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ORIGINAL RESEARCH ARTICLE

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Exercise rescues the immune response fine-tuned impaired by peroxisome proliferator-activated receptors γ deletion in macrophages

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Abstract

Background: Exercise is a powerful tool for prevention and treatment of many conditions related to the cardiovascular system and also chronic low-grade inflammation. Peroxisome proliferator-activated receptors γ (PPARγ) exerts an import role on the regulation of metabolic profile and subsequent inflammatory response, especially in macrophages.

Purpose: To investigate the effects of 8-week moderate-exercise training on metabolic and inflammatory parameters in mice with PPARγ deficiency in myeloid cells.

Methods: Twelve-week old mice bearing PPARγ deletion exclusively in myeloid cells (PPARγlox/lox Lys $Cre^{-/+}$, knockout [KO]) and littermate controls (PPARγlox/lox Lys $Cre^{-/-}$, wild type [WT]) were submitted to 8-week exercise training (treadmill running at moderate intensity, 5 days/week). Animals were evaluated for food intake, glucose homeostasis, serum metabolites, adipose tissue and peritoneal macrophage inflammation, and basal and stimulated cytokine secretion.

Results: Exercise protocol did not improve glucose metabolism or adiponectin concentrations in serum of KO mice. Moreover, the absence of PPARγ in macrophages exacerbated the proinflammatory profile in sedentary mice. Peritoneal cultured cells had higher tumor necrosis factor-α (TNF-α) secretion in nonstimulated and lipopolysaccharide (LPS)-stimulated conditions and higher Toll-4 receptor (TLR4) gene expression under LPS stimulus. Trained mice showed reduced TNF-α content in adipose tissue independently of the genotype. M2 polarization ability was impaired in KO peritoneal macrophages after exercise training, while adipose tissue-associated macrophages did not present any effect by PPARγ ablation.

Conclusion: Overall, PPARγ seems necessary to maintain macrophages appropriate response to inflammatory stimulus and macrophage polarization, affecting also whole body lipid metabolism and adiponectin profile. Exercise training showed as an efficient mechanism to restore the immune response impaired by PPARγ deletion in macrophages.

KEYWORDS
cytokines, energetic metabolism, aerobic training, transcriptional factor

1 | INTRODUCTION

Regular practice of physical exercise has been shown to exert many beneficial effects in the prevention and treatment of metabolic diseases such as obesity, cardiovascular diseases, and type 2 diabetes (Denham, O'Brien, & Charchar, 2016). Although the mechanism underlying these beneficial effects are still not completely defined, recent evidence indicates that exercise may protect from those diseases by attenuating the chronic low-grade inflammation, which affects many important metabolic organs in those conditions (Petersen & Pedersen, 2005). Indeed, chronic moderate-intensity exercise was shown to improve innate immune system function (Chen, Chen, & Jen, 2010), enhancing macrophages phagocytic ability and influencing its peritoneal phenotype (Kizaki et al., 2008).

Macrophages change their polarization depending on their microenvironment. Classically activated M1 macrophages mainly rely on glycolytic metabolism, display antitumoricidal and antibactericidal activities and enhanced secretion of proinflammatory cytokines and reactive oxygen species. Alternatively activated M2 macrophages, on the other hand, feature oxidative metabolism predominantly and are involved in tissue remodeling and immunomodulatory function (Tugal, Liao, & Jain, 2013). Previous studies have found that peroxisome proliferator-activated receptors γ (PPARγ), a nuclear receptor mainly expressed in adipocytes and macrophages (Lehrke & Lazar, 2005), is an important transcriptional factor involved in the determination of macrophage metabolism and function (Tugal et al., 2013). Indeed, PPARγ, an important regulator of lipid metabolism that exerts anti-inflammatory actions by transrepressing the nuclear factor κB (NF-κB; Rao & Lokesh, 2016), has been reported in macrophages to induce mitochondrial biogenesis and expression of genes involved in oxidative metabolism leading to alternative M2 polarization (Odegaard et al., 2007). In addition, macrophages lacking PPARγ showed prolonged inflammation and a delay in the healing process (Mirza et al., 2015), indicating that PPARγ is required for proper macrophages function.

Pharmacological activation of PPARs in macrophages has shown significant benefits in inflammation attenuation in vitro and in vivo (Paukkeri et al., 2013), furthermore, not only synthetic but endogenous PPARγ ligands have inhibitory effects on proinflammatory mediators production and secretion (Caito et al., 2008). Moderate intensity exercise evidently enhances lipolysis in white adipocytes (Ogasawara et al., 2015) and PPARγ may also be activated by lipid mediators released in the lipolysis acting in an anti-inflammatory manner (Capó, Martorell, Sureda, Tur, & Pons, 2016). Moreover, adipose tissue is an important regulator of metabolism by hormones and adipokines production and associated macrophages are crucial for the tissue homeostasis (Goh, Goh, & Abbasi, 2016).

Thus, due to this importance on exercise-mediated macrophages plasticity, and the PPARγ transcriptional control, the aim of this study was to analyze alterations on metabolic and inflammatory parameters in PPARγ knockout (KO) macrophages of mice submitted to 8-week moderate-exercise training.

2 | METHODS

2.1 | Animals

All procedures were approved by Ethics Committee on Animal Use (CEUA) of the Institute of Biomedical Science (Protocol 112/13CEUA). All mice were from The Jackson Laboratory (Bar Harbor, ME), on a C57BL6/J background and kept at 22 ± 1°C, 12:12-hr light–dark cycle, with free access to tap water and food ad libitum. Mice with PPARγ deletion in myeloid cells and littermate controls were produced by crossing PPARγLox/Lox (B6.129-Ppargtm2Rev/J) with lysozyme M-cre (B6;129P2-Lyz2 < tm1(cre)Ifo>/J) mice. Heterozygous PPARγLox/WT; lysozyme M-cre$^{+/-}$ offspring (WT) were then crossed with PPARγLox/Lox mice to obtain mice with the genotype PPARγLox/Lox; lysozyme M-cre$^{+/-}$ (referred as KO) and their littermates PPARγLox/Lox; lysozyme M-cre$^{-/-}$ (referred as WT). Genomic DNA from mouse tail was isolated for mice genotyping by polymerase chain reaction (PCR) using Taq polymerase purchased from Invitrogen (Carlsbad, CA). PCR products were analyzed by electrophoresis in 2% agarose gel (KASVI) and visualized by ethidium bromide staining, using a 100-bp DNA ladder marker (Invitrogen; Supporting Information Figure S1). Male mice were fed with standard rodent chow diet (13% kcal from fat; Nuvilab, Colombo, PR, Brazil) for 12 weeks. At the end of the study, mice were euthanized by decapitation; blood was collected, and serum was obtained by centrifugation at 3000rpm for 10 min at 4°C.

2.2 | Experimental groups

Mice were subdivided into four groups (8–10 mice each): Wild-type (WT) sedentary, KO sedentary, WT trained, and KO trained. All animals were adapted to a treadmill for 1 week (10 min—10 m/min) and performed a maximum test speed in three moments (week 1/8, week 4/8, and week 8/8). Only trained groups were submitted to the exercise training protocol (5 days/week, 1 hr/day 60% of maximum speed test). After 72-hr resting from the last maximum speed test the animals were euthanized (6-hr fasting).

2.3 | Metabolic profile analysis

Nonesterified fatty acids (NEFA; HR Series NEFA-HR; Wako Pure Chemical Industries, Ltd., Richmond, VA), glucose, triacylglycerol (TG), total cholesterol (TC), and high-density lipoprotein (HDL) were analyzed in serum by a colorimetric method (Labtest, Lagoa Santa, Brazil). Lipids were extracted from the liver with chloroform-methanol (Folch, Lees, & Sloane Stanley, 1957) and TG concentrations were determined by enzymatic assay (Labtest). All assays were performed in duplicates.

2.4 | Insulin (ITT) and glucose tolerance tests (GTT)

After 4-hr fasting, mice received an intraperitoneal injection of insulin (1 U/kg body weight) or D-glucose (2 g/kg body weight). Blood drops were collected from the tail vein before and at 10, 20, 30, and

40 min after the insulin injection. The constant for serum glucose disappearance (KITT) was calculated by linear regression of glycemic levels measured between 5 and 15 min after insulin injection (Bonora et al., 1989). For GTT blood samples were collected from the tail vein before and at 15, 30, 60, 90, and 120 min after the glucose injection. The glucose concentrations at each time point were used to calculate the area under the curve (AUC). Accu-Chek Performa (Roche, São Paulo, SP, Brazil) was used to assess the levels of glucose and the interval of 72 hr between the two test was respected.

2.5 | Enzyme-linked immunosorbent assay (ELISA)

Subcutaneous (inguinal) adipose tissue (80–100 mg) was homogenized in radioimmunoprecipitation assay buffer (0.625% Nonidet P-40, 0.625% sodium deoxycholate, 6.25 mM sodium phosphate, and 1 mM ethylenediaminetetraacetic acid [EDTA] at pH 7.4) containing 10 μg/ml of protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). After centrifugation, the supernatant was used to determine the protein concentration by the Bradford assay (Bio-Rad, Hercules, CA). Cytokines from the supernatant of cultured macrophages and from adipose tissue were assessed in duplicates by ELISA (DuoSet ELISA; R&D Systems, Minneapolis, MN).

2.6 | Peritoneal macrophage isolation and culture

Cytokine production was evaluated in macrophages obtained by washing the peritoneal cavity with 6 ml Roswell Park Memorial Institute (RPMI) culture medium (Sigma-Aldrich), enriched with 10% fetal bovine serum (FBS) and antibiotics. The lavage fluid was centrifuged at 100*g*, the supernatant aspirated, the cell pellet resuspended in 1 ml of complete medium, and a live cell count determined by using trypan blue exclusion and a hemocytometer (5×10^5). Macrophage-rich cultures were obtained by incubating peritoneal cells in 24-well polystyrene culture plates for 2 hr at 37°C in a 5% CO_2, humidified air environment. Nonadherent cells were removed by washing with RPMI. Adherent cells were then collected using cold phosphate-buffered saline (PBS) 2% simulated body fluid for further flow-cytometry analysis or incubated with 2.5 ng/ml of lipopolysaccharide (LPS; *Escherichia coli*, serotype 0111:B4; Sigma-Aldrich) for 24 hr (Zhang, Goncalves, & Mosser, 2008). The medium was collected for the determination of interleukin-6 (IL-6), IL-10, IL-1β, monocyte chemoattractant protein-1 (MCP-1), IL-1ra, and TNF-α by ELISA (R&D System) according to the manufacturer's instruction.

2.7 | Adipose tissue-resident macrophages and peritoneal macrophages flow cytometry

Adipose tissue was digested with collagenase type II (2 mg/ml; Sigma-Aldrich) in PBS with EDTA (2 mM; Sigma-Aldrich) and 0.5% FBS for 60 min at 37°C in a shaker. The suspension was filtered using a cell strainer 100 μm and subsequently centrifuged at 400 rpm for 10 min at 4°C. The supernatant was decanted and the pellet was resuspended in 3 ml lysis buffer (17 mM Tris–HCl + 0.144 M de cloreto de amônia, pH 7.2) for 2 min for purification of leukocytes. After centrifugation at 3,000 rpm for 10 min at 4°C, cells were washed with PBS and stained with the following antibody panel: Anti-CD11b, F4/80, CD11c, CD206, diluted 1:100 (BioLegend, San Diego, CA). M1 macrophages were characterized by expression of CD11c in adipose tissue-resident macrophages and CD86 in peritoneal macrophages concomitant with F4/80 and CD11b markers and M2 macrophages by expression of CD206 concomitant with F4/80 and CD11b markers (Supporting Information Table S2). The characterization of subpopulations of leukocytes was performed on the FACS CANTOII machine (Beckton Dickson, Franklin Lakes, NJ) and data analysis was performed with FlowJo 9.5.3 software Tree Star (Ashland, OR).

2.8 | Real-time reverse transcription PCR

Macrophage total RNA was extracted with TRIzol reagent (Invitrogen Life Technologies, Grand Island, NY), following the method described by Chomczynski and Sacchi (1987). Reverse transcription to complementary DNA (cDNA) was performed using the high capacity cDNA kit (Applied Biosystems, Foster, CA). Gene expression was evaluated by real-time PCR according to Higuchi, Dollinger, Walsh, and Griffith (1992), using Fast SYBR Green Master Mix (Applied Biosystems) as a fluorescent dye. Primer sequences are shown in Supporting Information Table S1. Quantification of gene expression was carried out using the glyceraldehyde 3-phosphate dehydrogenase gene as an internal control, as previously described by Liu and Saint (2002).

2.9 | Statistical analysis

Results are presented as mean ± SEM. All groups were compared in the effects of genotype (WT x KO) and exercise (Sedentary x Trained) by using two-way ANOVA followed by Bonferroni post hoc test using GraphPad Prism version 6.01 (La Jolla, CA). The significance level was set at $p < 0.05$.

3 | RESULTS

3.1 | Body composition and performance does not change by depleting PPARγ in myeloid cells

We first analyzed mice caloric ingestion during time protocol. Despite a considerable reduction in caloric ingestion in both genotypes of trained mice (Figure 1) no significant changes in body weight or adiposity index (Supporting Information Figure S2) were found. All animals were submitted to a maximum speed test in the moments of the protocol and there was a tendency to increase the maximum speed on Test 2 (4 weeks) in the trained groups but this tendency did not extended to the final speed test (8 weeks; Supporting Information Figure S3).

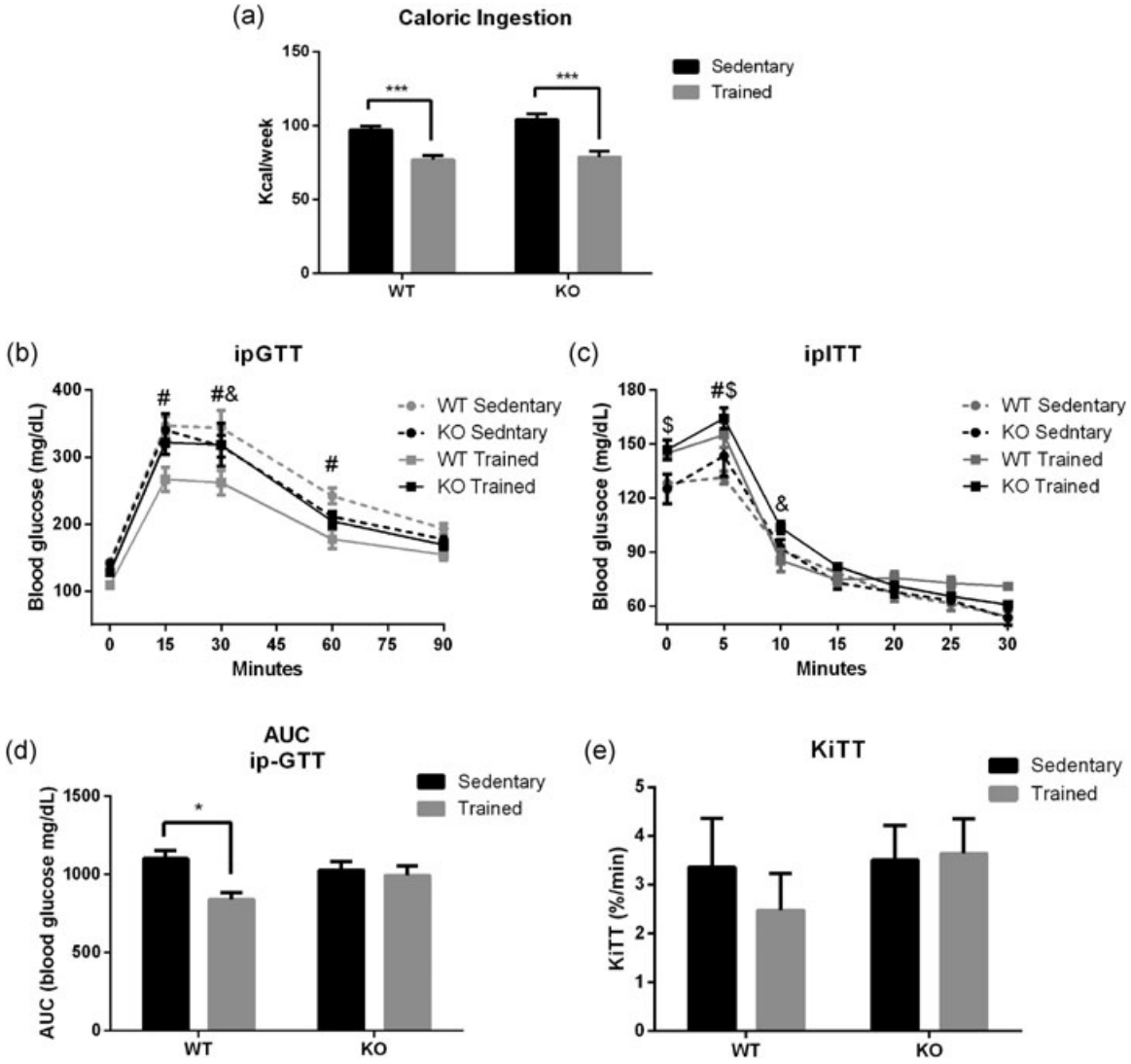

FIGURE 1 Effects of exercise on caloric ingestion and glucose homeostasis in mice with PPARγ deletion in myeloid cells (KO) and littermate controls (WT). Average of caloric intake within 12 weeks of the protocol (a), ipGTT (b), ipITT (c), AUC of the glucose tolerance test (d), and KITT (e). All values are mean ± *SEM* (n = 8–10/group); two-way ANOVA followed by Bonferroni test. $^{\#}$WT Sedentary versus WT trained; $^{\$}$KO Sedentary versus KO Trained; $^{\&}$WT Trained versus KO Trained; $p < 0.05$; $^{***}p < 0.001$. ANOVA: analysis of variance; AUC: area under the curve; ipGTT: intraperitoneal glucose tolerance test; ipITT: intraperitoneal insulin tolerance test; KITT: blood glucose lowering constant; KO: knockout; PPARγ: peroxisome proliferator-activated receptors γ; WT: wild type

3.2 | Training improved metabolic parameters in PPARγ KO mice

Regarding glucose homeostasis, we performed a GTT and ITT and observe that only WT when trained improved glucose uptake by decreasing the AUC (Figure 1d) and insulin sensibility by increasing KITT (Figure 1e). To access the lipid profile, we performed colorimetric methods using the isolated serum. When compared genotypic differences between sedentary animals, was observed the lower concentration of TG on KO mice (Figure 2d). On the other hand, exercise was able to balance this difference in the trained groups. Also, KO mice, when trained, reduced total glucose (Figure 2a) but increased TC when compared with WT trained (Figure 2b). Adiponectin was significantly higher only in WT mice when exercised (Figure 2c). HDLs and nonesterified fatty acids had no differences among genotypes or training (*data not shown*).

3.3 | Exercise boost immune response in the absence of PPARγ in myeloid cells

Flow cytometry was used to identify M1 and M2 surface markers in peritoneal and adipose tissue-associated macrophages. There was no difference between M1 and M2 counts from sedentary WT and KO mice within the two macrophages subpopulations (Figure 3a,b). Exercise enhanced M1 and M2 peritoneal macrophages in WT but

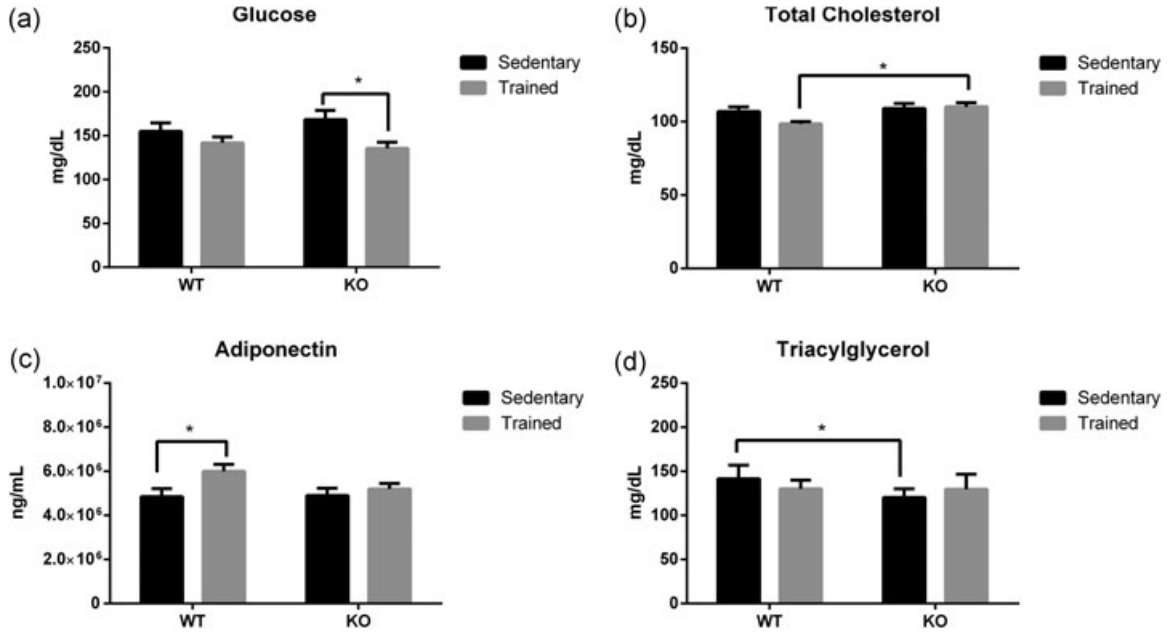

FIGURE 2 Effects of moderate exercise on metabolic variables in mice with PPARγ deletion in myeloid cells (KO) and littermate controls (WT). Serum glucose (a), total cholesterol (b), adiponectin (c), and triacylglycerol (d). All values are mean ± *SEM* ($n = 8–10$/group; two-way ANOVA followed by Bonferroni test; $^*p < 0.05$. ANOVA: analysis of variance; KO: knockout; PPARγ: peroxisome proliferator-activated receptors γ; WT: wild type

KO cells fail to boost M2 (Figure 3a) withal in adipose tissue macrophages there were a significant higher populations of M2 cells (Figure 3b).

We then analyzed pro- and anti-inflammatory cytokines content from the subcutaneous adipose tissue. No differences were observed between genotypes in sedentary groups. Exercise training was able to reduce the proinflammatory cytokine TNF-α in WT (Figure 4a). Exercised KO mice showed the tendency to raise MCP-1 ($p = 0.061$) when compared with sedentary KO (Figure 4c) and significant increased IL-6 and IL-1ra when compared with sedentary KO and trained WT. No modulations by genotype or exercise were observed in adipose tissue content of IL-1β, IL-10, and adiponectin (Figure 4d,e,g).

To determinate cytokines production from peritoneal macrophages the cells were challenged or not with LPS for 24 hr. Our results showed that KO macrophages from sedentary mice presented higher TNF-α concentrations under nonstimualted and stimulated conditions and the exercise was able to restore this profile (Figure 5a). WT peritoneal macrophages from trained mice increased expression of IL-6, IL-1β, IL-10, and IL-1ra under LPS stimulation. KO macrophages from trained mice presented higher IL-1ra in non-stimulated conditions (Figure 5f) but lower IL-6 (Figure 5b) and higher IL-1β and IL-10 when stimulated (Figure 5d,e).

To understand the mechanisms behind peritoneal macrophages profile and cytokines release we evaluated some inflammatory target genes by RT-PCR. HIF-1α was reduced in WT and KO trained when stimulated by LPS (Figure 6a). TLR4 from KO sedentary macrophages increased when stimulated with LPS but the exercise was able to restore this phenomenon (Figure 6b). There was no difference in NF-κB mRNA expression (Figure 6c).

4 | DISCUSSION

We investigated herein whether myeloid cell PPARγ is involved in the beneficial actions of moderate-intensity exercise training. We mainly found that exercise training reduces adipose tissue inflammation and cytokine secretion and stimulate macrophage polarization into an alternative M2 phenotype independent of myeloid cells PPARγ.

Improvements in glucose tolerance or insulin sensitivity are expected effects of exercise also in normal diet mice (Gollisch et al., 2009), although this outcome was not found in KO mice. Our results showed that exercise did not affect KO mice glucose tolerance measured by intraperitoneal GTT but they presented lower resting glucose levels after exercise protocol. These mice had normal insulin function as shown by no significant alterations on fasting insulin, HOMA-IR or insulin resistance test compared with the WT, thus our hypothesis is that their initial fasting glucose was slightly higher than the other groups so small alterations such as low caloric intake and exercise were able to incite a decrease.

Differences in lipid profile between the genotypes also have to be highlighted. Sedentary KO mice had lower serum TG concentrations and a slight but no significant higher TG content in the liver when compared with WT sedentary mice, indicating that possibly TG has been accumulating in KO mice liver. Lack of PPARγ in kupffer cells (liver macrophages) may lead to an M1 or classical activation, which is more glycolytic (Galvan-Pena & O'Neill, 2014). Additionally, previous studies observed upregulation of genes related to fatty acids biosynthesis (acetyl-CoA carboxylase and fatty acid synthase) and gluconeogenesis (G6PC and Fbp1; Moran-Salvador et al., 2011)

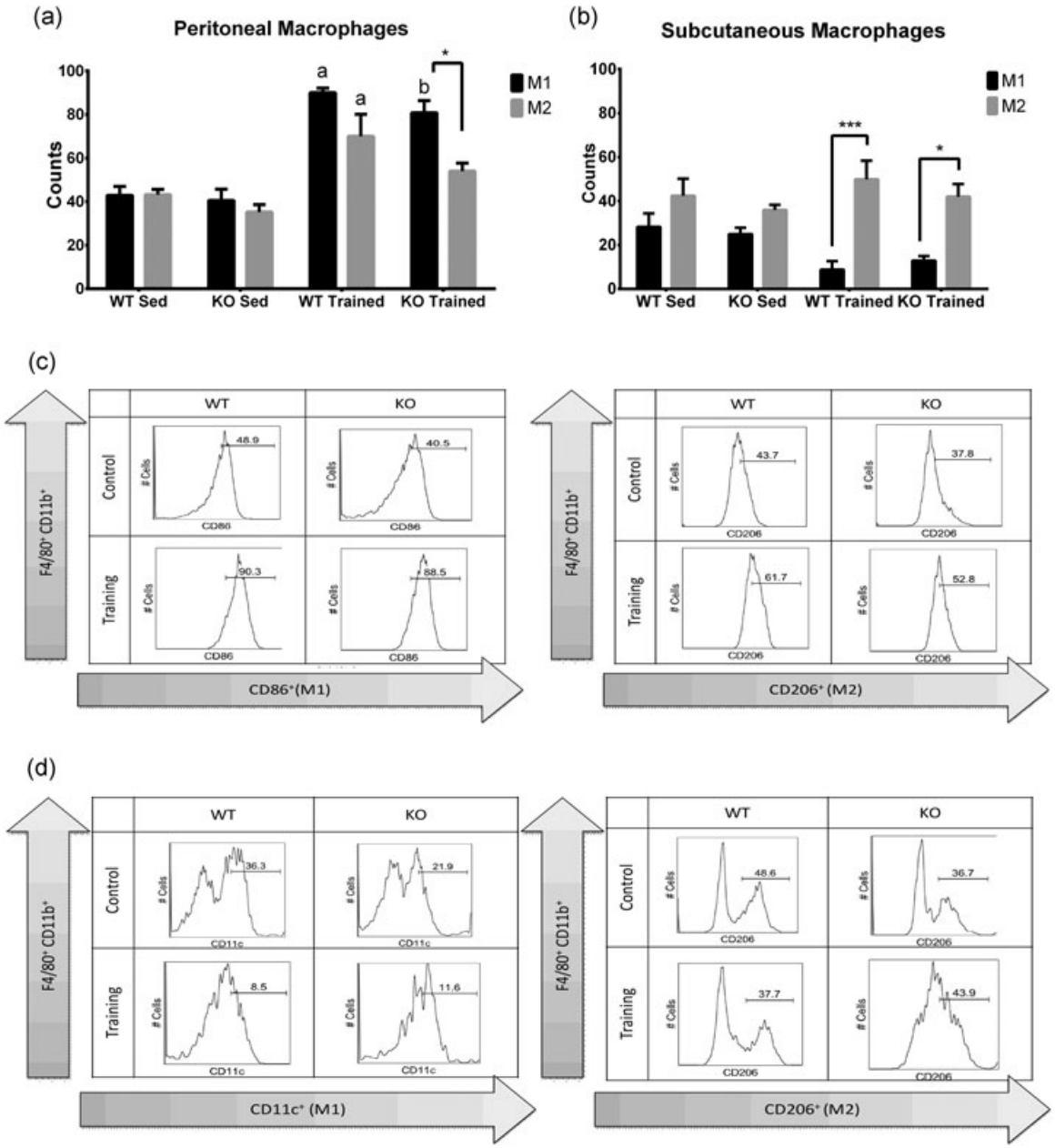

FIGURE 3 Flow cytometry of peritoneal macrophages (a) and a stromal portion from subcutaneous adipose tissue (b). Cells were isolated from the peritoneal cavity or enzymatically digested fat pads, and after the exclusion of doublets and debris, immune cells were identified by CD45 staining. A sequential gating strategy was first used to identify populations expressing specific markers ($F4/80^+CD11b^+$), followed by the identification of populations with overlapping expression patterns: Histogram of cell counts peritoneal macrophages M1 ($CD86^+$), M2 ($CD206^+$; c), and adipose tissue macrophages M1 ($CD11c^+$) M2 macrophages (CD206+; d). Two-way ANOVA followed by Bonferroni test. [a]Different from WT sedentary; [b]different from KO sedentary; ***$p < 0.001$, *$p < 0.05$. KO: knockout; WT: wild type

in the liver of macrophage-specific PPARγ KO mice, as a consequence, an accumulation of TG in the liver may occur.

An important adipokine that is involved in insulin sensitivity and glucose metabolism is adiponectin, which is produced by adipose tissue and controlled by PPARγ (Astapova & Leff, 2012). Our model lacks PPARγ in myeloid cell lineage but this mice phenotype also failed to increase adiponectin in the serum after exercise protocol. Studies have been suggesting that serum adiponectin can be a biomarker for PPARγ activation in vivo (Yang et al., 2004); moreover, in subcutaneous adipose tissue, the adiponectin content seems not be affected by the absence of PPARγ. TNF-α has been strongly suggested as one of the molecules responsible for insulin resistance, more specific in 3T3-L1 adipocytes, Maeda et al. (2001) found that TNF-α had suppressor effects in adiponectin secretion in a dose-dependent manner, corroborating with the outcomes we had in adipose tissue, no changes in TNF-α concentration nor adiponectin differences between WT and KO sedentary groups.

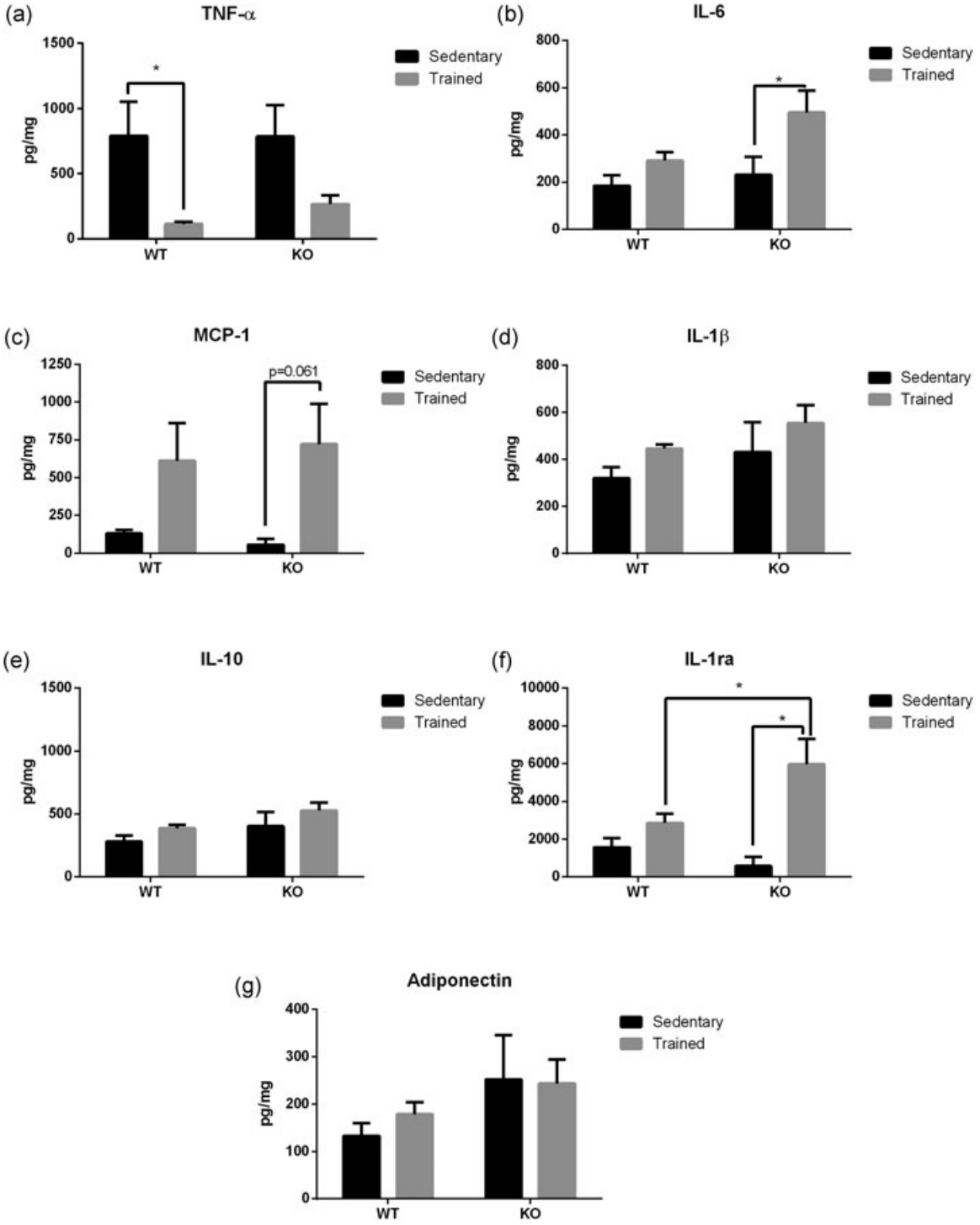

FIGURE 4 Effect of moderate exercise in subcutaneous adipose tissue cytokine content in mice with PPARγ deletion in myeloid cells (KO) and littermates controls (WT). Content of TNF-α (a), IL-6 (b), MCP-1 (c), IL-1β (d), IL-10 (e), IL-1ra (f), and adiponectin (g). Cytokine concentrations in the tissue were determined by ELISA ($n = 8–10$/group) and expressed by total protein content. Data are presented as mean ± *SEM*; two-way ANOVA followed by Bonferroni test. $^*p < 0.05$. ANOVA: analysis of variance; ELISA: enzyme-linked immunosorbent assay; IL-6: interleukin-6; IL-10: Interleukin-10; IL-1ra: interleukin-1 receptor antagonist; KO: knockout; MCP-1: monocyte chemoattractant protein-1; PPARγ: peroxisome proliferator-activated receptors γ; TNF-α: tumor necrosis factor-α; WT: wild type

Regarding macrophages switch, no difference between M1 and M2 subcutaneous and peritoneal macrophages from WT and KO sedentary mice was observed. However, when trained, both populations of peritoneal macrophages arose in WT, indicating that chronic exercise recruits macrophages to the peritoneum. A strong hypothesis for this exacerbated recruitment on peritoneal cavity may be due to an increase in gastrointestinal permeability that has been discussed as a plausible consequence of the mechanical stress caused by running

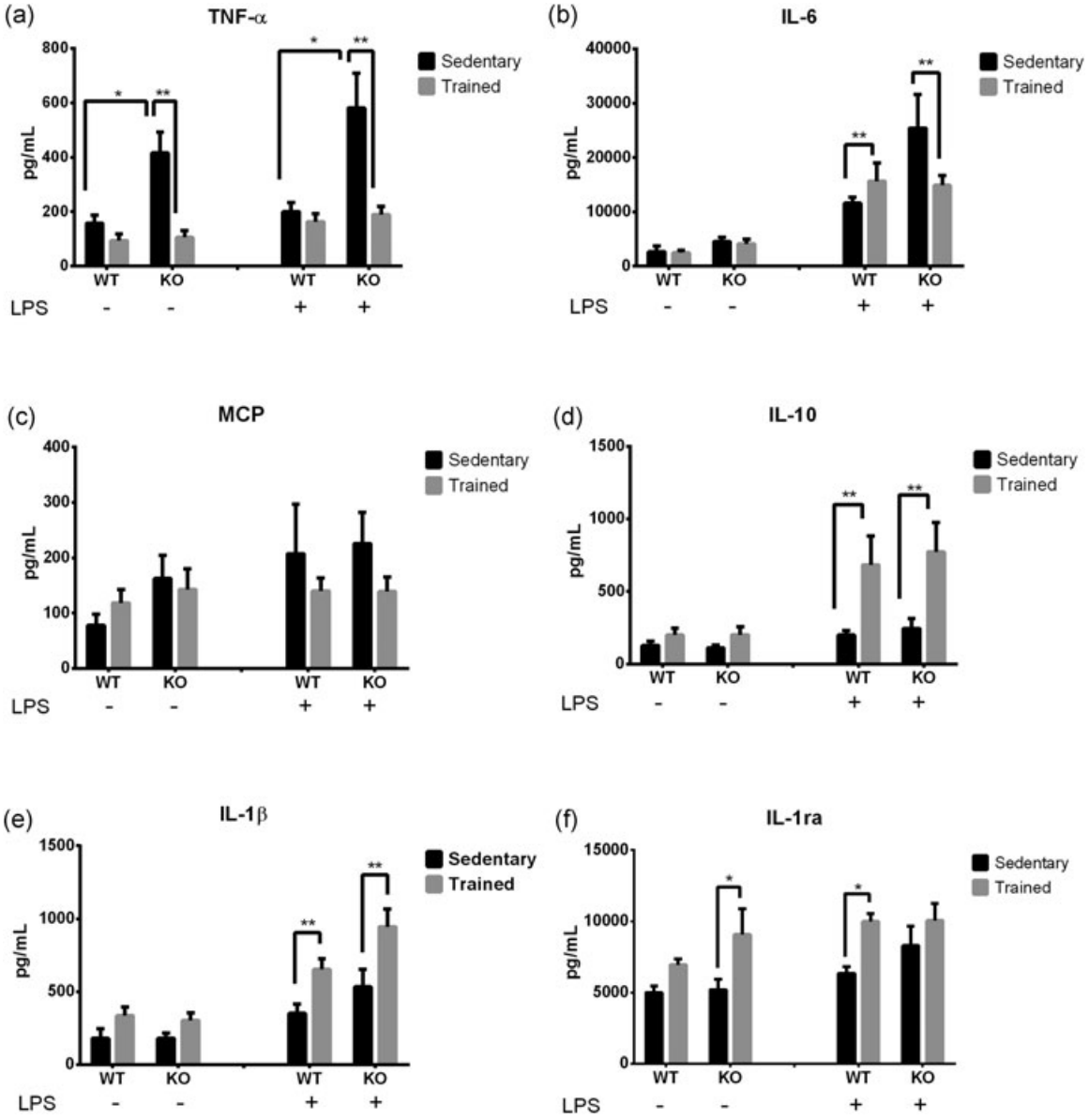

FIGURE 5 Effect of moderate exercise on basal and LPS-stimulated cytokine secretion by peritoneal macrophages of mice with PPARγ deletion in myeloid cells (KO) and littermates controls (WT). TNF-α (a), IL-6 (b), MCP-1 (c), IL-10 (d), IL-1β (e), and IL-1ra (f). Cytokines concentrations from the culture medium was determined by ELISA ($n = 8–10$/group). Data are presented as mean ± *SEM*; two-way ANOVA followed by Bonferroni test. $^{*}p < 0.05$, $^{**}p < 0.01$. ANOVA: analysis of variance; ELISA: enzyme-linked immunosorbent assay; IL-6: interleukin-6; IL-10: interleukin-10; IL-1ra: interleukin-1 receptor antagonist; IL-1β: interleukin-1β; KO: knockout; LPS: lipopolysaccharide; MCP-1: monocyte chemoattractant protein-1; PPARγ: peroxisome proliferator-activated receptors γ; TNF-α: tumor necrosis factor-α; WT: wild type

(Karhu et al., 2017). KO mice also enhanced M1 population by exercise stimulus, but fail to increase M2 subset, indicating that PPARγ might play a role in exercise-induced M2 peritoneal macrophages recruitment and polarization. M2 macrophages polarization as a result of exercise intervention has been shown in studies involving humans (Yakeu et al., 2010) and mice (Kawanishi, Yano, Yokogawa, & Suzuki, 2010), and the mechanisms involved in this are strongly associated with PPARγ (Ruffino et al., 2016).

In subcutaneous adipose tissue, no overall increase in macrophages populations as showed in peritoneum but an exercise-induced M2 subset prevalence in both WT and KO mice. It suggests that adipose tissue-resident macrophages from lean mice may not require PPARγ to assume M2 profile under trained condition. It is a topic that has been widely discussed in adipose tissue from obese animals (Ahn & Kim, 2014) although we could observe similar effects on the M2 phenotypic switch in lean mice. Further, this macrophages subset profile in subcutaneous adipose tissue was confirmed by cytokines content. No differences between sedentary genotypes but lower levels of a classic proinflammatory cytokine, TNF-α, was observed in WT trained animals. IL-6 protein content was elevated only in KO trained animals and a similar outcome

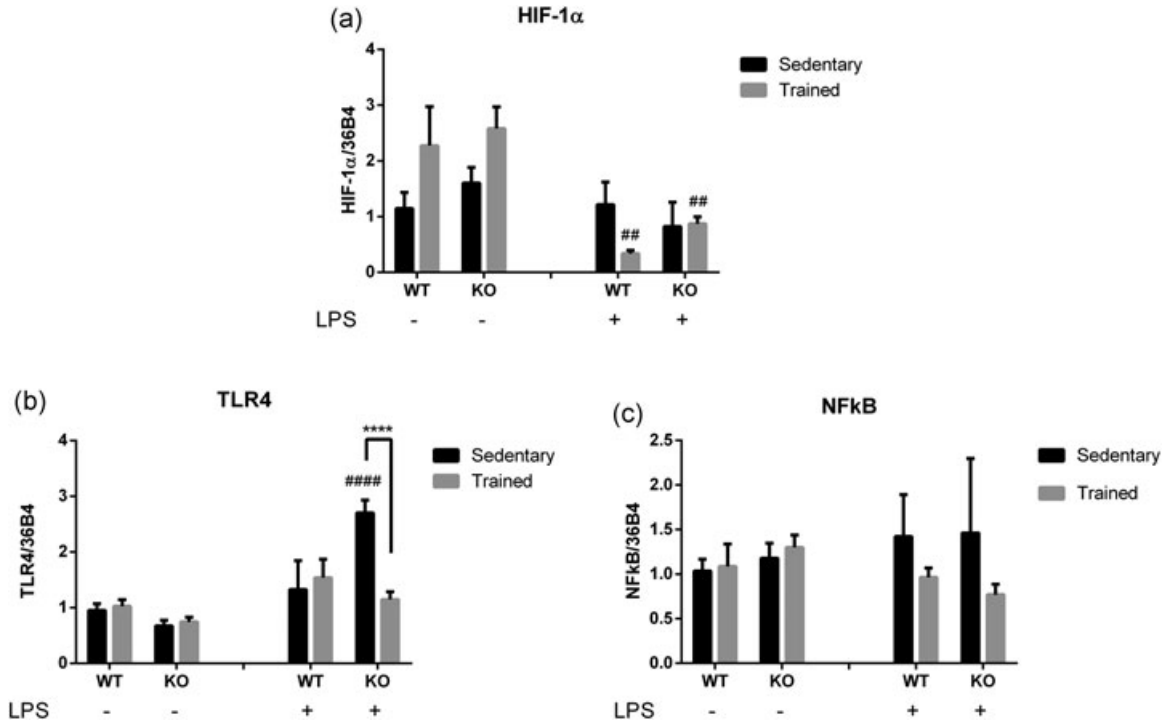

FIGURE 6 Gene expression from cultured macrophages. Expression of genes involved in inflammatory pathways in LPS-stimulated peritoneal macrophages. HIF-1α (a), TLR4 (b), and NF-kB (c). Data are shown as mean ± *SEM* folder; two-way ANOVA followed by Bonferroni test. ## Different from nonstimulated $p < 0.01$, ####Different from nonstimulated $p < 0.0001$; ****$p < 0.0001$. ANOVA: analysis of variance; HIF-1α: hypoxia-inducible factor 1-α; LPS: lipopolysaccharide; NF-kB: nuclear factor κB; TLR4: toll-like receptor 4

was reported by Van Pelt, Guth, and Horowitz (2017) in overweight rats after aerobic acute exercise.

From other perspective, IL-6 also plays dual role being proinflammatory when associated to diseases such as metabolic syndrome (Bao, Liu, & Wei, 2015) and anti-inflammatory when induced by exercise in muscle fibers (Petersen & Pedersen, 2005) and in a subcutaneous adipose tissue even for long periods postexercise (Lyngsø, Simonsen, & Bülow, 2002). It is also known that IL-6 effect is lipolytic under stress situation (Lyngso et al., 2002). We suggest that in this case, IL-6 might be released by adipocytes in response to exercise stress, once peritoneal macrophages do not present any alteration in this cytokine under basal conditions (non-LPS). MCP-1 and its receptor chemokine receptor type 2 CCR2 act as an attractant for monocytes and subsequently macrophages in adipose tissue and are considered as marker of inflammation (Lumeng, Bodzin, & Saltiel, 2007). We report a tendency in MCP-1 increase in KO trained animals but was not significant ($p < 0.06$), once more suggesting a susceptibility of KO mice to impaired anti-inflammatory effects of exercise.

Cytokine secretion from peritoneal macrophages showed that sedentary KO mice presented higher expression of TNF-α under control and LPS-stimulated conditions thus exercise training was able to lower it in both states, corroborating with studies performed in healthy and sedentary young male which observed decreases in serum TNF-α and interferon-γ levels (Jahromi et al., 2014). IL-6 levels from KO trained animals had a positive response to exercise under LPS stimulus, however, the same cytokine had an opposite response in adipose tissue macrophages. It suggests that exercise plays an immunomodulatory role, and not just an anti-inflammatory function as constantly described in the literature (Pellegrin, Aubert, Bouzourène, Amstutz, & Mazzolai, 2015; Petersen & Pedersen, 2005). Agreeing with the hypothesis of exercise being immunomodulatory, peritoneal macrophages from trained animals also increased anti-inflammatory cytokines under LPS stimulation. As an example, IL-10 secretion by macrophages via TLR4 stimulation (e.g., LPS) may be an attempt to control the excess of proinflammatory cytokine secretion (Iyer, Ghaffari, & Cheng, 2010).

To better understand the mechanisms behind cytokines production we evaluated mRNA expression of some inflammatory genes and we could observe that TLR4 dramatically increase in KO sedentary mice after LPS exposure but was restored by exercise. TLR4 is a receptor that recognizes microbial structures and induces to immune responses by NF-kB inflammatory pathway (Barton & Medzhitov, 2003). In this context, once again PPARγ has to be highlighted by inhibiting transcriptional activation of NF-kB in macrophages (Wang et al., 2015). It suggests that macrophages lacking PPARγ react excessively to LPS stimulus and increase inflammation but exercise training was able to reduce this hyper-inflammation caused by PPARγ deletion.

We conclude that PPARγ is necessary to maintain macrophage appropriate response to inflammatory stimulus and macrophage polarization, affecting also whole body lipid metabolism and adiponectin profile. Exercise training showed as an efficient mechanism to restore the immune response fine-tune impaired by PPARγ deletion in macrophages.

ACKNOWLEDGMENTS

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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REFERENCES

Ahn, N., & Kim, K. (2014). Combined influence of dietary restriction and treadmill running on MCP-1 and the expression of oxidative stress-related mRNA in the adipose tissue in obese mice. *Journal of Exercise Nutrition & Biochemistry*, *18*(3), 311–318.

Astapova, O., & Leff, T. (2012). Adiponectin and PPARgamma: Cooperative and interdependent actions of two key regulators of metabolism. *Vitamins and Hormones*, *90*, 143–162.

Bao, P., Liu, G., & Wei, Y. (2015). Association between IL-6 and related risk factors of metabolic syndrome and cardiovascular disease in young rats. *International Journal of Clinical and Experimental Medicine*, *8*(8), 13491–13499.

Barton, G. M., & Medzhitov, R. (2003). Toll-like receptor signaling pathways. *Science*, *300*(5625), 1524–1525.

Bonora, E., Moghetti, P., Zancanaro, C., Cigolini, M., Querena, M., Cacciatori, V., … Muggeo, M. (1989). Estimates of in vivo insulin action in man: Comparison of insulin tolerance tests with euglycemic and hyperglycemic glucose clamp studies. *Journal of Clinical Endocrinology and Metabolism*, *68*(2), 374–378.

Caito, S., Yang, S. R., Kode, A., Edirisinghe, I., Rajendrasozhan, S., Phipps, R. P., & Rahman, I. (2008). Rosiglitazone and 15-deoxy-Delta12, 14-prostaglandin J2, PPARgamma agonists, differentially regulate cigarette smoke-mediated pro-inflammatory cytokine release in monocytes/macrophages. *Antioxidants and Redox Signaling*, *10*(2), 253–260.

Capó, X., Martorell, M., Sureda, A., Tur, J. A., & Pons, A. (2016). Effects of dietary Docosahexaenoic, training and acute exercise on lipid mediators. *Journal of the International Society of Sports Nutrition*, *13*, 16.

Chen, M. F., Chen, H. I., & Jen, C. J. (2010). Exercise training upregulates macrophage MKP-1 and affects immune responses in mice. *Medicine and Science in Sports and Exercise*, *42*(12), 2173–2179.

Chomczynski, P., & Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analytical Biochemistry*, *162*(1), 156–159.

Denham, J., O'Brien, B. J., & Charchar, F. J. (2016). Telomere length maintenance and cardio-metabolic disease prevention through exercise training. *Sports Medicine*, *46*(9), 1213–1237.

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, *226*(1), 497–509.

Galván-Peña, S., & O'Neill, L. A. (2014). Metabolic reprograming in macrophage polarization. *Frontiers in Immunology*, *5*, 420.

Goh, J., Goh, K. P., & Abbasi, A. (2016). Exercise and adipose tissue macrophages: New frontiers in obesity research? *Front Endocrinol (Lausanne)*, *7*, 65.

Gollisch, K. S. C., Brandauer, J., Jessen, N., Toyoda, T., Nayer, A., Hirshman, M. F., & Goodyear, L. J. (2009). Effects of exercise training on subcutaneous and visceral adipose tissue in normal- and high-fat diet-fed rats. *American Journal of Physiology, Endocrinology and Metabolism*, *297*(2), E495–E504.

Higuchi, R., Dollinger, G., Walsh, P. S., & Griffith, R. (1992). Simultaneous amplification and detection of specific DNA sequences. *Biotechnology*, *10*(4), 413–417.

Iyer, S. S., Ghaffari, A. A., & Cheng, G. (2010). Lipopolysaccharide-mediated IL-10 transcriptional regulation requires sequential induction of type I IFNs and IL-27 in macrophages. *Journal of Immunology*, *185*(11), 6599–6607.

Jahromi, A. S., Zar, A., Ahmadi, F., Krustrup, P., Ebrahim, K., Hovanloo, F., & Amani, D. (2014). Effects of endurance training on the serum levels of tumour necrosis factor-alpha and interferon-gamma in sedentary men. *Immune Network*, *14*(5), 255–259.

Karhu, E., Forsgård, R. A., Alanko, L., Alfthan, H., Pussinen, P., Hämäläinen, E., & Korpela, R. (2017). Exercise and gastrointestinal symptoms: Running-induced changes in intestinal permeability and markers of gastrointestinal function in asymptomatic and symptomatic runners. *European Journal of Applied Physiology and Occupational Physiology*, *117*(12), 2519–2526.

Kawanishi, N., Yano, H., Yokogawa, Y., & Suzuki, K. (2010). Exercise training inhibits inflammation in adipose tissue via both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in high-fat-diet-induced obese mice. *Exercise Immunology Review*, *16*, 105–118.

Kizaki, T., Takemasa, T., Sakurai, T., Izawa, T., Hanawa, T., Kamiya, S., … Ohno, H. (2008). Adaptation of macrophages to exercise training improves innate immunity. *Biochemical and Biophysical Research Communications*, *372*(1), 152–156.

Lehrke, M., & Lazar, M. A. (2005). The many faces of PPARgamma. *Cell*, *123*(6), 993–999.

Liu, W., & Saint, D. A. (2002). Validation of a quantitative method for real time PCR kinetics. *Biochemical and Biophysical Research Communications*, *294*(2), 347–353.

Lumeng, C. N., Bodzin, J. L., & Saltiel, A. R. (2007). Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *Journal of Clinical Investigation*, *117*(1), 175–184.

Lyngsø, D., Simonsen, L., & Bülow, J. (2002). Interleukin-6 production in human subcutaneous abdominal adipose tissue: The effect of exercise. *Journal of Physiology*, *543*(Pt 1), 373–378.

Maeda, N., Takahashi, M., Funahashi, T., Kihara, S., Nishizawa, H., Kishida, K., … Matsuzawa, Y. (2001). PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes*, *50*(9), 2094–2099.

Mirza, R. E., Fang, M. M., Novak, M. L., Urao, N., Sui, A., Ennis, W. J., & Koh, T. J. (2015). Macrophage PPARgamma and impaired wound healing in type 2 diabetes. *Journal of Pathology*, *236*(4), 433–444.

Morán-Salvador, E., López-Parra, M., García-Alonso, V., Titos, E., Martínez-Clemente, M., González-Périz, A., … Clària, J. (2011). Role for PPARgamma in obesity-induced hepatic steatosis as determined by hepatocyte- and macrophage-specific conditional knockouts. *FASEB Journal*, *25*(8), 2538–2550.

Odegaard, J. I., Ricardo-Gonzalez, R. R., Goforth, M. H., Morel, C. R., Subramanian, V., Mukundan, L., … Chawla, A. (2007). Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. *Nature*, *447*(7148), 1116–1120.

Ogasawara, J., Izawa, T., Sakurai, T., Sakurai, T., Shirato, K., Ishibashi, Y., … Kizaki, T. (2015). The molecular mechanism underlying continuous exercise training-induced adaptive changes of lipolysis in white adipose cells. *Journal of Obesity*, *2015*, 473430–10.

Paukkeri, E. L., Leppänen, T., Lindholm, M., Yam, M. F., Asmawi, M., Kolmonen, A., … Moilanen, E. (2013). Anti-inflammatory properties of a dual PPARgamma/alpha agonist muraglitazar in in vitro and in vivo models. *Arthritis Research & Therapy*, *15*(2), R51.

Pellegrin, M., Aubert, J. F., Bouzourène, K., Amstutz, C., & Mazzolai, L. (2015). Voluntary exercise stabilizes established angiotensin II-dependent

atherosclerosis in mice through systemic anti-inflammatory effects. *PLOS One*, *10*(11), e0143536.

Van Pelt, D. W., Guth, L. M., & Horowitz, J. F. (2017). Aerobic exercise elevates markers of angiogenesis and macrophage IL-6 gene expression in the subcutaneous adipose tissue of overweight-to-obese adults. *Journal of Applied Physiology*, *123*(5), 1150–1159.

Petersen, A. M. W., & Pedersen, B. K. (2005). The anti-inflammatory effect of exercise. *Journal of Applied Physiology* (1985), *98*(4), 1154–1162.

Rao, Y. P., & Lokesh, B. R. (2016). Down-regulation of NF-kappaB expression by n-3 fatty acid-rich linseed oil is modulated by PPARgamma activation, eicosanoid cascade and secretion of cytokines by macrophages in rats fed partially hydrogenated vegetable fat. *European Journal of Nutrition*, *56*, 1135–1147.

Ruffino, J. S., Davies, N. A., Morris, K., Ludgate, M., Zhang, L., Webb, R., & Thomas, A. W. (2016). Moderate-intensity exercise alters markers of alternative activation in circulating monocytes in females: A putative role for PPARgamma. *European Journal of Applied Physiology and Occupational Physiology*, *116*(9), 1671–1682.

Tugal, D., Liao, X., & Jain, M. K. (2013). Transcriptional control of macrophage polarization. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *33*(6), 1135–1144.

Wang, L., Chen, K., Liu, K., Zhou, Y., Zhang, T., Wang, B., & Mi, M. (2015). DHA inhibited AGEs-induced retinal microglia activation via suppression of the PPARgamma/NFkappaB pathway and reduction of signal transducers in the AGEs/RAGE axis recruitment into lipid rafts. *Neurochemical Research*, *40*(4), 713–722.

Yakeu, G., Butcher, L., Isa, S., Webb, R., Roberts, A. W., Thomas, A. W., … Morris, K. (2010). Low-intensity exercise enhances expression of markers of alternative activation in circulating leukocytes: Roles of PPARgamma and Th2 cytokines. *Atherosclerosis*, *212*(2), 668–673.

Yang, B., Brown, K. K., Chen, L., Carrick, K. M., Clifton, L. G., McNulty, J. A., … Pahel, G. L. (2004). Serum adiponectin as a biomarker for in vivo PPARgamma activation and PPARgamma agonist-induced efficacy on insulin sensitization/lipid lowering in rats. *BMC Pharmacology*, *4*, 23.

Zhang, X., Goncalves, R., & Mosser, D. M. (2008). The isolation and characterization of murine macrophages. *Curr Protoc Immunol*, *Chapter 14 (Unit 14)*, 11.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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