Life Sciences 125 (2015) 79–87

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Perivascular adipose tissue and vascular responses in healthy trained rats

Hygor N. Araujo [a], Carmem P. Valgas da Silva [a], Amanda C.S. Sponton [b], Stefano P. Clerici [b], Ana P.C. Davel [b], Edson Antunes [c], Angelina Zanesco [a], Maria A. Delbin [b,*]

[a] Department of Physical Education, Institute of Biosciences, Univ. Estadual Paulista (UNESP), Rio Claro, SP, Brazil
[b] Department of Structural and Functional Biology, Institute of Biology, University of Campinas (UNICAMP), Campinas, SP, Brazil
[c] Department of Pharmacology, School of Medical Sciences, University of Campinas (UNICAMP), Campinas, SP, Brazil

ARTICLE INFO

Article history:
Received 14 July 2014
Accepted 30 December 2014
Available online 29 January 2015

Keywords:
Aerobic exercise training
Vascular response
Perivascular adipose tissue
Adipokines

ABSTRACT

Aims: The importance of perivascular adipose tissue (PVAT) in vascular function has recently been recognized. The aim of the study was to investigate the effects of exercise training on anticontractile responses of periaortic adipose tissue.
Main methods: Male Wistar rats were divided into sedentary (SD) and trained (TR). Running training was performed for 60 min/day, 5 days/week, for 8 weeks. Concentration–response curves to acetylcholine (ACh), sodium nitroprusside (SNP), phenylephrine (PHE) and serotonin (5-HT) were obtained in aortic rings without (PVAT−) or with (PVAT+) PVAT. The protein expressions of eNOS, AMPKα, pAMPKThr172 and mtTFA were determined in PVAT. The contents of adiponectin, leptin and TNF-α were evaluated systemically and locally.
Key findings: The PVAT+ rings did not modify the relaxing responses to ACh and SNP whereas it showed anticontractile effects for both PHE and 5-HT agents in the SD and TR groups. The amount of PVAT was markedly reduced in TR (3.6 ± 0.3 mg/mm) compared with SD (6.8 ± 0.6 mg/mm). Increased protein expressions of eNOS, pAMPKThr172 and mtTFA were observed in PVAT from TR animals, without modifications in PVAT-derived adiponectin, leptin and TNF-α. Circulatory leptin levels were reduced in TR without changes in adiponectin.
Significance: Our findings show that exercise training for 8 weeks did not alter the anticontractile effects induced by PVAT in rat-isolated aorta. Moreover, PVAT-derived adipokine, adiponectin and leptin levels were not different in trained healthy animals despite a significant metabolic adaptation and reduction in periaortic adipose tissue amount.

Introduction

It is well known that physical exercise promotes beneficial health effects in a variety of functional systems, preventing chronic disorders such as arterial hypertension, type 2 diabetes mellitus and obesity [5,49]. Exercise improves health span, enhances cardiovascular function and delays age-associated frailty in healthy animals [14,23].

Different from the old concept that the periorgan adipose tissue (pericardial, perimuscular, and perivascular) plays a mechanical role by protecting organs against injury impact, more recent evidence has shown that, particularly, perivascular adipose tissue (PVAT) releases a wide range of biologically active molecules [8] including adipocyte-derived relaxing factor (ADRF) [7,30], adiponectin [31], leptin [12], and nitric oxide (NO) [16]. It has been demonstrated that, functionally, PVAT exhibits an anticontractile effect, which might involve endothelial-dependent and/or endothelial-independent pathways [39]. In contrast, the anticontractile effect of PVAT is lost in experimental models of chronic obesity despite its higher amount. An imbalance between ADRF and vasocontractile agent production as well as a great releasing of proinflammatory substances might be the primary causes of the loss of the anticontractile effect of PVAT in obesity models [13,32]. On the other hand, increased contractile responses were observed in an experimental model of hypertension which was associated with a reduction in PVAT amount as well as a lower release of vasodilatory adipokines [11,12].

Besides their fundamental role in the regulation of the endocrine system, evidences show that leptin and adiponectin also participate in the regulation of the vascular tone [9,25]. Indeed, it has been demonstrated that leptin elicits an anticontractile effect that depends on an intact and functional endothelium [28,36,43]. Additionally, adiponectin derived from PVAT is considered a physiological modulator of local vascular tonus by increasing NO bioavailability and/or by inhibiting synthesis of inflammatory markers [26]. However, the number of studies that have evaluated the contribution of these adipocyte-derived adipokines on the vascular response is scarce.

* Corresponding author at: Department of Structural and Functional Biology, Institute of Biology University of Campinas (UNICAMP), P.O. Box 6109, Campinas, SP 13083-970, Brazil. Tel.: + 55 19 3521 6189.
E-mail address: nadelbin@hotmail.com (M.A. Delbin).

http://dx.doi.org/10.1016/j.lfs.2014.12.032
0024-3205/

Regarding exercise training and PVAT function, only two studies have examined this issue. However, the results were controversial [2, 35]. In addition, no one has investigated the anticontractile effects of PVAT in healthy trained animals which is crucial for a better understanding of the insight mechanisms by which exercise training promotes lower incidence of cardiometabolic diseases. Therefore, the aim of this work was to examine the effects of 8 weeks of aerobic exercise training on the vascular responses of isolated aortic rings and its relationship with the amount of the periaortic cushion fat. To further elucidate the paracrine control involved in the vascular responses, we focused on the mediators released from PVAT by measuring adiponectin, leptin and tumor necrosis factor-alpha (TNF-α). Given that adipose tissues play a key role in metabolism, we evaluated the protein expressions of endothelial nitric oxide synthase (eNOS), AMP-activated protein kinase α (AMPKα), phosphoAMPKThr172 (pAMPKThr172) and mitochondrial transcription factor A (mtTFA) in an attempt to detect the insight mechanisms by which exercise training might have a protective effect on cardiometabolic biomarkers. We also performed histology assessment in PVAT to analyze potential phenotype changes in adipose tissue. To exclude the contribution of systemic adipokines on the vascular responsiveness in reply to exercise training, we also evaluated circulating adiponectin, leptin, TNF-α and nitrite/nitrate (NO_x^-) levels.

Material and methods

Animals

This study was approved by the Ethical Committee for Animal Research (CEUA 014/2012) at the University of São Paulo State (UNESP) established by the Brazilian College for Animal Experimentation (COBEA).

Male Wistar rats (weighing 250–300 g) were obtained from the Animal Care Facility of the University of Campinas (UNICAMP) and were maintained in a room at 20–21 °C with a normal 12 h light/dark cycle. The animals were housed in groups of two/three and had free access to water and commercial chow (Nuvilab Radiated-CR1, Brazil). Animals were divided into two groups: sedentary (SD) and trained (TR). Body weight and food intake measurements were performed weekly during all periods of the study.

Aerobic exercise training

Animals were trained on a treadmill designed for small animals with individual lanes (Gesan, São Paulo—SP, Brazil). One week before starting the training program, the animals were adapted to the treadmill to minimize potential stress; during this week the duration and speed begun at 5 meters/minute (m/min) for 15 min and were progressively increased to 10 m/min for 20 min. Only the animals adapted to the treadmill were used in the present study.

After four days of adaptation, the animals performed an acute incremental exercise testing on the treadmill, where the intensity of exercise was increased by 5 m/min (5–40 m/min) every 3 min at 0% grade until exhaustion. The maximal speed was used to calculate the percentage corresponding to moderate intensity. At the beginning of the training program, the duration and speed started at 10 m/min for 30 min and were progressively increased to 60 min and at a speed of 60–80% of maximal capacity (15 m/min-17 m/min), 5 days/week, for 8 weeks and at 0% grade. All the animals were trained in the early morning, between 6:00 a.m. to 8:00 a.m.

At the last week of the training program, the effectiveness of exercise was evaluated by acute incremental exercise testing on the treadmill for both groups, SD and TR. This test provided the total distance, total time and the maximal speed performed for each animal.

Blood sample and epididymal fat pad collection

After 48 h of the last exercise session and 12 h of fasting, blood samples were collected from the tail vein and glycemia was measured using standard test strips (Accu-Chek Performa Roche Diagnostics, Indianapolis—IN, USA). Immediately after glycemia measurement, animals were anesthetized with sodium thiopental (40 mg/kg, i.p.) and arterial blood samples were collected from the abdominal aorta in different tubes (one for serum and one for plasma using EDTA as anticoagulant) and centrifuged (3000 rpm, for 15 min). Fresh serum was separated for lipid profile measurements and serum aliquots were also stored at −80 °C; all plasma supernatants were stored at −80 °C. After that, animals were euthanized and the epididymal fat pad was collected and weighted.

Concentration–response curves

Intact thoracic aorta was isolated carefully and placed in freshly prepared ice-cold Krebs solution containing (mM): NaCl, 118; $NaHCO_3$, 25; glucose, 5.6; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 1.1; and $CaCl_2 \cdot 2H_2O$, 2.5. Further, thoracic aorta was cut into 3 mm rings using a calibrated eyepiece with a dissecting microscope (Nikon Instruments, Melville—NY, USA) and rings were isolated without (PVAT−) or with (PVAT+) the perivascular adipose tissue (PVAT). Each ring was suspended between two wire hooks and mounted in an organ chamber (Panlab Harvard Apparatus, Barcelona, Spain) with 10 ml Krebs solution at 37 °C, pH 7.4 and continuously gassed with 95% O_2 and 5% CO_2 under a resting tension of 10 milliNewton (mN). The rings were allowed to equilibrate for 60 min, during this period the tension was verified every 15 min and washed with Krebs solution.

After the equilibration period, rings were precontracted with KCl 80 mM and washed with Krebs to verify their viability. Cumulative concentration–response curves to vasodilator agents: acetylcholine (ACh, 1 nM–30 μM) and sodium nitroprusside (SNP, 1 nM–100 μM) were obtained. Relaxing responses were plotted as percentage of the contraction induced by phenylephrine (in a concentration necessary to produce 50–70% of maximal response of KCl 80 mM). In accordance with standard in vitro analysis of vascular responses, concentration–response curves to phenylephrine (PHE, 1 nM–100 μM) were obtained in the presence of propranolol (100 nM; [42]). We also performed concentration–response curves to serotonin (5-HT, 10 nM–100 μM). Contractile responses were plotted according to the force and length from each ring measured as milliNewton/millimeter (mN/mm). Data acquisition was performed using PowerLab 8/30 (LabChart 7, ADInstruments, Sydney, Australia). After the concentration–response curves, PVAT of each ring was collected, the excess of Krebs solution was dried with a filter paper and the tissue was weighted wet and measured as milligram/millimeter (mg/mm).

All the concentration–response data were fit to a logistic function in the equation: $E = E_{MAX}/((1 + (10^c/10^x)^n) + \Phi)$, where E is the effect of

Table 1
Body weight, epididymal fat pad, food intake, glucose, total cholesterol and triglycerides in rats from the sedentary (SD) and trained (TR) groups.

	Groups	
	SD	TR
I-body weight (g)	281 ± 15 (8)	276 ± 14 (9)
F-body Weight (g)	445 ± 15 (8)	399 ± 11* (9)
Epididymal fat (g)	8.0 ± 0.7 (8)	4.5 ± 0.3* (9)
Food intake (g/rat/day)	29 ± 1 (8)	24 ± 1* (9)
Blood glucose (mg/dl)	86.6 ± 3.5 (8)	80.8 ± 1.9 (9)
Total cholesterol (mg/dl)	49.8 ± 2.9 (5)	55.1 ± 2.1 (5)
Triglycerides (mg/dl)	83.1 ± 6.7 (8)	56.9 ± 4.4* (9)

Initial (I) and Final (F). Data are mean ± SEM. The number of animals per group is indicated in parentheses.
* $p < 0.05$ compared with SD.