Original Article

Lifetime Overproduction of Circulating Angiotensin-(1-7) in Rats Attenuates the Increase in Skeletal Muscle Damage Biomarkers after Exhaustive Exercise

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Abstract

Angiotensin-(1-7) (Ang-[1-7]) can modulate glucose metabolism and protect against muscular damage. The aim of this study was to investigate the influence of lifetime increase of circulating levels of Ang-(1-7) at exhaustive swimming exercise (ESE). Sprague-Dawley (SD) and transgenic rats TGR(A1-7)3292 (TR) which overproduce Ang-(1-7) (2.5-fold increase) were submitted to ESE. The data showed no differences in time to exhaustion (SD: 4.90 ± 1.37 h vs. TR: 5.15 ± 1.15 h), creatine kinase, and transforming growth factor beta (TGF-β). Lactate dehydrogenase (SD: 219.9 ± 12.04 U/L vs. TR: 143.9 ± 35.21 U/L) and α-actinin (SD: 336.7 ± 104.5 U/L vs. TR: 224.6 ± 82.45 U/L) values were significantly lower in TR. There was a significant decrease in the range of blood glucose levels (SD: −41.4 ± 28.32 mg/dl vs. TR: −13.08 ± 39.63 mg/dl) in SD rats. Muscle (SD: 0.06 ± 0.02 mg/g vs. TR: 0.13 ± 0.01 mg/g) and hepatic glycogen (SD: 0.66 ± 0.36 mg/g vs. TG: 2.24 ± 1.85 mg/g) in TR were higher. The TR presented attenuation of the increase in skeletal muscle damage biomarkers and of the changes in glucose metabolism after ESE.

Keywords: Angiotensin-(1–7), exhaustive swimming exercise, glucose metabolism, muscle damage

Introduction

It is generally accepted that the renin–angiotensin system (RAS) is composed of two main axes: the classical angiotensin-converting enzyme (ACE)/ANG II/AT1 axis which is hypertrophic, proliferative, and vasoconstrictive and the novel ACE2/Ang-(1-7)/Mas axis which is antiproliferative and induces vasodilatory effects.[1] These two axes control homeostasis and promote different responses to pathophysiological and physiological stimuli.[2] Activation of the ACE2/Ang-(1-7)/Mas axis has been observed to be induced by physical exercise in rat aorta and cardiac muscle.[3,4] In skeletal muscle, the RAS regulates muscle mass, and angiotensin II (Ang II) is associated with deleterious effects such as muscle wasting.[5-7] In contrast, angiotensin-(1-7) (Ang-[1-7]) minimizes the muscle wasting effects of Ang II through the ubiquitin–proteasome pathway signaling, as well as by maintaining myosin heavy-chain protein levels.[2] In addition, Ang-(1-7), through Mas receptor, attenuates skeletal muscle atrophy and prevents insulin resistance, fibrosis, and autonomic dysfunction.[8-10] It was shown that components of the Ang-(1-7)-Mas axis play a critical role in skeletal muscle damage and fibrosis in muscular dystrophy through transforming growth factor beta (TGF-β) signaling inhibition.[11,12]

Studies have shown that Ang-(1-7) plays an important role in metabolic control.[13,14] Ang-(1-7) Mas receptor deficiency mice in Friend Virus B NIH Jackson (FVB/NJ)

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background have dyslipidemia, low glucose tolerance and insulin sensitivity, hyperinsulinemia, and hyperleptinemia.[14] Transgenic rats (TGR[A1-7]3292) (TR) express a fusion protein that leading lifetime chronically overproducing of Ang-(1-7). These TR show testicular-specific expression of a cytomegalovirus promoter-driven transgene, resulting in 2.5-fold of circulating Ang-(1-7) compared with nontransgenic control rats (31.5 ± 2.7 pg/ml vs. 14.1 ± 5.9 pg/ml). The plasma Ang II levels are slightly augmented in the TR.[15] It was shown that these TR present a lower activation of the gluconeogenesis pathway without evidence of alteration in the hepatic glycogenolysis.[16] The decrease of gluconeogenesis pathway was demonstrated by a pyruvate challenge. The levels of glycogen phosphorylase enzyme and glucose-6-phosphatase were not altered in these TR, but phosphoenolpyruvate carboxykinase expression in these TR hepatic tissue was decreased.[16] In addition, TR present an enhanced glucose tolerance and insulin sensitivity (*in vivo* experiments) accompanied by a prominent increase in insulin-stimulated glucose uptake by adipocytes (*in vitro* experiments).[14] The hypothesis is that Ang-(1-7) improves glucose metabolism through an activation of the insulin metabolic pathway (PI3K/AKT) that acts directly on glucose metabolism.[8,16]

Strenuous physical exercise can stimulate a systemic inflammatory response and induce increased expression of various extracellular matrix components such as connective tissue growth factor and TGF-β.[17] Other circulatory biological markers released into the blood are used to measure microtrauma induced by physical exercise such as creatine kinase (CK), lactate dehydrogenase (LDH),[18] and alpha-actinin.[19] Muscle damage induced by physical exercise has various etiologies, including mechanical and metabolic stress (such as lower adenosine triphosphate (ATP) values).[20] Ang-(1-7) plays a critical role in the metabolic control of glucose, and it has been demonstrated that this peptide is involved in skeletal muscle remodeling.[21] Thus, the aim of the present study was to investigate the effects of long-duration swimming exercise on glycogen storage and skeletal muscle damage biomarkers in rats with lifetime overproduction of circulating Ang-(1-7).

Materials And Methods

Animals

Male Sprague-Dawley (SD) and TGR(A1-7)3292 rats (TR), 4 months old, with body weight SD: 409.3 ± 46.96 g vs. TR: 295 ± 23.94 g, were obtained from the animal facilities of the university where the rats had been maintained in a temperature-regulated room (22°C to 24°C) under a light/dark 12/12 h cycle and received commercial rodent diets until the day of the experiment. The generation and characterization of TR have been previously described.[15] All experimental protocols were performed in agreement with the international guidelines for animal care and were approved by local authorities (n° 67/2007).

Study design

Exhaustive swimming exercise protocol

SD rats ($n = 14$) and TR ($n = 14$) were submitted to swimming exercises until they reached exhaustion. First, all animals underwent an adaptation period that consisted of 4 days of swimming exercise (without load) for 15 min/day. On the 5th day, the animals underwent an exhaustive exercise session. The exhaustion criteria adopted were the nonmaintenance of surface swimming and the loss of movement that sank the rat to the bottom of the tank and the moment when the rat was quickly rescued. The exhaustive swimming exercise was performed in a tank (200 cm × 50 cm) with individual dividers of 25 cm in diameter and depth in 50 cm and the water temperature was controlled from 30°C to 32°C. The load was equivalent to 4% of the body weight tied to the tail; this load was chosen because it is the one whose exercise is predominantly performed by oxidative pathways, considering that sedentary rats can maintain a stable anaerobic threshold at a workload of up to 5% of body weight.[22,23] The exercise until exhaustion aimed to evaluate the circulating levels of CK, TGF-β, LDH, α-actinin, glucose, and muscular and hepatic glycogen contents in SD and TR.

Blood collection and analysis

Blood samples were taken from the tail before the exhaustive exercise session to measure glycemic levels using a glucose monitor (Accu-Chek® Active, Roche Diagnostics Corp, Indianapolis, IN, USA). After exhaustive exercise, the second tail blood sample was collected, and the rats were euthanized by decapitation. Serum was obtained from the blood samples after centrifugation (840 g for 10 min at 4°C). Total serum CK and LDH were measured with a commercially available, standardized enzymatic assay (Labtest, Lagoa Santa, MG, Brazil, and Bioclin, Belo Horizonte, MG, Brazil). TGF-β and α-actinin serum concentrations were measured using a sandwich enzyme-linked immunosorbent assay (Research and Diagnostic Systems Inc., Minneapolis, MN, USA).

Muscle and liver glycogen content

The skeletal muscle (gastrocnemius) and liver were immediately collected after decapitation. The tissue samples were rapidly weighed, frozen on dry ice, and stored at −80°C for later analysis. Muscular and hepatic glycogen was extracted as glucose following acid hydrolysis as previously described.[24]

Statistical analysis

Statistical analyses were conducted using the statistical package GraphPad Prism, version 5.00 for Windows, (GraphPad Software, La Jolla, California, USA). A Kolmogorov-Smirnov test was used to verify normality of the data, which were expressed as mean ± standard derivation. The unpaired *t*-test was used to compare differences between the two groups, and $P < 0.05$ was considered statistically significant.

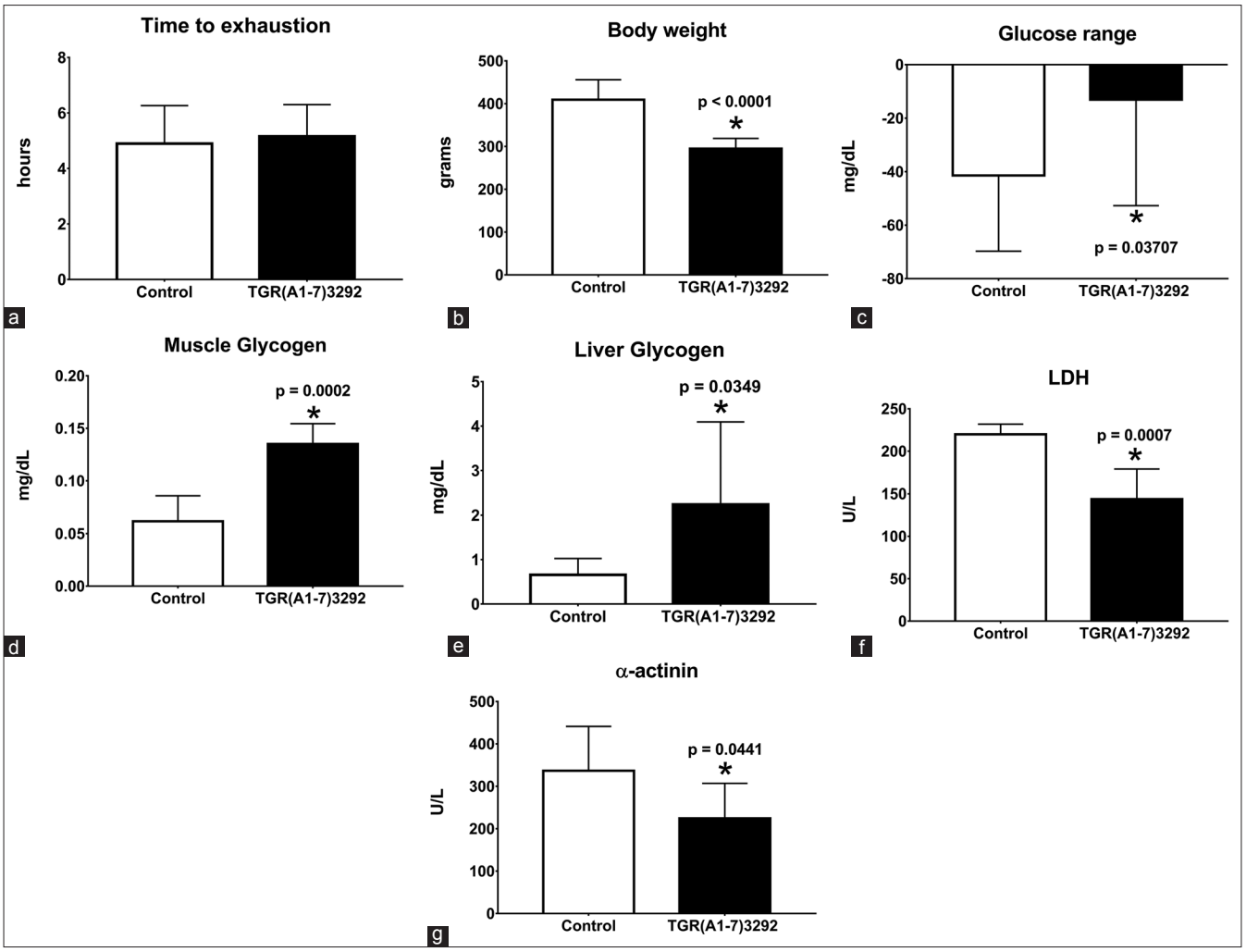

Figure 1: (a) Total exercise time to reach exhaustion, (b) body weight, (c) plasma glucose levels range (before exhaustive exercise values minus after exhaustive exercise values), (d) muscle glycogen, (e) liver glycogen, (f) lactate dehydrogenase, (g) α-actinin. The results of control (Sprague-Dawley) and transgenic rats (TGR[A1-7]3292) were expressed as mean and standard deviation, with the *P* values for unpaired *t*-test shown in the graph

Results

The total exercise time to reach exhaustion was not different between the SD (4.90 ± 1.37 h) and TR (5.15 ± 1.15 h) groups [Figure 1a]. TR had statistically significantly lower body weight (295 ± 23.94 g) compared to SD rats (409.3 ± 46.96 g) ($P < 0.0001$) [Figure 1b].

Glycemic and glycogen responses to long-duration swimming exercise

TR presented an attenuation in the postexercise fall of blood glucose as shown in Figure 1c (TR: −13.08 ± 39.63 mg/dL vs. SD: −41.4 ± 28.32 mg/dL, $P = 0.03707$), even though the basal concentration was not different (TR: 106.1 ± 13.79 mg/dL vs. SD: 97 ± 11.61 mg/dL, $P = 0.0653$). This result suggests that TR were able to preserve glycogen content. Muscle (TR: 0.13 ± 0.01 mg/g vs. SD: 0.06 ± 0.02 mg/g, $P = 0.0002$) [Figure 1d] and hepatic (TR: 2.24 ± 1.85 mg/g vs. SD: 0.66 ± 0.36, $P = 0.0349$) [Figure 1e] glycogen contents after exercise in TR were also significantly higher compared to those in SD rats.

Circulating muscle damage biomarkers after long-duration swimming exercise

The blood levels of muscle damage markers were evaluated after swimming exhaustive exercise. As shown in Figure 1f, total serum LDH values were statistically significantly lower in TR (143.9 ± 35.21 U/L) versus SD (219.9 ± 12.04 U/L) ($P = 0.0007$). In addition, the total α-actinin values in blood were also statistically significantly lower in TR (TR: 224.6 ± 82.45 U/L vs. SD: 336.7 ± 104.5 U/L, $P = 0.0441$) [Figure 1g]. On the other hand, CK values and TGF-β were not different between the two groups (CK: 468.3 ± 36.63 U/L for TR vs. 462.9 ± 34.87 U/L for SD, $P = 0.8026$) (TGF-β: 61.82 ± 8.99 μg/ml for TR vs. 56.46 ± 11.61 μg/ml for SD, $P = 0.8026$).

Discussion

The major findings of this study were that TR submitted to exercise until exhaustion presented higher glycogen content and lower glycemic range and lower circulating

LDH and α-actinin levels compared to SD rats. These results suggested that Ang-(1-7) plays an important role in glucose metabolism during physical exercise and could interfere with muscle damage induced by strenuous exercise. Chronically increased levels of circulating Ang-(1-7) prevent muscular and hepatic glycogen utilization in TR, which can maintain blood glucose levels and abolishing exercise-induced hypoglycemia [Figure 1].

Several studies show the Ang-(1-7) effect on glucose control. Chronic subcutaneous infusion of Ang-(1-7) in mice fed with high-fat diet has improved insulin sensitivity; this effect was associated with an increase in glucose transporter 4 (Glut4) levels in skeletal muscle.[25]

In a diabetic model induced by streptozotocin, the Ang-(1-7) treatment facilitated insulin production by β-cells, decreased fasting blood glucose and serum Ang II levels and homeostatic model assessment of insulin resistance (HOMA-IR) values, and increased fasting serum insulin levels.[26] In transgenic rat model (DM2) based on a reduction in insulin sensitivity in all peripheral organs, oral administration of an Ang-(1-7) formulation reverses hyperglycemia.[27] In a diabetic mouse model (AKITA mouse-autosomal dominant model of spontaneous Type 1 diabetes), the Ang-(1-7) administrated subcutaneously prevented hypertension, oxidative stress, and renal dysfunction.[28]

During aerobic exercise, metabolic fuel demand increases significantly partially through an increase in the glucose uptake/utilization and consequently the glycogen content falls progressively.[29] Muscular glycogen storage is an important fuel during physical activity, particularly in long-duration exercise. In the present study, interestingly, TR were able to save glycogen reserves probably due to optimized glucose metabolism that has been previously shown by our group.[14] Several studies indicate that Ang-(1-7) plays an important role in the metabolic control of glucose.[25,30] Our group showed that TR used in this experiment presented higher glucose tolerance and insulin sensitivity.[16,24] Our hypothesis is that Ang-(1-7) improves glucose metabolism through an activation of the insulin metabolic pathway (PI3K/AKT) that acts directly on glucose metabolism.[8,16] In addition, it was observed that in TG, total and phosphorylated AKT were increased in adipose tissue.[16] In addition, these animals have higher adiponectin levels, suggesting that the TR have a higher capacity to use lipid substrate during exercise and thus conserve glucose.[16] The limitation of the present study is that we were unable to analyze gene and protein expression in skeletal muscle after physical exercise. However, it has been demonstrated that Ang-(1-7) in rat skeletal muscle enhances AKT phosphorylation via a MAS receptor-dependent mechanism.[8]

In our study, the TR had lower body weight compared to SD rats [Figure 1]. Our group also previously described that TR present lower body weight, suggesting that Ang-(1-7) may be involved in body weight control.[24] It was also demonstrated that TR were protected from developing obesity although they were fed with cafeteria diet. Ang-(1-7) regulates food intake and body weight.[31] In addition, it was shown that in mice lacking ACE and with increased Ang-(1-7) plasmatic levels, higher energy expenditure and lower fat mass and body weight were observed.[32]

The increase in the levels of circulating Ang-(1-7) appears to affect exercise-induced blood biomarkers of muscle injury such as LDH and α-actinin. The present study showed that TR submitted to exercise had attenuation in LDH and α-actinin circulating values [Figure 1]. The activation of the ACE2/Ang-(1-7)/Mas axis improves skeletal muscle metabolism and induces antifibrotic and antiapoptotic effects. Recently, it was shown that components of the ACE2/Ang-(1-7)/Mas axis played a critical role in skeletal muscle remodeling in a mouse model for muscular dystrophy (Duchenne disease).[11,12] Several studies have shown the protective antiatrophic/antifibrotic effect of Ang-(1-7) in cardiac and skeletal muscles.[21,33]

The evaluation of muscle damage is largely based on blood level analysis of different sarcoplasmic enzymes such as CK and LDH. These enzymes act intracellularly, and a blood increase could be associated with membrane rupture.[18] The present results indicated lower circulating α-actinin levels in TR subjected to exhaustive exercise and no differences in CK and in TGF-β responses between the groups. Detection of α-actinin and myosin molecules is closely related to muscle contraction and involves a reliable technique that offers high sensitivity and specificity.[19] α-actinin levels were compared in athletic and sedentary men, which showed that α-actinins are better biomarkers for identifying muscle damage induced by exercise than CK.[19]

Conclusion

This is the first study suggesting that Ang-(1-7) plays an important role in the metabolic control of glucose during exhaustive exercise. In addition, this peptide can be involved in skeletal muscle damage and/or remodeling induced by exercise.

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Conflicts of interest

There are no conflicts of interest.

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