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Role of Ribose Deficit in Rat Testicular Metabolism under Conditions of Overtraining

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Acute disorders of purine metabolism develop in rat testes under conditions of overtraining. These disorders are characterized by enhanced catabolism and reduced reutilization of purine mononucleotides and activation of lipid peroxidation of membrane structures against the background of reduced activities of the pentose cycle and antioxidant system. Administration of D-ribose to rats subjected to overtraining improves purine reutilization, stimulates the pentose cycle work, inhibits lipid peroxidation in membrane structures of the testes, and saves the testicular incretory function.

Key Words: *ribose; energy metabolism; testes; exercise*

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 [1]; the incidence of endocrine diseases is increasing [7]. One of the factors which have a negative effect on the male reproductive function is fatigue caused by excessive exercise [7,8]. The means used for prevention and correction of fatigue are ineffective for sexual function recovery and have contraindications and numerous side effects [4]. 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 [3]. We suggested that this monosaccharide also possesses a gonadoprotective effect.

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

MATERIALS AND METHODS

The study was carried out on 30 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, 10 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 exhausting 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

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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 INC DSL kits on devices from BIO-RAD Laboratories. The testes were homogenized in 0.15 M potassium chloride (10% suspension) at 0-2°C. The suspensions were centrifuged (2000*g*, 20 min) in a C-80 centrifuge (Hospitex). Total protein was measured in the supernatant by the biuret method, MDA was evaluated by TBA reaction, uric acid was measured by spectrophotometry at 290 nm [2], glutathione was evaluated by reaction with 5.5-dithio-bis-(2-nitrobenzoic) acid, and testosterone by enzyme immunoassay. Activities of glutathione reductase (EC 1.6.4.2) and glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) were measured by spectrophotometry at 340 nm [6]. Randox reagents were used. Optical densities were measured on an SF-56 spectrophotometer and Screen Master biochemical analyzer (Hospitex).

The results were statistically processed using Statistica 6.0 software. The statistical significance of differences was evaluated by Student's *t* test.

RESULTS

The effects of overtraining caused by EE led to intensification of anaerobic glycolysis and β-oxidation of fatty acids in rats, which manifested by elevation of blood lactate and β-hydroxybutyrate levels (Table 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 cycle reactions. However, under conditions of our experiment activity of this metabolic chain was inhibited, which manifested by reduced activity of G6PDH (Table 2).

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 glutathione. The level of this tripeptide 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 [5]. 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. 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.

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 glutathione 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 antioxidant defense enzymes, for example, glutathione reductase, also contributes to more intense lipid peroxidation of testicular membranes (Table 2). Reduced glutathione

TABLE 1. Blood Biochemistry in Control Rats Subjected to EE or Treated with Ribose during EE (*M*±*m*)

Parameter	Group		
	1 (control)	2 (EE)	3 (EE+ribose)
Lactic acid, mmol/liter	6.53±0.21	10.80±0.43***	7.04±0.60^{+++}
β-Hydroxybutyric acid, μmol/liter	86±9	139±17*	97±10^{+}
Uric acid, μmol/liter	80.1±5.3	131.0±5.9***	94.7±5.8^{+++}
Total testosterone, nmol/liter	13.8±2.1	7.6±2.1*	14.1±2.0^{+}
Free testosterone, pmol/liter	5.98±0.64	4.11±0.57*	5.62±0.42^{+}
Luteinizing hormone, U/liter	457±81	739±97*	480±72^{+}

Note. Here and in Table 2: *p<0.05, **p<0.01, ***p<0.001 compared to the control; ^{+}p<0.05, ^{++}p<0.01, ^{+++}p<0.001 compared to group 2.

TABLE 2. Testicular Biochemistry in Control Rats Subjected to EE or Treated with Ribose during EE (*M*±*m*)

Parameter	Group		
	1 (control)	2 (EE)	3 (EE+ribose)
Uric acid, nmol/mg protein	143±11	194±10**	151±8++
G6PDH, U/mg protein	12.3±0.9	7.1±1.0***	11.3±0.5+++
Glutathione, nmol/mg protein	20.1±1.4	11.7±1.8**	17.8±1.9+
MDA, nmol/mg protein	10.2±0.9	17.6±1.2***	12.3±1.1++
Glutathione reductase, U/mg protein	93.3±3.5	64.2±6.1***	92.0±6.7++
Testosterone, pmol/mg protein	14.2±3.1	6.4±1.1*	15.6±3.0++

reductase activity, in turn, is caused by inhibition of NADPH generation in the G6PDH reaction. Carbohydrate deficit developing over the course of overtraining, 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 overtraining 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 ribose kinase and is then involved into metabolism [2].

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 excessive lipid peroxidation in the testicular membrane structures, which manifests by lesser accumulation of MDA and elevation of glutathione 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 cycle, exogenous ribose transforms into glucose-6-phosphate, thus stimulating activity of G6PDH (Table 2), which leads to improvement of NADPH generation. Sufficient supply of NADPH to the testes elevates activity of glutathione reductase, an enzyme participating in glutathione reduction and, along with alleviation of ADPM manifestation, promoting recovery of testosterone incretion. Its content in the gonads 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, ADPM develops in rat testicles under conditions of overtraining. This abnormality is characterized by intense catabolism and reduced reutilization of purine mononucleotides and by intensification of lipid peroxidation in membrane structures paralleled by reduced activity of the pentose cycle and antioxidant system. Daily oral ribose (50 mg/kg) treatment of rats subjected to overtraining during the last week of the experiment before and after exercise improved purine reutilization, pentose cycle work, reduced the intensity of testicular membrane lipid peroxidation, and saved the gonadal incretory function. This means that ribose deficit is one of the pathogenetic mechanisms of testicular metabolism disorders under conditions of overtraining.

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