Effects of treadmill exercise on sexual behavior and reproductive parameters in chronically stressed-male rats

Haluk Kelestimur[1,*], Ozgur Bulmus[2], Ihsan Serhatlioglu[3], Zubeyde Ercan[4], Seyma Ozer Kaya[5], Ahmet Yardimci[1], Nazife Ulker[1], Emine Kacar[1]

[1]Department of Physiology, Faculty of Medicine, Firat University, Elazig, Turkey

[2]Department of Physiotherapy and Rehabilitation, Faculty of Health Sciences, Balikesir University, Balikesir, Turkey

[3]Department of Biophysics, Faculty of Medicine, Firat University, Elazig, Turkey

[4]Department of Physiotherapy and Rehabilitation, Faculty of Health Sciences, Firat University, Elazig, Turkey

[5]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Firat University, Elazig, Turkey

[*]**Correspondence Address:**

Haluk Kelestimur

Firat University

Faculty of Medicine

Department of Physiology, TR23119, Elazig, TURKEY

hkelestimur@firat.edu.tr

+90 424 237 00 00/4671

Summary

Exposure to chronic stress stimulates the hypothalamic-pituitary-adrenal (HPA) axis and then simultaneously inhibits hypothalamic-pituitary-gonadal axis (HPG) axis activity. The inhibition formed by the HPA axis is the main mechanism of action of stress on reproductive function. HPG axis activity is known to be changed by various factors, including exercise. Exercise has been found to have a number of positive effects on sexual behavior, reproductive hormones, and sperm parameters in studies with animal models for many years. The main aim of this study is to investigate the effects of chronic treadmill exercise on chronically stressed-male rats' sexual behavior, reproductive hormones, and sperm parameters. A total of 40 sexually adult male rats were randomly and equally divided into four groups as control, stress, exercise, and stress+exercise. Animals in the exercise group were subjected to the chronic treadmill exercise (moderate intensity) for 33 days with a periodic increase in speed and duration. Animals in the stress group were exposed to restraint stress for 1 hour, 2 hours, and 3 hours during the first, second and third 15 days respectively. Sexual behavior parameters, hormone measurements, and sperm parameters were evaluated. The main effects of chronic exercise on sexual behavior were centered on a significant increase in the ejaculation frequency (EF) in the stress+exercise group. Also, sperm concentration and motility in the stress group significantly decreased, and then sperm motility was improved by exercise in the stress+exercise group. In sum, our results show that chronic treadmill exercise may improve the adverse effects of chronic stress on sexual behavior and sperm parameters in male rats in terms of some parameters.

Key words: Treadmill exercise, restraint stress, sexual behavior, sperm parameters, hormones

Introduction

Stress is a process that triggers systemic and behavioral responses associated with a series of physiological changes, leading to structural and functional abnormalities in many organs. Reproductive activity is one of the main functions that change and inactivate during the adaptive response to stress (Johnson *et al.* 1992). Especially, exposure to chronic stress results in hypothalamic-pituitary-adrenal (HPA) axis dysregulation, mood, and reproductive disorders (Bao *et al.* 2008, Florio *et al.* 2007). Several studies have shown that chronic stress causes morphological and functional changes in reproductive organs. It has detrimental effects on testis and various semen parameters including sperm concentration, sperm motility, and sperm morphology (Clarke *et al.* 1999, Demirci and Sahin 2019, Nordkap *et al.* 2016). In this sense, it has been revealed that the impairments in sperm production, decreased testosterone levels (García-Díaz *et al.* 2015, Hari Priya and Reddy 2012) and testicular weights were observed in rats exposed to chronic stress (Hari Priya and Reddy 2012, Rosmond and Björntorp 2000). Chronic restraint stress has been considered as one of the most commonly used stress-inducing models in rodents and produces detrimental effects in a variety of systems, including sexual dysfunction, such as those observed in humans (Bal *et al.* 2009). It has been reported that chronic restraint stress leads to suppression of testosterone secretion (Al-Damegh 2014), sexual motivation, and spermatogenesis (Almeida *et al.* 1998, Almeida *et al.* 2000a). Chronic restraint stress has been shown to cause delayed testicular maturation in male rats when mated. In addition, there is a 2-fold loss of pre-implantation and post-implantation pregnancy formation in female rats (Almeida *et al.* 2000b). Male rats exposed to chronic stress have been shown to prolong intromission and ejaculation latency as an indicator of impaired sexual activity and show less ejaculation than control rats (Grønli *et al.* 2005).

Exercise has many physical and psychological beneficial effects on the organism. Exercise has been found to have significant improvements in erectile function, sexual confidence, sexual desire, sexual satisfaction, and overall sexual health (Lamina *et al.* 2009, Maio *et al.* 2010, Khoo *et al.* 2013). However, there is still important contradictory in how exercise affects sex hormone levels and spermatogenesis. For example, moderate-intensity exercise attenuates the negative effects of age or obesity on spermatogenesis (You *et al.* 2013, Joseph *et al.* 2014), increases male sex hormone levels (Sato and Lemitsu 2015), and improves male reproductive capacity (Rosety-Rodriguez *et al.* 2014, Gaskins *et al.* 2015). In contrast, prolonged, high-frequency, high-intensity endurance exercise has been found to reduce male sex hormone levels and decrease sperm concentration and motility in animals and humans (Maleki and Tartibian 2015). Despite several reports on the effects of stress stimuli on the reproductive system, the effect of exercise on reversing these effects is not fully understood. Therefore, this study aims to examine the potential effects of chronic treadmill exercise on sexual behavior, sperm parameters, specific hormone levels, and reproductive organ weights in adult male rats exposed to chronic restraint stress.

Materials and Methods

Animals

The experimental protocol was approved by the Animal Experimental Ethics Committee of Firat University (Elazig, Turkey), performed according to the Guide for the Care and Use of Laboratory Animals. Sexually experienced Sprague-Dawley male rats (3 months old, n=40) were obtained from the Firat University Experimental Research Unit. Animals were housed in standard polycarbonate cages (2-3 rats per cage) under controlled conditions: 12:12-hr light-dark cycles (lights on 07:00-19:00 h), temperature 21 ± 1 °C, and relative humidity 55 ± 5%. They had access

to standard chow food and tap water *ad libitum*. All male rats used in this study received two or three sexual behavior tests before starting the experiments. Accordingly, the animals that ejaculated 2-3 times in a copulation test (30-min session) were considered sexually active (experienced) and selected for the study. The rats were randomly divided into 4 groups as follows: control (sedentary), exercise, stress, and stress+exercise groups (n=10, for each group).

Treadmill exercise procedure

The schema of the experimental protocol in the present study is demonstrated in Figure 1. The animals in exercise and stress+exercise groups were forced to run on a 5-channels treadmill apparatus (May TME 0805, St. Louis, Missouri, MO, USA) at 0° inclination in the exercise room during the light phase (10:00-13:30 h) for a total of 33 days of the experimental periods (moderate intensity). Running time and speed gradually increased from 15 min at 15 m/min to 50 min at 25 m/min during the experiment (5 days/week and except on the day of sexual behavior tests) on the basis of the procedure in Kim *et al.* (2011) with slight modifications. During the exercise training, the animals rarely received a mild electric shock (0.2-0.5 mA) through the grid located just behind the treadmill apparatus to motivate animals to run constantly. After completion of the daily exercise training, animals were placed back in their home cage. The treadmill apparatus was cleaned with 70% ethanol solution after each rat's exercise training.
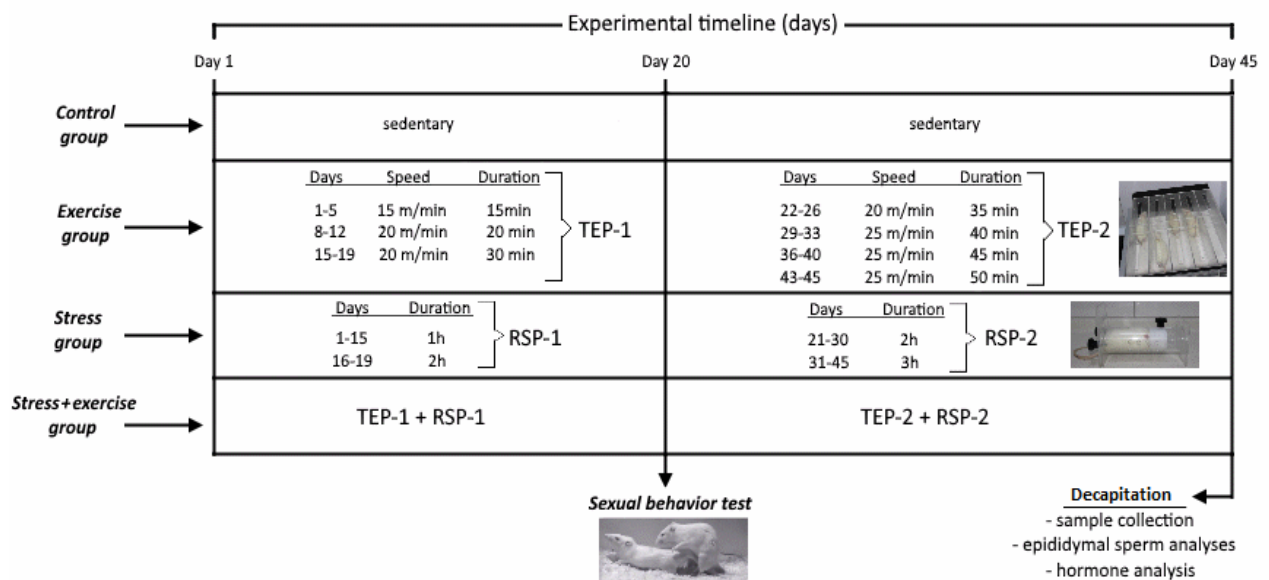
Fig 1. Schematic presentation of the experimental design. TEP: Treadmill Exercise Procedure and RSP: Restraint Stress Procedure.

Restraint stress procedure

All rats in stress and stress+exercise groups were subjected to chronic restraint stress for 45 days (except on the day of sexual behavior tests). Restraint stress was performed with clear plastic rat restrainers in a different room than the housing room of animals in normal room light (14:00-17:00 h). The rats were individually placed in the restrainers, and therefore restrained rats were allowed to breathe freely while their forward or backward movements were prevented (Wisłowska-Stanek *et al.* 2013). Animals were restrained daily for 1 h in the first 15 days, 2 h in the following 15 days, and 3 h in the last 15 days of the experiment. The animals were returned to their own cage after the daily stress procedure was completed, and the restrainer was cleaned and dried after each rat. Rats in the control group (sedentary rats) were not subjected to treadmill exercise or restraint stress during the experiment.

Sexual behavior testing procedure

To determine the effects of chronic exercise and/or stress on the sexual behavior of the male rats, sexual behavior test was conducted on all animals on the 20th day of the experimental studies as described in Canpolat *et al.* (2016) and Yardimci *et al.* (2020). Stimulus ovariectomized females used in the sexual behavior tests were brought into sexual receptivity by subcutaneous injections of estradiol benzoate (10 µg/rat/0.1 mL sesame oil), (Sigma Chemical Co, St. Louis, Missouri, MO, USA) and progesterone (500 µg/rat/0.1 mL sesame oil), (Sigma Chemical Co, St. Louis, Missouri, MO, USA) 48 and 4 h before the mating sessions, respectively (Jones *et al.* 2013).

All sexual behavior tests were performed in a test room during the reversed dark phase (14:00-16:00 h, under dim red light). On test day, male rats were individually placed to an empty Plexiglas testing cage (40 x 40 x 40 cm) in order to habituate the environment for 10 min. Thereafter, the sexually receptive female rat was introduced in the testing cage and sexual behavior test for each animal was carried out for 30 min. All sexual behavior tests were recorded and later scored by an observer blinded to the experimental conditions. During the 30-min test, the following parameters were recorded: *mount frequency* (MF; the number of mounts before the first ejaculation), *intromission frequency* (IF; the number of intromissions before the first ejaculation); *ejaculation frequency* (EF; the number of ejaculations during the test period), *mount latency* (ML; latency from the introduction of the female to the first mount), *intromission latency* (IL; latency from the introduction of the female to the first intromission), *ejaculation latency* (EL; latency from the first intromission/mount to the first ejaculation), *total mount frequency* (MFT; the number of mounts during the test period), *total intromission frequency (IFT;* the number of intromissions during the test period), and *postejaculatory interval* (PEI; latency from the first ejaculation to the next intromission).

Sample collection

At the end of the experiment (45 days), after the last restraint stress session was completed, animals were anesthetized with an intramuscular injection of ketamine-xylazine, and then decapitated. Following decapitation, trunk blood was immediately collected and centrifuged at +4°C (5 min at 4000 rpm), and the serum samples were stored at − 20°C until hormone analysis. The reproductive organs of rats including testis, epididymis, prostate, and seminal vesicle were removed, cleared from adhering connective tissue, and weighed as wet. Immediately after weighing the epididymis, the content of the right and left epididymis was used for epididymal sperm analyses.

Epididymal sperm analyses

Epididymal sperm concentration, sperm motility, and sperm morphology were determined by using the modified methods described by Türk *et al.* (2008). In brief, the epididymal sperm concentration in the right epididymis was counted with a hemocytometer. The percentage of forward progressive sperm motility in freshly isolated left cauda epididymis was evaluated using a light microscope with heated stage. The sperm morphologic examination was also performed in the left cauda epididymis. After preparing the slides stained with eosin-nigrosin (1.67% eosin, 10% nigrosine, and 0.1 M sodium citrate), all slides were viewed under a light microscope at 400x magnification for assessment of morphologically abnormal sperm. Finally, a total of 300 spermatozoa were examined on each slide (3000 cells in each group). The head, tail, and total abnormality rates of spermatozoa were expressed in percentages.

Hormone analysis

The serum luteinizing hormone (LH), testosterone, and corticosterone levels were measured by enzyme-linked immunosorbent assay (ELISA) method using the commercial rat-specific ELISA

kits provided by SunRed Biological Technology Company (Shanghai, China). Serum LH levels (Cat. No: 201-11-0180; sensitivity: 0.206 mIU/mL; and detection range of 0.3-60 mIU/mL), serum testosterone levels (Cat. No: 201-11-5126; sensitivity: 8.775 pg/mL; and detection range of 10-3000 pg/mL), and serum corticosterone levels (Cat. No: SRB-T-87473; sensitivity: 5.005 ng/ml; and detection range of 6-1000 ng/mL) were detected in accordance with the manufacturer's instructions. Concentrations were measured at 450 nm with an ELISA reader (Thermo Scientific, Waltham, Massachusetts, MA, USA).

Statistical analysis

The statistical analyses were performed by SPSS software, version 22 for Windows. All data were presented as mean ± standard error of mean (SEM) or in percentage and $p < 0.05$ was considered statistically significant. One-way analysis of variance (ANOVA), followed by *post hoc* Tukey's test was used to determine the differences among the groups in terms of all behavioral data, sperm characteristics (sperm concentration and motility), hormone levels, and reproductive organ weights. In addition, abnormal sperm morphology rates were analyzed with the Pearson Chi-square test.

Results

Effects of chronic exercise and/or stress on sexual behavior

Table 1 shows the effects of chronic exercise and/or stress on sexual behavior parameters in male rats when compared to the control group. It was observed that there was no statistically significant difference in mount responses (MF, ML, and MFT) in the exercise, stress, or stress+exercise group when compared to the control group. Similarly, no change was seen for intromission responses (IF, IL, and IFT) in both exercise group and stress plus exercise group in comparison to the sedentary rats. On the other hand, only restraint stress caused a significant increase in IF ($p < 0.05$, Table 1), while it did not change IL and IFT. Regarding ejaculation responses, only treadmill exercise or only restraint stress did not alter the EF of rats compared to sedentary rats. However, restraint stress combined with treadmill exercise significantly increased the EF when compared to the stress group ($p < 0.05$, Table 1). Treadmill exercise, restraint stress or restraint stress combined with treadmill exercise did not statistically affect the EL compared with the sedentary group. When compared to the sedentary rats, rats in both exercise group and stress plus exercise groups had a shorter PEI which were found to be statistically significant ($p < 0.05$ and $p < 0.01$, respectively, Table 1). However, it was determined that only restraint stress had no effect on the PEI of rats when compared to the control group.

Table 1. Effects of chronic exercise and/or stress on parameters of male sexual behavior. Data are expressed as mean ± SEM. * $p < 0.05$ and ** $p < 0.01$; when compared to control group and # $p < 0.05$; when compared to stress group (one-way ANOVA followed by *post hoc* Tukey's test, n=10 for each group).

Parameters	Control	Stress	Exercise	Stress+exercise
MF	4.8 ± 0.8	10.3 ± 3.8	7.5 ± 1.6	6.2 ± 1.3
IF	11.9 ± 1.5	21.3 ± 3.3*	18.4 ± 1.6	17.2 ± 1.3
EF	2 ± 0	1.8 ± 0.3	2.5 ± 0.2	2.6 ± 0.2#
ML (s)	84.9 ± 31.6	132.6 ± 43.3	69.8 ± 18.7	93.4 ± 32
IL (s)	23.4 ± 6.6	32.9 ± 9	24.4 ± 8.3	20.6 ± 5.8
EL (s)	354.1 ± 63.3	527.6 ± 93.9	501.7 ± 66.8	419.4 ± 51.1
MFT	13 ± 2	15.9 ± 3.5	14.5 ± 2.5	15.5 ± 2.6
IFT	27.2 ± 2.7	34.9 ± 4.6	34.5 ± 3.9	36.5 ± 4.1
PEI (s)	494.1 ± 31.8	392.9 ± 22.5	367.7 ± 29.8*	353.6 ± 31.2**

MF: Mount frequency; IF: Intromission frequency; EF: Ejaculation frequency; ML: Mount latency; IL: Intromission latency; EL: Ejaculation latency; MFT: Total mount frequency; IFT: Total intromission frequency; and PEI: Postejaculatory interval.

Effects of chronic exercise and/or stress on sperm parameters

The spermatological findings in all groups are given in Table 2. Both treadmill exercise and restraint stress combined with treadmill exercise did not significantly affect the epididymal sperm concentration, sperm motility, and abnormal sperm rates compared to the control group. On the other hand, sperm parameters were negatively affected by the restraint stress, and consequently, sperm concentration and sperm motility significantly decreased ($p < 0.05$

and $p < 0.001$, respectively) while the tail and total abnormality rates of spermatozoa significantly increased ($p < 0.05$, Table 2) compared to the control group. In addition, significantly decreased sperm motility in rats receiving restraint stress increased significantly with treadmill exercise in rats receiving stress plus exercise ($p < 0.001$, Table 2).

Table 2. Effects of chronic exercise and/or stress on sperm characteristics in rats. Data are expressed as mean ± SEMor in percentage. * $p < 0.05$ and ** $p < 0.001$; when compared to control group and # $p < 0.001$; when compared to stress group (one-way ANOVA followed by *post hoc* Tukey's test or Pearson Chi-square test, n=10 for each group).

Variable	Control	Stress	Exercise	Stress+exercise
Sperm concentration (10^6/mL)	145.3 ± 11.6	109.9 ± 8*	137.5 ± 8.5	142.5 ± 8.6
Sperm motility (%)	65.5 ± 2.3	36.3 ± 1.8**	55.6 ± 4.2	59.3 ± 4.6#
Abnormal sperm rate (%)				
Head	5	7	6	6
Tail	4	13*	5	9
Total	9	20*	11	15

Effects of chronic exercise and/or stress on serum levels of LH, testosterone, and corticosterone

Chronic restraint stress significantly decreased serum LH levels compared to the control group (mIU/mL; control group: 3 ± 0.1 and stress group: 2.7 ± 0.1, $p < 0.05$, Fig 2A). However, serum LH levels were not affected by treadmill exercise or restraint stress combined with treadmill exercise when compared to the control group (Fig 2A). The serum testosterone levels were significantly increased in the exercise group (pg/mL; control group: 118.4 ± 1.9 and exercise group: 134.9 ± 1.7, $p < 0.001$, Fig 2B), whereas they did not change in the stress group or stress+exercise group compared with the control group (Fig 2B). The exercise group and the stress group had significantly higher serum corticosterone levels than the sedentary rats (ng/mL; control group: 55.5 ± 1.2, exercise group: 63.3 ± 1.3, and stress group: 64.3 ± 1.5; $p < 0.01$ for exercise group and $p < 0.001$ for stress group, Fig 2C). On the other hand, the stress+exercise group did not differ significantly from the control group in terms of serum corticosterone levels (Fig 2C).
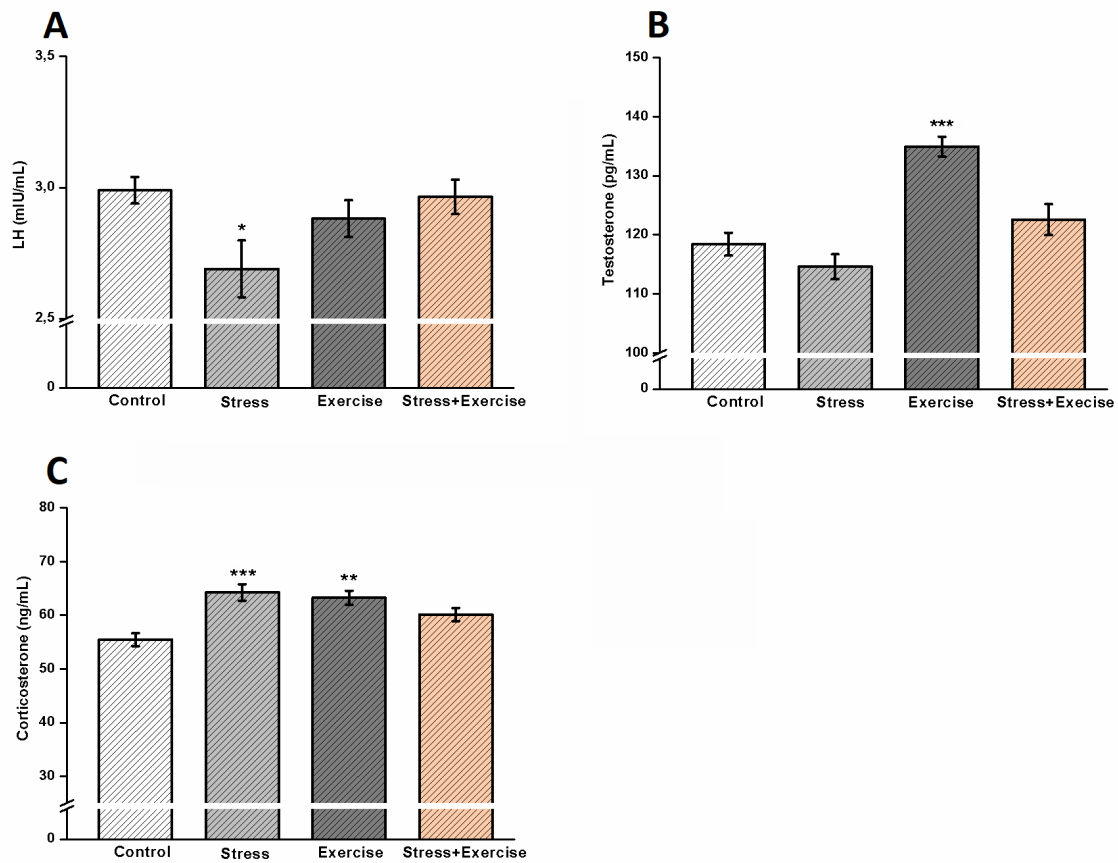
Fig 2. Effects of chronic exercise and/or stress on serum hormone levels in rats. A) Serum LH levels. B) Serum testosterone levels. C) Serum corticosterone levels. Data are expressed as mean ± SEM.* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; when compared to control group (one-way ANOVA followed by *post hoc* Tukey's test, n=10 for each group).

Effects of chronic exercise and/or stress on reproductive organ weights

Treadmill exercise, restraint stress or restraint stress combined with treadmill exercise did not significantly change the absolute weights of testis, epididymis, prostate, and seminal vesicle compared to the control group (Table 3).

Table 3. Effects of chronic exercise and/or stress on absolute reproductive organ weights. Data are expressed as mean ± SEM (one-way ANOVA followed by *post hoc* Tukey's test, n=10 for each group).

Variable	Control	Stress	Exercise	Stress+exercise
Testis (g) (Left + right/2)	1.4 ± 0.04	1.24 ± 0.04	1.42 ± 0.04	1.33 ± 0.03
Epididymis (g) (Left + right/2)	0.56 ± 0.01	0.54 ± 0.01	0.54 ± 0.01	0.52 ±0.02
Prostate (g)	0.5 ± 0.02	0.44 ± 0.01	0.44 ± 0.03	0.4 ± 0.05
Seminal vesicle (g)	0.73 ± 0.02	0.84 ± 0.07	0.83 ± 0.03	0.71 ± 0.03

Discussion

The effects of chronic exercise and/or stress on serum LH, testosterone and corticosterone levels, and sexual behavior

The effects of chronic treadmill exercise on chronically-stressed male rats' serum LH, testosterone, corticosterone levels, and sexual behavior were revealed, for the first time, in this study. Chronic restraint stress significantly decreased LH levels in the stress group while there was no change in LH levels in other groups. This result is in agreement with that chronic restraint stress in rats leads to a reduction in the hypothalamic corticotropin-releasing hormone (CRH) level, a rise in plasma levels of adrenocorticotropic hormone (ACTH) and glucocorticoids, as well as a general suppressing effect on the hypothalamic-pituitary-gonadal axis (HPG), by declining testosterone and LH (Retana- Márquez*et al.* 2003). However, in our study, there was no change in testosterone levels in the stress group, and such an effect may depend on the type and duration of the chronic restraint stress protocol (Retana-Márquez*et al.* 2014). On the other

hand, chronic treadmill exercise significantly increased testosterone levels in only exercise group in accordance with the previous studies (Fry *et al.* 1998, Raastad *et al.* 2000, Linnamo *et al.* 2005, Izquierdo *et al.* 2006). However, there was no change in testosterone levels in stress and stress+exercise groups. It has been reported that the changes in androgenic response are not always relevant to varied LH levels (Andersen *et al.* 2004), and hence, our testosterone result may not have changed for such a reason. Concerning corticosterone levels, it significantly increased in both stress and exercise groups. Increased corticosterone levels in the stress group confirmed the activation of the HPA axis by restraint stress (Retana- Márquez *et al*. 2014). In addition, unexpected increase in corticosterone levels in the exercise group may have resulted from exercise protocol causing sensitization of the HPA axis response. Some studies have indicated that treadmill exercise may act as a stressor activating the HPA axis (Contarteze *et al.* 2008, Radahmadi *et al.* 2017). Therefore, it has been suggested in the literature that voluntary wheel running has an advantage over treadmill exercise since it needs only minimal researcher intervention and does not require aversive stimuli (e.g., electric shocks) to motivate the animals to run (Poole *et al.* 2020). Nevertheless, we preferred to use treadmill exercise rather than voluntary wheel running as treadmill exercise in rats permits calculation and setting of external work and work rate as well as intensity and duration (Poole *et al.* 2020). Also, the animal has control over the quantity, intensity, and timing of its running behavior in the voluntary wheel running, which is variable and also may change on a large scale among animals (Poole *et al.* 2020).

Concerning the effects of chronic stress on sexual behavior in male rats, chronic restraint stress is known to disrupt sexual behavior parameters involving ML, IL, EL, and PEI (Brotto *et al.* 2001). However, whether chronic treadmill exercise may positively influence the effects of chronic

stress on sexual behavior is not known. In our study, we demonstrated that chronic restraint stress decreased sexual activity by increasing IF in agreement with the increase of IF, which may be considered as the sign of weakening of sexual activity (Grønli et al. 2005). It seems that a decrease in sexual activity by increased IF may be due to other stress-related mechanisms in the brain since sexual behavior is suggested to be not affected by the increase in corticosterone nor decrease in testosterone (Retana-Márquez et al. 2009). Also supporting this situation, one study has shown that stress can inhibit the consummatory part (including intromission) (Paredes et al. 1999) of male rat sexual behavior by increasing endogenous opioids, especially beta-endorphin (Retana- Márquez et al. 2014).

On the other hand, EF was significantly improved in the stress+exercise group compared to the stress group. Chronic treadmill exercise is suggested to increase sexual performance by increasing EF in previous studies in a similar way (Chaturapanich et. 2012, Lee et al. 2018). Retana-Márquez et al. (2003) reported that testosterone propionate treatment could not prevent the effects of stress on sexual behavior in castrated male rats even though the levels of testosterone were high in the treated animal. Male sexual behavior is known to be under the control of central neural mechanisms and testosterone (Chaturapanich et. 2012). However, in our study, improved sexual performance by increased EF may be related to central mechanisms rather than testosterone since there is no change in testosterone levels in the stress+exercise group.

Exercise and stress+exercise groups had significantly lower PEI compared to the control group. The PEI has been assessed as libido, index of potency, and the rate of recovery from exhaustion after the first series of mating (Yakubu and Akanji 2011). A PEI of more than 5400 sec shows

that the male is sexually tired, and the intensity of sexual behavior will be diminished in subsequent mating (Yakubu and Akanji 2011). Therefore, the significantly decreased PEI at exercise and stress+exercise indicated that treadmill exercise increased potency or libido while chronic restraint stress did not affect this parameter. Damassa *et al.* (1977) showed that there was no relationship between the circulating plasma testosterone levels and PEI in intact as well as castrated rats. Therefore, PEI may have changed independently of testosterone in our study.

The effects of chronic exercise and/or stress on sperm parameters and reproductive organ weights

Chronic restraint stress significantly decreased sperm concentration and motility and increased abnormal-tail and abnormal total sperm rate. To our knowledge, chronic treadmill exercise improved sperm motility in the stress+exercise group compared to the stress group for the first time in this study. However, chronic treadmill exercise did not affect sperm concentration and sperm morphology in the exercise or stress+exercise group. Chronic restraint stress was found to decrease sperm count (Hari Priya and Reddy 2012, Arun *et al.* 2016a, Arun *et al.* 2016b), motility (Hari Priya and Reddy 2012, Ribeiro *et al.* 2018), and increase abnormal sperm rate (Arun *et al.* 2016a, Arun *et al.* 2016b) in previous studies. Chronic restraint stress reduces testosterone release by inhibiting LH secretion (Nirupama *et al.* 2013a). The decrease in sperm concentration and motility may result from the decrease in testosterone levels in rats exposed to chronic restraint stress (Arun *et al.* 2016a, Ribeiro *et al.* 2018). However, there was no change in testosterone levels in the stress group in our study. Therefore, another possible mechanism may be that oxidative stress have a role in the deterioration of sperm parameters in the stress group. Arisha *et al.* (2019) reported that oxidative stress may lead to an increase of morphological abnormalities and a decrease in sperm concentration and motility as a result. To our knowledge, there is no mechanism of action explaining the effects of chronic

restraint stress or chronic treadmill exercise on sperm parameters without affecting testosterone levels, which needs to be investigated in further studies.

Concerning the effects of chronic exercise and/or restraint stress on the male rats' reproductive organ weights, chronic restraint stress did not cause any change of reproductive organs' weights in the stress group, and chronic treadmill exercise has no effect on these organs' weights in the stress+exercise group in our study. Sexual accessory glands are known to be androgen-dependent, and thus testosterone is necessary for them to sustain their functionality (Retana-Márquez *et al.* 2014). As a result, the weights of these glands may decrease due to low levels of testosterone (Hari Priya and Reddy 2012). Also, the weight of the testis is dependent on testosterone (Nirupama *et al.* 2013b). It was reported that chronic restraint stress caused a significant decrease in testis and epididymis weights' as well as testosterone levels in previous studies (Hari Priya and Reddy 2012, Arun *et al.* 2016a, Arun *et al.* 2016b, Ribeiro *et al.* 2018). In addition, it was reported that seminal vesicles' weight did not change in the same studies (Hari Priya and Reddy 2012, Arun *et al.* 2016b), while it decreased in another study (Arun *et al.* 2016a). In addition, prostates' weight did not change in the chronic restraint stress group (Hari Priya and Reddy 2012). However, in our study, there was no change in testosterone levels in stress or stress+exercise groups in line with unchanged accessory organs' and testis weights in the stress or stress+exercise group. On the other hand, among the accessory sex organs, prostate and seminal vesicles are suggested to be the most sensitive reproductive parts to stress, but it is indicated that epididymal weights may decrease, and testis weights are usually not affected in the rat. Epididymal weights (absolute and relative to body weight) may decrease when testosterone level is low; however, epididymal weights are suggested as not sensitive, which may be an indicator as to the weights of the seminal vesicles and prostate. In the same

study, it is emphasized that the effects of stress may be affected by several factors, involving the property of the stressor, period of the stress, species of animal. (Everds *et al.* 2013). By depending on these variables, our chronic restraint stress protocol may not have caused any changes in the weights of accessory sexual organs and testis.

In conclusion, chronic restraint stress may disrupt sexual behavior in terms of consummatory parameters of sexual behavior involving IF, and also sperm motility, and concentration. Furthermore, the ameliorative effect of chronic treadmill exercise on the disruption in sperm motility induced by chronic restraint stress has been revealed for the first time in this study. Since this study is based on an animal model and thus the present findings could partially reflect the characteristics of human sexual behavior. In addition, given the fact that the effects of stress include complex neuroendocrine mechanisms, further clinical studies are needed to clarify the effects of chronic stress on sexual behavior and reproductive parameters in men. Also, it can be concluded that treadmill exercise may also have a role as a stress factor. This is a methodological limitation that we will assess in further experiments.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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