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ORIGINAL ARTICLE

Male Endocrinology

The effect of flutamide on the physical working capacity and activity of some of the key enzymes for the energy supply in adult rats

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The aim of the study was to assess the effects of androgen receptor antagonists on the physical working capacity and activity of some of the key muscle enzymes for the energy supply in rats. Young adult male Wistar rats were divided into two groups. One group received 15 mg kg^{-1} of flutamide daily for 6 days a week and the other group served as control for 8 weeks. At the beginning and at the end of the experiment, all rats were subjected to submaximal running endurance (SRE), maximum time to exhaustion (MTE), and maximal sprinting speed (MSS) tests. At the end of the trial, maximum oxygen consumption (VO_{2max}) test was performed and the levels of testosterone, erythrocytes, hemoglobin as well as enzyme activity of succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), and NAD.H2-cytochrome-c reductase (NAD.H2) of the gastrocnemius muscle were measured. Serum testosterone of the flutamide-treated rats was higher than that of the controls, which verifies the effectiveness of the dose chosen. MTE and SRE of the anti-androgen-treated group were lower compared with the initial values. Flutamide treatment decreased the activity of SDH and NAD.H2 compared with the controls. We found no effect of the anti-androgen treatment on MSS, VO_{2max}, running economy, LDH activity, and hematological variables. Our findings indicate that the maintenance of the submaximal and maximal running endurance as well as the activity of some of the key enzymes associated with muscle oxidative capacity is connected with androgen effects mediated by androgen receptors.

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INTRODUCTION

Androgens play a pivotal role in male reproductive and sexual function and are responsible for anabolic effects observed during male adolescence and adulthood. Testosterone, the primary male sex hormone, exerts a widespread pattern of effects on metabolism, body composition, and physical performance.[1,2] Anabolic androgenic steroids, which are synthetic derivatives of the testosterone, are used in various sports for their ergogenic effects of increased muscle mass and enhanced athletic performance.[3] On the other hand, hypogonadal men lacking sufficient testosterone levels have particular physical and metabolic traits seen as alterations in lipid and glucose metabolism, which influence further fat depots, muscle mass and, ultimately, physical performance. The testosterone deficiency in man is associated with higher BMI and increased visceral fat mass, decreased muscle protein synthesis, higher fasting glucose and leptin levels, decreased insulin sensitivity, and increased incidence of metabolic syndrome and obesity,[4,5] while the androgen substitution has beneficial effects on these metabolic changes, muscle mass, and strength.[6,7] A positive correlation between testosterone levels and mitochondrial capacity assessed by measuring maximal aerobic capacity and expression of oxidative phosphorylation genes has been reported,[8] suggesting the important role of androgens for maintaining aerobic performance. It is well known that androgens enhance erythropoiesis and blood volume,[6] and physical abilities in androgen-deficient men could be further attenuated by lower oxygen supply due to decreased hemoglobin concentrations.

The mechanisms by which testosterone acts on pathways to control metabolism and physical working capacity are not fully elucidated.[2] Testosterone can act directly on target cells, or it can be converted to dihydrotestosterone by the enzyme 5α-reductase, or to estradiol by the enzyme aromatase in the peripheral tissues.[9] Testosterone, dihydrotestosterone, and other androgens exert most of their effects by binding to specific intracellular androgen receptors (ARs), which act as transcription factors that mediate the biological responses,[10] although rapid, nongenomic action of androgens have also been reported.[11]

Blockers of androgen receptors are used in medical practice as anti-androgens for the treatment of prostatic carcinoma. They belong to the group of nonsteroidal AR antagonists such as flutamide, bicalutamide, and nilutamide, and due to the complete blockage

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of AR in both peripheral and CNS tissues, they often trigger significant increases in luteinizing hormone release, which further stimulates higher serum testosterone concentrations. Therefore, in clinical practice, they are used primarily in combination with a gonadotropin-releasing hormone analog which shuts down testicular testosterone production.[12] Decreased physical working capacity of patients is observed after long-term androgen deprivation therapy,[13] but it is not clear what is the exact role of the disease itself, the age of the patient, and/or the therapy for the observed exercise intolerance.[14] Studying the changes that the indices of physical performance undergo during AR blocking of intact experimental animals will provide additional information about the underlying mechanisms related to these androgen effects. Therefore, the present study was undertaken to assess the effects of androgen receptor antagonists' treatment on the indices of physical working capacity and the activity of some of the key enzymes for energy supply in the gastrocnemius muscle of male rats.

MATERIALS AND METHODS

Test animals

Young adult male Wistar rats, weighing 200 ± 20 g at the beginning of the experiment (60–80 days of age), were housed in individual metabolic cages, fed standard rat chow and water *ad libitum*, and kept at a temperature of 21–24°C with a 12/12 h dark-light cycle. Body mass was measured weekly. The experimental protocol was approved by the Ethical Committee on Human and Animal Experimentation of the Medical University, Plovdiv, and the Commission for Ethical Treatment of Animals at the Bulgarian Food Safety Agency. The rats were reared and all experimental procedures were performed according to the recommendations of the European Commission for the protection and humane treatment of laboratory animals.

Training program and anti-androgen treatment

As running on a treadmill is a skill activity which the rats should develop and maintain to conduct the functional tests, prior to the experiment, all of them were trained 3 times a week on a treadmill (Columbus Instruments, Columbus, OH, USA) for 5 min daily, with a velocity of 27 m min^{-1}, at a track elevation of 5° for 2 weeks. Such a workload induces no training adaptations, but familiarizes the animals with treadmill running and allows a selection of rats which run spontaneously.[15]

Compliant animals were randomly divided into two groups (n = 8). One group received 15 mg kg^{-1} of the AR blocker flutamide (Sigma-Aldrich, Munich, Germany) dissolved in sterile sesame oil s.c. and the other group (controls) was given the sesame oil as vehicle once a day, 6 days a week, for 8 weeks. Both groups were exercised on a treadmill for 5 min 3 days a week with the same intensity as during the preliminary period to ensure familiarization with treadmill running. The chosen dose represents the lowest one being able to completely block the effect of testosterone replacement on the accessory sexual organ weights in adult castrated male rats according to previously reported data.[16,17] The mode of application was selected according to the previously reported chronic exposure to flutamide in adult rodents.[18]

Functional tests

At the beginning and at the end of the experiment, all rats were subjected to submaximal running endurance (SRE) test, maximum time to exhaustion (MTE) test, and maximal sprinting speed (MSS) test. At the end of the trial, both groups were subjected to a maximum oxygen consumption (VO_{2max}) test.

Submaximal running endurance (SRE) test

SRE of rats was determined by having them run at 27 m min^{-1} and 5° elevation (which is about 70%–75% VO_{2max}) until they could no longer maintain their position on the treadmill belt. The time taken to reach this stage was assessed as SRE.[15]

Maximum time to exhaustion (MTE) test

The protocol of the maximal performance test involved stepwise increase of the treadmill speed and elevation and each step was 3 min long. Rats were removed from the test when they could no longer maintain their position on the treadmill belt. The time taken to reach this stage was assessed as MTE.[15]

Maximal sprinting speed (MSS) test

The sprinting performance was determined by having them run at 27 m min^{-1} and 5° elevation for 3 min. The speed was then increased to 45 m min^{-1} for 30 s and again by 10 m min^{-1} every 30 s until the rat was unable to maintain the pace of the treadmill belt. The highest speed which the rat could maintain for 15 s was defined as MSS.[19]

Maximum oxygen consumption (VO_{2max}) test

The test was performed using the Oxymax gas analyzing system for small animals (Columbus Instruments, Columbus, OH, USA). The test protocol involved a stepwise increase of treadmill speed and elevation as follows: (I) 15 m min^{-1}, 5° elevation; (II) 19 m min^{-1}, 10°; (III) 27 m min^{-1}, 10°; (IV) 27 m min^{-1}, 15°; (V) 30 m min^{-1}, 15°. Each step of the exercise was 3 min long and oxygen consumption (VO_2) was measured every minute. Each rat was placed in the chamber 10 min before exercising and the lowest value during the last 4 min was taken as rest consumption. The highest estimated VO_2 at each workload was taken as a measure of each rat's running economy ($VO_{2submax}$) for the workload and, at the last step, as VO_{2max}. The test was discontinued either when the rats could no longer maintain their position on the treadmill belt or at the occurrence of the plateau phenomenon.[15,20]

Blood and serum testosterone analysis

Two days after the last exercise bout, all rats were sacrificed under narcosis with a dose of 30 mg kg^{-1} Thiopental i.p. Mixed blood was collected in tubes for investigation of the erythrocyte count, hemoglobin concentration, and total testosterone levels. The red blood cell parameters were determined by a hematological analyzer Coulter T660 (Coulter Electronics, Inc., Hialeah, Finland). Blood serum was separated, transferred to cones, and stored at −20°C. Testosterone levels of each animal were measured by the ELISA method using a Testosterone kit (BTAR-E-8000, Biotrend Chemikalien, GmbH, Cologne, Germany), with intra- and inter-assay coefficients of variation of 6.5%–11.07% and 9.3%–11.3%, respectively. Double tests were run using a SIRIO micro plate reader (SEAC srl, Calenzano, Italy) and the mean value of both tests was accepted as representative for each animal. According to the manufacturer of the kit, the normal values of the serum testosterone concentrations for male rats are between 0.66 and 5.4 ng ml^{-1}.

Enzyme histochemistry

The right gastrocnemius muscle from six rats in each group was dissected and stored in liquid nitrogen. Enzyme histochemical reactions were applied on cryostat sections (10 μm) of medial head of the gastrocnemius muscle (51% fiber type I and 49% fiber type II)[21] for the following key enzymes:[22] for the Krebs cycle – succinate dehydrogenase (SDH), for respiratory chains – NAD.H2-cytochrome-c reductase (NAD.H2), and for glycolysis – lactate dehydrogenase (LDH). The SDH activity was determined in an incubation medium

containing 1 mol l^{-1} sodium succinate and 1.5 mmol l^{-1} nitroblue tetrazolium, adjusted to pH 7.2 for 30 min. Then, it was transferred into 4% formaldehyde for 10 min and washed in distilled water.[23] The NAD.H2 activity was determined in an incubation medium containing nicotinamide adenine dinucleotide-reduced disodium salt (DPNH2-Na2). Sections were incubated at 37°C for 20 min. Then, they were transferred into 4% formaldehyde for 10 min and washed in distilled water.[24] The LDH activity was determined in appropriate incubating solution (1 mol l^{-1} lactate) at 37°C for 20 min. Then, they were transferred into 4% formaldehyde for 10 min and washed in distilled water.[24] All sections were counterstained with Schiff reagent according to Feulgen, washed in distilled water, and cover-slipped with Canada balsam. Visualization was accomplished with a colored insoluble reaction product (formazan deposits). The enzyme activity was assessed in cross sections at equal magnification (×200), and the mean color saturation of the reaction dye product (arbitrary units, AU) in 50 myofibers of random microscopic fields of each muscle was recorded. The analysis was performed using a Microphot microscope (Nikon, Japan) and special image-analysis software DP-Soft 3.2 (Olympus, Japan). The mean value of each field was taken for further calculations.

Statistical analysis

The results were analyzed using parametric tests because of normally distributed data, as assessed by the Kolmogorov–Smirnov test. The intergroup differences were assessed by unpaired Student's *t*-test and intragroup differences were assessed by paired Student's *t*-test, at a level of significance of $P < 0.05$ (SPSS 13.0; SPSS, Chicago, IL, USA). The mean results are presented with ± standard deviation.

RESULTS

Body mass

At baseline, the rats of both groups had similar body mass. The body weight of all rats gradually increased and the body mass of the anti-androgen treated group was not different from that of the controls throughout the experiment. At the end of the trial, the analysis failed to find any difference in their body mass (controls: 317.38 ± 33.84 g; flutamide-treated: 310.50 ± 30.53 g; $P > 0.05$).

Submaximal and maximal running performance and maximal sprinting speed

At baseline, the rats in the two groups had similar SRE (**Figure 1a**). At the end of the trial, submaximal performance of the flutamide-treated rats was lower compared with the initial values ($P < 0.01$) and tended to decrease compared with the controls ($P = 0.09$). We found no differences in MTE in the incremental maximal treadmill test between the groups at the beginning and at the end of the trial (**Figure 1b**). The maximal running performance of the anti-androgen treated group was lower compared with the initial values ($P < 0.01$), whereas no differences were found between initial and final levels of MTE of the controls ($P > 0.05$). There were no differences in MSS between the control and the flutamide-treated group at the beginning (66.25 ± 6.41 m min^{-1} *vs* 61.25 ± 5.18 m min^{-1}, $P > 0.05$) and at the end of the experiment (61.25 ± 7.44 m min^{-1} *vs* 63.75 ± 8.35 m min^{-1}, $P > 0.05$) as well as between initial and final levels of MSS in both groups ($P > 0.05$).

Maximum oxygen consumption and running economy

We found no difference between the groups in the VO_{2max} measured at the end of the experiment ($P > 0.05$) (**Figure 2**). The $VO_{2submax}$ measured during the first four steps of the test showed no differences between the control and flutamide-treated rats ($P > 0.05$). The $VO_{2submax}$ of the fifth step was not analyzed because of the small and unequal number of measured values in the groups. There was no effect of flutamide treatment on the running economy when it was analyzed as $\%VO_{2max}$ during the different steps of exercise intensity in comparison with control rats ($P > 0.05$).

The analysis of enzymohistochemical reactions (**Figure 3**) showed that the anti-androgen treated rats had lower activity of SDH and NAD.H2 in the medial gastrocnemius muscle compared with the controls (3.43 ± 0.53 AU *vs* 5.63 ± 0.47 AU, $P < 0.001$; 15.22 ± 1.66 AU *vs* 30.20 ± 5.40 AU, $P < 0.01$, respectively). We found no differences in LDH activity between the groups (9.69 ± 2.29 AU *vs* 10.87 ± 3.23 AU, $P > 0.05$).

Serum testosterone levels of the control group were normal for male rats. After 8 weeks of AR blocker treatment, the total testosterone was higher than those of the controls (9.13 ± 4.99 ng ml^{-1} *vs* 2.49 ± 0.76 ng ml^{-1}, $P < 0.01$). The testosterone levels of the anti-androgen treated group were higher than the normal values for male rats.

No differences in the studied blood hematological variables were found between the control and the flutamide group: red blood cell count (7.31 ± 0.61 × 10^{12} l^{-1} *vs* 7.73 ± 0.32 × 10^{12} l^{-1}, $P > 0.05$) and hemoglobin concentrations (148.00 ± 10.78 g l^{-1} *vs* 151.25 ± 5.23 g l^{-1}, $P > 0.05$).

DISCUSSION

To our knowledge, this is the first study which demonstrates that the blocking of AR leads to a decrease of the maximal and submaximal running endurance in intact male rats in comparison with the pretrial values. The flutamide treatment in our experiment has different effects on the indices of physical working capacity, which can be accounted by the fact that they are dependent on different mechanisms. For example, sprint training in rats increases their maximal sprinting speed and MTE, whereas it has no effect on SRE and muscle oxidative capacity.[25] On the other hand, differences in the degree and rate of increase of maximum time to exhaustion and submaximal running endurance in rats have been reported in submaximal treadmill training.[26] While we found no effect of the AR blocker on the maximal sprinting speed, which is dependent on the maximal anaerobic power and some neuromuscular and mechanical factors,[27] the treatment with flutamide decreased the maximum time to exhaustion, which is a good predictor of the aerobic–anaerobic working capacity of the rats. It appears that the AR blocking had a more profound negative effect on the aerobic performance, in that it decreased submaximal running endurance of flutamide rats not only in comparison with the initial values, but also tended to reduce submaximal performance compared with the controls. Our results suggest that AR-mediated effects of the androgens are more important for maintaining the parameters determining aerobic performance than those affecting the achievements in anaerobic-dependent physical activities by untrained individuals.

Aerobic performance is heavily dependent on aerobic re-synthesis of ATP and requires an adequate delivery of oxygen from the atmosphere to the mitochondrial electron transport chain and the fuel supply in the form of carbohydrates and lipids within the contracting muscle cells.[28] Therefore, VO_{2max}, exercise (running) economy, and muscle oxidative capacity are considered the key parameters determining submaximal running endurance.[28,29] Higher running economy (lower VO_2 at a specific intensity of submaximal exercise) is considered an advantage in endurance exercises as it leads to utilization of lower percentage of VO_{2max} and is a better predictor of performance

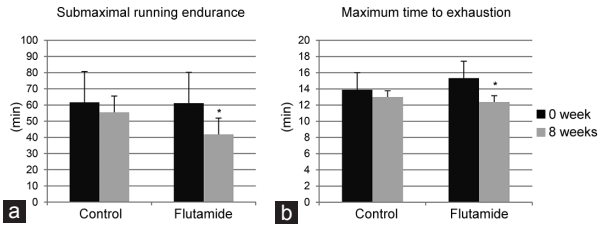

Figure 1: Submaximal running endurance (**a**) and maximum time to exhaustion (**b**) of the experimental groups at the beginning and at the end of the experiment. *$P < 0.01$ in comparison with the initial values of the flutamide-treated group.

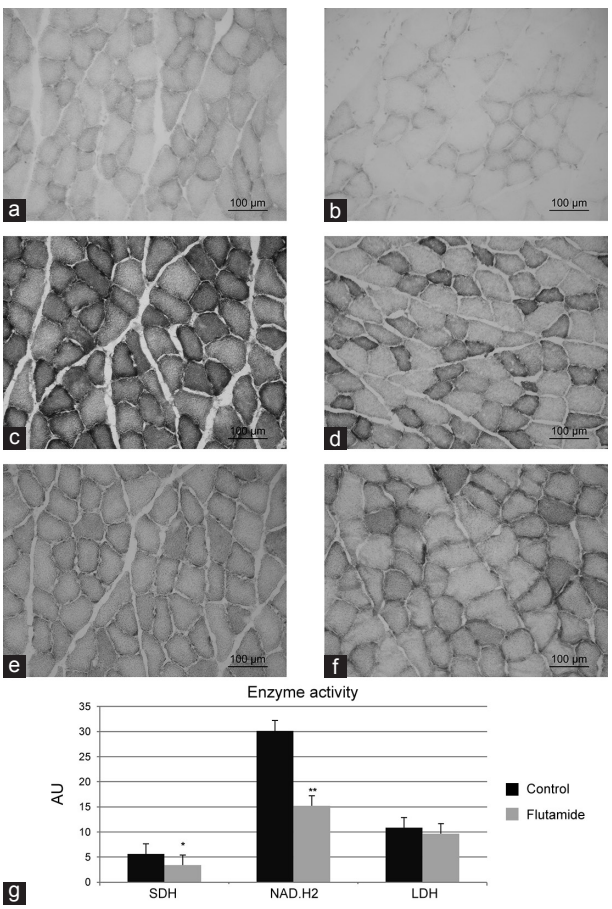

Figure 3: Microphotographs of the enzymohistochemical reactions for SDH (**a** and **b**), NAD.H2-cytochrome-c reductase (**c** and **d**) and LDH (**e** and **f**) in medial gastrocnemius of the controls (**a**, **c**, **e**) and flutamide-treated rats (**b**, **d**, and **f**). Enzyme activity of SDH, NAD.H2-cytochrome-c reductase, and LDH (AU) in medial gastrocnemius of the experimental groups at the end of the experiment (**g**). *$P < 0.01$, **$P < 0.001$ in comparison with the control group.

than VO_{2max}.[29] Our results indicate that flutamide treatment does not change aerobic power and running economy, and the negative effect of the anti-androgen on the SRE is not related to changes in variables determining oxygen transport to the working muscles. Therefore, our findings suggest that the decline of SRE is due to the induced peripheral alterations in the skeletal muscles. During submaximal prolonged exercises, the primary sites of fatigue appear to be within the muscle cell itself and are closely related to muscle glycogen depletion and lactic acid formation. A rise of mitochondrial enzyme levels and muscle oxidative capacity leads to an increased rate of utilization of fatty acids as energy

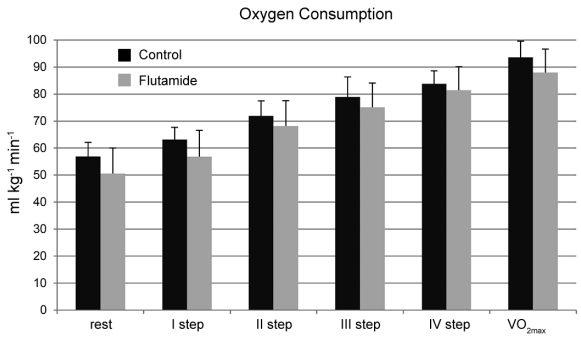

Figure 2: Oxygen consumption (ml kg^{-1} min^{-1}) at rest, at different steps of exercise intensity and VO_{2max} of the experimental groups at the end of the experiment. Each step lasted 3 min: I step (15 m min^{-1}, 5°); II step (19 m min^{-1}, 10°); III step (27 m min^{-1}, 10°); IV step (27 m min^{-1}, 15°).

and consequently has a glycogen-sparing effect, decreases lactate production, delays fatigue, and improves endurance performance.[29,30] We found no effect of flutamide on LDH activity, which indicates that AR is not involved in maintaining the muscle glycolytic capacity and correspond to the lack of effect of the anti-androgen on MSS. The decreased activity of SDH and NAD.H2 (the key enzymes for the Krebs cycle and respiratory chains respectively) in the mixed gastrocnemius muscle after anti-androgen treatment demonstrates that AR-mediated effects of the androgens are important for maintaining the normal muscle oxidative capacity of untrained subjects and suggests that the suppressed activity of these oxidative enzymes is one of the mechanisms by which AR blocking decreases SRE of flutamide-treated rats. The decreased submaximal performance and muscle oxidative capacity found in our experiment are in congruence with the established important role of testosterone in maintaining the normal level of activity of cytochrome-c oxidase in skeletal muscles of male mice[31] and mitochondrial oxidative phosphorylation gene expression in skeletal muscles in men.[8]

The results obtained show that flutamide treatment for 8 weeks has no effect on the oxygen carrying capacity of the rats as assessed by the red blood cell count and hemoglobin concentration. As oxygen carrying capacity is one of the factors determining VO_{2max},[29] these data are consistent with the absence of differences in the values of VO_{2max} between the groups at the end of experiment. Our findings indicate that the negative effect of AR blocking on SRE cannot be explained by changes in the parameters of the red blood cells. Our findings agree with data from studies on nonmetastatic prostate cancer patients, which report that at least 4–6 months of androgen deprivation therapy is required to suppress hemoglobin concentration[13] and that a statistically significant decline of hemoglobin is observed after a 2-year androgen suppression.[32]

In vivo studies in intact male rats showed that flutamide produces dose-related, marked increases in the serum luteinizing hormone and testosterone as a result of the central inhibition of the negative feedback effects of androgens on the hypothalamic-pituitary-testes axis.[33] Thus, the increased serum testosterone concentrations of flutamide-treated rats found in our experiment verify the effectiveness of the chosen dose. We may conclude that the role of androgens for maintaining the normal levels of MTE and SRE, and the activity of key enzymes involved in the aerobic energy supply in the skeletal muscles is mediated by AR and not by nongenomic actions of male sex hormones. These changes following anti-androgen treatment could be used to explain the observed decreased physical working capacity of the patients with nonmetastatic prostate carcinoma.

CONCLUSIONS

Our findings show that maintenance of some of the indices of the physical working capacity is connected with androgen effects mediated by androgen receptors. Blocking of androgen receptors leads to a decrease of submaximal running endurance, maximum time to exhaustion, and muscle oxidative capacity in intact male rats. Future studies are necessary to assess whether the effects of the anti-androgen treatment can be modulated by endurance training.

AUTHORS CONTRIBUTION

KNG conceived the study, arranged its design and coordination, helped to carry out the functional tests, performed the statistical analysis, and drafted the manuscript. PAA and MSSD carried out the functional tests. FDG and SDD carried out the enzymohistochemistry and software assessment of the saturation and SDD helped to draft the manuscript. DDT carried out the ELISA and hematological analysis. VVV helped in drafting the manuscript and offered a comprehensive assessment of the work. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests of any kind.

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REFERENCES

1 Zitzmann M. Effects of testosterone replacement and its pharmacogenetics on physical performance and metabolism. *Asian J Androl* 2008; 10: 364–72.
2 Kelly D, Jones T. Testosterone: a metabolic hormone in health and disease. *J Endocrinol* 2013; 217: R25–45.
3 Bahrke M, Yesalis C. Abuse of anabolic androgenic steroids and related substances in sport and exercise. *Curr Opin Pharmacol* 2004; 4: 614–20.
4 Basaria S, Muller DC, Carducci MA, Egan J, Dobs AS. Hyperglycemia and insulin resistance in men with prostate carcinoma who receive androgen-deprivation therapy. *Cancer* 2006; 106: 581–8.
5 Wang C, Jackson G, Jones TH, Matsumoto AM, Nehra A, *et al*. Low testosterone associated with obesity and the metabolic syndrome contributes to sexual dysfunction and cardiovascular disease risk in men with type 2 diabetes. *Diabetes Care* 2011; 34: 1669–75.
6 Wang C, Swedloff RS, Iranmanesh A, Dobs A, Snyder PJ, *et al*. Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. Testosterone Gel Study Group. *J Clin Endocrinol Metab* 2000; 85: 2839–53.
7 Brodsky IG, Balagopal P, Nair KS. Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men – A clinical research center study. *J Clin Endocrinol Metab* 1996; 81: 3469–75.
8 Pitteloud N, Mootha V, Dwyer A, Hardin M, Lee H, *et al*. Relationship between testosterone levels, insulin sensitivity, and mitochondrial function in men. *Diabetes Care* 2005; 28: 1636–42.
9 Wu F. Endocrine aspects of anabolic steroids. *Clin Chem* 1997; 43: 1289–92.
10 Haendler B, Cleve A. Recent developments in antiandrogens and selective androgen receptor modulators. *Mol Cell Endocrinol* 2012; 352: 79–91.
11 Foradori C, Weiser M, Handa R. Non-genomic actions of androgens. *Front Neuroendocrinol* 2008; 29: 169–81.
12 Gao W, Kim J, Dalton J. Pharmacokinetics and pharmacodynamics of nonsteroidal androgen receptor ligands. *Pharm Res* 2006; 23: 1641–58.
13 Alibhai SM, Breunis H, Timilshina N, Naglie G, Tannock I, *et al*. Long-term impact of androgen-deprivation therapy on physical function and quality of life. *Cancer* 2015; 121: 2350–7.
14 Jones L, Eves N, Haykowsky M, Freedland S, Mackey J. Exercise intolerance in cancer and the role of exercise therapy to reverse dysfunction. *Lancet Oncol* 2009; 10: 598–605.
15 Georgieva K, Boyadjiev N. Effects of nandrolone decanoate on $VO_{2max,}$ running economy and endurance in rats. *Med Sci Sports Exerc* 2004; 36: 1336–41.
16 Burton S, Trachtenberg J. Effectiveness of antiandrogens in the rat. *J Urol* 1986; 136: 932–5.
17 Roselli C, Resko J. Androgens regulate brain aromatase activity in adult male rats through a receptor mechanism. *Endocrinology* 1984; 144: 2183–9.
18 Penatti CA, Costine B, Porter D, Henderson L. Effects of chronic steroid cocktail on α_5-receptor-mediated gabaergic transmission and neural signaling in the forebrain of female mice. *Neuroscience* 2009; 161: 526–37.
19 Lambert M. The Effects of Training Overload on Treadmill Trained Rats. Doctoral Thesis. Cape Town, South Africa: University of Cape Town Medical School; 1990.
20 Bedford TG, Tipton CM, Wilson NC, Oppliger RA, Gisolfi CV. Maximum oxygen consumption of rats and its changes with various experimental procedures. *J Appl Physiol* 1979; 47: 1278–83.
21 Delp M, Duan C. Composition and size of type I, IIA, IID/X, and IIB fibers and citrate synthase activity of rat muscle. *J Appl Physiol* 1996; 80: 261–70.
22 Lojda Z, Gossrau R, Schiebler TH. Enzyme Histochemistry. A Laboratory Manual. Berlin: Springer-Verlag; 1979. p. 211–32.
23 Nachlas M, Tsou K, de Souza E, Cheng CS, Selligman A. Cytochemical demonstration of succinic dehydrogenase by the use of a new p-Nitrophenyl substituted diterazole. *J Histochem Cytochem* 1957; 5: 420–36.
24 Hess R, Scarpelli D, Pearse A. The cytochemical localizations of oxidative enzymes. II pyridine nucleotide linked dehydrogenases. *J Biophys Biochem Cytol* 1958; 4: 753–60.
25 Davies KL, Packer L, Brooks G. Exercise bioenergetics following sprint training. *Arch Biochem Biophys* 1982; 215: 260–5.
26 Lambert M, Noakes T. Dissociation of changes in VO2max, muscle QO2, and performance with training in rats. *J Appl Physiol* 1989; 66: 1620–5.
27 Psotta R, Bunc V. Reliability and validity of the intermittent anaerobic running test (IAnRT). In: Reilly T, Cabri J, Araújo D, editors. Science and Football V: The Proceedings of the Fifth World Congress on Sports Science and Football. London, New York: Routledge; 2005. p. 115–25.
28 Jones AM, Carter H. The effect of endurance training on parameters of aerobic fitness. *Sports Med* 2000; 29: 373–86.
29 Bassett D, Howley E. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc* 2000; 32: 70–84.
30 Holloszy JO, Kohrt WM. Regulation of carbohydrate and fat metabolism during and after exercise. *Annu Rev Nutr* 1996; 16: 121–38.
31 Koenig H, Goldstone A, Lu C. Androgens regulate mitochondrial cytochrome c oxidase and lysosomal hydrolases in mouse skeletal muscle. *Biochem J* 1980; 192: 349–53.
32 Choo R, Chander S, Danjoux C, Morton G, Pearce A, *et al*. How are hemoglobin levels affected by androgen deprivation in non-metastatic prostate cancer patients? *Can J Urol* 2005; 12: 2547–52.
33 Furr B, Tucker H. The preclinical development of bicalutamide: pharmacodynamics and mechanism of action. *Urology* 1996; 47: 13–25.