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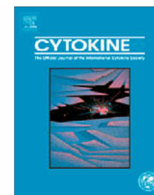

Endurance training induces depot-specific changes in IL-10/TNF-α ratio in rat adipose tissue

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ABSTRACT

White adipose tissue (WAT) is the source of pro- and anti-inflammatory cytokines and recently, it has been recognized as an important source of interleukin 10 (IL-10). Acute physical exercise is known to induce an anti-inflammatory cytokine profile, however, the effect of chronic physical exercise on the production of IL-10 by WAT has never been examined. We assessed IL-10 and TNF-α concentration in WAT of rats engaged in endurance training. Animals were randomly assigned to either a sedentary control group (S, $n = 7$) or an endurance trained group (T, $n = 8$). Trained rats ran on a treadmill 5 days/wk for 8 wk (55–65% VO_{2max}). Detection of IL-10 and TNF-α protein and mRNA expression, as well as the gene expression of PPAR-γ, and immunocytochemistry to detect mononuclear phagocytes were carried out. A reduction in absolute retroperitoneal adipose tissue (RPAT) weight in T (44%; $p < 0.01$), when compared with S was observed. IL-10 concentration was increased (1.5-fold, $p < 0.05$), to a higher extent than that of TNF-α (66%, $p < 0.05$) in the mesenteric adipose tissue (MEAT) of the trained group, while no change related to training was observed in RPAT. In MEAT, IL-10/TNF-α ratio was increased in T, when compared with S (30%; $p < 0.05$). PPAR-γ gene expression was increased in T (1.1-fold; $p < 0.01$), when compared with S in the same adipose depot. No monocyte infiltration was found. In conclusion, exercise training induced increased IL-10 expression in the mesenteric depot, resulting in a modified IL-10/TNF-α ratio. We also conclude that WAT presents a depot-specific response to endurance training regarding the studied aspects.

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1. Introduction

The current view of white adipose tissue (WAT) function considers its secretory properties in addition to the storage of lipids [1]. WAT actively secretes various bioactive peptides, termed "adipokines", which act locally and distally, with autocrine, paracrine and endocrine effects [2]. Nevertheless, the physiology, metabolism and function of WAT vary in a depot-specific manner, as reviewed by [3]. Therefore, marked differences in gene expression amongst depots are reported both for rodents [4] and humans [5], and cytokine secretion is also heterogeneous [3]. The anti-inflammatory interleukin 10 (IL-10), for instance, secreted by human subcutaneous and visceral adipose depots, is more expressed in the latter [6], while the secretion of tumor necrosis factor α (TNF-α), an important pro-inflammatory cytokine, with a major role in the regulation of cellular processes [7], is secreted in a similar way by the human subcutaneous and visceral adipose depots [8]. TNF-α is the most-studied cytokine in WAT, with the greatest mRNA expression being found in the adipocyte *per se* [9]. This cytokine is involved in metabolic, physiological and immunological regulation in this tissue, and plays a pivotal role in relation to the production of several cytokines (e.g. IL-10) and many other adipokines in WAT, such as leptin [10,11].

IL-10, secreted by adipocytes, stromal vascular fraction and tissue matrix of WAT [12], acts as a natural antagonist to TNF-α, inhibiting nuclear factor kappa B (NF-κB) signaling through the preservation of inhibitory factor kappa B (IκB) [13]. Thus, due to its anti-inflammatory potential, the therapeutic use of IL-10 has also been tested in many chronic inflammatory diseases in which TNF-α is believed to play a prominent role (e.g. rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease and cardiac allograft rejection) [14]. Interleukin 6 (IL-6-a cytokine related both to acute phase reaction and anti-inflammation) secretion [6], is also heterogeneous, being higher in the visceral depots. IL-6, together with TNF-α, have been the focus of most of the research related to cytokine production by the adipose tissue, as it also potentially appears as an important molecule in the communication between WAT and the hypothalamus, associated with the regulation of energy balance [15], in concomitance to leptin. The

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expression of interleukin 1β (IL1-β, another important pro-inflammatory product of WAT) also seems to present depot variation [16].

Several studies [17–19] have shown an anti-inflammatory effect of acute physical exercise, characterized through increased circulating concentrations of IL-10, IL-1 receptor antagonist (IL-1ra), soluble receptor of TNF (TNFRs), among other alterations. Fewer studies addressed the effect of chronic exercise on the same parameters with respect to the contribution of WAT, and no study, to our knowledge, has compared cytokine production in different WAT depots in trained healthy rats.

Therefore, considering the important role of WAT in the synthesis and secretion of cytokines, and the well established capacity of moderate intensity exercise to induce an anti-inflammatory response, we have sought to examine the effects of endurance training upon the expression of cytokines in rodent retroperitoneal and mesenteric depots. The results show that IL-10/TNF-α ratio in WAT, which has been pointed out as an indicator of inflammatory status [20,21] presents a depot-specific response to endurance training.

2. Materials and methods

2.1. Animals

A total of 15 male Wistar rats weighing ~250 g, obtained from the Animal Breeding Unit, Institute of Biomedical Sciences, University of São Paulo, were used. They were housed, five per cage, receiving food and water *ad libitum*, in an animal room under 12 h light–dark cycle, at 22 ± 1 °C and 60 ± 5% humidity. The experiments were carried out after acclimation for a wk.

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 (protocol no. 041/2005).

2.2. Experimental design

After 2 wk (this being the necessary interval to induce adaptation to treadmill running), animals were randomly assigned to either a sedentary (S, $n = 7$) or trained (T, $n = 8$) group.

2.3. Endurance training program

Rats in the training group ran 5 days/wk for 8 wk at a work rate that ranged between 55–65% VO_{2max}. VO_{2max} was determined for each rat at the end of 2 wk of training, and work loads were adjusted upward according to the increase found in VO_{2max}. On the first day of training, all rats ran for 30 min. On the subsequent days of training running time was extended 10 min each day until all rats were running 60 min/day^{-1}. Familiarization with the treadmill was maintained also in the sedentary groups by having each rat run on the treadmill (0% grade) 10 min/day, 2 days/wk at a speed of 15 m/min. After a rest period of 24 h after the last workout session, the animals were killed by decapitation without anesthesia.

2.4. Determination of citrate synthase maximal activity

Citrate synthase activity, an index of oxidative capacity, was determined in the soleus muscle of each rat. Tissue samples were homogenized 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. CS activity was measured according to the spectrophotometric method described previously in [22]. All assays were linear with respect to time and dilution, and each sample was analyzed in duplicate.

2.5. Immunoassays for cytokines

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 homogenized 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 μg/ml of protease inhibitor cocktail (Sigma–Aldrich, St. Louis, Missouri). Homogenates were centrifuged at 12,000*g* 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. Quantitative assessment of TNF-α, IL-1β, IL-6 and IL-10 proteins was carried out by ELISA (DuoSet ELISA, R&D Systems, Minneapolis, MN). For TNF-α (DY510), IL-1β (DY501), IL-6 (DY506) and IL-10 (DY522) assay sensitivity was found to be 5.0 pg/ml in the range of 31.2–2000 pg/ml. The intra- and inter-assay variability of the TNF-α, IL-1β and IL-6 kits were 2.7–5.2%, and 4.9–9.5%, respectively. Assay sensitivity for IL-10 was 10 pg/ml in the range from 31.2 to 2000 pg/ml. The intra-assay variability of the IL-10 kit was 2.0–4.2%, and its inter-assay variability was of 3.3–6.4%. All samples were run as duplicates and the mean value was reported.

2.6. Analysis of gene expression

Total RNA was obtained from aliquots of 100 mg of rat adipose tissue (RPAT and MEAT) with TRIZOL® (Invitrogen, Carlsbad, CA), following the manufacturer's recommendations. RNA concentration was determined spectrophotometrically (Beckman DU 640, Fullerton, CA, USA). A semi-quantitative reverse transcriptase/polymerase chain reaction (RT-PCR) method was used for the estimation of the concentration of TNF-α, IL-1β, IL-6, IL-10 and PPAR-γ mRNA. A 33 μl assay mix containing 3 μg RNA, 10 U placental RNAse inhibitor, 2 μl oligo(dt), 2 μl dNTP (10 nmol), 2 μl dithiothreitole, 10 U Moloney-murine leukaemia virus reverse transcriptase (Invitrogen, USA), and 4 μl 10× reaction buffer (100 mM TRIS–HCl pH 8.3, 500 mM KCl, 150 mM MgCl2 in nuclease-free water) were used to produce cDNA. The RT-mixture was incubated at 80 °C for 3 min, followed by 21 °C for 10 min, 42 °C for 30 min and then 99 °C for 10 min. The obtained product (2 μl) was fractionated in 1% agarose and ethidium bromide gel to assess the quality of the reaction. The primers were designed with regard to the published GeneBank sequences:

TNF-α (NM 012675.2 sense: 5′TCTCAAAACTCGAGTGACAAGC3′; antisense: 5′GGTTGTCTTTGAGATCCATGC3′),
IL-1β (NM 012589.1 sense: 5′GAGTCACAGAAGGAGTGGCTAA3′; antisense: 5′ACAGTGAGGAATGTCCACAAAC3′),
IL-6 (NM 012895.1 sense: 5′ATCTGCTCTGGTCTTCTGGA3′; antisense: 5′TGACCACAGTGAGGAATGTC3′),
IL-10 (NM 012854.1 sense: 5′GACCCATGAGAGTCTTCACAAC3′; antisense: 5′GATCTTAGCTAACGGGAGCAAC3′) and
PPAR-γ (NM 199267.2 sense: 5′GATCCTCCTGTTGACCCAGA3′; antisense: 5′TCAAAGGAATGGGAGTGGTC3′).

cDNA amplification was carried out in 37 cycles for TNF-α, IL-1β and IL-6, 38 cycles for IL-10 and 34 cycles for PPAR-γ, consisting of 45 s at 94 °C, 60 s at 60 °C (TNF-α, IL-1β and IL-6), 58 °C (IL-10) and 54 °C (PPAR-γ) and 60 s at 72 °C. The RPL19 gene was used as the internal control. Each PCR mixture contained 2 μl of cDNA, 0.5 U AmpliTaq Gold Polymerase (Perkin-Elmer, Foster City, CA, USA), 2.5 nmol each dNTP, and 1.0 μM of the primers in reaction buffer

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(10 mM TRIS–HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$). The PCR mixture (2 µl) was fractionated in polyacrylamide gel (agarose 1.0% and ethidium bromide). Semiquantitative analysis was carried out with the program Image QuaNT TM.

2.7. Immunocytochemistry

The different adipose tissue depots were removed, washed with 0.9% NaCl at 37 °C, minced with scissors and fixed for 4 h in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, at 4 °C. After washing (30 min with tap water) the samples were dehydrated with increasing concentration of ethanol (2 × 70%, 1 × 95%, 2 × 100%), incubated with xylol and infiltrated into Paraplast (EMS). After embedding, sections of 5 µm were obtained (microtome R. Jung, Heidelberg) and placed onto slides previously covered with polylysine (Sigma). Sections were submitted to successive xylol baths (3 × 1 h, 3 × 30 min, 3 × 20 min), dehydrated with decreasing concentration ethanol solutions and washed with distilled water. Endogenous peroxidase was blocked with 20 vol. hydrogen peroxide: methanol (1:1). After washing with water and phosphate buffer, the unspecific antigenic sites were blocked with 1% albumin in 0.1 M phosphate buffer/0.2% Triton X-100 (1:10). Samples were incubated with monoclonal primary antibody anti-rat RT1B (BD, Pharmigen) (1:50 in phosphate buffer/Triton X-100), or anti-rat mononuclear phagocyte (BD Pharmigen) (1:500 in the same buffer) for 16 h, at 4 °C, followed by incubation (2 h) with the secondary antibody (horse anti-mouse, Jackson Immunolab) diluted 1:200 in the same buffer. After washing with phosphate buffer, the samples were incubated with the biotin–anhydrous peroxidase tertiary complex (Kit ABC/Elite Vectastain, Vector) for 2 h at ambient temperature. The sections were finally incubated with diaminobenzidine (Sigma), and the slides mounted in glycerol carbonate for image obtainment (Olympus BX40 light microscope connected to a 3CCD camera (MTI). Fifteen sections of each adipose tissue depot of three animals of each experimental group (S and T) were studied.

2.8. Statistics analysis

Statistical analysis was performed by using a commercial statistical package from SigmaStat (version 3.1, SigmaStat, SYSTAT, Point Richmond, CA). Data are expressed as means ± SD. A primary observation showed that the results of experiments were distributed normally. After that, data (S × T) were compared using Student's T-test. The level of significance of at least $p < 0.05$ was chosen for all statistical comparisons.

3. Results

3.1. Body, tissue weight, and citrate synthase activity

Table 1 illustrates the values of body weight before and after the training period, as well as absolute retroperitoneal adipose tissue (RPAT) and mesenteric adipose tissue (MEAT) weight, and citrate synthase maximal activity in the soleus muscle. Exercise training attenuated body weight gain (46%; $p < 0.004$), when compared with the sedentary group (S). Absolute RPAT weight was decreased in T (44%; $p < 0.02$), in comparison with S (Table 1). On the other hand, absolute MEAT weight was not different between groups (Table 1). Exercise trained rats demonstrated enhanced maximal activity of citrate synthase (1.2-fold; $p < 0.003$), when compared with S (Table 1).

3.2. Local expression of cytokines

The local expression of IL-10 and TNF-α was assessed by Enzyme Linked Immuno Sorbent Assay (ELISA) and Semi-Quantitative RT-PCR. MEAT IL-10 concentration was higher (1.5-fold, $p < 0.04$) in S when compared with T (Table 3), although no effect of training on IL-10 expression was detected. The training protocol was unable to modify IL-10 mRNA expression or concentration in RPAT (Table 2). In MEAT training increased IL-10 protein, but not gene expression, while PPAR-γ mRNA expression in this depot was increased in T (80.1%; $p < 0.04$), when compared with S (Table 3).

Protein expression of TNF-α in MEAT was 66% higher (0.089 ± 0.006 vs. 0.148 ± 0.014 pg/µg protein, $P < 0.02$) in S when compared with T (Table 3), with no differences being found in RPAT (Table 2). MEAT and RPAT TNF-α gene expression in T (Table 2 and Table 3) showed no (undetectable) significant differences. PPAR-γ mRNA expression in RPAT was not modified in T (Table 2).

We evaluated IL-10/TNF-α ratio in both adipose depots of S and T. In MEAT, this ratio was increased in T, when compared with S (30%; $p < 0.02$) (Table 3). However, in RPAT, no differences were observed between the groups (Table 2). Table 2 and Table 3 show the relative contribution of each cytokine to the ratio, demonstrating a more prominent increase of IL-10 when compared with TNF-α in MEAT, as induced by the endurance training protocol. Interleukin

Table 1
General parameters.

	Sedentary	Trained	p
Initial body weight (g)	151 ± 0.30	149 ± 0.28	0.945
Final body weight (g)	382.0 ± 17.90	261.4 ± 12.24*	0.004
Absolute RPAT weight (mg)	2.56 ± 0.12	1.77 ± 0.13*	0.024
Absolute MEAT weight (mg)	0.92 ± 0.06	0.97 ± 0.06	0.578
Citrate synthase maximal activity			
In soleus muscle (nmol.min. protein)	183.4 ± 26.17	407.6 ± 28.85*	0.003

Results are expressed as mean value ± SEM.
* Significantly different from control values.

Table 2
Cytokine (IL-1β, TNF-α and IL-10) mRNA and protein expression in RPAT.

	Sedentary	Trained	p
Gene expression (Arbitrary units)			
TNF-α	0.34 ± 0.09	ND*	0.001
IL-1β	0.78 ± 0.19	0.27 ± 0.06	0.062
IL-10	1.34 ± 0.16	0.89 ± 0.18	0.101
PPAR-γ	1.48 ± 0.19	1.01 ± 0.11	0.127
Protein expression (pg. ug protein^{-1})			
TNF- α	1.79 ± 0.45	3.83 ± 0.71	0.140
IL-1β	2.58 ± 0.61	4.29 ± 1.03	0.235
IL-10	1.40 ± 0.40	2.93 ± 0.36	0.129
IL-10/TNF- α ratio	0.75 ± 0.04	0.80 ± 0.06	0.633

Results are expressed as mean value ± SEM. ND is not detectable.
* Significantly different from control values.

Table 3
Cytokine (IL-1β, TNF-α and IL-10) mRNA and protein expression in MEAT.

	Sedentary	Trained	p
Gene expression (Arbitrary units)			
TNF-α	ND	ND	
IL-1β	0.54 ± 0.09	0.69 ± 0.29	0.321
IL-10	0.85 ± 0.22	0.70 ± 0.21	0.524
PPAR-γ	0.65 ± 0.04	1.36 ± 0.35*	0.048
Protein expression (pg.ug protein^{-1})			
TNF-α	0.089 ± 0.006	0.148 ± 0.014*	0.023
IL-1β	0.668 ± 0.026	1.454 ± 0.180*	0.013
IL-10	0.190 ± 0.013	0.485 ± 0.088*	0.048
IL-10/TNF- α ratio	1.97 ± 0.124	2.55 ± 0.146*	0.022

Results are expressed as mean value ± SEM. ND is not detectable.
* Significantly different from control values.

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1β protein expression was found to be increased in MEAT of T ($p < 0.013$), in comparison with S, while its mRNA expression was unaffected by training in the studied depots. We were unable to detect Interleukin 6 gene or protein expression in the present model.

We found marked differences regarding protein expression of TNF-α ($p < 0.002$), IL-1β ($p < 0.008$), IL-10 ($p < 0.01$) and IL-10/TNF-α ratio ($p < 0.001$) and PPAR-γ mRNA expression ($p < 0.001$) between the two different fat pads in sedentary animals, reinforcing the concept of functional heterogeneity of the adipose tissue.

In order to detect the presence of infiltrating mononuclear phagocytes which could contribute to the secretion of the studies cytokines, immunocytochemistry was performed (Fig. 1). The results show the absence of noteworthy macrophage infiltration (MHCII labeling is not different among the studied groups).

4. Discussion

The effects of exercise training on cytokine expression in different rat adipose tissue depots (mesenteric and retroperitoneal, the two most central pads in the abdominal cavity) were studied. It is well known that intra-abdominal adiposity plays a fundamental role by secreting cytokines in many inflammatory conditions [6], yet little is known about cytokine synthesis profile under physiological conditions and how endurance training modifies one such profile. We chose to examine the protein and gene expression of TNF-α, IL-1β, IL-6, and IL-10, since plasma concentration of these cytokines is known to change under the stimulus of physical exercise [23], and it is well established that the adipose tissue is capable of synthesizing these factors [24].

We demonstrate that IL-10 and TNF-α concentration in WAT is affected by 8 wk of endurance training, the principal effect being a marked increase of IL-10 in the mesenteric depot. The changes comprise increased IL-10/TNF-α ratio, as well as enhanced gene expression of PPAR-γ in this fat pad. The IL-10/TNF-α ratio has been adopted as an indicator of the inflammatory status and disease-associated morbidity, with lower values being related to poorer prognosis [20,21]. We demonstrate that even in healthy animals, exercise is able to improve this parameter.

The training protocol was thus efficient in inducing increased IL-10 expression in MEAT, but not RPAT, and therefore a depot-specific response was found. It is interesting to note that recently, even differences between fat cells of the same adipose depot are reported [25], with at least two heterogeneous population of cells

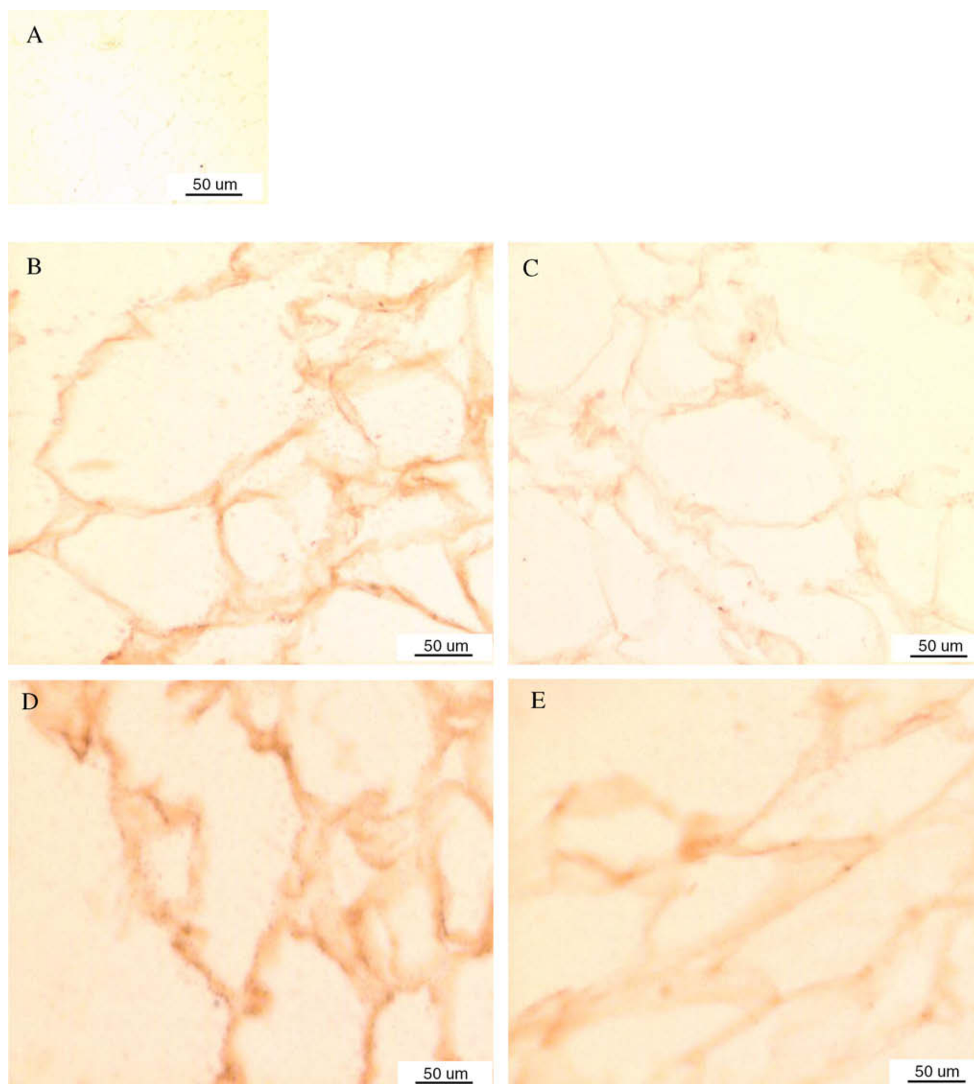

Fig. 1. Immunocytochemistry for RT1B (indicating the presence of MHCII). Negative control (A), RPAT of sedentary rat (B), RPAT of trained rat (C), MEAT of sedentary rat (D) and MEAT of trained rat (E).

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regarding expression of various genes and proteins in one single pad having been found. The present results contribute to the concept that each different fat pad should be studied separately, as it seems clear that there is great heterogeneity of response linked to the anatomical position of the tissue.

The increase of IL-10 protein expression in MEAT was not, however, accompanied by a similar augment in mRNA expression. It is known that IL-10 production is regulated by other transcriptional regulatory elements such as enhancers and/or post-transcriptional control mechanisms, a possibility that has been put foward after the finding that a T cell clone that was actively transcribing IL-10 did not yield detectable mature IL-10 mRNA [26]. Indeed, a combination of ubiquitous transcriptional and post-transcriptional mechanisms for IL-10 expression might be critical to the role of this cytokine [27], and may this way at least partially explain the absence of detectable changes in IL-10 gene expression at the time point analyzed.

Moreover, the alteration of IL-10 and TNF-α expression was not a result of mononuclear cell infiltration in WAT, a possibility which we examined by immunohistochemistry, as in a previous study, we found infiltrating cells in the adipose tissue of cachectic rats [28]. Thus the cytokine source is either adipocytes *per se*, or the stromal fraction of the tissue. It has been demonstrated [6] that mature human adipocytes are capable of secreting IL-10, although we have not investigated the specific contribution of each compartment of adipose tissue to the production of this cytokines.

There is no consensus in the literature about the effect of exercise training upon TNF-α production, as some studies show increase [29] decrease [30] or no change [31,32] of this parameter. Gomez-Merino et al. [32] demonstrated down-regulation in the rat visceral adipose tissue (retroperitoneal) of IL-1ra, IL-1β, and IL-12, with no changes in TNF-α and IL-10 tissue concentration after 7 wk of high intensity endurance training (treadmill running). In the mentioned study, decreased body weight gain and fat mass reduction were also found, results which are in agreement with those of our study, as no change in RPAT TNF-α concentration could be found, but we report reduced body weight gain and depot mass.

TNF-α expression was, nevertheless, enhanced in the mesenteric depot of T. This change in protein concentration was, similarly to what was found for IL-10, not accompanied by any detectable changes in TNF-α mRNA. The magnitude of one such increase in expression was inferior to that found for IL-10, causing a shift of the IL-10/TNF-α ratio towards a predominantly anti-inflammatory *milieu* as a response to training. TNF-α concentration increases in WAT (mesenteric and subcutaneous) have also been demonstrated to be induced by high and moderate intensity treadmill running training in Wistar rats, corroborating our results [29,30]. The present measurements were carried out 24 h after the last bout of exercise, an interval which we may consider sufficient to ensure that any changes measured are due to an effect of chronic training, and not to the actual exercise session [32]. In addition, it is known that the changes in immune system function following exercise normally return to pre-exercise values within 3–24 h [33].

IL-6 expression (protein and mRNA) could not be detected in the present study. Although typically IL-6 is the first cytokine to respond to exercise [17], it derives predominantly from the skeletal muscle. Gomez-Merino et al. [32] reported absence of difference in IL-6 concentration in RPAT between sedentary and trained animals, and in obese women, long-term exercise was also unable to affect IL-6 gene expression in the adipose tissue [31]. Gleeson [33] also points out to the lack of substantial increases in circulating IL-6 in light to medium-intensity exercise of non-prolonged nature.

No significant differences were found for IL-1β regarding the effect of exercise on RPAT (although a trend towards reduced gene expression was found). Gomez-Merino et al. [32] demonstrated down-regulation of rat retroperitoneal adipose tissue IL-1β concentration after 7 wk of high intensity endurance training. We attribute our different results to exercise intensity variation between the training protocols. Nevertheless, increased concentration of this cytokine was found in MEAT. To our knowledge this is the first report showing one such effect of endurance training.

Exercise training for 8 wk induced an increase in PPAR-γ mRNA in the mesenteric adipose tissue from trained rats. This nuclear factor is known to be related to anti-inflammatory actions [9]. Petridou et al. [34] demonstrated a significant increase of DNA binding activity of PPAR-γ in response to 8 wk of voluntary wheel running in the epididymal and subcutaneous fat depots, but found no changes in the protein expression. Despite the herein reported change in PPAR-γ gene expression, we have not investigated whether activation of this factor was induced by exercise. Activation seems to be more economical and flexible than induction and thus, may be the first strategy of the cell as a means of triggering any signal transduction pathway. It seems plausible, therefore, to speculate that if there is increased PPAR-γ expression, it is probably accompanied by enhanced activation. Additional studies should be carried out in order to test this hypothesis.

Taken together, our results suggest that the increase in IL-10 concentration and augmented PPAR-γ expression induced by exercise training in MEAT may lead to a shift towards decreased inflammation. Thus, in particular pathological conditions in which chronic infammation is present, moderate intensity exercise training could be adopted as a parallel therapeutical strategy, leading to lower IL-10/TNF-α ratio and improved prognosis. Previous studies by our group have shown that the anti-inflammatory effect of exercise seems to be, as expected, more evident under the presence of pathological conditions, such as heart failure [35], cancer cachexia [36,37] and malnourishment [38].

Finally, we also conclude that the mesenteric depot seems to be more responsive to moderate intensity exercise training than the retroperitoneal pad, and that heterogeneity of response is present also in healthy animals.

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References

[1] Cinti S. The adipose organ. Prostaglandins Leukot Essent Fatty Acids 2005;73:9–15.
[2] Pond C. Physiological specialisation of adipose tissue. Prog Lipid Res 1999;38:225–48.
[3] Lafontan M, Berlan M. Do regional differences in adipocyte biology provide new pathophysiological insights? Trends Pharmacol Sci 2003;24:276–83.
[4] Gesta S, Bluher M, Yamamoto Y, Norris AW, Berndt J, Kralisch S, et al. Evidence for a role of developmental genes in the origin of obesity and body fat distribution. Proc Natl Acad Sci USA 2006;103:6676–81.
[5] Vohl M-C, Sladek R, Robitaille J, Gurd S, Marceau P, Richard D, et al. A survey of genes differentially expressed in subcutaneous and visceral adipose tissue in men[ast][ast]. Obesity 2004;12:1217–22.
[6] Juge-Aubry C, Henrichot E, Meier C. Adipose tissue: a regulator of inflammation. Best Pract Res Clin Endocrinol Metab 2005;19:547–66.
[7] Cawthorn WP, Sethi JK. TNF-[α] and adipocyte biology. FEBS Letters 2008;582:117–31.
[8] Dusserrea E, Moulinb P, Vidal H. Differences in mRNA expression of the proteins secreted by the adipocytes in human subcutaneous and visceral adipose tissues. Biochim Biophys Acta 2000;1500:88–96.
[9] Montague CT. Adipose depot-specific effects of PPAR-γ agonists: a consequence of differential expression of PPAR-γ in adipose tissue depots? Diabetes Obes Metab 2002;4:356–61.
[10] Coppack S. Pro-inflammatory cytokines and adipose tissue. Proc Nutr Soc 2001;60:349–56.
[11] Trayhurn P, Wood IS. Signalling role of adipose tissue: adipokines and inflammation in obesity. Biochem Soc Trans 2005;33:1078–81.
[12] Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes

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from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology 2004;145:2273–82.

[13] Schottelius AJG, Mayo MW, Sartor RB, Baldwin Jr AS. Interleukin-10 signaling blocks inhibitor of κB kinase activity and nuclear factor κB DNA binding. J Biol Chem 1999;274:31868–74.

[14] Pretolani M. Interleukin-10: an anti-inflammatory cytokine with therapeutic potential. Clin Exp Allergy 1999;29:1164–71.

[15] Trayhurn P, Wood I. Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br J Nutr 2004;92:347–55.

[16] Juge-Aubry CE, Somm E, Giusti V, Pernin A, Chicheportiche R, Verdumo C, et al. Adipose tissue is a major source of interleukin-1 receptor antagonist: upregulation in obesity and inflammation. Diabetes 2003;52:1104–10.

[17] Petersen AMW, Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol 2005;98:1154–62.

[18] Pedersen BK. State of the art reviews: health benefits related to exercise in patients with chronic low-grade systemic inflammation. Am J Lifestyle Med 2007;1:289–98.

[19] Flynn MG, McFarlin BK, Markofski MM. State of the art reviews: the anti-Inflammatory actions of exercise training. Am J Lifestyle Med 2007;1:220–35.

[20] Kaur K, Sharma AK, Dhingra S, Singal PK. Interplay of TNF-α and IL-10 in regulating oxidative stress in isolated adult cardiac myocytes. J Mol Cell Cardiol 2006;41:1023–30.

[21] Leonidou L, Mouzaki A, Michalaki M, DeLastic AL, Kyriazopoulou V, Bassaris HP, et al. Cytokine production and hospital mortality in patients with sepsis-induced stress hyperglycemia. J Infect 2007;55:340–6.

[22] Batista Jr ML, Santos RVT, Lopes RD, Lopes AC, Costa Rosa LFBP, Seelaender MCL. Endurance training modulates lymphocyte function in rats with post-MI CHF. Med Sci Sport Exerc 2008;40:549–56.

[23] Peeling P, Dawson B, Goodman C, Landers G, Trinder D. Athletic induced iron deficiency: new insights into the role of inflammation, cytokines and hormones. Eur J Appl Physiol 2008;103:381–91.

[24] You T, Yang R, Lyles MF, Gong D, Nicklas BJ. Abdominal adipose tissue cytokine gene expression: relationship to obesity and metabolic risk factors. Am J Physiol Endocrinol Metab 2005;288:E741–747.

[25] Gesta S, Tseng Y-H, Kahn CR. Developmental origin of fat: tracking obesity to its source. Cell 2007;131:242–56.

[26] Naora H, Altin JG, Young IG. TCR-dependent and -independent signaling mechanisms differentially regulate lymphokine gene expression in the murine T helper clone D10.G4.1. J Immunol 1994;152:5691–702.

[27] Powell MJ, Thompson SAJ, Tone Y, Waldmann H, Tone M. Post-transcriptional regulation of IL-10 gene expression through sequences in the 3′-untranslated region. J Immunol 2000;165:292–6.

[28] Machado AP, Costa Rosa LFPB, Seelaender MCL. Adipose tissue in Walker 256 tumour-induced cachexia: possible association between decreased leptin concentration and mononuclear cell infiltration. Cell Tissue Res 2004;318:503–14.

[29] Nara M, Kanda T, Tsukui S, Inuka IT, Shimomura Y, Inoue S, et al. Running exercise increases tumor necrosis factor α secreting from mesenteric fat in insulin-resistant rats. Life Sci 1999;5:237–44.

[30] Ito Y, Nomura S, Ueda H, Sakurai T, Kizaki T, Ohno H, et al. Exercise training increases membrane bound form of tumor necrosis factor α receptors with decreases in the secretion of soluble forms of receptors in rat adipocytes. Life Sci 2002;71:601–9.

[31] Polak J, Klimcakova E, Moro C, Viguerie N, Berlan M, Hejnova J, et al. Effect of aerobic training on plasma levels and subcutaneous abdominal adipose tissue gene expression of adiponectin, leptin, interleukin 6, and tumor necrosis factor α in obese women. Metabolism 2006;55:1375–81.

[32] Gomez-Merino D, Drogou C, Guezennec CY, Chennaoui M. Effects of chronic exercise on cytokine production in white adipose tissue and skeletal muscle of rats. Cytokine 2007;40:23–9.

[33] Gleeson M. Immune function in sport and exercise. J Appl Physiol 2007;103:693–9.

[34] Petridou A, Tsalouhidou S, Tsalis G, Schulz T, Michna H, Mougios V. Long-term exercise increases the DNA binding activity of peroxisome proliferator-activated receptor γ in rat adipose tissue. Metabolism 2007;56:1029–36.

[35] Batista Jr ML, Santos RVT, Oliveira EM, Seelaender MCL, Costa Rosa LFBP. Endurance training restores peritoneal macrophage function in post-MI congestive heart failure rats. J Appl Physiol 2007;102:2033–9.

[36] Bacuau R, Belmonte M, Seelaender M, Costa Rosa L. Effect of a moderate intensity exercise training protocol on the metabolism of macrophages and lymphocytes of tumour-bearing rats. Cell Biochem Funct 2000;18:249–58.

[37] Lira F, Tavares F, Yamashita A, Koyama C, Alves M, Caperuto E, et al. Effect of endurance training upon lipid metabolism in the liver of cachectic tumour-bearing rats. Cell Biochem Funct 2008;26:701–8.

[38] Dos Santos Cunha W, Giampietro M, De Souza D, Vaisberg M, Seelaender M, Costa Rosa L. Exercise restores immune cell function in energy-restricted rats. Med Sci Sports Exerc 2004;36:2059–64.

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