Systematic Review

Impact of ACTN3 R577X Polymorphism on Muscle Damage Susceptibility Following Aerobic or Strength Exercises: A Systematic Review

Abstract

This study compares muscle damage levels in individuals with and without the ACTN3 R577X polymorphism after engaging in aerobic or strength exercises. A systematic review was performed using articles from PubMed, SciELO, ScienceDirect, Cochrane Library, Medline, and Lilacs. The keywords were "muscle damage" and "muscle injury" combined with "ACTN3," "R577X," and "alpha-actinin-3," following the guidelines of the Preferred Reporting Items for Systematic Reviews (PRISMA). A total of 421 articles were identified, of which 10 were considered eligible. In total, 411 individuals were investigated considering all studies included in this review. Of the five studies with strength exercises, four found no differences between genotypes post exercise. On the contrary, of the five studies that evaluated muscle damage in aerobic exercise, three (that monitored this aspect in long-duration and strenuous sporting events: marathon, half ironman, and ultra-endurance adventure race) observed that individuals with the ACTN3 R577X polymorphism presented higher levels of muscle damage (measured by creatine kinase, myoglobin, and lactate dehydrogenase). To conclude, the ACTN3 R577X polymorphism can make an individual more susceptible to muscle damage after more high-volume aerobic exercise. As far as strength exercises are concerned, such a relationship does not appear to be observed. In this sense, individuals with this polymorphism require a longer recovery time from aerobic training sessions with long duration, as well as specific strategies for distributing training throughout the week.

Keywords: *Alpha-actinin-3, muscle damage, polymorphism*

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Introduction

While monitoring physical exercise programs, parameters related to the intensity, volume, and frequency of physical effort and the consequent risk of injury can be measured by analyzing muscle damage markers. These markers indicate structural ruptures in the fibers caused by muscle contractions, particularly high-intensity eccentric muscle contractions.[1-3] Thus, indirect indicators such as creatine kinase, lactate dehydrogenase, and serum myoglobin can be evaluated to quantify muscle damage.[4-7]

These intramuscular proteins leak into the bloodstream during muscle damage. High concentrations of these proteins may indicate the rupture of a large number of sarcoplasmic membranes during the given physical effort.[4] Therefore, an individual who presents higher levels of these indicators may pose a greater risk of in response to the imposed stimuli injury than those who possess lower levels after the same effort. This risk depends on the training model, age, or specific genetic variations, one of which is the R577X polymorphism of the *ACTN3* gene.[5,6]

The *ACTN3* gene is responsible for encoding alpha-actinin-3, a structural protein present in type II muscle fibers, which anchors actin proteins in the Z line of sarcomeres.[5,8-10] The *R577X* gene is a common nonsense polymorphism (rs1815739) that alters the production of alpha-actinin-3 by exchanging a cytosine for thymine at position 1747 of exon 16 on chromosome 11. This transforms an arginine base to form a premature stop codon (577X) in the middle of the amino acid sequence, encoding a truncated and non-functional form of alpha-actinin-3.[8-10] This protein plays a role in stabilizing the contractile units of muscle cells with alpha-actinin-2, a protein present in all muscle fibers, including cardiac fibers.[5,9] The RR genotype, which expresses α-actinin-3,

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is associated with a greater capacity of skeletal muscle to produce high-intensity contractions, which may favor individuals with this genetic makeup in power and speed sports. In addition, this genotype appears to be linked to a better capacity to withstand muscle damage induced by physical exercise.[5]

It is known that the lack of alpha-actinin-3 can be compensated by alpha-actinin-2, which has a regulatory role in oxidative metabolism.[5,9,11] As observed in a study that used knockout mice for the *ACTN3* gene, the sarcomere Z lines are unstable in the presence of high concentrations of alpha-actinin-2, being more susceptible to muscle damage induced by contractions.[8,9,12] Cohorts of power and speed athletes have a higher prevalence of individuals who produce alpha-actinin-3.[5,9] Therefore, it would be pertinent to assume that individuals with the *ACTN3* R577X polymorphism, those who do not produce alpha-actinin-3, would perform better in endurance tests. However, this relationship has not been validated.[5,9]

Based on these facts, some authors argue that in the absence of alpha-actinin-3 to anchor actin filaments in the Z line, sports performance through indirect factors, such as a higher risk for injury or lower resistance to muscle-damaging exercise, may be affected.[4,5] This assumption has been discussed in several observational studies.[13-15] The absence or presence of alpha-actinin-3 has been proven to modify muscle phenotypes,[5,11] and different genotypes are observed in speed/sprint athletes and athletes of endurance.[9] However, little is known about the possible genetic association of this protein with exercise-induced muscle damage and related mechanisms.[6] A systematic review that specifically compares muscle damage between individuals with the *ACTN3* R577X polymorphism and those without the polymorphism after aerobic or strength exercise using indirect markers of creatine kinase, myoglobin, or lactate dehydrogenase does not exist. Hence, the following question was raised: is there a difference in the muscle damage variables after exertion in individuals with and without the R577X polymorphism?

Therefore, this systematic review aimed to compare the levels of muscle damage between individuals with and without the R577X polymorphism of *ACTN3* after aerobic or strength exercises. It was initially hypothesized that individuals with the alpha-actinin-3 polymorphism would present higher levels of muscle damage compared to those without the polymorphism, regardless of the nature of the exercise (aerobic or strength).

Methodology

Search strategy

This systematic review was designed using articles extracted from seven databases: PubMed, SciELO, ScienceDirect, Cochrane Library, Medline, and Lilacs. The keywords were "muscle damage" and "muscle injury" combined with "ACTN3," "R577X," and "alpha-actinin-3." The search was performed using combinations of these terms linked with the Boolean operators "AND" (Boolean intergroup operator) and "OR" (Boolean intragroup operator), following the guidelines of the Preferred Reporting Items for Systematic Reviews (PRISMA).[16-18] Thus, the following Boolean function was created: ((muscle damage OR muscle injury) AND (ACTN3 OR R577X OR alpha-actinin-3)). Following the search, the articles were filtered, and duplicates were removed. Two independent researchers evaluated the selected articles by analyzing the title, abstract, and full text. The references cited in the eligible articles were also carefully analyzed. The review was conducted up to 30/09/2022, without restriction of publication year.

Eligibility criteria

The inclusion criteria were as follows: (1) observational studies; (2) published in peer-reviewed scientific journals; (3) studies evaluating the *ACTN3* R577X polymorphism and muscle damage; (4) assessment of creatine kinase, serum myoglobin, and/or lactate dehydrogenase activities. The exclusion criteria were as follows: (1) animal studies; (2) review articles; (3) conference abstracts and unpublished studies; (4) methods that evaluated more than one polymorphism at a time; (5) no assessment of muscle damage after physical exercise; (6) articles whose sample presented muscle damage before exercise. Two reviewers independently assessed the full text of the articles. Disagreements regarding the inclusion or exclusion of studies were resolved by consensus. To this end, the studies in question were read jointly by two reviewers, with each one presenting the line of reasoning adopted to interpret the articles according to the eligibility criteria. If necessary, the participation of a third reviewer was requested. This researcher, in turn, read the study without any influence from the other two reviewers.

Data extraction

Two researchers independently extracted data from each eligible article. A standardized form containing the following information was used for data extraction: name of the first author, year and journal of publication, research objective, studied polymorphism and evaluation method, population studied, sample characteristics, exercise/imposed stimulus, parameters and assessment of muscle damage, main results, study conclusion, and references used. The studies that presented data only in graph form had their data extracted using WebPlotDigitizer version 3.8. Results of studies with a known sample size (N) that were presented as standard error (SE) were converted to standard deviation (SD), according to the formula $SD = SE \times \sqrt{N}$. Disagreements in data extraction were resolved by consensus.

Methodological quality assessment

The methodological quality of the studies was individually evaluated using a tool developed by the

Joanna Briggs Institute (JBI) for case-control studies, containing 10 items whose answers could be Yes, No, Not Clear, or Not Applicable.[19] The JBI tool was chosen as it allows the collection and presentation of relevant data in an easy way, allowing the identification and comparison of the quality of the studies included in this review. Two reviewers independently applied the quality score to each included study. Any scoring discrepancies were resolved by consensus between the two researchers.

Results

Study selection

A total of 421 articles were identified from PubMed, Science Direct, Cochrane, Cochrane Library, and Medline, and references of eligible articles. A total of 165 articles remained after filtering for duplicates, and 31 were selected after reviewing the title and abstract. Thereafter, the full texts of these articles were reviewed, and 10 were considered eligible. Figure 1 shows the PRISMA diagram that represents the selection process. Furthermore, 12 additional records were identified through other sources and analyzed.

Table 1 lists the authors, title, study design, study population, sample characteristics, and journal name of the studies eligible for this review.

Study attributes

The studies applied training protocols or followed a competitive event to verify whether the applied stimulus would result in different levels of muscle damage in participants. By way of classification, strength exercises were those performed in a timely manner with regular intervals, dominated by the anaerobic metabolic system. In contrast, aerobic exercises, also called endurance activities, are continuous exercises or tests that use the aerobic metabolic system predominantly.

Ten studies published between 2005 and 2021 were considered eligible for this review, with a total of 411 participants. These studies were conducted in Belgium,[20,21] Brazil,[15,22-24] Spain,[13,14] the USA,[25] and Lithuania.[26]

TaqMan single nucleotide polymorphism (SNP) genotyping test,[13-15,20,21,25] polymerase chain reaction (PCR) amplification followed by restriction fragment length polymorphism (RFLP) analysis,[24,26] or the method of using Proteinase K followed by salt precipitation (salting out)[22,23] was used to verify the individual genotypes.

The methods used for the evaluation of biochemical markers varied. For creatine kinase activity assessment, the used tests were the Enzyline creatine kinase with n-acetylcysteine (CK NAC) Optimize test (bioMerieux, France),[20] the Spotchem EZ SP-4430 biochemical analyzer test (Arkray,

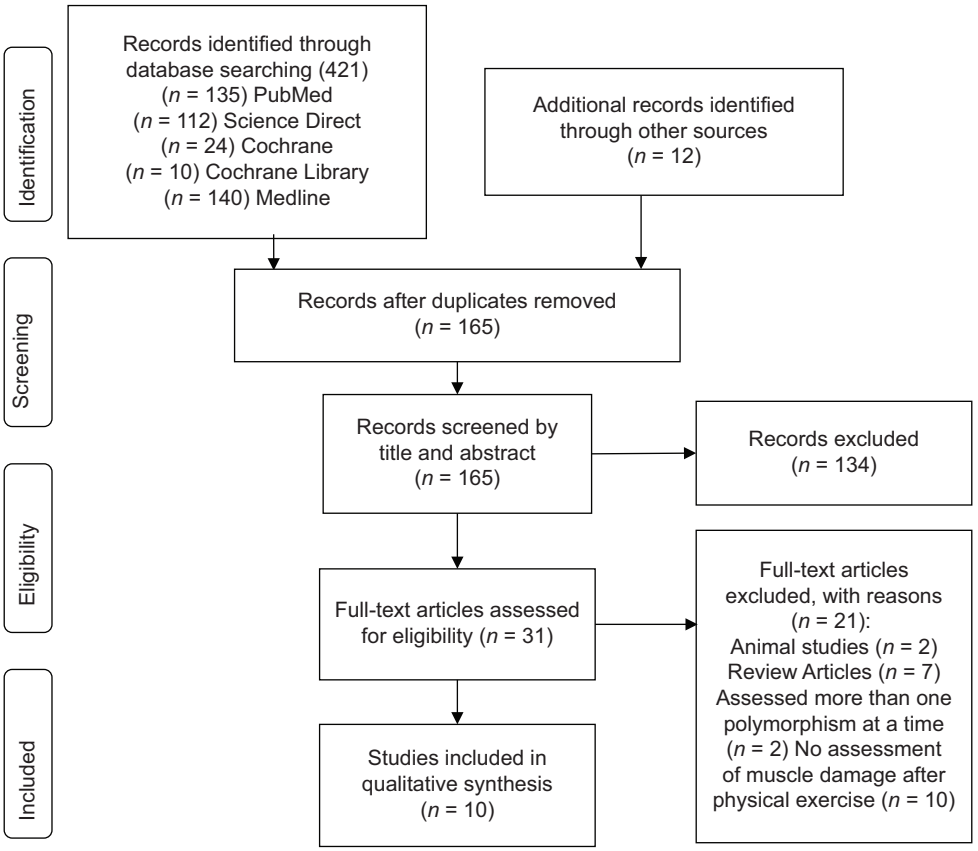

Figure 1: Organization of articles that analyzed the ACTN3 R577X polymorphism and muscle damage. Source: Adapted from Moher *et al*[18]

Table 1: Characteristics of studies included in this review (*n*=10)

Author	Title	Study design	Population	Sample characteristics						Journal
				RR	RX	XX	RR/RX	RX/XX	Total	
Clarkson *et al*.[25]	ACTN3 and MLCK genotype associations with exertional muscle damage	Case-control	Does not specify	*n*=35	*n*=78	*n*=41	-	-	*n*=157 24.1±5.2 y	J Appl Physiol
Vincent *et al*.[20]	Protective role of α-actinin-3 in the response to an acute eccentric exercise bout	Case-control	Healthy young men	*n*=9 21.3±0.5 y	-	*n*=10 21.08±0.7 y	-	-	*n*=19	J Appl Physiol
Venckunas *et al*.[26]	Human alpha-actinin-3 genotype association with exercise-induced muscle damage and the repeated-bout effect	Case-control	Young men	*n*=9 25.1±1.5 y	-	*n*=8 25.8±1.2 y	-	-	*n*=17	Appl. Physiol. Nutr. Metab.
Pimenta *et al*.[22]	The ACTN3 genotype in soccer players in response to acute eccentric training	Case-control	Soccer professional athlete	*n*=15 24.8±1.7 y	*n*=13 27.1±2.9 y	*n*=9 21.3±5.8 y	-	-	*n*=37	Eur. J. Appl. Physiol.
Del Coso *et al*.[13]	ACTN3 Xallele carriers had greater levels of muscle damage during a halfironman	Case-control	Experienced triathletes	*n*=10 36.5±7.3 y	-	-	-	*n*=13 36.4±5.2 y	*n*=23	Eur. J. Appl. Physiol.
Del Coso *et al*.[14]	ACTN3 genotype influences exercise-induced muscle damage during a marathon competition	Case-control	Experienced marathon runners	*n*=21 40.0±8.1 y	-	-	-	*n*=50 42.7±8.9 y	*n*=71	Eur. J. Appl. Physiol.
Belli, Crisp and Verlengia[15]	Greater muscle damage in athletes with ACTN3 R577X (RS1815739) gene polymorphism after an ultra-endurance race: a pilot study	Case-control	Ultra-endurance race athletes	-	-	*n*=4 44.0 (36.2-45.0) y	*n*=16 37.5 (31.5-42.7) y	-	*n*=20	Biol. Sport
Broos *et al*.[21]	The stiffness response of type IIa fibers after eccentric exercise-induced muscle damage is dependent on ACTN3 R577X polymorphism	Case-control	Non-athletic young men	*n*=4 20.1±0.9 y	-	*n*=4 21.7±1.1 y	-	-	*n*=8	Eur. J. Sport Sci.
Coelho *et al*.[23]	Alpha-Actinin-3 R577X Polymorphism Influences Muscle Damage And Hormonal Responses After A Soccer Game	Case-control	Soccer players U-16	-	-	*n*=10 15.83±0.25 y	*n*=20 15.5±0.50 y	-	*n*=30	J. Strength Cond. Res.
De Lima *et al*.[24]	The Impact of ACTN3 Gene Polymorphisms on Susceptibility to Exercise-Induced Muscle Damage and Changes in Running Economy Following Downhill Running	Case-control	Healthy men	*n*=10 22.4±3.7 y	-	-	*n*=19 22.0±2.2 y	-	*n*=29	Front. Physiol.

y=Years

Japan),[26] the enzymatic colorimetric method using the CK-NAC activated kit (Labtest Diagnóstica AS, Brazil),[22,23] the Access II auto-analyzer method (Beckman-Coulter Instruments, USA),[13,14] the BioClin 100 semi-automatic clinical analyzer method (BioClin, Brazil),[15] Vitros 4600 chemical system method (Ortho Clinical Diagnostics, USA),[21] or the triplicate method using spectrophotometry (Power Wave XS2, Biotek, Germany).[24] For serum myoglobin, the analysis was performed using the Access II autoanalyzer (Beckman-Coulter Instruments, USA),[13,14] the Abbott kit chemiluminescent microparticle immunoassay – Architect signal transducers and activators of transcription (STAT) Myoglobin (Architect system, Abbott Diagnostics, USA),[15] or the Cobas e411-1 (Roche Diagnostics, Switzerland)[21] instruments. Finally, a BioClin 100 semi-automatic clinical analyzer (BioClin, Brazil)[15] was used. In one study, the samples were forwarded to a specialized clinic, and the evaluation protocol was not described in detail.[25]

Assessment of the methodological quality of the studies

The studies evaluated by the JBI Appraisal tool for case-control presented an average score of eight points, of which four studies[13,14,20,22] scored 9 out of 9 possible points, two studies[23,26] scored 8, two studies[15,21,24] scored 7, and one study[25] scored 6 [Table 2].

Biochemical markers

All 10 studies[13-15,20-23,25,26] evaluated creatine kinase activity, five studies[13-15,21,24,25] evaluated myoglobin activity, and one

Table 2: Evaluation of the quality of studies using the JBI critical appraisal tool for case-control

Items	Clarkson *et al*.[25]	Vincent *et al*.[20]	Venckunas *et al*.[26]	Pimenta *et al*.[22]	Del Coso *et al*.[13]	Del Coso *et al*.[14]	Belli, Crisp e Verlengia[15]	Broos *et al*.[21]	Coelho *et al*.[23]	De Lima *et al*.[24]
1. Were the groups comparable other than the presence of disease in cases or the absence of disease in controls?	Un	Y	Un	Y	Y	Y	Y	Y	Y	Y
2. Were cases and controls matched appropriately?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
3. Were the same criteria used for identification of cases and controls?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
4. Was exposure measured in a standard, valid, and reliable way?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
5. Was exposure measured in the same way for cases and controls?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
6. Were confounding factors identified?	Y	Y	Y	Y	Y	Y	Un	Un	Y	Un
7. Were strategies to deal with confounding factors stated?	Un	Y	Y	Y	Y	Y	Un	Un	Un	Un
8. Were outcomes assessed in a standard, valid and reliable way for cases and controls?	Un	Y	Y	Y	Y	Y	Y	Y	Y	Y
9. Was the exposure period of interest long enough to be meaningful?	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
10. Was appropriate statistical analysis used?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Total score	6/9	9/9	8/9	9/9	9/9	9/9	7/9	7/9	8/9	7/9

Y=Contemplated item, N=Not contemplated item, Un=Not clear item, N/A=Not applicable, JBI = Joanna Briggs Institute. Source: Adapted from Moola *et al*[19]

study[15] evaluated lactate dehydrogenase activity. Table 3 presents the exercise protocol, muscle damage marker values by genotype, and conclusions of eligible studies.

The protocols with strength exercises involved those from one study involving maximal eccentric contractions of the elbow flexors on a Scott curl bench;[25] two studies involving maximal eccentric contractions of the knee extensors on a self-built programmable dynamometer;[20,21] one study involving stretch-shortening cycles (SSCs) through drop jumps;[26] and one study involving SSCs through a circuit containing jumps, changes in direction, and increases and decreases in speed.[22] These protocols followed post-exercise assessments that were taken at different time periods ranging from immediately at the end of the exercise to 14 days later. Among the included studies, the considered parameters were assessed before exercise and on days 4, 7, and 10 days after exercise in one study.[25] Assessments were conducted pre-exercise and 6, 24, and 48 h after exercise in another study.[20] In another study, assessments were made pre-exercise and on days 2, 7, and 14 after exercise.[26] One study assessed the parameters pre-exercise, immediately after, and 2 and 4 h after exercise.[22] One study assessed the parameters pre-exercise, and 5, 24, 72, and 120 h after exercise.[21]

Conversely, endurance protocols involved stimuli that approached the specificities of sports, where individuals participating in a triathlon race,[13] marathon race,[14] an adventure racing event,[15] or a soccer match[23] were monitored in each study. The individuals were evaluated after exercise in periods that varied from immediately at the end of the test until 4 h later. In three studies, the evaluation was conducted before and immediately after the tests.[13-15] In one study, pre-match evaluations and post-match evaluations immediately after, 2 h after, and 4 h after the match were conducted.[23] Only in one study, the muscle damage was assessed using the downhill running test (30 min of running downhill with a slope of −15% and speed equivalent to 70% of individual vVO2 max) for serum creatine kinase activity. The enzyme activity was assessed before, 2 days after, and 4 days after the downhill running.[24]

The results of the studies with strength activities revealed that from a total of five studies[20-22,25,26] in which creatine kinase activity was evaluated, four (80%)[20,21,25,26] did not show significant differences between individuals with and without polymorphism after physical exercise. One study[22] found that individuals with the XX genotype had higher CK values compared to those with the RR genotype 4 h after exercise. In addition, the single study[21] that evaluated myoglobin levels after force stimuli did not demonstrate significant differences between genotypes.

Regarding aerobic activities, five[13-15,23,24] assessed creatine kinase activity, three studies[13-15] assessed myoglobin activity, and one[15] assessed lactate dehydrogenase activity. Of these, three[13-15] (60%) of creatine kinase, two studies[14,15] (66%) of serum myoglobin, and one[15] (100%)

Table 3: Results of studies included in this review

Author (year)	Physical Exercise (Protocol)		Main Results		Conclusion
	Type	Intensity/duration			
Clarkson *et al*.[25]	Strength	Eccentric exercise 2×25 maximal eccentric contractions of elbow flexors 3s each rep 12s rest between reps 5 min rest between sets	CK (U/L)	Baseline: RR=106.2±9.9 RX=126.6±6.6 XX=99.1±9.1* (XX<RX) 4 days post: Data not reported by genotype 7 days post: Data not reported by genotype 10 days post: Data not reported by genotype	No difference between the genotypes post exercise
			Mb (ng/mL)	Baseline: Data not reported by genotype 4 days post: Data not reported by genotype 7 days post: Data not reported by genotype 10 days post: Data not reported by genotype	
Vincent *et al*.[20]	Strength	Eccentric exercise 4×20 maximal eccentric knee extensor contractions 45s rest between sets	CK (U/L)	Baseline: RR=153.29±153.29 XX=132.04±132.04 6 h post: RR=235.24±194.27 XX=271.67±271.67 24 h post: RR=291.4±227.66 XX=641.99±827.15 48 h post: RR=236.76±194.27 XX=377.91±493.25	No difference between the genotypes post exercise
Venckunas *et al*.[26]	Strength	Two drop jump bouts, separated by two weeks 50 drop jumps/bout 20s rest between reps	CK (% change)	Bout 1: Baseline: RR=100.0 (100.0 to 100.0) XX=100.0 (100.0 to 100.0) 2 days post: RR=172.1 (126.4-247.7) XX=173.1 (115.3-288.4) 7 days post: RR=175.6 (106.6-342.1) XX=102.4 (83.5-128.5) 14 days post: RR=99.3 (75.7-136) XX=72.3 (55.1-99.2) Bout 2: Baseline: RR=100.0 (100.0-100.0) XX=100.0 (100.0-100.0) 2 days post: RR=104.9 (69.5-176.6) XX=134.0 (102.4-183.1) 7 days post: RR=83.4 (67.9-105) XX=122.6 (91.3-173.3) 14 days post: RR=81.0 (63-107.8) XX=111.1 (94.8-132)	No difference between the genotypes post exercise
Pimenta *et al*.[22]	Strength	Circuit with jumps, changes in direction, and speed exercise 2x circuit of 5 stations, 3 min at each station 30s rest between stations Duration: 45 min	CK (U/L)	Baseline: RR=344.03 (285.37-403.69) RX=362.93 (314.4-411.65) XX=326.35 (260.91-392.59) Post: RR=438.49 (376.85-499.15) RX=472.39 (407.75-536.23) XX=394.98 (317.57-472.39) 2 h post: RR=380.82 (330.11-432.53) RX=448.45 (391.79-503.51) XX=403.76 (317.57-488.35) 4 h post: RR=344.03 (305.26-383.81) RX=386.2 (340.71-430.09) XX=523.46 (456.43-591.3)*	XX greater than RR at 4 h post eccentric exercise
Broos *et al*.[21]	Strength	Eccentric exercise 10 sets of 10 maximal eccentric contractions of the knee extensors at -30°/s 5 min rest, followed by one more set at -180º/s	CK (U/L)	Baseline: RR=159±49 XX=96±11* 5h post: RR=291±70 XX=188±77 24h post: RR=376±163 XX=957±1437 72 h post: RR=463±532 XX=18238±31347 120 h post: RR=296±146 XX=11450±22590	No difference between the genotypes post eccentric exercise
			Mb (µg/L)	Baseline: RR=45±15 XX=30±4 5h post: RR=98±48 XX=144±99 24h post: RR=46±19 XX=177±269 72 h post: RR=154±157 XX=1308±2203 120 h post: RR=79±52 XX=243±389	
Del Coso *et al*.[13]	Endurance	Triathlon Race Halfironman 1.9 km swimming 75 km cycling 21.1 km running	CK (U/L)	Baseline: RR=115±38 RX/XX=153±49 Post: RR=477±268 RX/XX=690±145*	For CK levels RX/XX greater than RR post triathlon race No difference in Mb levels between the genotypes post triathlon race
			Mb (ng/L)	Baseline: RR=17.9±3.1 RX/XX=20.2±5.8 Post: RR=441±248 RX/XX=591±275	

Contd...

Table 3: Contd...

Author (year)	Physical Exercise (Protocol)		Main Results		Conclusion
	Type	Intensity/duration			
Del Coso *et al*.[14]	Endurance	Marathon Race 42.195 km	CK (U/L)	Baseline: RR=187.72±296.49 RX/XX=172.81±64.91 Post: RR=358.77±188.6 RX/XX=507.89±855.26*	RX/XX greater than RR post marathon race
			Mb (ng/mL)	Baseline: RR=25.05±0 RX/XX=25.05±46.52 Post: RR=489.07±33.4 RX/XX=775.35±1141.55*	
Belli, Crisp and Verlengia[15]	Endurance	Ultra-endurance Adventure Racing 22.1 km mountain biking 10.9 km trekking 4.1 km water trekking 30 m rope course Orienteering	CK (U/L)	Baseline: RX/RR=129.99 (96.74-163.24) XX=136.03 (129.99-142.08) Post: RX/RR=822.25 (495.77-1015.72) XX=1278.72 (967.35-2046.55)*	XX greater than RR/RX post ultra-endurance adventuring racing
			Mb (ng/mL)	Baseline: RX/RR=23.2 (18.04-36.08) XX=23.2 (12.89-28.35) Post: RX/RR=682.99 (317.01-886.6) XX=1087.63 (920.1-2000)*	
			LDH (U/L)	Baseline: RX/RR=276.17 (246.73-305.61) XX=285.28 (277.57-296.5) Post: RX/RR=448.6 (413.55-477.34) XX=515.89 (512.38-519.39)*	
Coelho *et al*.[23]	Endurance	Soccer match Duration: 60 min	CK (U/L)	Baseline: RR/RX=417.61±466.37 XX=249.75±271.34* Post: RR/RX=591.74±664.18 XX=345.87±377.21* 2 h post: RR/RX=600.8±672.54 XX=352.84±391.14* 4 h post: RR/RX=540.9±625.87 XX=366.77±406.47	XX less than RR/RX at pre, immediately, and 2 h post soccer match
De Lima *et al*.[24]	Endurance	Downhill run/ treadmill Duration: 30 min Slope: -15% Speed=70% vVO2 max	CK (U/L)	Baseline: RR=112±54 RX/XX=115±43 2 days post: RR=398±120 RX/XX=273±121* 4 days post: RR=452±126 RX/XX=352±114*	RX/XX less than RR at 2 and 4 days post downhill run

*significant differences between groups at the same time point

of lactate dehydrogenase showed a significant increase in the levels of these markers in individuals with the X allele after physical exercise. Two studies (40%)[23,24] indicated a higher concentration of creatine kinase in individuals with the RR/RX[23] and RR[24] genotypes. In addition, it was observed that in all studies in which aerobic activity lasted longer, such as half-ironman,[13] marathon,[14] and ultra-endurance adventure running,[15] greater muscle damage was observed for those with the XX genotype.

Discussion

This systematic review identified studies that evaluated muscle damage in individuals with and without the *ACTN3* R577X polymorphism after strength and/or endurance exercises. There is an association between endurance exercises with high volume and an increase in muscle damage markers when the X allele is present, possibly due to the proximity of these activities to the specificity of the modality in question. Such an association was not evident in studies involving strength exercises.

The current literature indicates that the absence and/or low production of alpha-actinin-3 would make muscle fibers more susceptible to muscle damage.[4,5,9,12] However, in this review, most studies with strength exercises did not show statistically significant differences in biochemical markers between groups. Clarkson *et al*.[25] evaluated the R577X polymorphism of *ACTN3* and the C49T and C37885A polymorphisms of *MLCK*. The *MLCK* gene expresses the myosin light chain kinase protein that phosphorylates the regulatory myosin light chain. As a result, it is believed that these polymorphisms are also related to force production, tension, and consequent muscle damage. Thus, creatine kinase was analyzed as a biomarker for muscle damage in addition to evaluating the loss of muscle strength. The observed results did not demonstrate an association between the R577X polymorphism and the increase in creatine kinase levels after two sets of maximal eccentric contractions of the elbow flexors.

Clarkson *et al*.[25] found significant differences in creatine kinase values only at rest, where XX homozygotes showed lower values than those carrying the RX genotype. According to Baumert *et al*.[6] and Vincent *et al*.,[20] the difference in resting values can be explained by the difference in body composition of the groups studied, where individuals homozygous for the X allele would naturally have lower percentages of muscle mass. As alpha-actinin-3 is present in fast-twitch fibers and has the greatest capacity for hypertrophy, the limitation in its production and the compensation by alpha-actinin-2 would stimulate the formation of more oxidative and

slow contraction fibers (type I). These patients had lower hypertrophy capacity. Therefore, individuals with a lower percentage of muscle mass would present with lower levels of intramuscular proteins.

Continuing with eccentric exercise protocols, Vincent *et al.*[20] evaluated the results of maximal eccentric contractions of knee extensors. They collected data on muscle strength, creatine kinase level, pain perception, ribonucleic acid (RNA) content, and satellite cell immunohistochemistry. An increase in creatine kinase concentration was observed after exercise in XX homozygous individuals but without attaining significant levels. According to the authors, a conclusive result could be established if the protocol established higher contraction speeds in addition to evaluating other biochemical markers.

Similarly, Venckunas *et al.*[26] did not find significant differences in creatine kinase values after exercise with an SSC through drop jumps performed in two sets separated by 2 weeks each. The authors chose to perform SSC because of its characteristic combination of eccentric and concentric contractions. This protocol is associated with increased muscle damage.[27] In addition to muscle damage markers, pain perception, muscle strength, and the effect of repeated sets were analyzed.

Type II fibers from RR homozygotes appear more rigid, whereas those from XX homozygotes are more elastic.[28] Therefore, as SSC increases movement performance based on the elastic potential energy of the fiber, individuals with the R577X polymorphism could benefit from it without excessive muscle damage.[6] In addition, the protective effect of repeated sets may be more pronounced in individuals homozygous for the X allele, suggesting that these could support more frequent training sets.[5]

Pimenta *et al.*[22] also used SSCs in a circuit containing jumps, changes in direction, and increases or decreases in speed. In addition to analyzing creatine kinase for muscle damage, the authors evaluated testosterone, cortisol, alpha-actinin, and interleukin-6 (IL-6). However, unlike the findings from the study by Venckunas *et al.*,[26] it was possible to find a significant difference indicating that homozygous XX individuals would have higher concentrations of creatine kinase 4 h after exertion. According to Del Coso *et al.*,[5] the variety of exercises in this study by Pimenta *et al.*[22] may have favored the achievement of the result compared with those by Venckunas *et al.*,[26] in which only the drop jump was used.

In a recent study, Broos *et al.*[21] applied maximal eccentric contractions in knee extensors to analyze creatine kinase and serum myoglobin for muscle damage, with muscle biopsy for protein expression and messenger ribonucleic acid (mRNA) levels, pain perception, and muscle strength measurements. Similar to the others, there was no significant difference in the post-exercise levels of muscle damage markers between individuals with and without polymorphisms. A pre-exercise difference was observed, but similar to the results from the study by Clarkson *et al.*,[25] it could be justified by the lower levels of muscle mass in homozygous XX individuals.[21] The authors also found stiffening of type IIa fibers in homozygous RR individuals, confirming the findings of Broos *et al.*[28] on elasticity for individuals with the R577X polymorphism.

The protocols by Clarkson *et al.*,[25] Vincent *et al*.,[20] and Broos *et al*.[21] did not present an aspect focused on power and/or speed. As evidenced by Del Coso *et al.*,[5] the presence of alpha-actinin-3 does not appear to be advantageous for sports based on maximum strength as it is for power and/or speed modalities. Thus, the emphasis on maximal strength exercises in these articles could explain the lack of significance in the muscle damage results.

The articles with an emphasis on strength exercises evaluated biochemical parameters in intervals ranging from immediately after exercise to 14 days later. According to Clarkson *et al.*,[29] when evaluating eccentric high-strength exercises, they did not find an average increase in creatine kinase levels up to 2 days after exercise. Considering the selected studies, only the one by Pimenta *et al.*[22] reported the assessment at first 4 h and found significant differences. The results may depend on the time of evaluation.

Studies on endurance activities are more recent and converge on the same answer. All evaluated individuals in competitive situations may have contributed to greater motivation and performance. The modalities had a predominance of SSC in their execution, as explained by Byrne *et al*.[27]

The results of the study by Coelho *et al.*[23] demonstrated that after a soccer match, athletes with alpha-actinin-3 (XX) deficiency had lower levels of creatine kinase after 2 h of effort, which returned to normal levels after 4 h, compared with those without polymorphism, which was contrary to expectations. However, this significant difference also existed before the soccer match, thus indicating that the levels of creatine kinase may have increased equally for both groups. Therefore, the higher levels of creatine kinase pre- and post-exercise in RR and RX individuals may be due to the autoregulatory character of the modality, where stronger individuals (possibly RR/RX) would naturally show greater performance, resulting in greater muscle damage.

In addition, according to De Lima *et al*.,[24] X allele carriers were less susceptible to downhill running-induced muscle damage, as evidenced by greater strength loss and greater increases in serum creatine kinase activity observed in RR individuals. The higher involvement of type II muscle fibers during injurious attacks could explain the greater magnitude of creatine kinase in carriers of the R allele. Due to the greater proportion of type II muscle fibers

in R allele carriers than in the carriers of the X allele, the overexpression of type II muscle fibers in RR allele carriers may result in greater involvement of these fibers in eccentric contractions performed during harmful activities, culminating in greater susceptibility to muscle damage.

In the studies by Del Coso *et al.*,[13,14] levels of creatine kinase, serum myoglobin, and other blood markers and parameters of body mass, race time, muscle strength, perceived exertion, and pain perception were evaluated after triathlon[13] and marathon[14] tests. Del Coso *et al.*[13] found a statistical difference only in the increase of creatine kinase concentration in individuals with the X allele, while Del Coso *et al.*[14] found higher levels of creatine kinase and serum myoglobin in individuals with the X allele.

Finally, Belli, Crisp, and Verlengia[15] followed adventure-racing competitors during an event. They evaluated the level of physical activity, body composition, perceived exertion, creatine kinase, serum myoglobin, lactate dehydrogenase, and aspartate aminotransferase. The values showed significant differences before and after physical exercise, indicating a greater increase in the levels of all markers for individuals with the XX polymorphism.

According to Del Coso *et al.,*[14] protocols that use athletes in their real modalities can generate greater ecological validity for the results obtained, considering that muscle damage is related to the specific movement to be studied.

According to Moreno *et al.*,[8] a marathon is a challenging physical activity with a long duration (between 2 and 6 h) that involves constant eccentric and concentric muscular actions. These characteristics can be extrapolated to adventure racing and triathlon, which explains the greater variation in muscle damage between individuals with and without polymorphism.

Confounding factors in these studies may be related to body composition, diet, the influence of other polymorphisms, forms of exercise preparation, the temperature at the intervention site, and the relationship of biochemical markers with other tissues such as cardiac muscle.

For example, Clarkson's study[25] showed that individuals homozygous for the rare allele *MLCK 49T* and heterozygous for *MLCK 3788A* presented marked elevations in biomarkers (CK and Mb), suggesting a potential genetic impact on susceptibility to muscle damage and rhabdomyolysis. This finding may have critical clinical relevance, as excessive elevation of circulating Mb can precipitate in renal tubules, increasing the risk of acute renal failure, especially under conditions of heat stress and dehydration. These results highlight the need for genetic monitoring and personalized preventive strategies for individuals genetically predisposed to exacerbated muscle damage. In this sense, the divergence in the results may be related to the diversity of the population, age, gender, and training level presented.[6,9,15]

The primary limitations of the studies appear to be methodological, such as the impossibility of evaluating a greater number of polymorphisms, the sample size, and the evaluation moments. According to Ma *et al.,*[30] multiple genes influence multiple phenotypes that affect sports performance, such as muscle strength, tendon elasticity, skeletal structure, and heart and lung sizes. Furthermore, analysis of only one polymorphism may not be sufficient to identify significant differences in phenotypic changes.[31] Additionally, to establish solid conclusions in genetics, large samples would be required (greater than 200 participating individuals) to achieve sufficient statistical power.[32] Considering all the articles retrieved, the samples were small,[9] and the study with the largest number of individuals did not show significant differences in the evaluated parameters.[25] According to Del Coso *et al.,*[14] the variation in markers during the recovery phase, 24 to 48 h after endurance exercises may be assessed to obtain notable results. Therefore, future studies should focus on obtaining larger samples, performing multigenic analyses, as well as using longitudinal designs that evaluate muscle damage in different types of exercises. In addition, it is important for researchers to consider ecological validity, that is, that their findings are applicable to the real world.

The R577X polymorphism of the *ACTN3* gene could render an individual more susceptible to muscle damage after aerobic exercise, characterized by an increase in biochemical markers. However, these articles do not present a consensus regarding such an association after strength exercises.

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