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ORIGINAL ARTICLE

ACTN3 genotype influences exercise-induced muscle damage during a marathon competition

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Abstract
Purpose Exercise-induced muscle damage has been identified as one of the main causes of the progressive decrease in running and muscular performance in marathoners. The aim of this investigation was to determine the influence of the ACTN3 genotype on exercise-induced muscle damage produced during a marathon.
Methods Seventy-one experienced runners competed in a marathon race. Before and after the race, a sample of venous blood was obtained and maximal voluntary leg muscle power was measured during a countermovement jump. In the blood samples, the ACTN3 genotype (R577X) and the changes in serum creatine kinase and myoglobin concentrations were measured. Data from RX heterozygotes and XX mutant homozygotes were grouped as X allele carriers and compared to RR homozygotes.
Results At the end of the race, X allele carriers presented higher serum myoglobin (774 ± 852 vs 487 ± 367 U L^{-1}; $P = 0.02$) and creatine kinase concentrations (508 ± 346 vs 359 ± 170 ng mL^{-1}; $P = 0.04$) than RR homozygotes. Pre-to-post-race maximal voluntary leg muscle power reduction was more pronounced in X allele carriers than RR homozygotes (-34.4 ± 16.1 vs $-27.3 \pm 15.4\%$; $P = 0.05$). X allele carriers self-reported higher levels of lower limb muscle pain (7 ± 2 vs 6 ± 2 cm; $P = 0.02$) than RR homozygotes at the end of the race.
Conclusions In comparison to RR homozygotes, X allele carriers for the R577X polymorphism of the ACTN3 gene presented higher values for typical markers of exercise-induced muscle damage during a competitive marathon. Thus, the absence of a functional α-actinin-3 produced by the X allele might induce higher levels of muscle breakdown during prolonged running events.

Keywords α-Actinins · Rhabdomyolysis · Muscle pain · Endurance exercise · Genetics · Single-nucleotide polymorphism.

Abbreviations

Δ	Percentage of difference
ACTN2	α-Actinin-2 gene
ACTN3	α-Actinin-3 gene
A.U.	Arbitrary units
DNA	Deoxyribonucleic acid
ES	Effect size
PCR	Polymerase chain reaction
SNP	Single-nucleotide polymorphism

Introduction

Exercise-induced muscle damage is the deterioration of skeletal muscle caused by unusual or exhaustive exercise, especially involving eccentric and/or continuous muscle contractions. Exercise-induced muscle damage is characterized by a reduced capacity to generate force, delayed-onset muscle soreness, swelling, and increases in intramuscular proteins into blood stream (Clarkson and Hubal 2002;

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Baxter and Moore 2003). While several investigations have reported low-to-moderate levels of exercise-induced muscle damage in sports, such as soccer (Thorpe and Sunderland 2012), basketball (Chatzinikolaou et al. 2014), rugby (Oxendale et al. 2015), and badminton (Abian et al. 2015), greater levels of muscle damage have been typically related to long-lasting weight-bearing sports, such as the marathon (Del Coso et al. 2013b; Cuisinier et al. 2001) and half-ironman triathlon (Del Coso et al. 2012).

Specifically, it has been suggested that the physical challenges necessary to complete a marathon race might be "the perfect scenario" for the developing of exercise-induced muscle damage in amateur athletes (Clarkson 2007) because of the severe muscle fiber breakdown as the result of the continuous concentric and eccentric muscle actions necessaries for completing the distance. In fact, the muscle damage incurred during a competitive marathon race, as measured by blood markers, such as myoglobin and creatine kinase concentrations, has been identified as one of the key variables for the reduced muscular performance and reduced running pace at the end of the event (Del Coso et al. 2013a). Interestingly, the inter-individual level of muscle damage attained during a competitive marathon is highly variable, cannot be explained by factors, such as age or previous training status, and cannot be prevented with the ingestion of branched-chain amino acids (Areces et al. 2014) or the use of compression garments (Areces et al. 2015). The explanation as to why some individuals, and not others, incur severe exercise-induced muscle damage during a marathon is still unknown (Deuster et al. 2013), although it could be related to the presence/absence of α-actinin-3, a protein that plays a key role in the contractile apparatus of fast twitch skeletal muscle fibers (North 2008).

The α-actinins are a family of actin-binding proteins encoded by two genes: ACTN2 and ACTN3. The sarcomeric α-actinins are major components of the Z line and they crosslink the thin actin filaments, while they coordinate myofibril contraction (Yang et al. 2003). While the α-actinin-2 is present in all types of fibers, the α-actinin-3 is restricted to type 2 fibers. Despite the importance of α-actinin-3 to maintain the ordered myofibrillar array, there is a relatively common deficiency of the α-actinin-3 in some individuals due to homozygosity in a single-nucleotide polymorphism (SNP) in the ACTN3 gen (R577X, rs1815739). This polymorphism produces a premature stop codon that leads to the production of a non-functional α-actinin-3. Roughly, 18% of the world population is homozygous for this non-sense mutation (Ivarsson and Westerblad 2015) and it has been proposed that the positive selection of the ACTN3 X null allele during evolution has been related to increased cold acclimatisation and thermogenesis in populations living in cold environments (Head et al. 2015). Besides, it has been suggested that carriers of the X null allele compensate the absence of this protein with α-actinin-2 (Yang et al. 2003), which might confer different properties to the muscle fiber that can influence exercise performance (Eynon et al. 2011; Santiago et al. 2010).

Briefly, the presence of α-actinin-3 has a beneficial effect on the function of skeletal muscle to generate forceful contractions at high velocity (Yang et al. 2003). In fact, X allele carriers for the R577X SNP are much less frequently found in elite power- and sprint-like athletes (Eynon et al. 2013; Ma et al. 2013), although this is not always the case (Ruiz et al. 2011). Regarding exercise-induced muscle damage, it has also been found that the presence of α-actinin-3 in type 2 fibers might exert a protective role against acute eccentric muscle actions, because XX individuals presented higher serum creatine kinase activities and pain scores than RR individuals after exercise-induced muscle damage (Vincent et al. 2010). Similarly, XX soccer players showed higher levels of creatine kinase compared to RX and RR counterparts after eccentric muscle contractions and plyometric training (Pimenta et al. 2012). Likewise, X allele triathletes (e.g., XX and RX) presented higher values of jump height reduction, greater serum creatine kinase concentration, and a tendency for higher values of muscle pain than RR triathletes after a half-ironman triathlon, despite both groups have similar anthropometric, physical, and training characteristics (Del Coso et al. 2016). On the contrary, other investigations have failed to find an association between the R577X polymorphism and the damage induced to skeletal muscle fibers (Clarkson et al. 2005) or even a higher rate of force production after an exercise-induced muscle damage protocol in individuals with XX homozygosity (Venckunas et al. 2012).

Apart from the contradictory results obtained for the relationship between ACTN3 genotypes and exertional muscle damage, most of the aforementioned investigations (Clarkson et al. 2005; Pimenta et al. 2012; Venckunas et al. 2012; Vincent et al. 2010) have explored the role of the ACTN3 gene in the development of muscle breakdown using exercise activities that are not representative of any sport or competitive situations (eccentric elbow muscle actions, drop jumps, and eccentric knee extensions), while only one investigation has used an ecological context [real half-ironman competition; (Del Coso et al. 2016)]. Furthermore, it would be interesting to obtain information about the influence of the different ACTN3 genotypes in endurance runners, one of the groups of athletes more disposed to suffer exertional muscle damage, because of the characteristics of the competition (Clarkson 2007). For this reason, the aim of the current investigation was to determine the role of the ACTN3 genotype in the development of muscle damage after a competitive marathon in amateur runners. We hypothesized that runners with the null X allele would show greater losses in voluntary leg muscle

power and greater increases in intramuscular proteins–creatine kinase and myoglobin—in the blood when compared to homozygous RR counterparts.

Methods

Subjects

Seventy-one healthy experienced marathon runners volunteered to participate in this study. Most participants were contacted by phone and e-mail from a group of runners that had participated in the previous investigations, while other runners were recruited at the race registration. Inclusion criteria were as follows: age between 18 and 65 years, being free of any history of muscle, cardiac, or kidney disorders, participating in the marathon at maximal intensity, and having a running experience of at least 3 years with at least 3 marathons completed during this period. Exclusion criteria were as follows: taking medications during the two weeks prior to competing or having suffered a musculoskeletal injury in the 3 months previous to the competition. Participants underwent a pre-participation medical examination (including medical history and other routinary physical exams) carried out by a licensed physician to ensure the suitability of all participants to take part in the research protocols and to certify the fulfillment of inclusion/exclusion criteria. Participants also completed a questionnaire about the previous training, running experience, and best race time in the marathon. Age and main morphological and physical characteristics of the participants in this investigation are shown in Table 1.

Ethics statement

Each participant was informed of the risks and discomforts associated with this investigation and signed an informed consent document before the onset of the experiments. The study was approved by the Camilo Jose Cela University Ethics Committee in accordance with the latest version of the declaration of Helsinki. Participants' rights and confidentiality were protected during the whole experiment and the genetic information was only used for the purposes included in this investigation.

Experimental design

A case–control and ecological experimental design was used for this investigation. Initially, the participants were divided into three groups and established according to their individual genetic profile in the single-nucleotide polymorphism R577X of the ACTN3 gene (e.g., RR, RX and XX groups). However, X allele carriers (e.g., RX and XX) were clustered and considered as a single group due to a preliminary analysis that showed very similar phenotyping responses in RX and XX participants. In addition, this same collapsing strategy has previously been suggested for this SNP (Ma et al. 2013). The proportion of men and women in each group was very similar. Because the men and women responded in the same manner, we have analyzed all the data without considering the sex of the individuals.

For this investigation, all the participants underwent the same testing under the same experimental conditions. Participants completed the 2015 edition of the Rock 'n' Roll Madrid Marathon with no indications about running pace or fluid and food strategies. The marathon race was held in April on a rainy day with a mean dry temperature of 13.0 ± 1.0 °C (range from 11 to 14 °C, temperature readings at 30-min intervals from 0 to 5 h after the race onset) and a mean relative humidity of 88 ± 1% (range from 88 to 89%). The race started at 09:00 h and the race course altitude varied from 600 to 720 m above sea level.

Table 1 Age, anthropometric characteristics, running experience, and training status of marathoners with different ACTN3 genotypes (1 SNP tested R577X)

Variable (units)	RR	X allele carriers	*P* value
n	21	50	–
Men/women	19/2	45/5	–
Age (years)	40.0 ± 8.1	42.7 ± 8.9	0.26
Body mass (kg)	72.2 ± 10.0	73.2 ± 11.0	0.76
Body height (m)	1.73 ± 0.08	1.75 ± 0.08	0.58
Running experience (year)	9.4 ± 5.4	12.3 ± 9.9	0.22
Best race time in the marathon (min)	217 ± 37	216 ± 32	0.91
Completed marathons (number)	6.4 ± 7.0	8.9 ± 13.2	0.43
Average training distance/week (km)	56.0 ± 24.5	61.2 ± 22.8	0.59
Training sessions/week (number)	4.4 ± 0.9	4.5 ± 1.1	0.87

Data are mean ± SD

Experimental protocol

Participants were instructed to avoid pain-relieving strategies (e.g., analgesic medications), strenuous exercise, caffeine, and alcohol 48 h before the onset of the race. Twenty-four hours before the race, a 10-mLvenous blood sample was obtained from an antecubital vein after 10 min of supine resting. From the total, 3 mL were introduced into a tube with ethylenediaminetetraacetic acid, while the remaining blood was allowed to clot and serum was later obtained by centrifugation at 5000*g*. Then, participants underwent a standardized 10-min warm-up, including low-intensity running, and five submaximal countermovement jumps. At that point, participants performed two maximal countermovement vertical jumps on a force platform (Quattrojump, Kistler, Switzerland). For this measurement, participants began stationary in an upright position with their weight evenly distributed over both feet. Each participant placed their hands on their waist to remove the influence of the arms on the jump. On command, the participant flexed their knees and jumped as high as possible while maintaining the hands on the waist and landed with both feet. The pre-race jumps were performed with the competition clothes and shoes, and the attempts were separated by 1 min of rest. In each jump, maximal voluntary leg muscle power output during the concentric phase of the jump was determined from ground reaction forces. The jump with the highest height was used for statistical analysis. All participants had previously been familiarized with the jump test during the week prior to the competition.

The day of the race, participants had their usual pre-competition meal at least 3 h before the race which was not standardized among participants to avoid affecting their individual pre-competition routine. Runners were encouraged to ingest 500 mL of plain water 2 h before the start of the race to increase the likelihood of being euhydrated at the start line. During the race, participants wore a race bib with a time chip to calculate the actual amount of time that it took them from the start line of the race to the finish line (net time). Race time was also measured at 5-km intervals during the whole race. Participants completed the race at their own pace and drank *ad libitum* at the hydration stations placed at 5-km intervals. Within 2 min of the end of the marathon race, participants went to a research area located close to the finish line where they performed two countermovement vertical jumps, as described above. At this time, the rating of perceived exertion was assessed using the Borg scale [from 6 to 20 arbitrary units, A.U.; (Borg 1982)]. Participants then rested for 5 min and a venous blood sample was obtained. During this time, lower limb muscle pain was measured using a 10-cm visual analog scale where participants self-rated the score from 0 to 10 cm [0 cm meant no muscle pain and 10 cm meant unbearable muscle pain; (Portenoy and Tanner 1996)]. Participants were also asked about stoppages during the race to urinate or defecate, but none of the participants reported any of these types of stoppages. After that, participants were provided with fluid (water and sports drinks) and finished their participation in the study.

Genetic testing

To investigate the potential relationship between the ACTN3 genotype and the individual level of exercise-induced muscle damage during the marathon, genomic DNA was isolated from the whole blood obtained before the race (QIAamp® DNA Blood Mini Kit, QIAGEN, The Netherlands) according to the manufacturer's protocol. ACTN3 R577X (c.1858C>T; p.R577X; rs1815739) genotyping was performed using a TaqMan® SNP genotyping assay (Life Technologies™, USA) that employs the 5′ nuclease activity of Taq DNA polymerase to detect a fluorescent reporter signal generated during Real-Time PCR reactions. Amplification and detection were performed using a real-time PCR system (Applied Biosystems® Steponeplus™ Real-time PCR system, Life Technologies™, USA).

Blood samples

A portion of each blood sample was introduced *in situ* into a blood glucose analyzer (Accu-chek, Roche, Spain) to determine glucose concentration. The remaining blood was allowed to clot and serum was separated by centrifugation (10 min at 5000*g*) and frozen at −80 °C until the day of analysis. At a later date, the serum portion was analyzed for creatine kinase and myoglobin concentrations by means of an autoanalyzer (Access II, Beckman-Coulter Instruments, USA).

Statistical analysis

The normality of each variable was initially tested with the Kolmogorov-Smirnov test. Post-race creatine kinase and myoglobin concentration were the only variables that did not follow a normal distribution, and thus, they were analyzed with non-parametric statistics. The comparison between groups (RR homozygotes vs X allele carriers) was performed using Student's *t* test for independent samples. For the non-parametric variables, the Mann–Whitney *U* test was used for comparison between groups. The percentage of difference (Δ) and the effect size (ES) was calculated in all pairwise comparisons. The magnitude of the effect size was interpreted using Cohen's scale (Cohen 1988): an effect size lower than 0.2 was considered as small, an effect size around 0.5 was considered

as medium, and an effect size over 0.8 was considered as large. The data were analyzed with the statistical package SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). The significance level was set at $P < 0.05$. Data are presented as mean ± SD.

Results

R577X ACTN3 gene variants

From the total, 21 participants (29.6% of the sample) were RR homozygotes for the R577X single-nucleotide polymorphism. Forty-two participants (59.1%) were RX heterozygotes and 8 participants (11.3%) were XX mutant homozygotes. Thus, 70.4% of the sample were carriers of the null X allele for the production of α-actinin-3. There were no between-group differences for age, body mass, body height, best race time in the marathon, or training routines (Table 1; $P > 0.05$).

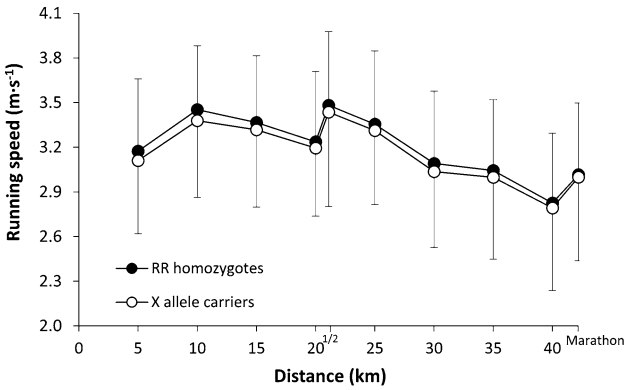

Fig. 1 Running pace during a competitive marathon in RR homozygote runners ($n = 21$) vs X allele carriers ($n = 50$) for the R577X polymorphism of the ACTN3 gene. Data are mean ± SD

Race time, leg muscle power, perceived fatigue, and muscle pain

Total race time was very similar for RR homozygotes and X allele carriers (225 ± 28 and 231 ± 36 min; Δ = 2.6%, ES = 0.2, $P = 0.26$). Running pace was similar between the two groups across the whole race (Fig. 1). From similar pre-race values, X allele carriers showed a more pronounced reduction in maximal voluntary leg muscle power than RR homozygotes at the end of the marathon (Table 2; Δ = 26.0%, ES = 0.5, $P = 0.05$). X allele carriers reported higher levels of perceived exertion (Δ = 8.2%, ES = 0.7, $P = 0.02$) and muscle pain (Δ = 19.1%, ES = 0.6, $P = 0.02$) than RR homozygotes (Table 2).

Serum responses

From similar pre-race values, X allele carriers showed higher serum creatine kinase (Δ = 41.8%, ES = 0.9, $P = 0.02$) and myoglobin concentrations (Δ = 58.9%, ES = 0.8, $P = 0.04$) than RR homozygotes at the end of the race (Fig. 2). The pre-to-post-race increase in serum myoglobin concentration (2937 ± 2752 vs 1908 ± 1307%; Δ = 54.0%, ES = 0.8, $P = 0.04$) was more pronounced in X allele carriers than RR homozygotes, although this change was not statistically significant for the creatine kinase concentration (462 ± 288 vs 375 ± 192%; Δ = 23.1%, ES = 0.5, $P = 0.20$). There were no between-group differences in blood glucose concentration before or after the race (Table 2).

Discussion

Strenuous exercise, such as running 42.2 km, can cause sarcomere disruption and muscle cell breakdown, but the level of muscle damage attained during this activity greatly varies among individuals despite similarities in age, running

Table 2 Maximal voluntary leg muscle power during a countermovement jump and blood glucose concentration before and just after a competitive marathon and ratings of perceived exertion and muscle pain after the marathon in RR homozygote runners ($n = 21$) vs X allele carriers ($n = 50$) for the R577X polymorphism of the ACTN3 gene

Variable (units)	RR homozygotes	X allele carriers	Δ	ES	*P* value
Maximal voluntary leg muscle power (W kg^{-1})					
Pre	23.9 ± 2.6	23.5 ± 3.8	−1.4%	0.1	0.36
Post	17.2 ± 3.7	15.4 ± 4.5	−10.8%	0.5	0.06
Change (%)	−27.3 ± 15.4	−34.4 ± 16.1	26.0%	0.5	0.05
Blood glucose concentration (mg dL^{-1})					
Pre	98.6 ± 13.1	109.4 ± 15.5	11.0%	0.8	0.01
Post	114.5 ± 23.7	119.0 ± 24.0	4.0%	0.2	0.24
Rating of perceived exertion (A.U.)	14 ± 2	16 ± 2	8.2%	0.7	0.02
Rating of perceived muscle pain (cm)	5.5 ± 1.8	6.6 ± 1.6	19.1%	0.6	0.02

Data are mean ± SD. The percentage of difference (Δ) and the effect size (ES) was calculated for each between-group pairwise comparison

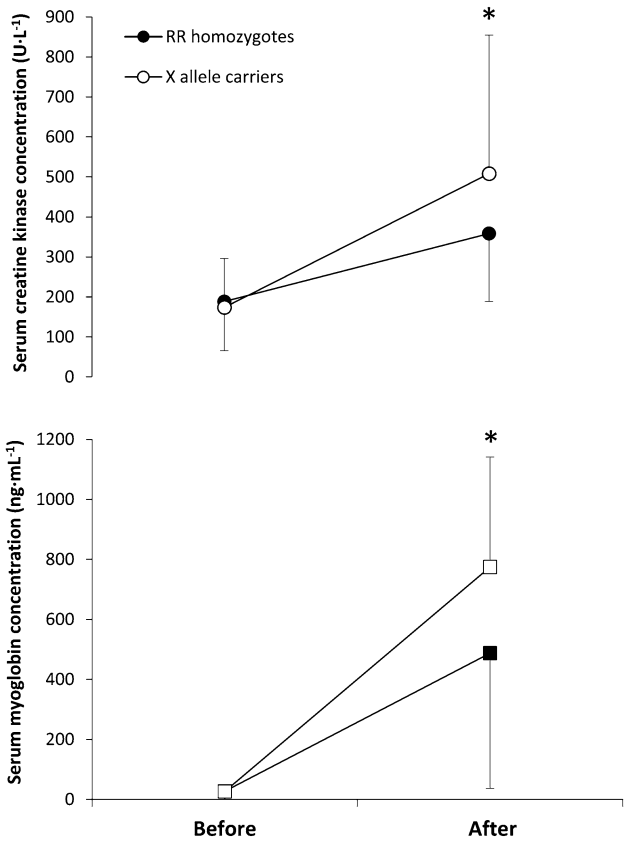

Fig. 2 Serum creatine kinase and myoglobin concentration before and just after a competitive marathon in RR homozygote runners ($n=21$) vs X allele carriers ($n=50$) for the R577X polymorphism of the ACTN3 gene. Data are mean ± SD. (*) Different from X allele carriers, $P<0.05$

experience, or previous training (Del Coso et al. 2013a, b). The main outcomes of this investigation were: (a) amateur runners that carried the X allele for the ACTN3 R577X (e.g., RX and XX) self-reported higher values of fatigue and lower limb muscle pain during the marathon (Table 2) while presenting higher post-race serum concentrations of creatine kinase and myoglobin than runners with RR homozygosity (Fig. 2); (b) X allele carriers also showed a higher muscle performance reduction after the marathon than RR homozygotes, as measured by pre-to-post-race changes in maximal voluntary leg muscle power production during a jump (Table 2); (c) X allele carriers and RR homozygotes had a very comparable running pace during the race (Fig. 1). These results indicate that the presence of the null X allele in the R577X SNP was associated with increased post-marathon values of muscle pain and blood concentration of muscle proteins, while it reduced the capacity to generate maximal voluntary muscle power during a jump. Thus, X allele carriers might be more prone to develop exercise-induced muscle damage during a competitive marathon.

The basis of exercise-induced muscle damage has been well established (Clarkson and Hubal 2002), although most investigations on this topic have been carried out in experimental settings that included muscle activities different from the ones produced in most sport situations (Sayers et al. 1999). The same has occurred to determine the role of genetics on the development of muscle damage during exercise. The ACTN3 gene has been targeted as the main gene responsible for the inter-individual variability found in situations that produce exercise-induced muscle damage. Specifically, it has been speculated that X allele carriers for R577X SNP might be more prone to exercise-induced muscle damage because of a deficiency in the production of α-actinin-3 in type 2 muscle fibers (Clarkson et al. 2005; Pimenta et al. 2012; Venckunas et al. 2012; Vincent et al. 2010). However, the influence of the R577X polymorphism on muscle damage is unclear, since XX homozygosity has been found to exert a deleterious (Pimenta et al. 2012; Vincent et al. 2010), neutral (Clarkson et al. 2005), or even positive effect (Venckunas et al. 2012) after an exercise activity that produced skeletal muscle deterioration. A recent investigation has determined the role of R577X polymorphism on muscle damage during a real half-ironman competition (Del Coso et al. 2016). In that investigation, the triathletes that carried the X allele presented a higher serum creatine kinase concentrations (total and the MM isoform), greater values of self-reported muscle pain, and a reduced capacity to generate muscle force during a jump when compared to RR counterparts with similar age, training routines, and race time in the half-ironman competition.

In the present investigation, we have used a real marathon competition as the experimental setting, a context comparable to the aforementioned investigation carried out in a half-ironman (Del Coso et al. 2016), because they increase the ecological validity of the outcomes obtained. In addition, comparable to the investigation with half-ironmen, it has been determined that the X allele carriers for the R577X SNP of ACTN3 showed higher indexes of muscle damage during an endurance competition—marathon—when compared to RR homozygotes with similar ages, running experience, and training habits. Thus, taking together the analogous outcomes of these two investigations, it is suggested that endurance athletes with the X allele might be more prone to suffer exertional muscle breakdown and its associated symptoms (muscle pain, reduced capacity to generate force, leakage of intramuscular proteins into blood circulation, and swelling) due to the dysfunctionality of α-actinin-3 in their muscle fibers.

Interestingly, X allele carriers presented clear symptoms of higher muscle breakdown than RR homozygotes without it affecting running pace during the whole race (Fig. 1), as it has been previously found in other endurance competitions (Del Coso et al. 2016). The presence

of the X allele affects the functionality of α-actinin-3 exclusively in type 2 fibers, while the SNP R577X does not influence the presence of α-actinin-2 in type 1 fibers. Besides, XX homozygotes tend to have a higher proportion of type 1 fibers than RR homozygotes (Vincent et al. 2007). Due to the distance, the muscle contractions necessary to complete a marathon race are mainly based on type 1 muscle fiber recruitment with a low influence of type 2 muscle fibers, even for elite marathoners (Skiba and Jones 2011). With this background, it is likely that X allele carriers were able to produce a running pace during the race similar to RR homozygotes, despite a higher level of exercise-induced muscle damage, because of the functionality of α-actinin-2 and a higher proportion of type 1 fibers. On the other hand, this information suggests that the higher values for muscle pain, the higher muscle power reductions, and the higher intramuscular proteins concentrations in the blood found in X allele carriers after the marathon were linked to muscle fiber damage specific for the type-2 fibers—rather than type-1 fibers—(Friden and Lieber 1992). These symptoms, connected to more severe exercise-induced muscle damage in X allele carriers, were evident even when the carriage of the 577X allele likely derived in a lower percentage of type 2 fibers, as previously suggested (Vincent et al. 2007).

The present investigation also presents some limitations derived from the experimental design selected. Because this study was carried out in a real competitive marathon, factors, such as age, pre-competition diet, training volume, and running intensity, during the race were not controlled. Nevertheless, we registered this information, and based on the absence of between-group differences (Table 1), we believe that these factors had a negligible influence on the outcomes of this investigation. It is well established that some of the symptoms of exercise-induced muscle damage are present 24 to 48 h after the cessation of the exercise activity (Clarkson 2007), but our results only extended to the end of the race. It would be interesting to investigate whether the influence of ACTN3 is also present during the recovery phase of exercise-induced muscle damage. The serum makers used in this investigation to evaluate the degree of skeletal muscle breakdown (e.g., CK and myoglobin) can also reflect damage in other tissues, such as cardiac muscle. Thus, it is also necessary to determine in future investigations that the effect of the ACTN3 on exercise-induced muscle damage is specifically related to the deterioration of skeletal muscle. Finally, the level of muscle damage attained during the race was measured by indirect markers, such as self-reported muscle pain, changes in leg muscle power, and serum concentrations of creatine kinase and myoglobin. Although these variables are trustable markers for determining skeletal muscle breakdown in a wide range of exercise activities, it would be interesting to confirm whether X allele carriers for the ACTN3 gene are more prone to muscle damage using muscle biopsies.

Conclusions

Marathon runners with the XX or RX genotype (e.g., X allele carriers) for the R577X SNP of the ACTN3 gene showed higher levels of exercise-induced muscle damage than RR counterparts, as measured by higher values of muscle pain, maximal voluntary leg muscle power reduction and blood concentrations of intramuscular proteins. Because the presence of the X allele in this gene is related to the assembly of a non-functional α-actinin-3 in type 2 muscle fibers, it is likely that the greater levels of muscle breakdown found in X allele carriers are related to the absence of this structural protein. Thus, the determination of the ACTN3 genotype of amateur marathoners could be advisable to establish specific training and diet routines that prevent X allele carriers from developing severe levels of muscle damage during endurance running competitions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest derived from the outcomes of this study.

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Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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