Serum Creatine Kinase activity in Indian Athletes and its Reference Intervals

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ABSTRACT

The serum concentration of Creatine Kinase (CK) is widely used as an index for skeletal muscle damage in sports and exercise. Since athletes have higher CK values than non-athletes, it is necessary to establish reference intervals for athletes. The purpose of the present study was to establish reference intervals for CK in Indian athletes. Serum samples from 104 male and 60 female athletes from various sports were collected and assayed for CK activity. The reference intervals (RI) were found to be 82-746 U/L in male and 25-478 U/L in female athletes. The upper reference limits were significantly higher in male athletes as compared to the female athletes thus supporting gender differences in muscle damage after strenuous exercise. The RI values proposed in this study would be useful to distinguish persistent hyperCKemia induced by exercise from any pathological condition or over training in athletes.

KEYWORDS: CK, persistent hyperCKemia, gender differences, Estradiol, antioxidant, Heat shock proteins

INTRODUCTION

Creatine Kinase is a dimeric globular protein consisting of two subunits with a molecular mass of 43 kDa. It buffers cellular ATP and ADP concentrations by catalysing the reversible exchange of high-energy phosphate bonds between phospho creatine and ADP during contraction. At least five isoforms of CK exist: three isoenzymes in cytoplasm (CK-MM, CK-MB and CK-BB) and two isoenzymes (non-sarcomeric and sarcomeric) in mitochondria. In normal serum, total CK is provided mainly by the skeletal muscle and is almost only of the MM fraction. The activity levels of CK is a marker of the functional status of muscle tissue and varies widely in both pathological and physiological conditions(Brancaccio et al., 2007) as it gives an indication of the

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degree of metabolic and muscular adaptation to physical training. The levels of CK in serum is normally very low but increases considerably after intensive exercise as a consequence of damage to sarcolemmal membrane and muscle pathology (Brancaccio et al., 2006).

Several studies have reported changes in serum activity of CK in normal subjects and athletes after strenuous exercise (Wolf et al, 1987; Priest et al, 1982; Munjal et al, 1983). Also, the serum level of CK changes according to different protocols and intensity, and level of training (Boros-Hatfaiudy et al, 1986). CK activity measured by needle biopsy displayed different behaviour before and after training (Kalpcinska et al, 2001; Ohkuwa et al, 1984).

High levels of post-exercise serum enzyme activities have been found after very prolonged competitive exercise such as ultra distance marathon running, triathlon events and weight-bearing exercises (Denvir et al, 1999). However, weight bearing exercises such as downhill running that include eccentric muscular contractions bring about the greatest increases in serum enzyme activities (Nuviala et al, 1992; Malm et al, 2004). The basal levels of CK have been reported to be higher in athletes when compared to normal sedentary subjects (Hortobagyi et al, 1989; Fallon et al, 1999) but post exercise CK response in trained athlete is considerably lower as compared to untrained subjects (Vincent et al,1997). In fact, studies performed by Garry et al (2000) and Karamizrak et al (1994) stated that the CK levels of athletes are lower than those recorded in matched healthy control subjects when same exercise protocol was followed. Study of CK in sport medicine allows to obtain information on the state of the muscle and high levels of enzyme in apparently healthy subjects may be correlated with physical training status , however persistent hyperCKemia at rest may be a sign of subclinical muscle disease (Angelini,2004).

There are marked sex differences in CK serum levels at rest, with higher values in males than females. After muscular exercise, sex-linked differences are still present (Amelink et al, 1998) and oestrogen may be an important factor in maintaining post-exercise membrane stability, thus limiting CK leakage from the damaged muscle (Amelink et al, 1990).

In athletes, the study of CK at rest and after exercise could be an important tool for coaches and clinicians. Athletes have higher resting CK when compared with untrained subjects (Koutedakis et al,1993), in addition a large increase in serum CK levels combined with reduced exercise tolerance could be a marker of overtraining (Hartmann et al, 2000). Total serum CK activity is markedly elevated for 24 hours after the exercise bout when subjects rest and remains elevated for 48 hours when subjects train in the first week post-exercise (Kratz et al, 2002). In the present study the authors have attempted to frame reference intervals for CK

activity levels after 24hours of rest from sports specific training.

METHODOLOGY

Subjects

104 male and 64 female Indian athletes aged 14-33 years (male 15-30 years; female14 -33years) were selected from different sports discipline such as Taekwondo, Hockey, Rowing, Athletics (sprint, middle distance and endurance), Volleyball, during their training season and the selected individuals have been training for 2-15years, undertaking 5–10 training sessions per week and exercising 1–2 hours per training session. Further, all the athletes are presently undergoing training in Sports authority of India, Bangalore. The details regarding the health status, history of muscle injuries, hospitalization, medication and supplementary usage were obtained through the questionnaire. The Study was approved by the institutional ethics committee and all the subjects provided written informed consent to participate in the study.

Specimen collection and assay

Blood samples were collected from Subjects in seated position from antecubital vein into plain evacuated tubes, in the morning, after the subjects had undergone overnight fast and refrained from training 24 hours prior to the test, to avoid acute exercise-induced shifts in plasma volume, and allow the CK concentration to reach post exercise peak value. The blood was left to coagulate at room temperature for 30 minutes and centrifuged at 1500 g for 10 minutes to separate the serum which was analysed for CK on same day, by using CK-NAC (Kinetic UV method) kit from Spin react on ERBA semi auto analyser at 37° C. The activity levels of the enzyme were expressed as U/L.

Statistics

Shapiro-Wilk test was used to check normal distribution, measurements of outliers was done by Tueky Test and 95% Reference limits were calculated as the 2.5th and 97.5th percentiles of the reference collectives by using Robust method according to CLSI Guidelines C28-A3.With the Robust method, 90% confidence intervals for the reference limits were estimated by using bootstrapping (percentile interval method, Efron & Tibshirani, 1993).The analysis was done by using Med Calc Software 2016.

RESULTS & DISCUSSIONS

Figure 1 & 2 depicts the reference intervals of CK in male and female athletes. The range of CK in male and female athletes is given in Figure 3 and the range of CK in male athletes from different sports is presented in figure 4.

Table 1 shows the 95 % double sided Reference intervals of CK for male and female athletes with 90% confidence intervals.

The Mean and SD levels of CK in male athletes from different sports have been depicted in Table 2.

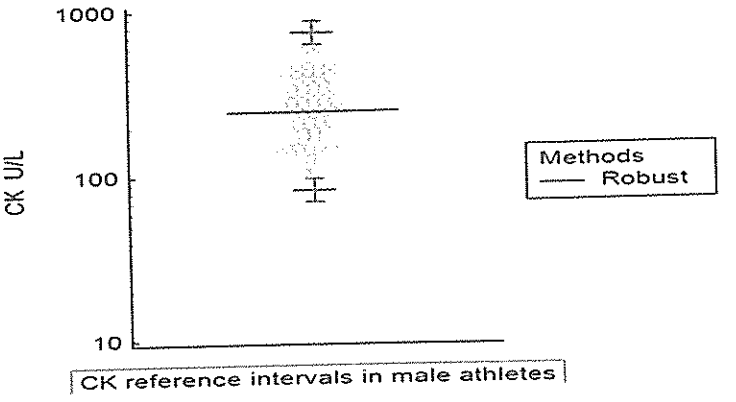

Fig. -1 : CK reference intervals in male athletes

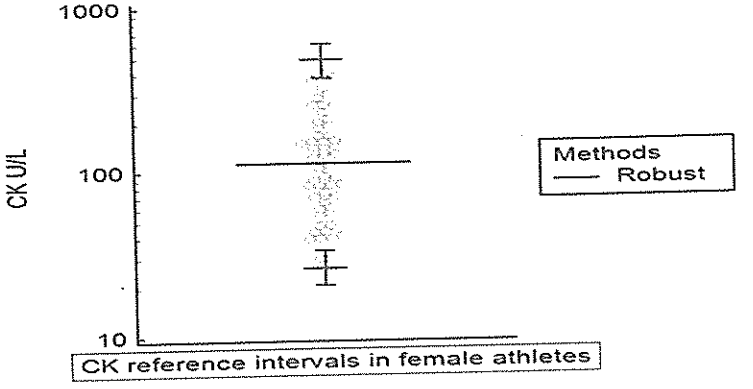

Fig. -2 : CK reference intervals in female athletes

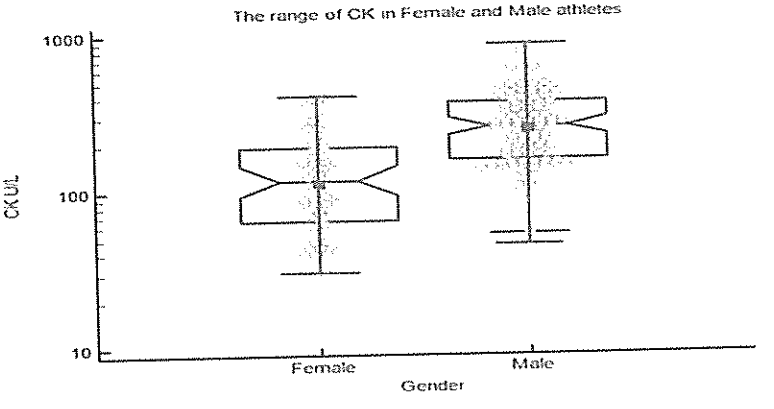

Fig. -3 : The range of CK in Female and Male athletes

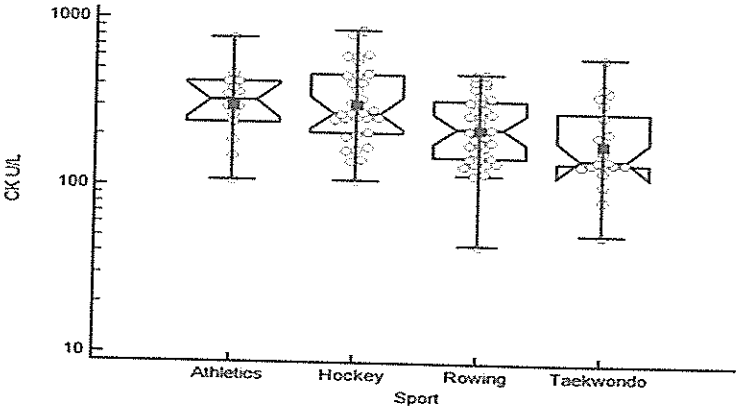

Fig.- 4 : The range of CK in male athletes from different sports

Table-1 : 95 %(double sided) Reference Limits and Lower and Upper limits of 90% Confidence intervals of serum CK activity (U/L,37o C) in male and female athletes

CPK / Group	Lower reference limit	Confidence Interval of lower reference limit	Upper reference limit	CI of upper reference limit
Male athletes	82	70 to 97	746	635 to 873
Female athletes	25	20 to 33	478	370 to 601

Table-2 : CK (U/L at 37oC) levels in male athletes from different sports

Sport	N	Mean ± SD
Athletics	14	336±162.5
Hockey	32	350±190.8
Rowing	37	246±110.3
Taekwondo	21	214±131.7
Total	104	284±158.6

Creatine kinase is used as a measure of exercise induced muscle damage. During exercise, interruption of muscle sarcolemma, sarcoplasmic reticulum and Z- lines results in the release of muscle protein and enzymes such as Creatine kinase (CK) into the interstitial fluid. Eccentric exercise results in more strain and muscle damage (Brancaccio et al, 2007; Clarkson et al, 2001) so CK is a useful marker in understanding membrane stability following strenuous exercise. Black men usually have higher values than Caucasians (Wong et al, 1983) but some studies have not approved the same.

The main aim of the present study was to monitor the basal levels of CK in Indian athletes during training season and set the reference intervals of CK in Indian

athletes. During the present investigation it was observed that the CK upper reference limits of the selected Indian athletes (male athletes 746 U/L; female athletes 478 U/L) were lower than the values suggested by Vassilis Mougios (2007) for athletes (male athletes 1083 U/L; female athletes 513 U/L) selected from Greek sports club. The reason for this might be due to variation in composition of muscle fibres in both the races. Moreover, this may also be because of the difference in training protocols and testing methodology. Furthermore, RI limits in athletes of the present study were found to be considerably higher than RI limits of non athletes (male non-athletes 491 U/L; female non-athletes 252 U/L) proposed by Vassilis Mougios (2007) and the same is evidenced in other studies (Koutedakis et al.1993).

Mean CK levels were found to be significantly higher in male athletes when compared to female athletes ($P < 0.001$). Numerous studies have depicted marked gender differences in CK serum levels at rest (Fu et al, 2002), with females showing lower values than males. Gender differences in muscle damage and creatine kinase response after exercise have been well documented in several studies (Tiidus, 2000; Clarkson &Sayers, 1999; Brancaccio et al, 2007). Researchers have suggested that the presence of Estradiol in women is the cause for this difference. Carter et al (2001), Clarkson & Sayers (1999) and Rothe et al (2001) have shown that Estradiol mitigates CK response after exercise in females. Estradiol has shown to protect the muscle membrane from damage caused by free radicals produced during strenuous exercise because of its structural similarity with Vitamin E, a well known antioxidant. Prorock et al., (2003) noted that oestrogen can influence Nitric Oxide (NO) and inducible nitric oxide synthase (iNOS) activity in a number of tissues and thereby influencing damage and recovery processes, as NO and iNOS are important regulators of skeletal muscle satellite cell activation (Anderson et al, 2004) and post exercise calcium homeostasis. Further, several studies have documented the ability of estrogen to attenuate the circulatory cytokines thereby reducing the inflammatory response (Puder et al, 2001; Tiidus P, 2003). Milne et al (2008) and _Bombardier et al (2009) have shown that Estradiol is able to enhance heat shock proteins (HSPs), important facilitators of post-synthesis muscle protein assembly and maintenance of protein structure, thereby providing protection against potential damage induced by exercise and also assist in the repair process.

As mentioned above, the present study tries to set RI levels for Indian athletes, however more studies need to be carried out in various sport disciplines in India to reach out to more acceptable CK reference intervals.

CONCLUSION

In this study the RI values were found to be in the range of 82-746 U/L for male and 25-478 U/L for female athletes, respectively. RI values proposed in this study will be useful for clinicians to avoid misinterpretation of persistent hyperCKemia induced by exercise as a pathological condition or over training in athletes.

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