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Estrogen and Gender Do Not Affect Fatigue Resistance of Extensor Digitorum Longus Muscle in Rats

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Summary

The effects of estrogen on skeletal muscle fatigue are controversial. To determine the effects of estrogen and gender on rat extensor digitorum longus (EDL) muscle, we either injected 40 μg β-estradiol 3/benzoate·kg BW^{-1} to female rats or sham injected male or female rats for 14 days. Subsequently a 90 min fatigue protocol consisting of electrical stimulation at 10 Hz delivered in 500 ms trains was administered. Force was recorded for a 5 s period at the start of the protocol (0 min) and at 5 min intervals until completion following 90 min of stimulation. After 90 min, EDL force generation at 10 Hz stimulation declined in all groups to between 50-60 % of initial values. However, no significant difference in fatigue rate or final 10 Hz stimulated force was seen between females administered estrogen, sham injected females or males. Hence, estrogen administration and gender did not significantly affect EDL muscle fatigue in this model.

Key words

Muscle fatigue • Estrogen • Gender • Extensor digitorum longus

Introduction

Recent reports on the efficacy of estrogen to affect skeletal muscle force generation and fatigue rates in humans have been contradictory. Phillips *et al.* (1993) measured age/related changes in maximal isometric force per cross-sectional area of adductor pollicis muscle in males and females aged 17-90 years. There was no difference between age/matched males and females until female menopause, when a significant decline in force relative to males occurred. This decline was not evident in females taking estrogen replacement therapy. Sarwar *et al.* (1996) reported menstrual cycle related variations in forearm and quadriceps fatigue resistance and strength in 19 to 24-year-old females, with peak strength and fatigue resistance corresponding to peak circulating estrogen levels. Females taking oral contraceptives exhibited no such menstrual cycle related variation in muscle fatigue resistance or strength. Skelton *et al.* (1998) also recently reported increased adductor pollicis strength in post-menopausal women taking hormone replacement therapy, when compared to untreated women.

In contrast, no differences were found in quadriceps or hand grip strength (expressed as an index of

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fat-free mass) between pre- and post-menopausal females, aged 45-54 years (Bassey *et al.* 1996). Greeves *et al.* (1997) also reported no effect of estrogen on force or fatigability of the first dorsal interosseus muscle in women supplemented with supra-physiological levels of estrogen as a part of *in vitro* fertilization procedures. Furthermore, there have not been any general trends toward differences in performance during various menstrual cycle phases of elite female strength or endurance athletes (Lebrun 1993). Hence, no clear answer has yet emerged as to the potential for estrogen to affect muscle fatigue during repeated contractions.

It is not currently known how estrogen may act by influencing muscle fatigue, although several potential mechanisms exist. Estrogen has the potential to spare muscle glycogen by enhancing fat metabolism during exercise (Kendrick *et al.* 1987), and to act as an antioxidant and prevent exercise-induced membrane damage (Bär *et al.* 1997, Tiidus 1999). Estrogen may also influence skeletal muscle mass and contractility (Fisher *et al.* 1998). However, the potential mechanism for its ability to delay fatigue resistance, if it indeed exists, is still unknown.

Since numerous extraneous factors may affect muscle fatigue rates in human experimental models, we attempted to determine the effects of estrogen administration and gender on muscle fatigue by using an electrically stimulated *in situ* rat muscle preparation.

Method

This study was approved by the University of Waterloo Committee on Animal Care and was performed in accordance to the guiding principles of the Canada Council on Animal Care. To assess the effects of estrogen administration on female muscle fatigue rates, sexually mature female Wistar (Charles River) rats (aged 12-14 weeks) were randomly assigned to either an estrogen injection group (n=7), or a sham injection group (n=6). In order to assess gender differences in muscle fatigue using this model, a group of 5 male Wistar rats were also used as a sham injected group. Estrogen treatment consisted of subcutaneous estrogen injections (40 μg β-estradiol 3-benzoate in virgin olive oil vehicle · kg BW^{-1}) (Sigma Chemical, St. Louis MO) on 14 consecutive days. The sham injected male and female animals received vehicle alone. The estrogen injected female rats were exposed to approximately twice their normal average physiological levels of estrogen (Kendrick *et al.* 1987). Bär *et al.* (1988) reported that 14 days of estrogen administration was effective in reducing *in vitro* enzyme leakage from electrically stimulated rat muscle. Hence this period of injection was assumed to be appropriate for establishing estrogen protection from exercise induced oxidative stress or damage in rat tissues.

Twenty-four hours after the final injection, animals were anesthetized *via* intraperitoneal injection of sodium pentobarbital (0.6 mg · 100 g BW^{-1}). Both hind limbs were prepared for *in situ* electrical stimulation and assigned to either control or stimulated conditions. External skin and fascia were removed, the distal tendon of the extensor digitorum longus (EDL) muscle was freed, tied with silk suture and the suture tied to a Grass FT03 force transducer (Quincy, MA). To stabilize the knee joint at 90° a stainless steel 18.5 gauge needle was inserted through the joint capsule. Muscle temperature was monitored *via* a surface electrode and maintained between 28-31 °C *via* a heat lamp. Moisture was maintained throughout the experiment by applying Ringer's solution to the muscles. Stimulation was accomplished with a Grass 584 stimulator (Quincey, MA) *via* electrodes inserted in the superior and inferior hind limb. Muscle force data were collected on line and analyzed using a Watscope data acquisition unit and software (Northern Digital, Waterloo CA). Optimal EDL muscle length (L_0) was determined as the muscle length at which maximal twitch force is produced. After determination of L_0, a 90 min fatigue protocol consisting of electrical stimulation at 10 Hz delivered in 500 ms trains was administered. This degree of stimulation will produce successive submaximal contractions. For the first 25 min of the protocol, the EDL was stimulated once every 3 s. This increased to once every 2 s between 25-45 min, once every 1.5 s between 45 and 75 min and once every second between 75-90 min. Force was collected for a 5 s period at the start of the protocol (0 min) and at 5 min intervals until completion following 90 min of stimulation. Muscle stimulation at the time of collection was the same as that of the fatigue protocol. The general state of the animal was monitored throughout the experiment. In addition, 10 Hz stimulated force was determined in the control leg at the beginning and end of the experiment to ensure that no significant change in muscle viability had occurred during the course of the experiment.

Two-way analysis of variance (ANOVA) with repeated measures was performed to determine statistical significance which was set at $P<0.05$.

Results

The results of the fatigue protocol to electrically stimulated submaximal EDL muscle force generation in female, male and estrogen injected female rats are depicted in Figure 1.

Following an initial potentiation, the force declined significantly ($P<0.05$) in all groups to approximately 50-60 % of initial values after 90 min of stimulation. There were no significant differences in the rate of EDL muscle fatigue or degree of force produced at any time point, between sham injected males and females or estrogen injected females ($P>0.05$) throughout the stimulation protocol.

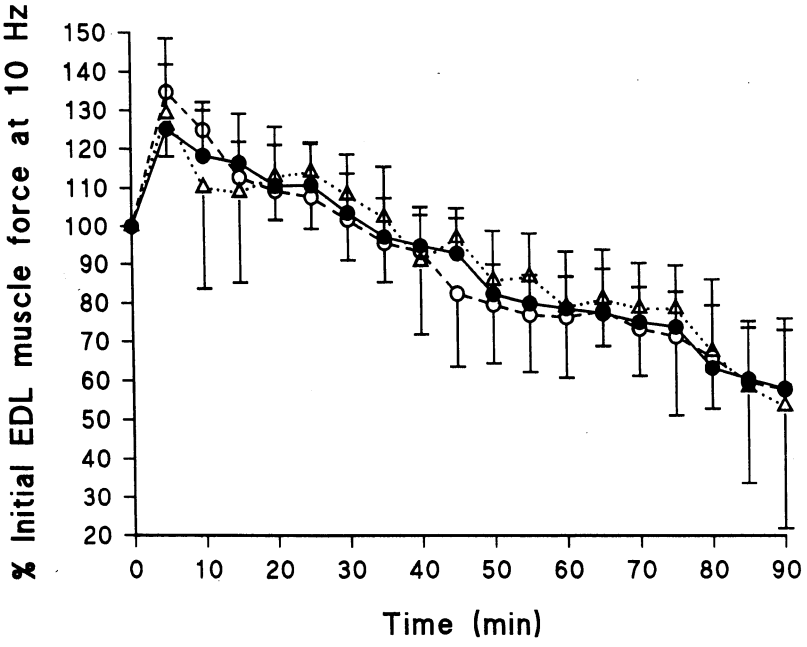

Fig 1. *Effect of gender and estrogen supplementation on submaximal (10 Hz) electrically stimulated EDL muscle fatigue rate in rats. Points are means ± S.D. Full dots represent estrogen-supplemented females (FE), open circles represent sham- injected females (FS), triangles represent sham injected males (MS). No significant differences were found between these groups (P>0.05).*

Discussion

The results of this study suggest that neither gender (male/female differences) nor prior estrogen administration to females affected the fatigability of rat EDL muscle under the concentric submaximal muscle stimulation protocol employed in this study. Warren *et al.* (1996) have also reported no difference in eccentric peak torque of EDL muscles following 150 eccentric contractions in ovariectomized controls or mice who were given estrogen for 21 days at a dosage similar to this study. These findings support the results of Greeves *et al.* (1997) who also did not find any effect of supra-physiological estrogen levels on the fatigue rate of the first dorsal interosseus muscle in young human females. On the other hand, these findings are in contrast to Sarwar *et al.* (1996) who reported a slower rate of fatigue in quadriceps muscle fatigue in young human females during mid-follicular and ovulatory menstrual phases than during luteal or early follicular phases. Greeves *et al.* (1997) have suggested that progesterone or a progesterone-estrogen interaction, rather than estrogen alone, may be responsible for any menstrual cycle or post- menopause related effects on human muscle function reported by studies such as Sarwar *et al.* (1996). These possibilities warrant further investigation. Alternatively, the cyclical nature of estrogen exposure associated with a normal menstrual cycle may be necessary to cause the changes in muscle fatigability reported by Sarwar *et al.* (1996). As Sarwar *et al.* (1996) noted, women taking oral contraceptives failed to demonstrate changes in muscle fatigue rate over the course of their menstrual cycle.

It should also be noted that Sarwar *et al.* (1996) induced muscle contraction *via* electrical stimulation of the motor nerve. Hence, it is possible that the effect of estrogen on muscle contraction may be manifested through neuromuscular interactions, since our study demonstrated no effect of estrogen on muscle fatigability induced by direct electrical stimulation.

Estrogen may theoretically also affect the muscle fatigue rate by altering muscle metabolism to greater fatty acid utilization and glycogen sparing (Kendrick *et al.* 1988), by reducing exercise-induced oxidative stress and related muscle fatigue and damage (Tiidus 1995, 1999, Bär *et al.* 1997), or by affecting muscle mass and contractility (Fisher *et al.* 1998). However, in the present

study, rat EDL fatigue rates were similar in male, female and estrogen-supplemented female rats. Hence, either these factors were not involved in the submaximal EDL contraction fatigue seen in this study or estrogen did not significantly influence these factors in this model. The lack of gender differences in EDL fatigue rate seen in this study also suggest that chronic exposure to normally higher estrogen levels as occurs in female rats when compared to males will also not influence skeletal muscle fatigue.

Recent findings in our laboratory suggest that estrogen may not greatly enhance antioxidant protection during *in vivo* exercise (Tiidus *et al.*1998). If this is the case, then estrogen may not be an effective agent in diminishing oxidative stress-induced muscle fatigue during exercise.

Since the rat EDL muscle is composed primarily of Type IIa and IIb fibers, the inability of estrogen to affect fatigue rate in this muscle may not necessarily apply to other muscles with different fiber compositions. It is possible that some of the variability in the reported effects of estrogen on muscle strength and fatigue in human females may be in part due to the potential differences in fiber type composition of the various muscles tested in previous studies. The possibility of differential effects of estrogen on the strength and fatigability of different muscle fiber types warrants further investigation.

In summary, this study did not demonstrate any difference in rat muscle EDL fatigue rate induced by 90 min of submaximal electrical stimulation between male and female rats. As well, there were no differences in EDL muscle fatigue rate between the sham injected male or female rats and female rats supplemented with estrogen for 14 days prior to the experiment. This suggests that neither gender nor estrogen supplementation influenced EDL muscle fatigue rates in the experimental model employed in this study and are in agreement with previous reports which used human females and also found no effect of estrogen or menstrual cycle on skeletal muscle fatigue.

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