International Journal of Medical Science and Clinical Invention 8(6): 5449-5457, 2021
DOI:10.18535/ijmsci/v8i06.04
e-ISSN:2348-991X, p-ISSN: 2454-9576

Research Article,

Effects of Estrogen and Exercise Stress on Adrenal Glands of Male Rats

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Abstract:
Consciously done exercises are beneficial for human health. However, unconscious and wrong practices (medication use, etc…) can cause various injuries and permanent damage to the human body. For reasons such as increasing muscle mass and/or to getting more performance, taking steroid hormones disrupts overall body hormone balance. Effects of exercise on oxygen and energy metabolism and estrogen as an exogenous steroid have significant effects on the adrenal gland. The aim of this study was to see the effects of 17β-estradiol on surrenal glands of rats that is put through regular physical exercises. 36 male Sprague-Dawley rats between the weights 220-250 gr, were put into thermal-controlled room with day-night cycles to stimulate an optimal day for the subjects. The experiment was modeled by dividing 36 animals into 6 groups in total according to the control, exercising and estrogen administration criteria. Experimental group animals received daily doses of 10 µg/kg/day 17β-estradiol during 30 days. Also exercises group animals ran at 20 m/min on a 15% grade for 90 minutes and rest 34 minutes. Then, TUNNEL and Hematoxylen & Eosin staining were performed to measure the damage on the adrenal glands. In group 2, dense presence of degenerative fibroblasts and inflammatuary cells infiltration in zona fasciculata were significantly different. In group 3, the degenerative areas were significant in all adrenal cortex zones. In group 4, necrotic areas were determined in zona reticularis. In group 5, zona fasciculata was severely degenerated. With group 6, Sinusoidal features were completely lost in zona reticularis. The results strongly show that exercise may affect the zona glomerulase in short time period. As a result, exposure to exercise and exercise stress with external administration of estradiol may cause cellular degeneration especially zona fasciculata and zona reticularis in the adrenal gland.

Keywords: 17β-estradiol, Surrenal gland, Eccentric exercise, Rat,

Introduction:

Exercising improves overall health and helps to maintain a stable figure with the use of physical activities which in turn makes a person mentally healthy. Regular exercising helpst to achive a longer lifespan, and delays the onset of 40 chronic diseases. By the 21st century, lack of exercise is the basis of many diseases and public health problems, and it is also the cause of chronic diseases and deaths (1, 2). It is known that most of scientific studies support the positive effects of exercise on muscles, heart, respiratory and circulatory systems while reducing the risk of diseases such as dyslipidemia, hypertension, hyperglycemia, visceral obesity, chronic proinflammation, insulin resistance (2, 3). Low-intensity regular exercise primarily causes an increase in the mitochondrial content of muscle

tissue, as well as regulating other cellular functioning (4). Conscious exercise is usefull for human health; however, malpractices (drug usage, eccentric exercise and balance problems) may lead several types of injuries and permenant damage in human body. Although there are many health benefits, sports activities also carry many risks of injury. In every age, incorrect application of exercise can cause physical injury, deformities in weight-bearing joints and limbs, including hip, knee and ankle, and osteoarthritis (OA) in the presence of many different injuries (5). People use many methods to increase their physical ability or to reduce the negative effects of exercise, such as oxidative stress. Steroid hormones have generally anabolic effections. They induce protein synthesis and play a key role on the growth of muscle and bone tissue. Androgens, because of their anabolic effects, are the most common drugs that are used by athletes. Taking some exogen hormones to get more performance from the body may disrupt the hormone balance (6).

The amount of androgens in the blood is directly parallel with the exercise intensity. In addition, the use of anabolic androgenic steroids and other anabolic doping agents can cause health problems such as cardiovascular disease, diabetes, cancer, mental health issues, virilization in women, and suppression of naturally produced androgens in men (7). Androgen hormone use has adverse effects on the cardiovascular system, kidney, endocrinological and reproduction. Normal steroid hormones are synthesized in the adrenal cortex, gonads (testicles and ovaries). For both gender, adrenal glands are one of the endocrine organs which are essential for the life (8). Androgens used as doping affect the hypothalamic-pituitary-ovarian axis. Exogenous hormones steroid hormones intake suppresses the hypothalamic-pituitary-testicular axis (HPT) and lowers the titles such as gonadotrophic (hence LH and FSH) hormones. Ultimately, the level of endogenous steroids decreases (9). Different physical activity characteristics (aerobic or anaerobic, agonistic or non-agonistic, duration of training sessions, frequency of weekly sessions), characteristics such as female age, menstrual cycle regularity, body mass index also affect ovarian dysfunction (10). In men, this situation can directly affect fertility events such as decreased sex hormone binding globulin, decreased testicular size, sperm count, sperm motility, and changes in sperm morphology, as well as gynecomastia and prostatic hypertrophy (7, 11). Some of the androgenic and estrogenic properties of steroids are also regulated in the adrenal glands. Individuals who want to increase their muscle mass encounter results that affect adrernal gland hormone production, such as HDL-C, insulin sensitivity and glucose tolerance, and the risk of Type 2 diabetes (12). Estrogen initiates various physiological proposals through two estrogen receptors ER_{α} and ER_{β}. 17β-estradiol can be bound at both estrogen receptors (13). Both androgen (AR) and estrogen (ER) receptor genes are expressed in all three layers of the adrenal cortex. More than 30 genes express estrogen receptors in the ZG layer, while more than 200 genes in ZF/R express estrogen receptors of the adrenal gland in both males and females (14). On the other hand, this ligand could affect a role in decreasing tissue damage occured depend on exercise (15, 16). The relationship of the adrenal gland with many issues such as its effects on systematic neuroendocrine and immune cascade, neuroprotective effects, and the functioning of the liver that regulates metabolism have been investigated (17, 18). But there is some controversy regarding estrogen supplementation effects. It is known to affect the use of estrogen hormones in exercise and the adrenal gland (19). We wanted to examine which layers of the adrenal gland are most affected by this situation and also which layer is most likely to be damaged. In this study, we tried to find the possible effects (positive and/or negative) of estrogen supplementation, is used as a doping for improving performance or muscle development, after exercise administration on male adrenal glands. Then, we are expecting the adrenal glands damage due to excessive work after the histological examinations depending on not only to compensate the excessive metabolic load occured during exercise but also eliminate the side effects of estrogen.

Material and Methods:

Animals

The study was carried out in facilities approved by the Atatürk University Institutional Animal Care and Use Committee (ATADEM-Approval No: 2008-05/39; 25.04.2008). Twelve weeks-old 36 male Sprague-Dawley rats (220-250 gr), were put into thermal-controlled room with day-night cycles to stimulate an optimal day for the subjects. Animals were allowed free access to food and water throughout the entire experiment. Rats were separated into two groups using random separation method. 18 rats were in experimental and remaining 18 were in control groups. Main experimental groups were divided into new six

groups as shown in Table 1.

Table1. Experimental groups and drug administration

Non-exercise group		Eccentric exercise group (dissected after 1 h)		Eccentric exercise group (dissected after 48 h)	
Group 1 (n:6)	Group 2 (n:6)	Group 3 (n:6)	Group 4 (n:6)	Group 5 (n:6)	Group 6 (n:6)
Control Groups **Plasebo solution, 30 days**	Experimental Groups Estrogen solution, 10 µg/kg/day, 30 days	Control Groups Plasebo solution, 30 days	Experimental Groups Estrogen solution, 10 µg/kg/day, 30 days	Control Groups Plasebo solution, 30 days	Experimental Groups Estrogen solution, 10 µg/kg/day, 30 days
Plasebo solution + Non-exercise	Estrogen solution + Non-exercise	Plasebo solution + Eccentric exercise (dissected after 1 h)	Estrogen solution + Eccentric exercise (dissected after 1 h)	Plasebo solution + Eccentric exercise (dissected after 48 h)	Estrogen solution + Eccentric exercise (dissected after 48 h)

Group 1 (Control group), Group 2 (Estrogen group), Group 3 (1 hour after exercise dissected group), Group 4 (1 hour after exercise dissected + estrogen group), Group 5 (48 hour after exercise dissected group), Group 6; (48 hour after exercise dissected + estrogen group)

Drug Administration:

Experimental group animals received daily doses of 10 µg/kg/day 17β-estradiol (3 mg 17β-estradiol + 50 ml absolute ethanol + 100 ml sesame oil). The injections were given subcutaneously for 30 consecutive days. On the other hand, control group animals received subcutaneously plasebo solution (50 ml absolute ethanol + 100 ml sesame oil) during 30 days in order to expose the same stress conditions.

Exercise Administration:

Acute exercise was performed on a motorized rodent treadmill (May Time 9805 Treadmill Exerciser). Group 3, 4, 5 and 6 were subjected to straight running for 5 minutes with 20 mt/min before one day from eccentric exercise administration. A day later, animals ran at 20 m/min on a 15% acute exercise on a motorized rodent treadmill. The procedure was 5 minutes running and 2 minutes resting. Totally, animals ran at 20 m/min on a 15% grade for 90 minutes and rest 34 minutes (20, 21).

Histological Procedures:

Adrenal glands were bilaterally taken from the rats to perform histological procedures. For classical histopathological examination, the surrenal tissue were fixed in 10% neutral formaldehyde solution for 1 week. Sections were obtained using a Leica RM2125RT microtome (Leica, Germany). In the following process, paraffin block was cut to 6 µm thickness. The sections were stained with Hematoxylin-Eosin (H&E) for microscope (Olympus BH 40) examination. Surrenal gland sections were examined histopathological evaluation. These parameters: degenerative areas, inflammatuary cells infiltration, necrotic area, fibrin plaques, endotelial cells features.

TUNNEL Staining:

According to the manufacturer's instructions, cell apoptosis rate was detected using In Suit Cell Death Detection kit (Roche Applied Science, Penzberg, Germany). Shortly, surrenal tissues sections were dewaxed, rehydrated by xylene and a graded series of ethanol. Then incubated proteinase K solution for 15 min at 37°C. Later, the sections were incubated at 37°C in dark for 1 h with 50 µL Converter-POD per sample for 30 min. Nuclei staining was done with hemagoxylene. Examination was evaluated under light microscope (Olympus BH 40). Immunpositive cells were counted in 10 randomly selected areas of X40 objective. Sections from each animal were counted and reported as semi-quantitatively. Following: none: −, mild: +, moderate: ++, severe: +++, and very strong: ++++.

Results:

All zones of adrenal gland have normal apparences in Group 1 (Control group). There were any pathological findings in this groups' section (Figure 1A). When immunohistochemical analyses were administered, there were no TUNNEL positive cells in capsule, cortex and medulla (Figure 2A).

The histopathological examination in Group 2 (Estrogen group), dense presence of degenerative fibroblasts and inflammatuary cells infiltration in zona fasciculata (ZF) were significantly different. There were irregular cords and focal necrotic focus which are surrounded with inflammatory cells, in ZF. Thickness of endothelial cell of medullar vein was decreased. Additionally, fibrin plaques were seen in medulla (Figure 1B). Immunohistochemically, there were moderate grade TUNNEL positive cells in capsule and zona glomerulosa (ZG), however weak TUNNEL positive cells were detected in ZF (Figure 2B, Table 2) In group 3 (1 hour after exercise dissected group), the degenerative areas were significant in all zones. Especially, fibrine plagues in sinusoids and hypercromatic endotelial cells were seen in zona reticularis (ZR). Inflamatory cells and necrotic debris were detected between medullary ganglion cells as well as destructive medullary veins (Figure 1C). In immunohistochemical analysis, TUNNEL positive cells were detected in all cortical layers. There were also dense TUNNEL positive cells in medulla. (Figure 2C, Table 2).

When histopathological examination was performed in Group 4, (1 hour after exercise dissected + estrogen group) necrotic areas were observed in ZR. Some cell had eosinophilic cytoplasm in ZF. Degenerative cells, transparent cells and intrasinusoidal inflamatory cells were appearented in ZF. (Figure 1D). There were moderate grade TUNNEL positive cells in all zone of adrenal gland (Figure 2D). In group 5 (48 hour after exercise dissected group), ZF was severely degenerated. Additionally, multi-nuclear giant cells and inflammatory cells were detected intrasunisoidal area. Accumulated ganglion cells and transparance cells were seen in medullary zone. Additinally, cytoplasmic brown granules were defined in medulla. (Figure 1E). In immunohistochemical analysis, there were moderate TUNNEL positive cells both in cortex and medulla (Figure 2E, Table 2).

Histopathological sections of group 6 (48 hour after exercise dissected + estrogen group), ZF had hyperchoromatic nucleus and pale cytoplasm. With group 6, Sinusoidal features were completely lost in ZR. In addition to, the presense of hyaline deposits were remarkable (Figure 1F). In immunohistochemical analysis, TUNNEL positive cells were detected in ZG and it's endothelial cells. Strong positivities were seen in ZR. On the other hand, a few TUNNEL positive cell were defined in ZF (Figure 2F, Table 2).

Table2. Semi-quantitative assessment of TUNNEL immunopositivity in surrenal glands

Groups	Capsule	Zona Glomerulosa	Zona Fasiculata	Zona Reticularis	Medulla
Group 1 (Control group)	-	-	-	-	-
Group 2 (Estrogen group)	++	++	+	++	+
Group 3 (1 h after exercise dissected group)	+	++	+	+	++
Group 4 (1 hour after exercise dissected + estrogen group)	+	++	++	++	+
Group 5 (48 hour after exercise dissected group)	++	++	++	+++	++
Group 6 (48 hour after exercise dissected + estrogen group)	++	+++	++	++++	++

none: −, mild: +, moderate: ++, severe: +++, and very strong: ++++.

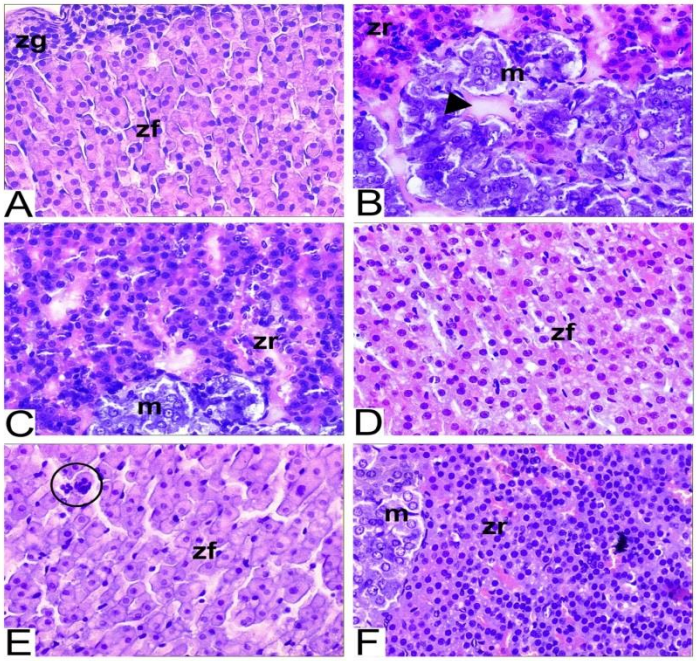

Figure1: Histopathological analysis of all experimental groups (H&E)

1A: In control group, healthy and normal appearance of capsule, zona glomerulosa and fasciculata. **B:** In estrogen group, appearance of fibrin plaques in the medulla. **1C:** In 1 hour after exercise dissected group, appearance of degenerative areas in zona reticularis. **1D:** In 1 hour after exercise dissected + estrogen group, appearance of eosinophilic cytoplasm in zona fasciculate. **1E:** In 48 hours after exercise dissected group, appearance of multi-nuclear giant cells in a black circle in the zona fasciculata. **1F:** In 48 hours after exercise dissected + estrogen group, appearance of the loss of sinusoidal features in the zona reticularis. *(**cap**; capsule, **zg**; zona glomerulosa, **zf**; zona fasciculata, **zr**; zona reticularis, **m**; medulla, **black arrowhead;** fibrin plaques. H&E staing. Mag X20)*

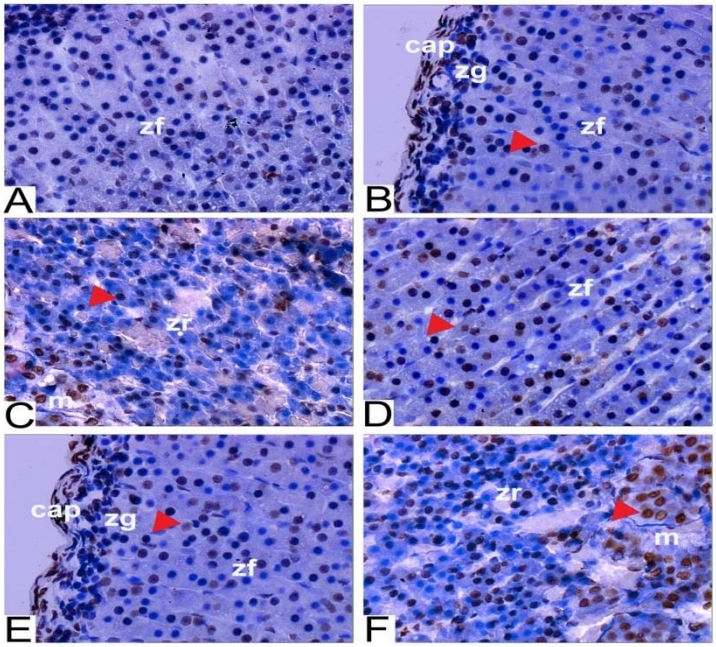

Figure2: Immunohistochemical analyses of all experimental groups (TUNNEL).

1A: In control group, appearance of no TUNNEL reaction in the adrenal cortex. **1B:** In estrogen group, appearance of weak TUNNEL staining of the zona glomerulosa and fasciculata. **1C:** In 1 hour after exercise dissected group, appearance of weak TUNNEL staining of the zona reticularis and medulla. **1D:** In 1 hour after exercise dissected + estrogen group, appearance of strong TUNNEL staining of the zona fasciculata. **1E:** In 48 hours after exercise dissected group, appearance of strong TUNNEL staining of the zona fasciculata. **1F:** In 48 hours after exercise dissected + estrogen group, appearance of intensive immunity in the adrenal medulla and zona reticularis. *(**cap**; capsule, **zg**; zona glomerulosa, **zf**; zona fascicula, **zr**; zona reticularis, **m**; medulla, **red arrowhead;** TUNNEL positive cells. H&E staing. Mag X20)*

Discussion:

Exercising is an activity which improves both human physical and mental healthalso helps to sustain it for the rest of the life span of the individual. However, it is well-known that incorrect training may cause destructive effects on whole organism. The aim of this study was to see the effects eccentric exercise on rat adrenal glands in acut/chronic terms by using histopathological methods. Additionally, it was analysed that whether exogen (external) estrogen has a positive or negative effect on this relationship or not. It showed that there was no abnormality in Group 1 (Control). It was seen the cells in ZF have degenerated and negatively affected by both inflammatory and fibrolabst. In group 2 (estrogen group). In group 3 (the group dissected 1 hour after exercise), degenerative areas were noticeable in all zones, while hyperchromatic endothelial cells were seen in ZR. In group 4, necrotic areas were detected in ZR on histopathological examination. A similar TUNNEL reaction was seen in the 1-hour groups (group 3 and group 4) in which the acute effects of exercise were seen. Immune activated was mild to moderate. In group 5, the ZF was severely degenerated and many nuclear giant cells and inflammatory cells were detected. In the histopathological sections of Group 6, the sinusoidal structural organization and cellular organization of ZF and ZR were lost. In addition, hyaline deposits were seen in both layers. Immune activity was very strong in 48 h groups (group 5 and group 6) in which chronic effects of exercise were observed. Especially in both groups, the most severe reaction was observed in ZR. Immune positivity was severe in the ZF layer in Group 5. Estrogens are steroid-structured hormones. They have key roles on cell growth and embryonic development (22, 23, 24). The analysis was performed between estrogen application and adrenal glands by comparing the data obtained from Group 1 (Control group) and Group 2 (Estrogen group) to understand the basic mechanism of estrogen application. With the use of these information, we can say that there were no abnormalities in Group 1 rats, whereas apoptosis was induced in zone glomerulosa of Group 2 rats. In one study, 8-weeks healthy male and female Sprague Dawley rats were subjected to 10-microgram estradiol application during a month. According to the findings of that study, it was detected that pituitary and adrenal glands weights increased in both genders. Also, the cell volumes of adrenal cortex zones had significant change (25). In another study was pointed out that long-term testosterone application led to cellular atrophy in especially ZR of adrenal cortex of male rats in a time-dependent manner. By the way, some abnormalitites were also detected in mitochondria and endoplasmic reticulum of this layer in the same study. Testosteron application inhibited the steroidogenic capacity of adrenocortical cells which may reduce the level of corticosterone. Therefore, adrenocorticotropic hormone (ACTH) level started to increase (26). The similar studies may be seen in current literature. The possible mechanism may be explained briefly as following: External estrogen application firstly reduces the capacity of corticosteroid synthesis and release in adrenal glands and then, increases the level of ACTH. Actually, it is estimated that increased level of ACTH provokes the steroidogenic capacity of adrenocortical cells. It is known that external estrogen application may block the mitochondrial and nuclear RNA synthesis. This blockage may cause a decrease in P450 c21-hidroxilase expression and the sensitivity of ACTH (27).

Acute and long-term effects of exercise on adrenal glands were analyzed by comparing the data obtained from eccentric exercise groups (Group1, 3, 5). Stress or other external stimulants activates otonom nervous system and this activation may induce Hypothalamic-pituitary-adrenal axis (HPA) (28). Plasma cortisol levels, released from adrenal glands, increased to co-op with stress (29). Most of scientific studies proved that ACTH sensitivity reduce depend on increased exercise time and severity (30). Sasse et al. (31) prolonged and repeated stress has a potential to occur a negative effect on HPA axis. As a consequent, they showed the reduced glucocorticoid level. In another study, Watabane et al performed (32), pointed out that ACTH and corticosterone plasma concentrations increased in acute exercise group as well as control and exercise groups. In contrast to this increase, ACTH response and Corticotropin Releasing Factor (CRF) were detected in low level in exercise group when compared with control group in same study.

Plasma levels of the stressor hormones that regulate the relationship with HPA should have been measured. In addition, the plasma steroids estrogen and testosterone should be evaluated. Estrogen receptors at the tissue mRNA level could not be examined at the molecular level. These analyzes, which cannot be done due to the budget

of the study, will be among the reference notes for the next studies. However, the destructive effects of exercise and exogenous estrogen intake have been clearly emphasized on the adrenal cortex and the relationship with the apoptotic pathway. As a result, when the modulating effects of estradiol treatment are examined after exercise training, it has negative results in the cortex of the adrenal gland. Especially ZF and ZR can cause cellular degeneration and cell death.

Conclusion:

The result if we take everything into account, the short-term effects of exercise can be seen in some areas of the adrenal glands. As a result, we can say that all zones in the adrenal cortex exercising a strong influence in the short term. In the end, the response of heavy exercise and physical activity to stress exposure can have a negative effect on the adrenal gland.

Conflict of Interest:

The authors have no conflict of interest. The authors have only responsible for the writing of this atricle.

References:

[1] Knapen, J., Vancampfort, D., Moriën, Y., & Marchal, Y. (2015). Exercise therapy improves both mental and physical health in patients with major depression. Disability and rehabilitation, 37(16), 1490–1495. https://doi.org/10.3109/09638288.2014.972579.

[2] Peixoto, T. C., Begot, I., Bolzan, D. W., Machado, L., Reis, M. S., Papa, V., Carvalho, A. C., Arena, R., Gomes, W. J., & Guizilini, S. (2015). Early exercise-based rehabilitation improves health-related quality of life and functional capacity after acute myocardial infarction: a randomized controlled trial. The Canadian journal of cardiology, 31(3), 308–313. https://doi.org/10.1016/j.cjca.2014.11.014.

[3] Adams, V., Reich, B., Uhlemann, M., & Niebauer, J. (2017). Molecular effects of exercise training in patients with cardiovascular disease: focus on skeletal muscle, endothelium, and myocardium. American journal of physiology. Heart and circulatory physiology, 313(1), H72–H88. https://doi.org/10.1152/ajpheart.00470.2016.

[4] Jacobs, R. A., Flück, D., Bonne, T. C., Bürgi, S., Christensen, P. M., Toigo, M., & Lundby, C. (2013). Improvements in exercise performance with high-intensity interval training coincide with an increase in skeletal muscle mitochondrial content and function. Journal of applied physiology (Bethesda, Md. : 1985), 115(6), 785–793. https://doi.org/10.1152/ japplphysiol. 00445.2013.

[5] Maffulli, N., Longo, U. G., Gougoulias, N., Caine, D., & Denaro, V. (2011). Sport injuries: a review of outcomes. British medical bulletin, 97, 47–80. https://doi.org/10.1093/bmb/ldq026.

[6] Birzniece V. (2015). Doping in sport: effects, harm and misconceptions. Internal medicine journal, 45(3), 239–248. https://doi.org/10.1111/imj.12629.

[7] Bird, S. R., Goebel, C., Burke, L. M., & Greaves, R. F. (2016). Doping in sport and exercise: anabolic, ergogenic, health and clinical issues. Annals of clinical biochemistry, 53(Pt 2), 196–221. https://doi.org/10.1177/0004563215609952.

[8] Green, M. R., & McCormick, C. M. (2016). Sex and stress steroids in adolescence: Gonadal regulation of the hypothalamic-pituitary-adrenal axis in the rat. General and comparative endocrinology, 234, 110–116. https://doi.org/10.1016/j.ygcen.2016.02.004.

[9] Maravelias, C., Dona, A., Stefanidou, M., & Spiliopoulou, C. (2005). Adverse effects of anabolic steroids in athletes. A constant threat. Toxicology letters, 158(3), 167–175. https://doi.org/10.1016/j.toxlet.2005.06.005.

[10] La Vignera, S., Condorelli, R. A., Cannarella, R., Duca, Y., & Calogero, A. E. (2018). Sport, doping and female fertility. Reproductive biology and endocrinology: RB&E, 16(1), 108. https://doi.org/10.1186/s12958-018-0437-8.

[11] Casavant, M. J., Blake, K., Griffith, J., Yates, A., & Copley, L. M. (2007). Consequences of use of anabolic androgenic steroids. Pediatric clinics of North America, 54(4), 677–x. https://doi.org/10.1016/j.pcl.2007.04.001.

[12] Bahrke, M. S., & Yesalis, C. E. (2004). Abuse of anabolic androgenic steroids and related substances in sport and exercise. Current opinion in pharmacology, 4(6), 614–620. https://doi.org/10.1016/j.coph.2004.05.006.

[13] Hamilton, K. J., Hewitt, S. C., Arao, Y., & Korach, K. S. (2017). Estrogen Hormone Biology. Current topics in developmental biology, 125, 109–146. https://doi.org/10.1016/bs.ctdb.2016.12.005.

[14] Trejter, M., Jopek, K., Celichowski, P., Tyczewska, M., Malendowicz, L. K., & Rucinski, M. (2015). Expression of estrogen, estrogen related and androgen receptors in adrenal cortex of intact adult male and female rats. Folia histochemica et cytobiologica, 53(2), 133–144.

[15] Enns, D. L., & Tiidus, P. M. (2010). The influence of estrogen on skeletal muscle: sex matters. Sports medicine (Auckland, N.Z.), 40(1), 41–58. https://doi.org/10.2165/11319760-000000000-00000.

[16] Ikeda, K., Horie-Inoue, K., & Inoue, S. (2019). Functions of estrogen and estrogen receptor signaling on skeletal muscle. The Journal of steroid biochemistry and molecular biology, 191, 105375. https://doi.org/10.1016/j.jsbmb.2019.105375.

[17] Fragala, M. S., Kraemer, W. J., Denegar, C. R., Maresh, C. M., Mastro, A. M., & Volek, J. S. (2011). Neuroendocrine-immune interactions and responses to exercise. Sports medicine (Auckland, N.Z.), 41(8), 621–639. https://doi.org/10.2165/11590430-000000000-00000.

[18] Marosi, K., Felszeghy, K., Mehra, R. D., Radák, Z., & Nyakas, C. (2012). Are the neuroprotective effects of estradiol and physical exercise comparable during ageing in female rats?. Biogerontology, 13(4), 413–427. https://doi.org/10.1007/s10522-012-9386-3.

[19] Kraemer, R. R., Francois, M., & Castracane, V. D. (2012). Estrogen mediation of hormone responses to exercise. Metabolism: clinical and experimental, 61(10), 1337–1346. https://doi.org/10.1016/j.metabol.2012.03.009.

[20] Tsivitse, S. K., McLoughlin, T. J., Peterson, J. M., Mylona, E., McGregor, S. J., & Pizza, F. X. (2003). Downhill running in rats: influence on neutrophils, macrophages, and MyoD+ cells in skeletal muscle. European journal of applied physiology, 90(5-6), 633–638. https://doi.org/10.1007/s00421-003-0909-0.

[21] Warren, G. L., Lowe, D. A., Inman, C. L., Orr, O. M., Hogan, H. A., Bloomfield, S. A., & Armstrong, R. B. (1996). Estradiol effect on anterior crural muscles-tibial bone relationship and susceptibility to injury. Journal of applied physiology (Bethesda, Md.: 1985), 80(5), 1660–1665. https://doi.org/10.1152/jappl.1996.80.5.1660.

[22] Fadini, G. P., Albiero, M., Cignarella, A., Bolego, C., Pinna, C., Boscaro, E., Pagnin, E., De Toni, R., de Kreutzenberg, S., Agostini, C., & Avogaro, A. (2009). Effects of androgens on endothelial progenitor cells in vitro and in vivo. Clinical science (London, England: 1979), 117(10), 355–364. https://doi.org/10.1042/CS20090077.

[23] Parborell, F., Irusta, G., Vitale, A., Gonzalez, O., Pecci, A., & Tesone, M. (2005). Gonadotropin-releasing hormone antagonist antide inhibits apoptosis of preovulatory follicle cells in rat ovary. Biology of reproduction, 72(3), 659–666. https://doi.org/10.1095/ biolreprod. 104.034454.

[24] Tomikawa, J., Homma, T., Tajima, S., Shibata, T., Inamoto, Y., Takase, K., Inoue, N., Ohkura, S., Uenoyama, Y., Maeda, K., & Tsukamura, H. (2010). Molecular characterization and estrogen regulation of hypothalamic KISS1 gene in the pig. Biology of reproduction, 82(2), 313–319. https://doi.org/10.1095/biolreprod.109.079863.

[25] Wernert, N., Antalffy, A., & Dhom, G. (1986). Effects of estradiol on adrenal cortex and medulla of the rat. Morphometric studies. Pathology, research and practice, 181(5), 551–557. https://doi.org/10.1016/S0344-0338(86)80148-0.

[26] Mazzocchi, G., Malendowicz, L. K., Robba, C., Rebuffat, P., Gottardo, G., Meneghelli, V., & Nussdorfer, G. G. (1983). Effects of testosterone on the zona fasciculata of the male rat adrenal cortex. A correlated stereological and biochemical study. Journal of submicroscopic cytology, 15(4), 991–1005.

[27] Su, Y., Carey, L. C., Rose, J. C., & Pulgar, V. M. (2012). Leptin alters adrenal responsiveness by decreasing expression of

ACTH-R, StAR, and P450c21 in hypoxemic fetal sheep. Reproductive sciences (Thousand Oaks, Calif.), 19(10), 1075–1084. https://doi.org/10.1177/1933719112442246.

[28] Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. Journal of psychosomatic research, 53(4), 865–871. https://doi.org/10.1016/s0022-3999(02)00429-4.

[29] Jung, C., Greco, S., Nguyen, H. H., Ho, J. T., Lewis, J. G., Torpy, D. J., & Inder, W. J. (2014). Plasma, salivary and urinary cortisol levels following physiological and stress doses of hydrocortisone in normal volunteers. BMC endocrine disorders, 14, 91. https://doi.org/10.1186/ 1472-6823-14-91.

[30] Kusuyama, J., Alves-Wagner, A. B., Makarewicz, N. S., & Goodyear, L. J. (2020). Effects of maternal and paternal exercise on offspring metabolism. Nature metabolism, 2(9), 858–872. https://doi.org/10.1038/s42255-020-00274-7.

[31] Sasse, S. K., Greenwood, B. N., Masini, C. V., Nyhuis, T. J., Fleshner, M., Day, H. E., & Campeau, S. (2008). Chronic voluntary wheel running facilitates corticosterone response habituation to repeated audiogenic stress exposure in male rats. Stress (Amsterdam, Netherlands), 11(6), 425–437. https://doi.org/10.1080/10253890801887453.

[32] Watanabe, T., Morimoto, A., Sakata, Y., Wada, M., & Murakami, N. (1991). The effect of chronic exercise on the pituitary-adrenocortical response in conscious rats. The Journal of physiology, 439, 691–699. https://doi.org/10.1113/jphysiol.1991.sp018688.