Advances in Research

21(11): 79-90, 2020; Article no.AIR.63992
ISSN: 2348-0394, NLM ID: 101666096

Hyperlipidic Diet-induced Obesity Increased Proliferative Signals- AR, ERK1/2- on Mice Prostate, Which can be Restored through Physical Training

João Vitor Pereira Leite[1], Daniela Cristina de Cario Calaça[1], Pedro Augusto Silva Nogueira[2], Renata Graciele Zanon[2] and Daniele Lisboa Ribeiro[1*]

[1]Department of Cell Biology, Histology and Embriology. Institute of Biomedical Sciences- ICBIM. Federal University of Uberlândia, Brazil. Av. Pará 1720 - Bloco 2B - Sala 2B221. Uberlândia - MG - CEP 38400-902, Brazil.
[2]Department of Anatomy. Institute of Biomedical Sciences. Federal University of Uberlândia- UFU, Brazil. Av. Pará 1720 - Bloco 2B - Sala 2B221. Uberlândia - MG - CEP 38400-902, Brazil.

Authors' contributions

This work was carried out in collaboration among all authors. Authors RGZ and DLR designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author PASN performed animal care and experimental groups. Authors JVPL and DCCC managed the analyses of this study and the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AIR/2020/v21i1130272
Editor(s):
(1) Dr. Soumendra Karmahapatra, Ohio State University, USA.
Reviewers:
(1) Alice Barros Câmara, Universidade Federal do Rio Grande do Norte, Brazil.
(2) Alan José Barbosa Magalhães, University of Campinas, Brazil.
Complete Peer review History: http://www.sdiarticle4.com/review-history/63992

Original Research Article

Received 20 October 2020
Accepted 27 December 2020
Published 31 December 2020

ABSTRACT

Aims: To evaluate the effects of hyperlipidic diet on the mouse prostate and also investigate if physical exercise is able to restore such effects.
Methodology: Adult male Swiss mice were fed with a balanced (ND) or hyperlipidic diet (45% saturated fat, HD) for 16 weeks. Half were submitted to a sedentary (NDS and HDS) or exercise routine (swimming- NDE and HDE) for 8 weeks. Then, the prostate was analyzed by immunoreactions (proliferating cell nuclear antigen- PCNA, androgen receptor- AR, and estrogen receptor-ERβ), western blotting (ERK 1/2), and caspase-3 activity.

**Corresponding author: E-mail: daniele.ribeiro@icbim.ufu, dl_ribeiro@yahoo.com.br;*

Results: We found that saturated fat uptake promoted 16% weight gain, increased fat-mass and hyperglycaemia, as well as reduced testosterone levels. In addition, HD atrophied prostate secretory epithelium and stimulated cell proliferation through higher expression of AR and activation of ERK signaling. Additionally, saturated fat reduced prostatic ERβ content. Physical exercise *per se* promoted an anabolic effect by increasing testosterone and stimulating cell proliferation in the prostate of sedentary animals. Finally, exercise was able to restore the proliferative signals caused by the hyperlipidic diet on prostate.
Conclusion: We suggest that the combination of hyperlipidic diet and sedentary lifestyle could negatively affected some prostate stimulating pathways that could trigger proliferative diseases in mice and physical exercise may be an interesting strategy to reverse such effects.

Keywords: Prostate; hyperlipidic diet; cell proliferation; androgen receptor; physical exercise.

ABBREVIATIONS

ND : Normal diet
HD : High-fat diet
NDS : normal diet and sedentary
NDE : normal diet and exercise
HDS : high-fat diet and sedentary
HDE : high-fat diet and exercise
PCNA : proliferating cell nuclear antigen
AR : androgen receptor
ERβ : estrogen receptor beta
ERK1/2 : extracellular signal-regulated kinases 1/2
AKT : Protein kinase B
MAPK : mitogen-activated protein kinase

1. INTRODUCTION

Obesity is a chronic disease and the most important nutritional disorder worldwide and global data showed that 39% of adults aged 18 years and over were overweight in 2016, and 13% were obese, demonstrating that obesity incidence has nearly tripled worldwide since 1975 [1]. The excessive fat accumulation causes disturbances such as hypertension, glucose intolerance (high sugar blood), insulin resistance (hyperinsulinaemia), diabetes, and multiple systemic disorders [2]. However, obesity also promotes important hormonal dysfunctions, affecting the hypothalamic-pituitary-gonadal axis. Hyperglycaemia and hyperinsulinaemia inhibits the synthesis of testosterone by the testes and also sex-hormone-binding globulins, decreasing the amount and the transport of testosterone in blood [3]. Thus, it is evident that obesity negatively affects gonadal hormone-dependent organs, such as prostate.

Over the past decade, the incidence of prostate cancer has risen to be the second most commonly diagnosed cancer for males, representing 15% of male cancer diagnosis and 3.8% of all deaths caused by cancer in men [2]. In this context, diet is believed to be an important factor related to cancer incidence. A hyperlipidic diet has been identified as one of the factors involved in the onset of prostate cancer and also benign prostate hyperplasia [4,5]. As well as the amount of fat, the quality of fat seems to be related to prostate carcinogenesis, since population studies have indicated an increased prostate cancer risk with high intake of saturated fats [6]. In this regards, several clinical and epidemiological studies have associated obesity to mortality or prostate cancer progression and such association is supported by the knowledge that adipokines, hyperinsulinaemia, and hyperglycaemia activate cell survival and proliferation signaling pathways such as AKT and MAPK [7].

While the benefits of physical exercise in improving global health and also obesity-related symptoms are unquestionable, the impact of physical activity *per se* on the prostate is virtually nonexistent on the literature. In contrast, the prescription of regular physical exercise in association with conventional cancer treatment has become common practice in the medical clinic, reducing the risk of various types of cancer, including prostate cancer [8,9]. The treatment of prostate cancer often uses hormonal ablation therapy, whose main long-term side effects are insulin resistance and obesity. Recent studies have provided support that physical exercise improves these side effects and quality of life among these patients besides to reduce high-grade prostate cancer progression and mortality [10]. Additionally, physical activity presents anti-inflammatory effects which could have a protective role against prostate cancer onset. Data from large longitudinal prospective cohort studies have provided strong evidence that physical activity correlates inversely with pro-

inflammatory and linearly with anti-inflammatory mediators [11].

The search for information about the effect of diet and/or obesity on prostate may help in the development of strategies to prevent further tissue alterations such as epithelial atrophy which could impair reproductive function; and increased cell proliferation that can stimulate the incidence of prostate proliferative disorders. Our research group has shown the negative effects of obesity in the prostate histology of rats treated with a 20% saturated fat diet in the short and long terms as well as the direct impact of saturated fatty acid on normal and prostate cancer cells [12-14]. In this study, we aim to evaluate the effects of a high fat-diet (45% saturated fatty acid) on prostate morphology and expression of steroidal receptors (AR and ER), its impact on a proliferative signaling pathway (ERK) and analyze if physical exercises may restore those changes.

2. METHODOLOGY

2.1 Experimental Design

Swiss mice were provided by the Bioterium Center of Federal University of Uberlandia and housed on a 12 hours light/dark cycle (inverted), at 22 ± 1°C with water and standard rations *ad libitum*. The animals were treated following the guidelines on ethics in the use of animals for biomedical experimentation and research of the National Council for Animal Experimentation Control (CONCEA). This study was approved by the Institutional Committee for Ethics in Animal Experimentation (CEUA/UFU, protocol 063/11). 40 male Swiss mice (5 weeks old) were separated in two groups: normal diet (ND)- treated with standard ration (4% saturated fat) and hyperlipidic diet (HD)- treated with 45% saturated fat (lard), during 16 weeks. After 8 weeks of diet, 50% of animals were submitted to physical exercise, while 50% remained sedentary. In this sense, the animals were classified in 4 groups according their diet and physical activity: NDS – normal diet sedentary; NDE – normal diet exercised; HDS – hyperlipidic diet sedentary; and HDE – hyperlipidic diet exercised. Table 1 shows the content of macronutrients of ND and HD.

The physical activity used in this protocol was swimming. Training sessions were performed between 7:00 a.m. and 7:00 p.m. and water temperature was maintained at 32°C±3°C. All animals were trained at the same period throughout the protocol. The first week included free swimming for only 10 min to animals adaptation. The next 7 weeks were followed by effective training protocol: 1 hour per day (without resting pause), for 5 days over 8 weeks. During the training period, the animals were weekly submitted to a progressive load test (incremental) to determine the most adequate load to be used. The test consisted of increasing load corresponding to 2% of animal's body weight, added every three minutes until animal exhaustion during swimming, according to Almeida et al., [15]. The intensity of the exercise was set at 50% of the maximum load obtained in the progressive load test. After the experimental period, animals were 12h fasted and anesthetized with a mixture of diazepam (Cristalia, São Paulo, Brazil), ketamine, and xylazine (Syntec, São Paulo, Brazil) (1:2:2). All mice were euthanized during the same period, at morning.

Table 1. Diet composition of normal diet (ND) and high-fat diet (HD)

Macronutrient	ND (kcal%)	HD (kcal%)
Protein	20	20
Carbohydrate	70	35
Fat	10	45
Total kcal/g	3.85	4.73

2.2 Biometrical and Hormonal Evaluation

Body weight was monitored during the experimental period. The glycemic level was measured (mg/dL, Contour Bayer glucometer, Bayer AG, Leverkusen, Germany) at the end of the experimental period. Adiposity index was determined by weighing the visceral (kidney, mesenteric) and epididymal fat deposits after euthanasia.

The plasma levels of testosterone were quantified by ELISA assay (Enzo life Sciences International, VT, USA) according to the manufacturer's instructions. Five animals per group were used in this analysis and the samples were tested in triplicate. The minimum sensitivity of the test was 5.67 pg/mL.

2.3 Morphological Analysis of prostate

A total of 5 ventral prostate were removed for each group, then we measured the prostate weight and fixed samples in methacarn solution (methanol, acetic acid, and chloroform, 6:3:1) for Paraplast embedding. The tissue sections was

stained with picrosirius-haematoxylin, and histological and stereological analysis were performed. Charts of microscopic figs. were performed using Powerpoint - Microsoft Office 365®.

For estimation of epithelial and luminal area, the sections stained by Picrosirius were submitted to stereological analysis using the Weibel method of counting points (Weibel, 1963), which consisted of a reticulum containing 100 points. Each point that touched epithelium or lumen was counted and data were expressed as a percentage of total area (Relative frequency).

2.4 Immunohistochemistry and Immunofluorescence

Briefly, paraffin sections of prostate were blocked for 10 minutes to prevent non-specific protein interactions through Background Sniper blocking reagent (Biocare Medical, Concord, MA, USA). Subsequently, the endogenous peroxidase was blocked by 3% H_2O_2 in methanol for 30 min. The primary antibodies (Santa Cruz Biotechnology, Dallas, TX, USA): mouse anti-PCNA (sc-59), mouse anti-AR (sc-32251) and rabbit anti- ERβ (sc-8974) were incubated at 1:100 dilution in 1% BSA at 4°C overnight or for 1 hr at 37°C (PCNA). The AR and PCNA reactions were revealed by the polymer Star Trekk (Biocare, Medical, Concord, USA) conjugated with horseradish peroxidase during 45 min at room temperature. Then, the reaction was visualized with diaminobenzidine (2 minutes) followed by haematoxilyn nuclei staining. ERβ was evaluated by immunofluorescence using an anti-rabbit secondary antibody (1:500) conjugated to Alexa Fluor 555 (Jackson Laboratories, West Grove, PA, USA) for 1 hr. Nuclei staining (green fluorescence) was performed by YOPRO (ThermoFisher Scientific, Whaltam, MA, USA) diluted 1:1000 in glycerol.

The quantification of PCNA, AR and ERβ - positive cells in the acinar epithelium of the ventral prostate was performed by using 20 microscopic fields at 400X, randomly selected from two different histological sections per animal. Five rats were employed, resulting in 100 fields per group. The index of immunoreaction was calculated as the number of positive nuclei divided by the total of epithelial cell nuclei and expressed as a percentage (Relative frequency of AR- and PCNA-positive cells). For ERβ data, only positive cells were counted.

2.5 Western Blotting

For western blotting, 5 prostate samples were pooled per group and homogenized in cell lysis buffer for protein extraction and quantification. Then, 50 µg of protein were separated by SDS-PAGE electrophoresis followed by transfer to nitrocellulose membrane. Afterwards, membranes were blocked by incubation in 5% BSA in Tris-HCl buffer plus 0.2% Tween 20 (TBST) for 60 minutes at 25°C. Then, membranes were incubated overnight with rabbit anti-human ERK 1/2 or rabbit anti-human-phospho-ERK 1/2 (Cell Signaling, Danvers, MA, USA) 1:1000 in 3% BSA. After washing, the membranes were incubated in anti-rabbit conjugated to peroxidase, diluted 1:10000 in TBST, for 1 hour. The immunoreactions were revealed by ECL kit (GE HealthCare, Chicago, IL, USA) and the chemiluminescence was detected in a photodocumentator Amersham Imager 600 (GE Heathcare). The protein bands were analyzed by densitometry using Image J (version 1.34; Wayne Rasband, Research Services Branch, National Institute of Health, Bethesda, MD, USA). The densitometry values of phosphorylated ERK 1/2 were normalized compared with total ERK 1/2. Five animals per group were used in this analysis and the blotting experiment was repeated 3 times.

2.6 Statistical Analysis

Statistical analyses were performed with GraphPad Prism® software (GraphPad Prism software, v.5.0). Data were tested for normal distribution using the Kolmogorov-Smirnov test. Differences between groups were assessed with One-Way ANOVA with post-test (Bonferroni Multiple Comparisons Test). In cases where data were non-parametric, Kruskal-Wallis was used for comparisons between groups. Statistical significance was considered when $p< 0.05$.

3. RESULTS

3.1 Physical Exercise without Diet Change did not Restore Body Adiposity and Hormonal Changes Caused by Hyperlipidic Diet

HD groups demonstrated an increase in body weight of nearly 12% compared to ND fed mice (Table 2). Surprisingly, the exercised groups presented no significant loss of body weight comparing to the sedentary ones. Physical activity without diet change had no impact on

body weight loss, as we can see in the HDE group compared to HDS. Increased body weight was associated with fat deposition, since HDS group presented an adiposity index approximately 58% higher than NDS. Our protocol of physical exercise did not decrease fat deposits in HD mice (Table 2). Higher glycemic levels were noted in the HDS group, which showed a rise of 41% when compared to NDS. Although a slight decrease in glucose levels was noted, physical exercise did not improve the effect of HD on mice (Table 2). The prostate weights of HDE and HDS were considerably lower than ND groups. Although the results were not statistically significant, this reduction in prostatic weight due to the hyperlipidic diet should be highlighted (Table 2). Considering testosterone plasma levels, we noted that exercised groups presented higher blood androgen levels in comparison to sedentary mices, with NDE showing the highest testosterone levels. Regarding diet, we found that HDS presented decreased testosterone levels when compared to NDS and physical exercise did not restore androgenic levels in the HD group (Table 2).

3.2 Hyperlipidic Diet can be Involved with Prostatatic Epithelial Atrophy, Which was Improved After Physical Exercise

Morphological and stereological analysis of the prostate showed that sedentary animals have decreased 31% (P=) secretory epithelial area after hyperlipidic diet (Figs. 1A, 1C and 1E). Physical exercise, regardless of diet, increased the relative frequency of epithelium by 40% and preserved the epithelial area in the HDS, as seen in HDE group (Figs. 1B, D and E). Regarding luminal compartment, there was a high possibility of lumen area expansion after hyperlipidic diet in the sedentary group, but this result was not statistically significant. Finally, physical exercise increased 166% and 70% the luminal relative frequency of the prostate in mice submitted to normal and high-fat diet, respectively (Fig. 1E).

3.3 Cell Proliferation is Positively Associated with High Expression of AR and ERK1/2 After Hyperlipidic Diet, being Reversed by Physical Training

The prostate of hyperlipidic group had increased levels of AR (Figs. 2A and C) and PCNA (Figs. 2B and C) when compared to normal diet. The same was found for physical exercise parameter, since the NDE group also presented high levels of these proteins when compared to sedentary group (NDS). However, there was a significant reduction in both AR expression and cell proliferation in the hyperlipidic diet group after physical training (Figs. 2A, B and C).

Related to cell proliferation, physical exercise promoted ERK upregulation in normal diet group since the phosphorylated levels of ERK1/2 were higher in the NDE group when compared to NDS. The hyperlipidic diet *per se* also stimulated ERK1/2 activation. In other hand, when physical exercise was evaluated in HD group, the activation of ERK1/2 signaling pathway was restored to normal levels (Fig. 2D). In summary, physical exercise can exert a promoter or inhibitor effect on AR and ERK1/2 expression, in a diet dependent manner.

Table 2. Biometrical, metabolic and hormonal parameters of mice treated with normal (ND) or hyperlipidic (HD) diet. -S represents sedentary mice (NDS, HDS) and -E represents exercised mice (NDE, HDE). All values are represented as the means ± SEM

	NDS	NDE	HDS	HDE
Body Weight (g)	40.9 ± 0.8	35.9 ± 1.2	45.8 ± 2.7*	45.9 ± 2.4*
Prostate weight (mg)	0.41 ± 0,08	0.47 ± 1,10	0.25 ± 0.01	0.20 ± 0.01
Adiposity index (%)	6.7 ± 0.5	3.3 ± 0.2*	10.6 ± 0.50*	9.0 ± 0.9*[a]
Glicaemia (mg/dL)	151 ± 10.7	146 ± 21.1	213 ± 22.1*	196 ± 25.8
Testosterone (ng/dL)	325± 38	410 ± 15*	219 ± 53*	270 ± 45*[a]

*Body weight were measured in the end of experimental period. Adiposity index was defined by the weight of visceral (kidney, mesenteric) and epididymal fat. * represents statistical difference compared to NDS and [a] represents statistical difference compared to NDE (P<0.05). One-way ANOVA with bonferroni multiple comparisons test, n=10 for all parameters, except for testosterone (n=5) where Kruskal–Wallis post-test was performed*

3.4 Protector Effect of ERβ is Reduced in the Prostate of Hyperlipidic Mice and it is Improved after Physical Exercise

Physical exercise *per se*, as well as hyperlipidic diet reduced the number of ERβ-positive cells compared to normal diet and sedentarism (Fig. 3A,B). On the other hand, physical training elevated ERβ-positive cells in prostate of HDE group, restoring the negative effect of hyperlipidic diet on estrogen receptor pathway (Fig. 3A,B).

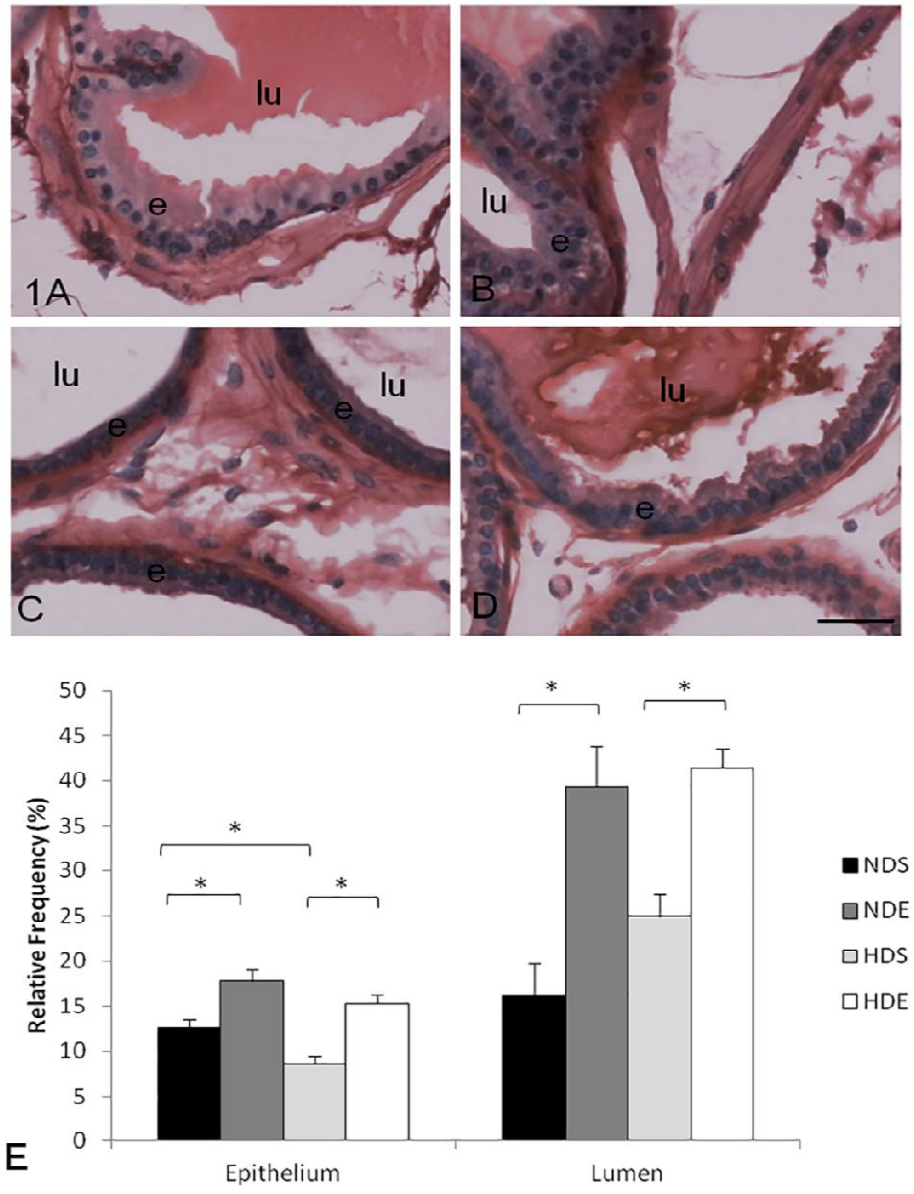

Fig. 1. Histological sections of mice prostate stained by Picrosirius-Hematoxylin showing epithelium (e) and lumen (lu). Mice were treated with normal (A and B) or hyperlipidic (C and D) diet / sedentary (A and C) or trained with physical exercise (B and D). This fig shows an atrophy of prostate epithelium and luminal enlargement after HD in sedentary groups. Physical exercise restored epithelial area, but increased the lumen region in a higher degree. E-Relative frequency (%) of prostatic epithelial and luminal area (means ± SEM). One-Way ANOVA with Bonferroni Multiple Comparisons Test, n=10. *Statistical difference (p<0.05). Scale bar: 50 µm

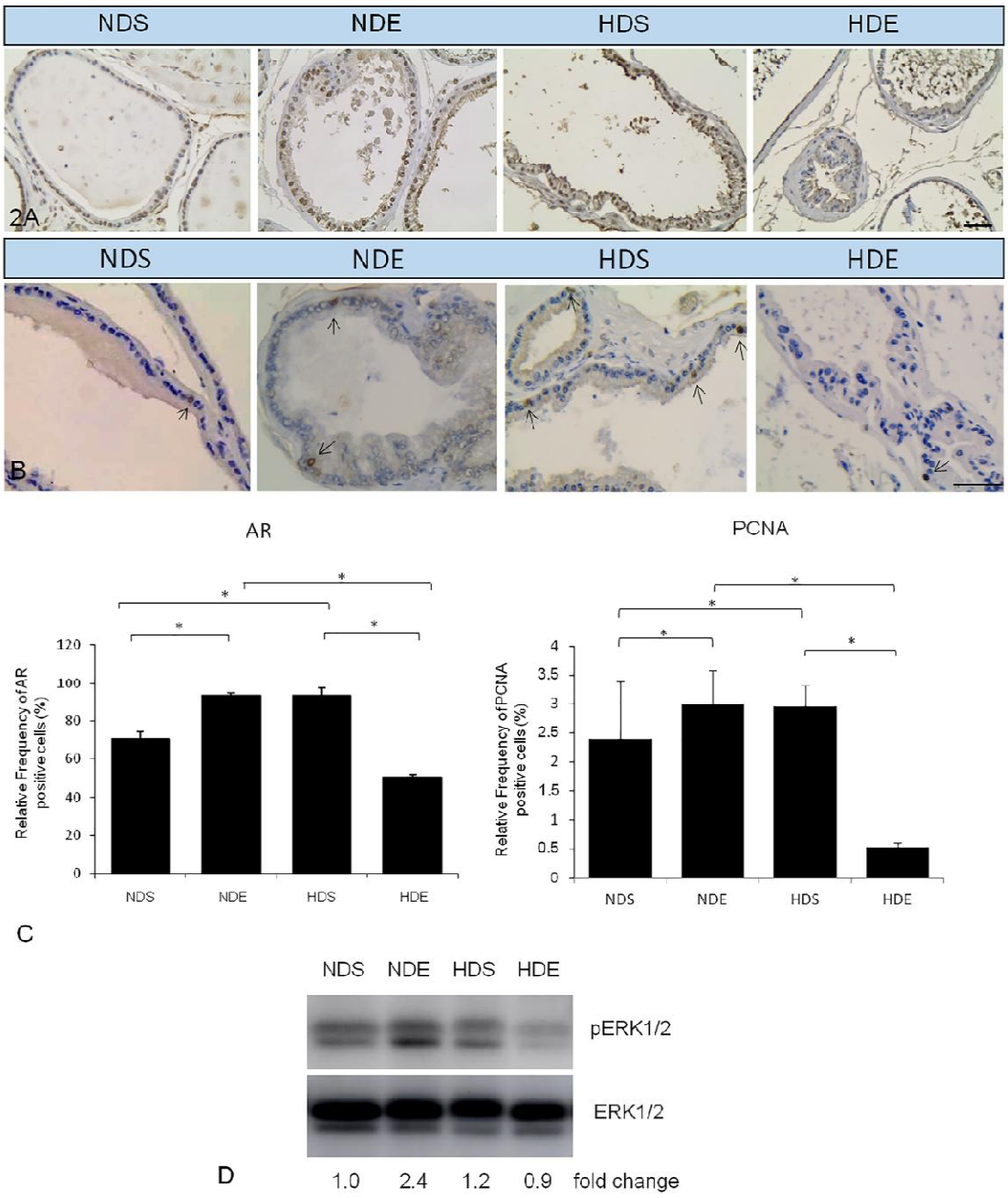

Fig. 2. Imunohistochemistry analysis for AR (A) and PCNA (B) on prostate of mice treated with normal (ND) or hyperlipidic (HD) diet. -S represents Sedentary mice (NDS, HDS) and -E represents Exercised mice (NDE, HDE). Swimming training *per se* and hyperlipidic diet increased AR expression and cell proliferation on mice prostate. Physical training restored these changes. C- Relative frequence of AR- and PCNA-positive cells (mean ± SEM). One-Way ANOVA with Tukey-Kramer Test, n=10 *Statistical difference ($p<0.05$) Scale bar: 50µm. D- Western blotting analysis of ERK1/2 (phosphorylated or not) demonstrate an increase of ERK1/2 activation after HD in sedentary mice, reducing after physical training. Swimming training *per se* also increased ERK1/2 activation

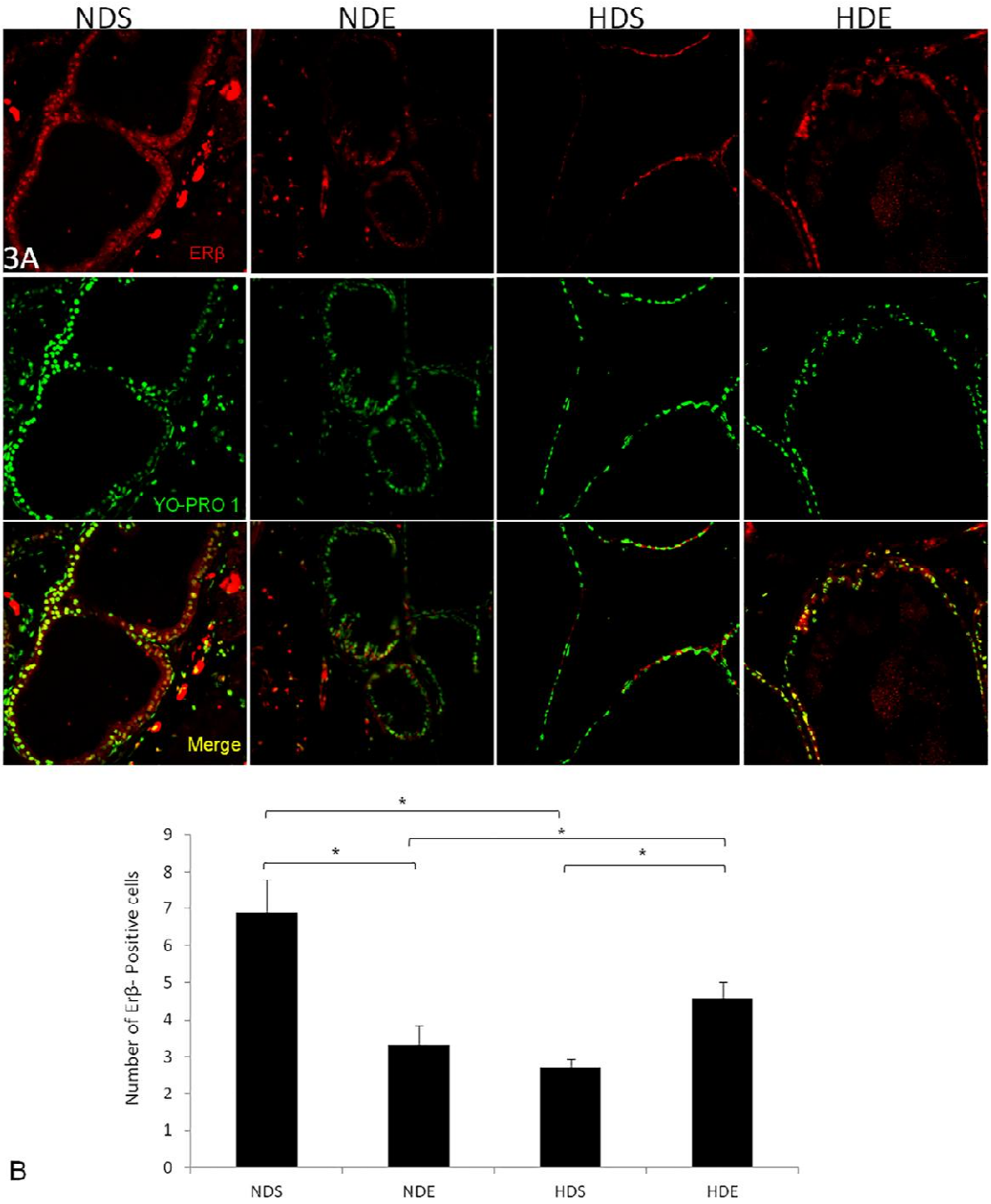

Fig. 3. A. Immunofluorescence of ERβ on prostate of mice treated with normal (DN) or hyperlipidic (HD) diet. -S represents Sedentary mice (NDS, HDS) and -E represents Exercised mice (NDE, HDE). ERβ is labeled in red by Alexa Fluor 555; nuclei is labeled in green by YOPRO and the third panel shows merged Fig. 3. B. Relative frequence of ERβ- positive cells in prostate sections (mean ± SEM) showing a significant decrease of ERβ expression caused by physical exercise in ND group and also by high-fat diet in sedentary group. Physical training protocol restored ERβ levels in HD group. *Statistical difference ($p<0.05$), One-Way ANOVA with Bonferroni Multiple Comparisons Test, n=5. Scale bar: 50 µm

4. DISCUSSION

In the present investigation, we evaluated whether a diet with an elevated content of saturated fat (45%) affected the prostate structure, androgenic response, as well as cell proliferation. We also evaluated if physical exercise was able to reverse such effects. This was the first study involving this goal in the prostate. The hyperlipidic diet increased body weight, adiposity, and glycaemia of sedentary mice. However, surprinsingly, these biometric and metabolic changes were not restored with physical exercise alone, at least not by swimming, without diet modification. Similarly, Riyahi & Riyahi showed that there was no loss of body weight after physical training by swimming in rats [16]. A routine of exercise helps to prevent positive energy balance, thus energy content of diet is one of the most importan factors in weight control even in a schedule of exercise [16]. Additionally, King & Tribble have reviewed several studies showing little or no weight loss in obese men after physical exercise without caloric restriction [17]. Thus, our data suggests the importance of dietary re-education concomitantly with the practice of physical exercise for achieving metabolic benefits and body weight reduction.

This investigation demonstrated a reduction in testosterone levels in HDS group after hyperlipidic feeding and prevent the body weight gain. In males, obesity alters the hypothalamic-pituitary-gonadal axis, reducing testosterone production [18,19]. In this scenario, Pinto-Fochi et al. demontrated that deregulation of leptin is the main factor related to steroidogenic impairment of Leydig cells in rats treated with hyperlipidic diet. Since the prostate is dependent on androgen for its function and tissue maintenance, the reduction of this hormone can led to glandular atrophy as we suggest in the present investigation [20]. The same effect can be found in different investigations involving metabolic syndrome, insulin resistence, and diabetes (common obesity-related disorders) where testosterone levels were also drastically decreased [12,21]. Regarding physical exercise, swimming *per se* was able to increase plasma testosterone in mice fed with normal diet. It is already known that intense physical exercise has an anabolic role, increasing blood testosterone levels [22]. However, in mice submited to hyperlipidic diet, the swimming protocol was not able to reverse completely the testosterone reduction caused by diet, since body weight remained high in these animals. In this context, Hebert et al. (1998) demonstrated that a low-fat diet associated with regular physical activity normalized serum testosterone levels and reduced the risk of developing prostate cancer in obese men [23]. Therefore, we suggest that an excessively lipidic diet can cause testosterone reduction and may be implied with prostate atrophy in mice. Moreover, swimming was not able to restore this effect, suggesting again the importance of a balanced diet associated the practice of physical activity on body weight reduction for androgenic normalization.

Epithelial atrophy was found in association to reduction in prostate weight in animals treated with hyperlipidic diet. In addition, the anabolic effect of physical exercise was clearly noted in the relative frequency of epithelium, since exercised groups presented higher values of epithelial cells, independently of diet. The same occurred for the lumen, a glandular region of secretion storage. Luminal enlargement in HF group could be related to secretion retention due to drop on androgenic stimuli and consequent reduced ejaculation, being a parameter for impaired reproductive function of the gland. Previous studies in rats have shown that situations of androgenic reduction such as castration, diabetes, and obesity can alter the histology of the prostate, causing atrophy of the epithelium and luminal enlargment [24,25]. Therefore, we suggest that an excessively hyperlipidic diet also has an atrophic effect on mice prostate and possibly may interfere with the reproductive function of the gland. Moreover, physical exercise could act as an anabolic factor in androgenic stimuli and prostate morphology, being able to restore the negative effect of HD on prostate secretory epithelium.

The prostatic levels of AR and cell proliferation were also increased by diet and exercise *per se*. The effects of diet on AR and PCNA expression probably reflect testosterone decline. Since prostate is androgen depentent, epithelial cells tend to increase AR levels to compensate for the lack of androgenic stimulus in the gland. Androgens, in addition to maintaining the function of the prostate, are the main regulators of prostate secretory function and growth [26]. Testosterone is the main androgen in blood binding to AR and stimulating proliferative androgenic action, being implied in cell growth and prostate cancer survival [27]. In this sense, it is well known that testosterone ablation causes prostate atrophy and cancer regression, due to

cell proliferation inhibition and cell death increase [28]. However, this benefit of androgen ablation is only initial, because basal cells (highly proliferative and androgen-independent) proliferate again, re-establishing a more agressive tumour over time. Thus, our data suggest that a hyperlipidic diet, in spite of reducing testosterone (which alone could have a testosterone-lowering effect and protect against carcinogenesis), has an important role, increasing AR expression and cell proliferation. In the long term, this situation may contribute to the onset of proliferative diseases such as hyperplasia and prostate cancer. Finally, physical exercise (particulary swimming) was beneficial when evaluating the improvement of AR and cell proliferation levels in those mice that received the hyperlipidic diet.

Besides androgenic signal improvement, cell proliferation can also be explained by the greater activation of the ERK 1/2 signaling pathway after hyperlipidic diet. ERK 1/2, participates in MAPK pathway which is related to cell survival and proliferation, being a strong cell signal of mitogenic activity [29]. It has been demonstrated that some factors associated with obesity, such as hyperglycaemia, hyperinsulinaemia, proinflammatory cytokines, and high amounts of fatty acids, can stimulate cell proliferation by mitosis [30]. Furthermore, Fernandez-Twinn et al. showed that the metabolic dysfunctions of obesity induce ERK signaling pathway activation, stimulating mitosis in cardiac tissue [31]. We also showed that physical exercise is able to reverse the increased cellular proliferation in the prostate after hyperlipidic diet through AR and ERK signaling pathways. Wang et al. recently demonstrated that aerobic exercise increases the expression of ERK and AMPK in mice liver, corroborating to our data [32]. Thus, our results indicate that obesity and its metabolic disorders can trigger cell proliferation in the prostate through ERK activation, even in situations of androgen deficiency, and physical exercise may reverse this effect.

In humans and also in rodents, androgen and estrogen can play a crucial role in the prostate development and functional maintenance. Furthermore, estrogen has a dual role in prostate cancer, being protective through ERβ signalling or carcinogenic promoter when ERα signal predominate [33]. In a previous work, we showed that the amount of ERβ expression increased considerably in rat prostate after 15 weeks of 20% hyperlipidic diet, reducing after long term diet (30 weeks) [12,13]. In the present investigation, we demonstrate that increasing the amount of saturated fat from 20% to 45% may have exacerbated this effect of diet on prostate ERβ. This HFD drastically decreased ERβ prostate content after 15 weeks of high-fat diet, a result that was found only after the long term in 20% hyperlipidic diet in rats. The decrease of prostate ERβ expression can reduce its antiproliferative and protective role on the glandular epithelium which also can explain the increased cell proliferation found in HDS group. In this context, this effect of an excessive high-fat diet on ERβ expression would be relevant in promoting proliferative diseases in the gland. As noted for AR and ERK, physical exercise also reversed the effects of the hyperlipidic diet on prostate ERβ expression, suggesting the beneficial influence of physical training on the maintenance of gland homeostasis.

5. CONCLUSIONS

The present study suggests that a diet of 45% saturated fat can be associated with a negative effect on mice prostate, atrophying the secretory epithelium and stimulating cell proliferation through increased AR and ERK 1/2 signaling and decreased ERβ expression. Considering a long term of a high-fat diet and a sedentary lifestyle, this effect may induce the establishment of proliferative diseases in the prostate. Furthermore, we also demonstrated for the first time that physical training, such as swimming, is able to reverse proliferative signals caused by high-fat diet, highlighting the importance of a non-sedentary routine for beneficial effects in prostate homeostasis, preventing some proliferative disorders in the gland.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care&quot" (NIH publication No. 85-23, revised 1985) were followed, as well

as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee. This study was approved by the Institutional Committee for Ethics in Animal Experimentation of the Federal University of Uberlandia (CEUA/UFU, # 063/11).

ACKNOWLEDGEMENTS

The authors are grateful for the technical assistance of Mr. Fabricio Faria Araujo, Mrs. Ester Cristina Borges Araujo. We also would like to thank to the Foundation to Support Research of the State of Minas Gerais (FAPEMIG) for funding this study (Grant number: APQ-02645-15) and the National Council for Scientific and Technological Development (CNPq). The sponsors had no involvement in the manuscript design or evaluation, but the provided funds to this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization. Obesity and overweight. Fact sheet, Last updated; 2017-09-27.
   Accessed: 4 December 2020.
   Available:http://apps.who.int/gho/data/view.main.BMI25AWBv?lang=en
2. Rawla P. Epidemiology of prostate cancer. World J Oncol. 2019;10(2):63-89.
3. Carvalheira JBC, Saad MJA. Insulin resistance/hyperinsulinemia associated diseases not included in the metabolic syndrome. Arq Bras Endocrinol Metabol. 2006;50(2):360-367.
4. Corona G, Vignozzi L, Rastrelli G, Lotti F, Cipriani S, Maggi M. Benign prostatic hyperplasia: A new metabolic disease of the aging male and its correlation with sexual dysfunctions. Int J Endocrinol. 2014;329456.
5. Vignozzi L, Maggi M. Prostate cancer: Intriguing data on inflammation and prostate cancer. Nat Rev Uro. 2014;11:369–370.
6. Pelser C, Mondul AM, Hollenbeck AR, Park Y. Dietary fat, fatty acids, and risk of prostate cancer in the NIH-AARP diet and health study. Cancer Epidemiol Biom Rev. 2013;22:697-707.
7. Zadra G, Photopoulos C, Loda M. The fat side of prostate cancer. Biochimica et Biophysica Acta. 2013;1831(10):1518-1532.
8. Liu YP, Hu F, Li D, Wang F, Zhu L, Chen W, Ge J, An R, Zhao Y. Does physical activity reduce the risk of prostate cancer? A systematic review and meta-analysis. Eur Urol. 2011;60:1029-1044.
9. Rundqvist H, Augsten M, Strömberg A, Rullman E, Mijwel S, Kharaziha P, Panaretakis T, Gustafsson T, Östman A. Effect of acute exercise on prostate cancer cell growth. PloS One. 2013;8(7):67579.
10. Nilsen TI, Romundstad PR, Vatten LJ. Recreational physical activity and risk of prostate cancer: A prospective population-based study in Norway (the HUNT study). Int J Cancer. 2006;119(12):2943-2947.
11. Tir AMD, Labor M, Plavec D. The effects of physical activity on chronic subclinical systemic inflammation. Arh Hig Rada Toksikol. 2017;68(4):276-286.
12. Ribeiro DL, Pinto ME, Rafacho A, Bosqueiro JR, Maeda SY, Anselmo-Franci JA, Taboga SR, Góes RM. High-fat diet obesity associated with insulin resistance increases cell proliferation, estrogen receptor, and PI3K proteins in rat ventral prostate. J Androl. 2012;33(5):854-65.
13. Silva SA, Gobbo MG, Pinto-Fochi ME, Rafacho A, Taboga SR, Almeida EA, Góes RM, Ribeiro DL. Prostate hyperplasia caused by long-term obesity is characterized by high deposition of extracellular matrix and increased content of MMP- 9 and VEGF. Int J Exp Pathol. 2015;96(1):21-30.
14. Landim BC, de Jesus MM, Bosque BP, Zanon RG, da Silva CV, Góes RM, Ribeiro DL. Stimulating effect of palmitate and insulin on cell migration and proliferation in PNT1A and PC3 prostate cells: Counteracting role of metformin. Prostate. 2018;78(10):731-742.
15. Almeida PW, Gomes-Filho A, Ferreira AJ, Rodrigues CE, Dias-Peixoto MF, Russo RC, Teixeira MM, Cassali GD, Ferreira E, Santos IC, Garcia AM, Silami-Garcia E, Wisløff U, Pussieldi GA. Swim training suppresses tumor growth in mice. J Appl Physiol. 2009;107:261-265.
16. Riyahi F, Riyahi S. Effect of moderate swimming exercise on weight gain in high fat diet rats. Annals of Military and Health Sciences Research. 2016;14(1):46-50.
17. King AC, Tribble DL. The role of exercise in weight regulation in

nonathletes. Sports Med. 1991;11(5):331-49.
18. Solomon TPJ, Haus JM, Kelly KR, Cook MD, Filion J, Rocco M, Kashyap SR, Watanabe RM, Arkoukis H, Kirwan JP. A low–glycemic index diet combined with exercise reduces insulin resistance, postprandial hyperinsulinemia, and glucose-dependent insulinotropic polypeptide responses in obese, prediabetic humans. Am J Clin Nutr. 2010;92(6):1359-1368.
19. Macdonald AA, Herbison GP, Showell M, Farquhar CM. The impact of body mass index on semen parameters and reproductive hormones in human males: A systematic review with meta-analysis. Hum Reprod Update. 2010;16(3):293-311.
20. Pinto-Fochi ME, Pytlowanciv EZ, Reame V, Rafacho A, Ribeiro DL, Taboga SR, Góes RM. A high-fat diet fed during different periods of life impairs steroidogenesis of rat Leydig cells. Reproduction. 2016;152(6):795-808.
21. Ehala-Aleksejev K, Punab M. The effect of metabolic syndrome on male reproductive health: A cross-sectional study in a group of fertile men and male partners of infertile couples. PLoS One. 2018;13:0194395.
22. França SCA, Barros Neto TL, Agresta MC, Lotufo RF, Kater CE. Divergent responses of serum testosterone and cortisol in athlete men after a marathon race. Arq Bras Endocrinol Metabol. 2006;50(6):1082-1087.
23. Hebert JR, Hurley TG, Olendzki BC, Teas J, Ma Y, Hampl JS. Nutritional and socioeconomic factors in relation to prostate cancer mortality: A cross-national study. J Nat Cancer Inst. 1998;90(21): 1637-1647.
24. Ribeiro DL, Caldeira EJ, Cândido EM, Manzato AJ, Taboga SR, Cagnon VH. Prostatic stromal microenvironment and experimental diabetes. Eur J Histochem. 2006;50(1):51-60.
25. Cakiroglu B, Hazar AI, Eyyupoglu SE, Can Balci MB, Sinanoglu O, Tuzlali P. The comparative evaluation of apoptosis produced by leuprolide or orchiectomy on rat prostate tissue. Arch Ital Urol Androl. 2016;87(4):265-269.
26. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nat Rev Cancer. 2001;1:34-45.
27. Cunha GR, Ricke W, Thomson A, Marker PC, Risbridger G, Hayward SW, Wang YZ, Donjacour AA, Kurita T. Hormonal, cellular and molecular regulation of normal and neoplastic prostatic development. J Steroid Biochem Mol Biol. 2004;92(4):221-36.
28. Denmeade SR, Lin XS, Isaacs JT. Role of programmed (apoptotic) cell death during the progression and therapy for prostate cancer. Prostate. 1996;28(4):251-265.
29. Sun Y, Liu WZ, Liu T, Feng X, Yang N, Zhou HF. Signaling pathway of MAPK/ERK in cell proliferation, differentiation, migration, senescence and apoptosis. J Recept Signal Transduct Res. 2015;35(6): 600-4.
30. Mistry T, Digby JE, Desai KM, Randeva HS. Obesity and prostate cancer: A role for adipokines. Eur Urol. 2007;52(1):46-53.
31. Fernandez-Twinn DS, Blackmore HL, Siggens L, Giussani DA, Cross CM, Foo R, Ozanne SE. The programming of cardiac hypertrophy in the offspring by maternal obesity is associated with hyper-insulinemia, AKT, ERK, and mTOR activation. Endocrinology. 2012;153(12): 5961-5971.
32. Wang M, Li S, Wang F, Zou J, Zhang Y. Aerobic exercise regulates blood lipid and insulin resistance via the toll-like receptor 4-mediated extracellular signal-regulated kinases/AMP-activated protein kinases signaling pathway. Mol Med. 2018;17(6): 8339-8348.
33. Prins GS, Korach KS. The role of estrogens and estrogen receptors in normal prostate growth and disease. Steroids. 2008;73(3):233-44.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/63992