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Research article

MUSCULAR OXIDATIVE CAPACITY IN OVARIECTOMIZED RATS DISCUSSION ON THE ENDURANCE PERFORMANCE OF FEMALE ATHLETES WITH SPORTS-RELATED-AMENORRHEA

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ABSTRACT

The purpose of this study was to evaluate the effects of ovariectomy on intramuscular energy metabolism in adult rats. Based on the results, we discussed the skeletal muscle metabolism in female athlete with sports related amenorrhea. Twenty-five adult (20-week-old) Sprague-Dawley female rats were used. Fifteen rats underwent ovariectomy (OVX group), and the other ten rats were sham-operated (Sham group). One and four weeks after surgery, muscular oxidative capacity was measured using ^{31}P-MR spectra of the gastrocnemius-plantaris-soleus (GPS) muscles group at rest and during electric stimulation. Wet weight and maximum tension of the whole GPS muscles group were also measured. From the MRS measurements, the muscle oxidative capacity in the OVX group was significantly lower than that in the Sham group ($p < 0.05$) at both one and four weeks after surgery. The muscle's wet weight one week after surgery in the OVX group was the same as the Sham group, while four weeks after surgery it was significantly greater than that in the Sham group ($p < 0.05$). There were no significant differences in maximum tension among the groups. In conclusion, in adult rats the oxidative capacity decreased due to ovariectomy despite the increase in muscle weight. It is suggested that the muscular endurance capacity in female adult athletes with sports related amenorrhea may deteriorate.

KEY WORDS: Sports-related-amenorrhea, skeletal muscle, oxidative capacity, ^{31}P-MRS, ovariectomy.

INTRODUCTION

The female athlete triad, i.e., disordered eating, osteoporosis and amenorrhea, has been well documented (Anderson, 1999; Beckvid et al., 2000; DeCree, 1998; Hobart and Smucker, 2000; Kopp-Woodroffe et al., 1999; Moen et al., 1998; Ramsay and Wolman, 2001; Sanborn et al., 1987; Teitz et al., 1997; Wade, 1972; West, 1998). The sports-related-amenorrhea (SRA) is generally considered a hypothalamic amenorrhea (Anderson, 1999; Loucks, 1990; Russel et al., 1984; Wade, 1972). Because of this condition, the serum concentration of estrogen was reported to be low in women with SRA (Baer, 1993; Russel et al., 1984; West, 1998). Estrogen has an antioxidant activity, thus, estrogen deprivation leads to increased production of free radicals which compromises mitochondrial function by depressing aerobic enzyme activity (e.g., aconitase). This leads to damaging effects on the mitochondrial DNA and mitochondrial membranes (Persky et al., 2000). Therefore, it is not difficult to assume that

hypoestrogenemia would induce functional changes on the oxidative capacity in skeletal muscles. However, the function of skeletal muscles and muscular energy metabolism of female athletes with SRA has not been studied in detail. If the function of skeletal muscles deteriorates under amenorrheic conditions, sports performance as well as trainability of female athletes with SRA will definitely deteriorate.

SRA induces hypoestrogenemia due to hypothalamic dysfunction, especially of the gonadotrophin-releasing hormone pulse generator (Anderson, 1999; Loucks, 1990; Russel et al., 1984; Teitz et al., 1997; West, 1998). So far, there have been no reports of animal models mimicking the hypoestrogenemic condition induced by SRA. Ovariectomy can produce hypoestrogenemia due to ovariogenic dysfunction (Chu et al., 1999). Thus, the ovariectomized (OVX) rat model can provide useful information on what happens in skeletal muscles under hypoestrogenemia.

As the skeletal muscles are essential for physical activity, it is important to examine the changes in energy metabolism of working muscles. Phosphorus-31 magnetic resonance spectroscopy (^{31}P-MRS) is a tool which can evaluate energy metabolism in real time on working muscle *in vivo* (Kato et al., 2000; Kemp et al., 1996; Sairyo et al., 1993, 2003; Sasa et al., 2001, 2004; Yoshida et al., 2001, 2003). The purpose of this study was to evaluate the effects of hypoestrogenemia on the skeletal muscles with reference to energy metabolism in the working muscles as assessed by ^{31}P-MRS using the OVX rat model. Based on the results obtained from this study, the skeletal muscular conditions in female athletes with SRA were discussed.

METHODS

Twenty-five 20-week-old Sprague-Dawley female adult rats were used in this study (Japan SLC Inc., Shizuoka, Japan). Fifteen of them underwent ovariectomy (OVX), and the other ten underwent a sham operation. The rats given OVX were assigned to two groups according to the date of measurements after the surgery; OVX-1 and OVX-4. Six rats in OVX-1 were tested one week after the surgery, and nine in OVX-4 four weeks after surgery. The rats that had undergone a sham operation were also assigned to two sub-groups, Sham-1 and Sham-4. Six rats in Sham-1 group were tested one week after the surgery, and four in Sham-4 four weeks after the surgery. They were housed in individual cages in a temperature ($22 \pm 1°C$) and humidity ($50 \pm 10\%$) controlled room on 12:12 hours light: dark cycle. The animals were allowed free access to food and water. All experiments were performed in accordance with the guidelines for animal experimentation (Orito et al. 1999).

Ovariectomy

After each rat was anesthetized with pentobarbital sodium (50 mg·kg^{-1} body weight), small incisions through the skin and the retroperitoneal area were made on the right and left sides over the lower back. The ovaries were then sectioned from the uterine horns and removed. For the sham operation, ovaries were approached through the incision, lifted out of the rat and placed back in their original position. Muscle and skin incisions were sewn separately with 4.0 silk sutures. The effects of ovariectomy were confirmed after MRS measurement by visual inspection for marked uterine atrophy in OVX rats. The gastrocnemius- plantaris- soleus (GPS) muscles group was subjected to the following measurements and the data from the OVX and Sham groups were compared.

Preparation of the animals for ^{31}P-MRS

After each rat was anesthetized with pentobarbital sodium (50 mg·kg^{-1} body weight), the right sciatic nerve was exposed at the gluteal region and a small bipolar electrode was attached to the sciatic nerve. The rat was immobilized on a small platform with both the knees and ankles in the full-extended position. An oval surface coil (20 x 14mm) for collecting MR spectrum was then placed on the right GPS muscles group. The distal tendon of the GPS muscles group was exposed, cut at its insertion to the calcaneal bone and attached to a strain gauge (T1-1000-240, Orientec Co. Ltd., Tokyo, Japan). The GPS muscles group were passively loaded (100-200g) by altering the position of the strain gauge, so that a supramaximal contraction was produced by a stimulus of 30-50V. After the preparation, the platform with rat was inserted into the bore of the MRS equipment.

Experimental protocol

Following a 3-minute rest, contraction of the GPS muscles group was induced by electrical stimulation (SEN-3301, Nihon Kohden Co. Ltd., Tokyo, Japan) of the sciatic nerve at 0.2 Hz for 10 min. Then, the stimulation frequency was increased to 0.4, 0.6 and 1.0 Hz every 10 min. This stimulation frequency was used to minimize anaerobic metabolism (Cieslar and Dobson, 2000). During the stimulation, twitch forces were recorded on a chart recorder, and ^{31}P-MRS was carried out simultaneously.

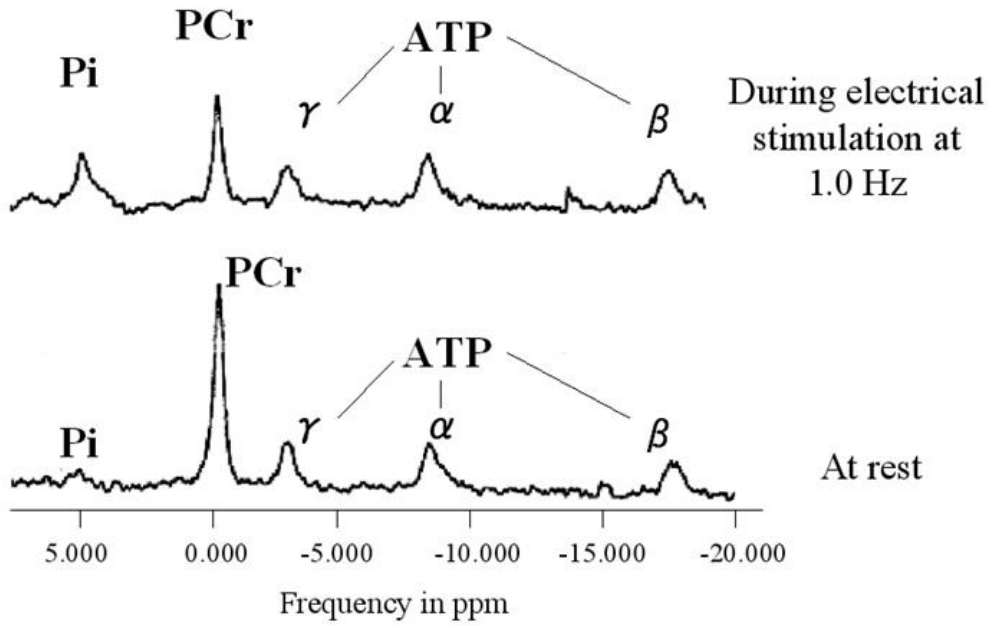

Figure 1. Representative ^{31}P-MR spectra at rest and during electrical stimulation.

^{31}P-MR spectra were recorded with a BEM 170/200 NMR instrument (Otsuka Electronics USA Inc., Fort Collins, CO) equipped with a horizontal 17-cm diameter bore magnet (4.7 Tesla) at 81.1MHz. The repetition time of pulse was 2.0 sec. The most suitable pulse duration (22 μs) was chosen to obtain the maximum signal to noise ratio. Radio-frequency was transmitted and the signal was detected using the surface coil (20 x 14mm). ^{31}P-MR spectra were recorded at rest and during muscle contraction.

Figure 1 represents the typical MR spectra at rest and during muscle contraction due to electrical stimulation at 1.0 Hz. In each spectrum, five major peaks are observed, i.e. inorganic phosphate (Pi), phosphocreatine (PCr), and three ATPs. At rest, the Pi peak is small. For muscle contraction, PCr decreases as it is utilized to produce energy, and Pi increases as energy is consumed. The three peaks of ATP remain stable under moderate exercise. The areas of the Pi and PCr were measured to calculate the PCr/(Pi+PCr) ratio, which indicates the level of available energy. Intracellular pH was also calculated based on the chemical shift (d), which is the distance between Pi and PCr peaks in ppm (Figure 1), using the following equation reported by Flaherty et al. (1982).

Intracellular pH= 6.90 – log [(d-6.81)/(3.29-d)]

The stimulus response in the last minute was regarded as the steady state under each electric stimulation condition. Therefore, we evaluated the energy metabolism at that time. We also calculated the momentum of muscle contractions during the steady state. The force times stimulation rate (F x R) product was used in this study as the indicator of the momentum, because the relationship between F x R and PCr/(Pi+PCr) ratio has been reported to indicate the muscle oxidative capacity (Kemp et al., 1996; McCully et al., 1989; Meyer, 1988; Sasa et al., 2001, 2004; Yoshida et al., 2001, 2003).

The body weight and wet weight of the whole GPS muscles group were determined after MRS measurements, and the maximum tension of twitch was also measured at the muscle contraction induced by 0.20 Hz.

Data analysis

Data were expressed as the means ± SD. One-way ANOVA and post hoc multiple comparison and analysis of covariance (ANCOVA) were used for statistical analysis. For comparison of regression lines, ANCOVA was used. Differences with a p value of less than 0.05 were taken as significant.

RESULTS

MR Spectrometry

MRS measurement was conducted at rest and during 40-min-electric stimulation. In all rats, intracellular pH did not decrease below 7.0 at rest and during muscular contractions, indicating that the muscular

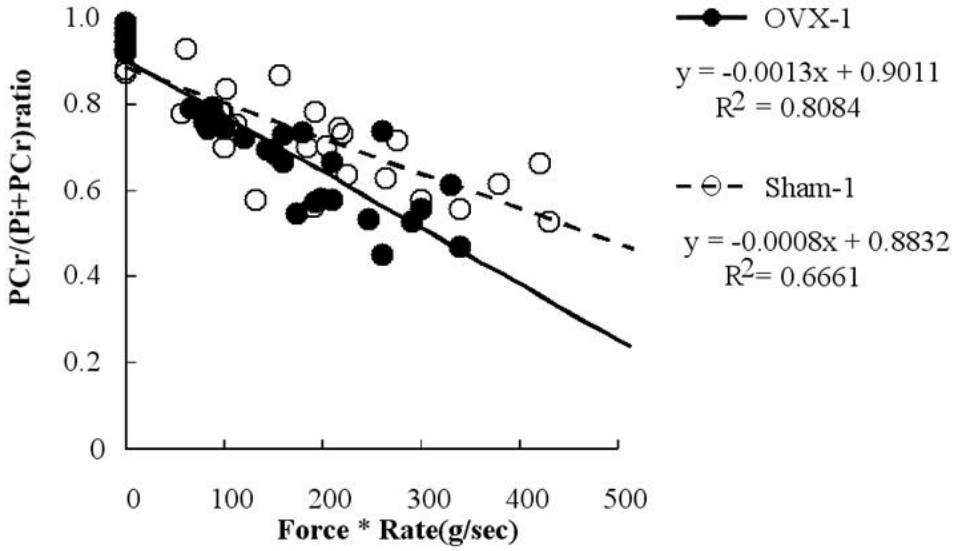

Figure 2. Relationship between PCr/(Pi+PCr) ratio and force time rate in the Sham-1 and OVX-1 groups PCr: phosphocreatine, Pi: inorganic phosphate.

exercise evoked by electric stimulation was aerobic. Significant ($p < 0.05$; ANCOVA) linear relationships between PCr/(Pi+PCr) and F x R were found in all groups during muscular contraction. Figure 2 shows the regression lines in the OVX-1 and Sham-1 groups, and the lines in Figure 3 indicate the relationship between OVX-4 and Sham-4. During aerobic exercise the slope indicates muscle oxidative capacity (Kemp et al., 1996; Meyer, 1988). The slope of the OVX group was significantly steeper than that of the Sham group ($p < 0.05$) one and four weeks after the surgery, indicating the oxidative capacity. It was shown that the oxidative capacity was deteriorated by ovariectomy.

Body and muscle weight

One week after the surgery, body weight was similar in both OVX and Sham groups, but four weeks after the surgery the body weight of the Sham group was significantly ($p < 0.05$) lower than that of the OVX group (Table 1).

The mean wet weight of the whole GPS muscles group in the OVX-1, OVX-4 Sham-1, and Sham-4 group was 1.79, 2.15, 1.77 and 1.94 (g), respectively. No significant difference was found between the OVX-1 and the Sham-1 group, but the weight of the OVX-4 group was significantly ($p < 0.01$) greater than that of the Sham-4 group (Table 1).

Maximum tension

The maximum tension was 425.0, 418.9, 431.7 and 412.5 (gw) in the OVX-1, OVX-4 Sham-1, and Sham-4 groups, respectively. The results showed no significant differences between the OVX and Sham groups (Table 1).

DISCUSSION

In this study, we investigated the effects of ovariectomy on skeletal muscles of adult rats using the ^{31}P-MRS system which can monitor the energy metabolism of skeletal muscle *in vivo* (Kato et al., 2000; Sairyo et al., 1993, 2003; Sasa et al., 2001, 2004; Yoshida et al., 2001, 2003), and clarified that the oxidative capacity deteriorated even though muscle wet weight increased after the ovariectomy.

Table 1. Body weight, muscle wet weight and maximum tension in each group.

	Body weight (g)	Muscle wet weight (g)	Maximum tension (gw)
Sham-1	273.0 (9.5)	1.77 (.06)	431.7 (107.2)
OVX-1	280.0 (14.3)	1.78 (.08)	425.0 (57.9)
Sham-4	278.0 (5.2) *	1.98 (.06) *	412.5 (35.9)
OVX-4	327.0 (11.0)	2.15 (.10)	418.9 (28.9)

* $p < 0.05$ compared with OVX-4.

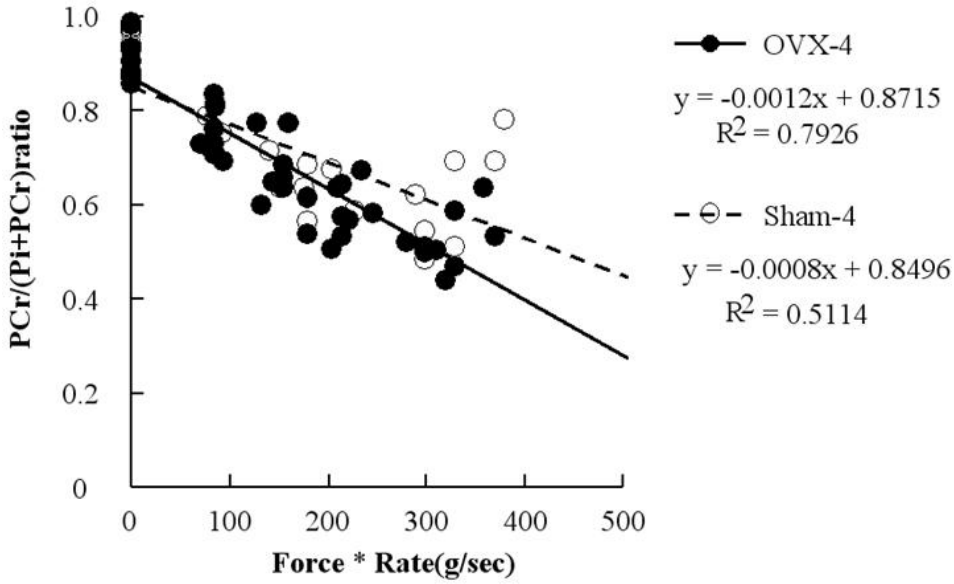

Figure 3. Relationship between PCr/(Pi+PCr) ratio and force time rate in the Sham-4 and OVX-4 groups PCr: phosphocreatine, Pi: inorganic phosphate.

In the literature, there are few *in vitro* studies showing the effects of hypoestrogenemia on energy metabolism of skeletal muscles. Most recently, Gigli and Bussmann (2001) demonstrated the effects of ovarian steroid hormone on mitochondrial respiration of skeletal muscles. They showed that state III oxygen consumption decreased in OVX rats during exercise, and suggested that ovariectomy induced a decrease of the mitochondrial respiration capacity. Moreover, they indicated that estradiol (E2) enhanced mitochondrial respiration. Persky et al. (2000) also suggested that estrogen deprivation led to deterioration of aerobic enzyme activity. Their *in vitro* studies and the present *in vivo* study clarify that hypoestrogenemia deteriorates the oxidative capacity of skeletal muscles.

Because their serum concentration of estrogen is reported to be low (Baer, 1993; Russel et al., 1984; West, 1998), these findings suggest that the oxidative capacity of female athletes with SRA may have deteriorated. For such athletes, endurance performance may not be optimal because of their deteriorated skeletal muscle oxidative capacity. In other words, in sports requiring higher endurance capacity, female athletes should avoid developing SRA.

Since top world-class female athletes are becoming younger, we previously examined the effects of hypoestrogenemia on muscle oxidative capacity using 7-week-old young rats (Sasa et al. 2001), which correspond to the age of human puberty (Toth et al., 2001). We found that muscle oxidative capacity did not change after ovariectomy in young rats, contrary to the results observed in the present study. Thus, the contribution of estrogen to skeletal muscle function may differ between immature and mature rats. Amelink and Bar (1986) examined the effects of OVX in different age group of rats on exercise-induced muscle damage. They found that after OVX in immature rats the muscle was as susceptible to muscle damage as male rats. Also, adult OVX rats had less muscle damage than immature OVX rats. Therefore, they concluded that effects of OVX were age-dependent. Their results are in good agreement with our studies.

McCormick et al. (2004) reported that ovariectomy for the 7 weeks old female rats did not change body weight and muscle fiber size. They concluded that estrogen may inhibit skeletal muscle growth when it is the ovarian hormone present. They, however, found that OVX induced deterioration of muscle contractile function. Toth et al. (2001) revealed that based on the results from the OVX of 7-8 weeks old rats ovarian hormones may influence skeletal muscle growth through their effects on skeletal muscle synthesis. Our previous study demonstrated that there were no effects on muscle wet weight and muscle oxidative capacity in this age groups of rats (Sasa et al. 2001). Thus, the effects of ovariectomy on skeletal muscle in the age group experiencing puberty have been controversial. To elucidate these inconsistent findings of the effects of ovariectomy on muscle, further study will be required.

The body weight of OVX-4 rats was heavier compared to that of the Sham group, and the muscle

weight in the OVX-4 group was also significantly larger than that in the Sham group. The results were in agreement with those of previous studies on OVX rats (Booth and Tipton, 1969; Borski et al., 1996, Toth et al. 2001), indicating that the skeletal muscles in this model were affected by ovariectomy, and we could investigate the effects of ovariectomy on skeletal muscles using this model.

However, the OVX rat model does not fully mimic the conditions necessary to investigate the effects of SRA on skeletal muscle, which was the limitation of this study. To solve this problem, two kinds of studies are proposed; i.e. animal study and human study. For the animal study, we need to establish the model with hypoestrogenemia due to hypothalamic dysfunction, mimicking the human SRA. For the human study, in vitro study using the biopsy samples from athletes with SRA, or in vivo study using this MRS system will be conducted to resolve the limitations of the present study and to fully understand the muscle condition of SRA athletes.

CONCLUSIONS

In conclusion, in adult rats, the oxidative capacity of the GPS muscles decreased due to ovariectomy-induced hypoestrogenemia despite the increase in muscles weight. This suggests that muscular endurance capacity may deteriorate in adult amenorrheic athletes.

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KEY POINTS

- In vivo measurement of muscular energy metabolism.
- Effects of ovariectomy on muscle function and volume.
- Muscle function of sports-related amenorrhea.

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