Physiol. Res. 52: 743-748, 2003

Estrogen Effect on Some Enzymes in Female Rats after Downhill Running

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Received October 25, 2002
Accepted January 2, 2003

Summary

The study investigates the effect of administered estrogen on plasma creatine kinase (CK) and lactate dehydrogenase (LD) levels in female ovariectomized rats after downhill running. Rats ovariectomized before sexual maturity were subcutaneously implanted with pellets containing 17β-estradiol or placebo. Three weeks later they were subjected to a 90-min intermittent downhill running protocol. Blood samples were obtained from the jugular vein immediately after and 72 h after exercise for determination of plasma CK, LD and 17β-estradiol levels. A two-way analysis of variance was used for data evaluation. Plasma CK and LD levels were significantly lower ($p<0.05$) in the estrogen-supplemented, ovariectomized animals which suggests that less muscle damage occurred compared to the controls immediately and 72 h after exercise. Estrogens may have a protective effect on muscle tissue possibly due to their antioxidant and membrane stabilizing properties.

Key words
CK • LD • Estrogen • Eccentric exercise • Muscle damage

Introduction

It is well known that exercise and especially eccentric exercise, such as downhill running, can cause muscle damage (Armstrong *et al.* 1983, Kyparos *et al.* 2001). The release of myocellular enzymes into the blood as the result of sarcolemmal disruption can generally be perceived as an indication of muscle damage (Evans and Cannon 1991). Muscle enzymes usually assayed include creatine kinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LD) (van der Meulen *et al.* 1991, Balnave and Thompson 1993). The release of CK and/or LD was mainly utilized as a criterion for muscle injury in 37 % of the animal studies reviewed by Warren *et al.* (1999). Creatine kinase leakage into the plasma is widely accepted as a semi-quantitative indicator of muscle injury (Brenner *et al.* 1999).

Gender is one of the most interesting factors affecting sarcolemma disruption and thus enzyme release. It has been reported that females have much lower basal and exercise-induced CK levels compared to the males as well as exercise-induced CK release after a comparable amount of work (van der Meulen *et al.* 1991). This gender difference results from skeletal muscle exposure to estradiol (Amelink and Bär 1986, Bär *et al.* 1988, 1990), which appears to protect muscles against enzyme release during basal and exercise conditions.

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Additionally, blood levels of muscle enzymes among females were found to be lower when the circulating estrogen concentration was higher, as was seen from the comparison of ovariectomized (OVX) with OVX estrogen-treated rats (Amelink *et al.* 1990, van der Meulen *et al.* 1991, Carter *et al.* 2001).

The purpose of this study was to test the hypothesis that the presence of estrogen reduces the blood CK and LD levels in ovariectomized rats immediately and 72 h after downhill running.

Methods

This project followed the guidelines for animal use established by the American Physiological Society and was approved by the local ethical committee according to 86/609 European Union Council Order. Forty adult (12 week-old) female Wistar rats, weighing 190-230 g, were used in the study. Animals were housed in a temperature-controlled room (22-24 ^{o}C) with 12:12 h light-dark cycle. Commercial rat chow and tap water were provided *ad libitum.* Ovaries were dissected out from rats before sexual maturity (i.e. before 24-26 days old) under anesthesia with 10 mg/kg xylazine and 50 mg/kg ketamine hydrochloride mixture injected intra-peritoneally. Ovariectomy was performed through two small dorsal paraspinal incisions between the iliac crest and the lower ribs. After carefully exposure of the ovaries, they were clamped between two mosquito clamps in order to prevent bleeding and removed after ligation of the surrounding tissue. Muscle and skin incisions were closed with a single stitch and treated with an aerosol antibiotic.

At the age of 2 months (9 weeks old) the animals were implanted subcutaneously (using a trochar) with a placebo or 17β-estradiol containing pellets (0.05 mg/pellet, 3 week release, Innovative Research of America, Sarasota, FL) under the same anesthesia as above.

Rats were randomly divided into four groups as follows: a) exercise group treated with estradiol, studied immediately after exercise (E_0), b) exercise group treated with estradiol, studied 72 h after exercise (E_{72}), c) exercise group treated with placebo, studied immediately after exercise (P_0) and d) exercise group treated with placebo, studied 72 hours after exercise (P_{72}).

Three weeks after the implantation all animals were subjected to the exercise protocol consisting of 90-min intermittent downhill running on a motor-driven treadmill. After allowing a few minutes to become accustomed to the procedure, the animals of the exercise groups performed 18 x 5-min running bouts at a speed of 16 m/min with –16^{o} inclination, separated by 2-min rest periods. When required, rats were encouraged to run by brushing their tails with a soft bristle brush.

Blood samples were obtained from the jugular vein of rats using the same anesthesia, immediately after exercise for the groups E_0 and P_0 and 72 h after exercise for the groups E_{72} and $P_{72,}$ in order to assess CK, LD activity and estradiol levels. Blood plasma samples were analyzed for 17β-estradiol using a commercially available RIA kit (Roche-Elecsys 1010/2010 Systems Elecsys Estradiol Immunoassay). Plasma CK and LD activity was determined spectrophotometrically with Dialab-Diapack CK-NAC and Dialab-Diapack LDH-P kits, respectively.

Data were analyzed using the SPSS program. Means ± S.E.M. were calculated for all data. To test for normal distribution of the variables, the Kolmogorov-Smirnov test was used. A 2 x 2 analysis of variance (2-way ANOVA) was used to determine the effects of treatment (E_2 vs P), time (0 h vs 72 h) or treatment vs time interaction. Statistical significance level was set at $p<0.05$.

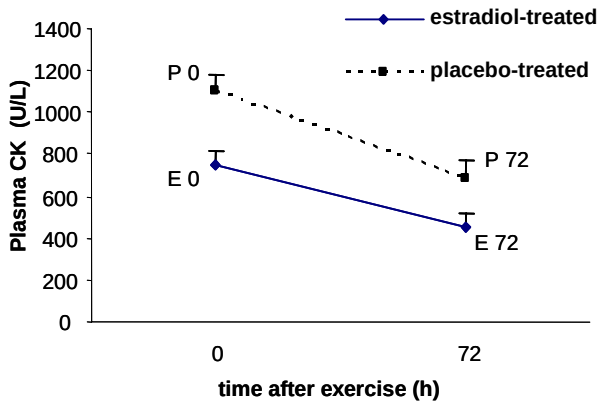

Fig. 1. *Schematic representation of creatine kinase data in Table 1. A significant main effect of estradiol observed in both groups, E_0 and E_{72} ($p<0.05$).*

Results

The results of the present study are shown in Table 1, while Figures 1 and 2 schematically represent the data of Table 1 for CK and LD, respectively. Estrogen treatment of OVX rats resulted in a significant decrease of plasma CK and LD activities ($p<0.05$), both immediately and 72 h after exercise. The analysis of

variance for CK (Fig. 1) and LD (Fig. 2) showed a significant ($p<0.05$) main effect of estrogen treatment. A positive correlation between CK and LD levels was also found ($r = 0.77$, $p<0.01$).

The estrogen-supplemented OVX animals had approximately 25- to 30-fold higher levels of circulating 17β-estradiol than the placebo-treated ones ($p<0.01$) (Table 1). Moreover the placebo-treated animals were 10 % heavier at the time of sacrifice than the estrogen-treated ones (Table 1), and this difference was significant ($p<0.05$).

Table 1. Effect of 17β-estradiol-treatment on plasma creatine kinase (CK), lactate dehydrogenase (LD), 17β-estradiol and on body weight of the rats.

	Placebo-treated (controls)		**Estradiol-treated**	
	0 h (n=10)	**72 h (n=10)**	**0 h (n=10)**	**72 h (n=10)**
CK (U/l)	1101±76.9	680±8.3	754±60.2*	449±65.8*
LD (U/l)	890±102.0	512±1.0	525±37.5*	396±32.5*
Estradiol (pg/ml)	3.1±0.9	3.0±1.2	78.5±8.7**	88.6±20.3**
Body weight (g)	224±5.7		206±9.7*	

*Data are means ± S.E.M. *Significantly lower ($p<0.05$) than the corresponding controls, **Significantly higher ($p<0.01$) than the corresponding controls.*

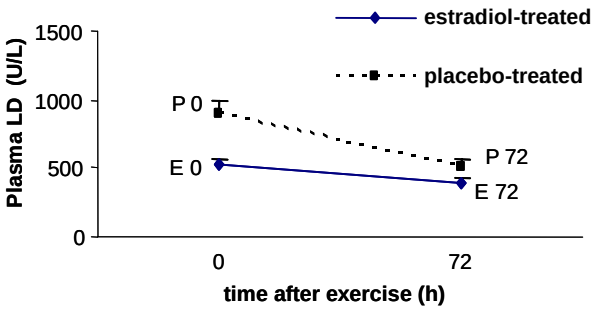

Fig. 2. *Schematic representation of lactate dehydrogenase data in Table 1. A significant mean effect of estradiol observed in both groups, E_0 and E_{72} ($p<0.05$).*

Discussion

The results of the present study indicated that the treatment of ovariectomized rats with estrogen induces less muscle damage after downhill running, as can be seen from the significantly lower levels of plasma CK and LD in the estrogen-treated rats compared with the placebo-treated rats. It is remarkable that CK levels are lower to the same extent for both time points (32 % immediately and 34 % 72 h after exercise), while LD levels are lower to a different extent for the two time intervals (41 % and 23 %, respectively).

The prolonged treatment of OVX rats for the period of 21 days with β-estradiol resulted in steadily high estradiol levels resembling those found in the phase of proestrus (Butcher *et al.* 1974) which are the highest in the rat cycle. Eccentric exercise, as downhill running, was preferred because it is considered to be the most appropriate kind of exercise to induce muscle damage (Armstrong *et al.* 1983). Using the same protocol as in the present study, these authors have reported a biphasic significant increase in plasma CK and LD activity immediately after exercise, associated with initial injury to the muscle, and 72 h post exercise, attributed to the infiltration with phagocytic cells, compared to the control unexercised rats.

The data of the present study are generally in agreement with the results of many other animal-based studies. Several investigators have demonstrated that rodents treated to have high estrogen levels sustain less muscle damage and disruption during and following exercise than those with lower estrogen levels (Carter *et al.* 2001). Dumke (1996) examined OVX estradiol-treated and OVX placebo-treated rats 2, 6, 12, 24 and 48 h after downhill running. His results exhibited greater

CK activity in placebo-treated rats at all time points with the exception of 2 h after exercise. In another study, a significantly higher CK activity in OVX placebo-treated rats was also found compared to OVX estrogen-treated rats, one hour after uphill running (Tiidus 2001)

Comparing male and female rats exposed to a similar running protocol, a higher CK release was observed in male animals (van der Meulen *et al.* 1991). Komulainen (1999) also reported that no dramatic changes occurred in the microarchitecture of muscle fibers immediately or even 6 h after the exercise in female compared with male rats.

The same results were also shown in an *in vitro* study (Amelink and Bär 1990), in which a significant reduction of CK efflux from rat soleus muscles after stimulation was observed in OVX estrogen-treated rats compared to OVX placebo-treated controls. It was also reported that there is a sex-linked difference in CK efflux under both basal and stimulated conditions. The CK efflux is higher in male rats because they have more serious muscle damage. On the contrary, they showed decreased indices of muscle tissue damage when they were treated with estrogen (Tiidus and Bobmardier 1999).

As far as humans are concerned, several studies have noted that women have lower plasma CK values than men, both at rest and following exercise. However, other studies, particularly those using weight lifting, exhibit the same or even higher post-exercise CK levels in women than in men (Miles *et al.* 1994, Clarkson and Sayers 1999). Following eccentric exercise, eumenorrheic women during the mid-follicular phase (low estrogen levels) exhibited greater CK responses (Carter *et al.* 2001) than oral contraceptive users during the mid-luteal phase (high estrogen levels), but no difference (Thompson *et al.* 1997) when both groups were compared during the mid-luteal phase (at similar estrogen levels).

As CK and LD activity gives an indication of membrane integrity, the research strongly suggests that estrogens play a major role in maintaining greater membrane stability. They diminish the postexercise inflammatory response by possibly reduction of the skeletal fiber membrane disruption and exert a protective effect on skeletal muscle tissue to attenuate the muscle damage process following exercise. 17β-estradiol is the primary estrogen with the greatest estrogenic properties, which is used in the majority of investigations. Estrogens are believed to have a high antioxidant capacity and membrane stabilizing properties (Subbiah et al. 1993).

The antioxidant characteristics of estrogen have been demonstrated *in vitro* as well as *in vivo* in both rat and human investigations (Tiidus 1995, Bär and Amelink 1997), although the mechanisms by which it acts as an antioxidant have not been fully determined. Estrogen may donate hydrogen atoms from the phenolic hydroxyl group, thus terminating the peroxidation chain reaction, in a way similar to vitamin E (Tiidus 1995, Sugioka *et al.* 1987)

The membrane stabilizing properties of estrogens are also due to their antioxidant capacity. As lipophilic hormones, estrogens intercalate into the bilayer of the cell plasma membrane and interact directly with the membrane phospholipids. They decrease membrane fluidity, increase membrane stabilization against peroxidation and finally diminish membrane disruption (Wiseman *et al.* 1993, Wiseman and Quinn 1994).

Another factor to be taken into consideration is the increased body weight of OVX placebo treated animals, which is consistent with other studies (Goldberg *et al.* 1984). This increases the metabolic load on postural muscles to support the additional body weight since the increase in total body weight does not reflect changes in muscle weight. The continuous recruitment to support the body weight leads to a higher metabolic load and therefore a higher oxygen consumption and free radical production (Persky *et al.* 2000), leading eventually to sarcolemmatic ruptures and release of CK and LD into the circulation.

In conclusion, the present results demonstrate that the CK and LD response immediately and 72 h after eccentric exercise in rats treated with estrogen is less than in those treated with placebo. From these findings it is suggested that estrogens may have a protective effect on muscle tissue and the mechanism of this effect may be related to the antioxidant characteristics and membrane stability properties associated with estrogens.

Acknowledgements

Sotiriadou Sofia is supported under a scholarship by the State Scholarships Foundation of Greece (IKY).

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