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# Combined Treadmill Running and Insulin Therapy Favors the Stabilization of Glycemic Metabolic Parameters and Avoids Increased Achilles Tendon Rigidity in Diabetic Rats

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## SUMMARY

**Background.** Hyperglycemia reduces tendon homeostasis. Effects of physical exercise on diabetic rats have been widely studied; however, the effects of a combined physical and insulin therapy on biomechanical properties of the Achilles tendon (AT) remain unclear. Therefore, the objective of this study was to evaluate the effects of the combination of moderate-intensity exercise on a treadmill and insulin therapy on metabolism, physical conditioning, and biomechanics of AT in diabetic rats.

**Methods.** Forty-eight Wistar rats were divided into six groups: Sedentary Control-SCG, Treadmill Control-TCG, Sedentary Diabetic-SDG, Sedentary Insulin Diabetic-SIDG, Treadmill Diabetic-TDG, and Treadmill Insulin Diabetic-TIDG. Diabetic animals were induced with streptozotocin diluted in sodium citrate buffer (50 mg/Kg; 10 mM; pH 4.5; intraperitoneally). All groups were subjected to the maximal effort test for pre ($MET_1$) and post ($MET_2$) maximal speed determination. The exercise protocol was administered for 5 weeks (1 h/day, 5 days/week). Blood glucose levels and biomechanical properties of AT (e.g., traction) were evaluated.

**Results.** Increased glycemia was observed in SDG ($p<0.001$; $p=0.003$), SIDG ($p=0.009$; $p=0.037$), and TDG ($p=0.002$; $p=0.009$); however, compared with SCG and TCG, TIDG showed no significant differences. Maximal force was reduced in TIDG ($p=0.009$) and SIDG ($p = 0.002$) and increased in SCG and TDG compared with in SIDG ($p=0.024$). The elastic modulus was reduced in TCG compared with in SCG ($p = 0.011$) and increased in SDG ($p<0.001$), SIDG ($p=0.019$), and TDG ($p=0.006$) compared with in TCG.

**Conclusions**. The combined physical exercise and insulin therapy favors the stabilization of glycemic metabolic parameters and avoids increased tendon rigidity in diabetic rats.

## KEY WORDS

*Achilles tendon; diabetes mellitus; maximal effort test.*

## BACKGROUND

Regular physical exercise concomitant with hyperglycemic control has been widely investigated for the treatment of type 1 diabetes mellitus (DM1) (1,2). Therefore, insulin therapy (IST) is commonly used to control hyperglycemia and other complications of diabetes, such as diabetic ketoacidosis and hyperosmolar states (2).

Insulin therapy is indicated for DM1 cases (3) because high glycemic indexes are associated with cardiometabolic (4) and musculoskeletal disorders (4-7), including tendon damage such as diabetic tendinopathy (5,8).

Evidence suggests that hyperglycemia reduces tendon homeostasis as a consequence of a series of changes, including vascular hyperplasia, disorganized collagen fibers, decrease collagen production, increased advanced glycation end products (AGEs) (9-11), and altered viscoelastic properties (12). Such changes may increase tendon stiffness, thereby inducing diabetic tendinopathy (8) and causing degeneration and rupture (5).

Meanwhile, when physical exercise was regularly performed, physiological and biomechanical benefits, such as increased collagen concentration, fibrillary density (13), elastic module, maximal tension, cross-sectional energy/area, and maximal force (14) as well as decreased thickness and neovascularization (15), were observed in the Achilles tendon (AT) of patients with calcaneus tendinopathy (15,16).

Recent studies in DM1-induced animals have reported positive effects of moderate-intensity aerobic exercise (running and swimming) on the restoration of the elastic characteristics of AT, preventing the progression of tendon degeneration (17,18). However, these studies did not implement IST during exercise. Assuming that IST can prevent the onset of musculoskeletal chronic complications (18) and optimize motor skills in DM1-induced rats (19), we hypothesized that IST concomitant with physical exercise can boost the biomechanical properties of AT.

Considering that some precautions should be ensured regarding exercise practice in patients with diabetes to appropriately prescribe safe exercise intensity, in the present study, a moderate-intensity treadmill exercise standardization model was adopted using the maximal effort test proposed by Brito et al. (20).

The present study aimed to investigate the effects of a combination of moderate-intensity aerobic exercise on a treadmill and IST on the biomechanical properties of AT in as well as the clinical status and physical fitness of DM1-induced rats.

## MATERIALS AND METHODS

### Experimental design

To evaluate the effects of the combination of IST and moderate-intensity treadmill exercise on AT in healthy and diabetic rats, this study was divided into four stages: (1) distribution of animals into six groups, induction of experimental diabetes, and onset of IST; (2) adaptation to the treadmill and administration of the first maximal effort test ($MET_1$); administration of the 5-week protocol of moderate-intensity treadmill exercise and the second maximal effort test ($MET_2$); material collection and administration of AT traction biomechanical test. The study was performed at the Neuromuscular Plasticity Laboratory of the Anatomical Department and the Biopolymer Laboratory of the Chemical Engineering Department of the Federal University of Pernambuco (UFPE).

Forty-eight male Wistar rats (234.4 ± 28.1 g) were housed in cages at 23 ± 1°C under a 12-h inverted light/dark cycle. Presence Food (Neovia, São Paulo, Brazil) and water were provided *ad libitum*. Animals that did not present a fasting blood glucose level >200 $mg.dL^{-1}$ on day 7 after the induction of experimental diabetes, did not continuously run during the adaptation week, and reached a $MET_1$ maximal speed <10 $m.min^{-1}$ were excluded. Ethical approval of the study was obtained from the Committee of the Federal University of Pernambuco Bioscience Center (number 23076.050209/2016-10) in accordance with the norms recommended by the Brazilian Committee for Animal Experimentation. The research was conducted ethically according to international standards and as required by the journal (21).

## PROCEDURES

### Animal distribution into groups and induction of experimental diabetes

At the age of 50 days, animals were randomly assigned to six groups (n = 8 per group): sedentary control group (SCG), treadmill control group (TCG), sedentary diabetic group (SDG), treadmill diabetic group (TDG), sedentary insulin diabetic group (SIDG), and treadmill insulin diabetic group (TIDG). Experimental diabetes was induced in animals in the diabetic groups at the age of 60 days, and after 12 h of fasting, measure body weight (FILIZOLA BP6 Digital Scale) and blood glucose (Accu-Chek Active Kit Glucometer) were measured. Experimental diabetes was induced

via the administration of streptozotocin (50 mg.kg$^{-1}$; STZ, Sigma Aldrich Co., St. Louis, MO) diluted in sodium citrate buffer (10 mM; pH 4.5; intraperitoneally [i.p.])(22). Animals in the control groups received an injection of sodium citrate buffer alone (23).

## Insulin therapy

From day eight after the induction of experimental diabetes, insulin (Eli Lilly and Company) was subcutaneously administered (24) (2 IU/day/mouse; HUMULIN® N) daily to animals in SIDG and TIDG, 4–6 h after the exercise sessions and throughout the experimental protocol (3).

## Treadmill adjustment period, MET, and moderate-intensity treadmill exercise protocol

All animals were subjected to treadmill adaptation nine days after the induction of experimental diabetes. Treadmill adaptation was performed for three days (10 min.day$^{-1}$; 5 m.min$^{-1}$; 0° inclination) on the multiple rodent treadmill (AVS Projects). On day 4, $MET_1$ was administered at an initial speed of 5 m.min$^{-1}$, with a progressive increase in speed by 5 m.min$^{-1}$ every 3 min. The maximum intensity for each rat was determined when the animal touched the back wall of the treadmill belt five times within 1 min (19,20,22). After 72 h of $MET_1$, the adapted protocol (19) was initiated at moderate intensity (20,25) in animals in TCG, TDG, and TIDG over 5 weeks (0° inclination; 1 h/day; 5 days/week). Each session comprised three periods: warm-up, moderate-intensity exercise, and recovery. During the first week, time and speed were progressively increased. To encourage the animals, a low-intensity electrical stimulus (1.5–2.0 mA) located at the end of each lane was used.

Approximately 48 h after the last exercise session, $MET_2$ was administered. All protocols are detailed in **table I**.

## Clinical and metabolic evaluation, material collection, and euthanasia

After the experimental period, weight and blood glucose levels were measured in all animals. This was followed by anesthesia with xylazine solution and ketamine hydrochloride (Anasedan®, 20 mg.kg$^{-1}$ and Dopalen®, 100 mg.kg$^{-1}$, respectively; 0.1 mL and 0.1 mL for each 100 g of animal weight, respectively, intraperitoneal). An incision was made on the upper surface of the right paw for the visualization and dissection of the myotendinous complex of the *triceps surae* muscle while preserving the distal fixation of the calcaneal bone and the animal's paw. The anatomical sample was moistened with saline and sent to the Biopolymer Laboratory at UFPE for biomechanical assay. After the collection of biological material and while still under the effect of anesthesia, animals were euthanized via the intracardiac injection of 1 mL potassium chloride (10%).

## Tendon traction mechanical test

Biomechanical testing was performed on the mechanical tensile testing conventional machine (EMIC, Model DL 500,

**Table I.** Treadmill exercise protocol applied in the diabetic treadmill group (TDG) and diabetic treadmill insulin group (TIDG).

| Protocol | Week or Day | Time (minutes) | Training |
|---|---|---|---|
| Adaptation | 1st day | 10 | Velocity: 5m.min$^{-1}$ |
| | 2nd day | 10 | |
| | 3rd day | 10 | |
| Maximum effort test | 4th day | Varies according to animal | Initial velocity of 5 m.min$^{-1}$ being increased every 3 min at 5 m.min$^{-1}$ until fatigue |
| Moderate intensity exercise | 1st day | 20 | 5min at 30%$_{MET1}$ + 10 min at 50%$_{MET1}$ + 5min at 30%$_{MET1}$ |
| | 2nd day | 30 | 5min at 30%$_{MET1}$ + 20 min at 50%$_{MET1}$ + 5min at 30%$_{MET1}$ |
| | 3rd day | 40 | 5min at 30%$_{MET1}$ + 30 min at 50%$_{MET1}$ + 5min at 30%$_{MET1}$ |
| | 4th day | 50 | 5min at 30%$_{MET1}$ + 40 min at 50%$_{MET1}$ + 5min at 30%$_{MET1}$ |
| | 5th day | 55 | 5min at 30%$_{MET1}$ + 45 min at 50%$_{MET1}$ + 5min at 30%$_{MET1}$ |
| | 2nd week | 60 | 5min at 30%$_{MET1}$ + 50 min at 60%$_{MET1}$ + 5min at 30%$_{MET1}$ |
| | 3rd week | 60 | |
| | 4th week | 60 | |
| | 5th week | 60 | |

Min - minutes; % - percentage; $MET_1$: Maximum Speed Test.

Brazil, 500 N). The ellipse formula was applied to measure the tendon cross-sectional area (26), as shown in Equation I. Subsequently, the sample was connected to the machine via two pieces that were previously made for this type of biological material, keeping the sample in the anatomical position. The length of the sample was then measured using a caliper (Vernier Calipers, 0.05 mm), and specimen traction was performed at a speed of 0.1 mm.s$^{-1}$ to its point of failure. Maximal force, maximal force tension, cross-sectional energy/area, maximal strain, specific strain, and elastic modulus were calculated using the stress vs. strain graph generated by the Tesc® software coupled with the biomechanical testing machine.

$$CSA = (D \times d / 4) \times \pi$$

Where:
CSA = the cross-sectional area;
D = the larger longest of the medial region of AT;
d = the shortest diameter of the medial region of AT;
and π = 3.1416

## STATISTICAL ANALYSIS

After verifying the data assumptions for parametric analysis using Kolmogorov–Smirnov test, one-way ANOVA was performed, followed by post hoc Bonferroni test, for the analysis of variables with normal distribution. When normality was not verified, Kruskal–Wallis test was used, followed by pairwise comparison. Parametric data were presented as mean and standard deviation and non-parametric data as median and interquartile range. To assess the effect of size, Cohen's *d* (27) was used. All analyses were performed using SPSS version 20. A significance level of 5% was adopted.

## RESULTS

Of the 32 animals with induced diabetes, two did not exhibit a blood glucose level >200 mg.dL$^{-1}$ on day 7 after the induction of experimental diabetes and were excluded from the study. At the beginning of the study, all animals presented homogeneous parameters of body weight (p = 0.367) and glycemia (p = 0.094). At the end of the exercise protocol, blood glucose levels and body weights in TIDG were similar to those in SCG (p = 1.000) and TCG (p = 1.000). However, compared with the nondiabetic groups (SCG and TCG), the diabetic groups (SDG, SIDG, and TDG) exhibited increased glycemic index (p < 0.05). Reduction in body weight was observed only in SDG (SDG vs. SCG: ↓ 24.03%, p < 0.001, *d* = 2.34 and SDG vs. TCG: ↓ 22.99%, p = 0.003, *d* = 2.41). Conversely, compared with the nondiabetic groups, the diabetic groups with IST alone or combined with treadmill exercise showed similarity in body weight (p > 0.05; **figure 1**). Increased blood glucose was observed in

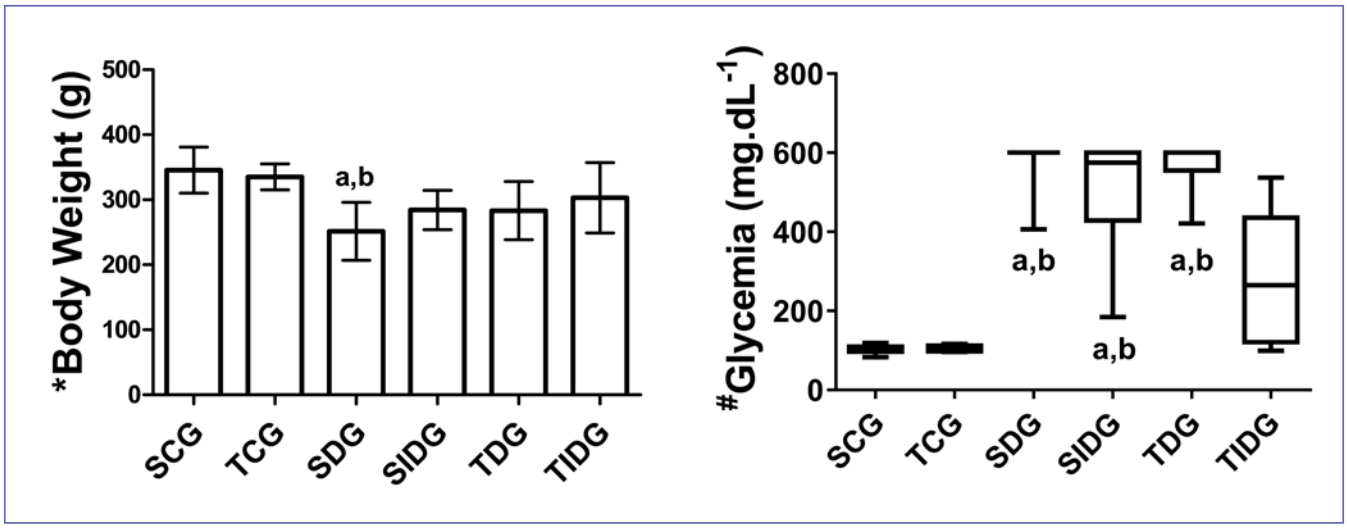


**Figure 1.** Body weight and glycemic indexes after five weeks of moderate treadmill exercise. SCG - Sedentary Control Group; TCG - Treadmill Control Group; SDG - Sedentary Diabetic Group; SIDG - Sedentary Insulin Diabetic Group; TDG - Treadmill Diabetic Group; TIDG - Treadmill Insulin Diabetic Group. Body weight values are expressed as mean ± standard deviation. (*) Anova with post hoc of Bonferroni, whereas final glycemia values are expressed in median and interquartile range (#) Kruskal Wallis with paired comparison to determine statistical difference between groups (p <0.05). Where (a) represents the difference in relation to SCG, (b) difference in relation to TCG.

all diabetic groups (SDG, SIDG, TDG, and TIDG) ($p < 0.05$) (**figure 2**).

In $MET_1$, no differences in maximal speed were observed among the evaluated groups. In $MET_2$, maximal speed was increased in TIDG compared with that in SDG (↑ 17.03%, $p = 0.002$, $d = 2.97$) and SIDG (↑ 23.82%, $p = 0.001$, $d = 2.35$) and decreased in SDG (↓11.11%, $p = 0.020$, $d = 3.88$) and SIDG (↓22.86%, $p = 0.011$, $d = 2.42$) compared with that in TCG (**figure 3**).

Regarding the biomechanical properties of AT, maximal force was reduced in TIDG (↓31.51%, $p = 0.009$, $d = 1.88$) and SIDG (↓32.23%, $P = 0.002$, $d = 1.84$) compared with

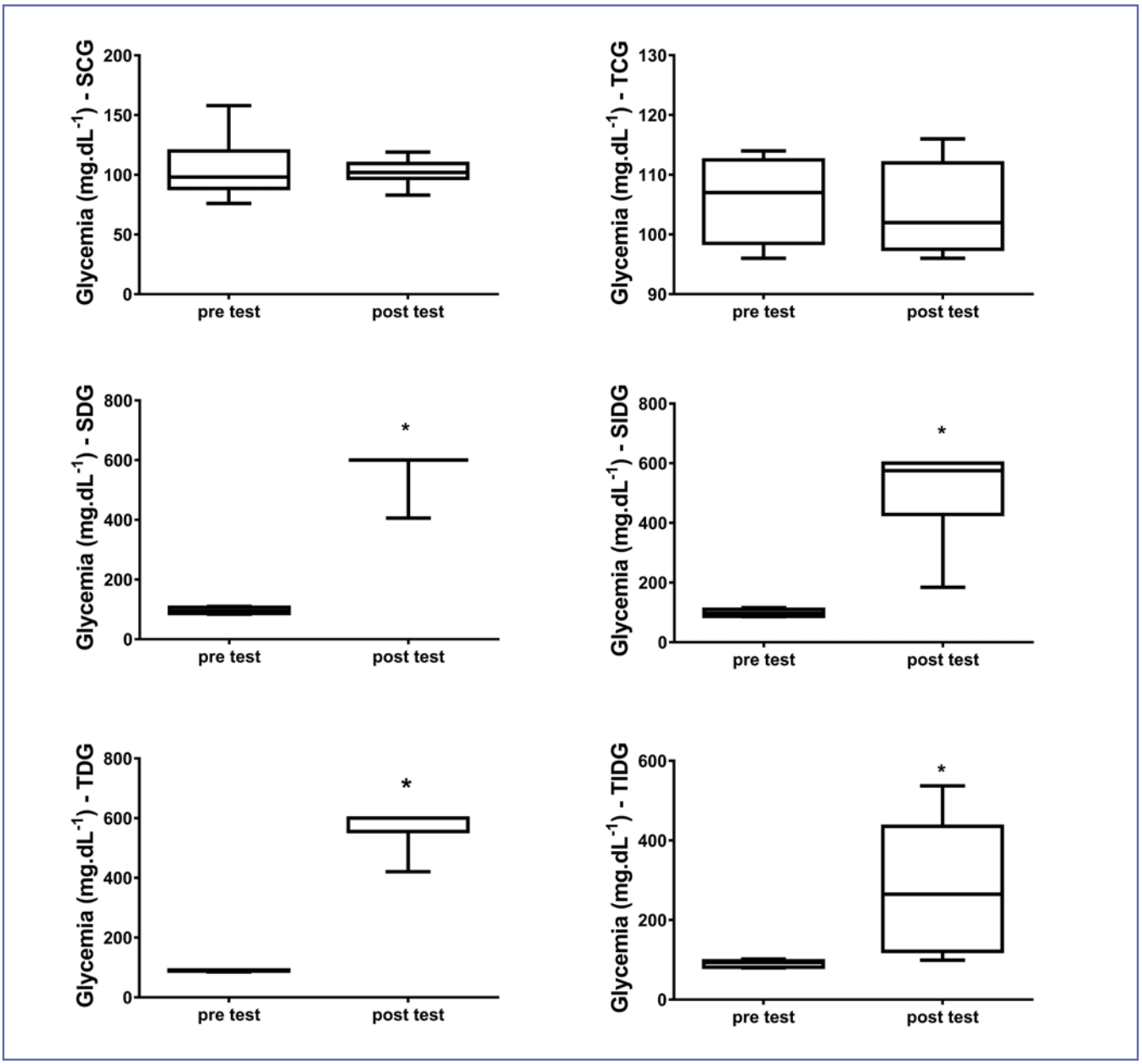


**Figure 2.** Evolution of Glycemia ($mg.dL^{-1}$) before and after five weeks of moderate treadmill exercise in each group. SCG - Sedentary Control Group; TCG - Treadmill Control Group; SDG - Sedentary Diabetic Group; SIDG - Sedentary Insulin Diabetic Group; TDG - Treadmill Diabetic Group; TIDG - Treadmill Insulin Diabetic Group. Body weight values are expressed as median and interquartile range. Wilcoxon test ($p < 0.05$).

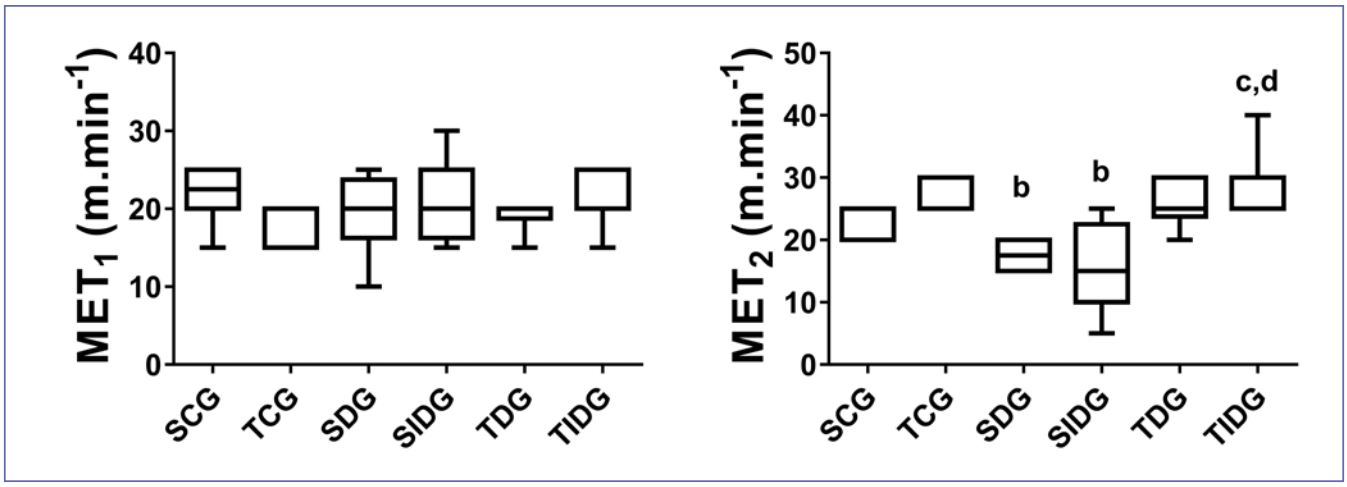


**Figure 3.** Maximum speed values (m.min$^{-1}$) achieved by the animals in the maximal stress tests before and after five weeks of moderate treadmill Exercise. MET1 - Maximum speed reached by animals in the maximum exercise test performed before the exercise protocol; MET2 - Maximum speed reached by the animals in the maximum exercise test performed after the exercise protocol; SCG - Sedentary Control Group; TCG - Treadmill Control Group; SDG - Sedentary Diabetic Group; SIDG - Sedentary Insulin Diabetic Group; TDG - Treadmill Diabetic Group; TIDG - Treadmill Insulin Diabetic Group. Values are expressed as median and interquartile range. Kruskal Wallis ($p < 0.05$) was used with paired comparison to determine statistical difference between groups. Where, (b) difference from TCG, (c) difference from SDG, (d) difference from SIDG.

that in SCG and increased in TDG compared with that in SIDG (↑47.78%, $p = 0.024$, $d = 1.40$). Moreover, elastic modulus was decreased in TCG compared with that in SCG (↓37.79%, $p = 0.011$, $d = 1.59$) and increased in SDG (↑28.32%, $p < 0.001$, $d = 2.06$), SIDG (↑ 24.22%, $p = 0.019$, $d = 2.32$), and TDG (↑ 53.23%, $p = 0.006$, $d = 1.06$) compared with that in TCG. There was no difference in the other biomechanical parameters of AT among the evaluated groups (**table II**).

## DISCUSSION

In this study, we aimed to investigate the biomechanical properties of AT as well as the clinical status and physical fitness of DM1-induced rats following a combination of IST and moderate-intensity aerobic exercise on a treadmill, with the exercise speed previously verified through the maximum effort test, thus ensuring the biological individuality and taking into account the pathological state of these animals. Given this, IST combined with five weeks of exercise prevented the increase in the elastic modulus of AT, favored glycemic balance, and increased physical fitness.

Similarly, the analysis of biomechanical and structural properties of AT indicated that IST without physical exercise may decrease maximal strength, and sedentary rats exhibited increased elastic modulus. Collectively, these results suggest an adaptive exercise response, which is optimized by IST; this is in accordance with the results of recent studies (3,28), which reported that regardless of IST being potentially relevant to the health of individuals with DM1, complementary associations and other therapies are necessary to counteract the triggered chronic complications.

Physical exercise aids glycemic control because it increases insulin-receptor sensitivity and facilitates glycolytic removal (29). Although the practice of moderate and regular physical activity alone is beneficial for DM1, we found that in combination with IST, it favored glycemic control, which was not observed in untrained diabetic animals. This implies that the induction of experimental diabetes results in hyperglycemia.

Our findings corroborate that even without the introduction of a low-carbohydrate diet, which is part of the adequate dietary planning for DM1 treatment (1), the combination of moderate-intensity aerobic exercise on a treadmill with IST benefits metabolic balance, possibly because of glycogenolysis and muscle energy production from physical exercise (30). Future studies should be conducted considering the results presented herein association with diet manipulation to test the hypothesis of benefits potentization.

Insulin combined with moderate-intensity exercise on a treadmill increased the physical fitness of DM1 animals, whereas sedentary animals exhibited decreased physical fitness regardless of IST use. Physical inactivity combined with a hyperglycemic state potentiates the chronic compli-

**Table II.** Biomechanical and structural parameters of the Achilles tendon in Wistar rats after five weeks of treadmill exercise with moderate intensity.

| Parameters | SCG (n = 8) | TCG (n = 5) | SDG (n = 8) | SIDG (n = 8) | TDG (n = 6) | TIDG (n = 8) | Kruskal-Wallis |
|---|---|---|---|---|---|---|---|
| **Cross-sectional Area (mm2)** | 1,26 (1,12-1,48) | 1,25 (1,10-1,71) | 0,93 (0,81-1,07) | 1,10 (1,03-1,30) | 1,28 (0,87-1,51) | 1,06 (0,91-1,19) | 0,074 |
| **Length of tendon (mm)** | 2,39 (1,84-3,21) | 1,78 (1,48-2,21) | 2,43 (1,86-2,77) | 2,40 (1,75-2,93) | 2,24 (1,96-2,99) | 1,63 (1,37-2,38) | 0,187 |
| **Maximum Force (N)** | 33,30 (29,79-35,07) | 30,74 (25,89-34,74) | 29,97 (25,91-32,80) | 25,16 (22,87-29,67)**a** | 32,32 (28,64-34,12)**d** | 26,84 (25,63-29,45)**a** | 0,022 |
| **Maximum Tension (MPa)** | 25,69 (23,17-29,60) | 24,59 (15,47-28,58) | 32,36 (26,08-38,06) | 23,02 (19,22-25,41) | 24,89 (22,19-35,86) | 25,61 (22,95-28,46) | 0,100 |
| **Deformation Maximum Strength (mm)** | 2,69 (2,18-3,19) | 2,87 (2,69-3,39) | 2,61 (2,03-3,04) | 2,43 (1,94-2,80) | 2,33 (1,73-2,77) | 2,10 (1,84-2,64) | 0,108 |
| **Specific Deformation (%)** | 103,39 (88,67-166,73) | 173,40 (127,25-214,10) | 97,07 (65,95-169,65) | 102,80 (92,82-133,03) | 93,73 (71,65-114,63) | 133,15 (88,91-155,58) | 0,175 |
| **Energy/Cross-sectional Area (N.mm/mm2)** | 48,65 (31,26-56,26) | 37,97 (24,98-50,52) | 49,58 (35,45-58,58) | 31,05 (28,30-42,73) | 31,55 (26,67-56,77) | 33,65 (28,86-39,79) | 0,229 |
| **Elastic Modulus (MPa)** | 35,52 (20,36-46,68) | 15,17 (11,21-19,41)**a** | 44,76 (26,48-61,27)**b** | 29,04 (25,43-37,33)**b** | 31,48 (25,82-69,03)**b** | 23,16 (17,97-50,96) | 0,018 |

SCG - Sedentary Control Group; TCG - Treadmill Control Group; SDG - Sedentary Diabetic Group; SIDG - Sedentary Insulin Diabetic Group; TDG - Treadmill Diabetic Group; TIDG - Treadmill Insulin Diabetic Group. The values are median and and interquartile range. The Krukal-Wallis test was used, followed by the paired comparison to determine the statistical difference between the groups ($p < 0.05$). Where (a) represents the difference in relation to SCG, (b) difference in relation to TCG.

cations of musculoskeletal (5,31) and cardiovascular (4,32) systems, which in turn can be mitigated by physical exercise (33,34). Together, that evidence reinforces the clinical benefits of physical exercise for diabetes.

In AT, IST did not counteract the loss of maximal strength, both in sedentary and diabetic animals that exercised on the treadmill. However, this analysis did not consider the intrinsic characteristics of the biomaterial under study, specifically the cross-sectional area and length of AT. However, these properties determine the maximal tension, which was calculated by the ratio of the maximal force supported by the tendon relative to the cross-sectional area and elastic modulus, as quantified based on the maximal tension vs. specific deformation curve (18).

Although IST did not promote any change in maximal stress, the cross-sectional area, maximal and specific deformation, and elastic modulus were increased in the diabetic groups. However, this effect was not observed in groups that received IST combined with physical exercise. These findings corroborate the results of the study by Silva, Santos (31), which showed that sedentary diabetic animals that did not receive IST showed increased elastic modulus. Although this result can be interpreted as the facilitation of the transmission of muscle energy to the tendon in healthy rats, this increase is related to the disorganization of collagen fibers in diabetic animals, with tendons being more prone to rupture (5,6).

In addition, increased elastic modulus is related to changes in the extracellular matrix components of the tendon because of prolonged hyperglycemia, with reduced proteoglycan levels and increased number of cross-links between collagen fibers being responsible for changes in the viscoelastic properties of the tendon (12,35). In turn, this decreases lubrication and increases slipping among collagen fibrils, consequently increasing tendon rigidity and promoting the development of diabetic tendinopathy (5). Furthermore, chronic hyperglycemia is associated with a significantly increased number of mast cells and vascular hyperplasia in the transverse area of blood vessels in AT as well as with the upregulation of the vascular endothelial growth factor, collagen type 1, inflammatory molecular indicators, such as nuclear factor kappa B (36), neutrophil

infiltration, increased basophilia of the tenocytes, increased nuclear size/rounding (37).

Achilles tendon plays an important biomechanical role, particularly in gait and body stability. The mechanical changes observed in the diabetic groups in this study were characterized by changes in the properties of rodent AT, notably due to hyperglycemia and consequent fragility of this structure (37). However, this condition could be attenuated by regular aerobic exercise of moderate intensity combined with IST.

In summary, the combination of IST and moderate-intensity exercise performed on a treadmill in DMI1-induced rats enhanced the stability of glycemic metabolic parameters, thus avoiding an increase in tendon stiffness, as demonstrated by the elastic modulus values. Therefore, the present study supports the importance of combining these two therapies in preventing tendon injuries in diabetic patients. In addition, it suggests that new works be developed combining these therapies with adequate food planning.

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## CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interests.

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