Effects of Ovariectomy on Intramuscular Energy Metabolism in Young Rats: How Does Sports-Related-Amenorrhea Affect Muscles of Young Female Athletes?

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Abstract The purpose of this study was to evaluate the effects of ovariectomy on intramuscular energy metabolism in young rats. Twenty-four Sprague-Dawley rats (7 weeks old) were used. Twelve of them underwent ovariectomy (OVX), and the others were sham-operated on. Seven OVX rats were examined 1-week after surgery (OVX-1 group), and the other five, 4 weeks after surgery (OVX-4 group). The gastrocnemius-plantaris-soleus (GPS) muscles group was subjected to the following measurements, and the data were compared with those of the sham group (Sham-1: n=7, or Sham-4 group: n=5). From the ³¹P-MR spectra of the GPS muscles group at rest and during electric stimulation, the muscular oxidative capacity was measured. Maximum tension and wet weight of the whole GPS muscles group were also measured. Body weight in the OVX-4 group was significantly (p<0.01) larger than that in the Sham-4 group. The weights of the whole GPS muscles group in the Sham-1, Sham-4, OVX-1 and OVX-4 groups were 1.17, 1.51, 1.25 and 1.71 (g), respectively. The muscle weight in the OVX group tended to be greater than that in the Sham group (p<0.10). The maximum tension and oxidative capacity did not differ significantly among the groups. These data indicated that in young rats, ovariectomy induced an increase in body and muscle weight, but did not affect the maximum tension nor oxidative capacity. J Physiol Anthropol 20 (2): 125-129, 2001 http://www.jstage.jst.go.jp/en/

Keywords: skeletal muscle, ³¹P-MRS, ovariectomy, oxidative capacity

Introduction

The female athlete triad, i.e., eating disorders, amenorrhea and osteoporosis, has been well documented (Anderson, 1999; Moen et al., 1998; Teitz et al., 1997). However, the skeletal muscle function and muscular energy metabolism of female athletes with sports-related amenorrhea has not been studied in detail. If the skeletal muscle function deteriorates under such conditions, sports performance as well as trainability of female athletes with sports-related amenorrhea will definitely deteriorate. Therefore, it is crucial to determine the effects of amenorrhea on skeletal muscles in female athletes. Although the effects of ovariectomy on skeletal muscle have been extensively investigated (Booth and Tipton, 1969; Borski et al., 1996; Fisher et al., 1998; Jansson et al., 1984; Kobori and Yamamuro, 1989; Roth et al., 1989; Santidrian and Thompson, 1981; Wade, 1972), little is known about energy metabolism.

Intramuscular energy metabolism needs to be measured in vivo, and it is important to examine the changes in metabolism in working muscles, because skeletal muscles are the organs for exercising and working. Since phosphorus-31 magnetic resonance spectroscopy (³¹P-MRS) was first applied to the skeletal muscle, numerous studies have been conducted and intramuscular energy metabolism has been evaluated under many skeletal muscle conditions (Iwanaga et al., 1992; Iwanaga et al., 1996; Kato et al., 2000; Sairyo et al., 1993; Sairyo et al., 2000). Recently, even younger girls are becoming top world-class female athletes. Therefore, to access what occurs in such young athletes, it is necessary to examine muscle metabolism in immature ovariectomized rats. The purpose of this study was to evaluate the effects of ovariectomy on skeletal muscles in young rats with reference to energy metabolism in the working muscles as assessed by ³¹P-MRS.
Materials and Methods

Materials

Twenty-four 7-week-old Sprague-Dawley female rats were used in this study (Japan SLC Inc. Shizuoka, Japan). They were housed in individual cages in a temperature (22 ± 1°C) and humidity (50 ± 10%) controlled room on a 12: 12 hours light-dark cycle. The animals were allowed free access to food and water.

Surgical procedure of ovariectomy

Twelve rats underwent ovariectomy (OVX group), and the other 12 were sham-operated on (Sham group). After each rat was anesthetized with sodium pentobarbital (50 mg/kg body weight), small incisions through the skin and the retroperitoneal area were made on the right and left sides over the lower back. After the ovaries were sectioned from the uterine horns, they were removed. For the sham operation, ovaries were approached through the incision, lifted out of the rat and replaced. Muscle and skin incisions were sewn separately with 4.0 silk. Seven OVX rats were examined 1-week after surgery (OVX-1 group), and the other five, 4 weeks after surgery (OVX-4 group). The gastrocnemius-plantaris-soleus (GPS) muscles group was subjected to the following measurements, and the data were compared with those of the sham group (Sham-1: n=7, or Sham-4 group: n=5).

Experimental preparation for measurement

After each rat was anesthetized with sodium pentobarbital (50 mg/kg body weight), the right sciatic nerve was exposed in the gluteal region and a small bipolar electrode was attached to the sciatic nerve. The rat was immobilized on a small platform with both knee and ankle in the full-extended position. Then, an oval surface coil (20 × 14 mm) was placed on the right GPS muscle at 0.2 Hz for 10 min. Then, the stimulation exercise at the last two minutes is considered as the steady state condition at each electric stimulation condition. Therefore, we evaluated the energy metabolism at such steady state condition. The force time rate (F × R) at the steady state obtained from the tension measurement was calculated as the indicator of the momentum of muscle contractions.

Intracellular pH was also calculated by the chemical shift (d) between Pi and PCr peaks as shown as follows (Flaherty et al., 1982):

\[ \text{Intracellular pH} = 6.90 - \log \left( \frac{(d-6.81)}{(3.29-d)} \right) \]

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Maximum tension of twitch was measured at the muscle contraction induced by 0.20 Hz, and muscle wet weight was also determined in whole GPS muscles group.

Data analysis

Data are expressed as the mean ± SD. One way ANOVA, post hoc multiple comparison and analysis of covariance were used for statistical analysis. A p value of less than 0.05 was taken as significant.

Results

Body and muscle weight

Fig. 1 shows the body weight in each group. It was 197 ± 12 and 202 ± 6 (g) in Sham-1 and OVX-1 group, respectively. No significant difference was found between them. However, the weight in Sham-4 was 231 ± 22 (g) and it was significantly (p<0.01) lower than that in the OVX-4 group, which was 273 ± 11 (g). The weight of the whole GPS muscles group in the Sham-1, Sham-4, OVX-1 and OVX-4 groups was 1.17 ± 0.01, 1.51 ± 0.24, 1.25 ± 0.05 and 1.71 ± 0.15 (g), respectively. The weight in the OVX-1 group tended to be greater than that in the Sham-1 group (p<0.08), and the same trend (p<0.09) was found between Sham-4 and OVX-4 group (Fig. 2).
Maximum tension
The maximum tension was 326 ± 55, 432 ± 37, 354 ± 43 and 468 ± 75 (gw) in the Sham-1, Sham-4, OVX-1 and OVX-4 group, respectively. The results showed no significant differences among the groups.

MRS measurements
Representative 31P-MR spectra are shown in Fig. 3. Intracellular pH did not decrease below 7.0 during muscle contractions, indicating that the muscle exercise was aerobic. Significant (p<0.05) linear relationships between PCr/(Pi + PCr) and F × R were found in all groups during muscle contraction. Fig. 4 shows the regression lines in the Sham-1 and OVX-1 group. During aerobic exercise the slope indicates muscle oxidative capacity (Kemp et al., 1996; Meyer, 1988). The slope of the regression lines did not show significant differences between two groups. Furthermore, as shown in Fig. 5, the
slope of the Sham-4 group was similar to that of the OVX-4 group.

Discussion

In this study, we investigated the effects of ovariectomy on skeletal muscles 1 and 4 weeks after surgery. One week after ovariectomy, no significant change was observed regarding body weight, maximum tension and muscle oxidative capacity, while muscles wet weight in OVX-1 groups tended to be larger \((p=0.08)\) than that of Sham-1 group. Four weeks after surgery although maximum tension and oxidative capacity did not change compared to the Sham group, the body and muscle wet weight tended to increase. These results indicated that energy metabolism and maximum tension of skeletal muscles were not affected by ovariectomy despite the tendency in the increase in muscle volume within 4 weeks.

It was reported that ovariectomized rats showed increased food intake, decreased physical activity, and elevated plasma growth hormone and insulin-like growth factor I concentrations (Borski et al., 1996; Jansson et al., 1984; Wade, 1972); thereby their body weight and organ weight including muscle mass (Booth and Tipton, 1969; Santidriand and Thompson, 1981) increased. Concerning body weight, in the present study we found significant differences between the OVX-4 group and Sham-4 group. Ovariectomized rats were heavier compared to the Sham group, and muscle volume in OVX-4 group tended to be larger than that in the Sham-4 group. The results were in good agreement with those of previous reports. Muscular energy metabolism was evaluated in vivo using 31P-MRS in this study. The intracellular pH during electrical stimulation at any frequency did not decrease 7.0 in all groups, and this indicated the exercise evoked by electrical stimulation was aerobic. Under such working conditions, most energy is supplied from the aerobic metabolism, that is, such from oxidative phosphorylation; therefore, the relationship between workload and Pi/PCr ratio reflects mitochondrial oxidative capacity (Chance et al., 1981; McCully et al., 1989). Experimental studies also demonstrated that during muscle twitch stimulation, where the intracellular pH does not decrease, the relationship indicates muscle oxidative capacity (Kemp et al., 1996; Meyer, 1988). As shown in Figs. 4 and 5, the slopes of the regression lines indicating the muscle oxidative capacity were similar in the OVX group and in the Sham group. Thus, skeletal muscle oxidative capacity was not impaired by ovariectomy in young rats within four weeks after surgery.

Roth et al. (1989) evaluated muscle metabolism in adult rats four weeks after ovariectomy and found that muscle oxidative capacity was worsened by ovariectomy. They speculated that ovarian sex hormones might be regulating the synthesis or degradation of respiratory enzymes or creatine kinase, because there had been no report concerning muscle metabolism in ovariectomized rats. The authors suggested that in situations, in which ovarian sex hormones are significantly decreased, such as in menopause, untreated ovariectomy, or amenorrhoeic athletes, the ability to perform repeated bouts of high intensity exercise or to sustain high-intensity exercise would be reduced. The authors did not investigate the effects of sports-related-amenorrhea on muscle energy metabolism. However, it has been well known that female athletes with the oligomenorrhoea shows decreased serum level of estradiol-17β \((E2)\) (Russell et al., 1984). Thus, those results might indicate the effect of such decreased serum E2 level on skeletal muscle in female athletes with sports-related-amenorrhea. Recently, top world-class female athletes are becoming younger. Therefore, in the present study we evaluated the effects of ovariectomy on skeletal muscles using 7-week-old young rats. The results showed that four weeks after surgery oxidative capacity of the skeletal muscle was not impaired by ovariectomy in young rats. The contribution of ovarian sex hormones on skeletal muscle between immature and mature rats may be different. Bar et al. (1988) suggested that effects of ovariectomy in immature rats on skeletal muscle were smaller than that in mature rats, since immature rats were never exposed to E2. The present results supported the suggestion.

In summary, 4-weeks after ovariectomy, although maximum tension and oxidative capacity did not change compared to the Sham group, the body and muscle wet weight tended to increase.

References


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Received: November 24, 2000
Accepted: January 9, 2001
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