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Tatara, Koki

Sato, Koji

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Aerobic exercise training and dehydroepiandrosterone administration increase testicular sex steroid hormones and enhance reproductive function in high-sucrose-induced obese rats

Koki Tatara*, Koji Sato*

* Equal contribution

Graduate School of Human Development and Environment, Kobe University,

Kobe, Hyogo, Japan

Running head: *Exercise and DHEA in reproductive function*

***Corresponding author**: Koji Sato, PhD;

Graduate School of Human Development and Environment,

Kobe University, 3-11 Tsurukabuto, Kobe, Nada-Ku, Hyogo, 657-8501, Japan

TEL: 81-78-803-7812

FAX: 81-78-803-7812

E-mail: sato712@people.kobe-u.ac.jp

E-mail: kokitatara@gmail.com

Keywords

Epididymis, sperm protection, sperm growth, steroid

Abbreviations

DHEA: dehydroepiandrosterone, DHT: 5α-dihydrotestosterone, HSD: hydroxysteroid dehydrogenase, GPx4: glutathione peroxidase 4

Abstract

This study assessed the effects of chronic dehydroepiandrosterone (DHEA) administration and exercise training on testicular sex steroid hormone levels and reproductive function in high-sucrose induced obese rats. After 14 weeks of a high-sucrose diet, Wistar male rats were assigned randomly to the control, exercise training (running at 25 m/min for 1 h, 5 days/week), DHEA administration, and combined exercise training and DHEA administration groups (n = 7 each group). Six weeks of DHEA administration and/or exercise training significantly increased plasma concentrations of DHEA and 5α-dihydrotestosterone (DHT) and epididymis DHEA concentrations; however, the expression of steroidogenic enzymes, such as 3β-hydroxysteroid dehydrogenase (HSD), 17β-HSD, and 5α-reductase, did not change following any interventions. Procathepsin L expression, which involved sperm maturation, was significantly lower in the DHEA and combination groups, and glutathione peroxidase 4 (GPx4) expression, which plays a role in protecting sperms from oxidative stress, was significantly increased in the DHEA administration group. Additionally, exercise training and/or DHEA administration-induced increase in procathepsin L expressions were significantly correlated with the epididymis DHEA concentrations. These findings suggest that exercise training and/or DHEA administration-induced increase in epididymis DHEA concentration may improve impairment of reproductive function in high-sucrose obese rats. Additionally, exercise training and/or DHEA administration-induced increase in DHEA concentration may have a role in testicular-specific action, which included protective role from exercise-induced oxidant damage as well as contributed to the enhancement of sperm modification and maturation in obese rats.

1. Introduction

Obesity and type 2 diabetes are risk factors for coronary artery disease, hypertension, and hyperlipidemia as well as impairment of reproductive function, and therefore may cause erectile dysfunction, especially in men. Individuals with higher body mass index (BMI) tend to have a lower progressive sperm count and a higher morbidity rate of oligozoospermia compared to individuals with normal BMI. Moreover, people with obesity have a lower sperm amount, count, concentration and motility [1]. Therefore, obesity is one of the risk factors of reduced number of motile sperm and increased occurrence of abnormal form sperm, and may cause impairment of reproductive function and erectile dysfunction.

Sex steroid hormones are mainly secreted by the ovaries, testes, and adrenal cortex, and regulate diverse physiological processes in the target tissues, such as the reproductive organs, bone, liver, cardiovascular system, brain, and skeletal muscle [2,3,4,5]. Dehydroepiandrosterone (DHEA) and its sulfate derivate (DHEA-S) is a precursor of sex steroid hormones and play physiological roles in maintaining steroidogenesis in peripheral tissues [2]. Obesity and type 2 diabetes patients have lower DHEA and all other sex steroid hormone concentrations [6]. In our previous study, chronic DHEA administration lowered fasting blood glucose levels through increased sex steroid hormone concentrations in tissues and enhanced muscle glucose metabolism in obese rats [7]. A single bout of DHEA administration was also shown to be able to improve hyperglycemia in streptozotocin-induced type 1 diabetes rat models [8]. Therefore, DHEA can enhance impaired glucose metabolism and utilization and may contribute to

improvement of glycemic control and insulin resistance in the obese. Additionally, sex steroid hormones play a major role in the maturation of sperm during the process of spermatogenesis and maintenance of sex steroid hormone concentrations in the Sertoli cells. This cell is important for improvement of adequate numbers of viable and mature sperm that is essential for fertility in men [9, 10]. However, whether the DHEA administration-induced increase in sex steroid hormones causes the enhancement of reproductive function in obesity is still unclear.

Aerobic exercise generally has numerous beneficial effects for improving cardiovascular function as well as preventing metabolic syndrome. However, there is a debate regarding the effects of exercise training on testicular and reproductive functions. According to a previous study, intensive exercise can result dysfunction in male reproductive function [11]. Another study found that chronic exercise training induced reduction in sex steroid hormone concentrations along with other reproductive hormonal abnormalities [12]. On the other hand, our previous studies reported that chronic exercise training increased sex steroid hormone concentrations in obese rats [7]. Thus, exercise induced the enhancement of impaired steroidogenesis in tissues for obese subjects. It is fully elucidated that mitochondria generate reactive oxygen species (ROS), and that production is a direct function of rate of oxygen utilization due to an increase in that utilization during exercise [13,14,15]. ROS damages almost all cellular micromolecules; however, studies on some testicular-specific antioxidant defense parameters revealed that glutathione peroxidase 4 (GPx4) plays a role in sperm protection from oxidative stress. GPx4 knockout in rodents induced a decrease in the mass of the testes and sperm count and an increase in abnormal forms of sperm [16]. According to previous study, increase in ROS reduced male sex

steroid hormone, especially testosterone levels and disrupt the hormonal balance that regulated male reproductive functions, and thus caused infertility [17]. On the contrary, exogenously DHEA administration induced decrease in ROS [18]. In addition, procathepsin L is one of the parameters that relates to sperm modification in the process of sperm maturation via proteolysis. However, the effects of the combination of chronic DHEA administration and exercise training on reproductive function, especially in testis function for obese model rats, remain unclear.

Therefore, this study aimed to investigate the effects of combination treatment on reproductive function in the epididymis for high-sucrose obese rats through the increase in plasma and testicular sex steroid hormone concentrations. We hypothesized that the combination of DHEA administration and exercise training may increase the level of sex steroid hormone more effectively than exercise training or DHEA administration alone, and ameliorate reproductive function in the testis for obesity rats. The enhancement of reproductive function through the increase in sex steroid hormone concentrations and steroidogenesis-related enzymes was investigated by measuring procathepsin L and GPx4 levels following the administration of DHEA and/or exercise training, as well as steroidogenesis-related enzyme expressions such as 3β-hydroxysteroid dehydrogenase (HSD), 17β-HSD, and 5α-reductase, which are enzymes for converting DHEA to testosterone. Additionally, testosterone to 5α-dihydrotestosterone (DHT) was measured to assess the ability of steroidogenesis in testes.

2. Materials and Methods

2.1. Animals and animal treatment

Male Wistar rats (220-250 g, 10 weeks old) were obtained from Charles River (Kanagawa, Japan), and cared for according to the *Guiding Principles for the Care and Use of Animals* based on the *Guide for the Care and Use of Laboratory Animals* (NIH). The study design and all experimental procedures were approved by the Committee on Animal Care of the University of Tsukuba, Japan.

The rats were housed individually in an animal facility under controlled conditions (12/12-h light/dark cycle). The rats were allowed *ad libitum* access to water and placed on a purified high-sucrose diet (68% of kcal from sucrose, 20% from protein, and 12% from fat) for 14 weeks, according to a previous study [7]. After 14 weeks of the high-sucrose diet, the animals were randomly assigned to one of the following groups: sedentary rats administered sesame oil (control group, n = 7), obese rats treated with DHEA (DHEA group, n = 7), obese rats subjected to exercise training (exercise group, n = 7), and obese rats treated with DHEA and subjected to exercise training (combination group, n = 7). DHEA was obtained from Wako Pure Chemicals Industries (Osaka, Japan). All animals continued on the high-sucrose diet during the 6-week experimental period. In the DHEA and combination groups, DHEA (1 mg/kg body weight) was dissolved in sesame oil and orally administered every day for 6 weeks. Body weight and dietary intake were measured every week during the experiment. After obtaining all other measurements, plasma samples were immediately centrifuged 1500g, for 15min at 4°C. the supernatant was immediately transferred to polypropylene tubes, and testes were quickly removed, weighed, rinsed in ice-cold saline, frozen in liquid nitrogen, and stored

at -80°C until further analyses.

2.2. Exercise Protocol

The obese exercise and combination groups were trained on a rodent treadmill (KN-73, Natsume Seisakusyo, Tokyo, Japan), at 10-15 m/min for 3 days before the initiation of the experiments. Afterward, the trained rats ran on the treadmill for 1 h at 25 m/min without incline, 5 days/week for 6 weeks. The intensity, duration, and time of the exercise was kept constant during the training period, as described previously [7].

2.3. Immunoblot Analysis

Epididymis specimens were homogenized in 20 mM Tris-HCl (pH 7.8), 300 mM NaCl, 2 mM EDTA, 2 mM dithiothreitol (DTT), 2% Nonidet P-40, 0.2% sodium dodecyl sulfate (SDS), 0.2% sodium deoxycholate, 0.5 mM phenylmethylsulfonyl fluoride, 60 μg/mL aprotinin, and 1 μg/ml leupeptin. Homogenates were rotated slowly for 30 min at 4°C and centrifuged at 12,000 ×*g* for 15 min at 4°C. The protein concentration of the resulting supernatant was determined. Samples (20 μg of total protein) were denatured at 96°C for 7 min in Laemmli buffer. Western blot analysis was performed in order to detect procathepsin L, GPx4, 3β-HSD, 17β-HSD, 5α-reductase type 1 and type 2 protein expressions [4, 7, 8]. Briefly, each sample was separated on a 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore Corp., Billerica, MA, USA). The membranes were treated with the blocking buffer (5% skimmed milk in phosphate-buffered saline (PBS) with 0.1% Tween 20 (PBS-T)) for 24 h at 4°C, and incubated in the blocking buffer containing polyclonal anti-procathepsin L antibody

(1:1000; Abcam, Cambridge, UK), anti-GPx4 (R&D systems, Inc., Minneapolis MN), anti-3β-HSD, anti-17β-HSD (Santa Cruz Biotec, Inc., Dallas, TX), 5α-reductase type 1 and type 2 (Abnova, Taiwan, China) antibodies. The membranes were washed 3 times with PBS-T and incubated in blocking buffer containing horseradish peroxidase-conjugated anti-rabbit immunoglobulin secondary antibodies (1:3000; Cell Signaling, Beverly, MA, USA) for 1 h at room temperature. Afterward, the membranes were washed again 3 times with PBS-T. Finally, procathepsin L, GPx4, 3β-HSD, 17β-HSD, 5α-reductase type 1 and type 2 levels were detected using an Enhanced Chemiluminescence Plus system with C-Digit Blot Scanner (M&S TechnoSystems, Osaka, Japan). All the western blotting data were corrected by internal control (α-tublin).

2.4. Sandwich Enzyme Immunoassay

The levels of DHEA (Enzo Life Sciences Inc., Farmingdale, NY) and DHT (IBL International, Hamburg, Germany) in plasma and epididymis extracts were determined using a sandwich enzyme immunoassay kit. The immobilized polyclonal antibodies were used against DHEA and DHT, whereas the secondary horseradish-peroxidase-coupled antibodies were monoclonal. Optical density at 450 nm was determined using a microplate reader (Bio-Rad; xMark, Tokyo, Japan). All samples were assayed in duplicate.

2.5. Statistical Analysis

All values are expressed as mean ± SE. Statistical evaluation of average dietary intake and body weight were performed using repeated measures ANOVA. A post-hoc

comparison test was used to correct for multiple comparisons (Bonferroni test), when significant differences were observed. For ANOVA, $P < 0.05$ was considered significant. $P < 0.01$ was considered to be significant for post hoc tests. Other data were analyzed using one-way ANOVA. Bonferroni post-hoc comparison test was used to correct for multiple comparisons when the analyses showed significant differences between the groups. For one-way ANOVA, $P < 0.05$ was considered significant.

3. Results

3.1. Animal characteristics

Body mass before the treatment period did not significantly differ among the groups. In the DHEA administration and/or exercise training groups, body mass was significantly lower than the control group after 6 weeks of treatment ($P < 0.05$, Table 1). At the point of 6 weeks treatment, body mass was significantly lower in the combination group than in the DHEA and exercise training groups ($P < 0.05$, Table 1). DHEA administration and/or exercise training induced a significant decrease in fasting blood glucose level compared with the control group, and the combination group was significantly lower than the DHEA group (Table 1). In addition, there was no significant difference in mass of testis among groups (Table 1).

3.2. Plasma and Epididymis Sex Steroid Hormone Concentrations

Plasma DHEA and DHT concentrations were lower in the control group than in any other groups where the plasma DHEA concentration was significantly increased by DHEA administration and exercise training (Fig. 1A), with increased plasma DHT concentrations (Fig. 1B). Epididymis DHEA concentration was also lower in the control group than in any other group, and DHEA concentration was significantly increased by DHEA administration and/or exercise training ($P < 0.05$, Fig. 2A). However, epididymis DHT concentration tended to be higher in the control but not statistically significant in exercise, DHEA and combination groups (Fig. 2B).

3.3. Steroidogenesis-related enzyme protein expression

Both 3β-HSD and 17β-HSD protein expression in the epididymis did not change among groups (Fig. 3A, B). Additionally, the protein expression of 5α-reductase type 1 and 2 also did not show any significant changes among groups (Fig. 4A, B).

3.4. Epididymis function-related protein expressions

GPx4 protein expression was measured as a testicular-specific antioxidant defense parameter. It was lower in the control group, and significantly higher in the DHEA group ($P < 0.05$). GPx4 protein expression also tended to be higher in the combination group but was not statistically significant. However, no significant change was seen in the exercise group (Fig. 5A). Moreover, procathepsin L is the parameter of sperm modification in the process of sperm maturation. The protein expression level of procathepsin L was higher in the control and exercise groups. On the contrast, procathepsin L protein expression was significantly lower in DHEA and combination groups than in the control group (Fig. 5B, $P < 0.05$). In addition, a negative correlation was seen between epididymis DHEA concentration and procathepsin L protein expression level (Fig. 6A, $P < 0.001$).

4. Discussion

This study demonstrated that DHEA administration and/or exercise training can induce an increase in plasma and epididymis DHEA and plasma DHT levels compared to obese controls. Additionally, GPx4 protein expression, which is a testicular-specific antioxidant defense parameter, significantly increased with DHEA administration. However, DHEA administration, and the combination of DHEA administration and exercise, induced a

significant decrease in procathepsin L protein expression, which is the parameter of sperm modification in the process of sperm maturation. Interestingly, epididymis concentration of DHEA was negatively correlated with the level of procathepsin L protein expression. Thus, DHEA administration and/or exercise training induced an increase in epididymis DHEA concentrations and may cause the defense of epididymis-specific anti-oxidation in high sucrose-induced obese rats. However, in the present study, DHEA administration-induced increase in testicular DHEA concentration had a protective role from exercise-induced oxidant damage and contributed to the enhancement of sperm modification and maturation.

Our previous studies reported that rodents with obesity and type 2 diabetes had significantly lower sex steroid hormone concentrations and steroidogenesis-related enzyme protein expressions in skeletal muscles [7, 19]. Moreover, DHEA administration and/or exercise training caused increase in sex steroid hormone levels in both plasma and tissues. Although the mechanisms which exercise induced increase in DHEA has not been fully elucidated, previous study reported that exercise stimulated production of DHEAS from adrenal cortex through the Hypothalamus-Pituitary-Adrenal axis, and it caused increase in DHEA production [20]. However, exercise did not increase DHEA concentration in individuals with higher amount of DHEA such as younger and healthy subjects in our previous study [21]. In the present study, plasma and epididymis DHEA levels were significantly lower in the obesity control group and were increased by DHEA administration and exercise training. However, both plasma and epididymis DHT as well as 3β-HSD, 17β-HSD and 5α-reductase protein expressions were not significantly lower in the obesity control group and did not change with any interventions. These results may

indicate that epididymis tissue has 100 to 1000 times higher sex steroid hormone concentrations than other tissues and could metabolize DHEA to testosterone and DHT through 3β-HSD, 17β-HSD and 5α-reductase enzymes in obesity rats. The present study demonstrated that both plasma and testicular DHEA concentrations were significantly lower in obese rats and with increased DHEA administration. In addition, only epididymis DHEA concentration was negatively correlated to procathepsin L protein expression level in the present study. Therefore, epididymis DHEA concentration may be important for sperm maturation.

GPx4 is part of the glutathione peroxidase (GPx) family, which catalyzes the reduction of hydrogen peroxide, organic hydroperoxidases, and lipid hydroperoxidases, and thereby protects cells against oxidative damage. Previous study reported that moderate intensity aerobic exercise induced a decrease in lipid hydroperoxidases and caused an increase in GPx activity [22]. On the contrary, high-intensity exercise induced a decline in plasma GPx activity, so the change in GPx activity may partly depend on exercise intensity. In the present study, GPx4 protein expression significantly increased only by DHEA administration and did not see any changes with exercise training. Moderate intensity exercise was conducted five days/week, which is more than is recommended for patients with obesity and type 2 diabetes [23], and might result in the increase in the production of reactive oxygen species (ROS). Consequently, in the present study, exercise did not increase in GPx4 protein expression. Although DHEA has a role of protection from oxidative damage, plasma or testicular DHEA concentration did not sufficiently increase by exercise training for protection from exercise-induced increase in ROS. Thus, increased by exogenously DHEA administration might protect from testicular-specific

ROS or any oxidative stress in obese rats. Additionally, exercise training significantly increased procathepsin L, the parameter of sperm modification and maturation. Secretion of procathepsin L by Sertoli cells has led to the proposal that secretion of this proenzyme is required to maintain the normal structure and function of the seminiferous epithelium. Previously, Okamura et al reported that procathepsin L was highly synthesized in the distal caput epididymis [25] and has been found to be unusually secreted into the epididymis fluid. Moreover, procathepsin L mRNA was expressed in various regions of the epididymis and the level of procathepsin L mRNA was greatly elevated in the distal caput epididymis. Thus, procathepsin L is absent or low in the proximal caput epididymal fluid and first appears in the distal caput epididymis. These contents gradually decrease with the passage through the epididymis, so the procathepsin L that was expressed high in the distal caput epididymis was secreted into the extracellular fluid instead being targeted to the lysosome [23]. In the present study, procathepsin L protein expression was significantly lower in the DHEA administration and combination groups and a negative correlation was seen between the level of procathepsin L protein expression and epididymis DHEA level. These results show that epididymis DHEA might regulate procathepsin L transport, which is transporting downstream such as sperm or any other epididymal regions from distal caput epididymis. However, procathepsin L protein expression in sperm was not measured in the present study, and further study is needed to investigate the level of procathepsin L transport in sperm from the distal caput epididymis as well as the condition of the sperm such as modification and maturation status.

In conclusion, this study demonstrated that 6-week DHEA administration and/or exercise training induced an increase in plasma and testicular DHEA concentration, and

that an increase in DHEA concentration may have a role in testicular-specific antioxidant defense. Additionally, DHEA administration induced the enhancement of procathepsin L protein expression, and it was negatively correlated with epididymis DHEA concentration. It may cause sperm modification and maturation through translocation from distal caput epididymis to sperm. Therefore, DHEA administration could be a new therapeutic candidate for sperm maturation in obesity. However, no additive effects were seen by the combination of DHEA administration and exercise training.

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Declarations of interest

None

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References

1. G.A. Ramaraju, S. Teppala, K. Prathigudupu, M. Kalagara, S. Thota, M. Kota, R. Cheemakurthi. Association between obesity and sperm quality, Androgia 50 (2018) e12888.

2. F. Labrie, A. Belanger, J. Simard, V. Luu-The, C. Labrie. DHEA and peripheral androgen and estrogen formation: Intracrinology, Ann N Y Acad Sci 187 (1995) 16-28.

3. K. Aizawa, M. Iemitsu, S. Maeda, S. Jesmin, T. Otsuki, C.N. Mowa, T. Miyachi, N. Mesaki. Expression of steroidogenic enzymes and synthesis of sex steroid hormones from DHEA in skeletal muscle, Am J Physiol 29 (2007) E577-E584.

4. K. Sato, M. Iemitsu, K. Aizawa, R. Ajisaka. Testosterone and DHEA activate the glucose metabolism-related signaling pathway in skeletal muscle, Am J Physiol 294 (2008) E961-E968.

5. S. Takeuchi, N. Mukai, T. Tateishi, S. Miyakawa. Production of sex steroid hormones from DHEA in articular chondrocyte of rats, Am J Physiol 293 (2007) E410-E415.

6. Y. Yamaguchi, S. Tanaka, T. Yamakawa, M. Kimura, K. Ukawa, Y. Yamada, M. Ishihara, H Sekihara. Reduced serum dehydroepiandrosterone levels in diaberic patients with hyperglycemia, Clin Endocrinol 49 (1998) 377-383.

7. K. Sato, M. Iemitsu, K. Aizawa, N. Mesaki, S. Fujita. Increased muscular dehydroepiandrosterone levels are associated with improved hypeglycemia in obese rats, Am J Physiol 301 (2011) E274-E280.

8. K. Sato, M. Iemitsu, K. Aizawa, R. Ajisaka. DHEA improves impaired activation of Akt and PKC ζ/λ-GLUT4 pathway in skeletal muscle andimproves hyperglycaemia in streptozotocin-induced diabetes rats, Acta Physiol 197 (2009) 217–225.

9. T.G. Cooper, M.C. Orgebin-Crist. The effect of epididymal and testicular fluids on the fertilizing capacity of testicular and epididymal spermatozoa, Andrologia 7 (1975) 85-93.

10. T.G. Cooper, M.C. Orgebin-Crist. Effect of aging on the fertilizing capacity of testicular spermatozoa from the rabbit, Biol. Reprod 16 (1977) 258-266.

11. T.J. Vasankari, U.M. Kujala, O.J. Heinonen, I.T. Huhtaniemi. Effects of endurance training on hormonal responses to prolonged physical exercise in males, Acta Endocrinol (Copenh) 129 (1993) 109-13.

12. M.R. Safarinejad, K. Azma, A.A Kolahi. The effects of intensive, long-term treadmill running on reproductive hormones, hypothalamus-pituitary-testis axis, and semen quality: a randomized controlled study. J Endocrinol 200 (2009) 259-71.

13. K.J. Davies, A.T. Quintanilha, G.A. Brooks, L. Packer L. Free radical and tissue damage produced by exercise. BiochemBiophys Res Commun 107 (1982) 1198-1205.

14. N.B. Vollaard, J.P. Shearman, C.E. Cooper. Exercise-induced oxidative stress:myths, realities and physiological relevance. Sports Med 35 (2005) 1045-1062.

15. A. Temiz, O.K. Baskurt, C. Pekçetin, F. Kandemir, A. Güre. Leukocyte activation, oxidant stress and red blood cell properties after acute, exhausting exercise in rats. Clin Hemorheol Microcirc 22 (2000) 253-259.

16. H. Imai, N. Hakkaku, J. Suzuki, T. Suzuki, Y. Tajima, K. Konishi, S. Minami, S. Ichinose, K. Ishizaka, S. Shioda, S. Arata, M. Nishimura, S. Naito, Y. Nakagawa. Depletion of selenoprotein GPx4 in spermatocytes causes male infertility in mice. J Biol Chem 284 (2009) 32522-32532.

17. M. Darbandi, S. Darabandi, A. Agarwal, P. Sengupta, D. Durairairajanayagam, R. Henkel, M. R. Sadeghi. Reactive oxygen species and male reproductive hormones.

Reprod Biol Endocrinol 16 (2018) 87.

18. J. P. G. Camporez, E.H. Akamine, A. P. Davel, C. R. Franci. L. V. Rossoni, C. R. de Oliveira Carvalho. Dehydroepiandrosterone protects against oxdative stress-induced endothelial dysfunction in ovariectomized rats. J Physiol 589 (2011) 2585-2596.

19. K. Sato, S. Fujita, M. Iemitsu. Dioscorea esculenta-induced increase in muscle sex steroid hormones is associated with enhanced insulin sensitivity in a type 2 diabetes rat model. FASEB J 31 (2017) 793-801.

20. R.H. Dressendorfer, C.E. Wade. Effects of 15-d race on plasma steroid levels and leg muscle fitness in runners. Med Scie Sports Exerc 23 (1991) 954-958.

21. K. Sato, M. Iemitsu, K. Matsutani, T. Kurihara, T. Hamaoka, S. Fujita. Resistance training restores muscle sex steroid hormone steroidogenesis in older men. FASEB J 28 (2014) 1891-1897.

22. C.P. Avula, G. Fernandes. Modulation of antioxidant enzymes and apoptosis in mice by dietary lipids and treadmill exercise. J Clin Immunol 19 (1999) 35-44.

23. S. Balducci, M. Sacchetti, J. Haxhi, G. Orlando, V. D'Errico, S. Fallucca, S. Menini, G. Pugliese. Physical activity as therapy for type 2 diabetes mellitus. Diabetes Metab Res Rev 1 (2014) 13-23.

24. I. Túnez, M.C. Muñoz, P. Montilla. Treatment with dehydroepiandrosterone prevents oxidative stress induced by 3-nitropropionic acid in synaptosomes. Pharmacology 74 (2005) 13-118.

25. N. Okamura, M. Tamba, Y. Uchiyama, Y. Sugita, F. Dacheux, P. Syntin, J.L. Dacheux. Direct evidence for the elevated synthesis and secretion of procathepsin L in the distal caput epididymis of boar. Biochim Biophys Acta 1245 (1995) 221-226.

Table 1: Animal charcteristics

	CON (n =7)	EX (n =7)	DHEA (n =7)	DHEA+EX (n =7)
Body weight (g)	710.4±10.7	610.1±12.6*	620.4±15.3*	569.3±11.2*†#
Fasting glucose (mmol/L)	8.2±2.4	5.4±2.5*	5.6±3.1*	4.6±2.1*
Testis (g/body mass)	0.9±0.5	1.1±0.8	1.1±0.3	1.1±0.4
Abodominal fat (g/body mass)	3.7±0.7	3.1±0.3	2.6±0.7*#	2.2±0.6*#
Soleus CS activity (µmol/g /min)	10.1±2.2	20.1±3.3*†	12.9±3.3	21.5±3.5*†

CON: Sedentary control group, EX: exercise training group, DHEA: *DHEA* administration group, DHEA +EX: *DHEA* administration and Exercise training group. CS: citrate synthase

* P<0.05, vs CON group

† P<0.05, vs DHEA group

♯ P<0.05, vs EX group

Figure legends

Figure 1: **Effect of DHEA administration and/or exercise training on plasma DHEA (A) and 5α-dihydrotestosterone (DHT) concentrations (B).** Data are means ± SE. * $P < 0.05$ compared with the control group.

Figure 2: **Effect of DHEA administration and/or exercise training on epididymis DHEA (A) and 5α-dihydrotestosterone (DHT) concentrations (B).** Data are means ± SE. * $P < 0.05$ compared with the control group.

Figure 3: **Effect of DHEA administration and/or exercise training on 3β-HSD and 17β-HSD protein expressions.** A: representative immunoblotting images for 3β-HSD protein expression in *top*. *Bottom*: statistical analyses of 3β-HSD protein expression level assessed by densitometry. B: representative immunoblotting images for 17β-HSD protein expression in *top*. *Bottom*: statistical analyses of 17β-HSD protein expression level assessed by densitometry.

Figure 5: **Effect of DHEA administration and/or exercise training on 5α-reductase type 1 and 2 protein expressions.** A: representative immunoblotting images for 5α-reductase type 1 protein expression in *top*. *Bottom*: statistical analyses of 5α-reductase type 1 protein expression level assessed by densitometry. B: A: representative immunoblotting images for 5α-reductase type 2 protein expression in *top*. *Bottom*: statistical analyses of 5α-reductase type 2 protein expression level assessed by densitometry.

Figure 6: **Effect of DHEA administration and/or exercise training on reproductive function-related protein.** A: representative immunoblotting images for procathepsin L protein expression in *top*. *Bottom*: statistical analyses of procathepsin L protein expression level assessed by densitometry. B: representative immunoblotting images for GPx4 protein expression in *top*. *Bottom*: statistical analyses of GPx4 protein expression level assessed by densitometry.

Figure5: **Correlations between epididymis DHEA, DHT concentrations and procathepsin L (A) and GPx4 (B) protein expression levels.**