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Resistance exercise modulates lipid plasma profile and cytokine content in the adipose tissue of tumour-bearing rats

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ABSTRACT

Cancer cachexia is a multifactorial syndrome characterised by progressive weight loss, frequently accompanied by anorexia, sarcopenia, and chronic systemic inflammation. The white adipose tissue is markedly affected by cachexia and contributes to this syndrome throught the secretion of pro-inflammatory factors which reach the adjacent tissues and the circulation. A nonpharmacologic intervention that may attenuate cancer cachexia is chronic physical activity, but the effect of resistance training upon adipose tissue inflammation in cachexia has never been examined. For that purpose we designed a protocol in which animals were randomly assigned to a control group (CT, $n = 7$), a Tumour bearing group (TB, $n = 7$), a Resistance Trained group (RT, $n = 7$) and a Resistance Trained tumour bearing group (RTTB, $n = 7$). Trained rats climbed a vertical ladder with an extra load attached to the tail, representing 75–90% of total body mass, 3 times per week, for 8 weeks. In the 6th week of resistance training, tumour cells (3×10^7 Walker 256 carcinosarcoma) were inoculated in the tumour groups. Body, adipose tissue, muscle and tumour mass was determined, as well a blood biochemical parameters, and the hormone and cytokine profile assessed. The glycogen content of the liver and muscle was measured. IL-10, IL-6 and TNF-α protein expression was evaluated in the mesenteric adipose tissue (MEAT) examined. Resistance training increased by 9% body weight gain in RTTB (final weight 310.8 ± 9.8 g), when compared with TB (final weight 288.3 ± 4.9 g). LDL-c levels were decreased in RTTB (0.28 ± 0.9 mmol/L) by 43% when compared with TB (0.57 ± 0.1 mmol/L). HDL-c levels were increased in RTTB (1.31 ± 0.12 mmol/L) by 15% in regard to CT (1.13 ± 0.7 mmol/L) and 22% as compared with TB (1.07 ± 0.07 mmol/L). RTTB testosterone levels (577 ± 131 ng/mL) were 55% higher when compared with CT (254 ± 41.3 ng/mL) and 63% higher when compared with TB (221 ± 23.1 ng/mL). Adiponectin levels were augmented in RT (23 μg/mL) by 43% when compared with TB (11 μg/mL). Protein expression of IL-6 was increased 38% in TB MEAT (5.95 pg/μg), as compared with CT (3.64 pg/μg) and 50% compared with RTTB (2.91 pg/μg). Similar results with respect to TNF-α TB (7.18 pg/μg) were observed: 39% and 46%, higher protein expression in comparison with CT (4.63 pg/μg) and RTTB (3.8 pg/μg), respectively. IL-10 protein expression was found to be increased in TB (4.4 pg/μg) and RTTB (3.2 pg/μg) 50% and 47%, respectively, in comparison with CT (1.2 pu/μg). The IL-10/TNF-α ratio was higher in RTTB in relation to all others experimental groups. The results show a robust effect of resistance exercise training in preventing important symptoms of cancer cachexia, thus strongly suggesting it may appear as an alternative to endurance exercise as a non-pharmacological therapy in the management of this syndrome.

1. Background

Cachexia is a multifactorial syndrome characterised by progressive weight loss, frequently accompanied by anorexia, sarcopenia, and chronic systemic inflammation [1]. It is today envisaged as a degenerative chronic inflammatory disease, being deeply related with the increase of pro-inflammatory factors, especially tumour necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) [2]. We have previously shown [3] than in addition to presenting morphological, biochemical and physiological alterations in response to cachexia, the white adipose tissue may function as an important contributor to cachexia-related inflammation, as it actively secretes pro-inflammatory factors which reach the adjacent tissues and the circulation [4].

To the present day, no specific interventions have proven effective in preventing or reversing cachexia. Interventions such as nutritional supplementation and appetite stimulants are only partially helpful. On the other hand, a most promising nonpharmacological intervention that may attenuate cancer cachexia is chronic physical activity [5]. The literature shows benefits that range from improvement of overall physical function, to reduce fatigue, and augment of life quality [6], and attenuation of the side

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effects associated with chemotherapy [7]. Furthermore, we have shown [8–11] that endurance exercise consistently ameliorates cachexia-related inflammation in a rodent model, presenting a systemic effect that is also conspicuous in regard to the adipose tissue.

Nevertheless, endurance exercise training may not be well accepted by all patients, as it demands more time than strength exercise sessions to be performed. Indeed, some reports suggest that resistance exercise may improve aspects of cachexia [5]. To our knowledge this is the first report to show the robust effects of resistance exercise training upon adipose tissue inflammation in cachexia, along with amelioration of many other symptoms of the syndrome.

2. Methods and materials

2.1. Animals

The investigation conformed to the Guide for the care and use of laboratory animals published by the US National Institute of Health (NIH Publication No. 85–23, revised 1996) and was approved by the Committee of Ethics in Animal Experimentation of the Institute of Biomedical Sciences, University of São Paulo. Wistar male rats (70 days old), from the Animal House of the Biological Science Building, University of São Paulo, were maintained under controlled temperature (23 °C), humidity (55% ± 10%), and a 12-h light/12-h dark cycle. The rats had free access to water and to a standard commercial chow (Nutrilab-CR1; Nuvital Nutrients Ltda) containing the following: carbohydrate (660 g/kg), protein (230 g/kg), fat (40 g/kg), fibre (60 g/kg), and vitamins plus minerals (10 g/kg). All animals were adapted to the laboratory environment for 1 week, before engaging the training program. Rats were randomly assigned to four experimental groups: control (*C*) ($n = 7$), tumour-bearing (TB) ($n = 7$), Resistance Trained (RT) ($n = 7$) and tumour-bearing RT (RTTB) ($n = 7$).

2.2. Resistance exercise training

The resistance training protocol was adapted from Hornberger and Farrar [12], During the 8 weeks of resistance training, climbing sessions were performed once every 3 days. Initially, the rats were adapted to the resistance training protocol, which required that the animals climbed a vertical ladder (1.1 × 0.18 m, 2-cm grid, 80° incline), with weights secured to their tails, as described below. The load apparatus was secured to the tail by wrapping the proximal portion of the tail with a self-adhesive foam strip. A Velcro strap was wrapped around the foam strip and fastened. With the load apparatus secured to the tail, the rats were placed at the bottom of the ladder and familiarised with climbing. The initial climb consisted of carrying a load that was 75% of the animal's body mass. After this, an additional 30 g weight was added until a load was reached with which the rat could not climb the entire length of the ladder. Failure was determined when the animal could not progress up the ladder after 3 successive stimuli to the tail. The highest load successfully carried the entire length of the ladder was considered the rat's maximal carrying capacity for that training session. Training sessions were 3–5 ladder climbs with 75%, 90%, and 100% of the rat's previous maximal carrying capacity, determined earlier. During subsequent ladder climbs, an additional 30 g load was added until a new maximal carrying capacity was determined.

2.3. Tumour injection

Walker 256 carcinosarcoma cells were inoculated (subcutaneous injection into the right flank as a sterile suspension of 3×10^7 cells) in groups TB and RTTB, in the beginning of the 6th week of the training protocol. Fifteen days after tumour inoculation (8 weeks after starting the protocol) the animals were killed by decapitation, 48 h after the last exercise session, as to avoid the acute effects of exercise. Body weight and caloric consumption were measured throughout the experimental period, and tumour weight was determined after euthanasia. Blood was collected and serum obtained for the measurement of the different parameters.

2.4. Determination of muscle and hepatic glycogen content

Muscle samples were digested in 30% KOH at 100° C and glycogen was precipitated with ethanol, between each precipitation the samples were centrifuged at 3000 rpm for 15 min. The precipitated glycogen was submitted to acid hydrolysis in the presence of phenol. The values expressed in mg/100 mg of wet weight, using the method described by Siu [13].

2.5. Plasma profile determination

All reagents and chemicals adopted were of analitical grade. Serum total cholesterol, triacylglycerol, glucose, lactate dehydrogenase, creatinine and high-density lipoprotein cholesterol were determined using Labtest® Blood samples from animals fasted for 12 h, were collected after euthanasia. The samples were or not anticoagulated with EDTA (1 mg/mL) and centrifuged at 2.500g at 4° C for 15 min to obtain plasma and serum and the tests were carried out in conformation with laboratory kit instructions. VLDL-cholesterol was calculated using the formula TG/2.2 mmol/L. Low density lipoprotein (LDL) cholesterol was determined by differential substraction of the sum of the cholesterol fractions from the total cholesterol. Liver TAG content was extracted with chloroform: methanol 2:1 (v/v) and measured in accordance with the method described by Folch [14].

2.6. Hormone and cytokine plasma levels

The samples were anticoagulated with EDTA (1 mg/mL) and centrifuged at 2.500g at 4° C for 15 min to obtain plasma for determination of testosterone, leptin and adiponectin with commercial kits and expressed in ng/mL. For leptin and adiponectin, the kits were obtained from Millipore Corp. Bedford, MA, USA and for testosterone, from Assay Designs, Inc., Ann Arbor, MI, USA.

2.7. Muscle lactate dehydrogenase activity

To characterise the metabolic effect of resistance exercise, lactate dehydrogenase activity, an index of glycolytic capacity, was determined in the gastrocnemius of the rats. Tissue samples were homogenised at 0 C in a volume of 1 mL of 100 mM KPO_4 buffer, so that a 1:20 (wt/vol) homogenate was obtained. LDH activity was measured according to the spectrophotometric method described by Fritz [15]. All assays were linear with respect to time and dilution, and each sample was analysed in duplicate.

2.8. Muscle protein content

Gastrocnemius protein concentration was determined by Bradford assay (Bio-Rad, Hercules, CA), with bovine serum albumin as a reference.

2.9. Immunoassays for cytokines in the adipose tissue

Adipose tissue samples were carefully rinsed in ice-cold 0.9% NaCl to remove any blood contaminants and snap frozen in liquid nitrogen and stored at 80 C. Frozen tissue (0.1–0.3 g) was

homogenised in RIPA buffer (0.625% Nonidet P-40, 0.625% sodium deoxycholate, 6.25 mM sodium phosphate, and 1 mM ethylenediamine tetraacetic acid at pH 7.4) containing 10 Lg/mL of protease inhibitor cocktail (Sigma–Aldrich, St. Louis, Missouri). Homogenates were centrifuged at 12,000g for 10 min at 4 C, the supernatant was saved, and protein concentration was determined by Bradford assay (Bio-Rad, Hercules, CA) with bovine serum albumin as a reference. For the TNF-α (DY510), IL-6 (DY506) and IL-10 (DY522) assays, sensitivity was found to be 5.0 pg/mL in the range of 31.2–2.000 pg/mL. Assay sensitivity for IL-10 was 10 pg/mL in the range from 31.2 to 2.000 pg/mL. All samples were run as duplicates, and the mean value was reported.

3. Statistical treatment

Data are presented as means ± standard deviation (SD). Statistical analysis was performed with *Student's T test* for resistance protocol progress and 2-way analysis of variance (2-way ANOVA) with exercise and tumour as factors. All variables presented normal distribution and homoscedasticity, when t difference was found to be significant, Tukey's *post hoc* test was adopted. A significance level of $p \leqslant 0.05$ was employed for all comparisons. Statistical analysis was carried out with GraphPad prism software, version 5.0 (GraphPad Software, San Diego, CA).

4. Results

4.1. Tissue weigtht

Table 1 illustrates the values of body weight before and after the training period, as well as the absolute retroperitoneal adipose tissue (RPAT), mesenteric adipose tissue (MEAT), epididymal adipose tissue (EPAT), and gastrocnemius weights. Resistance training increased body weight gain of RTTB, when compared with TB (9% $p < 0.048$), along with higher absolute gastrocnemius muscle weight (26% $p < 0.027$). RPAT showed statistical difference in TB and RTTB when compared with Control. (30%; $p < 0.002$ and 56% $p < 0.001$), showing that both the presence of the tumour and the exercise protocol influence this parameter. Tumour mass was decreased after exercise, yet the difference did not reach statistical significance.

4.2. Resistance training progress chart

Fig. 1 illustrates the progress of resistance training overload during the experimental period. A separate RT group was run to compare the maximal repetition (1-RM) evolution in the absence of the tumour. After tumour inoculation induced a decrease in total work performed (initial 6th week). RTTB presented reduced performance by 10% ($p < 0.05$), when compared with RT in the 6th, 7th and 8th weeks of the exercise protocol.

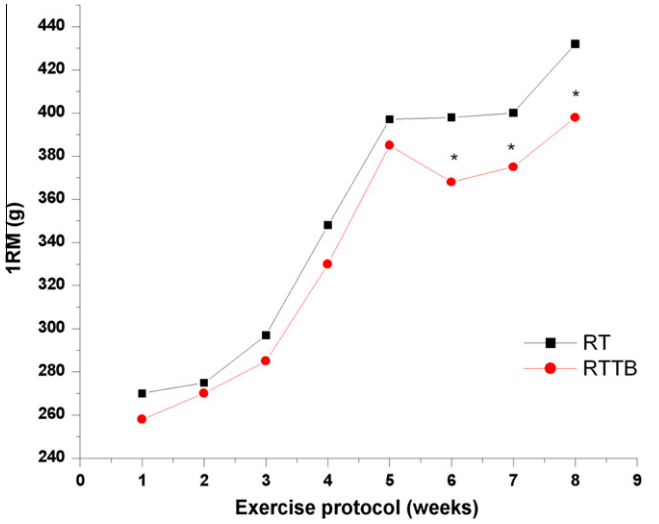

Fig. 1. RM evolution along the exercise protocol. Values are mean ± SD; RM (repetition maximum); RT (resistance training) and RTTB (resistance training tumour-bearing). Values that are significantly different by *test t*. $^{*}p < 0.05$ compared with RT.

4.3. Plasma biochemistry

The analysis of the plasma (Table 2) demonstrates a reduction of glycaemia in RTTB, when compared with CT (21%; $p < 0.006$) and TB (20%; $p < 0.008$). With respect to TAG, TB levels were 29% ($p < 0.02$) higher then CT's and no significant difference was detected regarding RTTB. The Lipoprotein profile presented heterogeneous results: in relation to VLDL-c, there was an increase of 30% ($p < 0.03$) in TB compared with CT. The parameter was not different among RTTB, CT and TB. LDL-c levels were decreased in RTTB by 43% ($p < 0.029$), when compared with TB, while no difference was found for CT. HDL-c levels were increased in RTTB by 15% ($p < 0.049$) compared with CT and 22% ($p < 0.005$), as compared with TB. Creatinine and LDH activity did not show significant variation among the experimental groups. With respect to hormones, testosterone was shown to be 55% ($p < 0.042$) and 63% ($p < 0.03$) higher, in RTTB compared with CT and TB, respectively.

Table 1
Body weight, adipose tissue and gastrocnemius weight.

Experimental groups	CT	TB	RT	RTTB
Initial body weight (g)	209.3 ± 3.2	216.5 ± 4.9	208.6 ± 7.9	211.2 ± 2.8
Final body weight (g)	335.6 ± 3.4	288.3 ± 4.9[a]	348.5 ± 10.9[b]	310.8 ± 9.8[b]
Final tumour weight (g)	–	32 ± 0.8	–	19,6 ± 0.5
Absolute EPAT (g)	3.6 ± 0.3	3.28 ± 0.7	2.96 ± 0.9	3.06 ± 0.3
Absolute MEAT (g)	1.74 ± 0.3	2.19 ± 0.4	1.89 ± 0.7	2.09 ± 0.4
Absolute RPAT (g)	3.16 ± 1.2	2.22 ± 0.2[a]	2.14 ± 0.4[a]	2.02 ± 0.7[a]
Absolute gastrocnemius (g)	2.65 ± 0.3	1.72 ± 0.1[a]	2.75 ± 0.4[ab]	2.30 ± 0.2[b]

Values are mean ± SD; initial and final body mass; absolute weight of the adipose tissues: Epididymal adipose tissue (EPAT), Mesenteric adipose tissue (MEAT) and Retroperitoneal adipose tissue (RPAT); tumour and gastrocnemius. CT (control), TB (tumour-bearing), RT (resistance training) and RTTB (resistance training tumour-bearing). Values that are significantly different by two-way ANOVA are indicated.
[a] $p < 0.05$ compared with control.
[b] $p < 0.05$ compared with TB.

Table 2
Plasma biochemical profile of experimental groups.

Parameters	CT	TB	RT	RTTTB
Glucose (mmol/L)	4.5 ± 0.2	4.6 ± 0.01	4.02 ± 0.2	3.8 ± 0.3[a,b]
Cholesterol (mmol/L)	2.49 ± 0.7	2.61 ± 0.4	2.37 ± 0.03	2.57 ± 0.2
TAG (mmol/L)	1.11 ± 0.07	1.44 ± 0.1[a]	1.03 ± 0.02[a,b]	1.24 ± 0.2
VLDL-c (mmol/L)	0.49 ± 0.03	0.64 ± 0.01[a]	0.46 ± 0.01[b]	0.55 ± 0.13
LDL-c (mmol/L)	0.49 ± 0.6	0.57 ± 0.1	0.24 ± 0.7[a,b]	0.28 ± 0.9[b]
HDL-c (mmol/L)	1.13 ± 0.7	1.07 ± 0.07	1.33 ± 0.13[a,b]	1.31 ± 0.12[a,b]
Creatinine (μmol/L)	18.96 ± 6.8	14.2 ± 3.2	17.85 ± 2.5	16.88 ± 2.9
Testosterone (ng/mL)	254 ± 41.3	221 ± 23.1	734 ± 88.2[a,b]	577 ± 131[a,b]

Values are mean ± SD; serum level: Glucose, Cholesterol total, TAG (triacylglycerol), VLDL-c (Very low-density lipoprotein), LDL-c (Low-density lipoprotein), HDL-c (High-density lipoprotein), Creatinine and testosterone; CT (control), TB (tumour-bearing), RT (resistance training) and RTTB (resistance training tumour-bearing). Values that are significantly different by two-way ANOVA are indicated by two-way ANOVA.
[a] $p < 0.05$ compared with control.
[b] $p < 0.05$ compared with TB.

Table 3
Gastrocnemius analysis.

Parameters	CT	TB	RT	RTTTB
Protein (μg/μL)	4.7 ± 0.1	4.5 ± 0.08	5.9 ± 0.03[b]	5.1 ± 0.3
Glycogen (mg/g)	3.8 ± 0.29	3.4 ± 0.33	5.3 ± 0.45[b]	4.8 ± 0.12[b]
IL-6 (pg/μg)	0.03 ± 0.02	0.08 ± 0.01	0.10 ± 0.01[a]	0.12 ± 0.03[a]
LDH (U/mg)	6.79 ± 0.9	7.03 ± 0.3	9.45 ± 0.9[a,b]	9.35 ± 0.6[a]

Values are mean ± SD; gastrocnemius profile: total protein, glycogen, IL-6 (interleukin-6) and LDH (lactate dehydrogenase); CT (control), TB (tumour-bearing), RT (resistance training) and RTTB (resistance training tumour-bearing). Values that are significantly different by two-way ANOVA are indicated.
[a] $p < 0.05$ compared with control.
[b] $p < 0.05$ compared with TB.

4.4. Muscle analysis

Gastrocnemius muscle protein content (Table 3) showed a statistical difference in RT, as compared with TB. IL-6 in RTTB was 90% higher ($p < 0.002$) when compared with CT and 20% higher ($p < 0.043$) compared with TB. In the gastrocnemius, glycogen content of RT and RTTB showed statistical difference when compared with TB. The activity of LDH in RTTB was increased 27% ($p < 0.044$) in relation to CT and showed no difference in comparison with TB.

4.5. Liver biochemistry

Glycogen content (Table 4) in the liver showed no differences among the experimental groups. Liver TAG content was lower in RT and RTTB, when compared with TB.

4.5.1. Plasma adipocytokine levels

In the Fig. 2, leptin levels were decreased in RTTB compared with CT ($p < 0.003$; 72%) and TB (69%; $p < 0.008$). Adiponectin concentration (Fig. 3) was higher in RT and RTTB (43% and 39%), yet only for RT ($p < 0.001$) statistical difference was found, when comparing with TB.

Table 4
Glycogen and tryacilglycerol content in the liver.

Parameters	CT	TB	RT	RTTTB
Glycogen (mg/g)	2.2 ± 0.02	1.8 ± 0.08	2.6 ± 0.1	2.3 ± 0.2
TAG (mg/ 100 mg liver)	52.9 ± 1.96	104.6 ± 22.11[a,b]	45.5 ± 9.93[b]	70.4 ± 9.01[a,b]

Values are mean ± SD; liver profile: glycogen and TAG (triacylglycerol); CT (control), TB (tumour-bearing), RT (resistance training) and RTTB (resistance training tumour-bearing). Values that are significantly different by two-way ANOVA are indicated.
[a] $p < 0.05$ compared with control.
[b] $p < 0.05$ compared with TB.

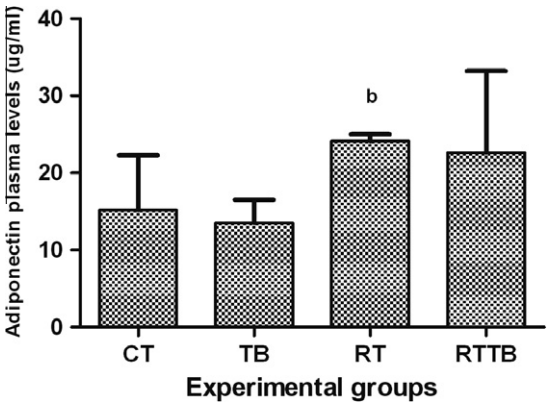

Fig. 3. Adiponectin plasma levels. Values are mean ± SD; CT (control), TB (tumour-bearing), RT (resistance training) and RTTB (resistance training tumour-bearing). Values that are significantly different by two-way ANOVA are indicated. $b = p < 0.05$ compared with TB.

4.6. Citokines in the mesenteric adipose tissue

The local tissue concentration of TNF-α, IL-6 and IL-10 was assessed by enzyme linked immuno sorbent assay (ELISA). TNF-α protein expression (Fig. 4) was higher 39% ($p < 0.04$) in comparison with CT and 46% ($p < 0.035$) higher, compared with RTTB. IL-6 in MEAT was higher in TB-38% ($p < 0.04$), when compared with CT and 50% ($p < 0.03$) when compared with RTTB (Fig. 5). The IL-10 expression (Fig. 6) was increased in TB and RTTB 50% and 47%, respectively when compared with CT. The IL-10/TNF-α ratio (Fig. 7) was higher in RTTB when compared with all other experimental groups

5. Discussion

Cancer cachexia afflicts around 80% of all hospitalised cancer patients and although directly causing up to 40% of all cancer deaths and markedly compromising cancer therapy outcome, has not so far, met a satisfactory treatment. Nutritional intervention has proven to improve some aspects of the syndrome, but still no nutrient or combination of nutrients is efficient in counteracting

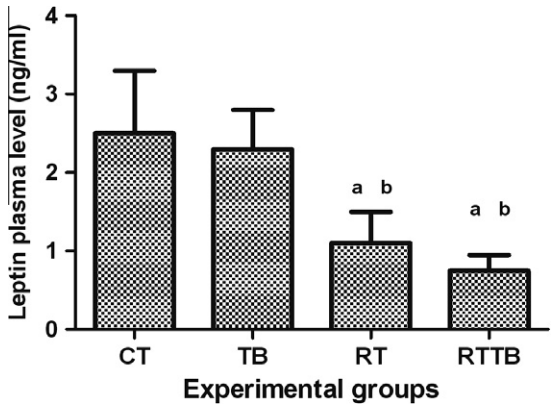

Fig. 2. Leptin plasma levels. Values are mean ± SD; CT (control), TB (tumour-bearing), RT (resistance training) and RTTB (resistance training tumour-bearing). Values that are significantly different by two-way ANOVA are indicated $a = p < 0.05$ compared with control and $b = p < 0.05$ compared with TB.

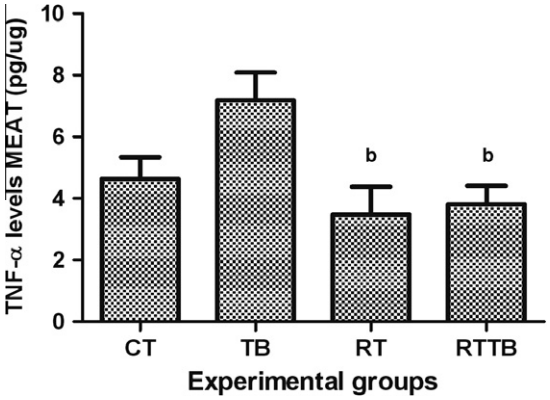

Fig. 4. TNF-α levels in the mesenteric adipose tissue (MEAT). Values are mean ± SD; CT (control), TB (tumour-bearing), RT (resistance training) and RTTB (resistance training tumour-bearing). Values that are significantly different by two-way ANOVA are indicated. $b = p < 0.05$ compared with TB.

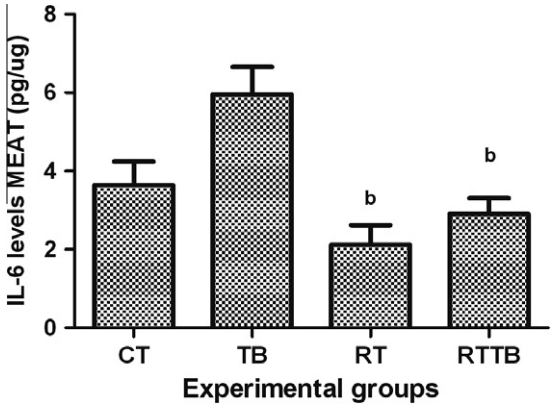

Fig. 5. IL-6 levels in the mesenteric adipose tissue (MEAT). Values are mean ± SD; CT (control), TB (tumour-bearing), RT (resistance training) and RTTB (resistance training tumour-bearing). Values that are significantly different by two-way ANOVA are indicated by two-way ANOVA $b = p < 0.05$ compared with TB.

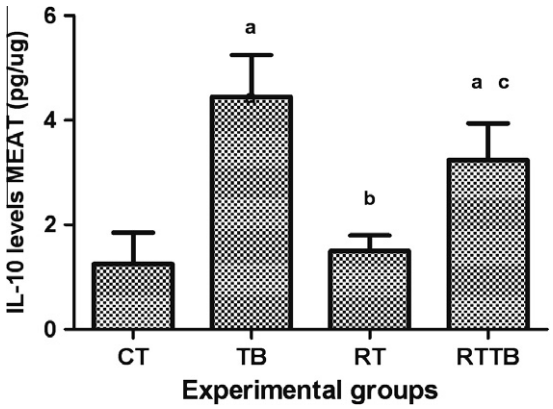

Fig. 6. IL-10 levels in the mesenteric adipose tissue (MEAT). CT (control), TB (tumour-bearing), RT (resistance training) and RTTB (resistance training tumour-bearing). Values that are significantly different by two-way ANOVA are indicated $a = p < 0.05$ compared with control, $b = p < 0.05$ compared with TB and $c = p < 0.05$ compared with RT.

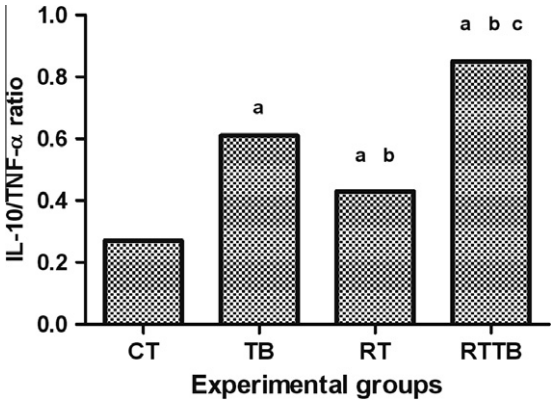

Fig. 7. IL-10/TNF-α ratio in the mesenteric adipose tissue. CT (control), TB (tumour-bearing), RT (resistance training) and RTTB (resistance training tumour-bearing). Values that are significantly different by two-way ANOVA are indicated $a = p < 0.05$ compared with control, $b = p < 0.05$ compared with TB and $c = p < 0.05$ compared with RT.

the plethora of symptoms of cachexia. Pharmacological therapy, on the other hand, may elicit more comprehensive results, yet is associated with deleterious side effects.

We have in the last years [8–11] addressed the potential of chronic physical exercise as a low cost, safe adjuvant therapy in cachexia, which is able to induce cachexia-attenuating effects in a systemic manner, thus affecting every and all compartments in the body. In particular, these previous studies clearly demonstrate the capacity of regular endurance exercise to reduce cachexia-related systemic inflammation. However, some patients may find endurance sessions too long to be performed on a daily basis, especially considering that cancer treatment is *per se* already time-consuming. Bearing this in mind we sought to examine whether a resistance exercise protocol would induce similar results, thus appearing as a less time-consuming, indoor alternative to submaximal intensity, long duration exercise. Resistance exercise has been associated with positive effects on the cardiovascular system, lean body mass and reported to decrease the risk of metabolic disorders [16].

In order to compare the two different exercise strategies, we adopted the same animal model of cachexia employed in our previous studies focusing on endurance exercise, the Walker 256 carcinosarcoma, known to induce most of the symptoms associated with human cachexia [17].

Resistance training exercise is known to promote physiological changes that may serve as markers of its effects, such as increase in muscle lactate dehydrogenase activity (the enzyme that that converts pyruvate to lactate in the glycolytic process [18] and augmented testosterone circulating levels [19]. The adopted resistance protocol was efficient in inducing such changes, as in RTTB increased LDH activity and testosterone levels were found, when compared with TB.

In a recent report, Prestes et al. [20] analysed the effect of RT upon muscle glycogen content in rats subjected to ovariectomy, having demonstrated a glycogen-sparing effect in the muscle. In our study model, a reduction in total muscle weight and in the amount of glycogen and the gastrocnemius was found for TB, but not for RTTB, pointing out to a positive effect of resistance exercise against sarcopenia and muscle glycogen depletion associated with cachexia.

Plasma lipid profile was also examined, because cachexia and resistance exercise exert opposite effects on these parameters. Hyperlipidaemia is a hallmark of the paraneoplastic syndrome, and serves as a means for its diagnosis [21]. This symptom is a reflex from the combination of diminished lipoprotein lipase (LPL) activity in white adipose tissue and muscle of tumour-bearing animals [22] increased free fatty acid release from adipose depots [23] and impaired hepatic lipoprotein metabolism [24]. Resistance exercise exerts, on another hand, opposite effects, decreasing circulating TAG and modulating lipoprotein metabolism [25].

We demonstrated that plasma glucose, TAG, HDL-c, LDL-c and VLDL-c were affected by 8 week of resistance training in tumour-bearing animals, who showed an improved lipid profile, as compared with the sedentary counterparts (TBs). Additionally, the protocol was able to reduce cachexia-related steatosis-liver lipid content was decreased in RT and RTTB, by 58% and 33%, respectively, in comparison with the control groups. In a recent study adopting the same resistance exercise protocol, Domingos et al. [26] demonstrated a down regulation of hepatic genes involved in the development of steatosis in rats submitted to ovariectomy.

The white adipose tissue is markedly affected by cachexia, but in addition, may contribute consistently to systemic inflammation [27]. Indeed this tissue is currently envisaged as a major endocrine organ, expressing a variety of pro and anti-inflammatory factors and hormones.

Amongst the latter, leptin communicates the status of energy reserves of the organism to the brain, thereby regulating feeding, substrate utilization, energy balance, and the endocrine, immune systems [28]. In advanced cancer patients, serum leptin

concentrations may be depressed as a consequence of reduced body fat mass [29] and rodents display the same symptom [3]. We report a marked decrease in the circulating levels of leptin as a consequence of the exercise protocol, ($p < 0.003$; 72% when compared with CT) and (69%; $p < 0.008$, in relation to TB), however this effect bears no direct relationship with diminished adipose tissue mass, as we found no difference among the experimental groups. Unfortunately, we did not measure this hormone protein expression in the adipose pads. Exercise elicits controversial effects on leptin secretion, depending on exercise type and duration [30].

We report for the first time, that resistance exercise has an anti-inflammatory effect upon the adipose tissue in cachexia, in a manner that does not greatly differ from that of endurance exercise. Our previous studies show that the anti-inflammatory effect of exercise is, as expected, more evident under the presence of pathological conditions, such as heart failure [31], cancer cachexia [8–11,32] and malnourishment [33]. Similarly, we found a more pronounced exercise-related shift towards an anti-inflammatory profile in the tumour-bearing animals.

TNF-α, a potent inflammatory cytokine, presents greatly increased concentration during cachexia and in addition to modulating adipose tissue lipolysis in this setting, is actively secreted by the white adipose tissue [3,34]. Therefore, we chose to examine adipose tissue protein expression of the pro-inflammatory cytokine TNF-α and of the anti-inflammatory cytokine IL-10, since plasma concentration of these cytokines is known to change under the stimulus of both physical exercise [35,36] and cachexia [37] and it is well established that the adipose tissue is capable of synthesising these factors [38], hence very likely contributing to circulating levels. Our studies with endurance exercise have shown a potent anti-inflammatory effect of physical activity [8–11]. We here in report a consistent change in the ratio between TNF-α and IL-10, indicating a clear shift, induced by resistance exercise training, favouring an anti-inflammatory milieu in the mesenteric pad. This ratio has been adopted by us [9] and others [37] as a means of assessing the degree of inflammation.

Another evidence corroborating the assumption that resistance exercise training has an anti-inflammatory effect is the fact that skeletal muscle IL-6 expression was increased by the protocol. Pedersen [39] showed that regular exercise modulates anti-inflammatory myokine expression. IL-6 has a negative feedback role in which it acts in the down regulation of cytokines stimulating inflammation at tissue level (e.g. inducing suppression of TNF-α expression) and thereby offering protection against exacerbation of inflammation. After 8 weeks of resistance training we found an increased IL-6 protein expression in the gastrocnemius of RTTB when compared with TB. When assessing IL-6 and TNF-α protein expression in the MEAT, the results were the opposite, with TB showing higher protein expression than RTTB in MEAT pad. Adipose tissue-derived IL-6, contrary to that secreted by the muscle, exerts a pro-inflammatory action [40].

Cachexia associated with cancer exhibits different effects on lipid metabolism in regard to the anatomical localisation of the fat depot [41]. These changes in lipid metabolism are associated with increased inflammation in the fat depots. Rosa Neto and colleagues [42] demonstrated that acute exhaustive exercise increases IL-6 protein content to a broader extent in RPAT as compared with mesenteric white adipose tissue (MEAT). These results add to the concept that different fat pads present a heterogeneous response to the same stimulus. In fact, as previously reported, RPAT hormone-sensitive lipase activity increases with exercise, while no effect was observed in MEAT [43]. In the present study, we show that MEAT is the main depot on which the effect of resistance exercise training is present.

Adiponectin is a cytokine produced and secreted exclusively by the adipose tissue. Decreased adiponectin levels are associated with the induction of metabolic syndrome, insulin resistance, and increased plasma lipid levels and circulating pro-inflammatory cytokines such as TNF-α [44–48]. In resistance exercise training positively modulated the concentration of plasma adiponectin.

Finally, the protocol was able to prevent cachexia-related steatosis. There is growing evidence that resistance training stimulates lipid oxidation in the liver and in the adipose tissue and it is tempting to speculate that resistance training could induce a decrease in the expression of transcription factors related to lipogenesis, improving liver fat oxidation and consequently reducing liver lipid content [49].

Taken together, the results reflect a potent effect of resistance exercise training in the prevention of systemic consequences of cancer cachexia, with a conspicuous down modulation of chronic inflammation, in a fashion that is similar to that induced by endurance exercise. Therefore we may suggest that this may be an advantageous low cost, low risk, time sparing strategy in the adjuvant treatment of the syndrome. This becomes of particular interest when the classification of cachexia into pre-cachexia, cachexia and refractory cachexia [50] is considered, since we might assume that patients presenting the early stages of the disease may benefit from engaging into a regular resistance exercise programme. It is also possible to suggest that the reported quality of life improvement in cancer patients who perform resistance exercise may be associated with its capacity to counteract systemic inflammation as shown by this study.

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