ORIGINAL

RESPONSE OF CREATINE KINASE TO ANAEROBIC SUPRAMAXIMAL EXERCISE IN ACTN3 GENOTYPES

RESPUESTA DE CREATINA QUINASA A UN EJERCICIO ANAEROBIO SUPRAMÁXIMO EN GENOTIPOS DE ACTN3

DOI: http://doi.org/10.15366/rimcafd2020.79.001

ACKNOWLEDGEMENTS: The authors would like to thank the National Council for Science and Technology (Consejo Nacional de Ciencia y Tecnología, CONACYT) for the predoctoral fellowship granted to Jaime Güereca Arvizuo (371390/249892).

Código UNESCO/UNESCO Code: 2409 Genética / Genetics; 5899 Deportes / Sports
Clasificación del Consejo de Europa/Council of Europe Classification: 2. Bioquímica del deporte / Biochemistry of sport

Recibido 28 de agosto de 2018. Received August 28, 2018
Aceptado 2 de febrero de 2019. Accepted February 2, 2019

ABSTRACT

The aim of the present study was to investigate the differences in the activity of the enzyme creatine kinase (CK) before and after supramaximal anaerobic exercise (SMAE) on alpha-actinin-3 gene (ACTN3) genotype carriers. Thirty-nine healthy
and physically active men (18-35 years) were recruited and underwent a 30-s SMAE (Wingate). The ACTN3 gene was determined from white blood cell DNA from peripheral blood and the CK activity was assessed in blood samples in basal conditions, 24 and 48h after SMAE. XX genotype carriers presented 1.4 times lower CK activity in basal conditions ($p<0.05$) and higher CK activity 24h after exercise ($p<0.05$) than RR carriers. One set of SMAE was enough to induce a significant increase in CK activity after 24h in XX genotype carriers.

**KEYWORDS:** Alpha-actinin-3, creatine kinase, sport genetics, gene polymorphism.

**RESUMEN**

El objetivo del presente estudio fue investigar las diferencias en la actividad de la enzima Creatina Quinasa (CK) en pre y post ejercicio anaerobio supramáximo (EASM) en portadores de los genotipos del gen de la alfa-actinina-3 (ACTN3). Se reclutaron 39 hombres sanos físicamente activos (18-35 años) y se sometieron a un EASM de 30 s (Wingate). El gen ACTN3 se determinó a partir del ADN de glóbulos blancos en sangre periférica y se evaluó la actividad de la CK en muestras sanguíneas en condiciones basales, a las 24 y 48 h post EASM. Los portadores del genotipo XX vs RR presentaron 1,4 veces menor actividad de CK en condiciones basales ($p<0.05$) y una mayor actividad de CK a las 24 h post ejercicio ($p<0.05$). Una serie de EASM fue capaz de causar un incremento significativo de la actividad de CK a las 24 h en los portadores del genotipo XX.

**PALABRAS CLAVE:** Alfa-actinina-3, creatina quinasa, genética deportiva, polimorfismo genético.

**INTRODUCTION**

Recent research on genetics has revealed that the expression of certain genes enhances physical capacities. Nevertheless, physical capacities are not determined by one single gene, but by the synergy and interaction of several genes and their response to environmental conditions (Saunders et al., 2007). Up till now, more than 200 genetic variations have been estimated to be related to sport performance (Bray et al., 2009), but only slightly more than 20 polymorphisms are closely related to the high performance athlete (Bray et al., 2009). The R577X polymorphism within the alpha-actinin-3 (ACTN3) gene is one of the most widely studied polymorphisms in the sport performance and physical capacity field.

The ACTN3 gene can present two alleles: R allele codes for the functional alpha-actinin-3 protein, while X allele produces a non-functional alpha-actinin-3 protein (MacArthur & North, 2004). Alpha-actinin-3 protein expresses only in the skeletal muscle, namely in fast-twitch fibres, and is one of the major structural components of sarcomeric Z-discs (Beggs et al., 1992). The combination of R and X alleles
generates the RR, RX and XX genotypes. These genotypes have been associated with sport performance, since there is evidence that they confer an advantage, either aerobic or anaerobic (MacArthur & North, 2004). In other words, RR and RX genotypes are associated with greater capacity to generate power (Orysiak et al., 2014), strength (Broos et al., 2015) and speed (Mikami et al., 2014), while XX genotype confers an advantage during endurance activities (Yang et al., 2003). The ACTN3 protein has been reported to have metabolic signalling functions in the skeletal muscle, at least in murine models (Lee, Houweling, North & Quinlan, 2016) and, therefore, to enhance muscle strength (MacArthur et al., 2008). By contrast, it is also well known that the absence of this protein (XX genotype) does not hinder muscle contraction (Zanoteli et al., 2002), although it produces enzymatic changes in knockout mouse skeletal muscle, altering the metabolism of fast fibres and turning them more oxidative (MacArthur et al., 2008). A decrease in the elastic properties of muscle proteins at the level of the sarcomeric Z-line has also been reported (Seto et al., 2011), what would lead to lower capacity to generate strong and fast contractions. From the above, we can deduce that athletes possessing XX genotype may be more susceptible to muscle damage induced by strong and fast contractions (Clarkson et al., 2005; Vincent et al., 2010), what would be reflected in an increase of creatine kinase (CK) activity.

Besides, inconsistent results have been recently reported on CK enzymatic activity after exercise among ACTN3 genotypes (Clarkson et al., 2005; Vincent et al., 2010; Venckunas et al., 2012). In the sports field, CK is used as biochemical marker of tissue damage (Landau et al., 2012; Wu et al., 2004). Its plasma concentration increases after aerobic (Hoffman, Ingwerson, Rogers, Hew-Butler & Stuemfle, 2012) or anaerobic exercise (Rodas et al., 2002; Vincent et al., 2010), being one single session of supramaximal anaerobic exercise (SMAE) enough for increase its level (Hammouda et al., 2012). Given all the above, the aim of the present study was to examine the differences in the activity of CK enzyme before and after SMAE (Wingate) between ACTN3 genotypes in physically active participants.

**MATERIAL AND METHOD**

**Participants**

This was a cross-sectional observational study with a sample of 42 healthy and physically active men (aged 18-35 years) who did sport activity three or more days a week. The inclusion criteria were: healthy and physically active men. The exclusion criteria were: elite athletes, and participants who were receiving any pharmacological medical treatment, or who suffered from any muscle injury that prevented them from doing physical exercise. Prior to the study, the procedure and the risks derived from all measurements were explained to the participants, who then signed an informed consent letter. A general health questionnaire and the PAR-Q & YOU questionnaire (Thomas et al., 1992) were applied in order to confirm the good health of the participants. The research protocol and procedures
were based on the recommendations of the Declaration of Helsinki and approved by the ethics committee of the Autonomous University of Ciudad Juárez (CBE.ICB/053.08-15).

All measurements were conducted between 8 and 10am. The participants were requested not to eat in the previous 8 hours and not to do intense exercise or physical activity in the three days prior to the study.

**Supramaximal anaerobic exercise**

Before the SMAE, the participants’ body mass wearing only a swimsuit was assessed using digital scales (SECA 876, Hamburg, Germany). Subsequently, they performed a general 10-min warm up including 5 min cycling and then they performed the anaerobic Wingate test as SMAE (Monark Ergomedic 884e, Sweden), according to the procedures already published (Bar-Or, 1987). The load added to the cycloergometer was 7.5% of the participant’s body mass.

**CK enzymatic activity assessment**

The participants were requested to attend on three consecutive days in order to determine plasma CK activity. A blood sample was obtained through antecubital venipuncture right before the SMAE, 24h and 48h after the SMAE. CK concentration was determined through a clinical chemistry analyzer (Cobas Integra 400 plus, Roche Instrument Center, USA).

**Genotypes**

Genomic DNA was extracted from leukocytes using a MasterPure commercial kit (Epicentre Biotechnologies, USA). An ACTN3 gene segment of 291 base pairs (bp) was amplified applying the polymerase chain reaction (PCR) technique with the following primers: direct: 5´-CTGTTGCTGTGGTAAGTGGA-3’ and reverse: 5´-GGTCACAGTATGCAGGAGGG-3’ (Eurofins MWG-Operon, Germany). The PCR reaction was conducted by mixing 19 µL sterile water, 2.5 µL buffer, 0.75 µL MgCl₂, 0.5 µL dNTPs, 0.5 µL direct/reverse primer, a DNA sample at 100 ng and 0.25 µL Taq polymerase enzyme during 35 cycles under the following amplification conditions: initial denaturation at 95°C for 10 min and a second denaturation at 95-°C for 1 min; annealing step at 58 °C for 30s ; extension step at 72° C for 1 min and final step at 72 °C for 10 min. In order to determine ACTN3 genotypes, PCR amplification and Ddel (Desulfovibrio desulfuricans) (New England BioLabs, United States) enzyme were combined. Incubation was conducted at 37 °C in humid heat for 4 h, followed by 20min of inactivation at 65°C. The products were viewed in polyacrylamide gel at 12%. The following bands were obtained for RR genotype: 205 and 86 bp; for RX: 205, 108, 97 and 86 bp; and, lastly, for XX genotype: 108, 97 y 86 bp (Figure 1).
Anthropometric measurements, somatotype and body composition

All these measurements were conducted according to a previously published protocol (Güereca et al., 2017) and following the standardized methodology of the International Society for the Advancement of Kinanthropometry (ISAK). LifeSize 2.0 software (Sydney, Australia) (Olds & Norton, 2000) was used to determine the somatotype components (Heath & Carter, 1990) and body composition.

Statistical analysis

A chi-squared test ($\chi^2$) for the complete population (42 participants) was conducted in order to determine the differences in genotype frequencies. Nevertheless, three participants did not complete all the measurements and were removed from the study. The sample size was calculated with G*power 3.1.9.2 software (Faul, Erdfelder, Lang & Buchner, 2007), using a size effect of 0.50, an alpha value of 0.05 and a power of 0.80, yielding a sample of 9 participants per group. Shapiro-Wilk test was performed in order to confirm the data normality and outliers were detected through a box-and-whisker plot. A multivariate analysis of the variance (ANOVA) and Tukey's post-hoc test were applied to search for differences between genotypes, anthropometric characteristics and CK activity. A repeated measures ANOVA was used to analyse the differences in CK activity between genotypes, between the different moments and to determine the potential effect of age and muscle mass. Genotype was selected as between-subject factor, age and muscle mass were the covariates and Bonferroni’s method was selected to compare the main effects. Lastly, a univariate ANOVA on the residuals (24h and 48h minus the basal concentrations) was conducted to remove the potential effect of the basal CK values. Statistical significance level was set at 0.05. The data were analysed with SPSS 22.0 software.

RESULTS

Allele and genotype frequencies are displayed in Table 1, showing that the complete sample conforms to Hardy-Weinberg ($X^2 = 0.095; p = 0.75$) equilibrium.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Genotype frequency n (%)</th>
<th>Allele frequency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n: 42)</td>
<td>RR 10(25) RX 22(50) XX 10(25)</td>
<td>R 0.50 X 0.50</td>
</tr>
</tbody>
</table>

$X^2 = 0.095, p = 0.75$.

The participants’ physical characteristics were similar among genotypes, except height (table 2), with RR-genotype participants being taller than their counterparts. The CK activity results are presented at the bottom of table 2.
The fragments obtained from enzymatic restriction with Ddel enzyme to determine ACTN3 genotypes are shown in Figure 1.
When analysing the starting CK concentrations (time 0) individually for each genotype, lower concentration was found in XX than in RR genotype (Figures 2 and 3). The simultaneous repeated-measures analysis on the CK differences between genotypes and moments only yielded differences in time, CK concentrations being higher after 24 h compared to the basal values ($p = 0.012$). However, the univariate analysis of the residuals revealed that CK was only significantly higher 24 h after SMAE in XX genotype compared to RR (Figure 3).
By removing the potential effect of basal CK activity through the analysis of the residuals, it was observed that XX genotype carriers presented higher activity than
RR carriers 24 h after SMAE, returning to the basal values 48 h after SMAE ($p < 0.05$). These results are similar to those previously reported by Vincent et al. (2010), who found a non-significant trend 24 h after eccentric exercise. The above suggests that XX genotype carriers could be more susceptible to muscle damage than RR carriers after one set of SMAE. The previous results are in accordance with the hypotheses, yet the XX genotype carriers were expected to present higher post-exercise CK activity than RR carriers. CK activity after exercise has been described to be higher in untrained individuals (Vincent & Vincent, 1997). Nonetheless, in the present study no differences were found in the participants' physical characteristics that could affect CK activity. It has been also observed that, in mice, XX genotype carriers presented reduced elastic properties of the muscle proteins at the level of the sarcomeric Z-line (Seto et al., 2011). This led to a decrease in interactions with other muscle proteins (Seto et al., 2011) and, therefore, to higher vulnerability to muscle damage during exercise (Clarkson et al., 2005). Furthermore, it is assumed that the presence of the R allele plays a protective role against muscle damage upon an exercise increase (Vincent et al., 2010).

Given the low CK concentrations observed 24 and 48 h after SMAE (< 500IU/L), the intensity of the applied exercise did not seem to be enough to induce muscle damage (Martínez-Abat et al., 2005). The highest CK value after exercise was 200.5 IU/L for XX genotype, close to the basal values found by other authors: 166-177 IU/L (Schumann & Klauke, 2003). For this reason, one possible limitation of the study was the fact that the best moment to assess the peak CK activity was not determined. However, there are contradictions regarding the time to peak CK after exercise. For example, Pimenta et al. (2012) reported the peak CK activity to be achieved 4 h after exercise, while other studies stated that peak activity occurred 24 to 48 h after exercise (Vincent et al., 2010; Venckunas et al., 2012).

The present study revealed that XX carriers presented (1.4 times) lower CK activity in basal conditions than RR carriers, similar to the data reported by Clarkson et al. (2005). The lower CK activity observed in XX genotypes could be due to the muscle fiber type. XX genotype carriers have been reported to possess greater proportion of type I fibers than RR and RX (Ahmetov et al., 2011), while RR carriers present greater proportion of type IIx muscle fibers (Vincent et al., 2010) and greater cross-sectional area of type IIa and IIx fibers than XX genotypes (Broos et al., 2016). Type II fibers contain the highest CK concentration (Jansson, & Sylvén, 1985) due to their energy metabolism.

CK activity has been proved to change with age, gender, race and muscle mass (Baird, Graham, Baker, & Bickerstaff, 2012; Meltzer, 1971). Age and muscle mass were included as covariates in the statistic model in order to determine their potential effect on CK activity. However, no effect of any of them was observed. Studies that address the relationship between CK activity and ACTN3 genotypes are scarce (Clarkson et al., 2005; Vincent et al., 2010; Venckunas et al., 2012). One of the major differences between those and the present study is the exercise
protocols applied to stimulate CK production. In the present study, a single session of SMAE was applied, while Vincent et al. (2010) used 20 maximal eccentric knee contractions, Clarkson et al. (2005) used 50 maximal eccentric elbow flexor contractions and Venckunas et al. (2012) applied 2 sets of 50 vertical jumps. Such protocols were based on not very common movements in elite sport practice. Nevertheless, in spite of using different anaerobic exercise protocols, none of them induced significant increases in CK activity in any of the genotypes.

The Wingate test conducted in this study revealed a little, but significant CK increase 24 h after exercise only in XX genotype carriers. Therefore, CK activity sampling at shorter intervals between 4 and 24 h after SMAE is suggested, as well as a longer and more intense exercise protocol, since plasma CK concentration has been observed to be much higher after exercise of longer duration (Belli et al., 2017; Del Coso et al., 2017).

CONCLUSIONS

The present study reveals that XX genotype carriers present lower CK activity in basal conditions and higher activity 24 h after SMAE than RR carriers. Nevertheless, the exercise type, intensity and duration were not enough to induce CK increases above 500 I U/L, value suggested as indicator of muscle damage.

REFERENCES


Schumann, G., y Klaue, R. (2003). New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum:


Número de citas totales / Total references: 37 (100%)
Número de citas propias de la revista /Journal’s own references: 0 (0%)