

EFFECTS OF AEROBIC TRAINING ON THE LIPID PROFILE OF RATS WITH HYPERTHYROIDISM



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ABSTRACT

The relationship between physical exercise, either acute or chronic, and metabolic changes resulting from hyperthyroidism has been little studied in the literature. The aim of this study was to analyze the effects of four weeks of aerobic training on the lipid profile of rats with experimental hyperthyroidism. 45 Wistar rats were randomly divided in four groups: Sedentary Control (SC) - administered saline solution during the experimental period and did not exercise (n = 12); Trained Control (TC) - administered saline solution and underwent physical training (n = 11); Sedentary Hyperthyroidism (SH) - induced hyperthyroidism and did not exercise (n=12) and Trained Hyperthyroidism (TH) - induced hyperthyroidism and underwent physical training (n = 10). The aerobic training lasted one hour per day, five times a week, during four weeks. After the training period, the rats were anesthetized in CO₂ chamber until their sedation. The blood was collected for total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol and serum T3 dosage. Additionally, heart, liver, gastrocnemius muscle and adipose tissue of the mesenteric, retroperitoneal and subcutaneous regions were collected for weighing and triglycerides dosage. Two-way ANOVA, followed by Fisher LSD Post-Hoc was applied for statistical analysis. Lower AGL values were observed in the SH group when compared with SC. The TH group presented lower weight of adipose tissue in the retroperitoneal compared with the SC group. The triglycerides concentrations in the mesenteric, gastrocnemius and retroperitoneal regions were higher in SH group compared with the SC and TC groups. Therefore, it can be concluded that the hyperthyroidism rats presented lipid profile different from the control rats and that aerobic training in rats may have altered the lipid profile of animals with experimental hyperthyroidism compared with the sedentary and control groups.

Keywords: physical activity, chronic effects, thyroid hormone, metabolism, metabolic substrates.

INTRODUCTION

Hyperthyroidism is characterized by increase in the thyroid hormone concentration, either caused by higher production by the glands or by the excessive consumption of the hormone through medication¹.

The excess of this hormone deeply affects metabolism, causing countless alterations such as increased energetic cost, excessive mobilization and use of metabolic substrates^{2,3}. Moreover, hyperthyroidism seem to significantly alter the lipid profile of the sick individual, causing alterations in the glycerol and circulating free fatty acids concentrations (FFA)², besides modulating the distribution of the high-density cholesterol (HDL)⁴ which, associated to alterations in the low-density cholesterol levels (LDL), result in a favorable HDL/LDL ratio⁵.

Studies show that morphological alterations such as muscular atrophy⁶, reduction in muscle mass and adipose tissue⁶ occur as consequence of hyperthyroidism, which compromise the performance of daily tasks and consequently quality of life of the individual^{6,7}.

In an attempt to attenuate and/or revert these effects; regular physical activity has been recommended, since it presents as one

of its main benefits improvement in the lipid profile in the long run⁸. Some studies have demonstrated that performance of acute physical exercise has presented positive effects on some physiological parameters, such as blood lipid concentrations, lipoproteins, cholesterol, blood pressure, glucose metabolism, immunological system and many other variables^{8,9,10}.

The observation of the energetic substrates used during exercise makes us suggest that the kind of exercise which acts the most in the lipid metabolism is the aerobic one^{11,12}.

However, there are still few studies about the correlation between physical exercise and lipid profile in hyperthyroidism patients, stressing hence the importance of investigations which verify the acute and chronic effects of physical exercise in the main metabolic alterations derived from hyperthyroidism.

Animal models using controlled conditions may contribute from the scientific point of view, allowing a broader analysis under several aspects, since studies involving endocrine and biochemical variables in humans present limitations due to the interfering factors as well as invasive character of many analyses. Thus, the aim of this study was assess the effect of four weeks of aerobic training on the lipid profile of rats with experimental hyperthyroidism.

MATERIAL AND METHODS

Sample

45 young Wistar rats from the Central Animal Facility of UNESP – Botucatu Campus were used and kept in the Animal Facility of the Biodynamics Laboratory of the Physical Education Department, Biosciences Institute of UNESP – Rio Claro Campus. The animals were placed in polyethylene cages (four rats per cage), kept at controlled room temperature of $23^{\circ}\text{C} \pm 1$, 12-hour light/dark photoperiod and fed with balanced standard food Purina^a for rodents and water ad libitum. The experiment was conducted according to the current Brazilian legislation and guidelines of the Brazilian College of Animal Experimentation-COBEA.

Experimental outlining

The animals were randomly distributed in four groups: Sedentary Control (SC) – rats which have been administered saline solution during the experimental period and did not perform physical exercise ($n = 12$); Trained Control (TC) – rats which have been administered with saline solution and performed aerobic physical training ($n = 11$); Sedentary Hyperthyroidism (SH) – rats induced to hyperthyroidism which did not perform physical exercise ($n = 12$); and Trained Hyperthyroidism (TH) – rats induced to hyperthyroidism which performed aerobic physical training ($n = 10$).

Daily doses of intraperitoneal levothyroxine sodium (T4) in saline medium ($25\mu\text{g}/100\text{g}$ of body weight), during 10 days prior to the minimum lactate test and during the entire experimental period were administered for induction of experimental hyperthyroidism¹³.

Training protocol

Initially, adaptation to the water medium was performed. On the two first adaptation days, the animals remained in shallow water during 10 minutes on the first day and 20 minutes on the second day. On the third day, the animals were kept during 10 minutes in deep water and on the fourth day during 10 minutes in deep water bearing a backpack with velcro attached to their thorax. Finally, on the fifth day, the animals were kept in deep water for 10 minutes with a backpack with led weight equivalent to 5% of their body weight.

After one rest day, all animals went through the minimum lactate test for determination of the training load, which was composed of hyperlactacidemia induction through two efforts with load of 13% of body weight, separated by 30 seconds of passive rest. The first effort lasted 30 seconds, while the second was performed until exhaustion (TLim). After the induction protocol, the peak lactate concentration was determined through the collection of blood samples in the minutes seven and nine during the passive rest. Immediately after these collections in the induction phase, the incremental test of eight stages with five-minute duration each (3%, 3.5%, 4%, 4.5%, 5%, 5.5%, 6% and 7% of body weight) started. The stages were separated by 30 seconds for blood samples collection (25ml) and lactacidemia. The lactacidemia values obtained in the incremental test were plotted and the load and lactacidemia values were obtained through adjustment of second order polynomial curve¹⁴.

The animals from the trained group started the physical training 48 hours after the minimal lactate test, in which their training load corresponded to 80% of the load obtained in the minimum lactate curve, where each load was individually adjusted. The training sessions were performed in a 100cm long, 70cm wide, 60cm high container with water temperature kept at $31 \pm 1^{\circ}\text{C}$ during the exercise performance, during one hour per day, five days per week for a total period of four weeks.

Previous and post-sacrifice animal evaluations

During the entire experimental period, body weight was weekly recorded and later analyzed through the trapezoid of area under a curve method¹⁵.

At the end of the experimental period all rats were anesthetized in CO_2 chamber until sedation and blood was collected through cardiac puncture with disposable needles and syringes. Blood was centrifuged at 3,000rpm for 15 minutes and the serum supernatant samples enabled analyses of total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol by colorimetric enzymatic method through commercial kits (Laborlab[®]), and T3 hormone through ELISA method (Bioclin[®]).

The adipose tissue of the mesenteric, retroperitoneal and subcutaneous posterior regions was removed for weighting. Excision of the different fat deposits was performed according description by Cinti¹⁶.

Heart, liver, gastrocnemius muscle and adipose tissue of the mesenteric, retroperitoneal and subcutaneous regions samples were removed for determination of the triglycerides concentrations. The samples were placed in tubes with 0.5ml of TritonX-100 at 0.1%. Subsequently, they were homogenized in Polytron[®] for 20 seconds in maximal velocity and after this procedure, the samples were centrifuged at 4,000rpm for 10 minutes. The supernatant was extracted for determination of triglycerides by spectrophotometry with a commercial kit¹⁷.

Data analysis

Firstly, data descriptive analysis presented in mean and standard deviation was performed. Since the Shapiro-Wilk test did not reject the normality hypothesis of data distribution, analysis of variance (two-way ANOVA), followed by Fischer LSD post hoc test were applied. Pre-set significance level of 5% was adopted.

RESULTS

Figures 1 and 2 present data of the area under the curve of body weight and nasoanal length, respectively. Statistical analysis did not point differences between groups for weight or length, demonstrating homogeneity between them.

Likewise, no difference was identified in the mean of the food ingestion (control: \pm ; hyperthyroidism: $\text{g}/100\text{g}$ of body weight) and water intake (control: \pm ; hyperthyroidism: $\pm \text{g}/100\text{g}$ of body weight) during the experiment. Concerning the levels of triiodothyronine (T3) significant statistical difference has been found with higher values for the SH ($p = 0.015$) and TH groups ($p = 0.008$) when compared to the SC (figure 3).

Table 1 presents the values concerning the lipid profile, as total cholesterol, HDL, LDL, AGL and triglycerides. Significant statistical difference with lower AGL values were found in the SH group when compared to the SC.

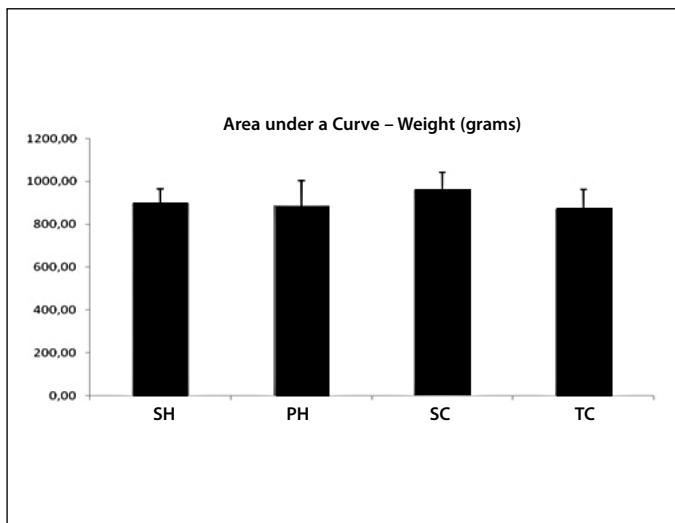


Figure 1. Area under a curve of body weight evolution of the groups during the four weeks of experiment (grams). Results expressed in mean \pm standard deviation. SH = sedentary hyperthyroidism; TH = trained hyperthyroidism; SC = sedentary control; TC = trained control.

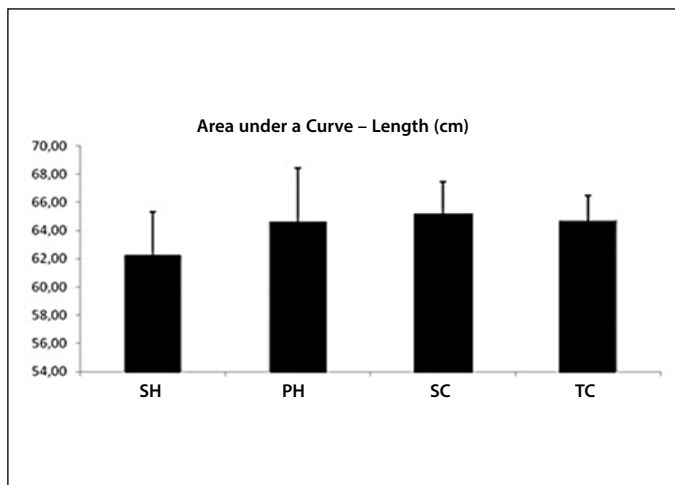


Figure 2. Area under a curve of the nasoanal length evolution of the groups during the four weeks of experiment (grams). Results expressed in mean \pm standard deviation. SH = sedentary hyperthyroidism; TH = trained hyperthyroidism; SC = sedentary control; TC = trained control.

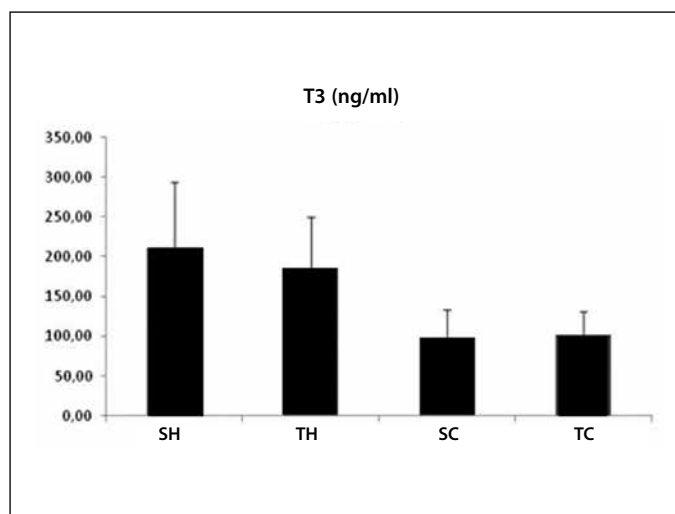


Figure 3. Area under a curve of the triiodothyronine levels (T3) of the groups during the four weeks of experiment (grams). Results expressed in mean \pm standard deviation. SH = sedentary hyperthyroidism; TH = trained hyperthyroidism; SC = sedentary control; TC = trained control.

Table 1. Mean and standard deviation (mg/dl) of the total cholesterol, low-density cholesterol (LDL), high-density cholesterol (HDL) and total triglycerides of the groups: sedentary control (SC), trained control (TC), sedentary hyperthyroidism (SH) and trained hyperthyroidism (TH).

	SH	TH	SC	TC
Cholesterol	112.58 \pm 14.15	104.16 \pm 14.43	101.47 \pm 18.20	112.11 \pm 14.21
LDL	91.54 \pm 18.83	88.72 \pm 13.22	88.62 \pm 13.39	90.56 \pm 15.90
HDL	67.67 \pm 9.81	65.59 \pm 8.82	58.81 \pm 8.68	62.76 \pm 8.34
Triglycerides	157.16 \pm 38.60	170.49 \pm 50.25	170.42 \pm 50.30	192.29 \pm 54.55

*Significant difference in comparison to SC.

Regarding the triglycerides levels of the regions presented in table 2, significant difference of the heart TG variable in which the TH group presented higher levels when compared to the SC ($p = 0.013$), TC ($p = 0.016$) and SH ($p = 0.032$) ones was observed. Statistical difference in the gastrocnemius region was also found with higher values for the SH group when compared to the SC ($p = 0.009$) and TC ($p = 0.002$) groups. The triglycerides levels of the mesentery were significantly higher for the SH group when compared to the SC ($p = 0.035$) and TC ($p = 0.016$) groups. Statistical difference was also found in the comparison where SH presented higher levels of triglycerides in the retroperitoneal region than the SC ($p = 0.024$) and TC ($p = 0.046$), and higher levels for the TH group compared to the SC ($p = 0.017$) and TC ($p = 0.035$) in the same region.

Concerning the weight of the adipose tissue collected in this study, the results are presented in table 3. Only the tissue removed from the retroperitoneal region presented significant differences, in which the group with hyperthyroidism which practiced physical activity presented lower weight of adipose tissue when compared to the SC ($p = 0.045$) and SH groups ($p = 0.024$).

Table 2. Levels of triglycerides (mg/dl) of specific regions, expressed in mean and standard deviation of the groups.

	SH	TH	SC	TC
TG liver	10.00 \pm 1.83	10.04 \pm 1.10	10.39 \pm 1.24	10.37 \pm 1.19
TG heart	2.38 \pm 0.26	2.79 \pm 0.74 ^{a,b,c}	2.33 \pm 0.39	2.28 \pm 0.24
TG gastrocnemius	2.75 \pm 0.66 ^{b,c}	2.04 \pm 0.61	1.91 \pm 0.56	1.88 \pm 0.31
TG mesenteric	29.2 \pm 06.0 ^{b,c}	28.1 \pm 4.5	22.8 \pm 6.2	22.1 \pm 4.4
TG retroperitoneal	22.5 \pm 3.2 ^{b,c}	22.9 \pm 4.8 ^{b,c}	18.7 \pm 4.9	19.2 \pm 1.2
TG subcutaneous	23.0 \pm 3.9	20.4 \pm 2.5	20.1 \pm 4.6	19.7 \pm 4.2

SC = sedentary control, TC = trained control; SH = sedentary hyperthyroidism, TH = trained hyperthyroidism. TG: triglycerides; ^aSignificant difference in the comparison to SH HS; ^bSignificant difference in the comparison to SC; ^cSignificant difference in the comparison to TC.

Table 3. Weight of the adipose tissue (mg/100mg) of the mesenteric, retroperitoneal and subcutaneous regions expressed in mean and standard deviation of the groups.

	SH	TH	SC	TC
Mesenteric	0,51 ± 0,16	0,44 ± 0,15	0,46 ± 0,12	0,48 ± 0,12
Retroperitoneal	0,51 ± 0,11	0,35 ± 0,14 ^{ab}	0,50 ± 0,13	0,45 ± 0,13
Subcutaneous	0,25 ± 0,07	0,26 ± 0,05	0,29 ± 0,08	0,28 ± 0,08

SC = sedentary control, TC = trained control, SH = sedentary hyperthyroidism, TH = trained hyperthyroidism. ^aSignificant difference in the comparison to SH; ^bSignificant difference in the comparison to SC.

DISCUSSION

Hyperthyroidism is caused by increase in the concentration of the thyroid hormone caused by higher production or exogenous administration of the hormone.

The results of the present study confirmed the effective induction of the rats to the hyperthyroidism condition since statistically significant difference of the T3 hormone was found in the treated groups when compared to the control ones.

On the other hand, significant differences have not been observed in any comparison with the TC, despite the mean value of the hormone had been higher for this group compared to the SC. Studies which tried to evaluate the chronic effect of physical exercise on the T3 levels of individuals with hyperthyroidism are rare or inexistent; however, there is consensus in the literature that alterations in the levels of triiodothyronine occur during and after aerobic exercise in healthy individuals^{18,19}. Nevertheless, the results seem controversial concerning the direction of these alterations, and these can be related to the variables of the implemented exercise^{18,19}. However, it cannot be stated that the aerobic training applied in the present study was able to significantly alter the circulating T3 levels for the control and hyperthyroidism groups. The treatment or physical training does not present interference on the body weight and length of rats during the experimental period, though.

Regarding the weight of the adipose tissue removed from the mesenteric, retroperitoneal and subcutaneous regions, the results of the present study demonstrated that most of the mean values were similar among groups, except for the trained hyperthyroidism which present significant difference in the comparison with the hyperthyroidism and sedentary control for the retroperitoneal region. Thus, the aerobic exercise applied in rats with hyperthyroidism led to significant reduction of adipose tissue of the retroperitoneal region. The results found in the literature did not try to evaluate alterations of the adipose tissue in trained and with hyperthyroidism individuals, but the effect of one or the other separately. Concerning the physical exercise, studies observed decrease of lipogenesis in the retroperitoneal region, suggesting that it can prevent the buildup of visceral fat in healthy individuals^{20,21}. Neves et al.⁵, and Haluzik et al.²² in a literature review found increase of lipolysis also in the subcutaneous adipose tissue for individuals with hyperthyroidism without evaluation of physical exercise practice. Moreover,

hyperthyroidism also seems to be related to decrease of adipose tissue measured through skinfolds⁶. Thus, physical exercise and hyperthyroidism separately seem to cause physiological alterations able to reduce visceral and subcutaneous fat, which have only been observed in our study when associated with these two variables.

The thyroid hormones stimulate the cholesterol synthesis²³, consequently increasing its levels in individuals with hyperthyroidism. In our study, significant differences have not been found about cholesterol. However, concerning physical training, it was expected that levels of total cholesterol chronically decreased²⁴. Since both training intensity and duration constitute important factors for improvement of the lipid profile, the training performed in this study may have not been sufficient to promote decrease of the total cholesterol mean levels²⁵.

Concerning the LDL cholesterol, it was expected that physical training provided decrease in its concentration²⁴; however, our study did not find significant alterations for the healthy and with hyperthyroidism rats. Our results corroborate a meta-analysis carried out by Leon and Sanches²⁶, in which the authors concluded that reduction in the LDL cholesterol, total cholesterol and triglycerides concentrations was less frequent compared to the increase in the HDL concentrations, in studies with period equal or longer than 12 weeks of intervention. Possibly, in training with shorter duration, this difference appears less frequently.

On the other hand, for the HDL cholesterol, studies have reported that regular physical training promotes increase in its plasma concentration^{10,24,27}. In our study, when the values of control rats were observed, a tendency of plasma HDL increase could be seen for all interventions. Nevertheless, no significant difference was found.

Despite not being significant, it is worth observing that when an aerobic training is applied in rats with hyperthyroidism their values are very close to the ones of healthy sedentary rats.

When we compare the triglyceride levels from specific body areas, significant differences were only found in the heart and retroperitoneal adipose tissue regions.

The hyperthyroidism group which practiced physical activity obtained higher triglycerides levels in the heart region when compared to the other groups. This finding clashes with the literature, since sedentary individuals with hyperthyroidism have accelerated metabolism with higher cardiovascular demand and consequently, lower fat concentrations in that region^{2,28}.

The sedentary with hyperthyroidism group obtained significantly higher triglycerides in the mesenteric, gastrocnemius and retroperitoneal regions when compared to the sedentary and trained control groups. The trained with hyperthyroidism group also presented higher values when compared with the control groups; not significant though. Possibly, the physical activity reduced the triglycerides values in that group, but not efficiently to generate these many metabolic alterations.

It can be concluded that the rats with hyperthyroidism presented lipid profile different from the control rats, and the aerobic training in Wistar rats may have altered the lipid profile of the animals with experimental hyperthyroidism when compared with the sedentary groups and control groups.

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