Reference intervals for serum creatine kinase in athletes

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ORIGINAL ARTICLE

Reference intervals for serum creatine kinase in athletes

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Background: The serum concentration of creatine kinase (CK) is used widely as an index of skeletal muscle fibre damage in sport and exercise. Since athletes have higher CK values than non-athletes, comparing the values of athletes to the normal values established in non-athletes is pointless. The purpose of this study was to introduce reference intervals for CK in athletes.

Method: CK was assayed in serum samples from 483 male athletes and 245 female athletes, aged 7–44. Samples had been obtained throughout the training and competition period. For comparison, CK was also assayed in a smaller number of non-athletes. Reference intervals (2.5th to 97.5th percentile) were calculated by the non-parametric method.

Results: The reference intervals were 82–1083 U/L (37˚C) in male and 47–513 U/L in female athletes. The upper reference limits were twice the limits reported for moderately active non-athletes in the literature or calculated in the non-athletes in this study. The upper limits were up to six times higher than the limits reported for inactive individuals in the literature. When reference intervals were calculated specifically in male football (soccer) players and swimmers, a threefold difference in the upper reference limit was found (1492 vs 523 U/L, respectively), probably resulting from the different training and competition demands of the two sports.

Conclusion: Sport training and competition have profound effects on the reference intervals for serum CK. Introducing sport-specific reference intervals may help to avoid misinterpretation of high values and to optimise training.

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Competitive sport imposes substantial energy, mechanical, mental and emotional burdens on the human. This reflects, among other things, on a number of biochemical and haematological properties, which display significant differences between athletes and non-athletes in blood samples collected at rest.[1–5] Most prominent among these properties is creatine kinase (CK, EC 2.7.3.2), which is believed to leak into the plasma from skeletal muscle fibres when they are damaged because of repeated and intense contractions.[6–8] Under such conditions the catalytic concentration of CK in the serum displays a far greater increase than the serum concentration of other muscle proteins.[9 10] As a result, and although its validity as a marker of exercise-induced muscle injury has been questioned,[11] CK is used widely as such a marker.[9 10 12 13] The serum concentration of CK peaks 1–4 days after exercise and remains elevated for several days.[9 12–14] Thus, athletes participating in daily training have higher resting values than non-athletes,[3 15] although this response to training is mitigated by the so-called repeated-bout effect. That is, the repetition of an exercise after several days or even weeks causes less muscle fibre damage than that caused by the previous exercise.[16]

Given the marked effects of exercise on the serum CK concentration and the association of high CK values with rhabdomyolysis and myocardial infarction,[17] one may wonder which values are reasonable to observe in an athlete and which are not. The answer to this question is of high practical importance, since, if a CK value, although high, is normal for athletes, one can increase the training load, according to the principle of progressive overload,[18] in pursuit of faster and larger adaptations to training, resulting in faster and larger gains in performance. If, on the other hand, a CK value is too high, even for athletes, one should decrease the training load to prevent severe muscle injury or the development of chronic fatigue leading to overtraining.[18] Evidently, the question of whether an athlete's CK value is reasonable cannot be answered by comparing it to the normal values established in the general population. To my knowledge, reference intervals (normal ranges) for serum CK in athletes have not been determined. Therefore, the aims of this study were:

- to propose such intervals based on the analysis of an adequate number of reference values from athletes
- to examine the dependence of the intervals on sex, age and sport
- to compare the intervals in athletes to those in non-athletes.

METHODS

Subjects

Reference values for serum CK were obtained from 483 male athletes, 245 female athletes, 115 male non-athletes and 122 female non-athletes, all being white, aged 7–44, who visited the laboratory for biochemical monitoring through the measurement of many relevant parameters over the course of 10 years. To be included in the study, subjects had to be apparently healthy, with no known diseases and no major injury or hospitalisation within the past 3 months. Additionally, they should have taken no prescription drugs during the week preceding blood sampling. These criteria were assessed through the administration of a questionnaire. All subjects provided written informed consent to participate in the study, which was conducted in compliance with the Helsinki Declaration of 1975, as revised in 1996, and was approved by the institutional ethics committee.

All athletes were members of Greek sport clubs and had been training for 2–25 years (median 8), undertaking 5–10 training sessions per week (median 6), and exercising 1–2 hours per training session (median 1.6). They practised a wide variety of sports encompassing both endurance and strength/power activities. The sports were running (sprint, middle distance and endurance), jumping, throwing, combined events (triathlon, heptathlon and decathlon), swimming (sprint and middle

Abbreviation: CK, creatine kinase

distance), cycling, rowing, kayaking, football (soccer), basketball, volleyball, handball, water polo, tennis, table tennis, gymnastics, judo, taekwondo, karate, boxing, weightlifting, bodybuilding, diving, motocross and snowboarding. Non-athletes did not participate in any sport training programme. They were mostly students or employees, performing light-to-moderate exercise up to three times a week. Physical activity was assessed through a questionnaire.

Specimens and assay

Subjects provided blood samples in a seated position from an antecubital vein into plain evacuated tubes in the morning after an overnight fast and sleep. They refrained from early morning training to avoid acute exercise-induced shifts in plasma volume that would affect the CK concentration. No other intervention in the daily training programme of the athletes was made. The blood was left to coagulate at room temperature for 30 minutes and was centrifuged at 1500 *g* for 10 minutes to separate the serum, which was analysed immediately or was stored at –20℃ for up to 1 week prior to analysis. Storage under these conditions does not affect the CK concentration.[17] CK was assayed spectrophotometrically through the use of commercially available kits that employed optimised conditions in accordance with the recommendations of the International Federation of Clinical Chemistry.[19 20] The catalytic concentration of CK was expressed as U/L at 37℃.

Calculations and statistics

Since many subjects, especially athletes, visited the laboratory regularly, each of them provided more than one sample, the maximum being 19 samples obtained from a swimmer over a period of 5 years, from adolescence into adulthood, at all phases of the yearly training and competition programme. In such cases the median of each individual's values was used in the calculation of reference intervals.

Potential outliers were identified by visual inspection of the reference distribution and were deleted if their distance from the next, more central value in the distribution exceeded one-third of the total range of values.[21]

Reference limits were calculated as the 2.5th and 97.5th percentiles of the reference collectives according to Solberg[21] using the non-parametric method, since most distributions were significantly different from normal ($p<0.05$ by the Kolmogorov-Smirnov test). The 90% confidence intervals (CI) of each reference limit were also calculated according to Solberg.[21] Reference limits for two groups were declared to differ if their CI did not overlap ($p<0.1$).

RESULTS

The distribution of CK values in the four study groups is shown in fig 1. Male athletes had the widest distribution followed by female athletes and male non-athletes.

Table 1 presents the reference limits and their CI for each group. Both the lower and the upper reference limits for male athletes were higher than the corresponding reference limits for male non-athletes, with non-overlapping CI. The same was the case for female athletes and non-athletes. Males had higher reference limits than females in both athletes and non-athletes, again with non-overlapping CI. In contrast, the lower and upper reference limits of female athletes and male non-athletes did not differ significantly (they had overlapping CI).

Dependence on age

Reference values tended to be higher in the centre of the age range of the subjects (from about 12 to about 30 years) in all four groups. However, the differences from the values at the edges of the age range were not large enough to justify any attempt to partition the data and establish separate reference intervals. In addition, values obtained from the same individuals over the course of up to 5 years showed no consistent tendency.

Dependence on sport

Within the group of male athletes two sports had high enough numbers of subjects to let one calculate specific reference intervals and thus partly examine the issue of dependence on sport. The sports were football, with 182 subjects, and swimming, with 93 subjects (fig 2). The lower reference limits (with CI in parentheses) were 83 (53–84) U/L for football players and 70 (61–89) U/L for swimmers. The upper reference limits were 1492 (924–1908) and 523 (435–543) U/L, respectively.

DISCUSSION

As presented in the introduction, the serum CK concentration serves as an index of both overexertion and adaptation of the muscular system to repeated bouts of exercise. As such, CK is one of the top choices of athletes and coaches when requesting a biochemical profile, although the interpretation of CK values is not always straightforward. This laboratory has previously shown that CK is higher in athletes than in non-athletes, whether they are male or female, juvenile or adult.[3] The aim of the present study was to contribute to the elucidation of what the serum CK concentration means for an athlete by providing reference intervals to be used mainly by sports medicine practitioners and coaches in daily practice. It is because of this that no intervention in the training programme of the athletes during the days preceding blood sampling was attempted. Given the fact that serum CK remains elevated for several days post-exercise, the values used in this analysis should be considered as the cumulative effect of recent training sessions in conjunction with the repeated-bout effect.[16]

The upper reference limits for CK in male and female athletes were twice those for male and female non-athletes. This is in agreement with the differences reported for the mean values in a previous study from this laboratory.[3] It is noteworthy that the upper reference limits in the non-athletes of this study are among the highest in the literature. In particular, Wu,[17] Wong *et al.*,[22] and Schumann and Klauke[23] have proposed limits that are under 350 U/L in males and under 200 U/L in females. Miller *et al.*[24] and Strømme *et al.*[25] have proposed limits (males, 391 and 398; females, 240 and 207 U/L, respectively) whose CI overlap with the CI of the limits in this study. Finally, the limits proposed by Lev *et al.*[26] are either slightly higher than (532 U/L in males) or very close to the ones in this study (248 U/L in females).

The major reason for the discrepancies among studies that have determined reference limits for CK in non-athletes (including the present study), as also suggested by Strømme *et al.*,[25] is probably the physical activity level of the subjects. Among the lowest upper reference limits were the ones determined in hospitalised subjects,[23] whereas the highest limits were determined in moderately active, physically fit military subjects.[26] As far as the present study is concerned, the non-athletes were all ambulatory, and, although not participating in any sport training programme, many were physically active, engaging in light-to-moderate physical activity up to three times a week, as mentioned in the Methods section. Such activity is widely advocated as beneficial to health. On this basis it may be necessary to revise the reference intervals for CK in the general population. These intervals should not be based on subjects who are physically inactive (or, what is more, hospitalised) but on subjects who lead a healthy lifestyle that includes regular physical activity. Thus, in addition to introdu-

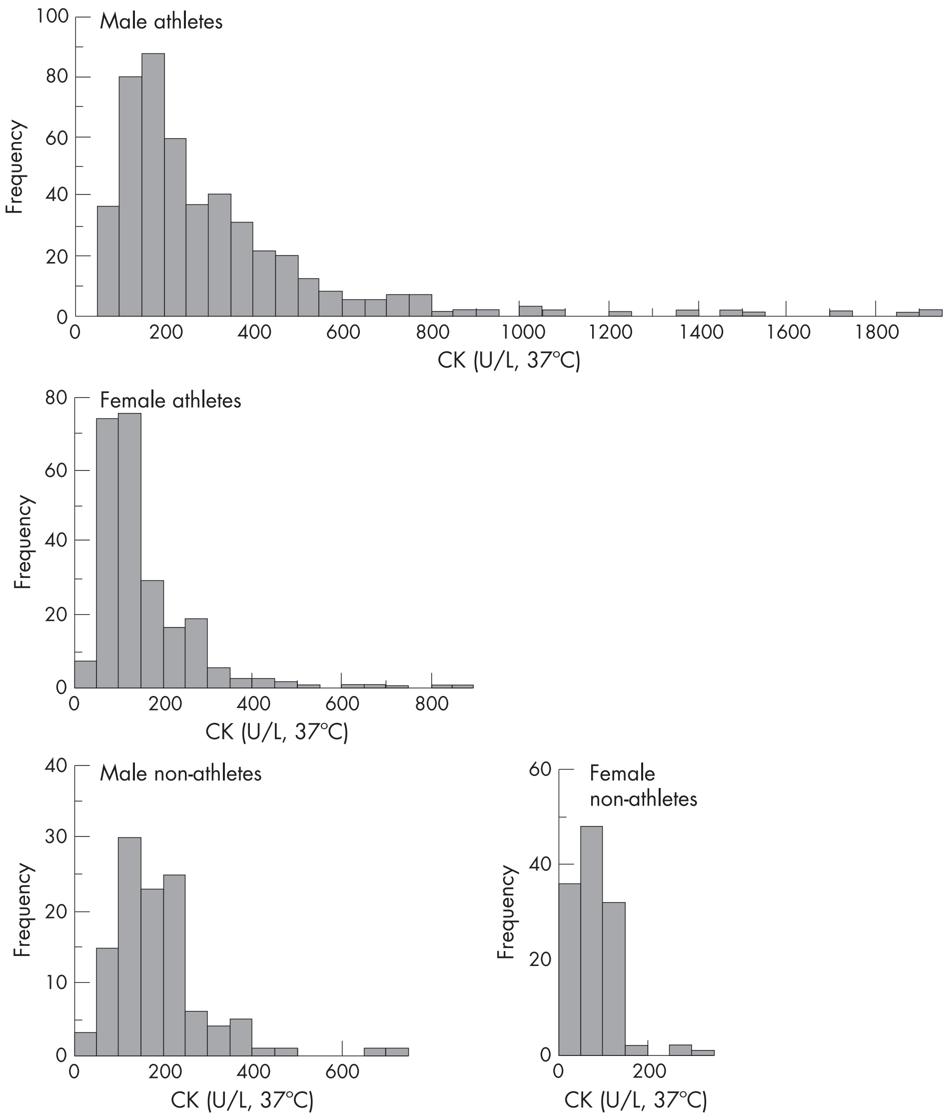

Figure 1 Distribution of serum CK values in male and female athletes and non-athletes.

cing reference intervals for athletes, the present study may contribute to establishing more relevant reference intervals for CK in the general population.

Gender affected the reference interval for CK in athletes, the upper reference limit for males being more than twice as high as that for females. This is in agreement with the differences in mean and maximal values seen in other studies[3 27] and is in line with the existence of sex-specific reference intervals for CK in the general population.[17 22–26] These differences may be explained by the higher CK content of men's muscle than that of women's muscle,[28] although other factors, such as muscle membrane permeability, CK clearance rate and lymph activity cannot be excluded.

Table 1 Reference limits and 90% confidence intervals (CI) for serum creatine kinase (U/L, 37°C) in male and female athletes and non-athletes

Group	Lower reference limit	CI of the lower reference limit	Upper reference limit	CI of the upper reference limit
Male athletes	82	73–86	1083	881–1479
Female athletes	47	39–55	513	404–836
Male non-athletes	45	39–72	491	369–728
Female non-athletes	25	17–30	252	141–345

Age (within the range of 7–44) did not seem to affect the reference interval for CK in athletes considerably. This is agreement with the statement that age does not appear to influence the degree to which serum enzyme concentrations increase with exercise[8] and is in line with the absence of age-specific reference intervals for CK in the general population within the aforementioned range.

Different sports have quite different demands in terms of strength, speed, endurance, flexibility and technique. Training programmes are tailored to meet these often conflicting demands and to provide the perfect mix of abilities that will enable an athlete to excel. Thus, although introducing reference intervals for CK in athletes is in itself an advance over having reference intervals in the general population only, asking for sport-specific reference intervals is a legitimate request. The available data permitted a partial response to this request, that is, the introduction of reference intervals in male football players and swimmers. Interestingly, the upper reference limit in football players was almost three times the upper reference limit in swimmers. This may be related to the nature of football. Football training and playing involves a great deal of

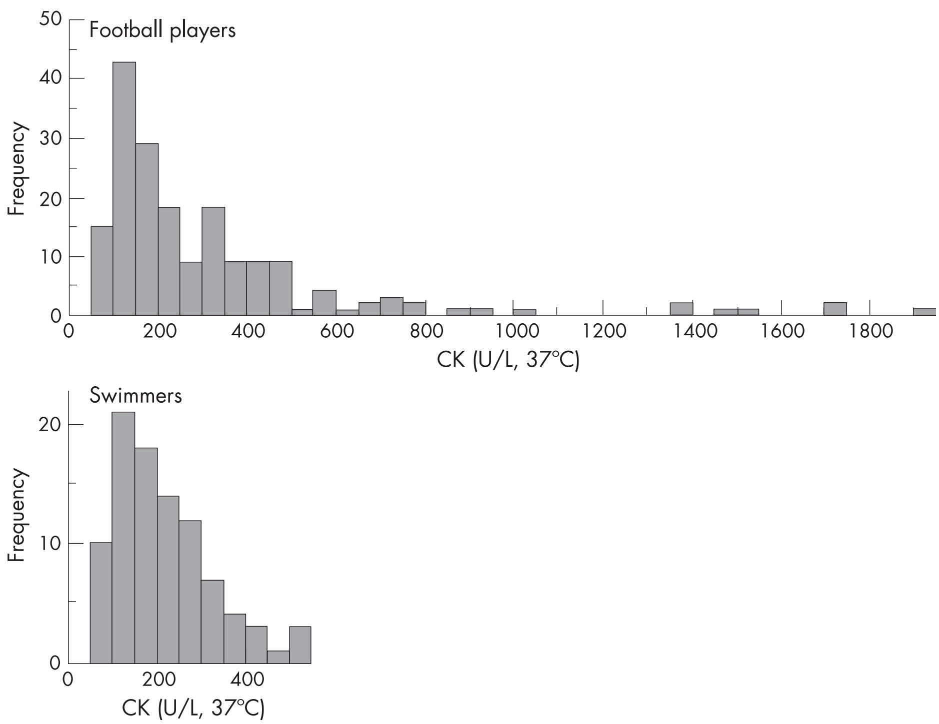

Figure 2 Distribution of serum CK values in male football players and swimmers.

weight-bearing activities, which include eccentric (lengthening) contractions of the leg muscles, such as during landing after a jump or when abruptly stopping while sprinting. Eccentric contractions are known to cause more muscle injury than concentric (shortening) contractions, resulting in higher increases of serum CK.[8 14] In addition, football playing can induce muscle damage due to mechanical impact with other players. Finally, football training and competition are often performed under harsh environmental conditions, and football games are among the longest (90 minutes) and most energy-demanding sporting activities. In contrast, swimming training and competition involve mainly non-weight-bearing activities and concentric contractions of the upper and lower limb muscles, causing minor muscle damage and only small increases in serum CK.[8] In addition, there is no muscle-damaging physical contact with other swimmers. Finally, swimming training and competition take place mostly in the protective environment of a swimming pool, and most swimming events last only a few minutes.

CONCLUSION

In summary, by analysing serum samples from 483 male athletes and 245 female athletes of a variety of sports, I have calculated the following reference intervals for the catalytic concentration of CK: males, 82 to 1083 U/L; females, 47 to 513 U/L. The upper limits of these intervals are twice the upper limits reported for moderately active non-athletes in the literature or calculated in the non-athletes of this study. The difference from the upper limits reported for inactive individuals in the literature (171 U/L for males and 145 U/L for females)[23] is more dramatic, being more than sixfold in males and threefold in females. The difference between physically active and inactive non-athletes suggests the need for a revision of the reference intervals in the general population in accordance with the guidelines for regular exercise as part of a healthy lifestyle. When reference intervals were calculated specifically in male football players and swimmers, a threefold difference in the upper reference limit was found (1492 vs 523 U/L, respectively), which may be explained by the heavier training and competition burden placed on football players.

The findings of the present study indicate profound effects of sport training and competition on the reference intervals for serum CK. Introducing specific reference intervals in athletes and, further, reference intervals according to sport may protect physicians from misinterpreting high CK values as pathological ones. Additionally, it may help coaches to establish decision limits for CK as guidelines in optimising training, in conjunction with other widely used parameters (such as blood lactate). In principle, coaches could maximise training adaptations and performance gains by employing training loads resulting in CK values close to the upper reference limits. However, exceeding these limits might signal an increased risk for overexertion or injury.

What is already known on this topic

- The serum concentration of creatine kinase (CK) is used as an index of skeletal muscle fibre damage in sport and exercise.
- Normal values have been established in the general population.
- Since athletes have higher values than non-athletes, comparing the values of athletes to the normal values established in non-athletes is pointless.

What this study adds

- This study introduces reference intervals for CK in male and female athletes, as well as specific reference intervals in male football (soccer) players and swimmers.
- These intervals may protect physicians from misinterpreting high CK values as pathological ones.
- They may help coaches to establish decision limits as guidelines in optimising training.

Finally, the findings of the present study may indicate introduction of reference intervals for CK in other sports and introduction of reference intervals for other blood parameters in athletes. Target parameters should be the ones upon which physical activity has a moderate-to-large effect, as identified in studies from many laboratories.[1–5] The establishment of specific reference intervals in athletes will set the framework for a more accurate evaluation of the training load and training status in the direction of both protecting an athlete's health and enhancing his or her performance. Additionally, it will increase the value of monitoring athletes through clinical laboratory tests and motivate sport clubs and individual athletes to seek such monitoring on a regular basis.

Competing interests: None declared.

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