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Creatine Kinase and the Isoforms of Creatine Kinase-MM Following a Competitive Swimming Workout

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Abstract

Serum levels of total creatine kinase (CK), CK-MM and the isoforms of CK-MM were measured in 14 male competitive collegiate swimmers. The purpose of this study was to observe changes in CK and the isoforms of CK-MM following a competitive swim training session. Venous blood samples were taken before and immediately following a 5550 yard training session. The main series in the workout consisted of a 27 minute, minimal rest interval set designed to obtain a moderately heavy physical effort. Total CK, composed predominantly of CK-MM, increased significantly ($p<0.05$) following the swim training session. CK-MM3 (skeletal muscle form) as well as the CK-MM3/CK-MM1 ratio increased significantly ($p<0.05$) from pre- to post-exercise. CK-MM2 (intermediate form) increased significantly ($p<0.05$) from pre- to post-exercise with CK-MM1 (serum form) decreasing significantly ($p<0.05$) from before to after the swim training session. These results indicate that significant changes will occur in serum CK and the isoforms of CK-MM following a competitive swimming workout.

Introduction

Creatine kinase (CK) catalyzes the phosphorylation of creatine by adenosine triphosphate (ATP). This rapid reaction creates creatine phosphate (CP) which is an immediate source of energy during exercise. Creatine kinase is found abundantly in skeletal muscle tissue and has demonstrated significant increases following different forms of exercise (9,14,19). Two factors seem to determine the amount of increase in serum enzymes following exercise. First, activities involving eccentric contractions as in downhill running have demonstrated significantly greater increases in serum CK when compared to equal amounts of level running (16). Second, there appears to be a high correlation between increases in serum enzymes and the degree of intramuscular pressure during a particular exercise (12). These increases have been associated with possible changes in skeletal muscle cell permeability (12).

Studies involving single bouts of swimming have demonstrated conflicting results when serum CK has been observed. Critz and Cunningham (7) and Haralambie and Senser (8) observed increases in serum CK immediately following a 12-minute maximal swimming effort and a 5,200 yard continuous swim, respectively. Conflicting results were reported by Kirwan et al. (10) and Symanski et al. (17) who demonstrated no significant changes in serum CK following swimming activity.

Three sub-types (isoforms) of the CK-MM isoenzyme have been identified and utilized as earlier and more sensitive indicators of acute myocardial infarction (AMI)

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(22), as an accurate noninvasive indicator of myocardial reperfusion following AMI (4), and as an effective means of more precisely predicting the time of necrotic onset following the diagnosis of AMI (13). The isoforms which are recognized in various ways in the literature will be identified in the present study as: CK-MM3 (muscle tissue form), CK-MM2 (intermediate form), and CK-MM1 (serum form). CK-MM3, the most cathodic form during electrophoresis, is released directly from muscle tissue into blood where it is converted to CK-MM2 and then to CK-MM1 (1). The CK-MM3 isoform is composed of two unmodified M subunit monomers designated as tissue (M_T) and serum (M_S) subunits by Wu (20). Serum carboxypeptidase-N (1) post-translationally cleaves a C terminal lysine group from M_T to form M_S. Different combinations of M_T and M_S create the CK-MM subtypes observed in serum. These alterations allow the CK-MM isoforms to be quantiated by different clinical laboratory methods including high voltage electrophoresis, chromatofocusing, and liquid chromatography (22).

Previous exercise investigations have indicated that the CK-MM isoforms will demonstrate significant changes following exercise activities (2,3,6). To date the isoforms of CK-MM have not been observed following competitive swimming. The purpose of this study was to observe indicators of change in skeletal muscle cells following competitive swim training by measuring total CK and the isoforms of CK-MM. This study also examined the discrepancies between previous investigations involving total CK following swimming.

Methods

Subjects/training. Fourteen male collegiate swimmers between the ages of 17 and 22 years (x age = 19.50 ± 1.40 yrs.) from a Division I AA university participated in this study. All subjects signed written forms of consent after being advised of the risks and benefits of participating in the study. This procedure is in accordance with the policy statement of the American College of Sports Medicine and the Human Subjects Protection Review Committee. Swim training was designed to include a gradual increase in volume (yardage swum per day) from September through December and included a progressive weight-training program. Data were collected following the first thirteen days of training which averaged 3500 yards swum per day. Blood samples were taken 36 hours following a previous weight-training session.

Protocol. The subjects in this study performed a regularly scheduled swim training session of approximately 90 minutes in duration. All subjects arrived at the pool at approximately 5:45 a.m. The training session began at 6:00 a.m. and ended at approximately 7:30 a.m. The subjects began and ended the training session as a group. The training session consisted of two warm-up swims, two main series, and a cool-down swim totaling 5550 yards. The main series were designed to obtain a moderately heavy physical effort.

Blood collection. Venous blood samples were taken before and immediately following the completion of the training session. Samples were drawn by two Registered Medical Technologists using routine venapuncture techniques within ten minutes (±3 minutes) following the swim training session Seven milliters of whole blood was obtained from each subject. Blood samples were obtained by a single, aseptic, nontraumatic venapuncture of an antecubital vein using a sterile 18 gauge Vacutainer brand needle. Blood was collected, allowed to clot for 15 minutes, and centrifuged at 3500 rpm to obtain a clear serum sample. The serum sample was then frozen at −190° F and transported to the institution laboratories conducting this research (approximately four hours away) where they were thawed at 37 °C and assayed immediately. Freezing and thawing serum samples have been demonstrated to have no effect on the isoform pattern (20).

Assay procedures. Single measures were taken for total CK and the Isoforms of CK-MM. Total CK was determined according to the method of Szasz et al. (18) using a Gilford™ CK assay kit (Gilford Diagnostics, Cleveland, Ohio 44135). Serum was incubated with NADH and observed at 60 second intervals for changes in absorbency at 340 nm. Total CK, in U/L were calculated from the changes in absorbency readings. The CK isoenzymes were determined electrophoretically using the Corning™ ACI System (Corning Laboratories, Palo Alto, CA 94360). Serum samples were applied to agarose gel plates using the formulation of Rosalki (15) and then electrophoresed at 90 volts for 20 minutes. The agarose plates were then incubated with fluorometric substrate for 20 minutes and dried. The dried plates were scanned with a recording fluorometric densitometer and quantified. The CK-MM isoforms were determined by high-voltage electrophoresis in accordance with procedures established by Helena Laboratories (Helena Laboratories, Beaumont, TX). Serum samples were applied to an acetate gel then electrophoresed at 800 volts for 12 minutes. The acetate plate was blotted and layered on a substrate plate (sandwich technique) and then incubated for six minutes at 45° C. Following incubation the plates were separated and dried at 56° C for five minutes. The isoforms were quantitated with a computer integrated scanning densitometer. Typical within-run %CV's for the CK-MM isoforms using high voltage electrophoresis range from 3% to 5% with a sensitivity of roughly 5 U/L per isoform band (21).

Units of activity. Activity (U/L) for the CK isoenzyme was derived by multiplying the percent for each isoenzyme fraction (CK-MM, CK-MB, & CK-BB), as

determined electrophoretically, times the total CK activity units. Activity (U/L) for the CK-MM isoforms was derived by multiplying the percent of each isoform (CK-MM3, CK-MM2, and CK-MM1) by the total CK-MM activity. This was also determined electrophoretically.

Statistical analysis. One-way analysis of variance (ANOVA) for repeated measures was used to determine any significant changes in pre- to post-data. Changes were considered significant at $p<0.05$.

Results

All samples contained exclusively the skeletal muscle isoenzyme CK-MM as determined by electrophoresis. Data for total CK and the isoforms of CK-MM are presented in Table 1. Total CK increased significantly from before to immediately following the swim training session. A significant difference was observed in total CK despite a wide range in both the pre and post samples. CK-MM3, CK-MM2, and the CK-MM3/CK-MM1 ratio also increased following the training session. There was a significant decrease in CK-MM1 following the training session.

Table 1
Total CK, CK-MM, CK-MM isoforms, Hematocrit, Hemoglobin before and immediately following the swim training session.

	Pre training session	Post training session	Prob. F
Total CK (U/L)	219.9 ± 81.6	238.8 ± 87.8	0.0081
CK-MM3 (U/L)	23.1 ± 12.8	39.1 ± 20.1	0.0219
CK-MM2 (U/L)	61.1 ± 30.2	77.3 ± 31.0	0.0005
CK-MM1 (U/L)	135.1 ± 48.6	123.8 ± 47.1	0.0355
MM3/MM1 ratio	0.18 ± .08	.30 ± .14	0.0170

Values are mean ± SE (N=14). CK = creatine kinase; CK-MM = creatine kinase-MM isoenzyme, $CK\text{-}MM_3$ = muscle isoform; $CK\text{-}MM_2$ = intermediate isoform; $CK\text{-}MM_1$ = serum isoform. All changes were statistically significant.

Discussion/Conclusions

Skeletal muscle structural alterations following exercise have been of interest to physiologists as well as athletic coaches for many years. Changes in the concentration of serum enzymes have become a common method of determining alterations of cellular membranes which possibly indicates damage to the muscle cell (12). These alterations of cell structure allow for the release of enzymes into general circulation increasing the concentration of those enzymes in sera which can be quantiated by common clinical laboratory methods (21). The conventional method of estimating damage to skeletal muscle cells has been to observe changes in serum levels of total CK (12).

Research to date indicates that serum CK concentrations will increase following most exercise activities (12). Conflicting results have been reported following swimming. Kirwan et al. (10) saw no significant changes in serum CK immediately following a sub-maximal (95% $\dot{V}O_2max$) front crawl swim while Symanski et al. (17) reported no significant change in serum CK following a one hour continuous submaximal (70% $\dot{V}O_2max$) tethered swim. Haralambie and Senser (8) reported marked increases in serum CK following a 5,200 yard (90 minute) continuous swim in agreement with Critz and Cunningham (7) who also reported a 16% increase in serum CK following a 12 minute maximal effort swim. The primary difference in the studies reporting conflicting results seems to be the training state of the swimmers observed. In those studies showing increases in total serum CK the subjects were either novice swimmers as in Critz and Cunningham (7) or classified as "generally well trained" as in Haralambie and Sensor (8). A closer look at the swimming speeds of the subjects in the latter study (0.99 m/sec for 5,500 yards) would indicate a less well trained group. In both investigations where no significant change was observed, the subjects were classified as "highly trained" (10,17).

The swimmers in the present study were all collegiate competitive swimmers. However, data were collected after only thirteen days of training. Our results agree with those studies showing an increase in serum CK following a competitive swimming workout. The present investigation also indicates that the training state of swimmers will greatly influence the amount of CK released into general circulation following swimming. Studies in which CK was observed in competitive swimmers during a complete season of training also demonstrated a decrease in resting and immediate post exercise samples as the season progressed (5,11). These studies also indicate that a diminished serum CK will accompany an increase in conditioning.

The present study was also designed to assess changes in serum levels of the isoforms of CK-MM following a competitive swim training session. Previous exercise studies observing the isoforms of CK-MM have demonstrated significant increases in both CK-MM3 and the CK-MM3/CK-MM1 ratio following repeated eccentric contractions of the forearm flexors (2) as well as after repeated isometric contractions of the knee extensor muscles (6). Changes in the CK-MM isoforms in these studies were observed two hours following the cessation of exercise with changes in total CK not occurring until six hours following the completion of the exercise protocol. The results of the present study demonstrated significant increases in CK-MM3, CK-MM2, and the CK-MM3/CK-MM1 ratio within ten minutes following the exercise intervention. A significant decrease in CK-MM1 following the swim training session was also observed.

The present study indicates that there will be a release of CK into serum during an intense swim training session in lesser trained swimmers. The increase in total CK, in this group, argues in favor of an increased release of CK from muscle cells, which is predominately CK-MM. It is, however, interesting to note that there was a concomitant decrease in the absolute amount in CK-MM1. Although it is tempting to hypothesize that there was increased clearance and/or inactivation of the CK-MM1 isoform due to increased metabolism during swimming, the research methodology did not reveal the mechanism. Additional research is needed to address these findings.

The results of the present study also demonstrated that there will be a significant change in serum levels of the isoforms of CK-MM. Also in this study both total CK and the isoforms of CK-MM demonstrated significant changes upon sampling. It is impossible to know whether the change in the isoforms could be detected before the change in total CK as has been demonstrated with other exercise studies (2,6). Serum carboxypeptidase-N, which is responsible for the conversion of the CK-MM isoforms, was not measured in the present study. The authors would like to point out that it is difficult to determine whether the changes in the percentages of the CK-MM isoforms were due in part to changes in serum carboxypeptidase-N. The increase in total CK indicates a release of CK-MM from muscle tissue and therefore indicates that the changes in the isoforms of CK-MM were due to this release.

In conclusion, total CK and the isoforms of CK-MM will demonstrate significant changes following competitive swim training. These changes will be more likely to occur in lesser trained swimmers. Although it is tempting to suggest that these analytes may be useful to the coach as a marker of conditioning, in most cases this would not be practical. More research that could directly quantitate skeletal muscle damage in relation to serum levels of total CK and the isoforms of CK-MM could bring more value to measuring these analytes.

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