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*

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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) entre 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

1

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.

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) between 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.

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 deduct 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 & Stuempfle, 2012) and anaerobic exercise (Rodas et al., 2002; Vincent et al., 2010), one single session of supramaximal anaerobic exercise (SMAE) being enough for its level to increase (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, 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 was amplified applying the polymerase chain reaction (PCR) technique with the following primers: direct: 5´-CTGTTGCCTGTGGTAAGTGGG-3' and reverse: 5´-GGTCACAGTATGCAGGAGGG-3' (Eurofins MWG-Operon, Germany). The PCR reaction was conducted by mixing 19µL sterile water, 2.5µL buffer, 0.75µL $MgCl_2$, 0.5µL dNTPs, 0.5µL direct/reverse primer, a DNA sample at 100ng and 0.25µL Taq polymerase enzyme during 35 cycles under the following amplification conditions: initial denaturation at 95ºC for 10min and a second denaturation at 95ºC for 1min; annealing step at 58ºC for 30s; extension step at 72ºC for 1min and final step at 72ºC for 10min. In order to determine *ACTN3* genotypes, PCR amplification and Ddel (*Desulfovibrio desulfuricans*) (New England BioLabs, United States) enzyme were combined. Incubation was conducted at 37ºC in humid heat for 4 hours, followed by 20min of inactivation at 65ºC. The products were viewed in

polyacrylamide gel at 12%. The following bands were obtained for RR genotype: 205 and 86bp; for RX: 205, 108, 97 and 86bp; and, lastly, for XX genotype: 108, 97 y 86bp (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 standardised 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 (χ^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 1. Genotype and allele frequencies of *ACTN3* gene.

Sample	Genotype frequency n (%)			Allele frequency %	
	RR	RX	XX	R	X
Men (n: 42)	10(25)	22(50)	10(25)	0.50	0.50

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

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.

Table 2. Anthropometric and physical characteristics divided by *ACTN3* genotype.

	RR (n=9)	RX (n=20)	XX (n=10)
Age (years)	25.4 ± 2.0	23.8 ± 2.7	24.9 ± 5.5
Body mass (kg)	80.5 ± 10.6	77.7 ± 13.4	74.1 ± 19.0
Height (m)	1.79 ± 0.05[ab]	1.73 ± 0.04	1.72 ± 0.08
BMI (kg/m^2)	24.8 ± 2.8	25.7 ± 3.7	24.5 ± 5.0
Fat mass (kg)	11.9 ± 4.8	12.5 ± 5.1	12.7 ± 8.7
Body fat (%)	15.4 ± 5.0	16.3 ± 4.8	15.5 ± 6.5
Muslce mass (kg)	31.7 ± 4.6	32.0 ± 4.2	31.4 ± 7.1
Mid-thigh (cm)	53.1 ± 2.4	53.4 ± 4.6	50.8 ± 6.0
Endomorphy	3.9 ± 1.5	4.9 ± 1.8	4.7 ± 2.9
Mesomorphy	4.2 ± 0.9	5.1 ± 0.8	4.9 ± 1.3
Ectomorphy	2.1 ± 0.9	1.5 ± 1.0	2.1 ± 1.7
Basal CK (U/L)	188.1 ± 62.7[b]	149.9 ± 48.8	129.9 ± 38.8
24-h CK (U/L)	188.9 ± 76.1	172.6 ± 63.4	200.5 ± 96.7
48-h CK (U/L)	200.6 ± 99.6	152.1 ± 52.3	145.4 ± 47.0

Values are presented as mean±standard deviation (SD). BMI = body mass index, CK = creatine kinase. [a] differences between RR and RX genotypes. [b] differences between RR and XX genotypes. $p < 0.05$.

The fragments obtained from enzymatic restriction with *Ddel* enzyme to determine *ACTN3* genotypes are shown in Figure 1.
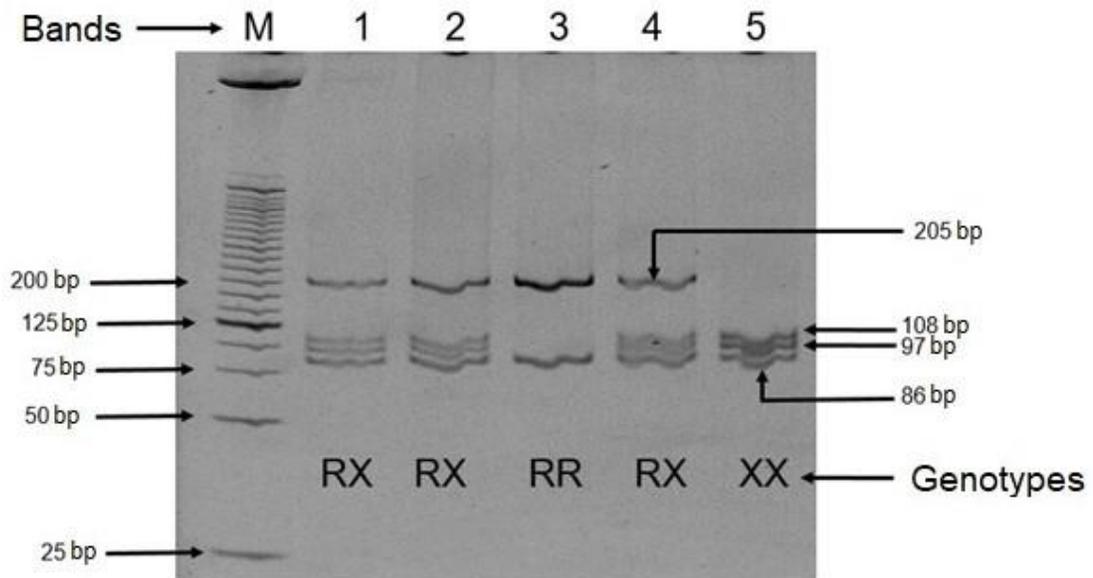
Figure 1. Fragments obtained from enzymatic restriction with *DdeI* enzyme. M band: markers of 25pb (1µg/µL). 2.5µL were loaded at 150 ng/µL. Bands 1-5: 20µL digest product of representative samples with 10X buffer load (bromophenol blue). Arrows: fragments of different sizes obtained from each amplification product digestion. Polyacrylamide/bisacrylamide gel at 12% stained with ethidium bromide (final concentration: 10µg/µL) in a big vertical electrophoresis system (25x25cm, Hoefer SB 600 Ruby, GE). Electrophoretic run: 145min at 300V. M: molecular marker. bp: base pair.

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 hours compared to the basal values ($p = 0.012$). However, the univariate analysis of the residuals revealed that CK was only significantly higher 24h after SMAE in XX genotype compared to RR (Figure 3).
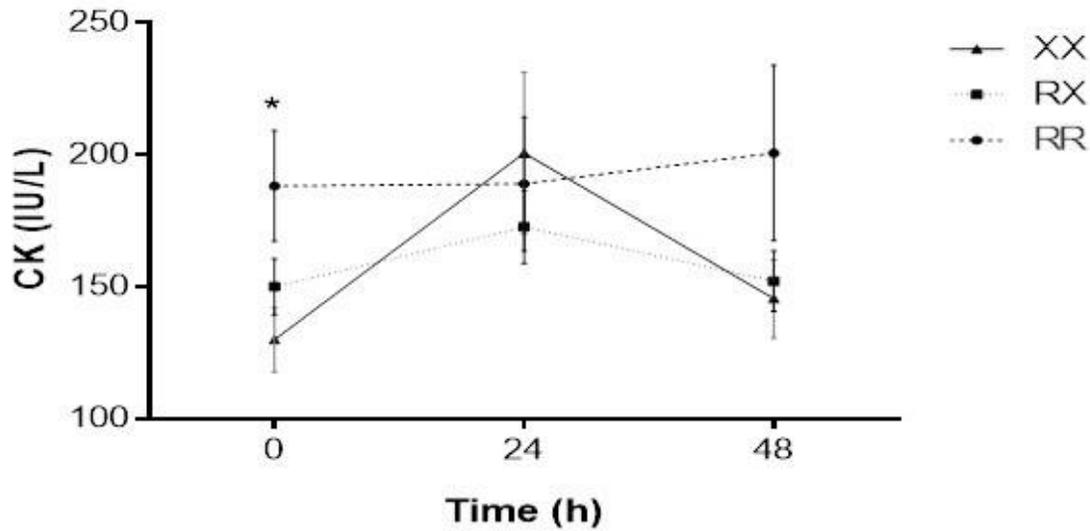
Figure 2. Comparison of CK activity between *ACTN3* genotypes at different moments after performing a Wingate test with a load of 7.5% of body weight. Values are presented as mean and standard deviation. * Statistical difference between XX and RR. CK: Creatine Kinase.
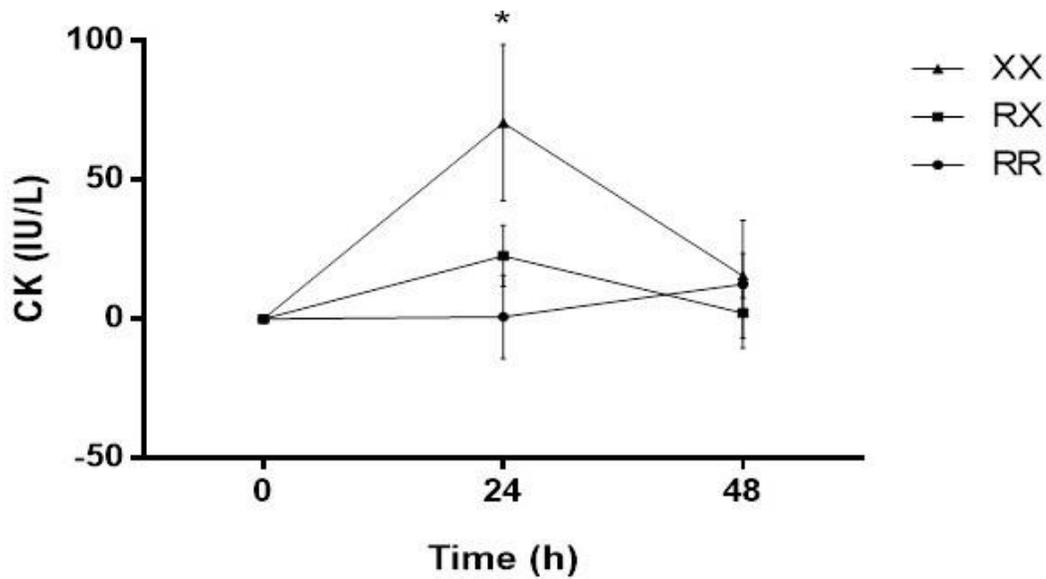

Figure 3. Comparison of CK activity between *ACTN3* genotypes at different moments after performing a Wingate test with a load of 7.5% of body weight. Values are presented as mean of the residuals and standard deviation. * Statistical difference between XX and RR. CK: Creatine Kinase.

DISCUSSION

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 24h after SMAE, returning to the basal values 48h after SMAE ($p < 0.05$). These results are similar to those previously reported by Vincent et al. (2010), who found a non-significant trend 24h 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 48h 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 200.5IU/L for XX genotype, close to the basal values found by other authors: 166-177IU/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 4h after exercise, while other studies stated that peak activity occurred 24 to 48h 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 24h after exercise only in XX genotype carriers. Therefore, CK activity sampling at shorter intervals between 4 and 24h 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 24h after SMAE than RR carriers.
Nevertheless, the exercise type, intensity and duration were not enough to induce CK increases above 500IU/L, value suggested as indicator of muscle damage.

REFERENCES

Ahmetov, I.I., Druzhevskaya, A.M., Lyubaeva, E.V., Popov, D.V., Vinogradova, O. L., and Williams, A.G. (2011). The dependence of preferred competitive racing distance on muscle fibre type composition and *ACTN3* genotype in speed skaters. *Exp Physiol. 96*(12): 1302–1310.

Baird, M.F., Graham, S.M., Baker, J.S., y Bickerstaff, G.F. (2012). Creatine-kinase-and exercise-related muscle damage implications for muscle performance and recovery. *J Nutr Metab*, 2012;2012:960363. doi: 10.1155/2012/960363.

Bar-Or, O. (1987). The Wingate anaerobic test an update on methodology, reliability and validity. *Sports Medicine*, *4(6),* 381-394.

Beggs, A. H., Byers, T.J., Knoll, J.H., Boyce, F.M., Bruns, G.A., y Kunkel, L.M. (1992). Cloning and Characterization of Two Human Skeletal Muscle Alpha-Actinin Genes Located on Chromosomes 1 and 11. *J Biol Chem*, *267*(13), 9281-9288.

Belli, T., Crisp, A. H., y Verlengia, R. (2017). Greater muscle damage in athletes with *ACTN3* R577X (RS1815739) gene polymorphism after an ultra-endurance race: a pilot study. *Biol Sport, 34*(2), 105-110.

Bray, M.S., Hagberg, J.M., Pérusse, L., Rankinen, T., Roth, S.M., Wolfarth, B., y Bouchard, C. (2009). The human gene map for performance and health-related fitness phenotypes: the 2006–2007 update. *Med Sci Sports Exerc, 41*(1), 35–73.

Broos, S., Malisoux, L., Theisen, D., van Thienen, R., Ramaekers, M., Jamart, C., …, and Francaux, M. (2016). Evidence for *ACTN3* as a speed gene in isolated human muscle fibers. *PLoS One.* 11(3): 1-11.

Broos, S., Van Leemputte, M., Deldicque, L., y Thomis, M. A. (2015). History-dependent force, angular velocity and muscular endurance in *ACTN3* genotypes. *Eur J Appl Physiol, 115*, 1637–1643.

Clarkson, P. M., Hoffman, E. P., Zambraski, E., Gordish-Dressman, H., Kearns, A., Hubal, M., … y Devaney, J. M. (2005). *ACTN3* and *MLCK* genotype associations with exertional muscle damage. *J Appl Physiol, (1985), 99*(2), 564-569.

Del Coso, J., Valero, M., Salinero, J.J., Lara, B., Díaz, G., Gallo-Salazar, C., … y Cacabelos. (2017). *ACTN3* genotype influences exercise-induced muscle damage during a marathon competition. *Eur J Appl Physiol*, 117(3), 409-416.

Faul, F., Erdfelder, E., Lang, A.G., y Buchner, A. (2007). G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods, 39*(2), 175-191.

Güereca-Arvizuo, J., Ramos-Jiménez, A., Flores-Martínez, N., Reyes-Leal, G y Hérnandez-Torres, R.P. (2017). *ACTN3* genotypes and their association with athletes somatotype: Results of a pilot study. *ECORFAN-Ecuador Journal 2017 4(6),* 10-17.

Hammouda, O., Chtourou, H., Chaouachi, A., Chahed, H., Ferchichi, S., Kallel, C., … y Souissi, N. (2012). Effect of short-term maximal exercise on biochemical markers of muscle damage, total antioxidant status, and homocysteine levels in football players. *Asian J Sports Med, 3*(4), 239-246.

Heath, B., y Carter, J.E. (1990). Somatotyping Development and Applications. Cambridge university press.

Hoffman, M. D., Ingwerson, J. L., Rogers, I. R., Hew-Butler, T., y Stuempfle, K. (2012). Increasing creatine kinase concentrations at the 161-km Western States Endurance Run. *Wildernees Environ Med*, *23*(1), 56-60.

Jansson, E., y Sylvén, C. (1985). Creatine kinase MB and citrate synthase in type I and type II muscle fibres in trained and untrained men. *Eur J Appl Physiol Occup Physiol, 54*(2), 207-209

Landau, M. E., Kenney, K., Deuster, P., Gonzalez, R. S., Contreras-Sesvold, C., Sambuughin, N., O'Connor, F. G., y Cambell, W. (2012). Investigation of the relationship between serum creatine kinase and genetic polymorphisms in military recruits. *Military Medicine*, *177*(11), 1359-1365.

Lee, F., Houweling, P., North, K., y Quinlan, K. (2016). How does alfa-actinin-3 deficiency alter muscle function? Mechanistic insights into *ACTN3*, the gene for speed. (2016). *Biochimica et Biophysica Acta,* 1863. 868-893.

MacArthur, D. G., y North, K. N. (2004). A gene for speed? The evolution and function of α-actinin-3. *Bioessays, 26(7),* 786-795.

MacArthur, D. G., Seto, J. T., Chan, S., Quinlan, K. G., Raftery, J. M., Turner, N., … y North, K. N. (2008). An Actn3 knockout mouse provides mechanistic insights into the association between alpha-actinin-3 deficiency and human athletic performance. *Hum Mol Genet, 17*(8), 1076-1086.

Martínez-Abat, A., Boulaiz, H., Prados, J., Marchal, J., Padial, P., Caba, O., Rodriguez-Serrano, F., Aranega, A. (2005). Release of A-Actin into serum after skeletal muscle damage. *Br J Sports Med, 39*(11), 830-834).

Meltzer, H.Y. (1971). Factors affecting serum creatine phosphokinase levels in the general population: the role of race, activity and age. *Clin Chim Acta, 33*, 165-172.

Mikami, E., Fuku, N., Murakami, H., Tsuchie, H., Takahashi, H., Ohiwa, N., … y Tanaka, M. (2014). *ACTN3* R577X Genotype is Associated with Sprinting in Elite Japanese Athletes. *Int J Sports Med, 35*(2), 172-177.

Olds, T., Norton, K., and Clark, J. (2000). LifeSize user manual release 1.0 Educational Software for Body Composition Analysis. Australia*:* Human Kinetics, Inc.

Orysiak, J., Busko, K., Michalski, R., Mazur-Różycka, J., Gajewski, J., Malczewska-Lenczowska, J., … y Pokrywka, A. (2014). Relationship between *ACTN3* R577X polymorphism and maximal power output in elite Polish athletes. *Medicina*, *50*(5), 303-308.

Pimenta, E. M., Coelho, D. B., Cruz, I. R., Morandi, R. F., Veneroso, C. E., de Azambuja Pussieldi, G., Carvalho, M. R., Silami-Garcia, E., De Paz Fernández, J. A. (2012). The *ACTN3* genotype in soccer players in response to acute eccentric training. *Eur J Appl Physiol, 112*(4), 1495-1503.

Rodas, G., Ventura, J. L., Cadefau, J. A., Cussó, R., y Parra, J. (2002). Un programa de entrenamiento intenso para un rápido mejoramiento tanto del metabolismo aeróbico como del anaeróbico. *Apunts Medicina de l'Esport*, *37(140),* 5-12.

Saunders, C. J., September, A. V., Xenophontos, S. L., Cariolou, M. A., Anastassiades, L. C., Noakes, T. D., y Collins, M. (2007). No association of the *ACTN3* gene R577X polymorphism with endurance performance in Ironman Triathlons. *Ann Hum Genet, 71*(Pt 6), 777-781.

Schumann, G., y Klauke, R. (2003). New IFCC reference procedures for the determination of catalytic activity concentrations of five ezymes in serum: preliminary upper reference limits obtained in hospitalized subjects. *Clin Chim Acta, 327*(1-2), 69-79.

Seto, J., Lek, M., Quinlan, K., Houweling, J., Zheng, X., Garton, F., … North, N. (2011). Deficiency of alfa-actinin-3 is associated with increased susceptibility to contraction-induced damage and skeletal muscle remodeling. *Human molecular genetics, 20*(15), 2914-2927.

Thomas, S., Reading, J., y Shephard, R.J. (1992). Revision of the Physical Activity Readiness Questionnaire (PAR-Q). *Can J Spt Sci*, *17*(4), 338-345.

Venckunas, T., Skurvydas, A., Brazaitis, M., Kamandulis, S., Snieckus, A., y Moran, C. N. (2012). Human *alpha-actinin-3* genotype association with exercise-induced muscle damage and the repeated-bout effect. *Appl. Physiol.Nutr. Metab*, *37*, 1-9.

Vincent, H.K., Vincent, K.R. (1997). The effect of training status on the serum creatine kinase response, soreness and muscle function following resistance exercise. *Int J Sports Med 1997, 18*(6), 431-437.

Vincent, B., Windelinckx, A., Nielens, H., Ramaekers, M., Van Leemputte, M., Hespel, P., y Thomis, M. A. (2010). Protective role of α-actinin-3 in the response to an acute eccentric exercise bout. *J Appl Physiol (1985), 109*(2)*, 564–573.

Wu, H. J., Chen, K. T., Shee, B. W., Chang, H. C., Huang, Y. J., y Yang, R. S. (2004). Effects of 24 h ultra-marathon on biochemical and hematological parameters. *World J Gastroenterol*, *10(18),* 2711-2714.

Yang, N., MacArthur, D.G., Gulbin, J., Hahn, A., Beggs, A.H., Eastel, S., y North, K. (2003). *ACTN3* Genotype Is Associated with Human Elite Athletic Performance. *Am J Hum Genet, 73*(3), 627–631.

Zanoteli, E., Lotuffo, R., Oliveira, A., Beggs, A. H., Canovas, M., Zatz, M., y Vainzof, M. (2002). Deficiency of Muscle α-Actinin-3 is compatible with high muscle performance. *J Mol Neurosci, 20*(1), 39-42.

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13