, this does not equate to a healthy muscle mass (1–5).

It is important to note that growing skeletal muscle exposed to type 1 diabetes will display a failure to accrue muscle mass/fiber area, and these findings are not the product of progressive atrophy resultant from prolonged type 1 diabetes exposure.
or neuropathic complications, because no change in muscle mass or fiber area was observed once into adulthood (a further 5 weeks of uncontrolled type 1 diabetes). Although we investigated the uncontrolled diabetic state in a rodent model of diabetes, it is worth considering that there are two situations in which pediatric type 1 diabetic populations may be under this stress: 1) before diagnosis (which may last a period of months) and 2) after diagnosis when glycemic control is difficult and suboptimal (43). Consistent with our results, findings in newly diagnosed juvenile type 1 diabetic humans demonstrate a reduced myofiber area compared with healthy age-matched control subjects (10).
Repair from muscle damage consists of multiple, overlapping stages (14). After the initial injury, an inflammatory phase ensues to remove damaged cells and debris. It is during this early phase that remodeling of the ECM begins, with a dramatic increase in ECM proteins, particularly collagen, which is needed as the structural integrity of the muscle is compromised while damaged muscle fibers undergo phagocytosis. As repair continues, so does ECM remodeling, with the excess of ECM proteins undergoing degradation as nascent myofibers form and mature.

Activity of the fibrinolytic system (PAI-1, uPA, MMPs) is critical during this ECM remodeling period (21–25). Newly formed myofibers will initially express immature myosin heavy chain isoforms, such as Myh3 (embryonic myosin heavy chain). As maturation continues, the muscle fibers will increase in size, returning to a preinjury state while replacing the immature contractile proteins with mature isoforms. We show in this study that in response to muscle damage, ECM remodeling is impaired in the type 1 diabetic state, and this is a direct result of elevated PAI-1. The increase in PAI-1 observed in regenerating muscle of type 1 diabetic mice decreased active uPA and MMP9 levels, FIG. 3. Pharmacologic treatment against PAI-1 improves fibrinolytic pathway activity, collagen degradation, and regeneration at 5 days post-CTX injury in type 1 diabetes. A: Treatment with PAI-039 caused an increase in free uPA in Ins2^WT/C96Y compared with untreated Ins2^WT/C96Y (t test: P = 0.004; N = 4). B: Similarly, active MMP9 was elevated in PAI-039–treated Ins2^WT/C96Y (t test: P = 0.025; N = 4). These findings are characteristic of restored fibrinolytic pathway activity, which presumably led to the (C and D) reduced collagen levels (t test: P < 0.001; N = 4) and (E and F) increased Myh3-positive area (t test: P = 0.011; N = 4) observed in PAI-039–treated Ins2^WT/C96Y compared with untreated Ins2^WT/C96Y (labeled Ins2, C and E). A–F: Black bars represent Ins2^WT/C96Y, and striped bars represent PAI-039–treated Ins2^WT/C96Y. Data are presented relative to the mean of the WT and WT + vehicle pooled data. *Differences between groups identified by t test. (A high-quality digital representation of this figure is available in the online issue.)
thereby attenuating ECM turnover (as noted by elevated intramuscular collagen) during the first 10 days postinjury. Restoration of the fibrinolytic system in type 1 diabetic mice via pharmacologic inhibition of PAI-1 restored active MMP9 expression, returned collagen levels to normative values, and ultimately allowed for nascent muscle fiber growth to occur. Consistent with these findings, mice deficient in uPA exhibit impaired muscle regeneration, whereas PAI-1–deficient mice exhibit augmented muscle repair (22,44). It is worth noting that, in the current study, PAI-039 treatment in Ins2WT/C96Y mice resulted in active uPA levels that significantly exceeded that measured in WT mice muscles. Although this may be considered “supraphysiologic,” tissues were collected 1 h after PAI-039 administration on the day of harvest, a time when the drug effects were at their peak. With a PAI-039 half-life of 4.1 h (29), it can be speculated that more time was spent below this elevated level than within it.

Although the evidence to date suggests that insulin administration does not restore function of the fibrinolytic pathway (because insulin therapy does not reduce PAI-1 levels in human pediatric populations) (4), and thus would not improve collagen degradation and de novo myofiber formation, insulin might facilitate the rate of myofiber growth (later stages of regeneration) by modestly improving protein turnover (5–8). Future studies are needed to clearly define if PAI-039 treatment, in combination with standard insulin therapy, provides a more optimal regeneration environment. Furthermore, we administered PAI-039 just before the injury; however, future studies should consider the clinically important issue of treatment after injury, in terms of both effectiveness and timeframe. A final clinical consideration of PAI-039 treatment is the long-term effects of its administration if it were to be given prophylactically or for prolonged periods of time. To date, there is limited information on this topic. One study administered PAI-039 for 42 days through addition to the rodent chow and found an acute protection against radiation-induced intestinal injury but noted no adverse effects of drug treatment (45), whereas a second study provided a 2-month administration of PAI-039 to AngII–salt–treated mice (46). In the latter study, PAI-039 was effective in decreasing aortic remodeling with no effect of PAI-039 alone on serum amyloid A levels (an index of systemic inflammation).

Although elevated PAI-1 has been linked to other complications usually associated with diabetes, such as coronary artery disease and nephropathy (18–20), it is somewhat surprising that this is the first time that a clinically relevant inhibition of PAI-1 (through pharmacologic means) has been used to treat a diabetes complication. In fact, to the best of our knowledge, only three other studies have investigated mitigating diabetes complications by altering PAI-1 levels. These studies used streptozotocin-induced diabetes in PAI-1–deficient rodents to investigate the role of PAI-1 in mediating the effects of type 1 diabetes on renal morphology and function (18,47,48). Collectively, these authors found that elevated PAI-1 was contributing to diabetic nephropathy (increased glomerular ECM, decreased glomerular filtration rate) and that amelioration of these symptoms occurred in the PAI-1–deficient background. Given the demonstrated (present study) (18,47,48) and proposed (19,20) linkage of PAI-1 with diabetes complications and the fact that PAI-1 is elevated in populations with pediatric type 1 diabetes regardless of the level of glycemic control (4,49,50), we propose that aggressive therapeutic approaches, including intensive insulin and PAI-1 inhibitor strategies, warrant further investigation for the treatment of young type 1 diabetic patients. This will not only ensure optimal accrual and maintenance of a healthy skeletal muscle mass but also will reduce the onset and progression of other diabetes complications. Clearly, future studies are needed to definitively demonstrate the causative role of PAI-1 in the impaired muscle regeneration of patients with diabetes, and these studies may also prove valuable in developing therapeutic strategies to ensure the most effective management of other diabetes complications.

ACKNOWLEDGMENTS

This work was supported by the Natural Science and Engineering Research Council of Canada (T.J.H.) and the Ontario Graduate Scholarship (M.P.K.).

No potential conflicts of interest relevant to this article were reported.

M.P.K. designed the study; interpreted the results; performed animal care, sample collection, and assays; performed all data analysis; and wrote the initial manuscript draft. J.M. designed the study, interpreted the results, and performed animal care, sample collection, and assays. A.A.N. performed animal care, sample collection, and assays. M.C.R. designed the study and interpreted the results. T.J.H. designed the study, interpreted the results, and performed animal care, sample collection, and assays. All authors contributed to the final version of the manuscript.

The authors thank April Scott and Janis MacDonald of the McMaster University CAF for technical assistance. The Myh3 Fl0.652 antibody developed by H.M. Blau was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biology (Iowa City, IA).

REFERENCES

1. Mozdzianz PE, Pulvermacher PM, Schultz E. Unloading of juvenile muscle results in a reduced muscle size 9 wk after reloading. J Appl Physiol 2000; 88:158–164
2. Wanek LJ, Snow MH. Activity-induced fiber regeneration in rat soleus muscle. Anat Rec 2000;258:176–185
3. Darr KC, Schultz E. Hindlimb suspension suppresses muscle growth and satellite cell proliferation. J Appl Physiol 1989:67:1827–1834
4. Zeitler P, Thiede A, Müller HL. Prospective study on plasma clotting parameters in diabetic children—no evidence for specific changes in coagulation system. Exp Clin Endocrinol Diabetes 2001;109:146–150
5. Bennet WM, Connacher AA, Smith K, Jung RT, Rennie MJ. Inability to stimulate skeletal muscle or whole body protein synthesis in type 1 (insulin-dependent) diabetic patients by insulin-plus-glucose during amino acid infusion: studies of incorporation and turnover of tracer L-[1-13C]leucine. Diabetologia 1990;33:43–51
6. Charlton MR, Balagopal P, Nair KS. Skeletal muscle myosin heavy chain synthesis in type 1 diabetes. Diabetes 1997;46:1336–1340
7. Godil MA, Wilson TA, Garlick PJ, McNurlan MA. Effect of insulin with concurrent amino acid infusion on protein metabolism in rapidly growing pubertal children with type 1 diabetes. Pediatr Res 2005;58:229–234
8. Nair KS, Ford GC, Ekberg K, Fernqvist-Forbes E, Wahren J. Protein dynamics in whole body and in splanchnic and leg tissues in type I diabetic patients. J Clin Invest 1995;95:2926–2937
9. Fricke O, Seewi O, Senier O, Tufleswki B, Stabrey A, Schoenau E. The influence of auxology and long-term glycemic control on muscle function in children and adolescents with type 1 diabetes mellitus. J Musculoskeletal Neuronal Interact 2008;8:188–195
10. Jakobsen J, Reske-Nielsen E. Diffuse muscle fiber atrophy in newly diagnosed diabetes. Clin Neuropathol 1986;5:73–77
11. Gordon CS, Serino AS, Krause MP, et al. Impaired growth and force production in skeletal muscles of young partially pancreatectomized rats: a model of adolescent type 1 diabetic myopathy? PLoS ONE 2010;5:e14002
12. Krause MP, Riddell MC, Gordon CS, Iman SA, Cafarelli E, Hawke TJ. Diabetic myopathy differs between Ins2Akita/+ and streptozotocin-induced type 1 diabetic models. J Appl Physiol 2009;106:1650–1659

13. Krause MP, Riddell MC, Hawke TJ. Effects of type 1 diabetes mellitus on skeletal muscle: clinical observations and physiological mechanisms. Pediatr Diabetes. 22 September 2010 [Epub ahead of print]

14. Hawke TJ, Garry DJ. Myogenic satellite cell physiology: to molecular biology. J Appl Physiol 2001;91:534–551

15. Gulati AK, Aguiuña E, Ren Y, et al. Plasminogen activator inhibitor-1 deficiency retards diabetic nephropathy. Kidney Int 2005;67:1297–1307

16. Lyon CJ, Hsueh WA. Effect of plasminogen activator inhibitor-1 on skeletal muscle regeneration capacity. Ann Thorac Surg 2009;33:1115–1119

17. Vignaud A, Ramond F, Hourdé C, Keller A, Butler-Browne G, Ferry A. Diabetes provides an unfavorable environment for muscle mass and function after muscle injury in mice. Pathobiology 2007;74:291–300

18. Nicholls SB, Aguiuña E, Ren Y, et al. Plasminogen activator inhibitor-1 deficiency impairs skeletal muscle regeneration. Am J Physiol Cell Physiol 2009;298:C217–C223

19. Naderi J, Bernreuther C, Grabinski N, et al. Plasminogen activator inhibitor-1 up-regulation is associated with skeletal muscle atrophy and associated fibrosis. Am J Pathol 2009;175:763–771

20. Suelves M, Vidal B, Ruiz V, et al. The plasminogen activation system in skeletal muscle regeneration: antagonistic roles of urokinase-type plasminogen activator (uPA) and its inhibitor (PAI-1). Front Biosci 2005;10:2978–2985

21. Sisson TH, Nguyen MH, Yu B, Novak ML, Simon RH, Koh TJ. Urokinase-type plasminogen activator increases hepatocyte growth factor activity required for skeletal muscle regeneration. Blood 2009;114:5052–5061

22. Yoshioita M, Kato Y, Ikeda T, Koizumi A. A novel locus, Mody4, distal to D7Mit189 on chromosome 7 determines early-onset NIDDM in nonobese C57BL/6 (Akita) mutant mice. Diabetes 1997;46:887–894

23. Johnston AP, Campbell JE, Found JG, Riddell MC, Hawke TJ. Streptozotocin-induced G2 arrest in skeletal muscle myoblasts and impairs muscle growth in vivo. Am J Physiol Cell Physiol 2007;292:C1033–C1040

24. Hawke TJ, Atkinson DJ, Kanatous SB, Van der Ven PF, Garry DJ. Xn, an actin binding protein, is expressed within muscle satellite cells and newly regenerated skeletal muscle fibers. Am J Physiol Cell Physiol 2007;293:C1636–C1644

25. Elokdah H, Abou-Gharbia M, Hennan JK, et al. Tiplaxtinin, a novel, orally efficacious inhibitor of plasminogen activator inhibitor-1: design, synthesis, and preclinical characterization. J Med Chem 2004;47:3481–3494

26. Oishi K, Okhura N, Kasamatsu M, et al. Tissue-specific augmentation of cardiac PAI-1 expression in mice with streptozotocin-induced diabetes. Thromb Res 2004;114:129–135

27. Cranick DL, Elsdon H, Li D, Lennan JK, Gorlatova NV, Lawrence DA. Characterization and comparative evaluation of a structurally unique PAI-1 inhibitor exhibiting oral-in vivo efficacy. J Thromb Haemost 2004;2:1422–1428

28. Krause MP, Liu Y, Yu V, et al. Adiponectin is expressed by skeletal muscle fibers and influences muscle phenotype and function. Am J Physiol Cell Physiol 2008;295:C203–C212

29. Hong EG, Jung DY, Ko HJ, et al. Nonobese, insulin-deficient Ins2Akita mice develop type 2 diabetes phenotypes including insulin resistance and cardiac remodeling. Am J Physiol Endocrinol Metab 2007;293:E1687–E1696

30. Andresen CS, Jakobsen J, Ringgaard S, Ejskaer Nielsen, Andersen H. Accelerated atrophy of lower leg and foot muscles—a follow-up study of long-term diabetic polyneuropathy using magnetic resonance imaging (MRI). Diabetologia 2009;52:1182–1191

31. Le Grand F, Rudnicki MA. Skeletal muscle satellite cells and adult myogenesis. Curr Opin Cell Biol 2007;19:628–633

32. Smith CK 2nd, Janney MJ, Allen RF. Temporal expression of myogenic regulatory genes during activation, proliferation, and differentiation of rat skeletal muscle satellite cells. J Cell Physiol 1994;159:379–385

33. Zimowska M, Brzoska E, Swierczynska M, Stremińska W, Moraczewski Z. Distinct patterns of MMP-9 and MMP-2 activity in slow and fast twitch skeletal muscle regeneration in vivo. Int J Dev Biol 2008;52:307–314

34. Khérif S, Laftuma C, Dehaupas M, et al. Expression of matrix metalloproteinases 2 and 9 in regenerating skeletal muscle: a study in experimentally injured and mdx muscles. Dev Biol 1999;205:138–170

35. Shortreed KE, Krause MP, Huang JH, et al. Muscle-specific adaptations, impaired oxidative capacity and maintenance of contractile function characterize diet-induced obese mouse skeletal muscle. PLoS ONE 2009; 4:e7203

36. Sikandaran P, Hevener AL, Karlamangla AS. Sarcopenia exacerbates obesity-associated insulin resistance and dysglycemia: findings from the National Health and Nutrition Examination Survey III. PLoS ONE 2010;5:e10605

37. Diabetes Control and Complications Trial Research Group. Effect of intensive diabetes treatment on the development and progression of long-term complications in adolescents with insulin-dependent diabetes mellitus: Diabetes Control and Complications Trial. J Pediatr 1994;125:177–188

38. Llusá F, Roma J, Suelves M, et al. Urokinase-dependent plasminogen activation is required for efficient skeletal muscle regeneration in vivo. Blood 2001;97:1703–1711

39. Abderrahmani R, François A, Buard V, et al. Effects of pharmacological inhibition and genetic deficiency of plasminogen activator inhibitor-1 in radiation-induced intestinal injury. Int J Radiat Oncol Biol Phys 2009;74:942–948

40. Weisberg AD, Albornoz F, Griffin JP, et al. Pharmacological inhibition and genetic deficiency of plasminogen activator inhibitor-1 attenuates angiotensin II/salt-induced aortic remodeling. Arterioscler Thromb Vasc Biol 2005;25:365–371

41. Wallis ME, Alexander SL, Lopez-Guisa JM, et al. Plasminogen activator inhibitor-1 deficiency has renal benefits but some adverse systemic consequences in diabetic mice. Nephron Exp Nephrol 2006;104:e23–e34

42. Lassila M, Fukami K, Jandeleit-Dahm K, et al. Plasminogen activator inhibitor-1 production is pathogenic in experimental murine diabetic renal disease. Diabetesology 2007;50:1315–1326

43. Gogittzde Joy N, Hedrington MS, Briscoe VJ, Tate DB, Ertl AC, Davis SN. Effects of acute hypoglycemia on inflammatory and pro-atherothrombotic biomarkers in individuals with type 1 diabetes and healthy individuals. Diabetes Care 2010;33:1529–1535

44. Small M, Kuhl C, MacCusich AC, Lowe GD. Tissue plasminogen activator inhibition in diabetes mellitus. Diabetes Care 1989;12:655–658