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Can low-level laser therapy when associated to exercise decrease adipocyte area?

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ABSTRACT

Obesity affects approximately 20% of the world population, and exercise is the primary non-pharmacological therapy. The combined use of exercise and low-level laser therapy (LLLT) may potentiate the effects promoted by exercise. The objective of this study was to investigate the effects of exercise in combination with phototherapy on adipocyte area, activity of the enzyme citrate synthase and muscle morphological analysis. We used 64 Wistar rats, which were divided into eight groups with 8 rats each: sedentary chow-diet (SC); sedentary chow-diet plus laser therapy (SCL), exercised chow-diet (EC); exercised chow-diet plus laser therapy (ECL); sedentary high-fat diet (SH); sedentary high-fat diet plus laser therapy (SHL); exercised high-fat diet (EH); exercised high-fat diet, laser therapy (EHL). The animals were submitted to a program of swimming training for 90 min/5 times per week for 8 weeks and LLLT (GA-Al-AS, 830 nm) at a dose of 4.7 J/point and a total energy of 9.4 J/animal, with duration of 47 s, which was applied to both gastrocnemius muscles after exercise. We conclude that the combined use of exercise and phototherapy increases the activity of the enzyme citrate synthase and decreases the white adipocyte area epididymal, retroperitoneal and visceral in obese rats, enhancing the effects of exercise.

1. Introduction

Obesity is a current global concern due to its increasing prevalence around the world. Furthermore, this chronic, low-grade inflammatory disease [1] is a precursor of other impairments, such as dyslipidemias, hypercholesterolemia, an increase in cardiovascular risks and diabetes mellitus type II [2,3].

The consequence of increasing serum triglycerides as result of hypercholesterolemia raises the risk for cardiovascular diseases. Although triglycerides constitute an important component of energy metabolism, excess serum triglycerides works as a negative factor and is redirected to fat deposits in adipose tissue [4]. Thus, the intracellular deposits of triglycerides are increased, enlarging the adipocyte area [4]. In general, the main cause of these fat deposits is a sedentary lifestyle and a poor diet that is usually rich in fatty nutrients [5]. An increase in the adipocyte cell area is harmful and is responsible for a chronic inflammatory condition that is characterized by abnormal cytokine production and activation of inflammatory signalling pathways, i.e., characteristics of obesity [6,7].

Diet-induced obesity is generally treated through controlled exercise [8], which is pivotal for improving the oxidative capacity of the mitochondria. Exercise results in the use of triglycerides as the main energy source to produce ATP [9]. This metabolic preference is dependent on mitochondrial activity. Several studies have shown that one of the effects of low-level laser therapy (LLLT) is mitochondrial alteration, increasing its size and oxidative capacity [10,11]. We hypothesised that the morphological and functional enhancements of mitochondria are associated with alterations in

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restricted mitochondrial enzymes, such as citrate synthase. This enzyme plays a central role in the oxidative capacity of mitochondria, and an increase of its activity after LLLT has been reported [12]. For this reason, we analysed the influence of LLLT on the adipocyte area in different white adipose tissues and the citrate synthase activity using obese rats in sedentary and exercised conditions. We expected that LLLT would increase both the citrate synthase activity and promote change in adipocyte area.

2. Materials and methods

2.1. Animals

All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996), and protocols were approved by the ethics committee of Universidade Federal de São Carlos-UFSCar (no. 067/2010). In this study, 64 male Wistar rats (90 days old and weighing 303.31 ± 19.83 g) were used. The animals were divided into eight groups with eight rats per group ($n = 8$) and assigned as: sedentary chow-diet (SC); sedentary chow-diet plus laser therapy (SCL), exercised chow-diet (EC); exercised chow-diet plus laser therapy (ECL); sedentary high-fat diet (SH); sedentary high-fat diet plus laser therapy (SHL); exercised high-fat diet (EH); exercised high-fat diet, laser therapy (EHL). Except for the SC, SCL, EC and ECL groups, all of the other groups were fed *ad libitum* with the high-fat diet (3 weeks) before experimental period for the development of exogenous obesity. The rats were kept in individual cages with food and water *ad libitum* for 8 weeks under a 12:12-h light–dark cycle at 23 ± 1 °C.

2.2. Diet

The rats were fed either a normocaloric diet (N), MP-77 standard rat chow diet provided in pellet form (Primor®, São Paulo, Brazil), which contained 23 g of protein, 49 g of carbohydrates, 4 g of total fat, 5 g of fibre, 7 g of ash, and 6 g of vitamins per 100 g of diet; or a high-fat diet, which consisted of the same commercial rat chow plus peanuts, milk chocolate and sweet biscuits in a ratio of 3:2:2:1. [13]. The quantities of caloric and total fat in diets are showed in Table 1.

2.3. Exercise and LLLT protocols

The exercise program consisted of swimming in individual tanks filled with water, which was maintained at 28–32 °C. The animals in the trained groups swam for 30, 60, and 90 min/day on the first, second, and third days, respectively, to become adapted to the program. The swimming period was then set to 90 min/day for 5 days/week for 8 weeks. All of the rats swam with an attached load of 3–5% of their body mass. This load was weekly adjusted and recorded. The values of loads were used to calculate of mean supported load per group. This exercise program is considered to be of moderate intensity [14].

The LLLT parameters are shown in Table 2. All applications were performed by the same person using Thera Laser (DMC Equipment, São Carlos, SP, Brazil). The probe was held stationary and contacted the skin at a 90° angle and with slight pressure. The laser was used for the transcutaneous irradiation of the muscle gastrocnemius in both legs (1 point in each). We decided not to use a sham group because we included the appropriate positive and negative controls to with respect to LLLT (positive, high-fat diet with LLLT; and negative, high-fat diet without LLLT) and diet (positive, high-fat; and negative, chow-diet; both with or without LLLT). The laser was applied after the daily exercise regimen because the stress induced from exercise results in the maximal absorption and metabolic effects. The same protocol was previously used by our laboratory [11,15]. All animals were handled similarly, promoting the same routine.

Table 1
Difference between chow-diet and high fat diet in calories and total fat.

	Chow diet	High fat diet
Calories	4.07 kcal/g	5.12 kcal/g
Fat	4 g	35 g

Table 2
Low-level laser therapy (LLLT) parameters.

Type	Ga-Al-As	Energy density	1.66 J/cm^2
Wavelength	830 nm (infrared)	Treatment time	47 s
Frequency	Continuous wave (CW)	Total energy delivered	9.4 J
Optical output	100 mW	Power density	35.36 W/cm^2
Spot diameter	0.6 mm	Energy per point	4.7 J/point

2.4. Sample preparation

At the end of the experimental period (8 weeks), the animals were euthanized by decapitation. The blood was immediately collected and centrifuged, and aliquots of the plasma were frozen at −80 °C for subsequent analyses. White adipose tissues (epididymal, retroperitoneal and visceral) were immediately excised, weighed and frozen at −20 °C. The gastrocnemius muscles were also removed and frozen at −80 °C.

2.5. Citrate synthase activity

The citrate synthase activity in samples of gastrocnemius muscle was assayed using the colorimetric method [16] and the CS Assay Kit (Sigma, St. Louis, Missouri, USA). The reaction protocol was performed using: 0.2 mM acetyl-CoA, 0.5 mM OAA, and 0.1 mM DTNB in 100 mM Tris–HCl (pH 7.5). The reaction was incubated for 1.5 min at 25 °C, and the absorbance was recorded at 412 nm.

2.6. Adipocyte area

Samples (100 mg) of epididymal, retroperitoneal and visceral adipose tissue were fixed in 0.2 M collidine buffer (pH 7.4) containing 2% osmium tetroxide at 37 °C. After 24 h, the samples were washed with warmed saline [17]. Histological preparations of the adipose tissue were photographed with a CCD-Iris camera (Sony Corp., Tokyo, Japan) interfaced with an Olympus BX60 optic microscope (Olympus Corp., New York, NY, USA) and a computer. The adipocyte area being 400 cells from each animal, totalling 2000 cells per group. It was measured using image analysis software (Image-Pro Plus 3.0; Media Cybernetics, Silver Spring, MD, USA) and was expressed in $\times 10^{-3}$ μm^2.

2.7. Morphological analysis

Animals (one per group, totalling 8 animals) were euthanized, and their right gastrocnemius muscles were immediately removed and perfused with Karnovsky's fixative solution (2.0% glutaraldehyde and 4.0% paraformaldehyde at a 1:1 ratio in 0.1 M sodium phosphate buffer [pH 7.4]). The samples were routinely processed for transmission electron microscopy and photographed using a Philips CM100 FEI. The index of the junctional measure was calculated as the product of the maximum length of the fifth junction in