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ORIGINAL ARTICLE

Protective effect of D-ribose against inhibition of rats testes function at excessive exercise

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An increasing number of research studies point to participation in endurance exercise training as having significant detrimental effects upon reproductive hormonal profiles in men. The means used for prevention and correction of fatigue are ineffective for sexual function recovery and have contraindications and numerous side effects. The search for substances effectively restoring body functions after overtraining and at the same time sparing the reproductive function, which have no contraindications precluding their long and frequent use, is an important trend of studies. One of the candidate substances is ribose used for correction of fatigue in athletes engaged in some sports.

We studied the role of ribose deficit in metabolism of the testes under conditions of excessive exercise and the potentialities of ribose use for restoration of the endocrine function of these organs.

45 male Wistar rats weighing 240±20 g were used in this study. Animals were divided into 3 groups (n=15): control; excessive exercise; excessive exercise and received ribose treatment. Plasma concentrations of lactic, β-hydroxybutyric, uric acids, luteinizing hormone, total and free testosterone were measured by biochemical and ELISA methods. The superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase activities and uric acids, malondialdehyde, glutathione, ascorbic acids, testosterone levels were estimated in the testes sample.

Acute disorders of purine metabolism develop in rat testes under conditions of excessive exercise. These disorders are characterized by enhanced catabolism and reduced reutilization of purine mononucleotides and activation of oxidative stress against the background of reduced activities of the pentose phosphate pathway and antioxidant system. Administration of D-ribose to rats subjected to excessive exercise improves purine reutilization, stimulates the pentose phosphate pathway work, inhibits oxidative stress of the testes, and saves the testicular endocrine function.

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The problem of male reproductive health has focused the attention of many scientists in recent years. Reduction of the mean concentrations and total content of spermatozoa in European men

amounts to 2% annually (Carvalho *et al.*, 2011); the incidence of endocrine diseases is increasing (Hackney, 2008). The prevalence of infertility has increased over the past 10 years, with approximately 10 million affected couples in the United States. Roughly 40% to 50% of infertility is either due to, or is contributed by, a male factor (Brant *et al.*, 2010). One of the factors which have a negative effect on the male reproductive function is fatigue caused by excessive exercise (Aitken and Roman, 2008; Eliakim and Nemet, 2006; Eliakim *et al.*, 2009; Hackney *et al.*, 2008; Liao *et al.*, 2010; Manna *et al.*, 2003).

The means used for prevention and correction of fatigue are ineffective for sexual function recovery and have contraindications and numerous side effects (Brant *et al.*, 2010). The search for substances effectively restoring body functions after overtraining and at the same time sparing the reproductive function, which have no contraindications precluding their long and frequent use, is an important trend of studies. One of the candidate substances is ribose used for correction of fatigue in athletes engaged in some sports (Berardi and Ziegenfuss, 2003; Kerksick *et al.*, 2005; Seifert *et al.*, 2009). Ribose is the rate-limiting compound regulating the activity of the purine nucleotide pathway of adenine nucleotide metabolism. Ribose plays a central role in the synthesis of ATP, coenzyme-A, flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NAD), vitamin B_2, DNA, RNA, and other important cellular constituents. Ribose is the only compound the body can use to perform this vital metabolic function (Zimmer, 1998). We suggested that this monosaccharide also possesses a gonadoprotective effect.

We studied the role of ribose deficit in metabolism of the testes under conditions of excessive exercise and the potentialities of ribose use for restoration of the endocrine function of these organs.

MATERIAL AND METHODS

The study was carried out on 45 male Wistar rats (240±20 g). The effects of exercise on rats were studied by the method of forced swimming with a load. Three groups were formed, 15 animals per group. Group 1 rats were controls swimming without the load during the mean period of 3-5 min every other day throughout the 5-week experiment. Group 2 rats were subjected to excessive exercise (EE): 10% body weight-loaded forced swimming until complete exhausting every other day during the first 3 weeks and then daily during the next 2 weeks. Group 3 rats were subjected to EE and received ribose treatment. D-ribose (SciFit) was dissolved in water and orally administered to rats before and after swimming, the single carbohydrate dose constituting 50 mg/kg. Importantly that ribose treatment was carried out only during the last week of the experiment. The study was carried out in accordance with the requirements of the European Convention on Experimental Animals Protection 86/609/EEC.

Plasma concentrations of lactic, β-hydroxybutyric, and uric acids were measured by universal methods. Luteinizing hormone (LH), total and free testosterone were measured by enzyme immunoassay using Vector-Best and Diagnostic Systems Laboratories, Inc. kits on devices from BIO-RAD Laboratories. The testes were homogenized in 0.15 M potassium chloride (10% suspension) at 0-2ºC. The suspensions were centrifuged (2000g, 20 min) in a C-80 centrifuge (Hospitex). Total protein was measured in the supernatant by the biuret method, malondialdehyde (MDA) was evaluated by TBA reaction, uric acid was measured by spectrophotometry at 290 nm, glutathione (GSH) was evaluated by reaction with

5.5-dithio-bis-(2-nitrobenzoic) acid, and testosterone by enzyme immunoassay. The superoxide dismutase (SOD; EC 1.15.1.1) (Sirota, 1999), catalase (CAT; 1.11.1.6) (Koroliuk *et al*., 1988) activities and ascorbic acids levels (Varley *et al*., 1988) were estimated in the testes sample. Activities of glutathione peroxidase (GPx; EC 1.11.1.9), glutathione reductase (GR; EC 1.6.4.2) and glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) were measured by spectrophotometry at 340 nm (Pashkov *et al*., 2005).

The results were statistically processed using Statistica 6.0 software. All the values are given as Mean ± SE. The statistical significance of differences was evaluated by Student's t-test. A difference of $p<0.05$ was accepted as significant.

Table 1 Blood biochemistry in control rats subjected to EE or treated with ribose during EE

Parameter	Group		
	1 (Control)	2 (EE)	3 (EE+ Ribose)
Lactic acid, mmol/l	6.53 ± 0.21	10.8 ± 0.43 ^{aaa}	7.04 ± 0.60 ^{bbb}
β-Hydroxybutyric acid, μmol/l	86 ± 9	139 ± 17 ^{a}	97 ± 10 ^{b}
Uric acid, μmol/l	80.1 ± 5.3	131 ± 5.9 ^{aaa}	94.7 ± 5.8 ^{bbb}
Total testosterone, nmol/l	13.8 ± 2.1	7.6 ± 2.1 ^{a}	14.1 ± 2.0 ^{b}
Free testosterone, pmol/l	59.8 ± 0.64	41.1 ± 0.57 ^{a}	56.2 ± 0.42 ^{b}
Luteinizing hormone, U/l	457 ± 81	739 ± 97 ^{a}	480 ± 72 ^{b}

Note. Here and in Table 2: ^{a} - $p<0.05$, ^{aa} - $p<0.01$, ^{aaa} - $p<0.001$ compared to the control;
^{b} - $p<0.05$, ^{bb} - $p<0.01$, ^{bbb} - $p<0.001$ compared to group 2.

Table 2 The level of uric acid, ascorbic acids, malondialdehyde (MDA), glutathione (GSH) and testosterone in testes in control rats subjected to EE or treated with ribose during EE

Parameter	Group		
	1 (Control)	2 (EE)	3 (EE+Ribose)
Uric acid, nmol/mg protein	143 ± 11	194 ± 10 ^{aa}	151 ± 8 ^{bb}
Ascorbic acids, μg/mg protein	138 ± 9	87 ± 12 ^{aa}	107 ± 10 ^{bb}
MDA, nmol/mg protein	10.2 ± 0.9	17.6 ± 1.2 ^{aaa}	12.3 ± 1.1 ^{bb}
GSH, nmol/mg protein	20.1 ± 1.4	11.7 ± 1.8 ^{aa}	17.8 ± 1.9 ^{b}
Testosterone, pmol/mg protein	14.2 ± 3.1	6.4 ± 1.1 ^{a}	15.6 ± 3.0 ^{bb}

RESULTS AND DISCUSSION

The effects of overtraining caused by EE led to intensifycation of anaerobic glycolysis and β-oxidation of fatty acids in rats. The level of lactate and β-hydroxybutyrate in the blood of the EE group rats increased by 65 and 62%, respectively, in comparison with the control (Tables 1). This promoted the development of lacto- and ketoacidosis, leading to acute disorders of purine metabolism (ADPM). This state is characterized by intense oxidation of purines to hypoxanthine and uric acid. The level of uric acid in the blood and testes of the EE group rats increased by 64 and 36%, respectively, in comparison with the control (Tables 1 and 2).

In addition, purine reutilization through the reaction catalyzed by hypoxanthine phosphoribosyl transferase was inhibited. This reaction is realized in the presence of a sufficient amount of ribose-5-phosphate generated in the pentose phosphate pathway reactions (Mikami and Kitagawa, 2006; Zolin and Konvai, 1997). However, under conditions of our experiment activity of this metabolic chain was inhibited, which manifested by

reduced activity of G6PDH. The activity of G6PDH in the testes of the EE group rats inhibited by 43% in comparison with the control (Tables 3).

Conversion of xanthine dehydrogenase into xanthine oxidase plays an important role in the development of ADPM. Several factors contribute to this. The first of them is partial proteolysis of xanthine dehydrogenase molecule by lysosomal enzymes and deficit of SH group donors, one of which is GSH. The level of GSH in EE rat testes decreased by 42% in comparison with the control (Table 2). One more factor promoting conversion is NAD deficiency observed under conditions of hypoxia (Finkel and Holbrook, 2000; Zhang *et al.*, 1998). This deficit is presumably caused by active cleavage of NAD for the construction of poly-(ADP-ribose) molecule, needed for repair of damaged DNA sites (D'Andrea, 2010). As a result of this, the electrons are transferred not to the NAD molecule during hypoxanthine oxidation to uric acid, but to O_2 with the formation of active oxygen metabolites (Ardan *et al.*, 2004; Saito and Nishino, 1989).

Table 3 The activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH) in testes in control rats subjected to EE or treated with ribose during EE

Parameter	Group		
	1 (Control)	2 (EE)	3 (EE+ Ribose)
SOD, U/mg protein	30.8 ± 3.1	19.4 ± 2.1 ^{aa}	31.1 ± 2.3 ^{bbb}
CAT, U/mg protein	65.3 ± 3.8	47.8 ± 4.2 ^{aa}	64.3 ± 2.7 ^{bb}
GPx, U/mg protein	337 ± 24	181 ± 21 ^{aaa}	262 ± 24 ^{b}
GR, U/mg protein	93.3 ± 3.5	64.2 ± 6.1 ^{aaa}	92.0 ± 6.7 ^{bb}
G6PDH, U/mg protein	12.3 ± 0.9	7.1 ± 1.0 ^{aaa}	11.3 ± 0.5 ^{bbb}

Intensive formation of active oxygen metabolites as a result of the xanthine oxidase and other reactions leads to excessive lipid peroxidation of the testicular membrane structures and accumulation of MDA level in them (Table 2). The developing GSH and ascorbate deficit observed in our experiments also contributes to this process. This shortage is explained by intensive involvement of this tripeptide in reactions of inactivation of peroxide compounds.

Inhibition of SOD, CAT, GPx and GR also contributes to more intense lipid peroxidation of testicular membranes (Table 3). SOD is the key antioxidant enzyme because superoxide is one of the main reactive oxygen species in the cell. SOD is responsible for the quenching of superoxide radicals which are released during the chemical reactions of the various metabolic pathways (Bloomer, 2008; Finkel and Holbrook, 2000). CAT functions to catalyze the decomposition of hydrogen peroxide to water and oxygen (Chelikani *et al.*, 2004). Hydrogen peroxide is a harmful byproduct of many normal metabolic processes. To prevent damage, it must be quickly converted into other, less dangerous substances. CAT is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules (Gaetani *et al*., 1996).

GR reduces glutathione disulfide to the sulfhydryl form GSH, which is an important cellular antioxidant. The activity of GR is used as an indicator for oxidative stress (Brancaccio *et al.*, 2010; Bykova *et al.*, 2007). Reduced GR activity is caused by inhibition of NADPH generation in the G6PDH reaction. Carbohydrate deficit developing over the course of excessive exercise, specifically,

shortage of ribose needed for *de novo* synthesis of NADP, promotes these events.

Combined effects of these factors on the testes lead to injuries of Leydig's cell membrane structures with subsequent inhibition of testosterone incretion by them. The content of this hormone in the testes of EE rats decreased by 55% in comparison with the control (Table 2). As a result, plasma levels of total and free testosterone decreased, while the concentration of LH increased (Table 1).

This means that ribose deficit developing under conditions of excessive exercise is a pathogenetic factor of testicular injury.

We attempted testicular protection by treatment with D-ribose, precursor in biosynthesis of ribose-5-phosphate and other metabolites containing this monosaccharide. Exogenous ribose is phosphorylated into ribose-5-phosphate under the effect of ribokinase and is then involved into metabolism (Bork et al., 1993). Exogenous ribose promotes reduction of the severity of lacto- and ketoacidosis and hence, of the intensity of purine mononucleotide catabolism. This manifests by reduction of blood levels of lactic, β-hydroxybutyric, and uric acids in rats subjected to EE and treated with ribose in comparison with the values in the EE group (Table 1).

By compensating for ribose-5-phosphate deficit in the testes, exogenous ribose promotes more effective reutilization of hypoxanthine in AMP, thus reducing the intensity of this substance oxidation to uric acid (Table 2). Reduction of active oxygen metabolites generation prevents the development of oxidative stress in the testes, which manifests by lesser accumulation of MDA and elevation of GSH and ascorbate level (Table 2). This promotes a reduction of xanthine dehydrogenase conversion to the oxidase form of this enzyme.

Involved in the plastic branch of the pentose phosphate pathway, exogenous ribose transforms into glucose-6-phosphate, thus stimulating activity of G6PDH (Table 3), which leads to improvement of NADPH generation. Sufficient supply of NADPH to the testes elevates activity of GR, an enzyme participating in GSH reduction and, along with alleviation of ADPM manifestation, promoting recovery of testosterone incretion. Its content in the testes of group 3 rats was 144% higher than in group 2 (Table 2). Plasma concentrations of total and free testosterone and of LH in group 3 were close to the control values (Table 1).

Hence, acute disorders of purine metabolism develops in rat testicles under conditions of excessive exercise. This abnormality is characterized by intense catabolism and reduced reutilization of purine mononucleotides and by intensification of oxidative stress paralleled by reduced activity of the pentose phosphate pathway and antioxidant system. Daily oral ribose (50 mg/kg) treatment of rats subjected to excessive exercise during the last week of the experiment before and after exercise improved purine reutilization, pentose phosphate pathway work, reduced the intensity of testicular oxidative stress, and saved the gonadal endocrine function. This means that ribose deficit is one of the pathogenetic mechanisms of testicular metabolism disorders under conditions of excessive exercise.

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