JEP**online**
Journal of Exercise Physiologyonline

Official Journal of The American Society of Exercise Physiologists (ASEP)

ISSN 1097-9751
An International Electronic Journal
Volume 4 Number 2 May 2001

Systems Physiology : Skeletal Muscle

EFFECTS OF CIRCULATING ESTRADIOL ON EXERCISE-INDUCED CREATINE KINASE ACTIVITY

STEPHEN M. ROTH[1], RICHARD GAJDOSIK[2] AND BRENT C. RUBY[1]

[1] Human Performance Laboratory, Department of Health and Human Performance & [2] Motor Control Research Laboratory, Department of Physical Therapy, The University of Montana, Missoula, MT 59812, USA

ABSTRACT

STEPHEN M. ROTH, RICHARD GAJDOSIK AND BRENT C. RUBY. **Effects Of Circulating Estradiol On Exercise-Induced Creatine Kinase Activity**. JEP**online**. 2001;4(2):10-17. The present investigation sought to determine the effects of circulating estradiol (E2) and oral contraceptive use on serum creatine kinase (CK) activity following acute eccentric exercise. Ten college-aged female subjects were grouped according to their use (OBC, N=5) or non-use (NOBC, N=5) of an oral contraceptive. Both groups performed strenuous eccentric muscle actions (5 sets with 10 repetitions/set; ≥100% of concentric 1RM) during the menstrual cycle phase corresponding to low E2 concentrations. The OBC group had significantly lower E2 concentrations than the NOBC group (11.6 versus 27.2 pg/mL, respectively; $p<0.01$) prior to exercise. The results indicated that the OBC group (lower E2) had significantly higher CK activity at 48 and 72 hr post-exercise when compared to baseline CK activity ($p<0.05$). Although the NOBC (higher E2) group showed a slight increase in CK activity at 24, 48 and 72 hr post-exercise, values were not significantly different from pre-exercise. Perceived soreness values were generally dissociated from CK activity, but demonstrated a similar post-exercise response between groups. The results indicate that pre-exercise concentrations of circulating estradiol can affect serum CK activity following damaging eccentric exercise.

Key Words: eccentric exercise, estrogen, muscle damage, oral contraceptive, soreness

INTRODUCTION

Strenuous exercise, especially exercise involving eccentric muscle actions, has been shown to elicit histological and ultrastructural muscle damage (1,2). One consequence of damaging exercise is the release of various skeletal muscle proteins into the blood. Increased serum creatine kinase (CK) activity has been used as a marker of muscle cell disruption, and has been indirectly associated with increased membrane permeability (2,3). However, other factors are known to affect membrane structure, including vitamin E and estradiol (E2), and thus these agents could affect serum CK activity following strenuous activity (4,5).

Lower levels of serum creatine kinase activity following strenuous exercise have been shown in females compared to males (6), indicating a possible role for estrogens in the CK response (7). Moreover, research in animals has indicated a role for estradiol in reducing membrane permeability following damaging exercise. For example, Amelink et al. (8) and others (9-11) have concluded that higher E2 concentrations decreased serum CK activity following stimuli that induced muscle damage. Recently, female rats demonstrated lower levels of histological muscle damage than male rats following eccentrically biased downhill running (1).

Research in humans on the direct role of estradiol in creatine kinase activity is limited. Buckley-Bleiler et al. (12) and Miles and Schneider (13) found no effect of E2 on CK activity following exercise, but both studies are associated with limitations including exercise intensity and E2 variability, respectively. Hayward et al. (14) reported that women taking an oral contraceptive had lower CK activity following eccentric muscle actions compared to controls, but the results were limited due to high variability in both CK activity and E2 concentration. Thompson et al. (15) reported that estradiol concentration was not related to CK release following stepping exercise, despite the fact that the group with higher E2 concentrations had a peak CK activity value six times lower than the low E2 group. In an attempt to clarify these discrepancies, the purpose of the present investigation was to assess the role of oral contraceptive (OBC) use and circulating estradiol concentration in creatine kinase activity and muscle soreness following strenuous eccentric exercise.

METHODS

Subject Characteristics

Ten college-aged (19-23 yr) females read and signed a written informed consent approved by the Institutional Review Board at The University of Montana. The subjects were moderately active physical education students who had not previously engaged in an eccentric exercise program or activities associated predominantly with eccentric muscle actions. The subjects were grouped according to their use (OBC, n=5) or non-use (NOBC, n=5, free from use for at least 6 months) of an oral contraceptive. All OBC subjects used a 28-day prescription containing ~30 mg Ethinyl E2. Exercise testing occurred according to the menstrual cycle phase associated with low E2 concentration. Thus, the OBC group was tested approximately 8-2 days prior to menses in accordance with the OBC-induced progestin phase. The NOBC group was tested approximately 0-2 days following menses in accordance with early follicular levels of E2.

Eccentric Exercise Protocol

All subjects completed a unilateral eccentric hamstring exercise protocol using a KIN-COM isokinetic dynamometer (Chattanooga, TN). Prior to the experimental protocol, all subjects performed a concentric one-repetition maximum (1RM) of the dominant hamstrings, which was used as a ‘goal’ force output for the eccentric exercise protocol. Before each test, the subjects performed a 3-min warm-up on a stationary cycle with light resistance and 5-10 min of static stretching of the hamstrings and quadriceps to help prevent injury. The 1RM consisted of repeated bouts (6-8) of one concentric repetition at steadily increasing resistance until failure, with 1-2 min rest periods between each bout. After one failed attempt at the final resistance, the highest resistance attained was defined as the 1RM.

Following the 1RM test, subjects rested for 5 min before performing five sets of 10 eccentric muscle actions with both the dominant and non-dominant hamstrings. Each repetition required that the subject maximally resist the KIN-COM's movement arm starting from full knee extension, through a range of motion approximating 90^{o} at an arm velocity of 60^{o}/s. The repetitions were performed in the seated position with the subject's non-dominant leg and upper body secured to the KIN-COM device. The graphical output corresponding to the elicited force output was in view of each subject and subjects were verbally encouraged to meet or surpass either the concentric 1RM force level or the level of the previous eccentric action throughout each set. Sets were separated by 2-3 min to allow recovery. The exercise protocol was repeated for the non-dominant leg after a 5-min rest period using the same conditions. Verbal encouragement was provided for all subjects by the

test administrator in an attempt to elicit maximal efforts at each muscle action, such that the resistance attained was equal to or higher than the dominant leg's concentric 1RM. The same individual administered all strength tests and was blind to the OBC condition of each subject.

Subjects were asked to refrain from physical activity for 48 hr prior to, and 72 hr following the exercise protocol to decrease the influence of physical activity on the serum CK activity elicited by the exercise protocol. Baseline serum CK activity of approximately 50 U/L indicated that no prior activity occurred in either group (16).

Blood Sampling and Analysis

Blood sampling occurred immediately before exercise (pre-exercise), immediately after exercise (post-exercise) and at 24, 48 and 72 hr post-exercise. Whole blood was allowed to clot at room temperature and the serum was stored at -20^{o}C until analysis. Serum CK activity was measured using standard spectrophotometric techniques (U/L at 30^{o}C, assay kit #47-20, Sigma Corp., St. Louis, MO). Serum E2 was measured using radioimmunoassay (pg/ml at 30^{o}C, assay kit # TKE21, Coat-A-Count, Diagnostic Products, Los Angeles, CA) with a detection limit of 8 pg/mL. All subjects had E2 concentrations >8 pg/mL. All analyses were performed in duplicate.

Muscle Soreness

Perceived muscle soreness was assessed pre-, immediately post- and 24, 48 and 72 hr post-exercise. The questionnaire used for this assessment was adapted from a questionnaire developed by Morgan et al. (17). The questionnaire required the subject to recall how her muscle 'felt' throughout the morning prior to the workout (pre-exercise value) or blood sampling (post-exercise) time points, and then asked the subject to assign a number from one to seven based on her perception of muscle soreness. Each subject assigned a numeric value corresponding to her perceived soreness, with '1' referring to 'very, very good' and '7' referring to 'very, very sore.' Subjects were allowed to use half points to more accurately express their level of soreness.

Statistics

Statistical analysis included *a priori* planned comparisons between cell means across time and OBC status for both CK activity and soreness. An *a priori* planned comparison for the main effect of time utilized a 2 x 5 split plot (1 within = time; 1 between = OBC status) repeated-measures ANOVA. Student's *t*-tests were performed on all other between group comparisons. Statistical significance was accepted at $p<0.05$. Data are presented as means±SE.

RESULTS

Circulating E2 concentrations were low in both groups compared to average mid-luteal concentrations (i.e. ~150 pg/mL) but were significantly higher ($p< 0.01$) in the NOBC group compared to the OBC group (Table 1).

Table 1. Descriptive data for OBC (n=5) and NOBC (n=5) groups

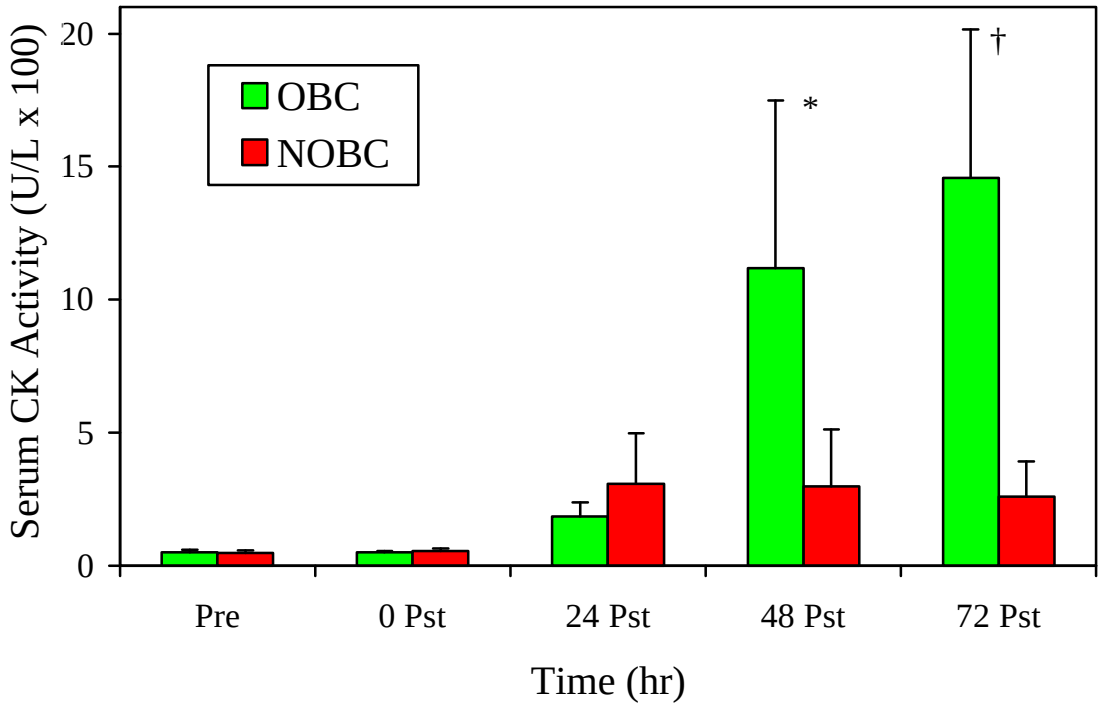
Group	*Mean E2*	*E2 Range*	*Eccentric Force*	*%Ecc/Con*
OBC	11.59±1.0*	10-14	240±17 N	100±11
NOBC	27.23±2.8	19.1-34.6	266±9 N	110±7

Data are means±SE. %Ecc/Con, percent of eccentric force versus dominant leg's concentric one-repetition maximum (1RM). Estradiol (E2) concentrations in pg/mL at 30^{o}C. *Significantly different from NOBC, $p<0.01$

For each leg, all subjects completed 50 unilateral eccentric muscle actions with mean force outputs at or above the dominant leg concentric 1RM (Table 1).

Serum CK activity remained at pre-exercise levels immediately post-exercise and began increasing at 24 hr post-exercise with peak values occurring at 24 hr post-exercise for the NOBC group and 72 hr post-exercise for the OBC group (Figure 1). The OBC group (lower E2) had significantly higher ($p<0.05$) CK activity at 48

Figure 1. Changes in serum CK activity before (Pre), immediately Post- (0 Pst), and 24, 48 and 72 hr following eccentric exercise. *Significantly different from pre-exercise, p<0.05; †Significantly different from pre-exercise, p<0.01; Data are mean CK (U/L at 30 ºC)±SE.



and 72 hr post-exercise when compared to the pre-exercise CK levels. CK values for the NOBC group (higher E2) declined slightly after 24 hr post-exercise and were not significantly different from pre-exercise values at any timepoint. The group x time interaction was significant (p=0.04).

Both groups reported a range of soreness ratings from 2.0 - 5.7 (scale=1-7), with the highest ratings occurring at 24 and 48 hr post-exercise (Figure 2). Soreness values increased significantly in both groups at all post-exercise timepoints (Figure 2; p<0.05). The NOBC group, which had a lower overall CK response to the exercise protocol, exhibited a higher mean rating of muscle soreness (4.4±0.3) following the exercise protocol compared to the OBC (higher CK response) group (3.8 ± 0.3) (p<0.01).

DISCUSSION

The results of the current investigation indicate that estradiol (independent of OBC use) has a role in creatine kinase activity in humans following damaging exercise, as the subjects with higher E2 levels exhibited no significant change in CK activity, while subjects with lower E2 levels exhibited a significant increase in CK activity at 48 and 72 hr post-exercise. Further, these results support prior research pointing to the lack of an association between perceived muscle soreness and CK activity (15,18).

CK activity has been shown to be lower following strenuous exercise in females compared to males (6,7). Following a marathon, serum CK activity was five-times higher in males versus females (7). The role of E2 was not directly assessed in those investigations. Amelink et al. (8-10) completed a series of investigations in rats both *in vivo* and *in vitro* indicating that E2 reduced the increase in serum CK activity normally observed following damaging exercise or electrical stimulation. Further, the data of Bar et al. (19) showed the protective effects of E2 treatment in male and ovariectomized female rats. Following a 2-hour treadmill run, CK activity

increased nearly 4-fold for the male and ovariectomized female rats; however, male and ovariectomized female rats treated with E2 showed less than a 1-fold increase in CK activity in response to the same protocol.

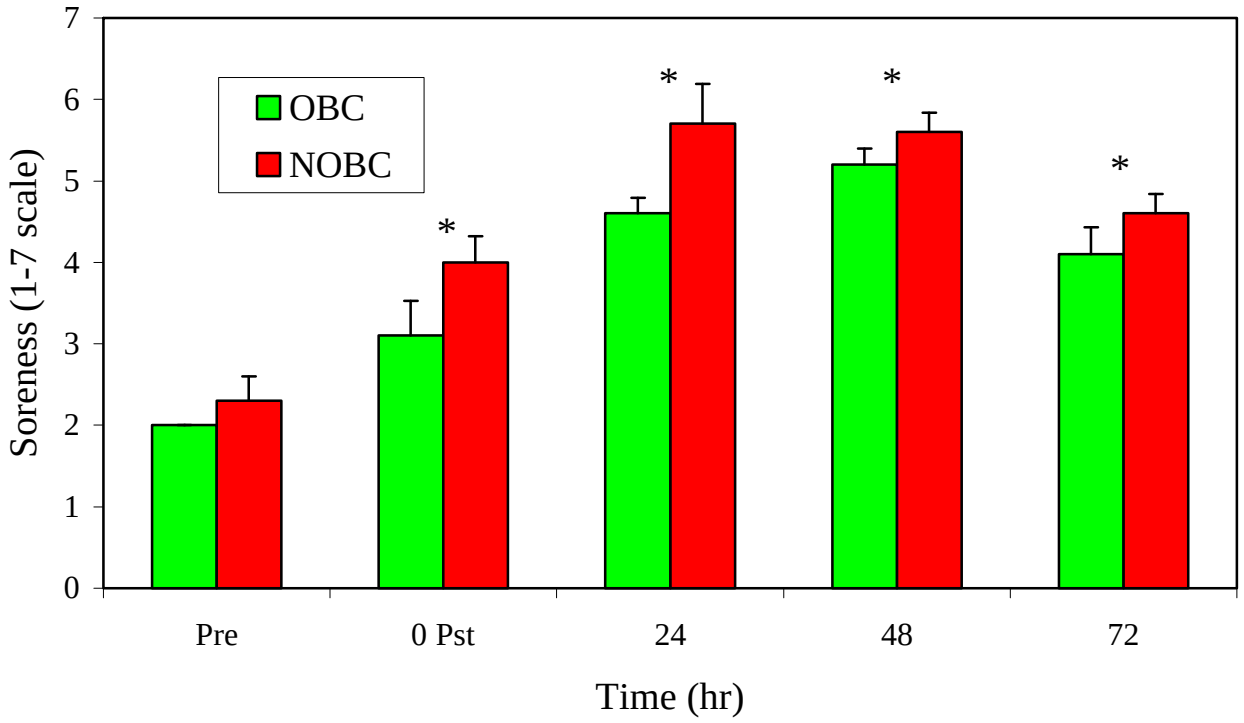


Figure 2. Mean soreness ratings (± SE) for both OBC and NOBC groups (scale = 1-7) at pre-exercise (Pre), immediately post-exercise (0 Pst) and 24, 48 and 72 hr post-exercise. *Significantly different from pre-exercise, $p<0.05$.

Recently, Komulainen et al. (1) reported the first direct evidence of gender differences in muscle damage following eccentric exercise, although estrogen levels were not assessed. They observed no changes in muscle microarchitecture immediately following and six hours after downhill treadmill running in female rats, while male rats demonstrated muscle damage immediately after and six hours after the exercise bout (1). Possible gender differences in muscle damage in response to strength training were recently reported in humans (20). Human research addressing the role of E2 in CK activity following exercise has been limited. Buckley-Bleiler et al. (12) found no effect of E2 on CK activity following an isometric exercise protocol. The results of that study are limited, as CK activity did not increase following the exercise protocol, staying within normal resting levels (highest post-exercise CK = 75 U/L, normal level <100 U/L) (12,16). Miles and Schneider (13) examined serum CK activity in 15 females following 40 min of stepping exercise. The exercise protocol was not standardized to menstrual cycle phase and E2 concentrations varied widely between subjects (10-359 pg/mL). The researchers reported no relationship between E2 and post-exercise CK activity, but noted that low sample size and data variability may have contributed to that result (13). In an abstract, Hayward et al. (14) reported 17 and 59-fold increases in CK activity in oral contraceptive users and non-users, respectively, following eccentric muscle actions. The variation in these values suggests a relationship between E2 and post-exercise CK activity; however, because of the variability in circulating E2 levels (50-220 and 60-400 pg/mL for OBC users and non-users, respectively) (14), the relationship between E2 and CK activity is difficult to interpret. Thompson et al. (15) examined the effects of OBC use on muscle damage following 50 min of stepping exercise. The researchers measured E2 concentrations in both OBC and NOBC groups, similar to the present

investigation. In contrast to the current study, the researchers tested subjects during the phase of the menstrual cycle that maximized the E2 environment. Similar to the results reported here, CK activity increased more in those women with low E2 (OBC, 17 pg/mL) compared to those with higher E2 levels (NOBC, 116 pg/mL). The peak CK activity was 715 vs 161 U/L for the two groups, respectively. However, baseline CK levels differed substantially in the two groups (123 vs. 51 U/L, respectively) and statistical significance was not attained leading the researchers to conclude that E2 was not related to CK activity following strenuous exercise (15).

The results of the current investigation indicate that estradiol can affect serum creatine kinase activity following damaging eccentric exercise. Subjects with low E2 concentrations (OBC - 11.6 pg/mL) demonstrated significantly higher CK activity than baseline at 48 and 72 hr post-exercise. The women with higher E2 concentrations (NOBC - 27.2 pg/mL) did not show a statistically significant increase in CK activity following exercise at any time point. The large increases in CK activity reported in the current study are much higher than those reported previously (12,15), likely the result of the strenuous nature of the exercise protocol used in the present study, which was designed to elicit muscle damage. One limitation of the current investigation is a low sample size; however, the sample size reported here is similar to that of Thompson et al. (15).

The results presented here also point to the limitations of CK as a marker of muscle damage. While CK has been associated with both soreness and muscle edema (21,22), direct associations between serum CK activity and histological and ultrastructural muscle damage remain unremarkable (2,23) and inter- and intra-subject variability are high (24,25). As such, some researchers have moved from associating CK with 'muscle damage' to 'membrane damage' or 'membrane permeability' (2,18). The results of the current study and those of Miles and Schneider (13) and Thompson et al. (15) suggest that CK variability limits the ability to assess statistical differences in CK activity.

The present results support prior research indicating a lack of association between perceived muscle soreness and serum CK activity (15,18). Perceived muscle soreness (mean value) was higher in the NOBC group compared to the OBC group (4.4 vs. 3.8; $p>0.05$), although both groups showed a similar significant increase over time compared to pre-exercise values (Fig. 2). These results contradict the CK activity results of each group, as the NOBC group showed a lower increase in CK response over time compared to the OBC group. The results, however, do support data presented by Thompson et al. (15) that indicated lower ratings of soreness (increased pain threshold) in women taking an OBC. The present results point to the inadequacy of CK as an indicator of muscle soreness, and show that the relationship between membrane damage (interpreted from serum CK activity) and actual soreness is tentative at best.

CONCLUSIONS

The present results indicate that circulating levels of estradiol affect post-exercise creatine kinase activity independent of oral contraceptive use. Further, serum CK activity does not necessarily correspond to perceived soreness levels following damaging exercise, although OBC status may be related to soreness ratings. Future work should determine the effects of E2 administration on serum CK activity in males following damaging exercise to verify the present results. The results presented here clearly suggest that investigators must carefully control for the possible effects of estradiol when assessing serum creatine kinase activity in females.

ACKNOWLEDGMENTS

The authors thank the volunteers for their participation in this study. Funding for this investigation was awarded to B. C. Ruby from the University Grant Program, Office of Research Administration, The University of Montana. S.M. Roth's current address is A300 Crabtree Hall, Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA 15261.

REFERENCES

1. Komulainen J, Koskinen SOA, Kalliokoski R, Takala TES, Vihko V. Gender differences in skeletal muscle fiber damage after eccentrically biased downhill running in rats. ***Acta Physiol.Scand.*** 1999;165:57-63.

2. Van Der Meulen JH, Kuipers H, Drukker J. Relationship between exercise-induced muscle damage and enzyme release in rats. ***J.Appl.Physiol.*** 1991;71:999-1004.

3. Goodman C, Henry G, Dawson B, Gillam I, Beilby J, Ching S *et al*. Biochemical and ultrastructural indices of muscle damage after a twenty-one kilometre run. ***Austral.J.Sci.Med.Sport*** 1997;29:95-98.

4. Van Der Meulen JH, McArdle A, Jackson MJ, Faulkner JA. Contraction-induced injury to the extensor digitorum longus muscles of rats: the role of vitamin E. ***J.Appl.Physiol.*** 1997;83:817-23.

5. Bar PR, Amelink GJ. Protection against muscle damage exerted by oestrogen: hormonal or antioxidant action? ***Biochem.Soc.Trans.*** 1997;25:50-54.

6. Shumate JB, Brooke MH, Carroll JE, Davis JE. Increased serum creatine kinase after exercise: a sex-linked phenomenon. ***Neurol.*** 1979;29:902-04.

7. Rogers MA, Stull GA, Apple FS. Creatine kinase isoenzyme activities in men and women following a marathon race. ***Med.Sci.Sports Exerc.*** 1985;17:679-82.

8. Amelink GJ, Kamp HH, Bar PR. Creatine kinase isoenzyme profiles after exercise in the rat: sex-linked differences in leakage of CK-MM. ***Pflugers Arch.*** 1988;412:417-21.

9. Amelink GJ, Koot RW, Erich WBM, Van Gijn J, Bar PR. Sex-linked variation in creatine kinase release, and its dependence on oestradiol, can be demonstrated in an in-vitro rat skeletal muscle preparation. ***Acta Physiol.Scand.*** 1990;138:115-24.

10. Koot RW, Amelink GJ, Blankenstein MA, Bar PR. Tamoxifen and oestrogen both protect the rat muscle against physiological damage. ***J.Steroid Biochem.Mol.Biol.*** 1991;40:4-6.

11. Persky AM, Green PS, Stubley L, Howell CO, Zaulyanov L, Brazeau GA *et al*. Protective effect of estrogens against oxidative damage to heart and skeletal muscle *in vivo* and *in vitro*. ***Proc.Soc.Exp.Biol.Med.*** 2000;223:59-66.

12. Buckley-Bleiler R, Maughan RJ, Clarkson PM, Bleiler TL, Whiting PH. Serum creatine kinase activity after isometric exercise in premenopausal and postmenopausal women. ***Exp.Aging Res.*** 1989;15:195-98.

13. Miles MP, Schneider CM. Creatine kinase isoenzyme MB may be elevated in healthy young women after submaximal eccentric exercise. ***J.Lab.Clin.Med.*** 1993;122:197-201.

14. Hayward, J. R., Dennehy, C. A., Rodearmel, S., Lennon, A., and Schneider, C. M. Effect of oral contraceptive on serum creatine kinase and CK MB following exercise-induced muscle injury. ***Med.Sci.Sports Exerc.*** 26(5 supp), S169. 1994.
Ref Type: Abstract

15. Thompson HS, Hyatt J-P, DeSouza MJ, Clarkson PM. The effects of oral contraceptives on delayed onset muscle soreness following exercise. ***Contraception*** 1997;56:59-65.

16. Hortobagyi T, Denahan T. Variability in creatine kinase: methodological, exercise, and clinically related factors. ***Int.J.Sports Med.*** 1989;10:69-80.

17. Morgan WP, Costill DL, FLynn MG, Raglin JS, O'Connor PJ. Mood disturbance following increased training in swimmers. ***Med.Sci.Sports Exerc.*** 1988;20:408-14.

18. Vincent HK, Vincent KR. The effect of training status on the serum creatine kinase response, soreness and muscle function following resistance exercise. ***Int.J.Sports Med.*** 1997;18:431-37.

19. Bar PR, Amelink GJ, Oldenburg B, Blankenstein MA. Prevention of exercise-induced muscle membrane damage by oestradiol. ***Life Sci.*** 1988;42:2677-81.

20. Roth SM, Martel GF, Ivey FM, Lemmer JT, Metter EJ, Hurley BF *et al.* High volume, heavy resistance strength training and muscle damage in young and older women. ***J.Appl.Physiol.*** 2000;88:1112-18.

21. Newham DJ, Jones DA, Edwards HT. Large delayed plasma creatine kinase changes after stepping exercise. ***Muscle Nerve*** 1983;6:380-85.

22. Nosaka K, Clarkson PM. Variability in serum creatine kinase response after eccentric exercise of the elbow flexors. ***Int.J.Sports Med.*** 1996;17:120-27.

23. Jones DA, Newham DJ, Round JM, Tolfree SEJ. Experimental human muscle damage: morphological changes in relation to other indices of damage. ***J.Physiol.*** 1986;375:435-48.

24. Nosaka K, Clarkson PM. Influence of previous concentric exercise on eccentric exercise-induced muscle damage. ***J.Sports Sci.*** 1997;15:477-83.

25. Brown SJ, Child RB, Day SH, Donnelly AE. Exercise-induced skeletal muscle damage and adaptation following repeated bouts of eccentric muscle contractions. ***J.Sports Sci.*** 1997;15:215-22.

Address for correspondence: Brent C. Ruby, Human Performance Laboratory, Department of Health and Human Performance, The University of Montana, Missoula, MT 59812, USA; email: ruby@selway.umt.edu