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Original Article

Comparison of physiological and biochemical changes in old and young hyperglycemic rats submitted to aerobic exercise and anabolic steroid use

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ABSTRACT

Prolonged hyperglycemia conditions are a risk factor for chronic degenerative diseases such as diabetes and obesity. Testosterone is known to cause muscle hypertrophy, reduced fat mass, and increased body strength. The study aimed to verify possible alterations and differences in the influence of testosterone on the physical performance in post-exercise conditions of young and old animals with alloxan-induced hyperglycemia. We randomly assigned 32 young Wistar rats to groups of untreated non-diabetic young, treated non-diabetic young, untreated diabetic young, and treated diabetic young rats, and 32 aged Wistar rats to groups of untreated non-diabetic elderly, treated non-diabetic elderly, untreated diabetic elderly, and treated diabetic elderly rats, with eight animals each group. The treated non-diabetic and treated diabetic groups received injections of 15 mg/kg weight Durateston™. All the trained groups performed aquatic training with an overload of 5% of the body mass. Following the experiment, we anesthetized and euthanized the animals after exercise (exhaustion). Hemoglobin, erythrocytes, and hematocrit values were higher in the treated groups. The treated diabetic elderly group had the highest leukocyte and neutrophil counts compared to the untreated young groups ($p < 0.05$). As for the lipid profile, untreated rats had the highest values. Glucose concentration was higher at rest and after exercise in the untreated diabetic groups ($p < 0.05$). Lactate was more elevated in the untreated diabetic groups, and the testosterone-treated groups performed the longest swimming time after the maximal test ($p < 0.05$). The use of testosterone in conjunction with physical exercise improved physical performance in water, blood glucose, and lipid profiles.

1. Introduction

Diabetes mellitus (DM) is a chronic disease primarily characterized by an increase in glucose concentration in the bloodstream due to a deficiency in the production of insulin by pancreatic β cells (type 1 DM) or in its action in the target tissues (type 2 DM). This hormone has several activities, such as promoting glucose transport into myocytes and adipocytes, accelerating metabolic pathways that consume glucose, and decelerating metabolic pathways that produce glucose, which explains hyperglycemia, the primary metabolic characteristic of the disease.[1]

The number of people with diabetes in the world has been increasing. From 1980 to 2014, this number quadrupled.[2] This current explosion in the epidemiology of the disease means an elevation of public spending on the population's health. In 2015, around 12% of global healthcare expenditures were devoted to treating type 2 DM and its complications.[3]

Concerning the people who develop the disease, the disease is associated with an 8-year reduction in their life expectancy.[4] In addition, DM harms their quality of life, as most patients also have secondary disease complications.

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Abbreviations

UNY	Untreated non-diabetic young
TNY	Treated non-diabetic young
UDY	Untreated diabetic young
TDY	Treated diabetic young
UNE	Untreated non-diabetic elderly
TNE	Treated non-diabetic elderly
UDE	Untreated diabetic elderly
TDE	Treated diabetic elderly
TN	Treated non-diabetic
TD	Treated diabetic
DM	Diabetes mellitus
AAS	Anabolic-androgenic steroids
COBEA	Brazilian College of Animal Experimentation
CEUA	Ethics Committee in the Use of Experimental Animals
UFAC	Federal University of Acre
K_3EDTA	Tripotassium ethylenediaminetetraacetic acid
Ht	Hematocrit
CK	Creatine kinase
AST	Aspartate aminotransferase
TGC	Triglycerides
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
LDL	Low-intensity lipoprotein
pH	Potential of hydrogen
mg	Milligrams
mg/kg	Milligram per kilogram
mg/dL	Milligram per deciliter
EPO	Erythropoietin
RBC	Red blood cell
Md	Median
IR	Interquartile range
Hb	Hemoglobin
Ht	Hematocrit
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
mL	Milliliters
μL	Microliter

The study of the disease is essential to find intervention strategies for prevention and treatment. Diabetes can be experimentally induced in animals by chemicals such as alloxan. This substance causes irreversible damage to the insulin-producing pancreatic cells, resulting in a considerable reduction in the production of this hormone and leading to diabetes.[5]

Insulin is a potent anabolic hormone that regulates glucose, lipid, and protein metabolism.[6] Insulin deficiency causes muscle mass loss due to proteolysis exacerbation and protein anabolism decline.[7] The loss of mass and muscle strength leads to sarcopenia.

Aging itself also aggravates the tendency to sarcopenia, generating in the elderly a state of frailty characterized by weight loss (4.5 kg in 1 year), self-reported exhaustion, weakness (20% decrease in grip strength), decrease (about 20%) in walking speed and amount of physical activity. Physical stimulation through resistance exercise can reduce the frailty arising primarily from muscle loss associated with aging.[8]

Exercise increases myofiber size[9] and attenuates the intermuscular infiltration of adipose tissue associated with aging,[10] improving muscle quality and function.[11] In addition, exercise can also prevent muscle resistance to insulin and decrease the mitochondrial capacity of this tissue.[12,13] Continuous training in the elderly maintains insulin sensitivity similar to young resistance-trained athletes.[14] High-intensity exercise can influence the immunological parameters related to neutrophil and lymphocyte quantity, activity, and cytokine production and increase glycogen use, decreasing steady-state blood glucose levels.[14]

Resistance exercise training lasting weeks to months has shown increased satellite cells in animals and older adults. In addition to the muscle regeneration seen in trained rats, a concomitant vascularization and inflammatory response improvement was observed.[15] However, it deserves to be noted that no single intervention could completely prevent the loss of muscle mass related to age, strength, and regenerative capacities, such as exercise without an adequate diet or hormonal treatment without the practice of training.[16]

In addition to insulin, testosterone also stimulates mass muscle gain.[7] For aging men, testosterone replacement is becoming a recognized treatment for maintaining libido, delaying age-related cognitive and physical function decline.[17] It favors protein synthesis in the musculature, increasing cell size, lean and bone mass, and strength.[18] Testosterone supplementation suppresses the activity essential to forming new osteoclasts, increasing bone density.[19] The use of anabolic-androgenic steroids (AAS) has also improved muscle regeneration and adaptability to overload.[20]

However, AAS therapy is not recommended for patients in any of the following situations: short-term fertility planning; breast or prostate cancer or risk factor for prostate cancer with PSA $>$ 3 ng/mL; prostate nodule with prostate-specific antigen $>$ 4 ng/mL; lack of previous urological evaluation; increase in hematocrit; untreated obstructive sleep apnea; symptoms indicating severe lower urinary tract disease; uncontrolled heart failure; myocardial infarction; and stroke in the last six months or history of thrombophilia.[21] It is also important to highlight that previous studies have shown harmful side effects related to the use of testosterone in athletes, but without emphasizing self-medication with steroids without prior evaluation and medical follow-up. Despite this, a growing increase in AAS prescriptions has occurred in treating hypogonadism, age-related sarcopenia, or acquired immunodeficiency syndrome.[22]

Thus, this study proposed to verify possible alterations and differences in the influence of testosterone on the physical performance in post-exercise conditions of young and old animals with alloxan-induced hyperglycemia.

2. Method

2.1. Experimental design

In this experiment, we used 64 male Wistar rats, 32 at three months (young rats) and 32 at 12 months (old rats). The animals were kept in collective cages with an average of four rats per cage at an ambient temperature of 22 °C–25 °C and alternating light/dark cycles of 12 hours (h). They received hypercaloric chow and water *ad libitum*. All care and procedures followed national and international laws and the resolutions of the Brazilian College of Animal Experimentation (COBEA) after approval by the Ethics Committee in the Use of Experimental Animals (CEUA) of the Federal University of Acre (UFAC) under the registration number 23107.018612/2016.31.

We divided the 64 rats into eight experimental groups: 1) Untreated Non-diabetic Young (UNY), 2) Treated Non-diabetic Young (TNY), 3) Untreated Non-diabetic Elderly (UNE), 4) Treated Non-diabetic Elderly (TNE), 5) Untreated Diabetic Young (UDY), 6) Treated Diabetic Young (TDY), 7) Untreated Diabetic Elderly (UDE) and 8) Treated Diabetic Elderly (TDE) rats, with eight animals per group. All groups received the same physical training protocol.

2.2. Treatment, adaptations, and training program

During six weeks of treatment, the TNY, TDY, TNE, and TDE groups received an intramuscular injection of 15 mg/kg of the testosterone ester mixture (Durateston™, Organon, Brazil) using 1 mL disposable syringes (BD, São Paulo, Brazil), twice a week, at 4:30 p.m. on Tuesdays and Fridays, for six weeks.[23,24] UDY, UDE, TDY, and TDE group rats received equal insulin doses. The animals in the control groups (UNY and UNE) received injections of a vehicle composed of peanut oil with 10% (v/v) of benzyl alcohol, as previously described.[25]

All injections used a deep intramuscular route in the quadriceps muscle, with weekly leg rotation.

Prior to the training period, the animals went through a period of adaptation consisting of swimming exercises in a 48 cm deep pool, water temperature between 30 °C and 36 °C, with lead spheres corresponding to 5% of body weight tied to the tail, for two weeks, three days a week, with an initial duration of 10 min and a maximum of 40 min at the end of two weeks. During the training period, animals were trained five times a week for six weeks, between 2 p.m. and 5 p.m., with lead spheres corresponding to 5% of body weight tied to the tail.[26,27]

2.3. Maximum test, animal Euthanasy, and blood sample collection

At the end of two weeks of adaptation and six weeks of training, the animals underwent intense training until complete exhaustion, with the time to exhaustion characterized when the animal could not keep its nostrils out of the water for more than 10 seconds (s). Then, they were quickly taken out of the water, placed on a bench, and had their body and tail carefully dried with sterile paper towels.[27]

After the last maximal test session, we anesthetized with 50 mg/kg (i.m.) xylazine and 50 mg/kg (i.p.) ketamine (VETEC, Rio de Janeiro, RJ, Brazil). Then we euthanized the animals to collect blood by cardiac puncture into vacuum tubes (Vacuum II™, São Paulo, SP, Brazil) for biochemical analytes determination and sterile tubes containing 0.1 mL of 10% potassium ethylenediaminetetraacetate (K_3EDTA) for the blood counts.

2.4. Blood tests

Determination of hematocrit (Ht) used the microhematocrit (FANEM, Model 211, São Paulo, SP, Brazil) and hemoglobin the cyanomethemoglobin method (CELM HB 520 hemoglobinometer, São Paulo, SP, Brazil). A complete blood count was performed using an automatic cell counter (CELM CC510, São Paulo, SP, Brazil). Differential or specific leukocyte counts were performed on blood smears stained by the May-Grünwald-Giemsa method in routine identification and counting in 100 cells using an optical microscope, then converted into relative values.

Creatine kinase (CK), aspartate aminotransferase (AST), total cholesterol, triglycerides (TGC), and high-density lipoprotein (HDL) cholesterol were determined by automated colorimetric methods using specific commercial kits (Labtest™, Lagoa Santa, MG, Brazil) and the Roche Cobas Mira Plus analyzer (Roche Diagnostics, Mannheim, Germany). Low-intensity lipoprotein (LDL) cholesterol was determined using the Friedewald formula (LDL = Total Cholesterol – [HDL-C + TGC/5]).

25 mL of blood were collected using properly calibrated heparinized glass capillaries directly into mini tubes containing 50 mL of 1% sodium fluoride to inhibit enolase and stop the glycolytic activity. These samples were used for lactate determination by an electroenzymatic method in the YSI 1500 Sport L-Lactate analyzer (USI Inc, Yellow Springs, Ohio, USA).

2.5. Induction of diabetes mellitus

To obtain the experimental DM, after fasting for 24 h, the rats received 45 mg/kg of body weight alloxan monohydrate (Sigma

Table 1
Erithrogram of rats subjected to six weeks of regular exercise followed by maximal exercise testing.

Varibles	Young						Elderly					
	Untreated			Treated			Untreated			Treated		
	Non-Diabetics (UNY)		Diabetics (UDY)	Non-Diabetics (TNY)		Diabetics (TDY)	Non-Diabetics (UNE)		Diabetics (UDE)	Non-Diabetics (TNE)		Diabetics (TDE)
	Md	*IR*	*Md*	*IR*	*Md*	*IR*	*Md*	*IR*	*Md*	*IR*	*Md*	*IR*	*Md*	*IR*
Hb (g/dL)	14.3^{a}	0.8	14.35^{b}	14.35	17.05^{c}	0.97	15.45^{d}	0.75	$13.7^{a,b,c,d,e}$	1.95	$14.1^{a,b,c,d}$	1.77	$16.6^{a,b,c,d,e}$	1.15	$16.75^{a,b,c,d,e}$	0.92
RBC (10^6/μL)	8.68^{a}	0.64	$8.13^{a,b}$	0.17	$8.9^{b,c}$	0.33	8.7^{d}	0.15	$8.06^{a,c,d,e}$	0.12	$8^{a,c,d,f}$	0.78	$8.79^{b,e,f}$	0.55	$8.67^{e,f}$	0.37
Ht (%)	45.9^{a}	3.17	45.65^{b}	2.07	$52.9^{a,b,c}$	3.77	$52.65^{b,d}$	2.12	$45^{c,d,e}$	4.05	$43.25^{c,d,f}$	4.17	$52.05^{e,f}$	5.37	$52.50^{b,e,f}$	1.9
MCV (μm^3)	54^{a}	1.5	55.5^{b}	2.75	$58.5^{a,c}$	2.25	56	2.25	$52^{c,e}$	1.5	55.5^{f}	3	57.5^{e}	3.25	$59^{a,b,e,f}$	1.75
MCH (pg)	8.68^{a}	0.64	8.14^{b}	0.17	8.89^{c}	0.33	8.7^{d}	0.15	$16.3^{a,b,c,d,e}$	1.3	$17.25^{a,b,c,d}$	0.47	$18.1^{a,b,c,d,e}$	0.95	$17.9^{a,b,c,d,e}$	0.3
MCHC (%)	32^{a}	0.32	$29.7^{a,b}$	1.58	$32.65^{b,c}$	1.95	31.1^{c}	1.17	$30.5^{a,c,e}$	0.6	31.3^{c}	1.2	$32.6^{b,e,g}$	1.17	$30.4^{c,g}$	2.25

Abbreviations: *Md*, median; *IR*, interquartile range; Hb, hemoglobin; RBC, Red blood cell; Ht, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.
Pairs of the same letter indicate significant differences ($p < 0.05$) between groups (Tukey's test): Untreated non-diabetic young [a]UNY, Untreated diabetic young [b]UDY, Treated non-diabetic young [c]TNY, Treated diabetic young [d]TDY, Untreated non-diabetic elderly [e]UNE, Untreated diabetic elderly [f]UDE, Treated non-diabetic elderly [g]TNE.

Table 2
Leukogram of rats submitted to six weeks of regular exercise followed by a maximal exercise test.

Varibles	Young								Elderly							
	No treatment				Treated				No treatment				Treated			
	Non-Diabetics (UNY)		Diabetics (UDY)		Non-Diabetics (TNY)		Diabetics (TDY)		Non-Diabetics (UNE)		Diabetics (UDE)		Non-Diabetics (TNE)		Diabetics (TDE)	
	Md	*IR*	*Md*	*IR*	*Md*	*IR*	*Md*	*IR*	*Md*	*IR*	*Md*	*IR*	*Md*	*IR*	*Md*	*IR*
Leukocytes (cells/μL)	4 450[a]	325	4 650[b]	275	5 500[a,c]	375	6 850[a,b,c,d]	900	4 000[c,d,e]	600	4 600[b,d,f]	325	6 000[a,b,e,f]	650	6 250[a,b,e,f]	575
Total neutrophils (cells/μL)	40[a]	5.5	41.5	9.25	42.5[c]	5,75	48.5[a,d]	2	33[d,e]	11	42.5	12,5	44.5[g]	7,75	53[a,c,e,g]	4
Rod neutrophils (cells/μL)	2,5[a]	1	3[b]	1	3.5[c]	1	4	0.5	3[e]	1,5	6.5[a,b,c,e,f]	9	1.5[f]	1,25	2.5[f]	1
Segmented neutrophils (cells/μL)	38[a]	3.25	40[b]	3	42	5,25	50.5[a,b,d]	3,5	31[d,e]	12	36.5[d,f]	12	42.5[d,g]	7,5	50[a,b,e,f,g]	4.25
Lymphocytes (cells/μL)	58.5[a]	4.5	57.5[b]	5	59[c]	3,75	60[d]	3	60[e]	4,5	45[a,b,c,d,e]	7,25	54.5	6,25	48[a,c,e]	4.25
Eosinophils (cells/μL)	1,5	1	2	1	2	1	2[d]	0.25	2	1	1[d]	1	1	1	1	1
Basophils (cells/μL)	118.5[a]	5.75	130.5[b]	10.75	167[a,c]	4,5	174.5[a,b,d]	9.5	115[c,d,e]	9	121.5[d,f]	30,75	171[a,b,e,f]	3,75	169.5[a,e,f]	23.25
Monocytes (cells/μL)	6[a]	1.25	7[b]	1.25	6.5[c]	1,25	8.5[d]	1.25	5	2	8[f]	3,5	4.5[g]	2	1.5[a,b,c,d,f,g]	2.25

Abbreviations: *Md*, median; *IR*, interquartile range.
Pairs of the same letter indicate significant differences ($p < 0.05$) between groups (Tukey's test): Untreated non-diabetic young [a]UNY, Untreated diabetic young [b]UDY, Treated non-diabetic young [c]TNY, Treated diabetic young [d]TDY, Untreated non-diabetic elderly [e]UNE, Untreated diabetic elderly [f]UDE, Treated non-diabetic elderly [g]TNE.

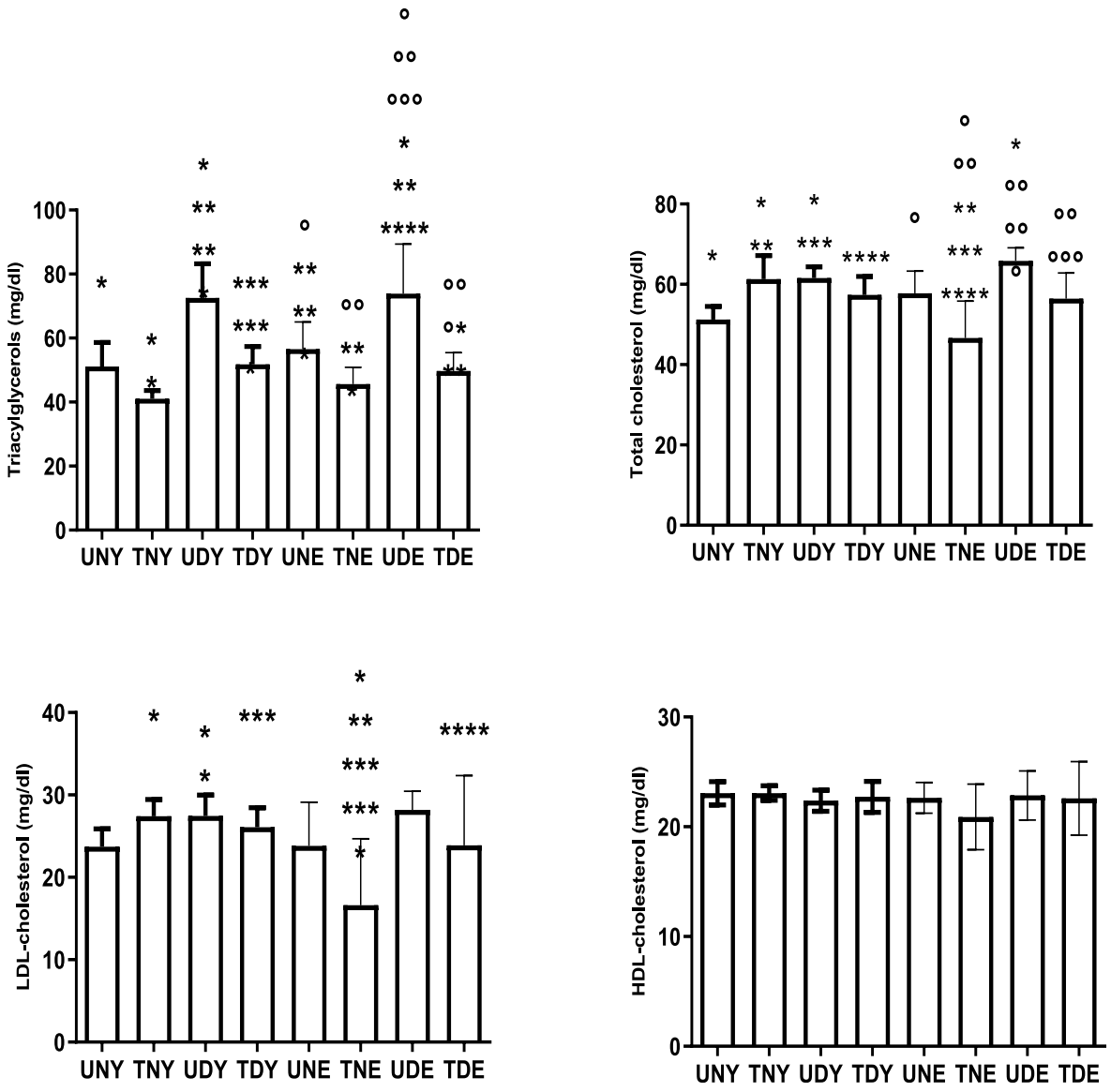

Fig. 1. Plasma concentration of triacylglycerols, total cholesterol, LDL-cholesterol, and HDL-cholesterol before and after the maximal exercise test. The symbols Untreated non-diabetic young UNY, Untreated diabetic young UDY, Treated non-diabetic young TNY, Treated diabetic young TDY, Untreated non-diabetic elderly UNE, Untreated diabetic elderly UDE, Treated non-diabetic elderly TNE, represent the groups: untreated non-diabetic young, testosterone-treated non-diabetic young, untreated diabetic young, testosterone-treated diabetic young, untreated non-diabetic elderly, testosterone-treated non-diabetic elderly, untreated diabetic elderly, and testosterone-treated diabetic elderly, respectively. Pairs of values with the same symbol are different ($p < 0.05$) from each other (Tukey's test).

Chemical Company™, Saint Louis, IL), dissolved in 0.01 M citrate buffer, pH 4.5, injected via intraperitoneal after anesthesia with xylazine (50 mg/kg i.m.) and ketamine (50 mg/kg i.p.) (Vetec Chemistry, Rio de Janeiro, Brazil). The animals also received antibiotics (Pentabiotic Veterinário™, Zoetis, São Paulo, SP, Brazil, 30 000 U/kg, intramuscular) and anti-inflammatory and antipyretic (Banamine® Shering-Plough, 1.1 mg/kg of body weight, subcutaneous). After treatment, the rats had free access to food and received a 30% glucose solution for 24 h.[28] Non-diabetic rats underwent similar manipulation but received injections of citrate buffer solution instead of alloxan.

The evidence of the development of diabetes was performed two weeks after the administration of alloxan through the determination of glycemic levels by the Accu-Chek device (Accu-Chek Advantage™, Roche, São Paulo, SP, Brazil). Rats with fasting glycemic levels equal to or greater than 126 mg/dL were considered diabetic and used in the study.[29]

2.6. Statistical analysis

After characterizing the sample results in terms of distribution, as most of the analyzed parameters did not present a normal distribution, we verified the difference between groups using the Kruskal-Wallis test for independent samples and Mann-Whitney posthoc with Bonferroni correction, with significant differences with p values < 0.05. All analyses used SPSS 15.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

Table 1 presents the median values obtained for each parameter of the erythrogram of each studied group. Hemoglobin, erythrocytes, and hematocrit values were higher in the treated groups. There was no difference between the testosterone-treated diabetic elderly group and the testosterone-treated diabetic young group for these blood parameters ($p > 0.05$). However, mean cell volume, mean cellular hemoglobin, and

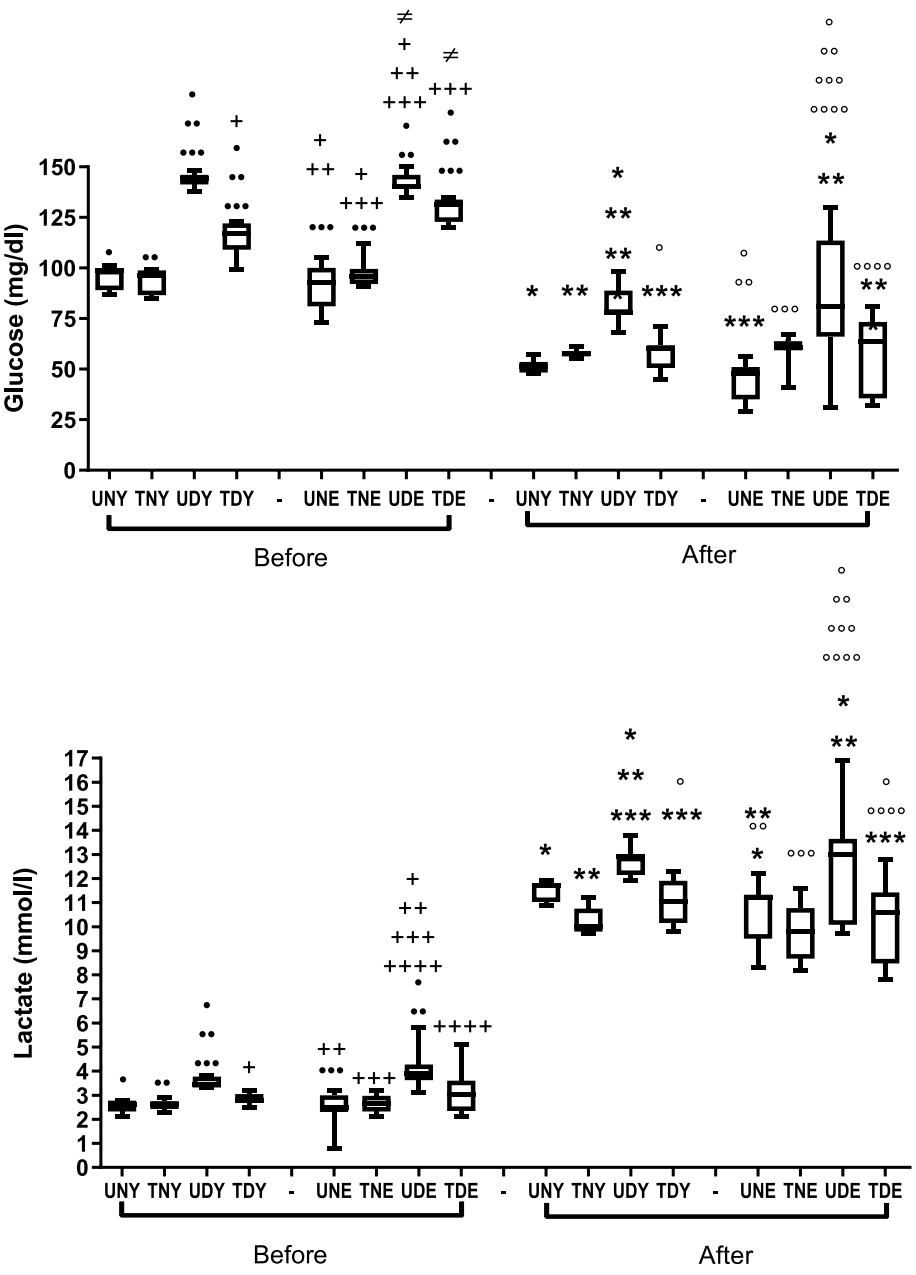

Fig. 2. Plasma glucose and lactate concentrations before and after the maximal exercise test. The symbols Untreated non-diabetic young UNY, Untreated diabetic young UDY, Treated non-diabetic young TNY, Treated diabetic young TDY, Untreated non-diabetic elderly UNE, Untreated diabetic elderly UDE, Treated non-diabetic elderly TNE, represent the groups: untreated non-diabetic young, testosterone-treated non-diabetic young, untreated diabetic young, testosterone-treated diabetic young, untreated non-diabetic elderly, testosterone-treated non-diabetic elderly, untreated diabetic elderly, and testosterone-treated diabetic elderly, respectively. Pairs of values with the same symbol are different ($p < 0.05$) from each other (Tukey's test).

mean cellular hemoglobin concentration had higher values in the treated diabetic elderly group when compared to young rats who exercised without using anabolic steroids.

Table 2 presents the median values obtained for the leukogram parameters of the different groups. The testosterone-treated group of diabetic elderly rats had higher leukocyte and total and segmented neutrophil counts than the untreated groups of non-diabetic young and elderly rats ($p < 0.05$). The testosterone-treated group of diabetic young rats had higher counts of lymphocytes, basophils, and monocytes when compared to the group of testosterone-treated diabetic elderly rats ($p < 0.05$).

As shown in Fig. 1, triacylglycerol concentrations were higher in the untreated diabetic groups for young and old rats. However, there was no significant difference between the non-diabetic young and non-diabetic elderly groups compared to the young and testosterone-treated diabetic elderly rats ($p > 0.05$). Total cholesterol concentrations were lower in the TNE group compared to the TNY, UDY, and UDE groups ($p < 0.05$). LDL-cholesterol had a lower concentration in the NET group when compared to the TDE and TNY groups ($p < 0.05$). HDL-cholesterol had no difference in comparison between all groups ($p > 0.05$).

As seen in Fig. 2, blood glucose levels decreased, and lactate concentrations increased after the maximal test ($p < 0.05$) in all groups. Glucose concentrations in the UDY and UDE groups had higher values at rest before the maximal test ($p < 0.05$).

After the maximal test, the lactate values of the UDY group were higher than those of the UNY and UNE groups, and the values of the UDE group were higher than those of the UNY group ($p < 0.05$). Lactate concentrations at rest before the maximal test were higher in the UDY group than in the UNY, TNY, and UNE groups, and the UDE group had higher values when compared to the UNY, TNY, UDY, UNE, and TNE groups ($p < 0.05$). Lactate values after the maximal test were higher for the UDY group when compared to TNY, TNE, and TDE ($p < 0.05$).

As shown in Fig. 3, AST levels were lower in the TNE group than in UDY, UDE, and TDE and higher in the UDE group than in the TNY group ($p < 0.05$). On the other hand, CK levels were lower in the TNE group when compared to the UDY and UDE groups ($p < 0.05$).

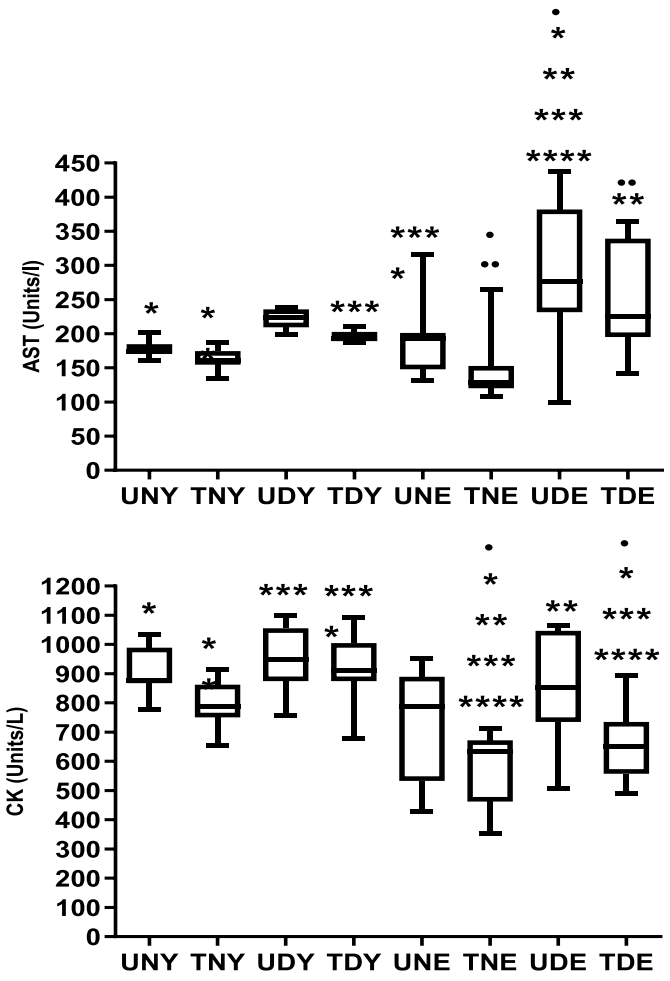

Fig. 3. Plasma levels of aspartate aminotransferase (AST) and creatine kinase (CK) activities after the maximal exercise test. The symbols Untreated non-diabetic young UNY, Untreated diabetic young UDY, Treated non-diabetic young TNY, Treated diabetic young TDY, Untreated non-diabetic elderly UNE, Untreated diabetic elderly UDE, Treated non-diabetic elderly TNE, represent the groups: untreated non-diabetic young, testosterone-treated non-diabetic young, untreated diabetic young, testosterone-treated diabetic young, untreated non-diabetic elderly, testosterone-treated non-diabetic elderly, untreated diabetic elderly, and testosterone-treated diabetic elderly, respectively. Pairs of values with the same symbol are different ($p < 0.05$) from each other (Tukey's test).

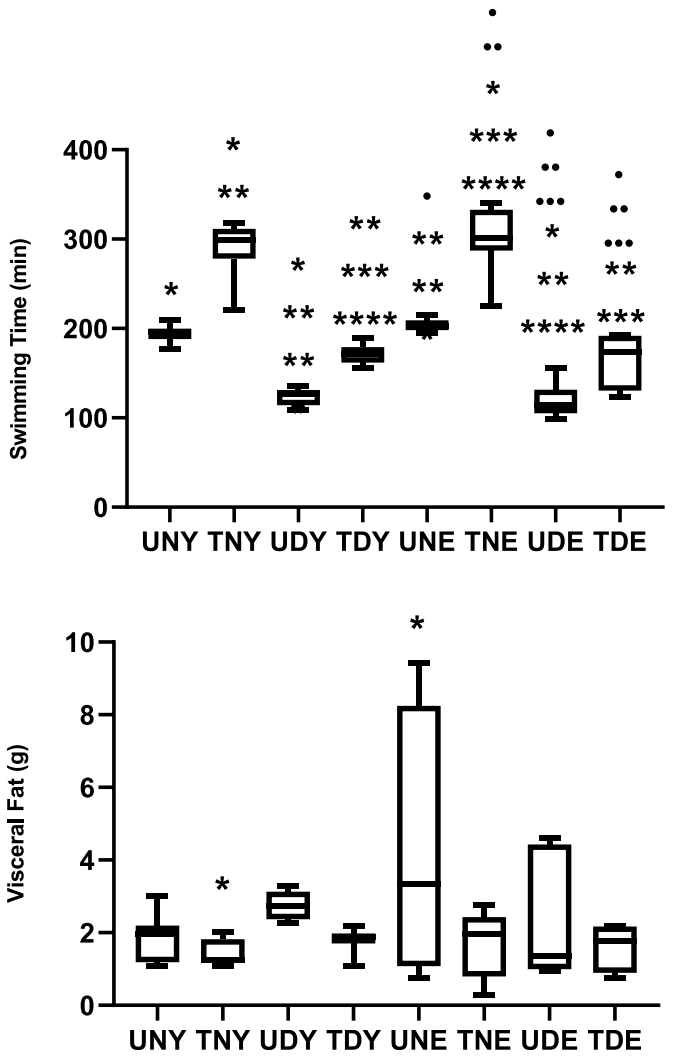

Fig. 4. Time of maximal exercise test and visceral fat of rats submitted to six weeks of regular exercise. The symbols Untreated non-diabetic young UNY, Untreated diabetic young UDY, Treated non-diabetic young TNY, Treated diabetic young TDY, Untreated non-diabetic elderly UNE, Untreated diabetic elderly UDE, Treated non-diabetic elderly TNE, represent the groups: untreated non-diabetic young, testosterone-treated non-diabetic young, untreated diabetic young, testosterone-treated diabetic young, untreated non-diabetic elderly, testosterone-treated non-diabetic elderly, untreated diabetic elderly, and testosterone-treated diabetic elderly, respectively. Pairs of values with the same symbol are different ($p < 0.05$) from each other (Tukey's test).

Fig. 4 shows the swimming time during the maximal test and the visceral fat mass of rats in all groups. The longest swimming time was in the TNY group compared to the UDY, TDY, UDE, and TDE groups and in the TNE group compared to the UDY, TDY, and UDE groups ($p < 0.05$). The shortest swimming times they have occurred in the UDY group compared to the TNY, UNE, and TNE groups and in the UDE group compared to the UNY, TNY, and TNE groups ($p < 0.05$). Visceral fat showed lower values in the TNY group compared to the UDY group ($p < 0.05$).

4. Discussion

In this study, we observed that the association of testosterone with aerobic training caused changes in the animal population's metabolic and physiological conditions.

These changes comprise a stimulatory effect of the anabolic steroid on the blood count in the treated groups of young (TDY) and elderly (TDE) diabetic rats. Although such an increase in the number of red blood cells may be directly related to thermoregulation, as a result of the increase in metabolic activity or the action of high-intensity exercise during forced swimming in rats, it is probably related to the stimulating effect of testosterone on the production of erythropoietin (EPO) in the kidneys and the consequent action of EPO on erythropoiesis in the bone marrow.[30–32] Indeed, Solheim et al. 2020[33] showed increased erythropoiesis in men with testosterone esters.

Due to this increased erythropoiesis, all testosterone-treated groups showed elevated hematocrit values. This elevation, however, may be far from a desirable change since increased hematocrit values have been associated with adverse effects on the cardiovascular system, as described in a study with diabetic men aged 50 and 74 years submitted to a hormone replacement and training program.[34] Indeed, increased red blood cell (RBC) count and hematocrit values may lead to a higher risk of venous thromboembolism.[35,36] However, the absence of difference in monocyte counts in elderly rats in hyperglycemia, and the most significant counts of monocytes in young rats in a situation of hyperglycemia can mean the presence of protection against venous thromboembolism. In addition, it is essential to highlight that the lowest blood glucose

values presented by all treated diabetic groups can constitute a protective factor against morphological dysfunctions in the arterial endothelium.[37]

In this study, the group of testosterone-treated diabetic elderly (TDE) rats had higher counts of leukocytes (neutrophils and segmented) than the untreated groups, which may have occurred due to a decrease in blood oxygen or an increase in the mitotic activity of bone marrow cells.[38,39]

The testosterone treatment protocol and physical exercise used in young and elderly diabetic rats positively affected the lipid profile compared to untreated diabetic rats. Positive impacts on the lipid profile were also observed in the 2017 study by Wen and Kang[40] when studying hormone replacement in elderly and middle-aged rats. However, in our research, the HDL-cholesterol values did not differ between the groups, unlike the 2001 study by Bhasin et al.,[18] which reported lower HDL-cholesterol values in the groups treated with testosterone.

Testosterone-treated rats in the present study had lower glucose values than untreated groups for younger and older rats. Pal and Gupta's 2016 study[41] also showed a positive effect of testosterone on glucose homeostasis.

After physical exertion, variations in lactate concentrations may predict improved physical performance.[42,43] Indeed, in this study, young and elderly diabetic rats not treated with testosterone had higher lactate values after maximal effort.

Furthermore, testosterone administration in young and elderly diabetic rats was protective against muscle damage since higher creatine kinase (CK) values occurred in rats without testosterone treatment. Although strenuous exercise can increase CK,[44] plasma levels of this enzyme did not change in testosterone-treated diabetic elderly rats compared to treated diabetic young rats. The slightest change or the presence of lower levels of CK in study groups that perform some physical effort may reflect a better adaptation of the muscle fiber to high levels of physical stress.

The effect of hepatic toxicity from testosterone use has been a significant concern for abusive or very high dose use, which has been evaluated by determining plasma levels of aspartate aminotransferase (AST), a marker of liver damage.[45,46] As AST levels did not increase in the groups of testosterone-treated young and elderly diabetic rats compared to the untreated diabetic groups, this indicates no deleterious effects on the liver.

Testosterone use was described as an intervention capable of decreasing visceral adiposity in both rats and humans,[47,48] although, in the present study, no differences have been found in visceral fat between young and elderly rats treated with testosterone.

Most groups of rats treated with testosterone could stay longer performing the swimming exercise. In the 2020 study by Sarchielli et al.,[49] rabbits treated with testosterone increased exercise performance.

We hope that the positive results described here for the effects of testosterone use combined with physical activity in hyperglycemic rats may encourage the deepening of this type of research in humans. This research seems necessary since none of the studies described to date recruited a population large enough and long enough to investigate the effects and risks of intervention with testosterone and physical activity.[50] The verification of efficacy and safety in this type of intervention may allow programs aimed at Men's Health to include hormone treatment with testosterone associated with physical exercise in both young and older adults, but especially in men with diabetes, under the supervision of medical professionals and physical educators, within the activities of Primary Health Care, as an option to improve the quality of life of the male population affected by this disease.

5. Conclusion

Using testosterone associated with physical exercise improved the hematological and immunological profiles and the physical performance of young and elderly hyperglycemic Wistar rats.

Authors' contributions

Carolina Freitas da Silva: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Morun Bernardino-Neto:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Thiago Montes Fidale:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Anibal Monteiro de Magalhães Neto:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **João Rafael Valentim-Silva:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Yuri Karaccas de Carvalho:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Formal analysis, Data curation, Conceptualization. **Rodrigo Daminello Raimundo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Formal analysis. **Luiz Carlos de Abreu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Formal analysis. **Romeu Paulo Martins Silva:** Writing – original draft, Visualization, Validation, Supervision, Project administration, Formal analysis, Data curation, Conceptualization. **Nilson Penha-Silva:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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