Received: 25 June 2022 | Revised: 1 September 2022 | Accepted: 7 September 2022

DOI: 10.1111/and.14600

ORIGINAL ARTICLE

First International Journal of Andrology ANDROLOGIA WILEY

Exercise improves testicular morphology and oxidative stress parameters in rats with testicular damage induced by a high-fat diet

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Funding information
Acibadem Mehmet Ali Aydinlar University Scientific Research Project Committee, Grant/Award Number: 2016/05/01; Marmara University Scientific Research Project Committee, Grant/Award Number: SAG-C-DRP-131016-0443; Scientific and Technological Research Counsil of Turkey-TUBITAK, Grant/Award Number: 216S280

Abstract

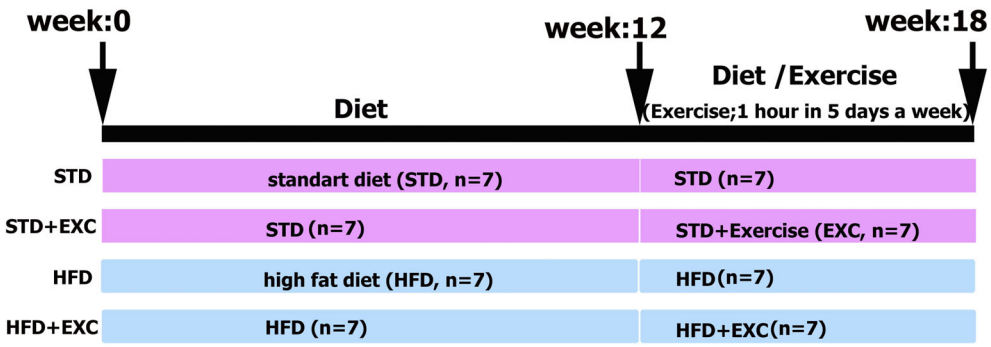
Obesity and male infertility are problems that affect population. Exercise is a nonpharmacological way to reduce the negative health effects of obesity. The purpose of this study was to examine the effects of exercise on hormone levels, blood-testis barrier, and inflammatory and oxidative biomarkers in rats that became obese due to a high-fat diet (HFD). Male rats received a standard diet (STD group) or a HFD (HFD group) for 18 weeks. During the final 6 weeks of the experiment, swimming exercises (1 h/5 days/week) were given to half of these animals (STD + EXC and HFD + EXC groups). Finally, blood and testicular tissues were analysed by biochemical and histological methods. Body weight, leptin, malondialdehyde, interleukin-6, TNF-alpha and myeloperoxidase levels, apoptotic cells and DNA fragmentation were increased, and testis weight, insulin, FSH, LH, testosterone, glutathione and superoxide dysmutase levels, proliferative cells, ZO-1, occludin, and gap junction protein Cx43 immunoreactivity were decreased in the HFD group. All these hormonal, morphological, oxidative and inflammatory biomarkers were enhanced in the HFD + EXC group. It is thought that exercise protected testicular cytotoxicity by regulating hormonal and oxidant/antioxidant balances and testicular function, inhibiting inflammation and apoptosis, as well as preserving blood-testis barrier.

KEYWORDS
blood testis barrier, exercise, high-fat diet, oxidative stress, ultrastructure

1 | INTRODUCTION

Obesity is a global problem in both developed and underdeveloped countries (Stefan et al., 2013). Male infertility has been seen to rise along with population body mass index (BMI) increases (Hammoud et al., 2012). Low semen volume and decreasing motile sperm count are associated with male obesity (Hammoud et al., 2012). Obesity decreases sperm quality and morphology, eventually impairs sperm maturation and function (Shukla et al., 2014).

The blood-testis barrier protects germ cells from the immune system. Loss of this barrier function causes infertility (Stanton, 2016). This barrier is made by tight junctions between Sertoli cells. Occludin, located in the tight junction barrier, plays a crucial role in controlling the blood-testis barrier. The linking components found between other junctional proteins are ZO-1 and ZO-2 (Contuk et al., 2012). Spermatogenesis occurs by well-organized interconnections between cells. Intercellular communication, provided in particular by connexin 43 (Cx 43) junctions, have a crucial function in both formation of fetal germ cells for the fetal period as well as the adult spermatogenic process (Stanton, 2016). Cx 43 is likely linked with Sertoli cell differentiation and Sertoli–Sertoli cell interactions (Günther et al., 2013).



SCHEME 1 Experimental design. Diagram illustrating the timeline of the diet and exercise applications to the experimental groups

Sex steroids regulate the highly specialized process of spermatogenesis. Sertoli cells have a testosterone-dependent effect on growing germ cells, thus as testosterone levels decrease, mature spermatids are phagocytosed, and the sperm count reduces (Kerr et al., 1993). Studies have shown the relationship between an increase in these hormone levels and BMI (Du Plessis et al., 2010). Hyperinsulinemia and hyperglycemia reduce sperm count and quality, thereby reducing fertility in obese men (Kasturi et al., 2008; La Vignera et al., 2012).

Oxidative stress is observed in male infertility and is activated by reactive oxygen species (ROS) (Hamada et al., 2013). Excessive ROS and oxidative stress in male reproductive system cause negative changes in sperm parameters and infertility (Khosrowbeygi & Zarghami, 2007).

Exercise can help to improve the morphology, motility, and function of sperm, particularly in obese animals (Palmer et al., 2012). Regular, non-strengthening exercise has been proven in studies to have beneficial effects on hormone balance (Palmer et al., 2012). With regular physical exercise, even if weight loss does not occur, total and visceral fat reduces as a result of exercise, which attenuates the negative effects of obesity on health parameters (Lee et al., 2005). Numerous experimental investigations have demonstrated that food consumption and/or physical activity can alter sperm characteristics in obese men, including motility, morphology, and biochemical characteristics such as DNA damage and mitochondrial ROS (Du Plessis et al., 2010). In obese men, diet and/or exercise have been shown to raise sperm quality, enhance fertilization, and significantly reduce sperm DNA damage and mitochondrial ROS levels (Palmer et al., 2012).

The effect of exercise on obesity- induced male infertility has been studied experimentally. However, in obese male rats, how moderate swimming exercise affects the integrity of the blood-testis barrier, which controls spermatogenesis, has not been studied. In this study, histological and biochemical techniques were used to determine the effect of swimming training on testicular damage induced by a high-fat diet in obese male rats.

2 | MATERIALS AND METHODS

2.1 | Experimental model

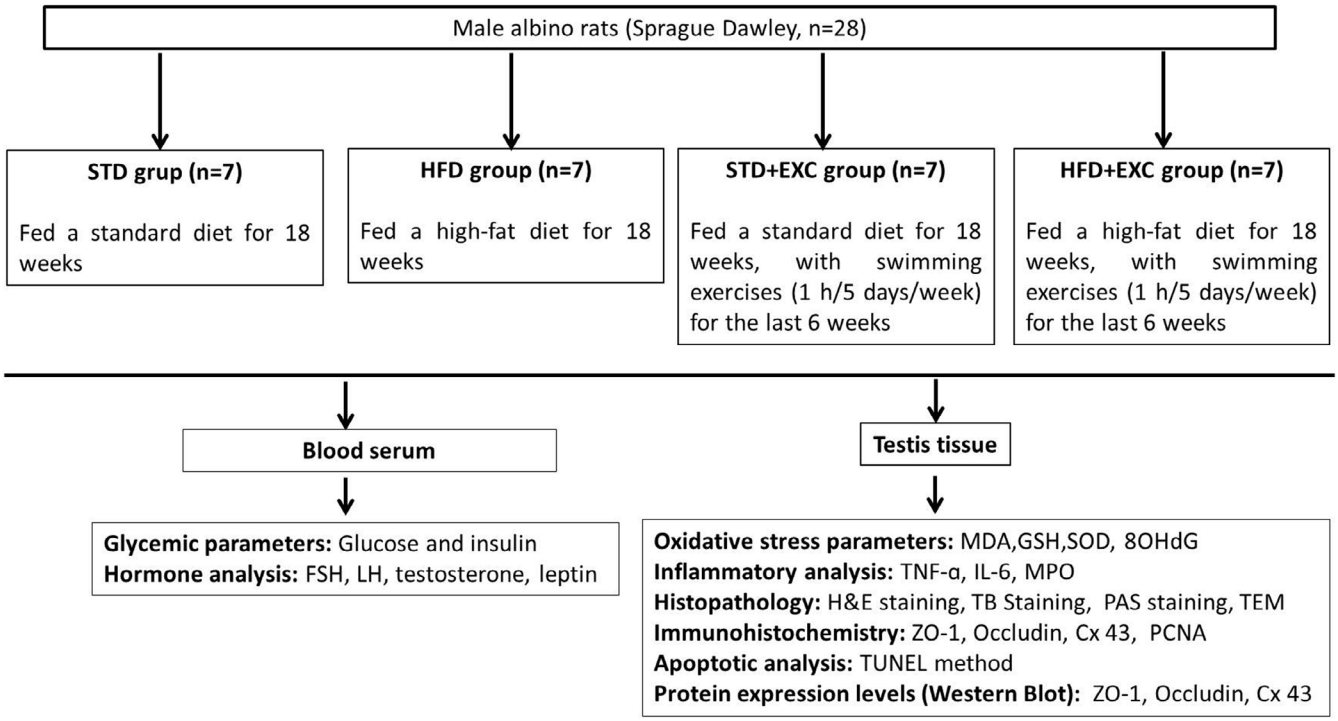
Sprague Dawley male rats ($n = 28$, 8 weeks old) were housed in a lab with a typical light/dark (12/12 h) cycle at 22 ± 2°C. Rats ($n = 7$, in each experimental groups) in the control (STD) and control + exercise (STD + EXC) groups were received a standard rat diet (STD; 57% carbohydrate, 24% protein, 5% fat). The high-fat diet (HFD) and HFD + exercise (HFD + EXC) groups were fed a high-fat diet. To induce obesity (Tuzcu et al., 2011), rats were fed a high-fat diet (35% carbohydrate, 20% protein, 45% fat). Rats were fed a STD or HFD for 18 weeks. The STD + EXC and HFD + EXC groups completed 6 weeks of swim training starting at week 12 of the study. Swimming exercises were performed (at 32 ± 1°C) in a plastic swimming pool (60 × 150 × 45 cm). The STD + EXC and HFD + EXC groups swam 5 days per week for 1 h from week 12 to week 18 (Scheme 1) (Burneiko et al., 2006; Ickin Gulen et al., 2015; Tuzcu et al., 2011). Finally, rats were anaesthetised with ketamine and xylazine injections. Blood was processed for biochemical examinations and testis tissue samples were used for microscopic and biochemical evaluations (Scheme 2). The Marmara University Ethics Committee for Experimental Animals gave approval to the project (014.2016. mar).

2.2 | Measurement of serum glucose and insulin concentrations and serum FSH, LH, testosterone and leptin levels

The serum glucose concentration and insulin levels were measured using a commercial glucose kit (Biolabo, Glucose GOD-PAP) and 'Rat Insulin ELISA kit' (Bioassay Technology Laboratory, China), respectively. Serum concentrations of FSH, LH, testosterone, and leptin were also estimated by commercial ELISA kits (Bioassay Technology Laboratory, China). Results were expressed in mg/dl for glucose, mIU/L for insulin, mIU/L for FSH and LH, and nmol/L for testosterone.

2.3 | Measurement of testicular malondialdehyde and glutathione levels and superoxide dismutase activity

The malondialdehyde (MDA) level was measured with the thiobarbituric acid (TBA) assay (Ohkawa et al., 1979). The glutathione (GSH) level was estimated by the Ellman (Ellman, 1959) method. Following the manufacturer's instructions, the SOD test Kit-WST (Sigma-Aldrich) was used to estimate the activity of superoxide dismutase (SOD). For MDA and GSH, the data were presented as nmol/g, and for SOD, as a percent inhibition.



SCHEME 2 Experimental analysis of blood serum and testis tissue. The diagram illustrates the analysed parameters in blood serum and testis tissue of the experimental groups

2.4 | Measurement of testicular tumour necrosis factor-α and interleukin-6 levels and myeloperoxidase activity

Tumour necrosis factor (TNF-α), interleukin-6 (IL-6), and myeloperoxidase (MPO) levels were estimated using commercially available rat ELISA kits (Bioassay Technology Laboratory). Results for TNF-, IL-6, and MPO were presented in pg/ml and ng/ml, respectively.

2.5 | Testicular 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels

A commercial DNA extraction kit was used to extract genomic DNA from the testicular samples (Invitrogen, USA) and ELISA kit was used to determine the 8-OHdG content of the tissue (OxiSelect Oxidative DNA Damage ELISA Kit, Cell Biolabs, USA). Data were presented as ng/ml tissue 8-OHdG.

2.6 | Light microscopic preparation

The right testis samples were prepared by the standard paraffin embedding technique. Sections stained with haematoxylin and eosin (H&E) and Periodic Acid-Schiff reaction (PAS) were used for morphological observations. The Image J tool (NIH-USA) was used to determine the epithelial thicknesses of hundred seminiferous tubules in each H&E-stained sections. These seminiferous tubules were also evaluated according to the modified Johnsen's scoring by using a scale ranging from 1 (absence of germinal epithelium of seminiferous tubule) to 10 (full spermatogenesis) (Arabacı Tamer et al., 2019).

2.7 | Proliferative cell nuclear antigen (PCNA) immunohistochemistry

Paraffin sections (5 μm thickness) were taken on the positively charged slides. After deparaffinization, endogenous enzyme blockade was applied by 3% hydrogen peroxide (H_2O_2, 20 min). Sections were incubated with a 300 W microwave in citrate buffer for antigen retrieval (20 min). The sections cooled at room temperature were treated with PBS (3 × 5 min) and then kept in blocking solution (10 min, Invitrogen). Sections kept in rabbit anti-PCNA (Novus) primary antibody (1: 2000) overnight (4°C) were treated with PBS (3 × 5 min) and then kept in biotinylated secondary antibody (30 min, Thermo). Later washing with PBS, they were treated with streptavidin peroxidase (10 min, Invitrogen), washed with PBS, washed with DAB chromogen (5 min), washed in PBS and counterstained with haematoxylin. Following the random selection of the first seminiferous tubule, 20 seminiferous tubules were investigated in each section. PCNA-positive cells were divided by the total number of cells to determine the proliferation index.

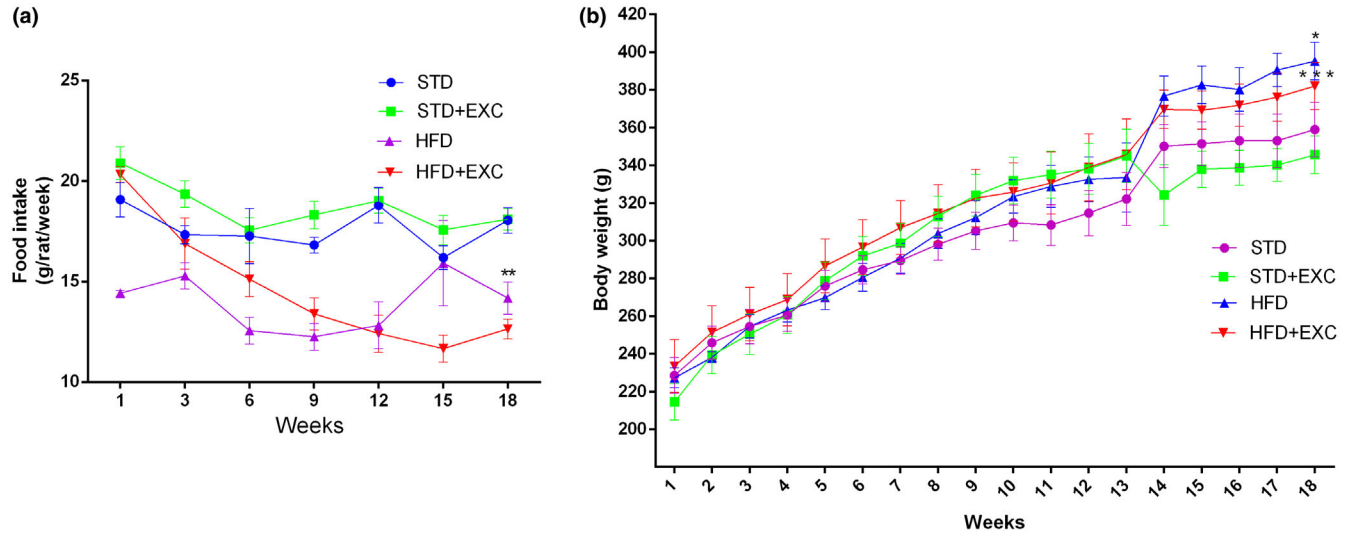


FIGURE 1 Food intake (a) and body weight (b) of the experimental groups. Body weight of the experimental groups ($n = 7$ per diet group). ANOVA revealed HFD > HFD + EXC > STD + EXC > STD. *$p < 0.05$ and **$p < 0.01$ versus STD group. Values are given as the mean ± SD

2.8 | Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) analysis

The TUNEL method was used according to the method in the user manual provided by the commercial kit (ApopTag Plus, In Situ Apoptosis Detection Kit, S7101, Millipore). TUNEL-positive cells were counted by examining 20 randomly selected cross-sectioned seminiferous tubules. The apoptotic index was estimated by dividing the total number of seminiferous tubules by the number of seminiferous tubules containing 3 or more TUNEL-positive cells.

2.9 | ZO-1, occludin, Cx 43 immunohistochemistry

For immunohistochemistry analyses, paraffin sections fixed with 4% paraformaldehyde were used. Paraffin sections were taken on the positively charged slides. After the deparaffinization procedure, sections were incubated in 3% H_2O_2 (20 min) for endogenous enzyme blockade. Sections were incubated in a 200-W microwave in ethylene diamine tetraacetic acid (EDTA) buffer (20 min) to retrieval antigen. Sections were cooled to room temperature, treated with PBS (3 × 5 min), and then stored in blocking solution (10 min, Invitrogen). Sections were treated with rabbit anti-ZO-1 (1: 100), anti-occludin (1: 150), and anti-Cx 43 (1: 50) primary antibodies (Novus) overnight (4°C). Sections were treated with PBS (3 × 5 min) and then treated with biotinylated secondary antibodies (30 min, Thermo). The sections received PBS washing, treated with streptavidin-peroxidase (10 min, Invitrogen), washed in PBS, washed with AEC chromogen (15 min), and treated with distilled water. Sections were then mounted with Keiser glycerol gel (Merck, 109242). Twenty seminiferous tubules in each section were analysed for the intensity of immunoreactivity of ZO-1, occludin, and Cx 43 using Image J software (NIH-USA).

2.10 | ZO-1, occludin, Cx 43 Western blotting analysis

Tissue protein concentrations were calculated by a Quant-iT™ protein analysis kit (Qubit; Molecular Probes, Invitrogen). Protein concentrations were measured with a Qubit™ fluorometer (Invitrogen Q32857). Samples were allocated with 12% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes. Membranes were treated with rabbit anti ZO-1 (1:500), occludin (1:500), and Cx43 (1:500) antibodies (Novus). Membranes were incubated in anti-rabbit secondary antibodies (IBlot Western Detection Chromogenic anti-rabbit Kit, Invitrogen, WB7105) for 1 h. After washing the membranes, the signal was detected with a western blot kit (IBlot Western Detection Chromogenic anti-rabbit Kit, Invitrogen-WB7105). Image J software was used to calculate protein levels (National Institutes of Health, USA).

2.11 | Transmission electron microscopic preparation

Testis samples were fixed with 2.5% glutaraldehyde in PBS and post fixed with 1% osmium tetroxide in PBS and processed for routine electron microscopy and embedded in Epon 812 resin. After contrasting, the sections were investigated with a transmission electron microscope.

2.12 | Statistical analysis

Histological and biochemical biomarkers were analysed by one-way or two-way analysis of variance (Prism 6.0 Graph Pad Software, San Diego, CA). Post hoc testing was done by Tukey's multiple comparisons test, and the data are expressed as the mean ± standard deviation (SD). The significance of differences was accepted as $p < 0.05$.

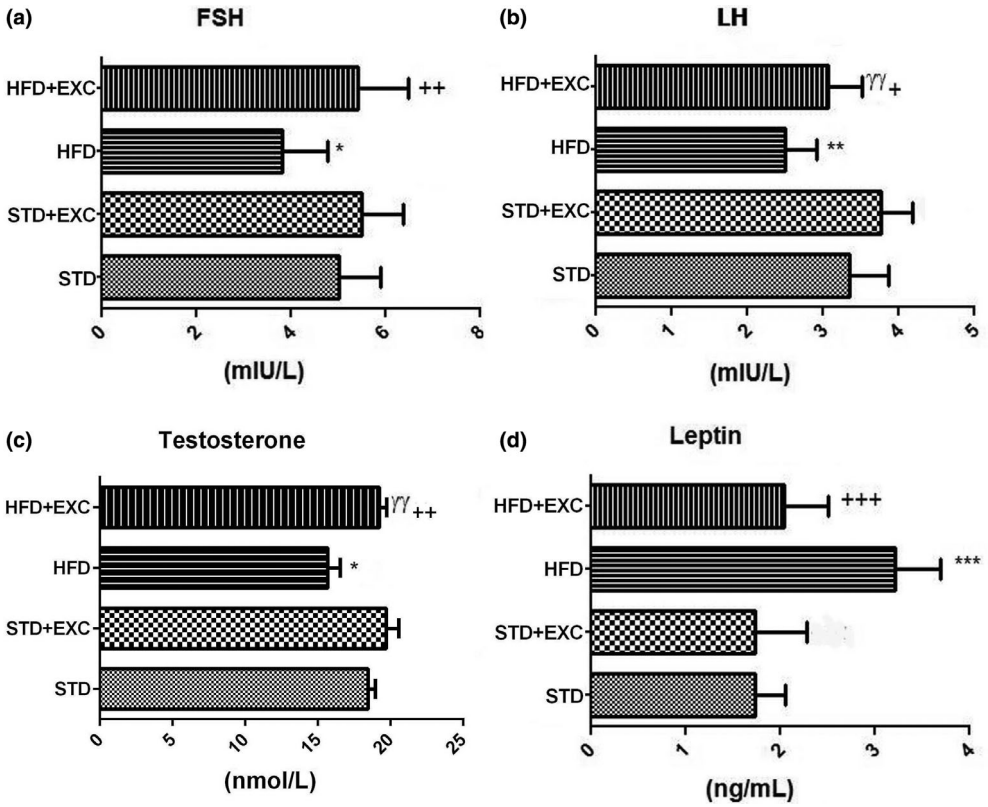


FIGURE 2 FSH (a), LH (b), testosterone (c), and leptin (d) values in the experimental groups. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus STD group; $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$, versus HFD group; $^{ɣɣ}p < 0.01$ versus STD + EXC group

3 | RESULTS

3.1 | Food intake, body and testis weight

Food intake of the experimental groups was analysed weekly and was expressed as grams. According to the food intake results, consumption in the HFD group was lower than that in the STD and STD + EXC groups (Figure 1a). The body weights of the HFD and HFD + EXC groups were higher than those of the STD group before and after the exercise training. All rats gained weight as anticipated, but compared to the other experimental groups, the HFD group put on more weight (Figure 1b). The testis weight of the HFD group was decreased compared to that of the STD group. Besides, the testis weight of the HFD + EXC group was increased than the HFD group (Figure 5e1).

3.2 | Serum glucose and insulin concentrations

The serum glucose level was 151.2 ± 28.47 mg/dl in the STD group, 146.3 ± 35.83 mg/dl in the STD + EXC group, 250.4 ± 36 mg/dl in the HFD group and 163 ± 35.16 mg/dl in the HFD + EXC group. Analysis of serum glucose showed that glucose concentration was risen in the HFD group ($p < 0.001$) compared with that in the STD and STD + EXC groups. In addition, glucose concentration was decreased ($p < 0.001$) in the HFD + EXC group encountered to that in the HFD group.

The serum insulin level was 5.74 ± 0.78 mIU/L in the STD group, 6.24 ± 0.46 mIU/L in the STD + EXC group, 5.10 ± 0.81 mIU/L in the HFD group and 6.68 ± 0.58 mIU/L in the HFD + EXC group. The insulin level was ($p < 0.05$) reduced in the HFD group than the STD group, and the insulin level was risen in the HFD + EXC group ($p < 0.01$) than the HFD group.

3.3 | Serum FSH, LH, testosterone and leptin levels

According to the hormone analysis, FSH, LH and testosterone levels in the HFD group were lower than the STD group. On the other hand, FSH, LH and testosterone levels were higher in the HFD + EXC group than the HFD group. Additionally, LH and testosterone levels were lower in the HFD group than the STD + EXC group. The leptin level of the HFD group was increased compared with that of the STD group. Besides, the leptin level of the HFD + EXC group was decreased than that of the HFD group (Figure 2).

3.4 | Testicular MDA and GSH levels and SOD and 8-OHdG activities

The MDA level and 8-OhdG activity were escalated in the HFD group than the STD group. Compared with the HFD group, MDA levels and 8-OhdG activity were lower in the HFD + EXC group (Figure 3a,d). When compared to the STD group, the GSH levels and SOD activity

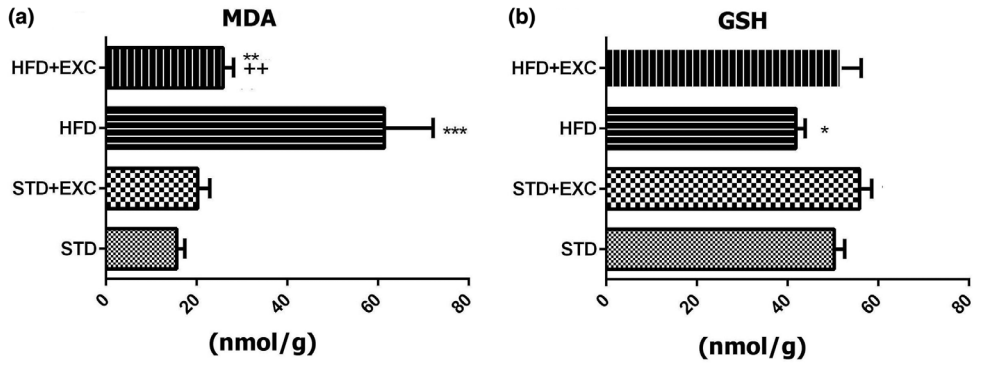


FIGURE 3 Tissue MDA (a) and GSH (b) levels and SOD (c) and 8-OHdG (d) activities in the experimental groups. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus STD group; $^{++}p < 0.01$ versus HFD group; $^{\gamma}p < 0.05$, versus STD + EXC group

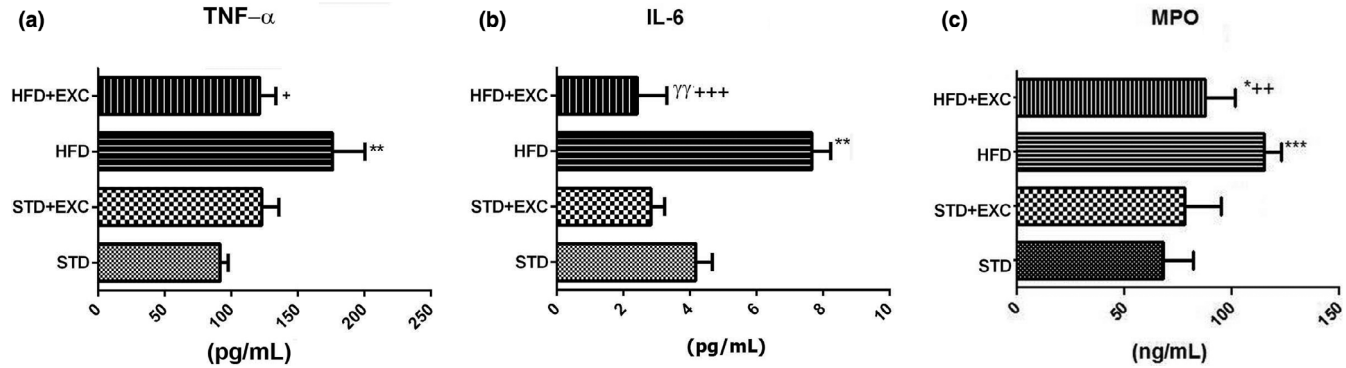


FIGURE 4 TNF-α (a) and IL-6 (b) levels and MPO (c) activity results in the experimental groups. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus STD group; $^{\gamma}p < 0.05$, versus STD + EXC group, $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ versus HFD group

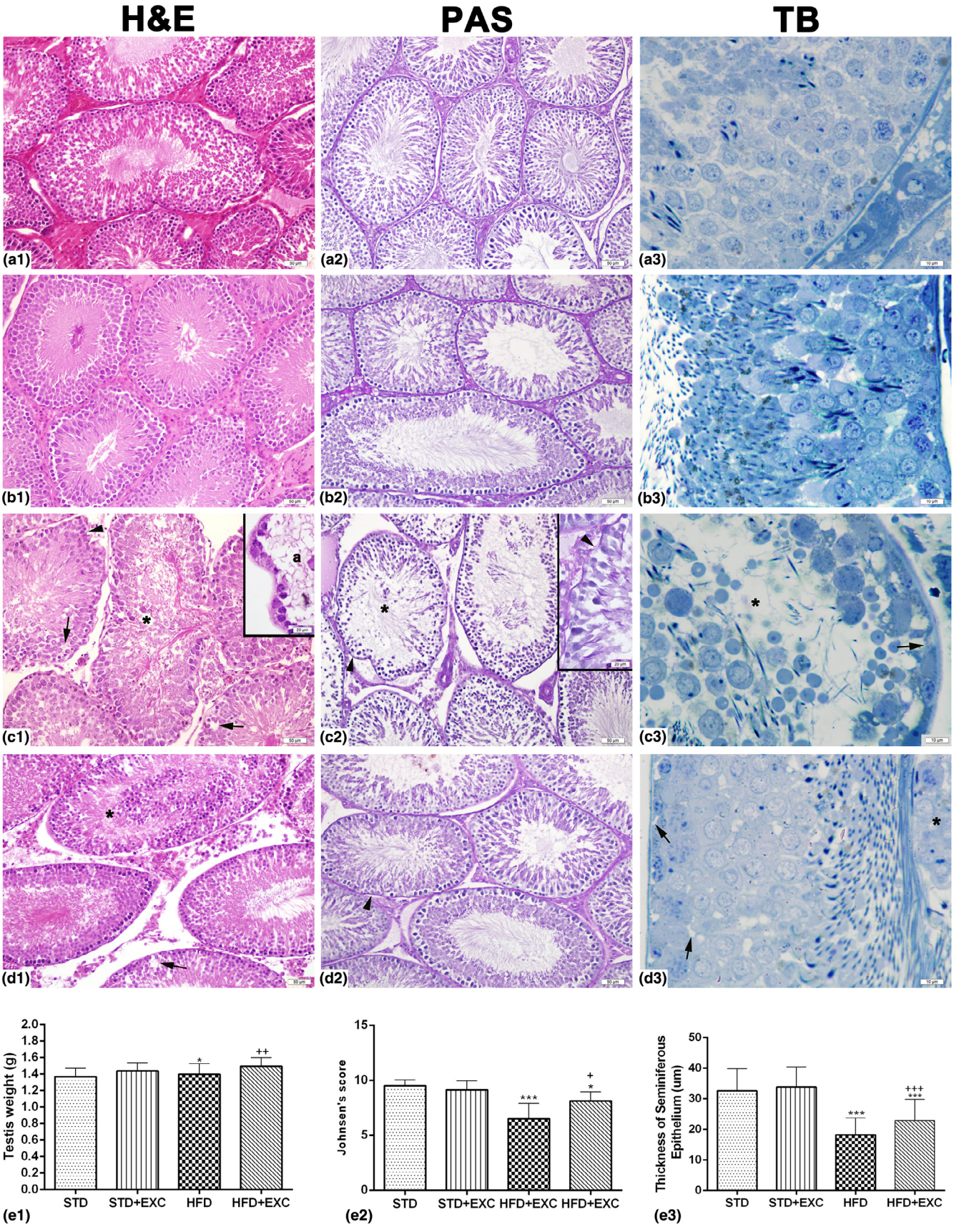
in the HFD group were lowered. GSH levels were higher in the HFD + EXC group than in the HFD group, but this difference was not significant. SOD activity was higher in the HFD + EXC group than the HFD group (Figure 3b,c).

3.5 | Measurement of TNF-α and IL-6 levels and MPO activity

Testicular TNF-α and IL6 levels and MPO activity were risen in the HFD group than the STD group. TNF-α and IL6 levels as well as MPO activity were lower in the HFD + EXC group than the HFD group (Figure 4).

3.6 | Histopathological results

Light microscopic analysis showed that seminiferous tubule morphology was normal in the STD (Figure 5a1–a3) and STD + EXC (Figure 5b1–b3) groups. Degenerated seminiferous tubules with degenerated basement membranes, dilatations between the germinal epithelial cells and cellular debris in the luminal region and atrophic tubules were seen in the HFD group (Figure 5c1–c3). Seminiferous tubule morphology was improved in the HFD + EXC group. However, some tubules had both dilatation between the epithelial cells and cellular debris in the lumen (Figure 5d1–d3). Histopathologic Johnsen's score and seminiferous tubule epithelium thickness were lower in the HFD group than in the STD



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group. Moreover, Johnsen's score and seminiferous tubule epithelium thickness were higher in the HFD + EXC group than in the HFD group (Figure 5e2,e3).

3.7 | PCNA immunohistochemistry results

Dark brown PCNA-positive cells were observed in all of the experimental groups. However, the lowest number of PCNA-positive cells was observed in the HFD group. The proliferation index was lower in the HFD group than the STD group and higher in the HFD + EXC group than the HFD group (Figure 6a1–e1).

3.8 | TUNEL results

TUNEL-positive cells were detected in the epithelium of the seminiferous tubules of all experimental groups, but the highest number of TUNEL-positive cells was observed in the HFD group. The apoptotic index was higher in the HFD group than the STD group. In addition, the apoptotic index was fallen in the HFD + EXC group than the HFD group (Figure 6a2–e2).

3.9 | ZO-1, occludin, Cx 43 immunohistochemistry results

ZO-1 and occludin immunoreactivity (ir) were observed in the basal region of the seminiferous tubule epithelium in the experimental groups. ZO-1 and occludin immunostaining intensity was lowered in the HFD group than the STD group, but ZO-1 and occludin immunostaining intensity were risen in the HFD + EXC group than the HFD group. Cx 43 ir was observed in the apical region of Sertoli cells, and the basal region of seminiferous epithelial cells and elongated spermatid convergence surface of the experimental groups. Cx 43 immunostaining intensity was lowered in the HFD group than the STD group, but this value was risen in the HFD + EXC group than the HFD group (Figure 7).

3.10 | Western blot results

ZO-1, occludin and Cx 43-ir were demonstrated as a band which were approximately 210, 65, and 43 kDa, respectively. The STD and STD + EXC groups showed darker bands than the HFD and HFD + EXC groups. The levels of the tight junction-related biomarker proteins ZO-1 and occludin and the gap junction protein Cx 43 were diminished in the HFD group than the STD group. Besides, these protein levels were increased in the HFD + EXC group than the HFD group (Figure 8).

3.11 | Transmission electron microscopy results

A regular ultrastructure of seminiferous tubules with tight junction structures between Sertoli cells, germinal epithelial cells and spermatozoa in the lumen was detected in the STD and STD + EXC groups. The seminiferous tubular epithelium was disorganized in the HFD group. Additionally, disrupted tight junctions between Sertoli cells and spermatogenic cells, lipid droplets in the cytoplasm and many apoptotic cells were seen in the HFD group. In the HFD + EXC group, a well-ordered ultrastructure of seminiferous epithelium with tight junction structures and spermatozoa in the luminal region was observed. However, lipid droplets were observed in some seminiferous tubules in the HFD + EXC group (Figure 9).

4 | DISCUSSION

This study showed that HFD-induced obesity increased leptin levels and decreased testosterone, FSH, and LH levels. Moreover, increased deteriorated seminiferous tubules with fewer proliferative cells, increased apoptotic cells and DNA fragmentation, and altered amounts and localizations of proteins related to the blood testis barrier (ZO-1 and occludin) and gap junction (Cx 43) proteins were detected in HFD-fed rats. The levels of oxidative stress parameters MDA, GSH, SOD and inflammatory markers TNF-α, IL-6, and MPO were altered in HFD-induced obese rats. All these hormonal, morphological, oxidative and inflammatory parameters were ameliorated in moderate swimming exercise-trained HFD-fed rats.

FIGURE 5 Representative light micrographs of testis samples (a1–d3), testis weight (e1), histopathological Johnsen's score (e2) and thickness of seminiferous tubules (e3) in experimental groups. The regular morphology of the germinal epithelium is observed in the STD (a1) and STD + EXC (b1) groups. Irregular border of basement membrane (arrowhead), vacuolated seminiferous epithelium (arrow), cell debris in lumen (asterisk) and atrophic (a) tubules (insert) are observed in HFD group (c1). Quite regular seminiferous tubules and some vacuole formations (arrow) and immature germ cells (asterisk) are seen in the HFD + EXC group (d1). PAS-positive regular basement membranes are seen in the STD (a2) and STD + EXC (b2) groups. PAS-positive stained irregular basement membranes (arrowhead) and degenerative tubules (asterisk) were observed in the HFD (c2) group. PAS-positive staining and an irregular basement membrane (arrowhead) are seen in some seminiferous tubules of the HFD + EXC group (d2). Regular morphology of seminiferous tubules is observed in the STD (a3) and STD + EXC (b3) groups. Degeneration and vacuolization (arrow) of germinal epithelial cells and immature germ cells (asterisk) in the lumen of the seminiferous tubule are observed in the HFD group (c3) and HD + EXC (d3) groups. H&E staining (a1–d1). PAS staining (a2–d2). TB staining (a3–d3). Original magnification: a1–d1, a2–d2: 20×, insets c1 and c2: 40×, a3–d3: 100×. Testis weight (e1), histopathological Johnsen's score (e2) and epithelial thickness (e3) of seminiferous tubules of experimental groups. *$p < 0.05$, ***$p < 0.001$, versus STD group. $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$ versus HFD group

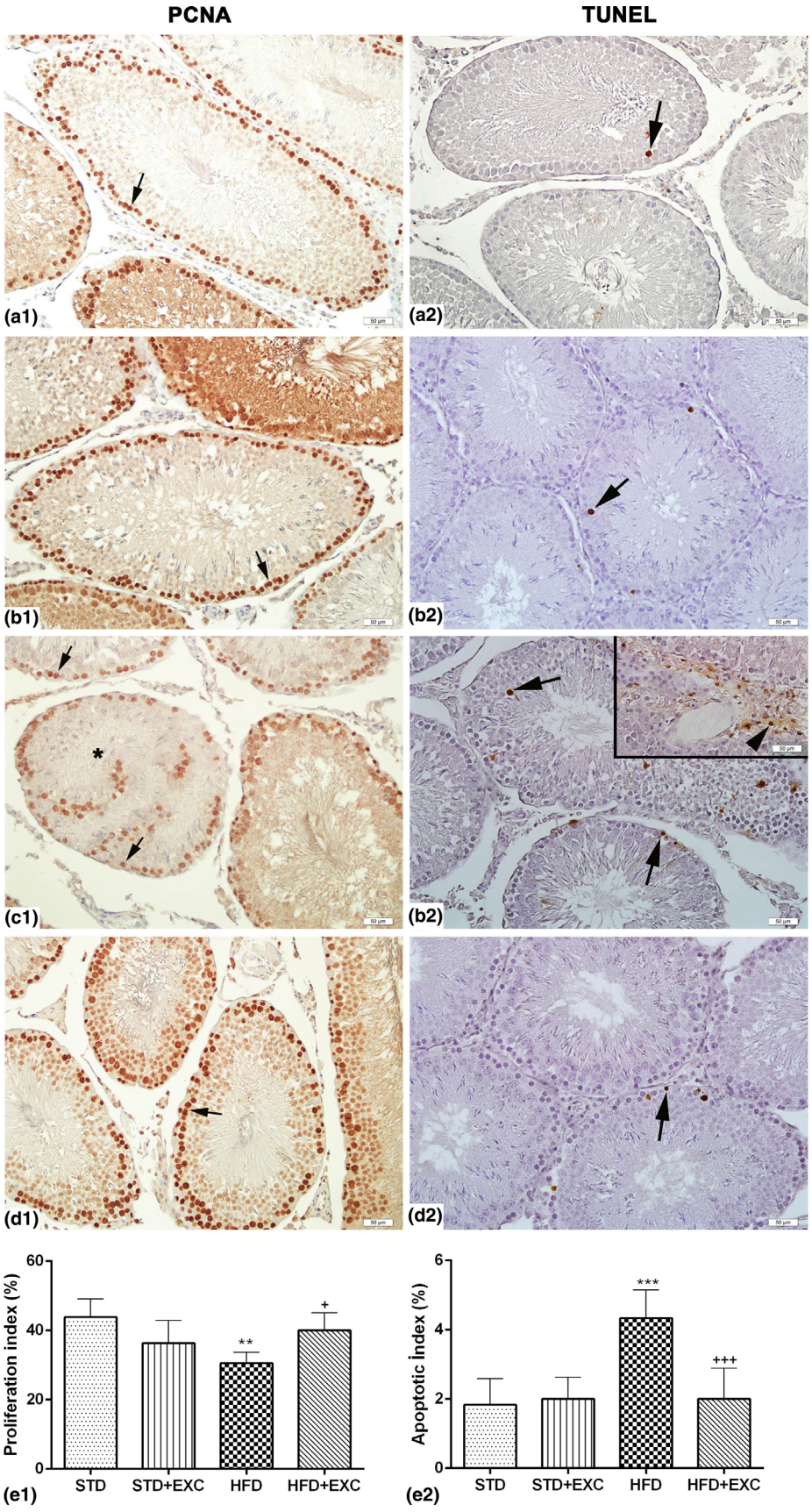
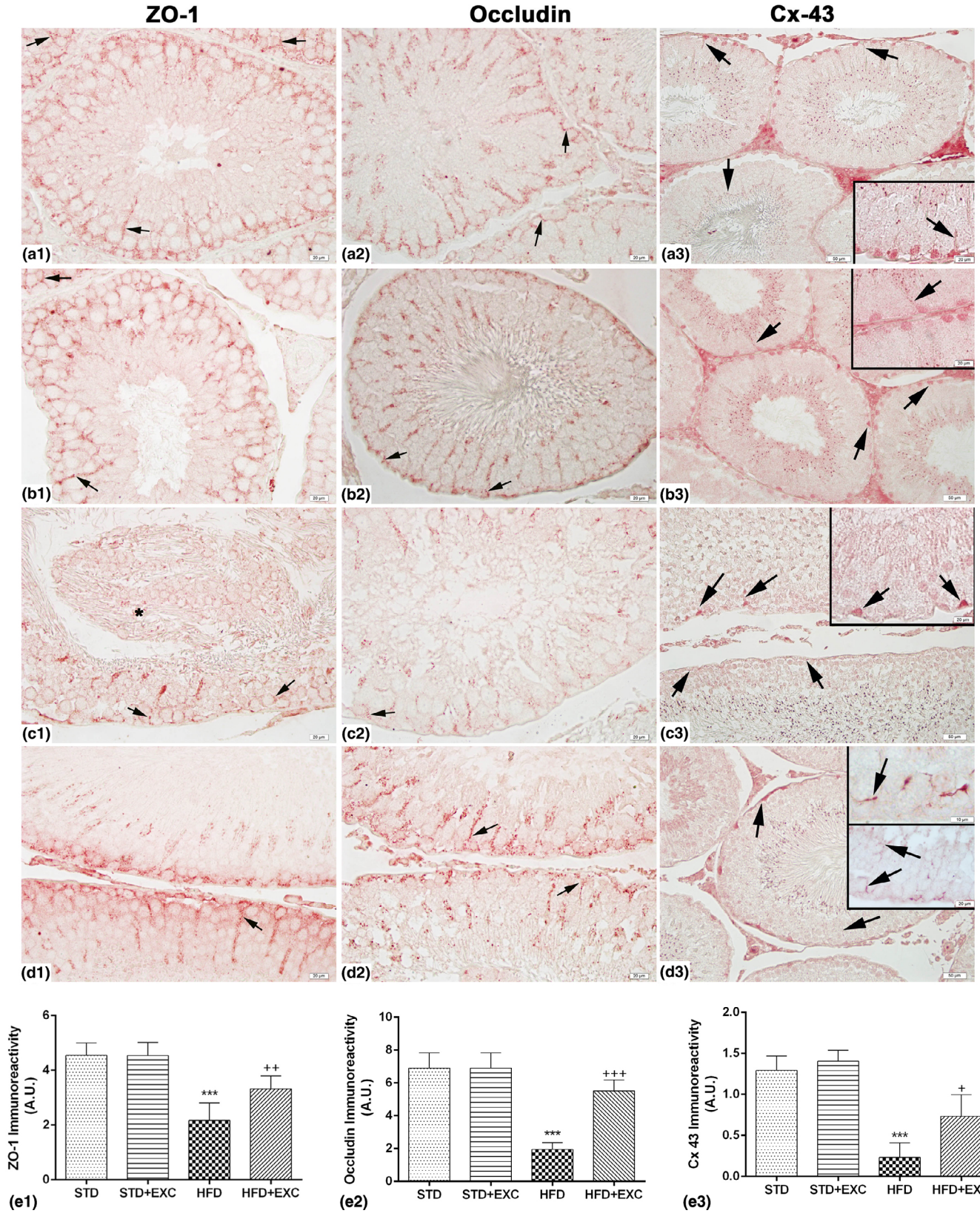


FIGURE 6 Representative photomicrographs of PCNA immunostained (a1–d1) and TUNEL stained (a2–d2) testis samples, proliferative (e1) and apoptotic (e2) indexes in experimental groups. Numerous PCNA-positive (arrow) spermatogenic cells are seen in seminiferous tubules of the STD (a1) and STD + EXC (b1) groups. Decreased PCNA-positive cells (arrow) in the seminiferous tubules and PCNA-positive spermatogenic cells (asterisk) in the lumen of seminiferous tubules are seen in the HFD group (c1). Numerous PCNA-positive spermatogenic cells (arrow) are seen in the seminiferous tubules of the HFD + EXC group (d1). A few TUNEL-positive spermatogenic cells (arrow) are seen in the STD (a2) and STD + EXC (b2) groups. An increased number of TUNEL-positive cells (arrow) in the seminiferous tubules and interstitial area (arrowhead) were observed in the HFD group (c2). A decreased number of TUNEL-positive cells (arrow) was observed in the seminiferous tubules of the HFD + EXC group (d2). Original magnification: 20×. Proliferative (e1) and apoptotic (e2) indexes of the experimental groups. $^{**}p < 0.01$ and $^{***}p < 0.001$ versus STD group, $^{+}p < 0.05$ and $^{+++}p < 0.001$ versus HFD group



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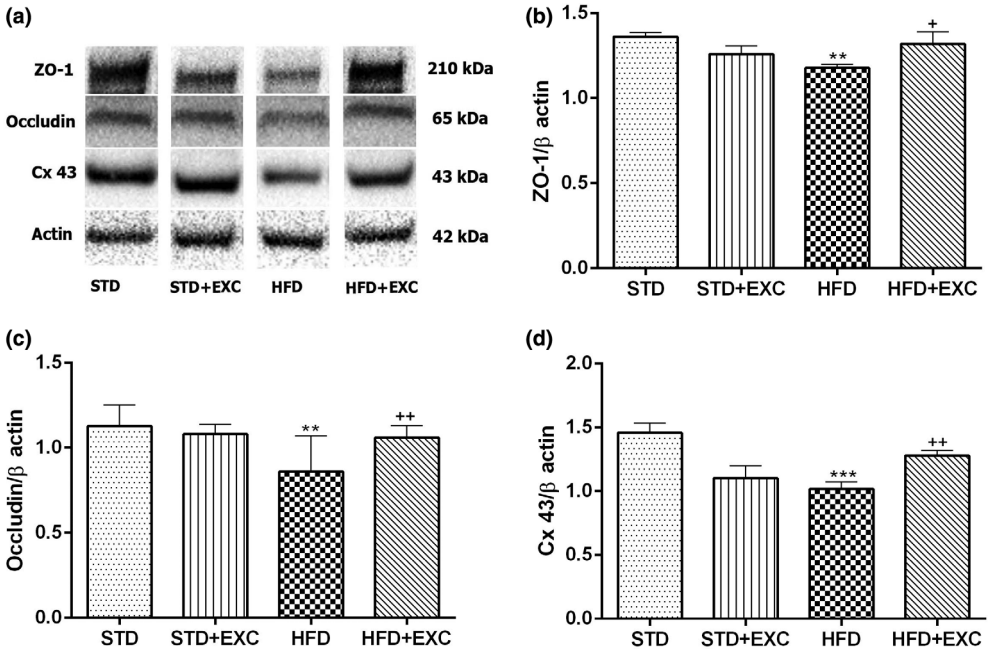

FIGURE 8 Western blot analyses of ZO-1, occludin, and Cx43 proteins (a) and their densitometric readings of the corresponding protein blots are presented as bar graphs (b–d) in the experimental groups. $^{**}p < 0.1$, $^{**}p < 0.01$ versus STD group, $^{+}p < 0.05$, $^{+++}p < 0.001$ versus HFD group. Values are given as the mean ± SD

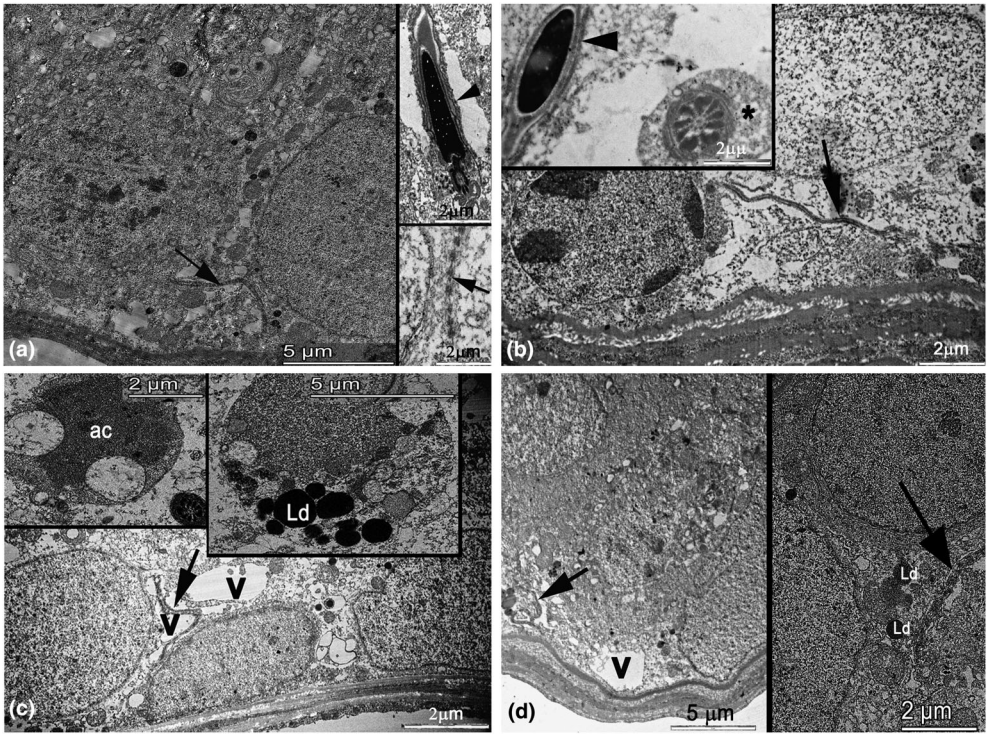

FIGURE 9 Representative electron micrographs of testis samples in the experimental groups. The regular morphology of tight junctions between adjacent Sertoli cells (arrow) is seen in the STD (a) and STD + EXC (b) groups. Degenerated tight junctions (arrow), lipid droplets (Ld) in the cytoplasm and apoptotic cells (ac) were observed in the HFD group (c). Quite regular tight junctions (arrow) and decreased lipid droplets (Ld) in the cytoplasm were observed in the HFD + EXC group (d)

FIGURE 7 Representative photomicrographs of ZO-1 (a1–d1), occludin (a2–d2), Cx 43 (a3–d3) immunostained testis samples, ZO-1 (e1), occludin (e2) and Cx 43 (e3) immunoreactivity levels in experimental groups. ZO-1 (a1, a2) and occludin (b1, b2) immunostained basolateral regions of Sertoli cells and Cx-43 (a3, b3) immunostained basolateral and apical regions of Sertoli cells are seen in the STD and STD + EXC groups, respectively. Decreased ZO-1 (c1), occludin (c2) and Cx-43 (c3) immunostaining intensities were observed in the basolateral regions of Sertoli cells in the HFD group. Increased ZO-1 (d1) and occludin (d2) immunostaining intensities in the basolateral regions of Sertoli cells and Cx-43 (d3) immunostaining intensities in the basolateral and apical regions of Sertoli cells were observed in the HFD + EXC group. Original magnification: a1–d1 and a2–d2: 40×; a3–d3: 20×, insets: a3–c3 and upper inset d3: 20×: Lower inset d3:100×. $^{***}p < 0.001$ versus STD group, $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ versus HFD

Environmental factors, decreased daily physical activity and excessive consumption of high-energy diets could be the cause of metabolic diseases and increased body fat mass. Increased physical activity is a non-pharmacological method of preventing obesity and weight gain. (Sene-Fiorese et al., 2008).

Experimental studies have shown that testis weight is reduced in obese rats compared to control groups (Miao et al., 2018). As BMI increases, testicular morphology is impaired (Jia et al., 2018). Similarly, this study showed that rise in body weight, and a decrease in testis weight with testicular damage presented by a high Johnsen's score, were seen in the HFD group, and testicular damage was enhanced in exercise-treated HFD-fed rats.

Obesity is related to hyper caloric food consumption and sedentary life. It was shown that rats fed a hyper caloric diet consumed less food than rats fed a control diet. Interestingly, their energy intake and weight gain were higher (Burneiko et al., 2006). Exercise frequency has been demonstrated to affect weight gain (Burneiko et al., 2006). Moreover, various experimental studies indicate that exercise is effective in reducing total and visceral fat, even if there is no weight loss, and thus may have positive effects on health parameters caused by obesity (Lee et al., 2005; Linden et al., 2014). Oztasan et al. have reported that 8 weeks treadmill training decreases the left gonadal fat pad in male rats (Oztasan et al., 2007). Additionally, it was shown that exercise ameliorated elevated levels of steatosis, lipid accumulation and ballooned hepatocytes in HFD-fed rats (Acikel Elmas et al., 2020). Similarly, in this study, it was observed that the HFD group gained more weight even though they ate less food than the standard diet-fed rats.

It has been shown that total cholesterol, serum glucose, and LDL values significantly increase in obese individuals with a sedentary lifestyle (de Lima et al., 2008; Silva et al., 2016). As a result of exercise, it was shown in this study that the glucose levels in obese rats were higher than those in the STD group.

Leptin effectively regulates appetite, reduces inflammation, and inhibits insulin secretion. Male infertility is considered to be caused by a reduction in leptin receptors in the testes of obese mice (Ghanayem et al., 2010). Leptin and testosterone are negatively correlated. It has been shown that serum leptin concentration and the irregularity of sex hormones in testis tissue were increased in HFD-fed mice (Yi et al., 2017). Similarly, in this study, it was observed that serum leptin levels were raised and testosterone levels were diminished in the HFD group, and that exercise restored leptin and testosterone levels to control levels.

Modulations in body metabolism stimulated by high-energy diets affect the hypothalamic–pituitary (HPT) axis and tend to alterations in signalling pathways that organize Sertoli cell metabolism (Rato et al., 2014). Similarly, in this study, the reduction in FSH and testosterone levels seen in the HFD group is thought to be related to the disruption of the HPT axis. A positive effect of exercise was detected as both hormone levels were restored.

Exercise, can increase reactive oxygen species production exceeding the capacity of antioxidant defences of the cells (Khanna et al., 1999; Oztasan et al., 2004). The glutathione-dependent antioxidant system plays a fundamental role in cellular defence against reactive oxygen species (Gul et al., 2000). The accumulation of free radical damage that is not adequately neutralized by antioxidants is referred to as 'oxidative stress,' and oxidative damage is increased by decreased activities of SOD, catalase, and glutathione S-transferases (Blokhina et al., 2003). SOD defends cells from ROS-induced free radical oxidative damage. Decreasing SOD and increasing MDA induce oxidative stress and cause cell damage and even death (Jia et al., 2018). The MDA/SOD ratio has been shown to be higher than normal in obese individuals (Acikel Elmas et al., 2019; Vincent & Taylor, 2006). Increased MDA levels may cause more ROS production and disrupt normal glucose metabolism in obese individuals (Yue et al., 2003). In studies with obese sedentary rats, MDA levels were higher and GSH levels were lower in various organs (Vincent & Taylor, 2006). Similar results were found in this study, where the HFD group had higher MDA levels and lower GSH and SOD activity. All these oxidant parameters were reversed by exercise. Exercise is thought to improve the balance between oxidants and antioxidants, which is disturbed by the effects of a high-fat diet.

Activation of MPO is linked to the formation of obesity (Wang et al., 2014). MPO affects various signalling pathways involved in cell signalling and cell–cell interactions, and thus can modulate inflammatory responses (van der Veen et al., 2009). Therefore, rise in MPO activity may be linked to diet-induced obesity and oxidative stress (Cardoso et al., 2018). In an exercise study with obese individuals, it was shown that moderate exercise reduces the MPO level (Koh et al., 2018). It is stated that the plasma concentrations of TNF-α and IL-6 increase in obesity, and the elevation of plasma TNF-α may cause insulin resistance (Chentouf et al., 2011). In this experiment, the HFD group showed an increase in TNF-α and IL-6 levels, whereas exercise improved testis morphology by diminishing these parameters.

Cell proliferation and cell death are regulated during the complicated process of spermatogenesis (Sakkas et al., 2003) and several studies have shown that cell proliferation is negatively affected in obese rats (Fukuta et al., 2017). Additionally, it has been stated that obesity causes male subfertility by significantly increasing the apoptotic index in spermatogenic cells (Jia et al., 2018). As a result, the incidence of spermatogenic cell apoptosis increases rapidly (Xin et al., 2011). The heat and oxidative stresses caused by obesity could lead to apoptosis, and apoptosis may induce the formation of autophagic vacuoles (Horibe et al., 2017). These apoptotic events and autophagic vacuoles could be related to increased oxidative stress, and the improvement in testicular morphology could also be related to decreased oxidative stress (Horibe et al., 2019).

In this experiment, proliferation of spermatogenic cells was decreased and apoptosis was increased in HFD-fed rats. Apoptotic cells and vacuole formation were also observed mainly in HFD-fed rats at stages VII–VIII of the spermatogenesis cycle. Exercise improved testicular morphology by reducing vacuoles and apoptotic cells at the same stage in rats. The positive effects of exercise in the HFD group were obviously observed in the proliferative and apoptotic indexes. Moderate swimming is thought to significantly restore the balance between altered cell proliferation and apoptosis brought on

by a high-fat diet. One of the most common free radical-induced oxidative lesions in nuclear and mitochondrial DNA is 8-hydroxy-2-deoxyguanosine (8-OHdG). Therefore, the measurement of 8-OHdG is considered a sign of oxidative damage to DNA (Helbock et al., 1999). 8-OHdG levels have been shown to be high in blood serum samples taken from obese individuals and animals (Qi et al., 2016). It was shown that the 8-OHdG level risen in the blood serum of rats fed a HFD, and the 8-OHdG level decreased in these rats after moderate exercise (Qi et al., 2016). Therefore, it can be concluded that high-intensity exercise increases oxidative damage in DNA in individuals with normal weight, while moderate-intensity exercise reduces oxidative damage in DNA in obese individuals. In this study, as the 8-OHdG level increased in the HFD group and the 8-OHdG level decreased in the testis with exercise sessions, it was concluded that exercise had a reducing effect on the oxidative damage in DNA.

Proteins which are occludin, claudin-3, -5 and -11 and ZO-1, -2, and -3 and the adhesion binding molecules (Junctional Adhesion Molecule -JAM)-A and -B primarily maintain the integrity of the blood-testis barrier. Various studies have revealed that male mice lacking the barrier proteins claudin-11 or occludin are infertile (Saitou et al., 2000). ZO proteins are adaptor proteins that bind claudin and occludin to actin in the cytoskeleton. It has been shown that impairment of the integrity of the blood-testis barrier causes a decrease in fertility (Xu et al., 2009). It was shown that obese mice have lower testosterone levels and tight junction proteins (Fan et al., 2015). Similarly, in our study, decreased testosterone levels and altered amounts and distributions of ZO-1 and occludin were observed in HFD-fed rats. In addition, degenerated tight junctions between Sertoli cells were detected under an electron microscope in the HFD group. Swimming exercise improved both the testosterone level, morphology of the blood-testis barrier and the distribution and amount of blood-testis barrier proteins. However, exercise effectively reversed this impairment of the regulation of the blood-testis barrier integrity in HFD-fed rats.

By regulating the flow of small molecules between neighbouring cells, gap junctions play crucial role in controlling cell development and differentiation (St-Pierre et al., 2003). Cx 43 is localized in the blood-testis barrier in the epithelium of the seminiferous tubules together with the tight junction protein occludin (Cyr et al., 1996). Studies show that the differentiation of Sertoli cells and the arrangement of this differentiation by thyroid hormones are also associated with Cx 43 interconnections between Sertoli cells (Cyr, 2011). Cx 43 acts as a regulator of blood testis barrier formation and function (Gerber et al., 2014). ZO-1 is crucial for determining Cx 43's location (Giepmans & Moolenaar, 1998). In this study, Cx 43 was observed in the normal amount and distribution in the groups fed a standard diet, while it was observed that the distribution and amount of Cx 43 as well as ZO-1 and occludin in the HFD group were disrupted. Exercise improved the distribution and the amount of Cx43 protein in the HFD + EXC group. These results are important to show the disruption of the distribution and amount of gap junction proteins in obese rats. Exercise reversed the gap junction protein Cx43 distribution and amount in HFD-fed rats.

It is concluded that moderate swimming alleviates the testicular degeneration induced by a high-fat diet in rats by regulating ROS formation and the HPT axis, maintaining the balance between the proliferation index and apoptotic index, and preserving the integrity of the blood-testis barrier. In addition, simple lifestyle changes, such as exercise in obesity, are thought to restore spermatogenesis-related parameters to normal levels, increase fertility, and help improve obesity-induced pathophysiology.

CONFLICT OF INTEREST

All authors declare that they do not have any conflict of interest that could inappropriately influence this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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

How to cite this article: Elmas, M. A., Ozakpinar, O. B., Kolgazi, M., Sener, G., Arbak, S., & Ercan, F. (2022). Exercise improves testicular morphology and oxidative stress parameters in rats with testicular damage induced by a high-fat diet. *Andrologia*, e14600. https://doi.org/10.1111/and.14600