Physical Exercise Mitigates the Disturbs Caused in Ventral Prostate by High Fat Diet, Clarifying the Role of PPAR-Alpha

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Research Article

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Abstract

Body fat resulting from the consumption of fatty foods develops inflammation, a precursor to obesity, diabetes mellitus and prostatic diseases, causing tissue changes. On the other hand, aerobic physical exercise in conjunction with PPARα promotes an increase in energy expenditure, modulating cellular metabolism by altering prostate cell metabolism. This study aimed to elucidate the effect of the gene transcription factor PPARα as a prostatic regulator associated with the effects of aerobic physical exercise in mice fed to a high-fat diet. Wild Type and PPARα $^{-/-}$ mice (C57BL/6J) were fed a high-fat diet and submitted to aerobic physical training for 8 weeks. Prostates of male mice were analyzed histologically and metabolically for PIN progression. These findings suggest that the absence of PPARα reduces adiposity and but does not modify the expression AR and GR, up-regulate prostate inflammation, and suppresses Fas (CD95/Apo-1) increase in TNF-α, induced by prostatic lesions. On the other hand, aerobic physical exercise was effective in protecting prostatic damage induced by a high-fat diet, both in wild type and in PPARα$^{-/-}$ animals. The absence PPARα induces an inflammatory process and prostatic changes and associated with a high-fat diet, however, these inflammatory processes induce increased apoptosis and may further inhibit cell growth associated with physical exercise.

Introduction

Obesity is a multifactorial disease characterized by increased visceral and subcutaneous fat, as a result of the imbalance between energy consumption and caloric expenditure [1]. Obesity develops mainly due to an inadequate diet rich in fats, causing chronic inflammation, which is responsible for acting on various metabolisms and alterations in the prostatic microenvironment, promoted by cellular disturbances in the metabolism of glucose and lipids [2,3]. Besides genetic factors and advanced age, it is described in the literature that insufficient physical activity associated with irregular eating habits are risk factors not only for obesity [4], but also for prostate cancer (PCa) [5–7]. Data show prostate cancer as the cancer with the highest incidence of new cases and the second-highest death rate in the USA in the year 2020 [8] as well as in Brazil, according to data from INCA, 2020 [9]. Prostatic changes induced by obesity may be due to hormone dysregulation and induction of inflammatory processes [10,11]. There is support for an association between chronic inflammation and PCa, confirming the proliferative response resulting from an inflammation potentiating epithelial growth in neoplasia [12,13].

The alpha peroxisome proliferator-activated receptor (PPAR) is a nuclear hormone receptor that acts as a lipid ligand-activated gene transcription factor responsible for the balance in cellular metabolism, energy metabolism in nutritional response, and inflammatory signaling [14,15]. It is characterized in the literature as negatively regulating the activation of pro-inflammatory pathways, as observed in models of systemic inflammation, atherosclerosis, and non-alcoholic steatohepatitis [16], and recently involved in the control of pathogens through the antioxidant protective factor and regulation of the apoptotic process [17]. Changes in the activity of these nuclear receptors play a key role in prostate pathologies such as benign prostatic hyperplasia and prostate cancer [18]. Refaie et al., [19] showed that administration of different

doses of testosterone induces tissue damage and prostate enlargement in rats, inhibiting the expression of caspase 3, however, administration with fenofibrate, a PPAR agonist, improved prostate morphology and caspase-3 expression. Studies demonstrate that negative regulation of PPARα can increase cell proliferation and differentiation in the prostate, liver, and heart, and in the prostate is linked to deregulated androgen expression [20,21]. Su et al., [22]found that PPAR knockout mice presented reduced mRNA from β-oxidation enzymes, impairing lipid metabolism. In an animal model fed with high fructose content, an accumulation of hepatic lipid droplets was observed, suggesting mitochondrial metabolic stress due to reduced oxidation.

Aerobic physical exercise increases caloric expenditure, and at the same time, a balanced diet reduces fat and body weight [23]. Batatinha et al., [24] found that aerobic exercise modifies the parameters of body composition and lipid storage in PPAR$^{-/-}$obese animals. On the other hand, PPARαknockout mice showed lower basal glycemia compared to WT. From the same perspective Silveira et al., [15] suggested that PPAR is required for glucose metabolism after aerobic exercise because it plays an important role in the anti-inflammatory response mediated by physical exercise and its absence induces overexpression of pro-inflammatory cytokines. Physical exercise has already been shown to regulate the prostate environment. Data from our laboratory demonstrated that physical training in Wistar rats modulates the lipid and hormonal profile of the ventral prostate [25,26] and improves body composition, expression of steroid hormone receptors, and cellular apoptosis in the prostate [27,28]. In animals fed with a high-fat diet (HFD) and submitted to strength training, reduced cell proliferation was observed as well as an increase in hormone receptors, modifying prostatic homeostasis [29]. However, it is not yet known which mechanisms activate the apoptotic and anti-inflammatory factors of physical exercise and the consumption of a high-fat diet.

Nonetheless, studies on the activity of PPARα are incipient, mainly on the molecular and physiological mechanisms modulated by the absence of PPARα and associated with aerobic physical exercise protocols to modulate the prostate. Therefore, the aim of the current study was to verify the participation of PPARα as a possible regulator in the protective effects of aerobic physical exercise, through prostatic alterations in mice fed a standard diet and a high-fat diet.

Results

Absence of PPARα reduces prostate weight and weight gain of mice fed with HFD

Figure 1 demonstrates that mice fed HFD chow presented an increase in weight gain in the WT-HF group when compared to the WT-SD (2.85-fold) and KO-HF (1.97-fold) groups. The WT-HFT group showed an expected reduction in weight gain when compared to the WT-HF group. The PPARα KO mice, even when fed the HFD, showed only a slight increase in weight gain when compared to the KO-SD group. The

association of aerobic physical exercise and HFD in PPARα KO mice led to a reduction in weight gain when compared to WT-HFT and KO-HF (Fig. 1A).

Loss of PPARα does not modify the expression of prostatic AR and GR under conditions of lipid consumption and aerobic physical exercise

To identify the effects of the absence of PPARα on the prostate of mice fed with HFD, we observed that the metabolic increase induced by the high-fat diet led to an increase in prostate weight in the WT-HF group compared to the WT-SD ($p = 0.018$) and WT-HFT groups ($p = 0.0092$). Likewise, the weight of the prostate was higher in the WT-HF group when compared to the KO-HF group ($p = 0.01$). However, we can verify that the aerobic physical training was able to reduce the weight of the prostate of mice fed with HFD in comparison to the WT-HF group. The absence of PPARα in KO animals led to no significant difference in prostate weight between treatments (Fig. 1B).

Representative images of AR immunohistochemistry expression in the prostate of PPARα and wild and knockout mice are presented (Fig. 1C-D). It is possible to verify that in knockout animals, the KO-SD group showed less expression of AR compared to wild-type animals, WT-SD ($p = 0.0028$, Fig. 2). The groups supplemented with high-fat diet, WT-HF and KO-HF, of both genotypes presented higher values of AR in the prostate, in comparison with the trained groups, WT-HFT ($p = 0.0009$), KO-HFT ($p = 0.0008$), and control WT-SD ($p = 0.0255$) and KO-SD ($p < 0.0001$), respectively. Similarly, knockout and wild mice fed with HFD showed the same pattern of GR expression (Fig. 2E –F). The WT-HF and KO-HF groups showed higher expression of GR in the prostate compared to the other groups. Aerobic physical exercise reduced GR expression in the prostate with or without PPARα (Fig. 1).

Loss of PPAR to up-regulate prostate inflammation, but does not change with HFD and exercise

We observed that aerobic physical exercise increased the IL-10 immunolabeling in the KO-HFT and WT-HFT animals in relation to KO-HF and WT-HF, respectively, showing a significant difference in HFD groups, without differences between wild type and knockout animals (Fig. 2). To identify whether physical exercise can mitigate the effects of the absence of PPARα associated with HFD on prostatic inflammation, we investigated the labeling of IL-6, TNF-α, and NF-kB. The immunostaining of IL-6 and TNF-α in the knockout animals demonstrated no differences between treatments, however, the expression showed higher levels when compared with the WT-SD and WT-HFT groups (Fig. 2). The high-fat diet significantly increased the levels of IL-6, TNF-α, and NF-kB in wild-type animals when compared to WT-SD, WT-HFT, KO-SD, and KO-HF groups. Physical training, in turn, was efficient in reducing the labeling of IL-6, TNF-α, and NF-kB in relation to the WT-HF group (Fig. 2).

Absence of PPARα suppresses Fas (CD95/Apo-1) -induced BCL-2 expression

To further understand the molecular mechanisms induced by loss of PPARα with relation to expression levels of proteins involved in the regulation of apoptosis, we evaluated the Fas/CD95, BAX, and BCL-2 (Fig. 3). The increase in Fas/CD95 protein expression was observed in KO-HF compared with KO-SD ($p = 0.0005$, Fig. 3). The KO-SD group showed decreased expression of Fas/CD95 in relation to WT-SD and other groups (Fig. 3). Similarly, PPARα knockout mice presented reduced pro-apoptotic protein expression of BAX (Fig. 3). The WT-HF and WT-HFT showed greater expression of BAX when compared to WT-SD ($p = 0.0373$ HF, $p = 0.0298$ HFT), and KO-HF ($p = 0.0048$). Thus, the BAX/BCL-2 ratio could indicate the spread of prostatic lesions. Our results are in line with a previous study which suggested that a low level of the BAX/BCL-2 ratio may result in an increase in proliferative tissue as observed in KO-SD (Fig. 3). Physical exercise increased the BAX/BCL-2 ratio, improving apoptosis in the same way as a high-fat diet (Fig. 3). On the other hand, we observed that the lower the levels of Fas/CD95 the greater the expression of BCL-2. BCL-2 exerts inhibitory effects on apoptosis by preventing mitochondrial cytochrome c release in the prostate. The absence of PPARα significantly increased BCL-2 expression in the KO-SD group when compared to KO-HF ($p = 0.0112$), KO-HFT ($p = 0.0141$), and WT-HF groups ($p = 0.7233$, Fig. 3). Physical exercise reduced protein expression of BCL-2 in the prostate of WT and KO mice fed with a high-fat diet (Fig. 3).

Loss PPARα increased lesions prostatic and reduced glycogen intercellular

The histopathological analyses showed glandular acini containing secretions in the lumen and lined by columnar epithelium with basal nuclei, rough endoplasmic reticulum, apical secretory granules, and microvilli (Fig. 4). In the WT- HF and KO-HF groups, it was observed that the epithelium had become thick with agglomerated nuclei of various heights and evident nucleoli, a profile that characterized areas of focal hyperplasia and PIN (Fig. 4). However, physical exercise reduced PIN in the prostate (Table 1). Animals treated with HFD showed chronic inflammatory processes in the prostate (Table 1). In the KO groups, we observed that the absence of PPARα leads to an imbalance in the inflammatory response, with 10% of the incidence of PIN in SD and HF groups. These data can be completed by the analysis of protein expression of cytokines, where KO animals already presented this inflammatory imbalance. However, in the results of inflammatory focus, we observed that the HFD group showed an increase, while the physical exercise group together with the SD group, presented a reduction in the incidence of inflammatory focus in prostatic tissues (Table 1).

Table 1
Frequency of lesions (%) identified in the ventral prostate

	WT			PPARα KO		
	SD	HF	HFT	SD	HF	HFT
Prostatic intraepithelial neoplasia (PIN) Histological fields analyzed (n = 40–50)	23 (40) 52.50%[b]	42 (50) 84%	28 (50) 56%[b]	28 (40) 70%	29(40) 72.5%[b]	22 (50) 44%
Inflammatory focus Histological fields analyzed (n = 40–50)	3 (40) 7.50%	8 (50) 16%	1 (50) 2%	4 (40) 10%	5 (50) 10%	3 (40) 7.50%

The data were expressed as absolute numbers and occurrence percentages. One Way ANOVA by contingency table, $p < 0.05$. [b] vs WT-HF. WT Mice: wild type; PPARα KO Mice: knockout; SD: standard diet; HF: high fat diet; HFT: high fat diet and physical exercise.

The histological studies of the WT and KO groups fed with HFD showed an increase in the epithelial volume (Fig. 4). The stroma volume was decreased in all groups when compared to the SD groups, however in exercised KO animals, this decrease was accentuated (Fig. 4). In percentages of the prostate lumen, physical exercise increased lumen volume (Fig. 4). On the other hand, HFD showed a decrease in the lumen, due to increased epithelium. However, physical exercise modulated the prostatic stroma. Even with less stromal volume in trained groups. In WT-HF increased collagen fibers among the acini, which enlarged the space among the glandular portions, corroborated the stereological analyses in stromal volume. Aerobic physical exercise reduced the percentage of collagen fibers as shown in stroma volume (Fig. 4). The WT-HF and WT-HFT groups presented reduction in intracellular glycogen, compared to WT-SD ($p = 0.0224$ and $p = 0.1139$). In the KO groups, the accumulation of glycogen was lower in relation to the WT. The KO-HFT showed lower glycogen when compared to the WT-HFT group ($p = 0.0495$, Fig. 4)

Granulated and degranulated mast cells were found in the prostatic stroma, close to the epithelium (Fig. 4). The observed data show that there was an increase in the number of mast cells and a significant difference between the control group WT-SD when compared to the WT-HF group ($p = 0.0048$).However, the aerobic physical exercise was not able to increase the number of mast cells in the prostate when compared to WT-SD ($p = 0.0008$) and WT-HF ($p = 0.0649$). In KO animals, there was a significant increase in the KO-SD group compared to the WT-SD ($p = 0.0014$). The loss of PPAR increased the number of mast cells, however, there were no significant differences between treatments (Fig. 4).

Discussion

Although epidemiological studies have pointed to a relationship between obesity and prostate cancer, these data come mainly from clinical studies and identify hyperglycemic and inflammatory factors as key factors for inducing prostate injuries. Although PPARα promotes strong anti-inflammatory properties,

lipoprotein metabolism, and apoptosis in various animal models [30], PPAR-null mice treated with HFD led to greater suppression of endogenous glucose production as revealed by hyperinsulinemia, indicating less insulin resistance in the absence of PPARα. In the current study, we seek to understand the action of the gene transcription factor PPARα in the ventral prostate of mice fed a high-fat diet or standard diet, and in what ways aerobic exercise modulates the prostatic microenvironment in the absence of this nuclear receptor.

The high-fat diet (HFD, 59% kcal fat) is related to the development of obesity, glucose tolerance, and inflammation. Metabolic alterations related to obesity are strong stimulators of the development of prostate cancer [31]. HFD was efficient to promote an increase in weight gain and the adipose index compared to animals fed a standard diet in WT mice. First, to relate the effect of PPARα null and diet-induced changes in fatty acid oxidation, we observed that PPARα null mice fed with HFD showed reduced weight gain and adipose index compared to the wild-type mice. Data already published by Batatinha et al., [24] the PPARα null reduced glycemia and increased level of triacylglycerol, cholesterol, and FFA in relation to WT. The expression of PPARα is maintained by the presence of endogenous ligands, such as Fas, which is important for the maintenance of basal FA metabolism [32]. FFA has been reported to inhibit glucose uptake, causing insulin resistance [33]. As shown in the current study, cholesterol and FFA are increased in the absence of PPARα. Nevertheless, the absence of PPARα reduces the oxidation of FA, favoring the use of glucose by the tissues, even in the HFD-fed mice with less glucose availability and, thus, the excess calorie intake is not stored in the white adipose tissue, leading to a reduction in weight gain and the adipose index of PPARα null animals.

On the other hand, aerobic exercise can regulate energy metabolism via PPARα and mitochondrial enzymes, preventing obesity and lipid disorders in rats fed with HFD [34]. We know that the absence of PPARα associated with aerobic physical exercise and HFD increased energy expenditure and did not regulate the energy balance, using lipid as an energy source [24]. In this way, animals of the PPARα KO group submitted to aerobic physical exercise had a lower fat index and higher levels of circulating FFA and marked glucose reduction. The action mechanism of reduced fatty acid oxidation in PPARα null mice is accompanied by twice the concentration of malonyl CoA and decreased activity of Malonyl-CoA decarboxylase [35]. Silveira et al., [15] demonstrated that after 24 hours, a moderate intensity session of aerobic physical training, performed in PPARα KO mice promoted a reduction in glucose and increase in non-esterified fatty acids, cholesterol, and triglycerides, indicating that the absence of PPARα impairs β-oxidation and, consequently, NEFA and the accumulated acetyl-CoA from glycolysis can be converted to triacylglycerol. Thus, the lack of PPARα even in conditions of physical demand with aerobic training reduces the transport of fatty acids to the mitochondria and the oxidation capacity.

The role of PPARα in the prostate has been little discussed, although there is evidence of greater expression of PPARα in prostatic adenocarcinomas [36]. Similarly, the impact of HFD on tumor growth is well documented [37]. Our results support the idea that PPARα null has an incidence of prostatic intraepithelial neoplasia in 70% of the analyzed group and when we associated HFD with PPARα null it

increased to 72% PIN, and when we analyzed the effect of HFD in WT mice we verified a higher incidence (84% PIN). The high expression of PIN in animals fed with HFD is related to increased expression of prostatic AR, however, the increase in PIN in the PPARα null group fed the standard diet is via AR independent routes. Lin et al., [38] found that $AR^{-/-}$ negatively alters PPARα expression in the liver, indicating that PPARα is androgen dependent. The absence of PPARα does not interfere with HFD-induced AR activation. On the other hand, aerobic physical exercise has the potential to reduce prostatic changes, as demonstrated by Teixeira et al [25,39], by reducing the expression of AR. Our results showed lower expression of AR in the groups submitted to aerobic exercise and HFD in both genotypes, with a reduction from 72% (PPARα KO HF) to 44% (PPARα KO HFT) in the incidence of PIN. The protective action of physical exercise on the prostate is independent of the presence of PPARα.

PPARα is best known as a critical regulator of lipid metabolism and inflammation [40] and is expressed in tissues that catabolize fatty acids, such as prostatic epithelial cells [36]. Prostatic inflammation is related to the development and progression of prostate cancer [41]. The HFD induces stromal infiltrates of inflammatory cells such as macrophages, T cells, monocytes, and mast cells in the prostate [42,43]. Studies show that the activation of NF-kB is dependent on PPAR□, consequently animals PPAR□ $^{-/-}$ present overexpression of NF-kB in the liver, stimulating the inflammatory pathway [44,45]. In the prostate, there was an increase in mast cells, IL-6, and TNF-□, but not NF-kB in response to low PPAR□ expression in animals fed a standard diet compared to WT. The absence of PPAR□ may induce greater expression of PPARγ in the liver [24]. The increase in PPARγ induced by the absence of PPAR□ may have reduced the expression of NF-kB but did not alter the expression of inflammatory cytokines, suggesting that there is a compensatory effect. One mechanism would be the greater induction of PPAR□$^{-/-}$ recruiting inflammatory macrophages (M1) in the prostate, modulating the secretion and expression of the cytokines IL-6 and TNF-α, regardless of NF-kB activation [46,47].

The increased expression of IL-6, an NF-kB inducible gene, is an autocrine growth factor for prostate cancer cells. However, the increase in the inflammatory response to an HFD occurs due to the increase in the expression of MCP-1, IL-6,and TNF-□ and inhibits the anti-inflammatory effect of GR [48]. The absence of PPAR□ did not interfere with the expression of GR, however, the HFD up-regulated GR in the prostate. Activation of GR seems to act in synergism with the activation of PPAR□ [49]. GR inhibited the activity of numerous transcription factors, including Activating Protein-1 (AP-1), NF-kB, signal transducer, and activator of transcription 1 (STAT1), many of which are regulated via the MAPK cascade [50,51]. Inflammatory mediators such as TNF-a and growth signals such as insulin-like growth factor-1(IGF-1) may activate the transcription factors AP-1 and NF-kB, thus inhibiting the GR-mediated transactivation effects in the prostate [52]. Our study suggested that a high-fat diet increases the expression of GR, NF-kB, and TNF-□ in both phenotypes. On the other hand, inflammation was reduced by the presence of aerobic exercise that mitigated the effects of HFD on the prostate regardless of the presence of PPAR□, inducing the highest expression of IL-10.

Fas (CD95/Apo-1) is a cell membrane glycoprotein that belongs to the TNF-α family, with the function of triggering proteolytic cleavage of caspases, culminating in the process of cell apoptosis [53].The extrinsic pathway of cell apoptosis induced by Fas activation (CD95/Apo-1) initiates caspase 8-mediated cleavage of proteins such as BID, which translocates to mitochondria to activate BAX and BAK, resulting in membrane permeability and release of cytochrome C [54]. The absence of PPAR promoted up-regulation of the BCL-2 protein and down-regulation of BAX and Fas (CD95/Apo-1). HFD induced an increase in Fas (CD95/Apo-1) in wild type mice, possibly induced by an increase in BCL-2. An increase in Fas (CD95/Apo-1) has been related to the increase in liver disease and insulin resistance and failure in mitochondrial oxidation [55]. The inhibition of PPAR modulates proteins related to mitochondrial apoptosis in the ventral prostate with the high consumption of lipids in HFD provoking lipotoxicity, promoting an increase in Fas expression (CD95/Apo-1). The downregulation of Fas (CD95/Apo-1) is a promising result of regulation and insulin resistance and mitochondrial alteration induced by aerobic physical exercise [28]. In the current study, aerobic training reduced Fas (CD95/Apo-1) levels, reinforcing the potential effect of exercise on prostatic lipid regulation, and increased the BAX/BCL-2 ratio.

Given the accumulated findings pointing to the importance of developing prostatic neoplasms of non-cancerous tissues, such as androgenic modifications, inflammation, and other functions mediated by stroma and infiltrating cells, our results add a new element to the emerging paradigm that the formation of neoplasms can be metabolically induced by the absence of PPARα and the great importance of energy regulation for the prostate environment. Inhibition of PPAR reduces the energy balance of prostatic epithelial cells and increases inflammation, inducing anti-apoptotic stimuli.

The increase in lipid intake due to the consumption of HFD in PPAR null animals induces a higher percentage of PIN and, consequently, an increase in NF-kB, which in turn increases the expression of GR as an attempt to restore the prostate environment by increasing cell apoptosis. The practice of aerobic exercise as a metabolic control therapy, associated with the absence of PPAR reduces the impact of HFD on prostatic lesions, increases the expression of anti-inflammatory proteins regulating the expression of AR and GR, and induces greater apoptotic expression via BAX and less activation via Fas (CD95/Apo-1), promoting a better prostatic mitochondrial environment.

The absence of PPARα promotes reduced adaptations to aerobic physical exercise in prostatic regulation through prostatic alterations in mice fed a standard diet and HFD, indicating that PPAR is a key factor in the effects of physical training on the prostate (Fig. 5). Therefore, the action of genes involved in prostate cell metabolism must be seen in a broader context. Several pro-inflammatory factors stimulate the tumor growth. Herein, we report that PPARα null induces an inflammation process and prostatic changes, however, these inflammatory processes induce the increase in apoptosis and may inhibit cell growth even more when associated with physical exercise, thus expanding the spectrum for anticancer therapies that aim to interfere with prostatic stromal processes.

Materials And Methods

Ethics Statement

Experiments all animals procedures, were conducted in accordance with the ethical principles in animals research adopted by the Brazilian College of Animal Experimentation (COBEA) and the study protocol was approved by the Animal Experimentation Ethics Committee ICB/USP (Protocol CEUA 112/13) approved the experimental procedures of this study. We confirm that this study is reported in accordance with ARRIVE guidelines.

Animals

Mice (background C57BL/6J), male, wild type (WT) and knockout PPARα (KO) were used, maintained in a light/dark cycle of 12h (23 ± 2ºC), with a normal diet (Nuvital food from Nuvilab, Colombo, PR) and water *ad libitum* until the start of treatments (70 days of age). The PPARα knockout animals were provided by the Cell Signaling Laboratory of the Department of Physiology and Biophysics at USP.

60 mice Wild and PPARα $^{-/-}$ mice (background C57BL/6J) were used, divided into 6 groups (n = 10/group): wild animals fed a standard diet (WT-SD); wild animals fed a high-fat diet (WT-HF); wild animals fed a high-fat diet and submitted to aerobic physical exercise(WT-HFT); PPARα $^{-/-}$ animals fed a standard diet (KO-SD); PPARα $^{-/-}$ animals fed a high-fat diet (KO-HF); PPARα $^{-/-}$ animals fed a high-fat diet and submitted to aerobic physical exercise(KO-HFT) (Fig. 1G).

Supplementation with the high-fat diet started at 70 days of age. Animals in the HFD groups were supplemented during the 12 weeks of the protocol, maintained on a high-fat diet composed of 26% carbohydrates, 59% lipids, and 15% protein. Animals in the SD group were kept on a balanced diet consisting of 76% carbohydrates, 9% lipids, and 15% protein.

Aerobic Physical Training

The maximum capacity test was performed to determine the training load, where the animals warmed up for 5 minutes on the treadmill at a speed of 10 meters/minute (m/m).The test began from the sixth minute, with increases in speed of 3m/m every minute, until exhaustion. Aerobic physical training was performed on a treadmill at 60% of maximum speed, 5 days a week, 10m/min for 60 minutes, from the 5th to the 12th week of diet supplementation [56]. In the 4th week of diet supplementation, the animals were adapted to the treadmill for 5 days. The maximum test was performed in the 4th week, before the training started, to determine the maximum load, in the 8th week to adjust the workload, and in the 12th week to check for any changes.

Euthanasia Protocol

At 155 days of age, the mice were anesthetized by inhalation of isoflurane and euthanized by beheading 24 hours after the final physical training session. The mice were weighed, and an abdominal-pelvic laparotomy was performed to expose the prostate and adipose tissue which were removed, weighed, and

processed for histological and immunohistochemical analysis. The fat index was composed of the total sum of retroperitoneal, epididymal, and subcutaneous adipose tissues.

Immunohistochemistry Data

Prostatic samples from five animals from each experimental group were used for immunostaining, according to the characteristics of each antigen [25]. Subsequently, protein block was performed with bovine serum albumin (BSA) diluted in TBS-T. In the next step, the sections were incubated with specific primary antibodies; AR (sc-816), GR (sc-1004), Fas/CD95 (sc-1024), BCL-2 (sc-492), BAX (sc-526), IL-6 (sc-1265), Il-10 (sc-73309), TNF-α (sc-1350), and NF-kB/p65 (sc-109) and incubated overnight. Subsequently, the sections were incubated with anti-rabbit secondary antibody or anti-goat secondary antibody at room temperature, developed with diaminobenzidine (DAB), and counterstained with Harris' hematoxylin The cuts were photographed in a Zeiss Axiophoto photomicroscope.

Quantification of Immunoreactivity

The percentage of intensity of immunolabeling of BAX, BCL-2, Fas/CD95, TNF-α, IL-6, and NF-kB antigens were evaluated in 5 animals per group examined in 10 fields per animal/antigen, using ImageJ software (version 1.50i). The cellular labeling of AR and GR was quantified in 1000 cells per animal, in five animals per group, thus, the percentage for totalization of positive epithelial cells was analyzed in 10 different sections of the prostate of5 animals per group [28].

Histopathological Analysis

The ventral prostate was collected, weighed, and fixed by immersion in Bouin and embedded in paraneoplastic for histological and histochemical analysis. The sections of five animals per group (5 µm) were stained with Hematoxylin & Eosin (H&E) for histopathological analysis. Slides of the ventral prostate (10 photos per animal) were analyzed for the incidence of the following lesions: inflammatory focus and PIN (prostatic intraepithelial neoplasia) [57,58].

Statistical Analysis

For these analyses, the Shapiro-Wilk normality test was used. When normal parameters were identified, the One Way ANOVA variance test was used for group analysis, followed by the Tukey post hoc. Data are presented as mean ± standard error of the mean, considered significant when $p \leq 0.05$. The statistical analysis and production of the graphs were performed using *GraphPad Prism* software version 8.0.

Abbreviations

PPARα - Peroxisome proliferator-activated receptor - alpha;

GR - Glucocorticoid receptor;

AR - Androgen receptor;

HFD - High fat diet;

WT-SD - Wild type animals fed a standard diet;

WT-HF - Wild type animals fed a high-fat diet;

WT-HFT - Wild type animals fed a high-fat diet and submitted to aerobic physical exercise;

KO-SD - Knockout animals fed a standard diet;

KO-HF - Knockout animals fed a high-fat diet;

KO-HFT - Knockout animals fed a high fat diet and submitted to aerobic physical exercise;

FFA - Free Fatty Acid;

IL-6 - Interleukin 6;

IL-10 - Interleukin 10;

TNF-α - Tumor Necrosis Factor Alpha;

NF-kB - Nuclear factor kappa B;

Fas/CD95 - Fas cell surface death/ cluster of differentiation 95;

BAX - BCL-2-associated protein X;

BCL-2 - B-cell lymphoma 2;

PCa - Prostate cancer;

BPH - Benign prostatic hyperplasia;

PIN - Prostatic intraepithelial neoplasia;

PPARγ - Peroxisome proliferator-activated receptor - gamma;

PAS - Periodic acid-reactive Schiff;

INCA - Nacional Cancer Institute, Ministry of Health, Brazil;

KO - knockout;

WT - Wild Type.

Declarations

Author contributions

Study concept and design: Teixeira GR and Baptista DB; Acquisition of data: Tavares MEA, Teixeira GR, Baptista DB, Veras ASC. Analysis and interpretation of data: Teixeira GR, Tavares MEA, Veras ASC. Drafting the manuscript: Tavares MEA, Veras ASC, Teixeira GR. Critical revision of the manuscript: Teixeira GR. Study supervision: Teixeira GR. All authors approved the final version of the manuscript.

Declaration of Competing Interest

None.

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Figures

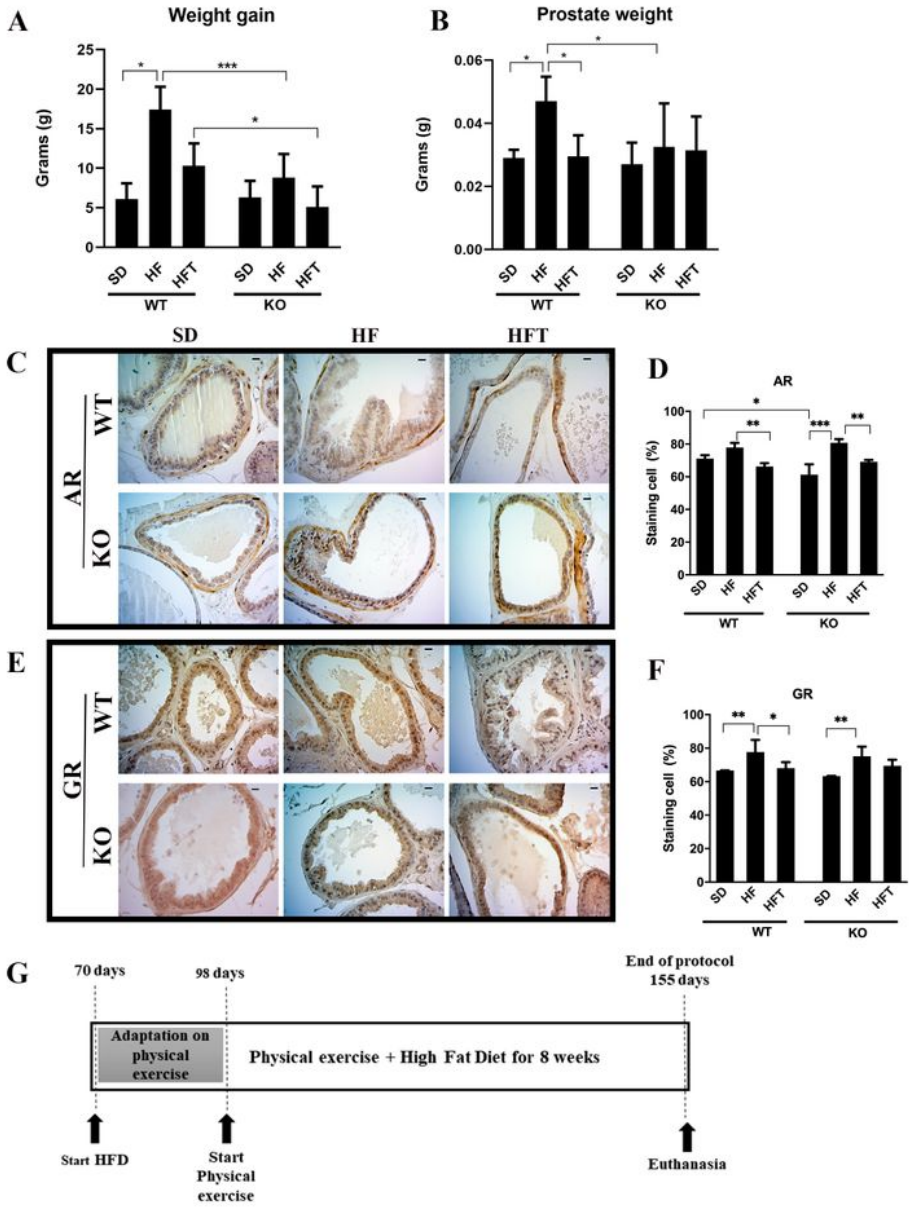

Figure 1

Graphs referring Weight gain (A), absolute weight of the ventral prostate (B), experimental design of the high-fat diet and aerobic exercise protocol (G) and Immunohistochemistry of the ventral prostate of Wild Type and KO PPARα mice submitted to aerobic exercise and a high-fat diet under the expression of AR-receptor androgen (C - D), and GR-glucocorticoid receptor (E –F). Bar = 20μm, 40x resolution. The

significant differences between the HF and HFT both WT and KO. ** p <0.05 and compared to the control group #p<0.05.

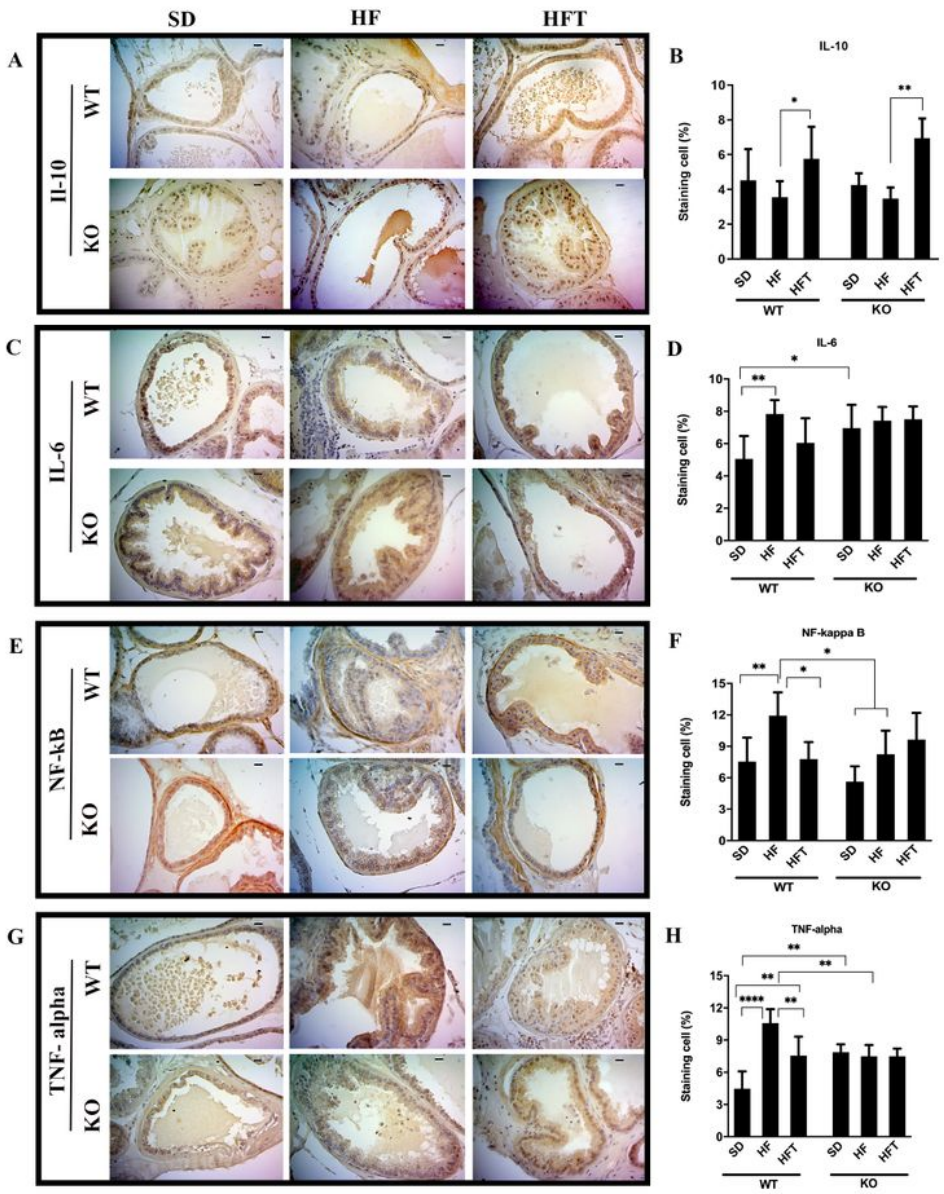

Figure 2

Immunohistochemistry of the ventral prostate of wild type and KO PPARα mice submitted to aerobic physical exercise and a high-fat diet under the expression of interleukin-10 IL-10 (A-B), interleukin-6 - IL-6 (C-D), of the factor of genetic transcription kappa B - NF-kB (E-F) and tumor necrosis factor-□ - TNF-□ (G-

H). Bar = 20μm, 40x resolution. Significant differences between the HF and HFT groups for both WT and KO. #P <0.03 compared to the control group. (One-Way ANOVA followed by the Fisher-LSD test). (n = 5 for all groups).

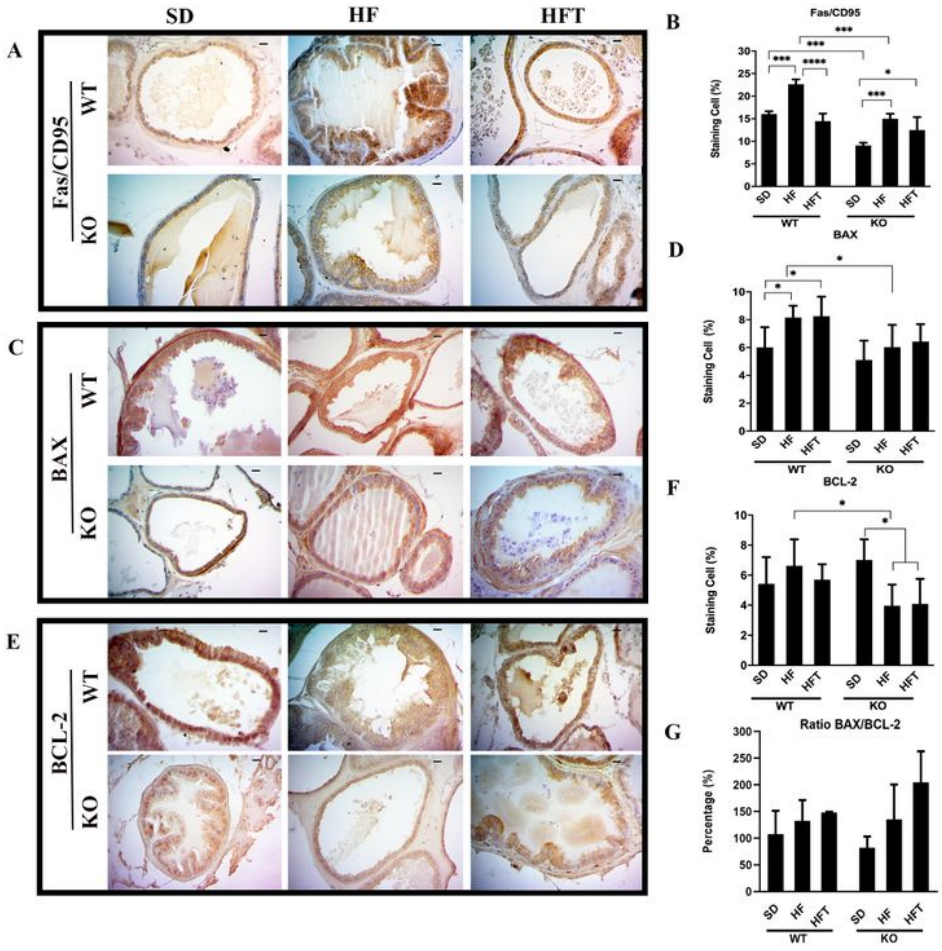

Figure 3

Immunohistochemistry of the ventral prostate of wild type and KO PPARα mice submitted to aerobic exercise and a high-fat diet under the expression of Fas/CD95 (A-B), BAX (C-D) and BCL-2 (E-F) and

BAX/BCL-2 Ratio (G). Bar = 20μm, 40x resolution. Significant differences between groups. ** P <0.001, # P <0.003, ## P <0.021 (One-Way ANOVA followed by Fisher-LSD). (n = 5 for all groups).

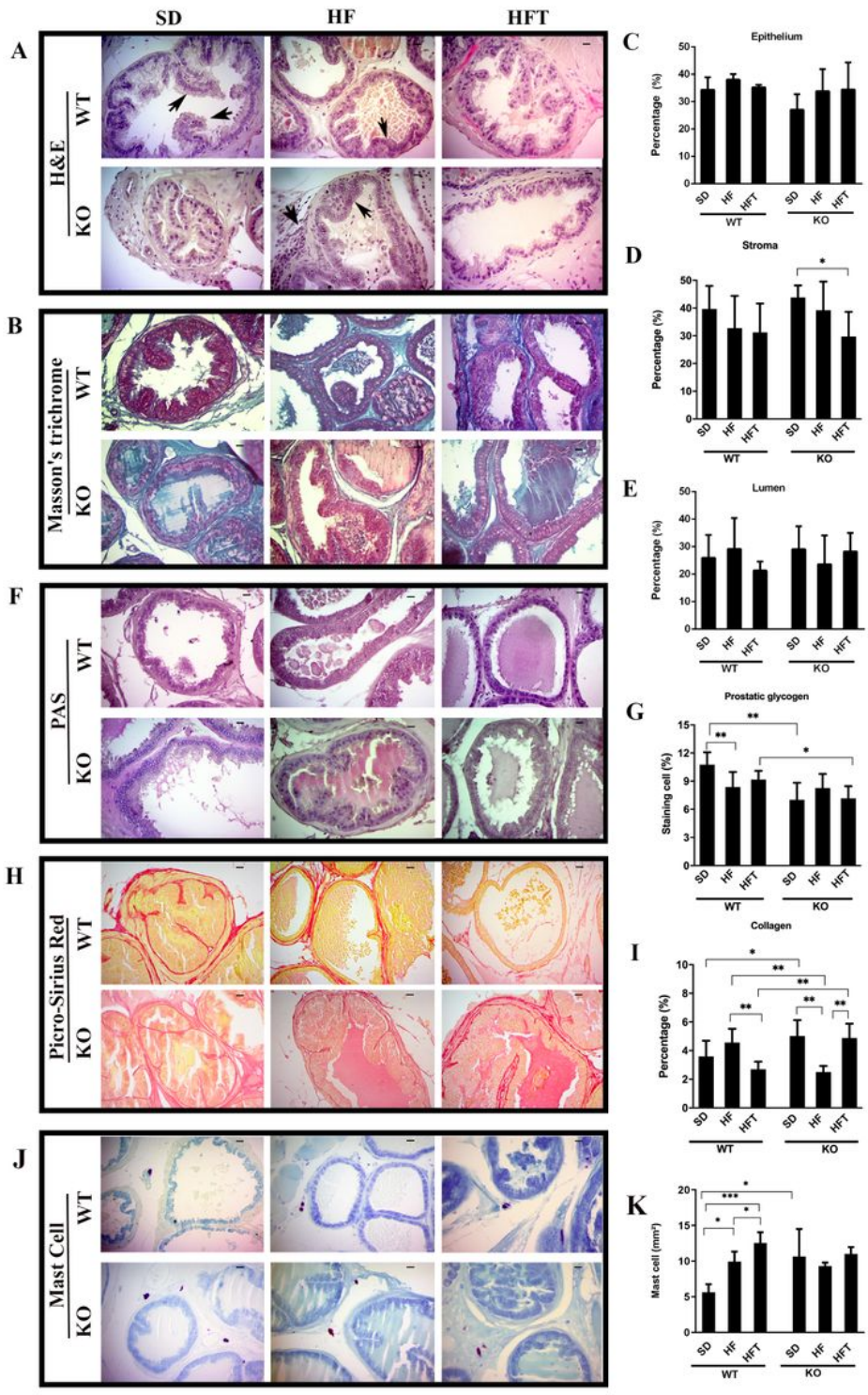

Figure 4

Histopathological technique of hematoxylin and Eosin (H&E) for histopathological analyzes (A), and Masson's trichrome for stereological analyzes (epithelium, stroma, and lumen) (B-C, D e E), periodic acid-Schiff staining for quantification of intracellular glycogen (F-G) and picrosirius red staining for stromal

collagen quantification (H-I) and mast cell staining (J - K). Bar = 20μm, 40x resolution. Arrows are changes in the prostatic epithelium (PIN and invaginations). #P <0.03 compared to the control group, ** P <0.001, *P < 0.28. (One-Way ANOVA followed by Fisher-LSD). (n = 5 for all groups).

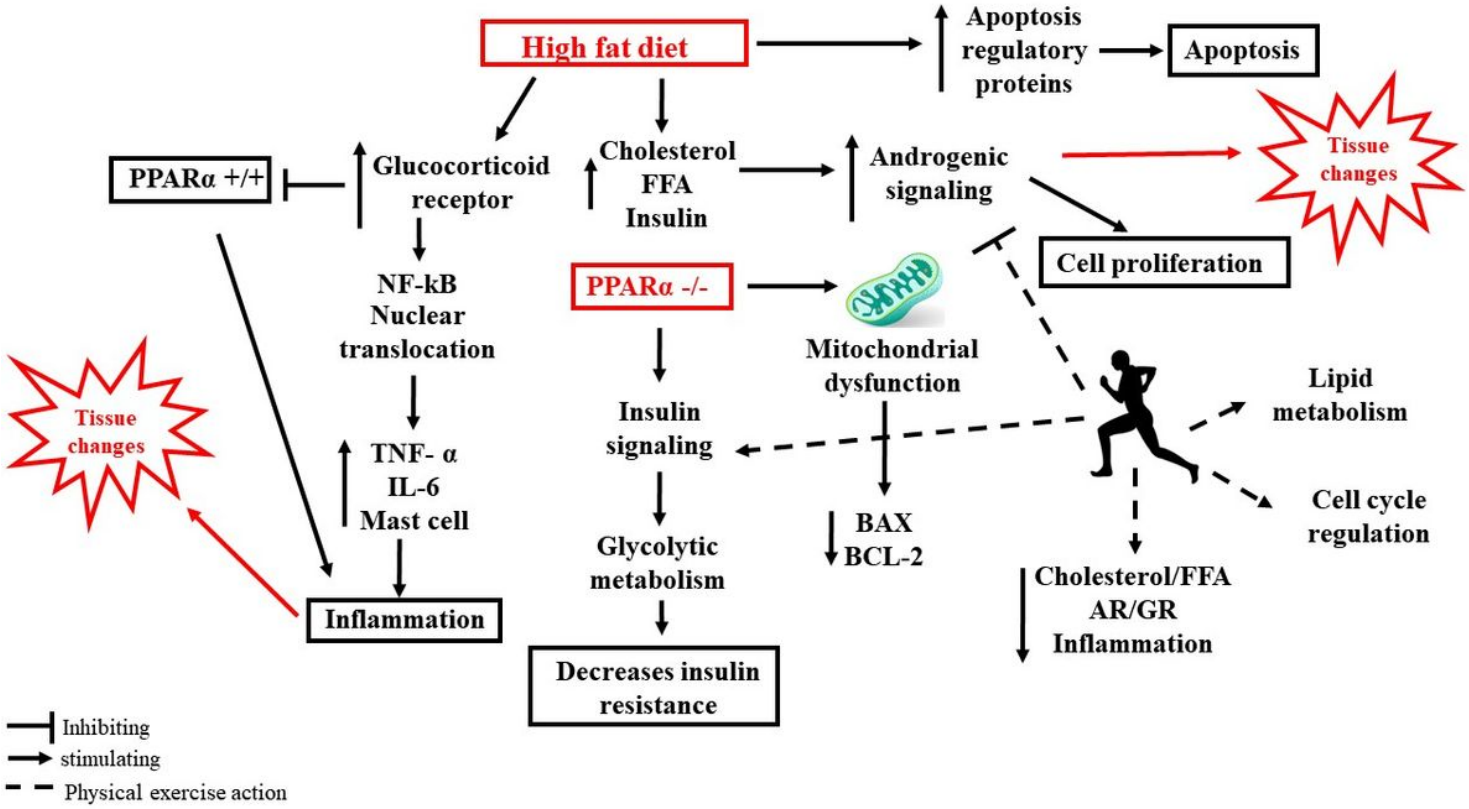

Figure 5

The role of 8 weeks of aerobic exercise in physiological and metabolic pathways associated with HFD and the PPARα knockout. The high-fat diet favors increased inflammation and proliferation in the prostate. On the other hand, aerobic physical exercise provided opposite effects in reducing inflammation, proliferative and metabolic pathways.