Age-Related Variations of Serum CK and CK MB Response in Females

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Catalog Data

**Key words:** exercise-induced muscle damage, estradiol, menarchial, premenarchial, postmenopausal

*Mots clés:* lésion musculaire causée par l'exercice, œstradiol, premières règles, postménopause

**Abstract/Résumé**

The objective was to determine whether serum creatine kinase (CK) and serum CK MB activity following exercise-induced muscle damage activity differs among females of varying menarchial status and to determine whether there is a relationship between serum estradiol (E2) concentration, CK, and CK MB activity. Fifteen menarchial (M), 15 premenarchial (P), and 10 postmenopausal (PM) females participated in the study. Exercise consisted of eccentric hamstring contractions. Estradiol concentrations were significantly higher in M women ($p = .0001$; $M, 125.0 \pm 20.8 \text{ pg/mL}$, $P, 54.6 \pm 38.6 \text{ pg/mL}$, $PM, 46.2 \pm 34.6 \text{ pg/mL}$). Menarchial women had lower resting CK and CK MB activity and responded with a higher efflux of CK and CK MB post exercise ($p = .0001$). An inverse relationship was found between E2 concentration and baseline CK ($p = .02$) and CK MB activity ($p = .006$). No relationship existed between post exercise efflux of CK and CK MB and E2 concentration. At rest, E2 influenced CK and CK MB activity across menarchial levels. However, E2 did not significantly reduce the level of CK and CK MB activity following this intense bout of eccentric exercise.

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Le but de cette étude est d'établir si, suite à un exercice ayant lésé le muscle, l'activité de la créatine kinase sérique (CK) et de la CK MB sérique diffère chez les femmes ayant eu leurs premières règles de celle ne les ayant pas eues. L'objectif est aussi d'établir la relation entre la concentration d'estradiol sérique (E2) et l'activité de CK et de CK MB. Quinze femmes ayant eu leurs premières règles (M), quinze autres ne les ayant pas eues (P) et dix femmes postménopausées (PM) se portent volontaires. L'exercice consiste en des actions pliométriques des ischiojambiers. Les concentrations d'estradiol sont significativement plus élevées chez les femmes du groupe M (p = 0.0001) (M, 125.0 ± 20.8 pg/mL, P, 54.6 ± 38.6 pg/mL, PM, 46.2 ± 34.6 pg/mL). L'activité de CK et de CK MB est plus faible chez les femmes du groupe M au repos et la sortie de CK et de CK MB est plus important suite à l'exercice (p = 0.0001). On observe une relation inverse entre E2 et les niveaux de base de CK (p = 0.02) et de CK MB (p = 0.006). Il n'y a pas de relation entre la sortie de CK et de CK MB suite à l'exercice et la concentration de E2. Au repos, E2 affecte l'activité de CK et de CK MB des trois groupes de femmes. Cependant, E2 n'abaisse pas significativement l'activité de CK et de CK MB suite à des actions pliométriques intensées.

Introduction

Exercise-induced skeletal muscle damage results from excessive tension (Armstrong, 1990) produced during unaccustomed eccentric contractions (Armstrong, Ogilvie, and Schwane, 1983; Child et al., 1998). The efflux of intracellular proteins, particularly CK, from muscle fibers into the blood is generally accepted as indication of muscle membrane hyperpermeability or damage (Noakes, 1987).

A dimorphic sex difference in serum CK activity has been observed at rest and following acute eccentric exercise. Amelink and colleagues (1986, 1988) and Shumate and colleagues (1979) suggested that endogenous estrogen might be responsible for this difference. Postmenopausal (PM) women and premenarchial (P) girls both display low levels of estrogens and correspondingly high serum CK activity (Lane and Roses, 1981; Smith et al., 1979). Menarchial women have high levels of circulating estrogens and low serum CK activity at rest (Lane and Roses, 1981; Smith et al., 1979).

Estrogens may exert a stabilizing effect on the muscle membrane, thereby reducing the level of CK activity related to muscle damage. However, Buckley-Bleiler and colleagues (1990), using menarchial (M) and PM women, and Webber and colleagues (1989), using P girls and M women, suggested that estrogens did not provide protection to muscle membrane integrity. The Buckley-Bleiler and colleagues (1990) investigation used an isometric exercise treatment. However, exercise-induced muscle damage is most severe following bouts of eccentric exercise, and the level of plasma CK activity and its isoenzyme CK MB are more notable (Armstrong, Ogilvie, and Schwane, 1983; Clarkson and Tremblay, 1988; Newham et al., 1986). Furthermore, serum E2 concentrations were not reported, and, therefore, it is unknown if the groups of women had different serum E2 concentrations.

Age and growth may have an effect on serum CK and CK MB activities. Webber and colleagues (1989) used an eccentric exercise treatment to compare adults to children of both genders. The investigators reported no pre-exercise serum CK activity difference between adults and children. Serum CK activity increased 24 hr post exercise in adults and children. A difference was reported
between children and adults; however, no difference was reported after serum CK activity was adjusted for body weight. These findings suggested that differences in the levels of serum CK activity due to growth might be due to weight differences. The study did not report results of women and girls separately. In contrast, Cannon and colleagues (1990, 1994) reported a higher amplitude of plasma CK activity in young compared to older adults. These results indicated that age might be a factor in CK response. These studies also did not report results of M and PM women separately. Furthermore, E2 concentrations were not reported.

Limited research is available on CK and CK MB activity in P girls, M women, and PM women who have been subjected to an acute bout of eccentric exercise. Therefore, the purpose of this study was to determine whether serum CK and CK MB activity following exercise-induced muscle damage differ among females of varying age and menarchial status and to determine whether there is a relationship between serum E2 concentration and serum CK and CK MB activity.

Methods

Forty Anglo-American females, grouped according to their menarchial history, volunteered to participate in this study. Fifteen participants were identified as menarchial (M, sexually mature; 23.4 ± 6.9 yr), 15 participants were premenarchial (P, sexually immature; 10.5 ± 1.1 yr), and 10 participants were determined to be post menopausal (PM; 59.4 ± 10.9 yr). The means and standard deviations for body mass and lean body mass of P, M, and PM females are reported in Table 1. Participants were excluded if they used oral contraceptives (Thompson et al., 1997), hormones, if protein intake was not between 10 to 15% of total caloric intake (Hayward et al., 1999; Schneider et al., 1993), and if participants had engaged in eccentric exercise of the hamstring in the past 6 months (Clarkson et al., 1985). All participants read and signed an informed consent approved by the Institutional Review Board at the University of Northern Colorado outlining the purpose, procedures, benefits, risks, and confidentiality of the study.

Serum CK, CK MB activity, and serum E2 concentration were measured for all participants pre-exercise. Additionally, serum CK and CK MB activity were assessed at 24, 48, 72, and 96 hr post exercise. Exercise treatment for the three groups consisted of eccentric contractions of the hamstrings on a leg curl machine.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Means and Standard Deviations for Body Mass (kg), Lean Body Mass (kg), and Work per Lean Body Mass (kgm/kg) of Premenarchial, Menarchial, and Postmenopausal Females</th>
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<tr>
<td></td>
<td>Premenarchial</td>
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<td></td>
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<tr>
<td>Body mass</td>
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<tr>
<td>Lean body mass</td>
<td>33.2</td>
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<td>Work/lean body mass</td>
<td>17.5</td>
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Each participant performed 6 sets of 10 repetitions at 110% of their concentric one repetition maximum (IRM). The contraction lasted 10 s with 20 s between repetitions. A 1-min rest period was taken between each set. The investigator manually raised the arm of the leg machine to the curled position to assure that participants only lowered the arm, thereby assuring only eccentric contractions (Clarkson et al., 1987). The mean and standard deviation for the IRM of P, M, and PM were 11.5 ± 1.8, 18.3 ± 3.9, and 11.1 ± 2.1 kg, respectively. Participants were encouraged to complete the set or continue until failure. If participants did not complete the previous set, 1.1 kg were removed, and this process continued until the participants completed the 6 sets. Total work per kilogram of lean body mass was determined from the ratio of work completed during the six sets by lean body mass (see Table 1).

Blood samples were collected, allowed to clot at room temperature, centrifuged, and serum frozen at −70 °C until analyzed. Serum was analyzed for total CK activity by an enzymatic method according to Rosalki (1967; Sigma Chemical Co., St. Louis, MO). The fraction of CK MB isoenzyme was determined using a UV method (Sigma Chemical Co., St. Louis, MO), which utilized an antibody specific to the CK M subunit. The activity of CK B, which is not inhibited by the antibody, is subsequently measured. The rate of change in absorbance is directly proportional to CK and CK B activity, respectively. Absorbance was measured at a wavelength of 340 nm with a Varian SuperScan 3 spectrophotometer. Serum E2 concentration was measured by radioimmunoassay technique (ICN Biomedical, Costa Mesa, CA). A liquid scintillation counter (Packard Tri - carb 4000 series) was used to measure 125Iodine-labelled E2.

Serum E2 concentrations were compared between groups by a one-way ANOVA. The 3 X 5 (group X time) ANOVA with repeated measures was used to determine differences in serum CK and CK MB activity between groups of females and across time. Bivariate correlations were assessed by Pearson’s correlations and used to identify potential covariates. The relationship between serum CK and CK MB activity and serum E2 concentration was determined utilizing a Partial correlation with potential covariates factored out. Differences were considered significant at the 95% level of confidence (p < .05). Owing to the small sample size within the investigation, magnitude of effect was examined using (Mean1 - Mean2)/ SDpooled. By reporting effect size along with the test of significance, due to the small sample size, results are protected against type II error (concluding that the treatment had the same effect on all groups when it did not).

Results

A one-way ANOVA on serum E2 concentration revealed a significant difference between groups, F(2, 37) = 25.23, p < .0001. A Bonferroni post hoc analysis further revealed that mean serum E2 concentration were significantly greater in M participants (125.0 ± 20.79 pg/mL) than in P (54.6 ± 38.62 pg/mL) and PM participants (46.20 ± 34.61 pg/mL). The mean difference between M and P, M and PM, and P and PM of 70, 78.8 and 8.4 pg/mL of serum E2 concentration reflected an effect size of 2.19, 2.47, and 0.26, respectively. The average M women’s serum E2 concentration was 2.19 and 2.47 standard deviations above the mean of the P and PM females serum E2 concentration. This mean difference reflected a large
effect size. The average P girl’s serum E2 concentration was 0.26 standard deviations above the mean PM women’s serum E2 concentration reflecting a small effect size.

Premenarchial girls had serum CK activity that ranged from 49.0 ± 30.2 U/L prior to exercise to 137.0 ± 185.8 U/L at 24 hr after exercise. Following the peak 24 hr post exercise, serum CK activity declined (see Figure 1). The mean difference between baseline and peak CK of 86 U/L reflected a large effect size of 2.85. Menarchial women had serum CK activity that ranged from 32.0 ± 14.0 U/L prior to exercise to a peak of 3386.0 ± 3913.9 U/L 72 hr after exercise. Serum CK activity increased 24, 48, and 72 hr after exercise. A 96-hr sampling revealed that serum CK activity was returning to resting levels (2673 ± 3533; see Figure 1). The mean difference between baseline and peak CK of 3354 U/L reflected a large effect size of 239.6. Post menopausal women had serum CK activity that ranged from 102.0 ± 38.5 U/L, prior to exercise, to 622.0 ± 692.3 U/L, 72 hr after exercise. Serum CK activity increased 24, 48, and 72 hr post exercise (see Figure 1). A 96-hr sampling revealed that serum CK activity was returning to resting levels (529 ± 536). The mean difference between baseline and peak CK of 520 U/L reflected a large effect size of 13.

A repeated measures ANOVA on serum CK activity revealed a significant interaction between group and time F(8,148) = 4.304, p < .0001. Bonferroni post

![Figure 1](image_url)  
Figure 1. Serum CK activity in premenarchial, menarchial, and postmenopausal females. Values are means ± SD.
hoch analysis revealed pre-exercise serum CK activity was significantly greater in PM women than in either P girls or M women. The mean difference in CK between P and M, PM and P, and PM and M at baseline of 17, 53, and 70 U/L reflected effect sizes of 0.55 (moderate), 1.43 (large), and 2.58 (large), respectively. The baseline serum CK activity of the three groups of participants was within the normal baseline range (Hortobagyi and Denehan, 1989). Post exercise, M women had significantly greater serum CK activity than P girls at 24, 48, 72, and 96. Menarchial women had significantly greater serum CK activity than PM women 72 and 96 hr after exercise (see Figure 1). The mean difference in CK between M and P, M and PM, and PM and P at peak of 3249, 2764, and 485 U/L reflected large effect sizes of 1.2, 0.9, and 1.06, respectively.

Premenarchial girls had serum CK MB activity that ranged from 3.6 ± 2.2 U/L, prior to exercise, to 5.2 ± 3.6 U/L, 48 hr following exercise (see Figure 2). Menarchial women had serum CK MB activity that ranged from 0.8 ± 1.2 U/L, prior to exercise, to 70.2 ± 97.9, 72 hr following exercise. Serum CK MB activity peaked at 72 hr and decreased toward resting values 96 hr post exercise (51.7 ± 93.7 U/L; see Figure 2). Postmenopausal women had serum CK MB activity that ranged from 2.8 ± 2.4 U/L prior to exercise to 8.5 ± 9.8 U/L 72 hr after exercise and decreased after 96 hr post exercise (7.3 ± 9.7; see Figure 2). Similar to serum

![Figure 2](image-url)

Figure 2. Serum CK MB activity in premenarchial, menarchial, and postmenopausal females. Values are means ± SD.
CK activity, large intersubject variability was found at all evaluation periods in all three groups. The mean difference between baseline and peak CK MB of the P, M, PM of 1.6, 69.4, and 5.7 U/L reflected large effect sizes of 0.7, 57.8, and 14.2, respectively.

A repeated measures ANOVA on serum CK MB activity revealed a significant interaction between group and time $F(8,148) = 3.76, p = .0001$. Pre-exercise serum CK MB activity was significantly lower in M women compared to pre-exercise serum CK MB activity of PM women and P girls. The mean difference in CK MB between P and M, PM and M, and P and PM at baseline of 2.8, 2.0, and 0.8 U/L reflected effect sizes of 1.6 (large), 1.1 (large), and 0.35 (small), respectively. Baseline serum CK MB activity of the three groups of participants was within the normal resting range (Hortobagyi and Denehan, 1989). Following the eccentric hamstring exercise, the three groups had increased serum CK MB activity. However, the increased serum CK MB activity of M women was greater than either P girls or PM women. The mean difference in CK MB between M and P, M and PM, and PM and P at peak of 65, 62, 3.3 U/L reflected effect sizes of 0.94 (large), 0.81 (large), and 0.49 (moderate), respectively. Serum CK MB and CK activity were significantly related ($p = .0001$) at 24, 48, 72, and 96 hr post exercise ($r = .76, r = .89, r = .82, \text{ and } r = .77$, respectively) after factoring out body mass, lean body mass, and work per kilogram of lean body mass. This suggests that the changes in serum CK activity were paralleled by the response of serum CK MB activity following exercise.

Since body mass, lean body mass, and work per kilogram of lean body mass correlated with serum CK activity and serum CK MB activity over time, a repeated measures ANCOVA was used to assess differences in serum CK activity and serum CK MB activity over time with body mass, lean body mass, and work per kilogram of lean body mass as covariates. When serum CK activity and serum CK MB activity was adjusted for the covariates, results still revealed a significant interaction between group and time $F(8,136) = 6.54, p = .0002$ and $F(8,136) = 2.72, p = .008$, respectively. Menarchial women had significantly greater serum CK activity and serum CK MB activity than P girls and PM women 24, 48, 72, and 96 hr post exercise. No difference was seen in P girls and PM women. Pre-exercise serum CK activity and serum CK MB activity was significantly greater in PM women than in either P girls or M women.

Partial correlation coefficients, with body mass, lean body mass, and work per kilogram of lean body mass factored out, among serum CK activity, serum CK MB activity, and serum E2 concentration revealed a significant inverse relationship between resting serum CK activity, serum CK MB activity, and concentrations of serum E2 ($r = -.38, p = .02, r = -.44, p = .006$). This finding indicated that 14% and 19% of the variability in serum CK activity and serum CK MB activity, respectively, was accounted for by serum E2 concentration. However, serum E2 concentration was not related to any post exercise efflux of CK or CK MB.

**Discussion**

The general conclusion concerning this investigation is that differences in serum E2 concentration have no significant effect on the level of CK or CK MB activities following strenuous eccentric exercise. Estradiol appears to be related to the level
of CK or CK MB activities at rest. However, if stress on muscle fibers is overly severe, E2 does not appear to reduce the level of serum CK and CK MB activities.

The higher resting serum CK and CK MB activity in P girls and PM women could be explained by the significantly lower serum E2 concentration compared to the M women. Statistically, this was shown by an inverse relationship, the higher the serum CK and CK MB activity, the lower the serum E2 concentration. This may suggest a possible influence of estradiol on membrane integrity during rest. This inverse relationship is supported by Smith and colleagues (1979). They reported that M and pregnant women with high serum E2 concentrations had low resting CK activity, whereas PM women and P girls exhibit low serum E2 concentrations and high resting serum CK activity. Meltzer (1971) and Gale and Murphy (1979) reported the highest resting serum CK activity in PM women, which is in agreement with our findings. In the present study, body mass and lean body mass were not related to pre-exercise level of CK and CK MB activities and, thus, was not responsible for the reported differences in P, M, and PM females. Miles and Schneider (1994) have also reported no significant correlation between measures of body composition and serum CK activity and serum CK MB activity. Another explanation for the resting level of CK and CK MB activities difference could be the clearance rate. Apple and colleagues (1986) and Van Der Meulen and colleagues (1991) have indicated similar clearance rates in human and rodent adult males and females; however, this has not been examined in females at various times in their lifespan.

In the present study, the P, M, and PM females did not respond with the same magnitude of serum CK and CK MB activities or time to peak serum CK and CK MB activities. Statistically, no relationship existed among postexercise serum CK and serum CK MB activities and serum E2 concentrations. Menarchial women responded with the highest level of CK and CK MB activities. In contrast, results of the Buckley-Bleiler and colleagues (1990) study indicated that M and PM women's magnitude of plasma CK activity and time to peak plasma CK activity were similar. Miles and Schneider (1994) reported no significant increase in serum CK MB activity over 48 hr in M women. A possible explanation for the difference in our findings may be due to the type and intensity of the exercise—women in the Buckley-Bleiler and colleagues (1990) study performed maximal isometric exercise, and participants in the Miles and Schneider (1994) study performed bench stepping at 60% of VO2 max. In the present study, participants performed eccentric leg curls at 110% of the participant's 1RM.

High intensity eccentric contractions produce more tension and cause greater injury to skeletal muscle than isometric and low intensity eccentric contractions (Armstrong et al., 1983). Webber and colleagues (1989) had P girls and M women perform downhill running and reported M women had a greater magnitude of serum CK activity 24 hr post exercise. However, after factoring out body mass, no difference existed in serum CK activity. In the present study, body mass, lean body mass, and work per kilogram of lean body mass were related to serum CK and CK MB activities over time; however, after factoring out these covariates, M women still responded with the highest level of serum CK activity. Furthermore, training levels could not explain the level of serum CK and CK MB activities difference, as no participants had performed eccentric exercise of the hamstring
for 6 months.

Byrnes and colleagues (1985) reported no significant difference in serum CK activity between the baseline bout and 9-week bout of exercise. Participants in the present study had not performed eccentric exercise for the hamstring for at least 6 months. In support of the present study, Cannon and colleagues (1994) reported diminished plasma CK activity in older participants (61–72 yr) compared to younger participants (20–32 yr) after eccentric exercise of the hamstrings. Furthermore, the increase in plasma CK activity was related to increases in circulating neutrophils. However, exercise-induced neutrophilia in the older subjects was significantly less than that observed in the younger subjects. It has been postulated that age-related loss of leukocyte function may result, in part, from a decline in endogenous antioxidant defenses (Harmon, 1984). Cannon and colleagues (1990) revealed that the antioxidant vitamin E significantly increased exercise-induced neutrophilia in older participants and increased plasma CK activity to levels reported in younger participants. Differential antioxidant status between M women and P girls and PM women may be one possible explanation for the observed difference in serum CK activity post exercise in the present study. Estradiol has antioxidant properties that are similar to vitamin E (Burton and Ingold, 1989; Sugioka et al., 1987). Therefore, the greater concentration of E2 in M women may be responsible for the greater level of serum CK activities post exercise suggesting optimum membrane function and low E2 concentrations may be responsible for the subdued CK and CK MB response in P girls and PM women.

References


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