EXPERIMENTAL INFECTION WITH *TRYPANOSOMA CRUZI* IN MICE: INFLUENCE OF EXERCISE *VERSUS* STRAINS AND SEXES

EXERCISE AND SPORTS SCIENCES

ORIGINAL ARTICLE

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ABSTRACT

Background: Chagas disease is an infection caused by *Trypanosoma cruzi* that affects eight million people in Latin America. One factor linked to the lifestyle that significantly interferes in the response to infection is physical exercise, depending on the kind, intensity and frequency of the activity practiced. Objective: To evaluate the influence of pre-infection chronic moderate aerobic exercise in the development of experimental infection with T. cruzi in mice of two distinct lineages from both sexes. Methods: 30-day old Swiss and BALB/c mice (male and female) were divided into four groups for each strain and sex (total 16) and named as follows: SM (Swiss males), SF (Swiss females) BM (BALB/c mice) and BF (BALB/c mice). The groups were: NT NI (untrained uninfected) T NI (trained not infected); NT I (untrained infected), TI (trained infected). The aerobic exercise pre-moderate chronic infection training was performed with one daily session for eight weeks, five times a week. The inoculum was 1,400 blood trypomastigotes of Y strain of T. cruziintraperitoneally. The peak of parasites, parasitemia total and average measurements of the serum activities of CK and CK-MB were evaluated. Results and Conclusions: The physical training promoted reduction in peak, parasitemia parasites and total average in animals infected with T. cruzi in both strains and sexes. Physical training induced reduction in serum activities of CK and CK-MB in animals infected with *T. cruzi* of both sexes and from the two strains, except for females in the Swiss CK activity.

Keywords: physical training, *Trypanosoma cruzi,* creatine kinase (CK), creatine kinase-MB (CK-MB), parasite

INTRODUCTION

Chagas disease is an infection caused by *Trypanosoma cruzi* which affects eight million people in Latin America[1]. A factor linked to the lifestyle that significantly interferes in the response to infection is physical exercise, depending on the kind, intensity and frequency of the activity practiced[2,3].

The literature has referred that one or few exercise sessions, performed at moderate intensity, either extenuating or long duration, inhibit type 1 T helper lymphocytes (Th1), of inflammatory character and stimulate type 2 T helper lymphocytes (Th2), of anti-inflammatory character[4]. The difference between exercise performance intensity is in the fact that moderate physical exercise, even during infection, may not alterorimprove the immunological response of the host, both in viral infections[5] and in parasitic infections[3,6], while extenuating exercise promotes higher risk for the development of infections[7]and worsens the immunological response of the host[8].

A recent study using a physical training program with moderate intensity for BALB/c mice concluded that chronic exercise is able to improve the body's response to infection by the *Trypanosoma cruzi*, significantly decreasing the parasite peak and increasing survival[3].

The difference of response between sexes is another factor which affects the correlation between exercise and infection. In the majority of studies with parasitic diseases, including with the *T. cruzi*, the female sexual hormones seem to be directly related to better response ofthe body to infection, while the male sexual hormones seem to be associated with increase of susceptability[9]. In rodents, ovariectomy was related to lower resistance of females to infection by the *T. cruzi*[10], while orchiectomy was related to improvement of the male response to infection[11,12]. In the infections by virus estrogen presents reduction of the pro-inflammatory response activity and the anti-inflammatory response stimulation which is related to better response to infection[13].

Creatine phosphokinase (CPK) is a great protein found in two or more presentations. These ways – dimericisoenzymes–are constituted by two distinct polypeptidic subunits, M and B. Three CPK isoenzymes are naturally found in human tissues: CK-MM (skeletal muscle), CK-MB (cardiac muscle) and CK-BB (brain)[14,15].

Due to the restrict size of the CKs which hampers them to be released from the host tissue to the blood flow, except for in the case of membrane injury, the presence of this protein in the serum is used to diagnose and evaluate the cellular damage caused by factors such as diseases or physical exercise. Thus, CK and CK-MB

are the most widely used enzymes as markers in the diagnosis of myocardial and skeletal muscle injury[16].

In the experimental infection by *T. cruzi* a positive correlation between the plasma levels of CK-MB and the inflammatory infiltrates and a non-correlation of this enzyme with the parasite nests was observed. Such information suggests that the myocardial injury occur due to the inflammatory response and not the straight effect of the *T. cruzi* and that the measurement of the CK-MB activity may be used as a marker of cardiac injury[17].

There are no references in the literature about evaluation of correlation of CK and CK-MB activity, *T. cruzi*, physical exercise, and sex and strain differences in mice or rats. The information obtained is found in works comparing only two out of the three variables, and in some cases, using another kind of intervention and/or treatment.

The study of the aerobic physical exercise in the evolution of the experimental infection by *T. cruzi* related to sex, CK and CK-MB activity and genetics of the host presents countless interesting points which can be elucidated, bringing new information both concerning the exercise per se as new alternatives for the treatment and quality of life of patients infected by the *T. cruzi*.

Thus, the aim of this investigation was to evaluate the influence of moderate physical exercise in the evolution of the experimental infection by the *T. cruzi* in male and female mice from two distinct strains.

MATERIAL AND METHODS

The study was approved by the Ethics Behavior in the Use of Animals in Experiments Committee (CEAE/UEM) under the legal resolution 076/2008.

Animals

Mice from the Swissand BALB/c strains (male and female), with approximately 30 days of life were used. Four groups for each strain and sex (total of 16) were sorted for the experiments: NT+NI (not trained +not infected), T+NI (trained + not infected), NT+I (not trained + infected) and T+I (trained + infected) (table 1).

The animals were placed in polypropylene boxes (414 x 344 x 168mm) covered with zinc fencing with a central depression for placement of food and a bottle of water. The boxes were kept in a climatized animal facility (temperature between 22 and 24º C) with light/dark cycle of 12 hours, with wood shaving and cleaned three times per week, with water (chloride) and food (Nuvilab Cr-1® by Nuvital®) ad libitum.

Table 1. Total number of animals used in the experiments with mice from the Swiss and BALB/c strains (male and female), submitted or not to eight weeks of chronic moderate aerobic physical exercise pre-infection and infected or not with the Ystock of *T. cruzi*.

	Strain			
Experiments	SWISS		BALB/c	
Groups	Male	Female	Male	Female
NT+NI	17	13	10	29
NT+I	10	30	25	38
T+NI	19	14	10	46
T+I	16	28	10	41
Total	62	85	55	154

In order to evaluate the infection evolution, 50% of the animals were sacrificed on the eighth day of infection and the other half was kept alive for follow-up of parasitemia.

Physicalexerciseprotocol

The animals were submitted to chronic moderate aerobic physical exercise pre-infection on treadmill (Inbrasport® model Classic CI®) with an adapter for small animals training and with a system which enabled the programing of training sessions and digital control of velocity with sensitivity of two meters per minute (m/min). The physical exercise protocol used corresponds to a moderate exertion[3,18]. Shock or other similar mechanisms have not been used to induce the animals to exercise. An initial period of week of training was considered for exclusion of the animals considered inapt for physical exercise.

The physical exercise program was conducted during eight weeks, being composed of a daily training session, five times per week, with duration of 30 to 45 minutes with velocity of six to 14m/min on the first week, 45 to 60 minutes and velocity of eight to 16m/min on the second week and 60 minutes with velocity of 10 to 20m/min on the remaining ones (mean velocity of 13m/min on the first four weeks and of 17.5m/min on the four last ones).

Infection

The inoculum used was of 1,400 blood trypomastigotes units from the Y stock from the *T. cruzi*[19], intraperitoneally.The animals were infected three days after the end of the pre-infection chronic physical exercise program.

Parasitemia curve

The parasitemia was evaluated using the Brener technique[20]collecting 5µL of blood from the tail and examining 50 fields between the mounting and the slide (22mm x 22mm), daily, from the fourth to the 11th day of infection. The parasitemia curve was traced using the mean of the parasites counting of the inoculated animals for each group.

Collection of biological material

On the eighth day of infection, 50% of the animals were sacrificed through deepening of inhaling anesthesia with ethyl ether (ethoxyethane). Subsequently, cardiac punction was performed for blood collection though a heparinized syringe for collection of about 1mL for BALB/c mice and 2mL for Swiss mice. The blood was kept in ice bath in assay tubes (hemolysis type) until being centrifuged at 2,000 RPM, the supernatant was removed and centrifuged at 4,000 RPM in a centrifuge refrigerated at 4ºC and then divided in 85µL aliquots in Eppendorf tubes, being frozen at –70ºC for evaluation of the CK and CK-MB serum activities.

Determination of the CK and CK-MB serum activities

Commercial kits for CK-NAC FS IFCC and CK-MB FS dosing, both manufactured by DiaSys Diagnostic Systems GmbH & Go. KG, imported and distributed by Biosys Ltda were used for the analysis. An automatic photometric biochemical analyzer from the Selectra E machine was used for the tests performance.The reading method for this instrument is done through the UV test optimized according to DGKC (German Society of Clinical Chemistry) and IFCC (International Federation of Clinical Chemistry) expressing the results in U/l.

STATISTICS ANALYSIS

The statistics comparisons were performed through the Assistat program version 7.5 (made available by the Federal University of Campina Grande, Brazil), using variance analysis (ANOVA), followed by Tukey test (for results which presented normality), Mann-Whitney test (U test) (for parasitemia results which did not present normality), Kruskal-Wallis test (for the remaining results which did not present normality), and also the Microsoft Excel program, version 2007 (Microsoft). The data were expressed as mean ± standard deviation, being adopted significance level of 10%.

RESULTS

Both in the Swiss and BALB/c strains, for both sexes, the trained and infected groups (T+I) presented parasite peak lower than their respective controls (NT+I). The difference was significant for male Swissand female BALB/c mice (figures 1 and 2).

It can be seen in figures 3 and 4 that the parasite peak was statistically higher in male BALB/c animals from the trained and infected group (T+I) than in Swiss animals of the same sex ($p< 0.10$). Concerning the female comparison, the parasite peak was higher in Swiss animals (SF and BF/T+I) ($p < 0.05$). Swiss females (SF) trained and infected (T+I) presented higher parasite peak than males from the same strain and group (SM/T+I)($p < 0.05$).

The mean total parasitemia was statistically lower for Swiss mice of both sexes from the trained and infected group (T+I) (table 2).

Regarding the male not trained and infected animals (NT+I), mean total parasitemia was higher for the Swiss strain ($p < 0.10$).

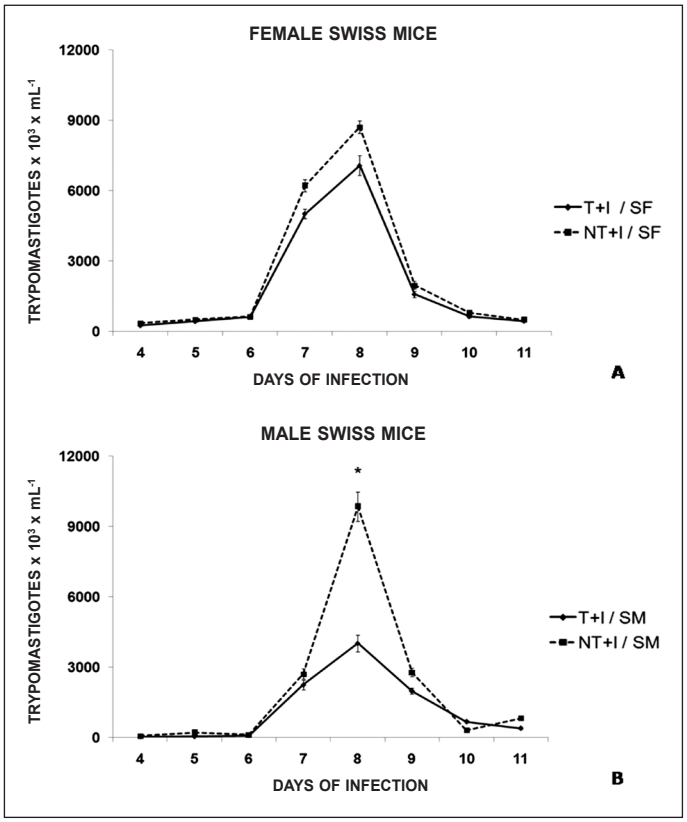

Figure 2. Parasitemia curve (mean ± standard deviation) demonstrating the parasite peak observed in Swiss mice (male and female), submitted or not to eight weeks of pre-infection chronic moderate aerobic physical exercise and infected with Y stock of the *T. cruzi*. Comparison performed between the groups NT+I and T+I, isolatedly, for each strain and sex. NT+I, not trained+infected; T+I, trained+infected. A) SF, Swiss female mice B) SM, Swiss male mice. * = $p < 0.05$.

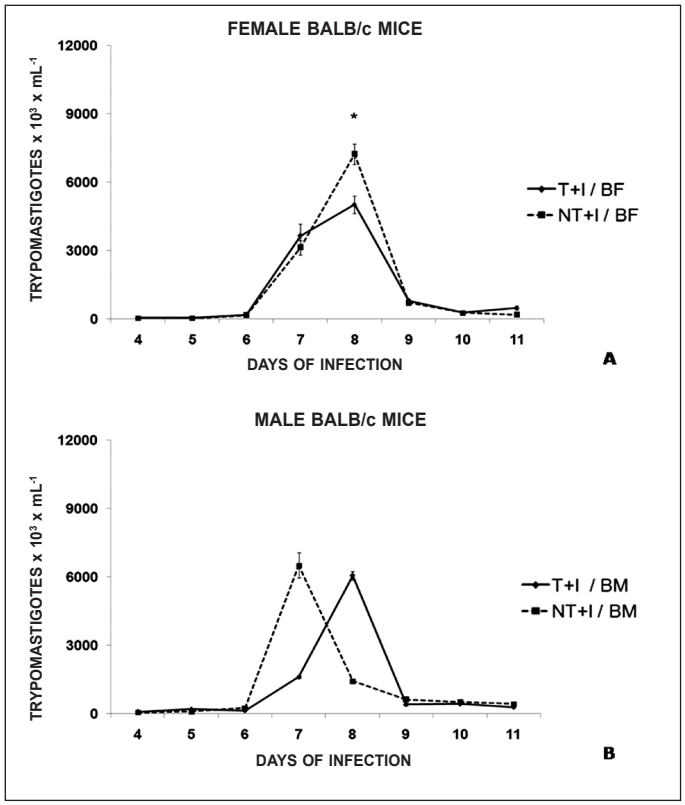

Figure 1. Parasitemia curve (mean ± standard deviation) demonstrating the parasite peak observed in BALB/c mice (male and female), submitted or not to eight weeks of pre-infection chronic aerobic physical exercise and infected with Y stock of the do *T. cruzi*. Comparison performed between the groups NT+I and T+I, isolatedly, for each strain and sex. NT+I, not trained+infected; T+I, trained+infected. A) BF, female BALB/c mice. B) BM, male BALB/c mice. * = $p < 0.05$.

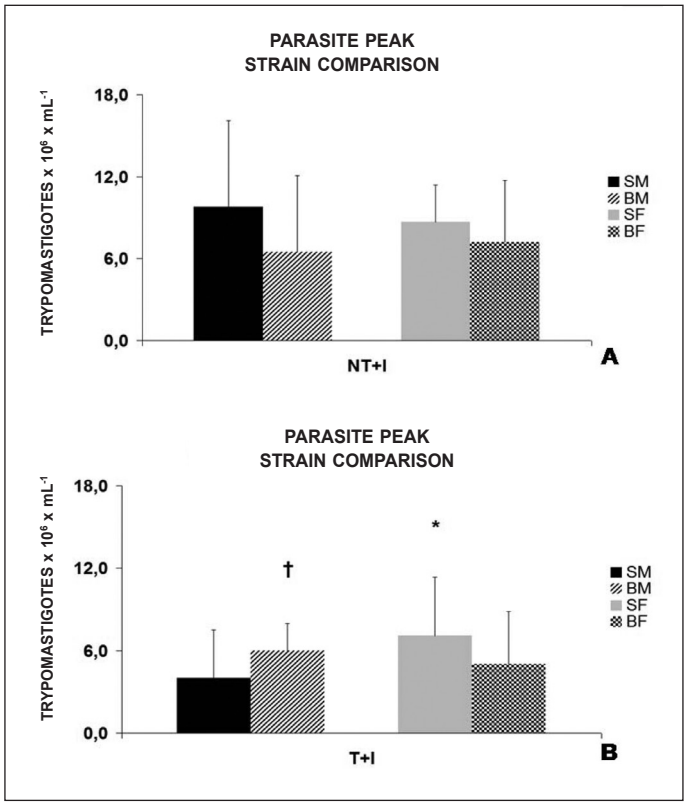

Figure 3. Parasite peak (mean ± standard deviation) observed in Swiss and BALB/c mice (male and female), submitted or not to eight weeks of pre-infection chronic aerobic physical exercise and infected with Y stock of the *T. cruzi*. NT+I, not trained+infected; T+I, trained+infected. SM – male Swiss; SF – female Swiss; BM – male BALB/c; BF – female BALB/c.A and B) Comparison between strains (Swiss and BALB/c) of mice of same sex.* = $p < 0.05$; † = $p < 0.10$.

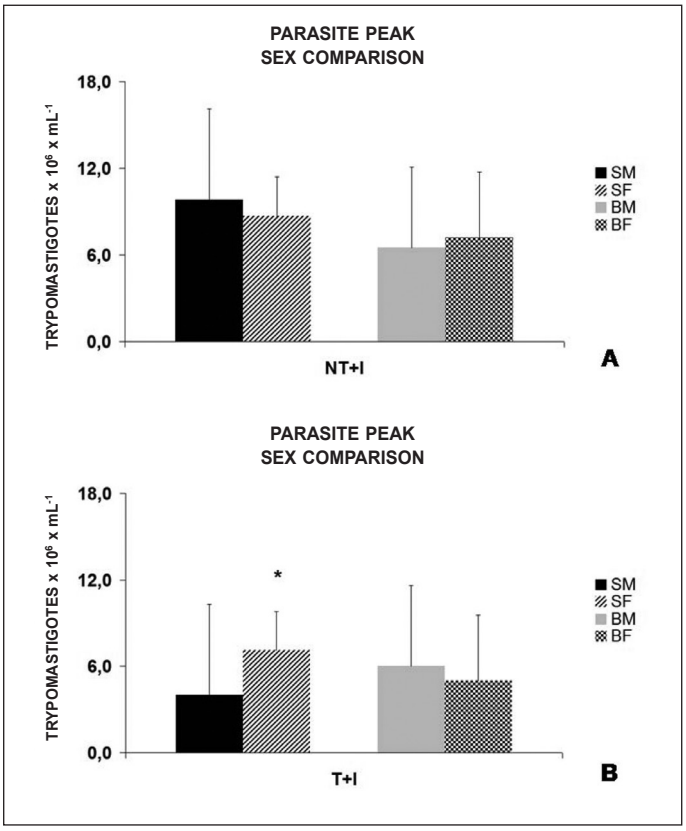

Figure 4. Parasite peak (mean ± standard deviation) observed in Swiss and BALB/c mice (male and female), submitted or not to eight weeks of pre-infection chronic aerobic physical exercise and infected with Y stock of the *T. cruzi*. NT+I, not trained+infected; T+I, trained+infected. SM – male Swiss; SF – female Swiss; BM – male BALB/c; BF – female BALB/c. A and B) Comparison between sexes (male and female) fromthe same strain. * = p < 0.05.

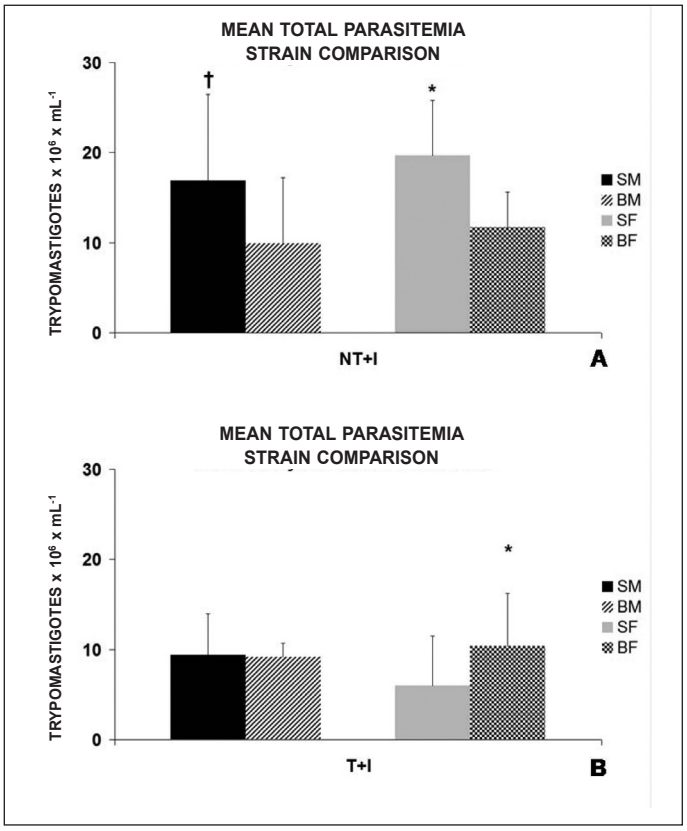

Figure 5. Mean total parasitemia (mean ± standard deviation) observed in Swiss and BALB/c mice (male and female), submitted or not to eight weeks of pre-infection chronic aerobic physical exercise and infected with Y stock of the *T. cruzi*. NT+I, not trained+infected; T+I, trained+infected. SM – male Swiss; SF – female Swiss; BM – male BALB/c; BF – female BALB/c.A and B) Comparison between strains (Swiss and BALB/c) of mice of the same sex.* = p < 0.05; † = p < 0.10.

Table 2. Mean total parasitemia (mean ± standard deviation) observed in mice from the Swiss ans BALB/c strains (male and female), submitted or not to eight weeks of chronic moderate aerobic physical exercise pre-infection and infected with the Y stock of T. cruzi.

	NT+I	T+I	p
Mean total parasitemia (trypomastigotes x 106 x mL-1)			
SM	16.9 ± 9.6	9.4 ± 4.6	< 0.05
SF	19.7 ± 6.1	6.0 ± 5.5	< 0.05
BM	9.9 ± 7.3	9.2 ± 1.5	NS
BF	11.7 ± 3.9	10.4 ± 5.8	NS

SM – Swiss male; SF – Swiss female; BM – BALB/c male; BF – BALB/c female. NT+I – non-trained + infected; T+I – trained+infected. Comparison performed between groups NT+I and T+I, isolatedly, for each strain and sex. NS = not significant.

Among female mice, mean total was also statistically higher for the Swiss strain of the not trained and infected group (SF/NT+I – p< 0.05). Concerning the trained an infected groups (SF and BF/ T+I), parasitemiawas higher for the BALB/c strain (p< 0.05) (figure 5).

In the trained and infected animals (T+I), the comparison between sexes presented higher mean total parasitemia only for male Swiss mice (SM) (p< 0.05) (figure 6).

The physical training promoted statistical reduction in the CK and CK-MB serum activities for the two strains in both sexes, except for female Swiss mice in the CK and CK-MB activity (table 3).

Swiss female mice presented statistically lower CK and CK-MB activity levels in all groups (figure 7).

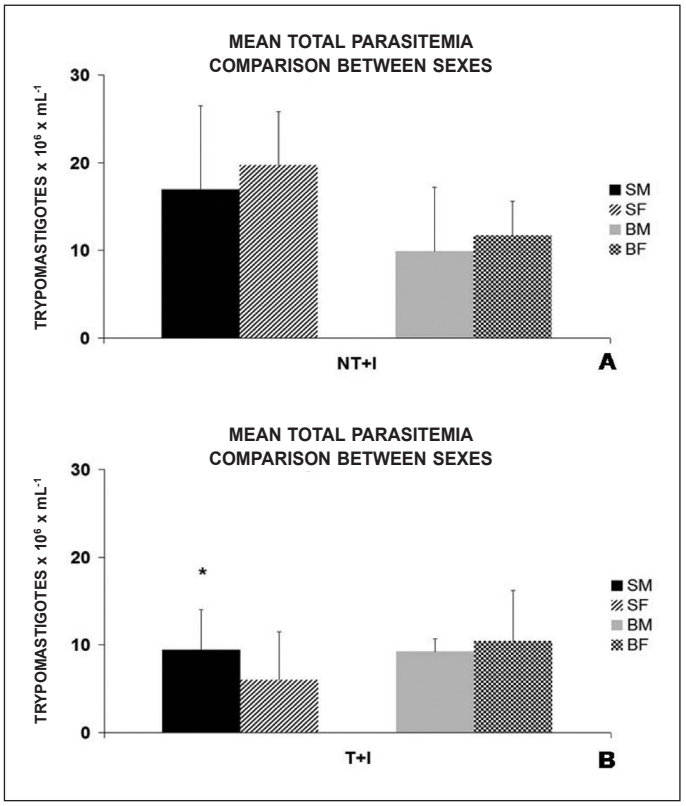

Figura 6. Mean total parasitemia (mean ± standard deviation) observed in Swiss and BALB/c mice (male and female), submitted or not to eight weeks of pre-infection chronic aerobic physical exercise and infected with Y stock of the T. cruzi. NT+I, not trained+infected; T+I, trained+infected. SM – male Swiss; SF – female Swiss; BM – male BALB/c; BF – female BALB/c;.A and B) Comparison between the two sexes (male and female) from the same strain.* = p < 0.05.

Table 3. CK and CK-MB serum activities (mean ± standard deviation) observed in Swiss and BALB/c mice (male and female), submitted or not to eight weeks of pre--infection chronic moderate aerobic physical exercise, and infected or not with Y stock of the T. cruzi.

	NT+NI	T+NI	NT+I	T+I	p
CK (U/l)					
SM	1.9 ± 0.5	1.2 ± 0.4	62.0 ± 54	2.2 ± 1.2	< 0.01
SF	2.5 ± 2.8	0.8 ± 0.5	1.2 ± 0.5	1.5 ± 0.3	N.S.
BM	53.1 ± 75.2	1.9 ± 2.4	97.4 ± 85	0.1 ± 0.0	< 0.01
BF	42.9 ± 40.0	12.5 ± 14.6	50.8 ± 51.2	28.6 ± 36.9	< 0.01
CK-MB (U/l)					
SM	0.9 ± 0.7	0.7 ± 0.4	32.4 ± 28.5	1.3 ± 0.8	< 0.01
SF	2.1 ± 2.8	0.4 ± 0.3	0.8 ± 0.4	1.1 ± 0.5	< 0.05
BM	44.5 ± 63.5	1.3 ± 1.8	73.3 ± 65.9	0.0 ± 0.0	< 0.01
BF	32.7 ± 30.5	10.0 ± 11.6	38.8 ± 38.3	23.0 ± 27.9	< 0.01

SM – Swiss male; SF – Swiss female; BM – BALB/c male; BF – BALB/c female. NT+NI – not-trained + not-infected; T + NI - trained + non-infected; NT + I - non-trained + infected; T + I - trained + infected. Comparison performed between non-trained and trained isoladetly for each strain and sex. N.S. = non-significant.

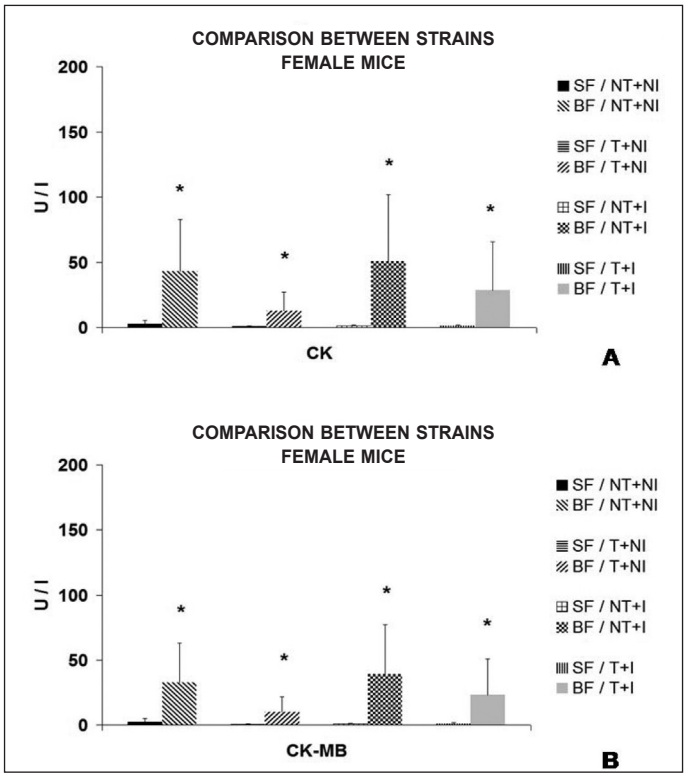

Figure 7. CK and CK-MB serum activities (mean ± standard deviation) observed in Swiss and BALB/c mice, submitted or not to eight weeks of pre-infection chronic moderate aerobic physical exercise and infected or not with Y stock of the T. cruzi. BF – female BALB/c; SF – female Swiss. NT+NI – nor trained+not infected T+NI – trained+notinfected; NT+I – not trained+infected T+I – trained+infected. A) Comparison between strains (Swiss and BALB/c) of the CK serum activity of female mice. B) Comparison between strains (Swiss and BALB/c) of the CK-MB serum activity of female mice. * = $p < 0.05$.

Male BALB/c animals (BM) trained and infected (T+I) presented significantly lower CK and CK-MB serum activity than male Swiss mice (SM) from the same group (figure 8).

In the BALB/c strain, female not trained and infected (NT+I) mice showed significantly lower CK and CK-MB activity levels than the male from the same group (figure 9).

Female Swiss mice, not trained and infected (NT+I), presented significantly lower CK and CK-MB activity levels than the male Swiss mice from the same group (figure 10).

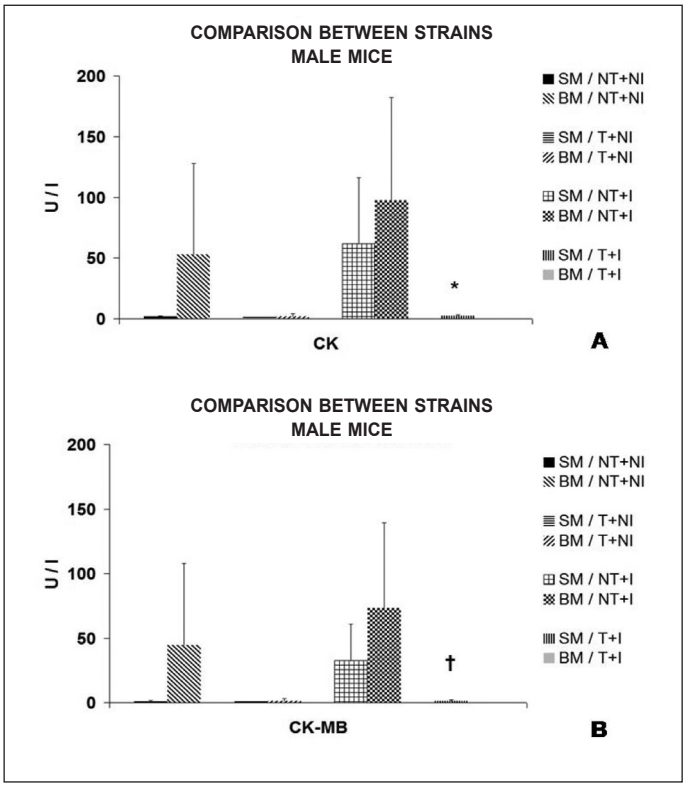

Figure 8. CK and CK-MB serum activity (mean ± standard deviation) observed in Swiss and BALB/c mice submitted or not to eight weeks of pre-infection chronic moderate aerobic physical exercise, and infected or not with Y stock of the *T. cruzi*. BM – male BALB/c; SM – male Swiss. NT+NI – not trained +not infected; T+NI – trained+not infected; NT+I – not trained+infected; T+I – trained+infected. A) Comparison between strains (Swiss and BALB/c) of the CK serum activity of male mice. B) Comparison between strains (Swiss and BALB/c) of the CK-MB serum activity of male mice. * = $p < 0.05$; † = $p < 0.10$.

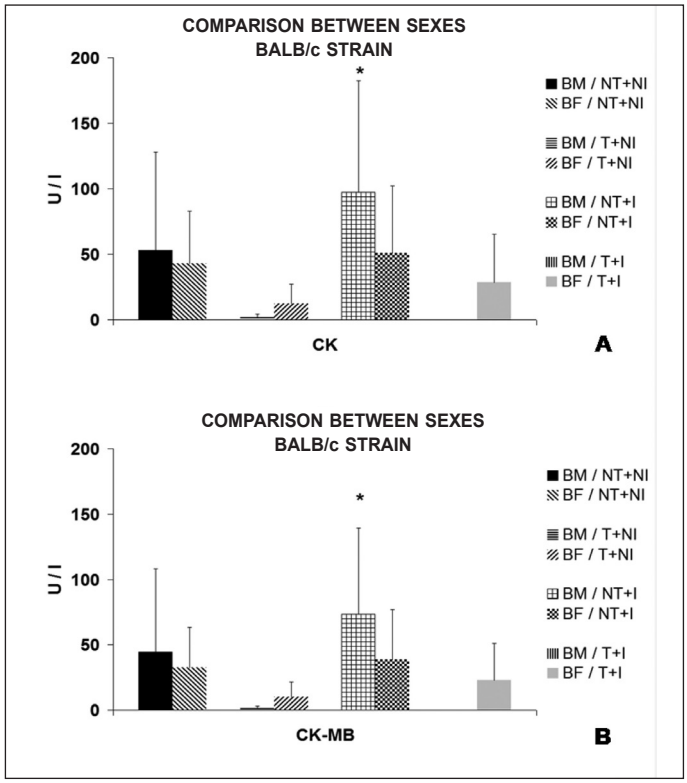

Figure 9. CK and CK-MB serum activities (mean ± standard deviation) observed in BALB/c mice (male and female), submitted or not to eight weeks of pre-infection chronic moderate physical exercise, and infected or not with Y stock with the *T. cruzi*. BM – male BALB/c; BF – female BALB/c. NT+NI – not trained+not infected; T+NI – trained+not infected; NT+I – not trained+infected; T+I – trained+infected. A) Comparison between sexes, of the CK serum activity of BALB/c mice. B) Comparison between sexes of the CK-MB serum activity of BALB/c mice. * = $p < 0.05$.

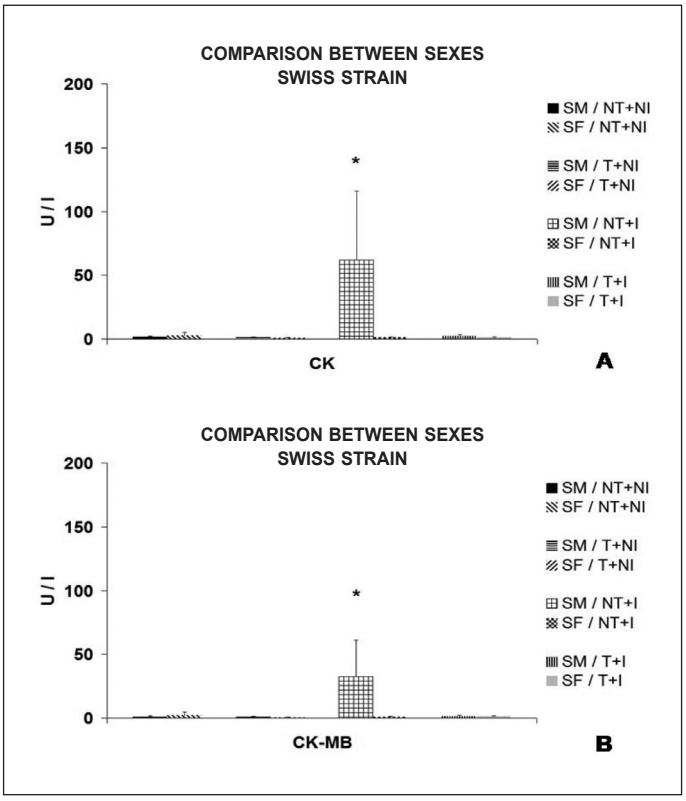

Figure 10. CK and CK-MB serum activity (mean ± standard deviation) observed in Swiss mice (male and female), submitted or not to eight weeks of pre-infection chronic moderate aerobic physical exercise, and infected or not with Y stock of the T. cruzi. SM – male Swiss; SF – female Swiss. NT+NI – not trained+not infected; T+NI – trained+not infected; NT+I – not trained+infected; T+I – trained+infected. A) Comparison between sexes, of the CK serum activity of Swiss mice. B) Comparison between sexes of the CK-MB serum activity of Swiss mice. * = $p < 0.05$.

DISCUSSION

This study evaluated the influence of an aerobic physical training program performed on treadmill for eight weeks before the infection in both sexes of two mice strains, being one strain isogenic (BALB/c mice) and the other non-isogenic (Swiss mice).

The comparison between sexes in the two strains demonstrated that physical training reduces the parasitic load for male and female mice in both strains.In studies evaluating parasitic diseases, including by the *T. cruzi*, positive correlations have been observed in the body response to infection for the female animals[9-12]. However, for this study, females submitted or not to the physical training did not present lower parasitic load when compared to the male mice.

The studies which assess the correlation between physical exercise and infection by the *T. cruzi* have pointed out that aerobic physical training performed before the infection may interfere on the response of the host to the parasite; however, the manner and the extent the such alterations will interfere in the infection course are not well explained yet[3,21]. The data obtained for parasitemia present significant differences and tendency observed in other studies recently carried out[3], in which the animals which are submitted to physical training and then to infection respond better to the infectious agent. Sex[22], age[23] and genetic constitution of the host can influence the infection course by the *T. cruzi* in vertebrates[24].

The genetic response of each strain and sex present interesting points to be elucidated in further research approaching the immunegenetics involved in defense mechanisms of the animals.

The great individual variation in the CK and CK-MB serum activity levels observed in this work is a point to be cleared. It is worth highlighting that methodological problems should not be considered, since all recommendations in the instructions of the enzyme kits were followed and the temperature range of the serum storage used was respected.

The high level of the CK and CK-MB activities for the not trained and infected groups of mice and decrease of activity of these enzymes for the trained and infected groups, for both strains, except for female Swiss ones, shows that the infection interferes in the circulating levels of these enzymes and that physical training provides decrease in their activity levels. This phenomenon could be explained by an adaptation of the body of animals submitted to training in purifying the enzyme in the blood[25].

Newhamet al.[26] suggested three explanations for the adaptation effect of training: an alteration in the recruiting pattern of muscle fibers, which, as the exercise sessions pass by, would preserve damaged muscle fibers; muscle fiber adaptation, making it more resistant to stress caused by exercise; and end of the growth cycle and substitution of the fibers. These explanations would answer for the result found in this study, in which the animals submitted to physical training and infection did not add to the inflammation response in the tissues caused by the infectious agent and physical training, being the exercise able to have caused tissue protection effect.

The CK activity levels also presented differences when sexes were compared in humans. The female sex presents lower serum activity of this enzyme at rest compared with the male sex. This difference excessively in creases after exercising[27]. The same authors observed this difference when compared sedentary men and women performing test on ergometric bicycle using 50% of O_2 maximum. The men presented CK serum levels five times higher, from the initial value (at time zero), going from 122mU/mL to 664mU/mL, while the women obtained increase of two times the initial value (72mU/mLto 152mU/mL).

Many theories have already been proposed to explain the differences observed in the CK serum activity for the sexes. One of them discusses the variations in the recruiting of muscle fibers or the difference in muscle mass observed between men and women[28,29]. The role of the estrogen and its derivatives has also been implied in the CK levels reduction in women compared to men. Estrogen seems to protect the muscle from the injury derived from exercising[30]. In the study under consideration, both female Swiss and BALB/c mice, generally speaking, presented this difference compared with the male sex.

The results obtained in these experiments suggest the involvement of physiological alterations derived from genetics of the host and sexes. The physical training influence promoted in the two strains and in both sexes reduction in the parasite peak, in the mean total parasitemia and in the CK and CK-MB serum activities in animals infected by the *T. cruzi*, except for female Swiss mice in the CK and CK-MB activity.

All authors have declared there is not any potential conflict of interests concerning this article.

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