Eur J Appl Physiol
DOI 10.1007/s00421-016-3507-7

ORIGINAL ARTICLE

# ACTN3 X-allele carriers had greater levels of muscle damage during a half-ironman

**Juan Del Coso[1] · Juan José Salinero[1] · Beatriz Lara[1] · César Gallo-Salazar[1] · Francisco Areces[1] · Carlos Puente[1] · David Herrero[1]**

Received: 29 August 2016 / Accepted: 24 November 2016


**Abstract**
*Purpose* Alpha-actinin-3, encoded by the ACTN3 gene, is an actin-binding protein with an important role in myofibril contraction and muscle force output. In humans, there is a relatively common deficiency of the α-actinin-3 due to homozygosity in a polymorphism of the ACTN3 gene (R577X, rs1815739), that has been related to decreased resistance to strain during voluntary muscle contractions. The purpose of this study was to investigate the influence of the ACTN3 genotype on the level of exercise-induced muscle damage attained by 23 experienced triathletes during an official half-ironman competition.
*Methods* Before and after the race, a sample of venous blood was obtained and jump height was measured during a countermovement jump. The changes in serum creatine kinase (CK-MM isoform) were measured in the blood samples and muscle pain was measured with a visual analogue scale (0–10 cm). Data from RX heterozygotes and XX mutant homozygotes were grouped as X-allele carriers ($n = 13$) and compared to RR homozygotes ($n = 10$).
*Results* Race time was very similar between groups (313 ± 31 vs. 313 ± 25 min; $P = 0.45$); however, pre-to-post-competition reduction in jump height was greater in X-allele carriers than RR homozygotes (−18.4 ± 11.4 vs. −8.2 ± 6.9%; $P = 0.04$). At the end of the race, X-allele carriers presented higher serum CK-MM concentrations (682 ± 144 vs. 472 ± 269 U/L; $P = 0.03$), and there was also a tendency for higher self-reported values of lower limb muscle pain (7.7 ± 1.1 vs. 6.3 ± 2.3 cm; $P = 0.06$).
*Conclusions* X-allele triathletes in the ACTN3 R577X polymorphism presented greater signs of exercise-induced muscle damage during a half-ironman race than RR homozygotes.

**Keywords** Rhabdomyolysis · Muscle fatigue · Triathlon · Endurance performance · Single nucleotide polymorphism

**Abbreviations**

| | |
|---|---|
| ACTN3 | α-Actinin-3 |
| A.U. | Arbitrary units |
| CK | Creatine kinase |
| DNA | Deoxyribonucleic acid |
| ES | Effect size |
| PCR | Polymerase chain reaction |
| SD | Standard deviation |
| SNP | Single nucleotide polymorphism |

## Introduction

The α-actinins are a group of cytoskeletal proteins located in the Z-disc of skeletal, cardiac and smooth muscle fibres. Their main role is the anchoring of the myofibrillar actin filaments to the Z-disc, and thus they aid the production of force during sarcomere contraction (Venckunas et al. 2012; Yang et al. 2003). Interestingly, some individuals lack one specific protein from this family in their type II skeletal muscle fibres, α-actinin-3, due to a polymorphism in the gene that codifies this protein (i.e., ACTN3 gene). The presence of the X-allele in this single nucleotide polymorphism (R577X, rs1815739) produces a premature stop codon that leads to the production of non-functional α-actinin-3,

Communicated by Fabio Fischetti.

✉ Juan Del Coso
jdelcoso@ucjc.edu

[1] Exercise Physiology Laboratory, Camilo José Cela University, C/Castillo de Alarcon, 49, Villafranca del Castillo, 28692 Madrid, Spain

Published online: 02 December 2016

ultimately affecting the functionality of skeletal muscle to generate forceful contractions at high velocity (Ivarsson and Westerblad 2015; Vincent et al. 2010; Yang et al. 2003). Although it has been proposed that carriers of the X null allele compensate the absence of α-actinin-3 with a higher presence of α-actinin-2—another cytoskeletal protein with a similar function—, several investigations have indicated that the absence of this protein can affect exercise performance (Alfred et al. 2011; Eynon et al. 2011, 2013; Ma et al. 2013; Orysiak et al. 2015; Santiago et al. 2010; Yang et al. 2003).

Specifically, 577X allele carriers are found much less frequently in elite power- and sprint-like sport disciplines than RR homozygotes (Alfred et al. 2011; Eynon et al. 2013; Ma et al. 2013; Yang et al. 2003), although this is not always the case (Ruiz et al. 2011). On the other hand, the proportion of athletes with the X-allele in the ACTN3 gene is higher in endurance-based sports than the proportion of RR homozygotes (Orysiak et al. 2015), although the evidence for the benefits of the X-allele vs RR homozygosity on endurance performance is less well supported in humans (Alfred et al. 2011; Ma et al. 2013) than in animal models (North 2008). Apart from exercise performance, the lack of functional α-actinin-3 due to the 577X allele has also been related to the degree of muscle damage attained during different exercise protocols (Baumert et al. 2016). ACTN3 XX homozygotes presented higher serum creatine kinase concentrations and self-reported pain scores than RR homozygotes after 20 maximal eccentric knee extensions with both legs (Vincent et al. 2010). Similarly, XX homozygous soccer players showed higher serum creatine kinase concentrations than RX and RR counterparts after an eccentric training practice that included jumps, changes of direction, accelerations and decelerations (Pimenta et al. 2012). On the contrary, other investigations have failed to find an association between the X-allele and higher levels of exercise induced muscle damage after an eccentric elbow flexion exercise protocol (Clarkson et al. 2005) or after the performance of 50 drop jumps (Venckunas et al. 2012).

These previous studies (Clarkson et al. 2005; Pimenta et al. 2012; Venckunas et al. 2012; Vincent et al. 2010) have investigated the importance of α-actinin-3 to prevent excessive damage to the skeletal muscle fibre using very diverse exercise activities. However, the exercise protocols chosen are not representative of any sport or competitive situations, a fact that likely explains in part the contradictory outcomes found in these investigations. On the contrary, there is no information about the consequences of the lack of α-actinin-3 due to the 577X allele in endurance competitions, despite the fact that this type of sport activities predisposes individuals to high levels of exercise-induced muscle damage (Del Coso et al. 2012, 2013a, b, 2014b). Thus, the aim of this investigation was to determine the role of the 577X allele in the development of exercise-induced muscle damage during a triathlon competition. We hypothesized that, after a half-ironman, triathletes with the null X allele would show greater reductions in countermovement jump height (Del Coso et al. 2012) as a sign of greater muscle fatigue, and higher serum concentrations of creatine kinase and myoglobin, together with higher levels of self-perceived local muscle pain as signs of muscle damage, than RR counterparts.

## Methods

### Subjects

Twenty-three (19 men and 4 women) healthy and experienced triathletes volunteered to participate in this study. We have analyzed all the data without considering the sex of the individuals. Inclusion criteria were as follows: age between 18 and 50 years, being free of any history of muscle, cardiac or kidney disorders, participating in the competition at maximal intensity, and having triathlon experience of at least 3 years and more than 5 days of training per week in the previous 3 months. Exclusion criteria were as follows: taking medications or supplements during the two weeks prior to competing or having suffered a musculoskeletal injury in the previous 3 months, and not finishing the competition or all the experimental procedures. Detailed information on the participants in this investigation is shown in Table 1. Each participant was informed of the risks and discomforts associated with this study and signed a written informed consent 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 prospective, case–control and ecological experimental design was used for this investigation. Initially, three groups of individuals were established according to their genetic profile for the ACTN3 R577X polymorphism as follows: RR, RX and XX. However, participants with X-allele carriage (e.g., RX and XX) were considered as a single group and subsequently compared to RR homozygotes. This grouping was based on previous investigations that found a higher proportion of sprint/power athletes with RR homozygosity when compared to the percentage of sprint/power athletes that carried the X-allele [RX + XX;

**Table 1** Sex, age, body mass, triathlon experience and training habits of participants according to their ACTN3 genotype (R577X, rs1815739)

| Variable (units) | RR | X-allele | *P* value |
|---|---|---|---|
| *n* | 10 | 13 | – |
| Men/women | 8/2 | 11/2 | – |
| Age (years) | 36.5 ± 7.3 | 36.4 ± 5.2 | 0.74 |
| Body mass (kg) | 67.6 ± 6.1 | 71.2 ± 8.8 | 0.31 |
| Triathlon experience (years) | 5.2 ± 0.3 | 3.7 ± 0.3 | 0.27 |
| Completed half-ironman competitions (number) | 3.6 ± 0.4 | 3.0 ± 0.7 | 0.78 |
| Swimming training (km/week) | 6.4 ± 3.6 | 5.4 ± 2.4 | 0.44 |
| Cycling training (km/week) | 157 ± 86 | 157 ± 38 | 1.00 |
| Running training (km/week) | 27.7 ± 11.9 | 32.5 ± 11.8 | 0.34 |
| Training sessions/week (number) | 5.5 ± 1.4 | 5.3 ± 1.0 | 0.76 |

Data are mean ± SD for each group of individuals

(Alfred et al. 2011; Ma et al. 2013)] or when compared to the distribution of this polymorphism in control individuals (Yang et al. 2003). These previous investigations suggest a comparable phenotype, in terms of sports/muscle performance, for X-allele carriers (RX and XX) and opposed to the phenotyping produced by the RR homozygosity. Thus, the experimental design of the current investigation was based on the assumption that X-allele carriage would not produce α-actinin-3 or at least would produce less functional α-actinin-3 affecting the capacity of skeletal muscle to resist muscle strain during the triathlon competition.

## Experimental protocol

For this investigation, all the participants underwent the same testing under the same experimental conditions. Participants completed the 2015 edition of the Ecotrimad triathlon with no indications about training (in the days before the competition), pace or fluid and food strategies, before and during the competition. The race started at 12:00 h and consisted of 1.9 km of swimming, 75 km of cycling (1100 m net increase in altitude) and 21.1 km of running. The triathlon race was held in June on a sunny day with a mean ± SD dry temperature of 19.7 ± 1.0 °C and a mean relative humidity of 68 ± 1%. During the cycling section, all participants used aero-road bikes with carbon frames. The swim section was performed in a watercourse with water temperature at 16 ± 1 °C and all participants wore a neoprene wetsuit during the swim section.

The day before the race, participants were instructed to avoid pain-relieving strategies (e.g., analgesic medications or massage), strenuous exercise and caffeine and alcohol ingestion. Participants were also instructed to adopt a pre-competition diet that included at least 7 g/kg/day of carbohydrate, freely choosing the sources of carbohydrates to avoid interfering with their competition routines. Compliance with these standards was confirmed by self-reported training and food/drink diaries.

The day of the race, participants were encouraged to ingest 500 mL of plain water 4 h before the start of the race to increase the likelihood of being euhydrated at the start line. Participants arrived at an area close to the start line 3 h before the onset of the race and a 10-mL venous blood sample was obtained from an antecubital vein after 5 min of supine rest. 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 in the competition clothes and running shoes and the attempts were separated by 1 min of rest. In each jump, maximal leg power output during the concentric phase of the jump was determined from ground reaction forces. The highest jump was used for statistical analysis (Del Coso et al. 2014a). All participants had been previously familiarized with the jump test during the week prior to the competition. Just 15 min before the onset of the race (and after their habitual warm-up but prior to wearing the wetsuit), participants were weighed in their competition clothes (±50 g scale; Radwag, Radom, Poland) and then headed for the start line to initiate the competition.

During the race, participants wore an ankle strap with a timing-chip to calculate race time and the time employed in each triathlon sector and transitions. Participants completed the race at their own pace and drank ad libitum at the hydration stations during the race. Just after the end of the race, participants went to a finish area where they performed two countermovement vertical jumps, as described above. At this time, the rating of perceived exertion at the

end of the half-ironman competition was assessed using the Borg scale [from 6 to 20 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 analogue 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. The estimated time for these stoppages was subtracted from the race time. Participants also filled out a detailed questionnaire about fluid and food intake during the race (Del Coso et al. 2016). Data on this questionnaire was used to calculate fluid intake during the race using the nutritional facts on the products consumed and nutrition software (PCN software, Cesnid, Spain). Participants were then provided with fluid (water and sports drinks) and finished their participation in the study.

### Genetic testing

Genomic DNA from the whole blood sample obtained before the race was isolated (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). The three different types of creatine kinase isoenzymes (CK-MM, CK-MB and CK-BB) were measured in each serum sample.

### Calculations

Sweat loss volume (in L) was calculated as the pre-to-post-race change in body mass plus the amount of fluid and food ingested during the race. Sweat rate (in L/h) was calculated from sweat loss volume and race time. Fluid intake rate (in L/h) was calculated from fluid intake volume and race time.

### Statistical analysis

The normality of each variable was initially tested with the Kolmogorov–Smirnov test. All the variables presented normal distributions, and thus were analyzed with parametric statistics. The comparison between groups (RR homozygotes vs. X-allele carriers) was performed using Student's *t* test for independent samples. Moreover, for the variables obtained twice during the experiment (e.g., before and just after the race), a two-way analysis of variance (time × group) was performed. The effect size was calculated in all pairwise comparisons and 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 20.0 (SPSS Inc., Chicago, IL). The significance level was set at $P < 0.05$. Data are presented as mean ± SD for each group.

## Results

### ACTN3 R577X gene variants

Ten triathletes (43.5% of the sample) were RR homozygotes for the ACTN3 R577X, nine were RX heterozygotes (34.7% of the sample) and five were XX mutant homozygotes (21.7% of the sample, Table 1). Thus, 56.5% of the triathletes in the sample were carriers of the null X-allele. There were no between-group differences for age, body mass, body height, experience or training habits (Table 1; $P > 0.27$ in each case).

### Race time, jump height, body mass and self-reported information

Total race time was very similar for RR homozygotes and X-allele carriers (Table 2; $P = 0.45$). In addition, there were no between-group differences for the swimming, cycling and running sectors ($P > 0.41$ in each case). During the race, RR homozygotes had similar sweat rates (0.9 ± 0.4 vs. 0.8 ± 0.3 L/h; ES = 0.1, $P = 0.76$) and fluid intake rates (0.6 ± 0.5 vs. 0.5 ± 0.3 L/h; ES = 0.2, $P = 0.60$) to X-allele carriers, and thus body mass loss after the race was very similar (Table 2; $P = 0.79$). The amount of energy (1693 ± 822 vs. 1663 ± 650 kcal; ES = 0.0, $P = 0.93$) and carbohydrate (371 ± 198 vs. 376 ± 144 g; ES = 0.0, $P = 0.95$) consumed was very similar between

**Table 2** Triathlon race time, body mass change, jump height change and ratings of perceived exertion and muscle pain during a competitive half-ironman triathlon in RR homozygote triathletes ($n = 10$) and X-allele triathletes ($n = 13$) for the R577X polymorphism of the ACTN3 gene

| Variable (units) | RR | X-allele | Δ (%) | ES | *P* value |
|---|---|---|---|---|---|
| Race time (min) | 313 ± 31 | 313 ± 25 | 0.0 | 0.0 | 0.45 |
| Swimming sector time (min) | 37.9 ± 4.3 | 39.8 ± 4.7 | 5.0 | 0.4 | 0.77 |
| Cycling sector time (min) | 158 ± 16 | 159 ± 17 | 1.0 | 0.1 | 0.42 |
| Running sector time (min) | 110 ± 14 | 106 ± 12 | −3.5 | 0.3 | 0.49 |
| Body mass change (%) | −2.5 ± 1.2 | −2.6 ± 1.0 | 5.3 | 0.1 | 0.79 |
| Jump height change (%) | −8.2 ± 6.9 | −18.4 ± 11.4 | 124 | 1.5 | 0.04 |
| Rating of perceived exertion (A.U.) | 17 ± 2 | 17 ± 3 | 1.9 | 0.2 | 0.75 |
| Rating of perceived muscle pain (cm) | 6.3 ± 2.3 | 7.7 ± 1.1 | 21.2 | 0.6 | 0.06 |

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

groups. However, from similar pre-race values (28.8 ± 3.0 vs. 30.4 ± 4.4 cm; ES = 0.5, $P = 0.46$), X-allele carriers showed a more pronounced reduction in jump height than RR homozygotes at the end of the marathon with a very large ES (Table 2; $P = 0.04$). X-allele carriers reported similar levels of perceived exertion ($P = 0.75$) and a tendency for higher perceived muscle pain ($P = 0.06$) than RR homozygotes.

### Serum responses

There were no between-group differences in blood glucose concentration before or after the race (Table 3; $P > 0.52$). X-allele carriers presented a lower pre-race serum sodium concentration than RR ($P = 0.02$) but this difference was not present at the end of the race. The responses of serum chloride and potassium concentrations during the race were very similar between groups. From similar pre-race values, X-allele carriers showed a tendency for a higher post-race serum myoglobin concentration ($P = 0.10$) than RR homozygotes. X-allele carriers showed a tendency for a higher pre-race serum creatine kinase concentration ($P = 0.06$) than RR counterparts and these differences reached statistical significance at the end of the race, with a large ES (Table 3; $P = 0.02$). This same pattern was followed by the MM isoform of the creatine kinase, while the changes in the MB and MM isoforms in the race were very similar between groups. Of note, changes from pre-race to post-race were similar between the two groups in all the serum variables.

## Discussion

The aim of the current investigation was to determine the function of α-actinin-3 during the development of

**Table 3** Blood responses during a competitive half-ironman triathlon in RR homozygote triathletes ($n = 10$) and X-allele triathletes ($n = 13$) for the R577X polymorphism of the ACTN3 gene

| Variable (units) | RR | X-allele | Δ (%) | ES | *P* value |
|---|---|---|---|---|---|
| Glucose (mg/dL) | | | | | |
| Pre | 102 ± 19 | 100 ± 11 | −2.7 | 0.1 | 0.68 |
| Post | 116 ± 35 | 108 ± 21 | −6.8 | 0.2 | 0.52 |
| Sodium (mEq/L) | | | | | |
| Pre | 141 ± 1 | 139 ± 1 | −1.0 | 1.2 | 0.02 |
| Post | 142 ± 2* | 142 ± 1* | 0.0 | 0.0 | 0.95 |
| Chloride (mEq/L) | | | | | |
| Pre | 105 ± 1 | 105 ± 1 | −0.4 | 0.6 | 0.32 |
| Post | 104 ± 2 | 104 ± 1* | −0.3 | 0.2 | 0.66 |
| Potassium (mEq/L) | | | | | |
| Pre | 4.3 ± 0.4 | 4.4 ± 0.3 | 0.9 | 0.1 | 0.79 |
| Post | 4.7 ± 0.5* | 4.9 ± 0.3* | 2.3 | 0.2 | 0.56 |
| Myoglobin (μg/L) | | | | | |
| Pre | 17.9 ± 3.1 | 20.2 ± 5.8 | 12.7 | 0.7 | 0.28 |
| Post | 441 ± 248* | 591 ± 275* | 33.9 | 0.6 | 0.10 |
| Creatine kinase (U/L) | | | | | |
| Pre | 115 ± 38 | 153 ± 49 | 33.1 | 1.0 | 0.06 |
| Post | 477 ± 268* | 690 ± 145* | 44.9 | 0.8 | 0.02 |
| Creatine kinase MM (U/L) | | | | | |
| Pre | 115 ± 38 | 149 ± 44 | 29.8 | 0.9 | 0.07 |
| Post | 472 ± 269* | 682 ± 144* | 44.4 | 0.8 | 0.03 |
| Creatine kinase MB (U/L) | | | | | |
| Pre | 0.2 ± 0.6 | 0.2 ± 0.5 | −17.2 | 0.1 | 0.29 |
| Post | 2.5 ± 2.2* | 2.4 ± 2.3* | −7.3 | 0.1 | 0.85 |
| Creatine kinase BB (U/L) | | | | | |
| Pre | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 | 1.00 |
| Post | 1.7 ± 1.9* | 2.4 ± 0.5* | 40.2 | 0.4 | 0.51 |

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

* Different from pre values within the same group, at $P < 0.05$

exercise-induced muscle damage in a triathlon competition. For this purpose, we compared the levels of muscle damage attained during a competitive half-ironman race by RR homozygotes for the ACTN3 R777X polymorphism—with functional α-actinin-3 to triathletes carriers of the X null allele—e.g., RX and XX, with less/non-functional α-actinin-3. The main outcomes of this investigation were: (a) although the anthropometric, physical and training characteristics of both groups of triathletes were very similar and the total race time was almost identical, triathletes that carried the X-allele for the ACTN3 gene presented higher post-race serum concentrations of creatine kinase (total and MM isoform) than the triathletes with RR homozygosity (Table 3). (b) X-allele carriers showed a greater muscle performance reduction after the race, as measured by pre-to-post-race changes in countermovement jump height (Table 2). (c) X-allele carriers also showed a tendency for greater values of perceived lower limb muscle pain after the race (Table 2). These results indicate that possessing the 577X allele, likely resulting in less functional α-actinin-3, increased the concentration of blood markers of muscle damage, self-reported muscle pain values and the reduction in the capacity to generate muscle force in a jump, at least when compared to RR counterparts.

It is well documented that strenuous exercise can cause sarcomere disruption and muscle cell breakdown affecting the capacity to generate force, increasing the appearance of intramuscular proteins into the blood stream and producing delayed-onset muscle soreness (Baxter and Moore 2003; Clarkson and Hubal 2002). Although most investigations on this topic have been carried out in experimental settings that included eccentric muscle activities different from the ones produced in most sport situations (Clarkson and Tremblay 1988; Sayers et al. 1999), recent investigations have reported that endurance sports can produced moderate-to-high levels of muscle damage (Cordova Martinez et al. 2015; Del Coso et al. 2012, 2013a). Perhaps one of the most representative features of exercise-induced muscle damage is the great inter-individual differences in the levels attained after a specific activity that cannot be explained by age, training, experience or other individual characteristics (Areces et al. 2015; Del Coso et al. 2013a, b, 2014b). Specifically in long-distance triathlons, slower triathletes presented higher levels of muscle damage and showed greater impairments in muscle performance than faster triathletes (Del Coso et al. 2014b). In addition, the level of muscle damage has been identified as one of the main causes of muscle and overall fatigue (Del Coso et al. 2012), and the use of compression stockings was ineffective to reduce it (Del Coso et al. 2014a). After the competition, exercise-induced muscle damage symptoms peak one or two days after the race and then decrease afterwards, although low-grade muscle damage persists until at least 5 days post-race, possibly reflecting incomplete muscle recovery (Neubauer et al. 2008; Prou et al. 1996).

Previously, the ACTN3 gene has been targeted as one of the key genes responsible for the inter-individual variability found in exercise situations that produce exercise-induced muscle damage (Baumert et al. 2016). Specifically, it has been speculated that X-allele carriers for R577X might be more prone to exertional rhabdomyolysis because of a deficiency in the production of α-actinin-3 in type II muscle fibres (Clarkson et al. 2005; Pimenta et al. 2012; Venckunas et al. 2012; Vincent et al. 2010). However, the influence of the 577X allele on exercise-induced 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) on the levels of muscle damage after ad hoc exercise protocols that included eccentric muscle contractions.

In the present investigation, we used a more ecologically valid experimental setting because we assessed the influence of the 577X allele during a real sports situation: a competitive half-ironman race. Under this experimental setting, triathletes that carried the X-allele showed higher indexes of muscle damage when compared to RR homozygotes with similar ages, triathlon experience and training routines. Jump height reduction and serum creatine kinase concentration after the race were greater in X-allele carriers while a tendency for statistical significance was found in perceived muscle pain and serum myoglobin concentration. In all the cases, the effect sizes of possessing the X-allele were classified as medium-to-large and the between-group percentage of change was very high (Tables 2, 3). Another novelty of this investigation is that we measured the different isoforms of creatine kinase to identify the source of the muscle damage between skeletal, cardiac and brain tissues. Although MM, MB and BB isoforms significantly increased after the race, the only isoform that was different between X-allele carriers and RR homozygotes was MM, suggesting that the difference in the ACTN3 genotype specifically affected the capacity of the skeletal muscle to resist strain (Sorichter et al. 1999). Thus, triathletes with the X-allele, and likely with less functional α-actinin-3 in their type II fibres (Ma et al. 2013; North 2008), might be more prone to suffering exercise-induced muscle damage and its main symptoms, such as muscle pain, reduced capacity to generate force, and leakage of intramuscular proteins into the circulating blood.

The present investigation also presents some limitations derived from the experimental design selected that have to be discussed to improve the scope and applicability of the outcomes. This study was carried out on a real competitive half-ironman and thus factors, such as age, pre-competition diet, training volume and running intensity, during the race

were not controlled. We registered this individual information and based on the lack differences between X-allele and RR triathletes (Table 1) we speculate that these factors had a negligible influence on the outcomes of the investigation. It is well established that some of the symptoms of exertional rhabdomyolysis are present 24–48 h after the cessation of the exercise activity (Neubauer et al. 2008; Prou et al. 1996) but our results only extended to the end of the race. It would be interesting to investigate whether the influence of the ACTN3 gene is also present during the recovery phase of exercise-induced muscle damage. Finally, the level of muscle damage attained during the race was measured by indirect markers, such as self-reported muscle pain, changes in jump height and changes in serum concentrations of creatine kinase and myoglobin. Although these variables are trustable markers for determining exercise-induced muscle damage in a wide range of exercise activities (Banfi et al. 2012; Byrne et al. 2004), it would be interesting to confirm whether X-allele carriers for the ACTN3 gene are more prone to muscle damage using muscle biopsies.

The current investigation suggests that triathletes with XX or RX genotypes (e.g., X-allele carriers) for the R577X polymorphism showed higher levels of exercise-induced muscle damage than RR counterparts, as measured by greater values of muscle pain, jump height reductions and blood concentrations of creatine kinase, specifically the skeletal muscle isoform. Because the presence of the X-allele in the R577X ACTN3 polymorphism is related to the assembly of a non-functional α-actinin-3 in type II muscle fibres, it is likely that the greater symptoms of muscle damage found in this investigation are related to a lower capacity of skeletal muscle to satisfy the physiological demands imposed by a half-ironman competition. Thus, the determination of the ACTN3 genotype of amateur athletes could be advisable to establish specific training and/or supplementation routines that prevent X-allele carriers from developing clinical conditions associated with exertional rhabdomyolysis in endurance competitions.

**Acknowledgements** The authors wish to thank the participants in this study and the organizing committee of the Ecotrimad triathlon.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest derived from the outcomes of this study. They also declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

**Financial support** The study was part of the DAMUS project supported by a Grant-in-aid from the Vice-Rectorate of Research and Science, at the Camilo José Cela University.

## References

Alfred T, Ben-Shlomo Y, Cooper R, Hardy R, Cooper C, Deary IJ, Gunnell D, Harris SE, Kumari M, Martin RM, Moran CN, Pitsiladis YP, Ring SM, Sayer AA, Smith GD, Starr JM, Kuh D, Day IN (2011) ACTN3 genotype, athletic status, and life course physical capability: meta-analysis of the published literature and findings from nine studies. Hum Mutat 32:1008–1018

Areces F, Gonzalez-Millan C, Salinero JJ, Abian-Vicen J, Lara B, Gallo-Salazar C, Ruiz-Vicente D, Del Coso J (2015) Changes in serum free amino acids and muscle fatigue experienced during a half-ironman triathlon. PLoS One 10:e0138376

Banfi G, Colombini A, Lombardi G, Lubkowska A (2012) Metabolic markers in sports medicine. Adv Clin Chem 56:1–54

Baumert P, Lake MJ, Stewart CE, Drust B, Erskine RM (2016) Genetic variation and exercise-induced muscle damage: implications for athletic performance, injury and ageing. Eur J Appl Physiol 116:1595–1625

Baxter RE, Moore JH (2003) Diagnosis and treatment of acute exertional rhabdomyolysis. J Orthop Sports Phys Ther 33:104–108

Borg GA (1982) Psychophysical bases of perceived exertion. Med Sci Sports Exerc 14:377–381

Byrne C, Twist C, Eston R (2004) Neuromuscular function after exercise-induced muscle damage: theoretical and applied implications. Sports Med (Auckland, NZ) 34:49–69

Clarkson PM, Tremblay I (1988) Exercise-induced muscle damage, repair, and adaptation in humans. J Appl Physiol 65:1–6

Clarkson PM, Hubal MJ (2002) Exercise-induced muscle damage in humans. Am J Phys Med Rehabil 81:S52–S69

Clarkson PM, Hoffman EP, Zambraski E, Gordish-Dressman H, Kearns A, Hubal M, Harmon B, Devaney JM (2005) ACTN3 and MLCK genotype associations with exertional muscle damage. J Appl Physiol 99:564–569

Cohen J (1988) Statistical power analysis for the behavioral sciences. Lawrence Erlbaum Associates, Hillsdale

Cordova Martinez A, Martorell Pons M, Sureda Gomila A, Tur Mari JA, Pons Biescas A (2015) Changes in circulating cytokines and markers of muscle damage in elite cyclists during a multi-stage competition. Clin Physiol Funct Imaging 35:351–358

Del Coso J, Gonzalez-Millan C, Salinero JJ, Abian-Vicen J, Soriano L, Garde S, Perez-Gonzalez B (2012) Muscle damage and its relationship with muscle fatigue during a half-iron triathlon. PLoS One 7:e43280

Del Coso J, Fernandez de Velasco D, Abian-Vicen J, Salinero JJ, Gonzalez-Millan C, Areces F, Ruiz D, Gallo C, Calleja-Gonzalez J, Perez-Gonzalez B (2013a) Running pace decrease during a marathon is positively related to blood markers of muscle damage. PLoS One 8:e57602

Del Coso J, Salinero JJ, Abian-Vicen J, Gonzalez-Millan C, Garde S, Vega P, Perez-Gonzalez B (2013b) Influence of body mass loss and myoglobinuria on the development of muscle fatigue after a marathon in a warm environment. Appl Physiol Nutr Metab 38:286–291

Del Coso J, Areces F, Salinero JJ, Gonzalez-Millan C, Abian-Vicen J, Soriano L, Ruiz D, Gallo C, Lara B, Calleja-Gonzalez J (2014a) Compression stockings do not improve muscular performance during a half-ironman triathlon race. Eur J Appl Physiol 114:587–595

Del Coso J, Gonzalez C, Abian-Vicen J, Salinero Martin JJ, Soriano L, Areces F, Ruiz D, Gallo C, Lara B, Calleja-Gonzalez J (2014b) Relationship between physiological parameters and performance during a half-ironman triathlon in the heat. J Sports Sci 32:1680–1687

Del Coso J, Gonzalez-Millan C, Salinero JJ, Abian-Vicen J, Areces F, Lledo M, Lara B, Gallo-Salazar C, Ruiz-Vicente D (2016) Effects of oral salt supplementation on physical performance during a half-ironman: a randomized controlled trial. Scand J Med Sci Sports 26:156–164

Eynon N, Ruiz JR, Oliveira J, Duarte JA, Birk R, Lucia A (2011) Genes and elite athletes: a roadmap for future research. J Physiol 589:3063–3070

Eynon N, Hanson ED, Lucia A, Houweling PJ, Garton F, North KN, Bishop DJ (2013) Genes for elite power and sprint performance: ACTN3 leads the way. Sports Med 43:803–817

Ivarsson N, Westerblad H (2015) alpha-Actinin-3: why gene loss is an evolutionary gain. PLoS Genet 11:e1004908

Ma F, Yang Y, Li X, Zhou F, Gao C, Li M, Gao L (2013) The association of sport performance with ACE and ACTN3 genetic polymorphisms: a systematic review and meta-analysis. PLoS One 8:e54685

Neubauer O, Konig D, Wagner KH (2008) Recovery after an Ironman triathlon: sustained inflammatory responses and muscular stress. Eur J Appl Physiol 104:417–426

North K (2008) Why is alpha-actinin-3 deficiency so common in the general population? The evolution of athletic performance. Twin Res Hum Genet 11:384–394

Orysiak J, Sitkowski D, Zmijewski P, Malczewska-Lenczowska J, Cieszczyk P, Zembron-Lacny A, Pokrywka A (2015) Overrepresentation of the ACTN3 XX genotype in elite canoe and kayak paddlers. J Strength Cond Res 29:1107–1112

Pimenta EM, Coelho DB, Cruz IR, Morandi RF, Veneroso CE, de Azambuja Pussieldi G, Carvalho MR, Silami-Garcia E, De Paz Fernandez JA (2012) The ACTN3 genotype in soccer players in response to acute eccentric training. Eur J Appl Physiol 112:1495–1503

Portenoy R, Tanner R (1996) Visual analog scale and verbal pain intensity scale: from pain management: theory and practice. Oxford University Press, New York

Prou E, Margaritis I, Tessier F, Marini JF (1996) Effects of strenuous exercise on serum myosin heavy chain fragments in male triathletes. Int J Sports Med 17:263–267

Ruiz JR, Fernandez del Valle M, Verde Z, Diez-Vega I, Santiago C, Yvert T, Rodriguez-Romo G, Gomez-Gallego F, Molina JJ, Lucia A (2011) ACTN3 R577X polymorphism does not influence explosive leg muscle power in elite volleyball players. Scand J Med Sci Sports 21:e34–e41

Santiago C, Rodriguez-Romo G, Gomez-Gallego F, Gonzalez-Freire M, Yvert T, Verde Z, Naclerio F, Altmae S, Esteve-Lanao J, Ruiz JR, Lucia A (2010) Is there an association between ACTN3 R577X polymorphism and muscle power phenotypes in young, non-athletic adults? Scand J Med Sci Sports 20:771–778

Sayers SP, Clarkson PM, Rouzier PA, Kamen G (1999) Adverse events associated with eccentric exercise protocols: six case studies. Med Sci Sports Exerc 31:1697–1702

Sorichter S, Puschendorf B, Mair J (1999) Skeletal muscle injury induced by eccentric muscle action: muscle proteins as markers of muscle fiber injury. Exerc Immunol Rev 5:5–21

Venckunas T, Skurvydas A, Brazaitis M, Kamandulis S, Snieckus A, Moran CN (2012) Human alpha-actinin-3 genotype association with exercise-induced muscle damage and the repeated-bout effect. Appl Physiol Nutr Metab 37:1038–1046

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

Yang N, MacArthur DG, Gulbin JP, Hahn AG, Beggs AH, Easteal S, North K (2003) ACTN3 genotype is associated with human elite athletic performance. Am J Hum Genet 73:627–631