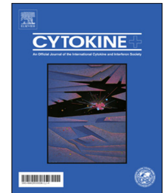
Cytokine 134 (2020) 155207

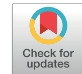
Contents lists available at ScienceDirect

Cytokine

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



Moderate aerobic exercise-induced cytokines changes are disturbed in PPARα knockout mice



Carolina Cabral-Santos[a], Loreana Sanches Silveira[b], Patricia Chimin[c], José Cesar Rosa-Neto[b,*], Fábio Santos Lira[a]

[a] Exercise and Immunometabolism Research Group, Department of Physical Education, Post-Graduation Program in Movement Sciences, State University of São Paulo (UNESP), Presidente Prudente, São Paulo, Brazil

[b] Immunometabolism Research Group, Department of Cell and Developmental Biology, University of São Paulo, São Paulo, Brazil

[c] Universidade Estadual de Londrina, Centro de Educação Física e Desportos, Departamento de Fundamentos da Educação Física, Londrina, Brazil

ARTICLE INFO

Keywords:
Peroxisome proliferator activated receptor
Lipid metabolism
Inflammatory response
Cytokines

ABSTRACT

The nuclear transcriptional factor peroxisome proliferator activated receptor alpha (PPARα) plays a role in regulating genes involved in lipid metabolism, adipogenesis and inflammation. We aimed to assess the role of PPARα on exercise-mediated locally produced cytokines in adipose fat deposits and skeletal muscle. C57BL/6 (WT) and PPARα knockout (PPARα$^{-/-}$) mice were examined. Each genotype was randomly subdivided into three groups: non-exercised, and euthanized 2 or 24 h after a moderate aerobic exercise session (run on a treadmill at 60% of maximum speed for 1 h). Fat content in gastrocnemius muscle and lipolytic activity in isolated adipose tissue from mesenteric (MEAT) and retroperitoneal (RPAT) adipose tissue were evaluated. In addition, Interleukin 6 (IL-6), interleukin 10 (IL-10), tumor necrosis factor α (TNF-α) and monocyte chemoattractant protein 1 (MCP-1) content were evaluated by ELISA. WT mice showed a maximum lipolysis rate, as well as higher IL-6, IL-10, and IL10/TNF-α ratio values 2 h post-exercise (RPAT only) compared with PPARα$^{-/-}$ mice. Taken together, our data suggests that PPARα knockout mice exhibited reduced lipolysis and anti-inflammatory response in adipose tissue following exercise, PPARα appears to play an important role in immunomodulatory and lipolysis signaling after acute moderate exercise.

1. Introduction

Acute exercise promotes profound perturbations in metabolites and lipids mediators, induces gene transcription and proteins synthesis that has direct influence in the production of myokines and adipokines and leads to both local and systemic inflammatory response [34]. Adipocytes are highly secretory cells and the heterogeneity of adipose tissue depots differently contribute to homeostasis and modulation of inflammation. Adipose tissue can communicate with other tissues and the immune system secreting peptides, lipids, and miRNAs, which affect systemic metabolism [19]. Supporting the idea of developmental and functional heterogeneity of white adipose tissue, studies have showed that individual adipose tissue depots might differ in metabolism (insulin-stimulated glucose uptake, maximal lipogenic rate, response to catecholamines, and uptake of free fatty acids) as well as response differently to inflammatory cytokines and growth hormones [21]. Compared with subcutaneous, visceral adipose tissue secretes more fatty acids and pro-inflammatory cytokines and has a higher infiltration of cytotoxic T cells and macrophages in obese adipose tissue of mice and humans [5]. It is important to highlight that adipose tissue is comprised of many cell types in addition to adipocytes, including preadipocytes, mesenchymal stem cells, vascular cells, and inflammatory cells, which also contribute to its physiology.

The increased release of cytokines such as interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) was postulated to be the driving forces behind the lipolytic process, in a hormone-like fashion - through a GR130R/IL-6R homodimers and soluble TNF-receptors (sTNF-R), respectively. This receptor initiates the activation of AMP-kinase and/or phosphatidylinositol 3-kinase to increase glucose uptake, fat oxidation and also increase hepatic glucose production during exercise increasing the availability of glucose and fatty acids (FA) in the bloodstream, directing this substrate to the skeletal muscles to maintain the contractile activity [9,22,25,27,28,29].

A previous study conducted by our group showed that exhaustive

* Corresponding author at: Immunometabolism Research Group, Department of Cell and Developmental Biology, University of São Paulo (USP), Av. Prof. Lineu Prestes, n° 2415, Butantã - São Paulo/SP - CEP 05508-000, São Paulo, Brazil.
E-mail address: jrosaneto@usp.br (J.C. Rosa-Neto).

https://doi.org/10.1016/j.cyto.2020.155207
Received 5 November 2019; Received in revised form 22 May 2020; Accepted 11 July 2020
1043-4666/ © 2020 Published by Elsevier Ltd.

exercise promoted an anti-inflammatory effect in skeletal muscle, especially in type 2 fibers, on the other hand, promoted a pro-inflammatory response in adipose tissue [29]. We have postulated that, higher cytokine concentrations (mainly IL-6 and TNF-α) could lead to increased lipolysis in order to provide energy supply to skeletal muscle contraction [4,5,22].

Peroxisome proliferator activated receptors (PPAR) are nuclear transcriptional factor that plays key roles in the regulation of genes involved in the glucose metabolism and are postulated to mediate metabolic responses to acute and chronic exercise [2]. PPARs, especially the alpha isoform (PPAR-α), also regulate proteins and enzymes required for inflammatory response, fatty acid oxidation and lipid metabolism, during both fed and fasted states, as well as ketogenesis [12]. This transcription factor is highly expressed in cells with high catabolic rates of FA, such as heart, liver, skeletal muscle and brown adipose tissue [11,15].

Our group has found that PPARα knockout mice submitted to acute exercise showed impaired glucose homeostasis and abolished anti-inflammatory response in macrophages [31]. However, the involvement of PPARα in adipose tissue lipolysis and cytokines release in response to acute exercise has not been investigated. Therefore, we aimed to understand the influence of PPARα on acute exercise-mediated metabolic and cytokine profiles in adipose tissue and skeletal muscle.

2. Methods

2.1. Animals

This study was carried out with male mice with whole body deletion of PPARα (PPARα$^{-/-}$) and their control C57BL/6J wild type (WT). Mice were housed in a temperature-controlled room (22 ± 2 °C) with a 12 h light cycle, fed with balanced chow pellet diet (Nuvilab CR1, Nuvital, PR, Brazil) and water supply *ad libitum*. All experimental protocols comply the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and were approved by the Animal Care Committee of the Institute of Biomedical Sciences, University of Sao Paulo, Brazil (112/13 CEUA).

2.2. Experimental protocol

Mice from PPARα$^{-/-}$ and WT groups were randomly subdivided in 3 experimental groups: a) Non-exercised, b) 2 h post-exercise and c) 24 h post-exercise. All animals were submitted to exercise adaptation in a treadmill for 5 days (running 10 min at 10 m·min^{-1}) and an incremental test consisting of 5 min warm-up at 10 m·min^{-1} followed by increments of 3 m·min^{-1} until exhaustion [1] aiming to determine the maximal speed. After 72 h rest, the exercise groups performed an acute session of 60% of maximum speed for 1 h. The animals were anaesthetized by isoflurane inhalation and euthanized by decapitation at rest, 2 or 24 h after acute exercise session. This exercise protocol was chosen in accordance to a previous study demonstrating increased IL-6 output and lipolysis post-exercise in subcutaneous adipose tissue [23].

2.3. Lipid extraction in gastrocnemius

Lipid extraction from muscle was performed according to Folch's method [14]. Briefly, gastrocnemius muscles were homogenized with chloroform and methanol (2:1 v/v), and later centrifuged at 2.000 rpm for 10 min. The organic lower phase was collected, and 2 ml of water was added. This mixture was centrifuged at 1.500 rpm for 5 min. Then the extracted lipids were collected and dried at room temperature. Samples were reconstituted in 300 ml of Triton 3% for storage. The concentration of triacylglycerol (TAG) was analyzed by colorimetric method (Labtest® Company, Brazil).

2.4. Lipolysis assay

Lipolytic activity was measured in isolated mesenteric (MEAT) and retroperitoneal (RPAT) in visceral fat pads from both genotypes and grouped into rest and 24 h pos-exercise conditions. Briefly, a pool of 50 mg tissue were incubated for 1 h on Krebs Ringer bicarbonate buffer (118 NaCl, 4.8 KCl, 1.25 CaCl2, 1.2 KH2PO4, 1.2 MgSO4, 25 NaHCO3, 5 glucose, supplemented with 2.5% BSA mmol/l) and were incubated in the absence (basal) or presence (maximum) of β-adrenergic agonist isoproterenol, as previously described [20]. The medium of adipose tissue explants were frozen until measurement of glycerol released (Sigma Aldrich, Oakville, ON, Canada) and used as an index of lipolysis rate.

2.5. Sample collection and cytokines production

MEAT and RPAT fat pads, as well as gastrocnemius muscles were excised and processed for cytokines measurement in immunoassay assay buffer and, after centrifugation, the supernatant was used to determine the protein concentration by Bradford assay (Bio-rad, Hercules, CA). The concentration of IL-6, IL-10, TNF-α and MCP-1 were quantified using an enzyme-linked immunosorbent assay commercial kit following the manufacturer instructions (R&D Systems, 614 McKinley Place NE, Minneapolis, MN 55413, USA).

3. Statistics/Calculation

The normality of the variances was verified using the Kolmogorov-Smirnov test and descriptive data are shown as mean and standard error of the mean (Mean ± SEM). To assess the basal and maximum stimulated glycerol release, the normal variables were analyzed using Student's *t* test and effect sizes (Cohen's d) were calculated. Two-way ANOVA was used to compare the differences between genotypes and different times of data collection. When appropriate, a Bonferroni post-hoc test was conducted. The effect size (eta-squared; η^2) of each test was calculated for all analyses.

For nonparametric data, Kruskal-Wallis Analysis of variance was used to compare the difference from IL to 10 in gastrocnemius muscle and all pairwise comparisons were performed using the Dunns method. Statistical significance was set at 5% and all data were analyzed using GraphPad Prism, version 6.1 (CA, USA).

4. Results

4.1. IL-6 content in gastrocnemius decrease 24 h post-exercise regardless PPARα

Our data showed elevated baseline content of triacylglycerol in the gastrocnemius muscle of PPARα$^{-/-}$ mice (Fig. 1A), but with no statistical difference between WT mice ($p > 0.05$). No differences were observed on TNF-α ($p = 0.673$; $\eta^2 = 0.024$, Fig. 1B) content at baseline and after exercise between genotypes. For intramuscular IL-6, it was observed a decrease 24 h post-exercise compared with rest in both genotypes ($p = 0.047$; $\eta^2 = 0.174$, Fig. 1C).

Despite a significant difference on basal levels of IL-10 between groups ($p = 0.032$, $\eta^2 = 0.240$), there was an increase in IL-10 in gastrocnemius muscle after aerobic exercise only in PPARα$^{-/-}$ group with higher values 2 h post-exercise compared with rest ($p = 0.023$, Fig. 1D).

4.2 WT. MEAT depot showed acute exercise-induced inflammatory response

Our data showed no significant differences on cytokines profile from WT and PPARα$^{-/-}$ animals in baseline conditions. Fig. 2 presents the inflammatory profile analyzed in both adipose depots. No significant

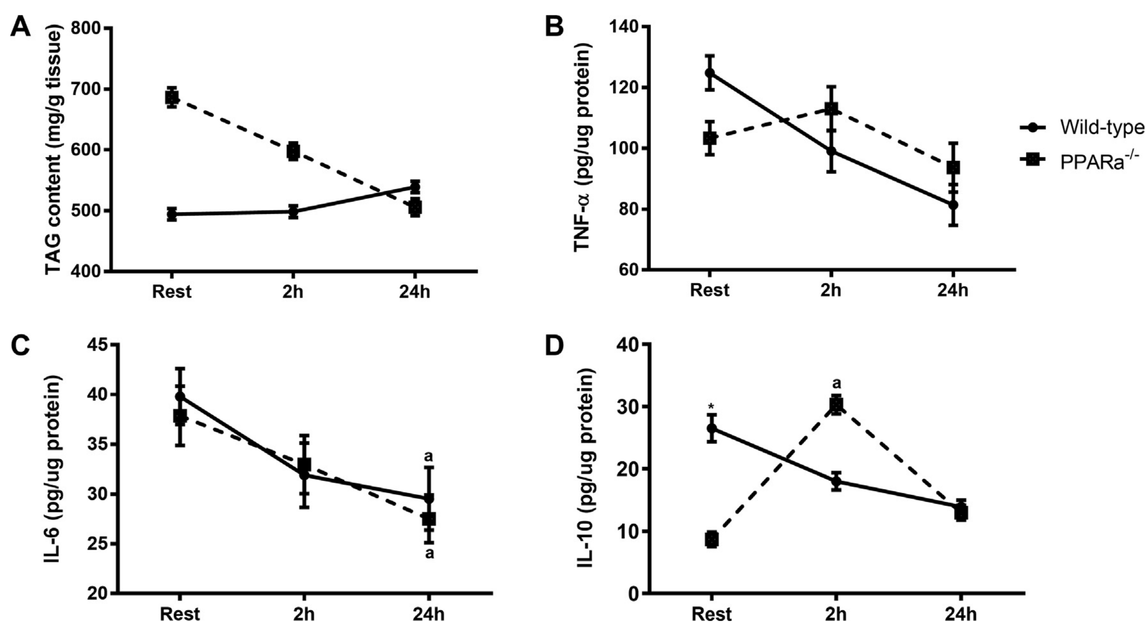


Fig. 1. Content of triacylglycerol (A), TNF-α (B), IL-6 (C), and IL-10 (D) in gastrocnemius muscle in WT (n = 8 per group) and PPARα$^{-/-}$ (n = 8 per group) mice at different timepoints (Rest, 2 h and 24 h after moderate exercise protocol). Legend: Results are represented on mean ± standard deviation. Anova Two-way followed by the post-test Bonferroni was used and the level of significance was set at $p < 0.05$. * = statistically difference between WT and PPARα$^{-/-}$ group; a = significant different from rest. IL-6 = Interleukin 6; TNF-α = Tumor necrosis factor alpha; IL-10 = Interleukin 10.

changes in IL-6 levels overtime were observed in MEAT depot between genotypes ($p > 0.05$). There was a significant increase in TNF-α levels at 2 h post-exercise compared to rest in both genotypes, but it was significant only in WT group ($p = 0.003$, $\eta^2 = 0.541$, Fig. 2A). A significant genotype x exercise interaction in MCP-1 ($p = 0.014$) with significant differences in the WT group at 2 h post-exercise compared with PPARα$^{-/-}$ mice.

Regarding the anti-inflammatory profile, MEAT showed a genotype x exercise interaction in IL-10 ($p = 0.001$, $\eta^2 = 0.503$) with important differences in the WT group at 2 h and 24 h post-exercise compared with PPARα$^{-/-}$ mice. For IL10/TNF-α ratio, WT mice also showed genotype x exercise interaction ($p = 0.001$, η2 = 0.553, Fig. 3B) with higher values at rest and 24 h post-exercise compared with PPARα$^{-/-}$ mice, suggesting a local anti-inflammatory microenvironment. There was no differences on this ratio in PPARα$^{-/-}$ mice for time ($p = 0.55$, η2 = 0.076, Fig. 3B).

4.3. RPAT depot inflammatory response are disturbed in PPAR α$^{-/-}$ mice

For RPAT fat pad, WT mice showed increased IL-6 content after 2 h post-exercise compared to rest ($p = 0.001$, $\eta^2 = 0.344$, Fig. 2E). For TNF-α and MCP-1 levels, there were no differences between groups overtime ($p > 0.05$, Fig. 2D and F).In RPAT, there was an increase in IL-10 levels 2 h after exercise in WT group ($p < 0.001$, $\eta^2 = 0.595$), which returned to basal levels after 24 h. This pattern was not observed in PPARα$^{-/-}$ mice (Fig. 3C).

For IL10/TNF-α ratio on RPAT there was a significant genotype x exercise interaction ($p = 0.001$, $\eta^2 = 0.420$). Post-hoc test revealed a significant increase in the WT group at 2 h post-exercise compared to rest, with no significant differences across time for the PPARα$^{-/-}$ mice. There was no significant difference on IL-10/TNF-α ratio for PPARα$^{-/-}$ mice.

4.4. Lipolysis

The rates of basal and maximum (isoproterenol) stimulated glycerol release are illustrated in Fig. 4. In both groups, measurement of basal lipolysis were similar in the MEAT, showing no difference between genotypes ($p = 0.64$, Cohen's D = 0.26). However, maximum lipolysis rates markedly increased in WT group ($p = 0.039$, Cohen's D = 1.13, Fig. 4) while the PPARα$^{-/-}$ group showed a restrained ability to increase of lipolytic rates in response to maximum lipolysis stimulus.

The same pattern was observed for RPAT, the basal lipolysis showed no significant difference between genotypes ($p = 0.25$, Cohen's D = 0.78, Fig. 4), however maximum lipolysis rates markedly increased in WT group ($p = 0.001$, Cohen's D = 3.21) when compared with the PPARα$^{-/-}$ group.

5. Discussion

In the present study, our data demonstrated that PPARα was involved in the regulation cytokines profile after acute moderate-intensity aerobic exercise, confirming our initial hypothesis. However, this response was different between adipose tissue depots as more drastic differences in IL-10 and IL-10/TNF-α ratio were seen in retroperitoneal compared to mesenteric adipose tissue. Here, we showed for the first time that PPARα modulates the immunometabolic events in adipose tissue after acute exercise. Most of energy reserves are stored in the form of triacylglycerol (TAG) in adipose tissue and skeletal muscles and supplied as FA during exercise. It is well known that in humans that intramuscular TAG utilization is stimulated at intensities between 65% and 85% of VO_2max [17]. De Souza et al. [7] showed that PPARα activation, especially in type I fibers, increase fatty acid uptake and β-oxidation in skeletal muscle and it was associated with a lowered concentration in plasma TAG and increased insulin sensitivity. In the present study, we observed that PPARα$^{-/-}$ mice exhibited approximately 1.4-fold higher TAG concentration in gastrocnemius muscle at rest when compared to WT animals (although not statistically significant, $p = 0.23$). Interestedly, Silveira et al. [31] showed PPARα$^{-/-}$ mice had a better performance during the maximum speed test (5 min of warm-up increasing 3 m/minutes each minute until exhaustion) reaching highest average of maximum speed than wild type mice.

When activated, PPARα increases fatty acid oxidation, mitochondrial biogenesis, promotes ketone body synthesis, and glucose sparing and many lipid-related alterations in the balance between lipid storage and degradation by oxidative catabolism [12,21]. It has been suggested

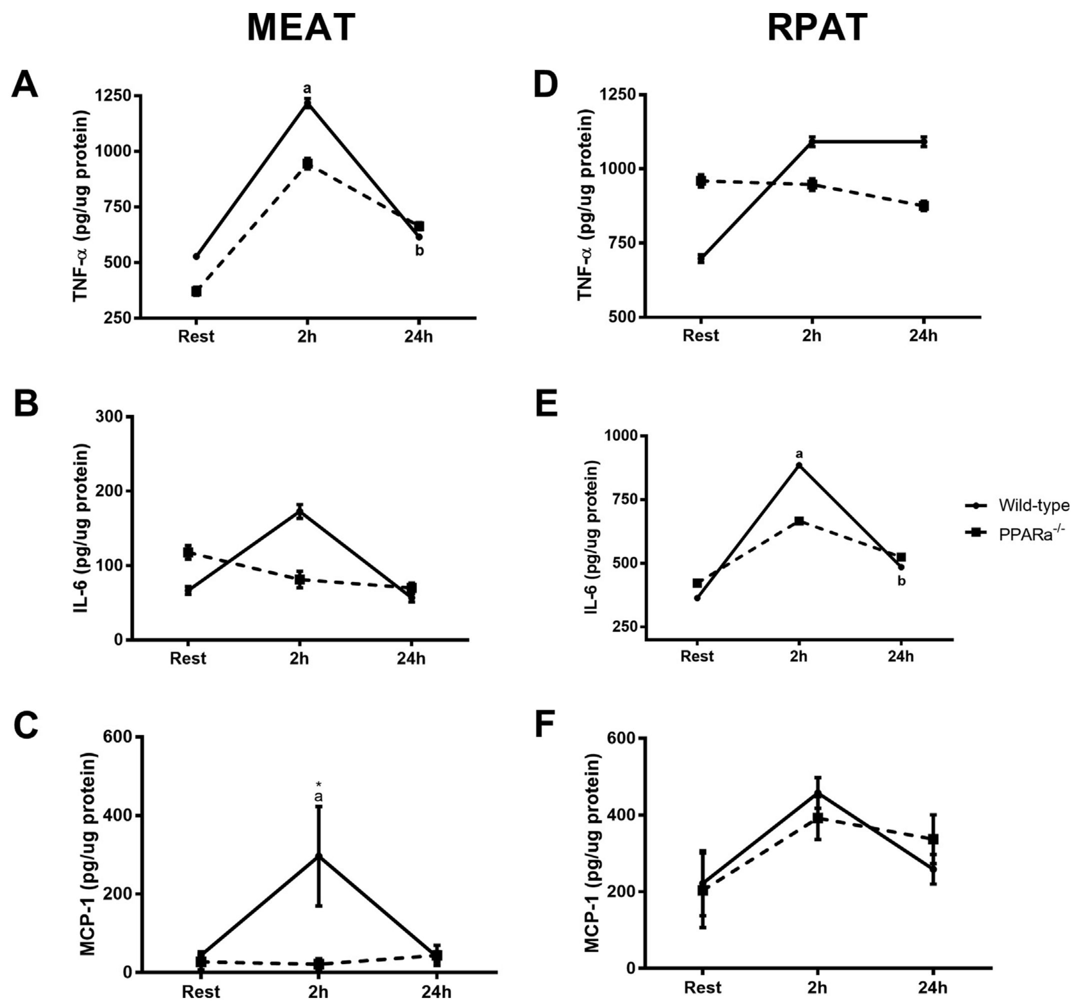


Fig. 2. Cytokines TNF-α, IL-6, and MCP-1 content on mesenteric (A, B, C) and retroperitoneal (D, E, F) adipose tissue in WT (n = 8 per group) and PPARα$^{-/-}$ (n = 8 per group) mice at different timepoints (Rest, 2 h and 24 h after moderate exercise protocol). Legend: Results are represented on mean ± standard deviation. Anova Two-way followed by the post-test Bonferroni was used and the level of significance was set at $p < 0.05$. * = statistically difference between WT and PPARα$^{-/-}$ group; a = significant different from rest; b = significant different from post-2 h. TNF-α = Tumor necrosis factor alpha; IL-6 = Interleukin 6; IL-10 = Interleukin 10.

that PPARα act as a sensor of overall tissue lipid supply and after evaluate the metabolic alterations, using lipolysis as base, we observed a restrained ability to stimulate the release of lipolytic products in response to maximum induction, suggesting that lipolysis capacity activity of adipose tissue was affected by the lack of PPARα. In addition, we suggest that the higher skeletal muscle storage of TAG observed in PPARα$^{-/-}$ group could be an adaptation to lower release of FA during exercise in order to provide sufficient substrate supply to the organism.

It is well known that the activation of β-adrenergic receptors on adipocytes mobilizes FA for oxidation and triggers acute changes in metabolism that can alter transcription of oxidative genes. These observations suggest that the deletion of PPARα could represent a limitation in adipose tissue in supporting the FA demand to active muscle, and it could be due to a reduced stimulation of the lipolysis process [17]. Ferré (2004) [12] showed that PPARα activation favors fatty acid oxidation, and induces glucose sparing, either directly by inducing the gene expression of pyruvate dehydrogenase kinase 4 (PDK4) or indirectly through the synthesis of ketone bodies, increasing fatty acid oxidation capacity; Thus PPARα is important to decrease lipid accumulation in local tissue and, consequently, may impact the inflammation. In addition, Li et al. [21] observed that white adipose tissue remodeling is an adaptive response to chronically excessive FA mobilization and PPARα and its downstream targets are key regulator in expanding β-oxidation in adipocytes and limiting adipose tissue inflammation, and its impact on pro-inflammatory signaling.

Recently studies have demonstrated that white adipose tissue is comprised of at least three distinct subpopulations of preadipocytes and adipocytes. Subtypes 1 and 2 are widely distributed and found in pericardial, mesenteric, perirenal, and perigonadal fat and have been characterized by highly glycolytic metabolism, in addition to reduced triglyceride accumulation and highly responsive to the cytokines (in the present study characterized by MEAT and RPAT). Whereas subtype 3 is primarily found in subcutaneous depots, are insulin sensitive and display an attenuated response to cytokines [19,21]. Subtypes 1 and 2 showed increased TNF-α activation by the phosphorylation of c-Jun N-terminal kinases (JNK) while subtype 3 were unresponsive and these differential responses were not due to differences in receptor expression [19,21]. In this study the acute induction of pro-inflammatory cytokines was highly similar in both WT and PPARα$^{-/-}$ mice. TNF-α is an immunomodulatory cytokine and it is important in insulin-mediated glucose uptake, as it impairs the phosphorylation and activation of insulin receptor substrate proteins (IRS-1), the activity of phosphatidylinositol-3-kinase (PI3K), and decreases the translocation of glucose transporter (GLUT-4) to the membrane [26]. Its activation culminates in the phosphorylation of transcription nuclear factor-kappa B (NF-κB), that plays the major role in the regulation of inflammatory response, becoming an important mechanism linking obesity and insulin resistance [30]. In our study, PPARα$^{-/-}$ mice showed a restrained ability to release MCP-1 2 h after exercise in MEAT only when compared with WT mice. However, Takahashi et al. [33] demonstrated that the

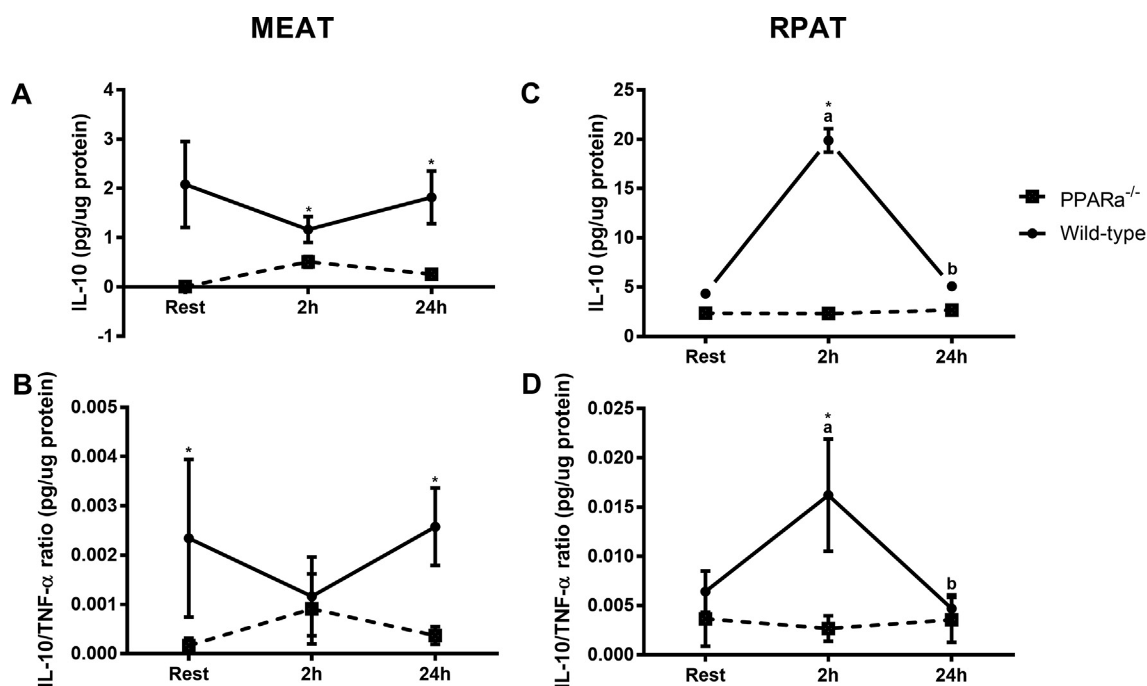


Fig. 3. IL-10 and IL-10/TNF-α ratio production on mesenteric (A, B) and retroperitoneal (C, D) adipose tissue in WT (n = 8 per group) and PPARα$^{-/-}$ (n = 8 per group) mice at different periods (Rest, 2 h and 24 h after moderate exercise protocol). Legend: Results are represented on mean ± standard deviation. Anova Two-way followed by the post-test Bonferroni was used and the level of significance was set at $p < 0.05$. * = statistically difference between WT and PPARα$^{-/-}$ group; a = significant different from rest; b = significant different from post-2 h. TNF-α = Tumor necrosis factor alpha; IL-10 = Interleukin 10.

expression of IL-6, TNF-α and MCP-1, which promote obesity-induced inflammation, were decreased by adipose tissue specific PPARα overexpression in mice, thereby contributing to the improvement of insulin sensitivity.

In addition, Pedersen [26] showed that the cytokine cascade response to exercise markedly differs from that elicited by infections - with an increase in anti-inflammatory response via IL-6 released during exercise, IL-6 produced during exercise is also postulated to induces lipolysis in adipose tissue and promote of hepatic gluconeogenesis to provide fuel for the contractile activity [26]. These data demonstrated that an acute exercise session activates the production of pro-inflammatory cytokines, mainly IL-6 and TNF-α, and we postulated that such rise favor the lipolysis process, increasing the availability of FA oxidation to directing the substrate to maintaining skeletal muscle activity.

It is well known that the main stimulus for the synthesis and release of IL-6 during exercise is muscle contraction per se, although study showed that the decrease in muscle glycogen content increases intracellular calcium levels, and an increased formation of reactive oxygen species (EROS) could also activate the transcription factors that are known to regulate IL-6 synthesis [13]. Previous studies conducted by our group showed that exercise promoted an anti-inflammatory effect in skeletal muscle, especially in type 2 fibers, while promote a pro-inflammatory response in adipose tissue [29]. Visceral adipose tissue cell cultures showed this depot produces ~ 3-fold more IL-6 than cells from subcutaneous depot [10]. We have postulated that, higher cytokines concentrations (mainly IL-6 and TNF-α) can lead to increased lipolysis to provide energy for the exercising skeletal muscle [4,5,22], and since PPARα$^{-/-}$ mice exhibited decreased cytokine release in response to acute exercise, this could explain the lack of response in maximum lipolysis.

Acute inflammatory response is essential for adipose tissue protection, extracellular matrix remodeling, angiogenesis and adipogenesis, therefore facilitating the return to homeostasis and subsequently allowing the inflammation to reach resolution as opposed to becoming chronic [35]. It is clear that immune cells are recruited to individual fat depots through the release of potent chemotaxic cytokines such as MCP-1. This chemotaxis protein is produced predominantly by endothelial cells and macrophages to promote immune cells recruitment into inflamed tissue via an activation of the NF-κB pathway. Bruun et al. [3]

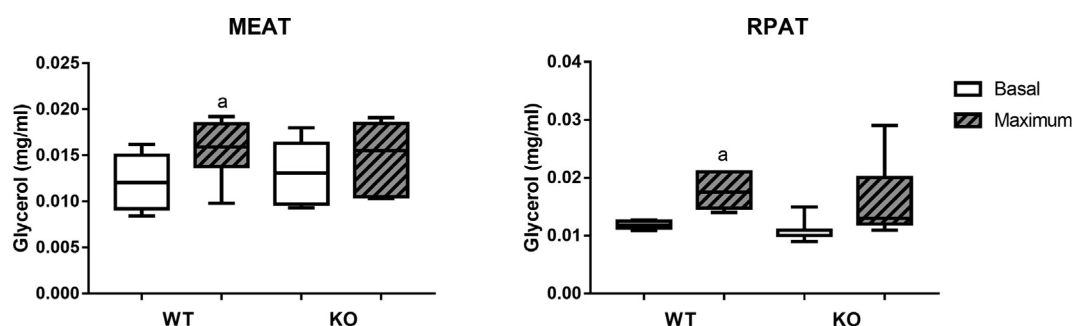


Fig. 4. Basal and isoproterenol-stimulated rates of glycerol release of basal and maximal lipolysis in mesenteric and retroperitoneal adipose tissue in WT (n = 6) and PPARα$^{-/-}$ mice (n = 7) grouped into rest and 24 h pos-exercise conditions. Legend: Results are represented on mean ± standard deviation. T-student test was used to compare mean and the level of significance was set at $p < 0.05$. a = statistically difference from basal rates from same genotype.3.

identified that visceral adipose tissue displayed a 2-fold higher MCP-1 release compared with subcutaneous in both lean and obese subjects. This production seems to be related to the number of resident macrophages in the human adipose tissue and it is associated with a general low-grade inflammatory process and an increased risk of developing type 2 diabetes and cardiovascular disease [3]. In the Fig. 2, we showed that MCP-1 release appears to be suppressed in MEAT of PPARα$^{-/-}$ mice. Supporting the idea that this pro-inflammatory signaling may be required for healthy response, an impaired pro-inflammatory response in adipocytes can lead to ectopic lipid accumulation and glucose intolerance in mice on high fat diet [35].

In addition, IL-6 exerts pleiotropic functions in the immune system, stimulating the synthesis of anti-inflammatory cytokines such as IL-1ra, IL-10 and soluble TNF receptor that acts downregulating the synthesis of pro-inflammatory cytokines [27]. IL-10 produced during exercise has strong down regulatory effects on the secretion of pro-inflammatory cytokines, inhibiting cytokine synthesis at the level of gene transcription, and blocking a possible persistent inflammatory status [25]. Classically, exercise leads to an inflammatory response that seems to be tissue dependent, as demonstrated by Rosa Neto et al. [29]. We have been shown that after exhaustive exercise (50 min running on a treadmill at 60% maximum speed and subsequently increase at 1 m/min until exhaustion), the IL-10/TNF-α ratio increased in skeletal muscle compared with baseline, and the improvement in the balance of pro- and anti-inflammatory cytokine induced by exercise prevents skeletal muscle inflammation and tissue damage. However, they observed decreased IL-10/TNF-α ratio in adipose tissue - showing that pro-inflammatory effect prevails - possibly contributing to increased lipolysis to provide energy for the exercising muscle. The present study showed increased IL-10/TNF-α ratio only in MEAT 2 h post-exercise protocol for WT group, corroborating with previous literature. This response may contribute to an increase in lipolysis in order to provide energy for active muscle [29]. Furthermore, the balance between anti- and pro-inflammatory cytokines is important for immune homeostasis maintenance.

6. Conclusion

Our data showed that the inflammatory profile is disturbed in PPARα$^{-/-}$ mice after acute moderate-intensity aerobic exercise, observed by the absence of IL-10 induction in skeletal muscle and adipose tissue. Induction of lipolysis also appeared blunted in PPARα$^{-/-}$ mice. PPARα therefore seems to influence the inflammatory response by regulating local cytokines and lipid metabolism. Future studies aiming to understand the mechanism and consequences of the inhibitory effects of PPARα$^{-/-}$ mice on IL-10 production derived from acute exercise are necessary.

CRediT authorship contribution statement

Carolina Cabral-Santos: Methodology, Formal analysis, Writing - original draft. **Loreana Sanches Silveira:** Methodology, Formal analysis, Writing - original draft. **Patricia Chimin:** Writing - review & editing. **José Cesar Rosa-Neto:** Conceptualization, Supervision. **Fábio Santos Lira:** Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Professor Dr Jonathan P Little (The University of British Columbia - Okanagan, BC, Canada) for his contributions to the revision of the manuscript. Sao Paulo Research Foundation (FAPESP) supported this work under grant number 2014/01246-6, 2015/26148-0 and 2018/17648-7.

References

[1] A.V. Bacurau, M.A. Jardim, J.C. Ferreira, L.R. Bechara, C.R. Bueno Jr., T.C. Alba-Loureiro, C.E. Negrao, D.E. Casarini, R. Curi, P.R. Ramires, A.S. Moriscot, P.C. Brum, Sympathetic hyperactivity differentially affects skeletal muscle mass in developing heart failure: role of exercise training, J. Appl. Physiol. 106 (2009) 1631–1640.

[2] H. Bays, L. Mandarino, R.A. DeFronzo, Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach, J. Clin. Endocrinol. Metab. 89 (2004) 463–478.

[3] J.M. Bruun, A.S. Lihn, S.B. Pedersen, B. Richelsen, Monocyte chemoattractant protein-1 release is higher in visceral than subcutaneous human adipose tissue (AT): implication of macrophages resident in the AT, J. Clin. Endocrinol. Metab. 90 (2005) 2282–2289.

[4] C. Cabral-Santos, C.I. Castrillón, R.A. Miranda, P.A. Monteiro, D.S. Inoue, E.Z. Campos, P. Hofmann, F.S. Lira, Inflammatory Cytokines and BDNF Response to High-Intensity Intermittent Exercise: Effect the Exercise Volume, Front. Physiol. 7 (2016) 509.

[5] C. Cabral-Santos, J. Gerosa-Neto, D.S. Inoue, V.L. Panissa, L.A. Gobbo, A.M. Zagatto, E.Z. Campos, F.S. Lira, Similar Anti-Inflammatory Acute Responses from Moderate-Intensity Continuous and High-Intensity Intermittent Exercise, J. Sports Sci. Med. 14 (2015) 849–856.

[7] A.T. De Souza, P.D. Cornwell, X. Dai, M.J. Caguyong, R.G. Ulrich, Agonists of the peroxisome proliferator-activated receptor alpha induce a fiber-type-selective transcriptional response in rat skeletal muscle, Toxicol. Sci. 92 (2006) 578–586.

[9] T.A. Diniz, J.C.J. Aquino Júnior, F.C. Mosele, C. Cabral-Santos, E.A. Lima Junior, A.A.S. Teixeira, F.S. Lira, J.C. Rosa Neto, Exercise-induced AMPK activation and IL-6 muscle production are disturbed in adiponectin knockout mice, Cytokine 119 (2019) 71–80.

[10] S.K. Fried, D.A. Bunkin, A.S. Greenberg, Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid, J. Clin. Endocrinol. Metab. 83 (1998) 847–850.

[11] P. Escher, O. Braissant, S. Basu-Modak, L. Michalik, W. Wahli, B. Desvergne, Rat PPARs: quantitative analysis in adult rat tissues and regulation in fasting and refeeding, Endocrinology 142 (2001) 4195–4202.

[12] P. Ferré, The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity, Diabetes 53 (2004) 43–50.

[13] C.P. Fischer, Interleukin-6 in acute exercise and training: what is the biological relevance? Exerc. Immunol. Rev. 12 (2016) 6–33.

[14] J. Folch, M. Lees, G.H.S. Stanley, A simple method for the isolation and purification of total lipides from animal tissues, J. Biol. Chem. 226 (1957) 497–509.

[15] M. Grabacka, M. Pierzchalska, M. Dean, K. Reiss, Regulation of Ketone Body Metabolism and the Role of PPARα, Int. J. Mol. Sci. 17 (2016) E2093.

[17] J. Jeppesen, B. Kiens, Regulation and limitations to fatty acid oxidation during exercise, J. Physiol. 590 (2012) 1059–1068.

[19] C.R. Kahn, G. Wang, K.Y. Lee, Altered adipose tissue and adipocyte function in the pathogenesis of metabolic syndrome, J. Clin. Invest. 1 (2019) 3990–4000, https://doi.org/10.1172/JCI129187.

[20] M. Laplante, W.T. Festuccia, G. Soucy, Y. Gélinas, J. Lalonde, J.P. Berger, Y. Deshaies, Mechanisms of the depot specificity of peroxisome proliferator-activated receptor gamma action on adipose tissue metabolism, Diabetes 55 (2006) 2771–2778.

[21] P. Li, Z. Zhu, Y. Lu, J.G. Granneman, Metabolic and cellular plasticity in white adipose tissue II: role of peroxisome proliferator-activated receptor-alpha, Am. J. Physiol. Endocrinol. Metab. 289 (2005) E617–E626.

[22] F.S.D. Lira, T. Santos, R.S. Caldeira, D.S. Inoue, V.L.G. Panissa, C. Cabral-Santos, E.Z. Campos, B. Rodrigues, P.A. Monteiro, Short-Term High- and Moderate-Intensity Training Modifies Inflammatory and Metabolic Factors in Response to Acute Exercise, Front. Physiol. 8 (2017) 856.

[23] D. Lyngsø, L. Simonsen, J. Bülow, Interleukin-6 production in human subcutaneous abdominal adipose tissue: the effect of exercise, J. Physiol. 543 (2002) 373–378.

[25] B.K. Pedersen, The diseasome of physical inactivity–and the role of myokines in muscle–fat cross talk, J. Physiol. 587 (2009) 5559–5568.

[26] B.K. Pedersen, Muscular interleukin-6 and its role as an energy sensor, Med. Sci. Sports Exerc. 44 (2012) 392–396.

[27] B.K. Pedersen, M.A. Febbraio, Muscle as an endocrine organ: focus on muscle-derived interleukin-6, Physiol. Rev. 88 (2008) 1379–1406.

[28] J.C. Rosa, F.S. Lira, R. Eguchi, G.D. Pimentel, D.P. Venâncio, C.A. Cunha, L.M.D. Oyama, M.T. Mello, M. Seelaender, C.M. do Nascimento, Exhaustive exercise increased inflammatory response via Toll Like Receptor 4 and NF-kBp65 pathway in adipose tissue in rats, J. Cell. Physiol. 226 (2010) 1604–1607.

[29] J.C. Rosa-Neto, F.S. Lira, L.M. Oyama, N.E. Zanchi, L.S. Yamashita, M.L.J. Batista, C.M.O. Nascimento, M. Seelaender, Exhaustive exercise causes an anti-inflammatory effect in skeletal muscle and a pro-inflammatory effect in adipose tissue in rats, Eur. J. Appl. Physiol. 106 (2009) 697–704.

[30] M. Shibasaki, K. Takahashi, T. Itou, S. Miyazawa, M. Ito, J. Kobayashi, H. Bujo, Y. Saito, Alterations of insulin sensitivity by the implantation of 3T3-L1 cells in nude mice. A role for TNF-alpha? Diabetologia 45 (2002) 518–526.

[31] L.S. Silveira, G.D. Pimentel, C.O. Souza, L.A. Biondo, A.A.S. Teixeira, E.A. Lima, H.A.P. Batatinha, J.C. Rosa Neto, F.S. Lira, Effect of an acute moderate-exercise session on metabolic and inflammatory profile of PPAR-α knockout mice, Cell Biochem. Funct. 35 (2017) 510–517.
[33] H. Takahashi, K. Sanada, H. Nagai, Y. Li, Y. Aoki, T. Ara, S. Seno, H. Matsuda, R. Yu, T. Kawada, T. Goto, Over-expression of PPARα in obese mice adipose tissue improves insulin sensitivity, Biochem. Biophys. Res. Commun. 493 (2017) 108–114.
[34] A.A. Teixeira, F.S. Lira, G.D. Pimentel, C. Oliveira de Souza, H. Batatinha, L.A. Biondo, A.S. Yamashita, E.A. Junior, J.C. Neto, Aerobic Exercise Modulates the Free Fatty Acids and Inflammatory Response During Obesity and Cancer Cachexia, Crit. Rev. Eukar. Gene 26 (2016) 187–198.
[35] I. Wernstedt Asterholm, C. Tao, T.S. Morley, Q.A. Wang, F. Delgado-Lopez, Z.V. Wang, P.E. Scherer, Adipocyte inflammation is essential for healthy adipose tissue expansion and remodeling, Cell Metab. 20 (2014) 103–118.