CK-MM gene polymorphism does not influence the blood CK activity levels after exhaustive eccentric exercise

CK-MM gene polymorphism does not influence the blood creatine kinase activity after exhaustive eccentric exercise

Chen Yamin[1], José Oliveira[2], Yoav Meckel[2], Nir Eynon[2], Moran Sagiv[2], Moshe Ayalon[2], Alberto Jorge Alves[1], José Alberto Duarte[1]

[1] Genetics and Molecular Biology Laboratory Sports Medicine Div The Zinman College of Physical Education and Sport Sciences at Wingate, Netantya, Israel.

[2]CIAFEL, Faculty of Sport, University of Porto, Portugal.

Abstract

Gene variants, such as creatine kinase (CK) polymorphisms, have been suggested to explain the inter-individual CK response. However, this association is still unclear. Therefore, the purpose of this study was to analyze the association between the magnitudes of the CK response to exercise with the occurrence of muscle-CK-MM NcoI polymorphism in young healthy subjects. Blood CK activity was assessed in 70 subjects immediately before and 3, 24, 48, 72, 96, 120, 168 hours after an unusual and eccentric exhaustive exercise. Based on the amount of CK release by each subject, sample was distributed in quartiles being the genotype and allele frequency distribution compared among quartiles. Despite the inter-individual variability of CK response observed between subjects, there were no differences in genotype and allele frequencies among quartiles. The results allowed us to conclude that CK response after exhaustive eccentric exercise is not associated with CK-MM Ncol polymorphism.

Key-words: Quartiles, genotype, inter-individual variability, muscle damage

Introduction

Exercise-induced muscle damage is a common human condition observed after the practice of unusual and/or exhaustive exercise, especially when it is required a high percentage of eccentric contractions, such as in military training, weight lifting or marathon running [21].

Exercise-induced muscle damage is associated with the enhanced permeability of the muscle cell membranes promoting the increased blood levels of specific proteins usually localized within the muscle fibers [6, 17]. Among these proteins, creatine kinase (CK) is one of the most used indicators of muscle damage [4, 14] since it is found almost exclusively in the skeletal and cardiac muscles.

Markedly high blood levels of CK (300-10174 U/L) are often seen following prolonged weight carriage activities (e.g.2-20 hours marching or running), and particularly following short exercises that involve intense resistive eccentric contractions (e.g. push-ups, squats etc.) [4, 8]. Nevertheless, there is a wide inter-individual variability in the magnitude and time course of plasma CK response related with exercise-induced muscle damage being the subjects usually classified as low, medium and high CK responders [5]. The reasons underlying this great inter-individual variability remain unclear, but there is growing evidence for a genetic contribution to the exertional muscle damage vulnerability [7, 8]. In fact, it is known that several inherited genetic muscular disorders, such as McArdle's disease and Duchenne's muscular dystrophy, may predispose individuals to develop exercise-induced muscle damage [28]. Additionally, it has been suggested that healthy subjects who were exposed to similar

resistive exercise load and environmental conditions presented different susceptibility to develop clinical symptoms and signs of muscle damage [28]. Moreover, it is also accepted that the wide variability in the CK response induced by exercise cannot just be predicted solely based on age [13, 16], race [20, 21], body composition [19] and the individual's level of physical activity or inactivity [24]. Therefore, other factors, particularly genetic factors, should also be considered as contributors for the development and severity of exercise-induced muscle damage [26, 27].

Indeed, it was recently suggested a close association between the inter-individual variability of blood CK response to exercise and a CK polymorphism affecting the CK-MM isoenzyme [10], the most prominent CK isoform found in skeletal muscle (98%), [9]. In the study of Heled et al. [10] the authors conclude that participants with the CK-MM AA genotype had a six-fold higher risk of being high-responders as compared to GG and AG genotypes. However, some weaknesses related with the data analysis, the reduced aggressiveness of the performed exercise and the cutoff point used to define high CK responders might compromise this study's conclusion. Indeed, it may be argued that in the work of Heled et al. [10] the magnitude of muscle damage induced by exercise was extremely reduced as expressed by the cutoff point used to define subjects as "high CK responders" (above the 90th percentile or presented CK activity higher than 230 U/L). This response, however, is extremely reduced when compared to that usually described in literature after eccentric exercise, where plasma CK levels can be ~10 fold higher reaching values above 10,000 U/L [34]. Additionally, as a result of the data analysis employed by Heled et al. [10] mixing homozygous (GG) and heterozygous

(GA) subjects, it was not clear if there is or there is not a gene dose effect on the reported CK response.

Therefore, since in our point of view the association between the CK-MM Nco I genotype and the CK response to eccentric exercise-induced muscle damage is still uncertain, the main purpose of this study was to utilize demanding eccentric contractions to induce indubitable muscle damage in healthy young individuals, associating the magnitude of plasma CK response with the occurrence of CK-MM Nco I gene polymorphism. Base on the results of Heled et al. [10], even using a different approach, we hypothesized that the CK-MM Ncol polymorphism may contribute to explain the inter-individual variability of CK response following exercise.

Materials and Methods

Subjects

Seventy physically active students (42 males and 28 females; aged 25 ± 3 years old; height 171 ± 8 cm; and weight 67 ± 10 kg) volunteered for the study. Participants were healthy, non-smokers, and not receiving any medical treatment nor involved in competitive sports. Moreover, subjects were not committed with heavy weight lifting or resistance training programs in the previous 6 months, and presented baseline blood values of CK activity within the normal range (males: 24-195U/L, females: 24-170U/L). Participants were all Israeli Caucasians, with an equivalent ratio of Ashkenazi and Non-Ashkenazi descent. The study was approved by the Institution Review Board (Helsinki

Committee) of the "Hillel-Yafe" Medical Center, and all participants gave written informed consent before inclusion in the study and the start of any study related procedures.

Exhaustive eccentric exercise protocol

Based on the elbow flexor maximal eccentric contraction assessed 2 months before the beginning of the experimental protocol, subjects performed one set of 50 maximal eccentric contractions of the elbow flexor muscles of their non-dominant arm at 120° s -1 (Each contraction lasted 3 s), starting with the elbow flexed at 50° and ending at an angle of 170°, using the BIODEX dynamometer (BIODEX System 3). Subjects were seated with the arm supported and were stabilized at the waist and the chest. During each movement, subjects were verbally encouraged to produce a maximal effort to resist the ability of the dynamometer to extend the elbow. Subjects were given a 10 s rest between each contraction, during which time the dynamometer arm returned passively to the starting position. Subjects were instructed to drink water before the exercise session, and encouraged to maintain hydration and to monitor their urine color throughout the study. They were also instructed to call the 24-h study phone to report whether their urine changed from clear or yellow to a brownish color (no subject experienced darkened urine). Subjects were followed-up and instructed not to participate in any strenuous physical activity until their CK values had returned to near

normal.Analysis of CK activity and genotyping for CK-MM Ncol polymorphism Blood samples were drawn from an antecubital vein by venipuncture before and 3, 24, 48, 72, 96, 120 and 168h post-exercise to determine whole blood CK activity, which was determined with a commercially available Reflotron@ CK assay using the Reflotron@ system [11]. Blood samples obtained before exercise were also used for genotypic analysis. Genomic DNA was extracted from peripheral blood leukocytes using a standard protocol [18]. We used a PCR amplification that was conducted to amplify 1170 base pair (bp) DNA fragment localized in the gene followed by restriction digestion using the NcoI restriction endonuclease to distinguish between the A and G alleles [15]. The allele without the NcoI restriction site was designated as the G allele (1170 bp) whereas the allele with the polymorphic NcoI site was designated as the A allele (985 bp + 185 bp). This DNA fragment was amplified using the specific primer pairs: 5'-GTGCGGTGGACACAGCTGCCG-3' (Forward) and 5'-CAGCTTGGTCAAAGACATTGAGG-3' (Reverse). PCR fragments were amplified from 20 ng of each DNA sample used as a template in 20µl polymerase chain reactions (PCR) containing 0.2U Taq polymerase, 1× reaction buffer, 0.2mmol/L concentration of Each deoxynucleotide triphosphate, and 10 pmol of each primer: The initial denaturation at 95°C for5 minutes was followed by 35 cycles of 94°C for 30 seconds, 58°C annealing for 30seconds, and 65°C elongation for 45 seconds.

Data analysis

The SPSS statistical package version 15.0 was used for statistical evaluation (SPSS Inc,Chicago, IL, USA). Data are presented as mean ± standard deviation (SD) or otherwise stated. Normality of each quantitative variable was tested using the Shapiro-

Wilk normality test. CK values were analyzed across time of the experimental protocol, and CK peak was identified for each subject as the highest value attained after exercise. Individual "maximal CK response" (Δ_{CK}) was determined by the difference between CK peak and that recorded at baseline (before exercise). The Δ_{CK} of male and female was compared using the Man-Whitney U test (asymmetrical data distribution). Taking into account the huge interindividual variability of maximal CK response in both genders, samples were separated by gender and further divided into quartiles of Δ_{CK}. Subjects located in the 1st quartile were the lowest CK responders while those placed in the 4th quartile comprised the highest responders. For each quartile the allele frequencies were determined by gene counting. A X^2 test was used to confirm that the observed genotype frequencies were in Hardy-Weinberg equilibrium, and to compare alleles and genotype frequencies between quartiles. The significant level was set for a $p<0.05$.

Results

The individual CK absolute values for each gender along the experimental protocol are depicted in Figure 1, which shows a great interindividual variability of CK levels following eccentric exercise. For men and women the average values of CK at baseline were, respectively, 119.9±39.7 U/L and 109.9±32.4 U/L while the Δ_{CK} was, respectively, 4,859.7±6,809.9 U/L and 3,515.5±4,163.5 U/L. No significant differences were found between genders for both baseline ($p=0.31$) and Δ_{CK} ($p=0.95$).

Insert Figure 1

The quartile distribution of Δ_{CK} for males and females is presented in Fig.2. Despite the different quartile cutpoints, a similar pattern of Δ_{CK} can be observed in both genders. Consequently, further analysis regarding the genotype and allele frequency distributions between Δ_{CK} quartiles were performed joining all subjects of each quartile from both genders.

Insert Figure 2

The genotype and the allele frequency for all sample and for each gender are shown in table 1. Males [42 (60%)] and females [28 (40%)] did not differ in CK-MM Ncol genotype and allele frequencies.

Insert Table 1

There was no deviation from the Hardy-Weinberg equilibrium (allele frequency CK-MM Ncol = 0.27/0.73, expected genotype frequencies % GG/AG/AA = 7%/40%/53%, X^2=0.25, P =0.61). The genotype and the allele frequency for Δ_{CK} quartiles are shown in table 2.

Insert Table 2

It was not found any significant differences between genotype/allele frequencies and the different prototypes of CK release after exercise, which were defined by quartiles. In particular, there were no differences in genotype/allele frequencies between the lowest CK responders (subjects of 1st quartile) and the highest CK responders (4th quartile) (X^2 = 1.91, df = 2, P = 0.38), between the highest responders and the remained sample (X^2 =

2.79, df = 2, P = 0.25), or even between the lowest responders and the remained sample (X^2 = 4.05, df = 2, P = 0.13).

Discussion

The present study clearly shows a marked increase of CK activity in blood following exhaustive eccentric exercise, with peak values reaching as high as ≈26,000 U/L in male. Nevertheless, as expected, the blood CK activity showed a huge interindividual variability, with several subjects presenting post-exercise CK levels identical to those registered at baseline. Despite the apparently lower peak CK values observed in female compared to male, the distribution pattern of blood CK activity was similar in both gender along time post-exercise. Surprisingly, in contrast to that previously reported in the literature [10]. our data does not support a role of CK-MM Ncol polymorphism to explain the CK variability between subjects.

Exercise-induced muscle damage is characterized by the breakdown of skeletal muscle cells accompanied by the leakage of muscle contents into the circulation, which is explained by the occurrence of intrinsic and extrinsic degenerative processes to the fibers, triggered by mechanical and/or metabolic overload [2]. As a result, an elevated CK activity is normally observed in blood, which is frequently used to assess the severity of muscle damage [28]. Comparatively to men, it is widely assumed the lower CK activity levels following strenuous exercise in women, which is traditionally explained by the role of estrogens in membranes stabilization and in the attenuation of inflammatory reaction [22]. Indeed, despite not being significantly different, the scatter plot data depicted in Figure 1 shows a general trend of women to present lower peak

CK values as compared to men. However, it is important to note that even presenting apparently lower CK values, women showed a pattern of CK responses along the time post exercise identical to male. These findings are in agreement with a previous study, which showed an identical time course post exercise in both genders, despite the lower CK levels observed in females [23]. Independent of the influence of hormonal environment, the CK response showed in our study a considerable interindividual variability in both genders as already reported in literature [8]. Since CK variability cannot be predicted just by the age, race, body composition, physical activity or inactivity of subjects [13, 16, 19, 20, 21, 24], several authors have suggested a genetic influence to explain the reason why subjects submitted to the same degree of effort may release different amounts of CK [26, 27]. Several genes might be proposed as potential candidates to explain the above-referred variability, such as those encoding metabolic enzymes, structural proteins of cytoskeleton or of the T–Tubules structure as well as those involved in the control of muscle proteolysis or inflammatory reaction.

One of those candidates was recently proposed by the study of Heled et al. [10] reporting that CK-MM NcoI polymorphism is related with individual differences in blood CK after moderate exercise. The authors found that subjects with the AA genotype had six-fold higher risk to be high responders than those with the GG and AG genotype. In a notorious contrast with the results reported by Heled et al. [10], the present study showed no differences in CK-MM NcoI genotypes and alleles frequency between high vs. low CK releasers.

This kind of disagreement is not uncommon in population-association studies [26] and may be attributable to different experimental designs and/or studied population genetic

characteristics. For instance, in the study by Heled et al. [10], subjects performed alternated submaximal concentric-eccentric exercise within a given time, while we requested our subjects to performed 50 maximal eccentric contractions. As a result of higher mechanical/metabolic overload imposed to skeletal muscle in the present study, the average increase in peak serum CK in high responders was almost 18 times higher in our study than that of study by Heled et al. [10], Therefore, based on CK as a marker of muscle damage, it is reasonable to question the severity or even the occurrence of muscle damage in the former study. On the other hand, using a more demanding exercise, the present work raises little doubts about the real existence of muscle damage, making more notorious the interindividual variability and, consequently, allowing a clear distinction between low and high CK responders. Furthermore, another non-negligible methodological issue in the study of Heled et al. [10], was the procedure used to compare the CK response between groups of genotype (AA vs. AG+GG), which precluded the demonstration of the existence of an additive genetic effect of the CK-MM NcoI polymorphism on CK response, required in this type of reports. In opposition, using a different methodological approach based on the level of CK response, we have analyzed the genotype and allele frequency distribution among groups with a wide range of CK response. Assuming a polygenetic influence on exercise-induced muscle damage [7, 8, 26, 27), it cannot be excluded the possibility that other genes differently expressed in the populations of the two studies could have contributed to the divergence of our results and those reported by Heled et al [10].

Nevertheless, apart the unsettled issue regarding the association of CK-MM NcoI polymorphism with CK response, another conceptual problem is yet to be solved: is CK

blood level a reliable marker of exercise-induced muscle damage? Do CK blood levels really parallel the degree of muscle damage? Although a favorable response to these questions has been provided by several authors [1, 3] others have established that differences in CK release after exercise do not consistently reproduce differences in the degree of histological muscle damage [23] Thus, while the doubt still persists, one should not consider the use of CK activity alone as marker of muscle damage. Consequently, in order to ascertain the real influence of gene polymorphisms on exercise-induced muscle damage, future studies should consider the use of more rigorous methodologies to assess the true degree of muscle damage.

In summary we did not find any association of CK-MM NcoI polymorphisms with the behavior of CK response. However, since CK is not consensually accepted as a good and reliable marker of muscle damage, our results cannot safely exclude the hypothetical influence of CK-MM NcoI polymorphism on the degree of muscle damage induced by exercise.

References:

1. Amelink GJ, Bär PR. Exercise-induced muscle protein leakage in the rat. Effects of hormonal manipulation. J Neurol Sci. 1986;76 :61-8.
2. Armstrong RB, Warren GL, Warren JA. Mechanisms of exercise-induced muscle fibre injury. Sports Med 1991; 12: 184-207.

3. Bär PR, Amelink GJ, Oldenburg B, Blankenstein MA. Prevention of exercise-induced muscle membrane damage by oestradiol. Life Sci. 1988;42: 2677-81.
4. Brancaccio P, Maffulli N, Limongelli FM. Creatine kinase monitoring in sport medicine. Br Med Bull 2007; 81-82: 209-230
5. Chen TC. Variability in muscle damage after eccentric exercise and the repeated bout effect. Res Q Exerc Sport 2006; 77: 362-371
6. Clarkson PM, Hubal MJ. Exercise-induced muscle damage in humans. Am J Phys Med Rehabil 2002; 81: S52-69
7. Clarkson PM, Hoffman EP, Zambraski E, Gordish-Dressman H, Kearns A, Hubal M, Harmon B, Devaney JM. ACTN3 and MLCK genotype associations with exertional muscle damage. J Appl Physiol 2005; 99: 564-569
8. Devaney JM, Hoffman EP, Gordish-Dressman H, Kearns A, Zambraski E, Clarkson PM. IGF-II gene region polymorphisms related to exertional muscle damage. J Appl Physiol 2007; 102: 1815-1823
9. Echegaray M, Rivera MA. Role of creatine kinase isoenzymes on muscular and cardiorespiratory endurance: genetic and molecular evidence. Sports Med 2001; 31: 919-934
10. Heled Y, Bloom MS, Wu TJ, Stephens Q, Deuster PA. CK-MM and ACE genotypes and physiological prediction of the creatine kinase response to exercise. J Appl Physiol 2007; 103: 504-510
11. Henriksen EJ, Jacob S, Kinnick TR, Youngblood EB, Schmit MB, Dietze GJ. ACE inhibition and glucose transport in insulinresistant muscle: roles of bradykinin and nitric oxide. Am J Physiol 1999; 277: R332-336

12. Hornemann T, Stolz M, Wallimann T. Isoenzyme-specific interaction of muscle-type creatine kinase with the sarcomeric M-line is mediated by NH(2)-terminal lysine charge-clamps. J Cell Biol 2000; 149: 1225-1234
13. Horska A, Fishbein KW, Fleg JL, Spencer RG .The relationship between creatine kinase kinetics and exercise intensity in human forearm is unchanged by age. Am J Physiol Endocrinol Metab 2000; 279: E333-339
14. Houmard JA, Costill DL, Mitchell JB, Park SH, Fink WJ, Burns JM. Testosterone, cortisol ,and creatine kinase levels in male distance runners during reduced training. Int J Sports Med 1990; 11: 41-45
15. Lindpaintner K, Pfeffer MA, Kreutz R, Stampfer MJ, Grodstein F, LaMotte F, Buring J, Hennekens CH. A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. N Engl J Med 1995; 332: 706-711
16. Manfredi TG, Fielding RA, O'Reilly KP, Meredith CN, Lee HY, Evans WJ. Plasma creatine kinase activity and exercise-induced muscle damage in older men. Med Sci Sports Exerc 1991; 23: 1028-1034
17. Nosaka K, Clarkson PM, Apple FS. Time course of serum protein changes after strenuous exercise of the forearm flexors. J Lab Clin Med 1992; 119: 183-188
18. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning. Cold Spring Harbor 1989; NY.
19. Salvadori A, Fanari P, Ruga S, Brunani A, Longhini E. Creatine kinase and creatine kinase-MB isoenzyme during and after exercise testing in normal and obese young people. Chest 1992; 102: 1687-1689

20. Schwane JA, Buckley RT, Dipaolo DP, Atkinson MA, Shepherd JR. Plasma creatine kinase responses of 18- to 30-yr-old African-American men to eccentric exercise. Med Sci Sports Exerc 2000; 32: 370-378
21. Sherwood RA, Lambert A, Newham DJ, Wassif WS, Peters TJ. The effect of eccentric exercise on serum creatine kinase activity in different ethnic groups. Ann Clin Biochem 1996; 33 (Pt 4): 324-329
22. Tiidus PM. Influence of estrogen on skeletal muscle damage, inflammation, and repair. Exerc Sport Sci Rev 2003; 31: 40-44
23. 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
24. 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: 431-437
25. Willoughby DS ,Taylor M, Taylor L. Glucocorticoid receptor and ubiquitin expression after repeated eccentric exercise. Med Sci Sports Exerc 2003; 35: 2023-2031
26. Yamin C, Amir O, Sagiv M, Attias E, Meckel Y, Eynon N, Sagiv M, Amir RE. ACE ID genotype affects blood creatine kinase response to eccentric exercise. J Appl Physiol 2007; 103: 2057-2061
27. Yamin C, Duarte JA, Oliveira JM, Amir O, Sagiv M, Eynon N, Sagiv M, Amir RE. IL6 (-174) and TNFA (-308) promoter polymorphisms are associated with systemic creatine kinase response to eccentric exercise. Eur J Appl Physiol 2008; 104: 579-586

28. Zager RA .Rhabdomyolysis and myohemoglobinuric acute renal failure. Kidney Int 1996; 49: 314-326

Table 1: Genotype and allele frequencies of CK-MM Ncol polymorphisms in the studied population (n = 70). Values are absolute and relative (in parenthesis) frequencies.

		Genotype [a]			Allele frequencies [b]	
		AA	GA	GG	AlleleA	AlleleG
All	n=70	5(7)	28(40)	37(53)	38(0.27)	102(0.73)

Male	n=42	21(50)	18(43)	3(7)	60(0.71)	24(0.29)
Female	n=28	16(57)	10(36)	2(7)	42(0.75)	14(0.25)
GG (rare allele) homozygous for the CK-MM Ncol; AA homozygous and GA heterozygous for the CK-MM Ncol allele. [a] - $X^2 = 0.376$, df = 2, P = 0.83 for CK-MM Ncol genotype frequencies in males vs. females. [b] - $X^2 = 0.07$, df = 1, P = 0.79 for CK-MM Ncol allele frequencies in males vs. females .						

Table 2: Genotype and allele frequencies of CK-MM Ncol polymorphisms in each quartile of CK response. Values are absolute and relative (in parentheses) frequencies.

		Genotype [a]			Allele frequencies [b]	
Δ_{CK}	Sample	AA	GA	GG	AlleleA	AlleleG
1st quartile	n=17	3(17.6)	7(41.2)	7(41.2)	13(0.38)	21(0.62)
2nd quartile	n=18	0(0)	10(55.6)	8(44.4)	10(0.28)	26(0.72)
3rd quartile	n=18	0(0)	7(38.9)	11(61.1)	7(0.19)	29(0.81)
4th quartile	n=17	2(11.8)	4(23.5)	11(64.7)	8(0.24)	26(0.76)

GG (rare allele) homozygous for the CK-MM Ncol; AA homozygous and GA heterozygous for the CK-MM Ncol allele. [a] - $X^2 = 9.32$, df =6, P = 0.15 for CK-MM Ncol genotype frequencies between quartiles.
[b] - $X^2 = 3.42$, df = 3, P = 0.33 for CK-MM Ncol allele frequencies between quartiles.

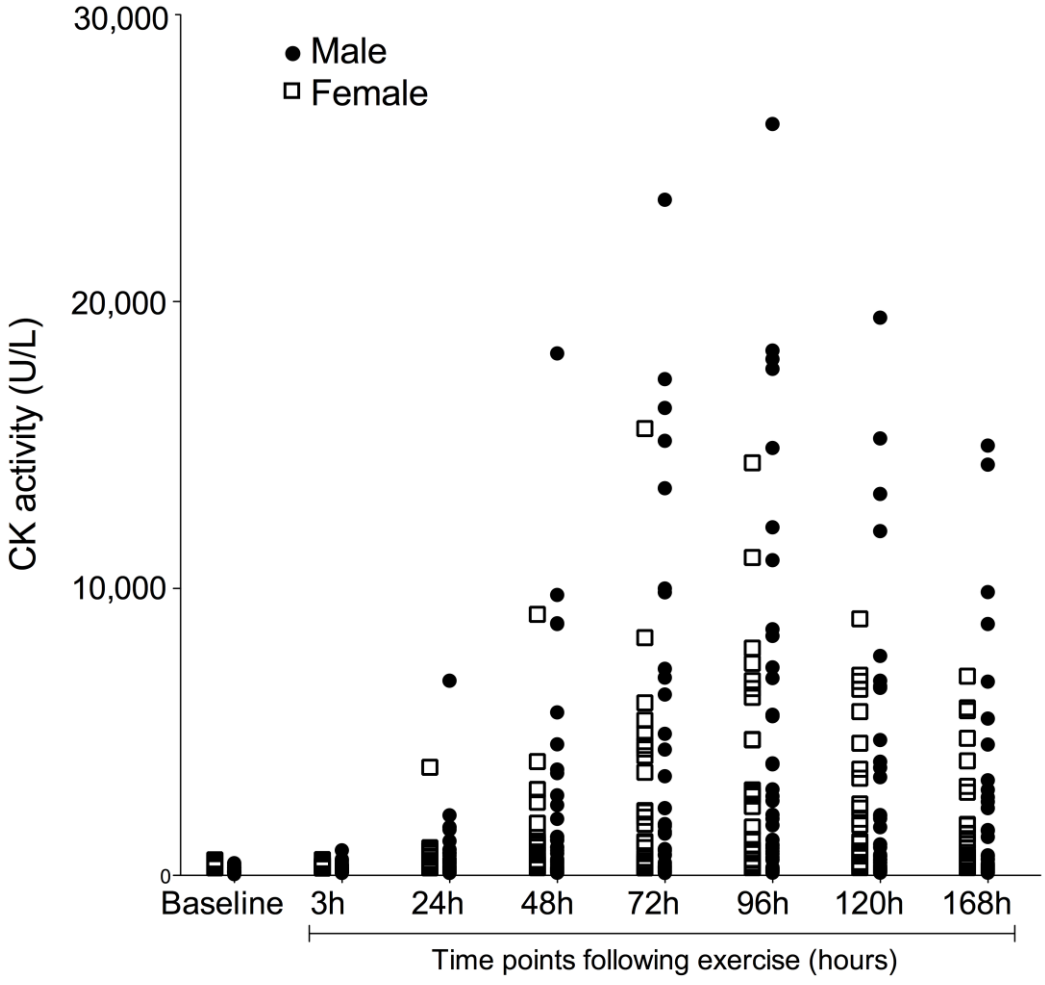

Figure 1 – Distribution in males and females of individual CK (creatine kinase) activity at baseline (before exercise) and along different time points (hours) following exercise.

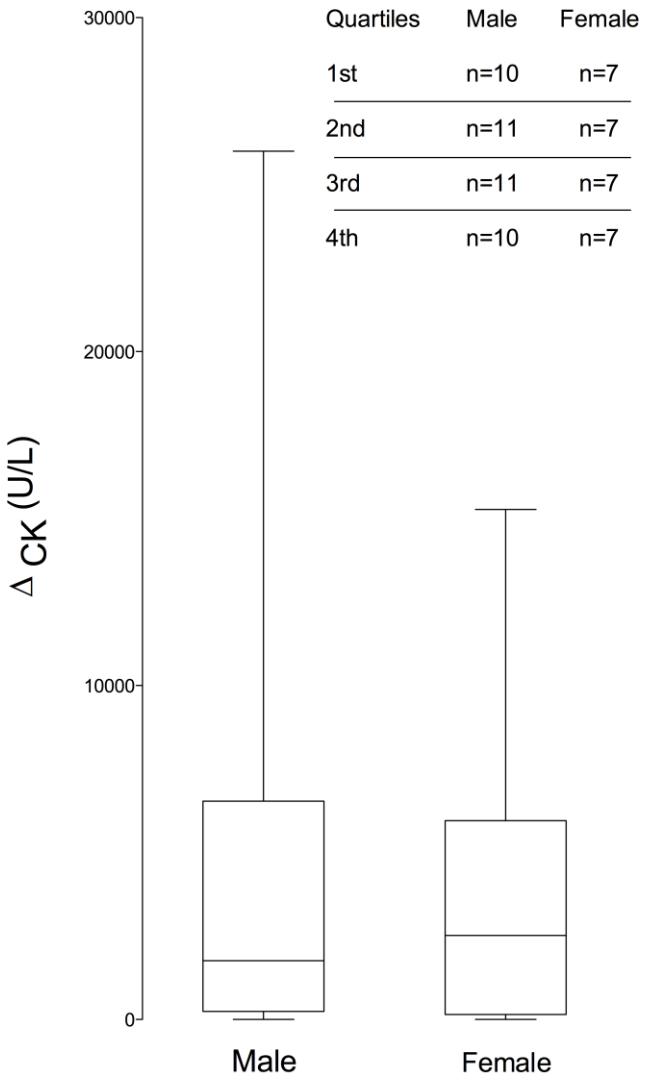

Figure 2 – Quartiles of maximal CK response (Δ_{CK}) to exhaustive eccentric exercise in male and female. It is also presented the sample size within each quartile.