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The ACTN3 R577X polymorphism is associated with metabolic alterations in a sex-dependent manner in subjects from western Mexico

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Abstract

Background: The *ACTN3* gene is primarily expressed in fast skeletal muscle fibres. A common nonsense polymorphism in this gene is *ACTN3* R577X (rs1815739), which causes an absolute deficiency of α-actinin-3 protein and alterations in muscle metabolism. Considering metabolic alterations are influenced by nutrition and genetic factors, as well as lifestyle factors, we hypothesise a possible association of the *ACTN3* R577X polymorphism with metabolic alterations.

Methods: In this cross-sectional study, 397 adults met the inclusion criteria. Body composition was measured by electrical bioimpedance. Dietary data were analysed using Nutritionist Pro™ software. Biochemical variables were determined by dry chemistry. Genomic DNA was extracted from peripheral leukocytes and genotyping of the ACTN3 R577X polymorphism was determined by allelic discrimination using TaqMan probes. The statistical analyses were performed using SPSS statistical software. $p < 0.05$ was considered statistically significant.

Results: The ACTN3 577XX genotype was associated with high glucose, triglyceride and very low density lipoprotein-cholesterol levels and a higher frequency of hypertriglyceridaemia and insulin resistance in women. In males, the genetic variant showed a trend towards significance for insulin resistance.

Conclusions: The ACTN3 R577X polymorphism was associated with metabolic alterations in women and a tendency was observed in men variant carriers. Thus, this common genetic variant could be implicated in the development of chronic metabolic diseases.

KEYWORDS

ACTN3, chronic disease, metabolic alterations, muscle, polymorphism

Highlights

- The ACTN3 R577X polymorphism is associated with metabolic alterations in women from western Mexico.
- Insulin resistance by the triglyceride-glucose index was associated with the XX genotype in women, and there was a significant trend towards a higher proportion in XX genotype males compared to the RR/RX genotype.
- The XX ACTN3 genotype could be a predictor for metabolic alterations in a sex-dependent manner.
- The present study does not exclude the potential role of ACTN3 R577X on gene–nutrient or gene–gene interactions related to metabolic alterations.

INTRODUCTION

The prevalence of chronic disorders such as obesity, type 2 diabetes mellitus and cardiovascular disease represents a serious public health problem and they contribute to mortality rates worldwide. In recent years, 71% of all global deaths can be attributed to some metabolic disorder and it is estimated that this number will continue to rise.[1] The difficult management of these complex diseases has lead physicians and health researchers to investigate and understand the most important factors involved in improving health. The interaction of environmental, nutritional and genetic factors can explain the pathogenesis of these metabolic diseases and their clinical manifestations.[2] Environmental factors and lifestyle factors such as sedentary behaviour, frailty or muscle damage have been associated with susceptibility to metabolic alterations[3] because skeletal muscle has an important role in whole-body metabolism.[4,5]

A group of muscle proteins known as α-actinins 1–4 have a role in the integrity of the sarcomere by binding to actin filaments in skeletal muscle. Furthermore, α-actinin proteins mediate the binding of some glycolytic enzymes to actin filaments.[6,7] α-actinin-2 is expressed in fast (glycolytic) and slow (oxidative) fibres, whereas α-actinin-3 is expressed only in fast fibres, particularly type IIX related to explosive contraction.[7,8]

A single nucleotide polymorphism (SNP) was identified in the α-actinin-3 (*ACTN3*) gene, which converts an arginine residue (R) to a premature stop codon (X) at amino acid position 577 (R577X).[9] Approximately 16% of the worldwide population is completely deficient in α-actinin-3 protein[10] and the allelic frequency varies between different ethnic groups (ranging from 0.09 to 0.55).[8,11]

The absence of α-actinin-3 protein in skeletal muscle does not cause muscle disease or damage[12] because the ACTN2 isoform can partially compensate for the lack of this protein at the sarcomeric Z-disk in fast muscle fibres.[11] However, this absence has been associated with a higher activity of mitochondrial enzymes to enhance oxidative pathways in fast muscle fibres.[7]

Therefore, beyond its structural function, the complete deficiency of α-actinin-3 plays an important role in the metabolic profile of muscle.[8,10,13] The alterations in sarcomeric binding proteins consequently modulate the calcineurin signalling pathway[13] and reduce the activity of glycogen phosphorylase in response to α-actinin-3 deficiency.[5,14] A limited number of studies have associated the *ACTN3* R577X polymorphism with human metabolism, such as cardiometabolic fitness,[15] healthy aging,[16] haematological parameters and iron metabolism,[17] metabolic syndrome,[18] lipid profile,[19,20] obesity,[21] and glucose tolerance.[5] In this sense, we suggest that the metabolic shift generated in skeletal muscle fibres by the *ACTN3* R577X polymorphism increases the risk of metabolic alterations in carrier subjects. Furthermore, considering the muscle mass difference between women and men,[22,23] the present study aimed to associate the *ACTN3* R577X polymorphism with metabolic alterations analysed by sex in non-athletic subjects from western Mexico.

METHODS

Subjects and study design

In this cross-sectional study, 415 non-athlete individuals were enrolled from March 2017 to February 2020. The study was carried out at the Institute of Translational Nutrigenetics and Nutrigenomics of the University of Guadalajara Health Sciences Center, Jalisco, Mexico, and the Civil Hospital of Tepic 'Dr Antonio González Guevara', Tepic, Nayarit, Mexico. All participants were recruited through flyers and social media invitations. The main inclusion criteria were mestizos from western Mexico aged over 18 years with a body mass index (BMI) ≥ 18.5 kg m^{-2} and subjects without any history of chronic medication, such as lipid-lowering and anti-hyperglycaemic drugs for at least 1 year. The main exclusion criteria included pregnant or breastfeeding women; cardiovascular, liver, autoimmune or pancreatic disease; cancer; alcohol consumption ≥40 g for men and ≥20 g for women per day; or tobacco consumption. All participants signed a written informed consent. This study was approved by the Ethics Committee for Human Research of the University of Guadalajara (Registration number: CI/019/2010) and the Committee for Research and Ethics in Health Research from Tepic, Nayarit (Registration number R-2017-1801-12). All procedures were performed according to the updated version of the Declaration of Helsinki, on the '64th WMA General Assembly from, Fortaleza, Brazil, 2013'.[24]

Anthropometric analysis

The anthropometric variables of each participant were measured wearing light clothes after fasting for 8–10 h with validated methods and standardised procedures by previously trained nutrition professionals. Body composition was assessed using an electric bioimpedance equipment (InBody 3.0; Biospace Co.). Height measurements were taken with a stadiometer (Rochester Clinical Research) and waist circumference (WC) was measured using a Lufkin Executive® tape. BMI was calculated as weight divided by height squared (kg m^{-2}).

Nutritional evaluation

One-on-one, the interviews were carried out by the nutrition staff with a previously structured questionnaire where clinical, socio-demographic and nutritional data were collected. The validated 24-h diet record was used to collect dietary information. Participants were instructed to provide the quantity and correct description of their food choices during the interview and the nutritionist showed food scale models from Nasco® to enhance the accuracy of the portion

sizes. Trained nutritionists analysed the dietary data using the Nutritionist Pro™ software (Axxya Systems) to obtain the average intake of nutrients.

Biochemical measurements

Venous blood sample was taken after fasting overnight for 8–12 h. Immediately, the serum was separated and frozen at −80°C until analysis. The biochemical variables (glucose, total cholesterol, high density lipoprotein cholesterol [HDL-C], and triglycerides) were determined by dry chemistry on the Vitros 250 Analyser (Ortho Clinical Diagnostics; Johnson & Johnson Co.). Very low density lipoprotein-cholesterol (VLDL-C) was estimated by dividing total triglycerides/5 and low density lipoprotein-cholesterol (LDL-C) was calculated using the Friedewald formula except when triglyceride levels were higher than 400 mg dL^{-1}.[25] The non-high-density lipoprotein-cholesterol (non-HDL-C) was estimated as total cholesterol – HDL-C.

Insulin resistance was determined by the triglyceride-glucose index (TyG). The TyG index was calculated as ln (fasting triglycerides (mg dL^{-1}) × fasting glucose (mg dL^{-1})/2). The cut-off points used to establish the main metabolic alterations were: hypercholesterolaemia >200 mg dL^{-1} (5.18 mmol L^{-1}), hypertriglyceridaemia ≥150 mg dL^{-1} (1.69 mmol L^{-1}), high LDL ≥ 100 mg dL^{-1} (1.12 mmol L^{-1}), hyperglycaemia ≥100 mg dL^{-1} (1.12 mmol dL^{-1}) and insulin resistance by the TyG index value ≥ 4.68.[26]

DNA extraction and ACTN3 genotyping

Genomic DNA (gDNA) was extracted and purified from peripheral leukocytes using the modified Salting-Out method as described previously,[27] and quantified by spectrophotometry in a Nanodrop 2000c (Thermo Scientific). The genotyping of the *ACTN3* R577X polymorphism (rs1815739) was determined by the allelic discrimination method using TaqMan® SNP Genotyping Assays (catalogue number 4351376; Applied Biosystems). The amplification reaction was performed with 50 ng gDNA under standard polymerase chain reaction (PCR) conditions: 95°C for 60 s, then 40 cycles of amplification at 95°C for 15 s and annealing/extension at 60°C for 60 s in a Real-Time PCR LightCycler 96 model (Roche). The accuracy of *ACTN3* genotyping was verified using positive controls of DNA samples corresponding to the three possible genotypes and negative controls in each 96-well plate assay. As a quality control measure, 20% of the samples were tested in duplicate, and the same result was obtained.

Statistical analysis

The sample size was calculated using *OpenEpi* online software, version 3.01. The population genetic data was obtained from the 1000 Genomes Project. To consider a confidence level of 95%, a sample size of at least 372 participants was required. The normal distribution of quantitative variables was analysed with the Shapiro–Wilk test. Variables with non-normal distribution were log-transformed and then normal distribution was confirmed. Quantitative variables were expressed as the mean ± SD and qualitative variables as frequencies (*n*) and percentages (%). All variables were analysed by sex and according to the R577X polymorphism considering the dominant model (RR/RX vs. XX genotype). Differences between genotype groups were analysed with Student's *t* test and the frequencies of metabolic alterations between genotypes were analysed using chi-squared. A logistic regression model was used to evaluate the association of hypertriglyceridaemia and insulin resistance with the R577X polymorphism of the *ACTN3* gene and, because triglycerides and insulin resistance are influenced by age, BMI and carbohydrate intake, these variables were used to fit the model. Analysis of covariance was used to assess the interaction between genotype and carbohydrate intake as a categorical variable (the cut-off point was 50% of total energy intake) considering triglyceride levels as the dependent variable, and age, BMI and energy intake as covariates, using the Bonferroni test for multiple post-hoc comparisons. This SNP was in Hardy–Weinberg equilibrium ($p > 0.05$). The analyses were performed using SPSS statistical software, version 21 (IBM Corp.). $p < 0.05$ was considered statistically significant. Illustrations were prepared using Prism, version 8.0.0 (GraphPad Software Inc.). The graphical abstract was created using Biorender.com.

RESULTS

General characteristics of participants

In the present study, 397 subjects met the inclusion criteria. Sixty-nine percent were females with a mean ± SD age of 38.0 ± 12.5 years and, for males, the mean ± SD age was 36.6 ± 12.7 years. The genotype frequencies for the R577X polymorphism were: RR = 13.1%, RX = 46.1% and XX = 40.8%. Allelic frequencies were 36% and 64% for R and X alleles, respectively. This SNP was in Hardy–Weinberg equilibrium (*p*-value 0.977).

Anthropometric, biochemical and nutritional variables

We compared anthropometric, biochemical and nutritional variables between genotypes. In females, the values of age, VLDL-C, triglycerides, glucose and TyG index were significantly higher in those females with the XX genotype compared to the RR/RX genotype. In men, LDL-C was higher in those individuals with the XX compared to the RR/RX genotype. These results are shown in Table 1. Regarding nutritional variables, no differences were found in neither women nor men (Table 2).

TABLE 1 Anthropometric and biochemical characteristics of participants according to the R577X polymorphism of the *ACTN3* gene

Variables	Females RR/RX genotype ($n = 165$)	Females XX genotype ($n = 109$)	p	Males RR/RX genotype ($n = 70$)	Males XX genotype ($n = 53$)	p
Anthropometric						
Age (years)*	35.2 ± 12.7	38.8 ± 12.5	**0.020**	38.0 ± 12.3	38.0 ± 12.8	0.943
Weight (kg)*	67.8 ± 13.3	69.8 ± 10.6	0.094	80.2 ± 13.8	80.7 ± 13.2	0.756
BMI (kg m^{-2})	26.6 ± 5.4	27.7 ± 4.4	0.070	26.7 ± 4.0	27.2 ± 3.9	0.484
MM (kg)	11.2 ± 1.5	11.4 ± 1.2	0.247	15.6 ± 2.4	15.3 ± 2.4	0.615
BFM (kg)	23.4 ± 9.1	25.0 ± 7.7	0.149	19.8 ± 9.4	21.5 ± 9.4	0.342
BFP (%)	33.9 ± 7.2	35.1 ± 6.6	0.167	23.4 ± 6.5	25.1 ± 6.1	0.139
WC (cm)	83.2 ± 13.0	86.1 ± 11.5	0.086	93.2 ± 10.4	96.5 ± 11.2	0.133
HC (cm)	102.6 ± 9,9	105.0 ± 8.8	0.067	101.4 ± 7.1	104.0 ± 7.4	0.081
Biochemical						
Cholesterol (mg dL^{-1})	183.7 ± 43.4	189.2 ± 38.6	0.285	187.4 ± 41.6	197.8 ± 43.0	0.177
HDL-C (mg dL^{-1})	47.9 ± 16.5	44.8 ± 12.2	0.109	38.4 ± 11.8	38.8 ± 6.7	0.785
LDL-C (mg dL^{-1})	112.3 ± 33.6	112.7 ± 31.9	0.930	108.4 ± 35.4	122.9 ± 34.5	**0.035**
VLDL-C (mg dL^{-1})*	25.4 ± 16.7	33.1 ± 38.7	**0.006**	39.1 ± 36.9	36.0 ± 17.5	0.444
Non-HDL-C (mg dL^{-1})*	136.9 ± 37.9	143.9 ± 41.6	0.199	147.9 ± 43.3	159.6 ± 42,2	0.112
Triglycerides (mg dL^{-1})*	122.6 ± 66.9	169.0 ± 194.6	**0.002**	202.4 ± 187.4	179.9 ± 87.5	0.617
Glucose (mg dL^{-1})	87.3 ± 10.9	90.9 ± 9.8	**0.006**	87.7 ± 8.6	89.8 ± 11.1	0.238
TyG index	4.6 ± 0.2	4.7 ± 0.3	**0.001**	4.7 ± 0.4	4.8 ± 0.3	0.515

Note: Bold values are statistically significant.

Abbreviations: BFM, body fat mass; BFP, body fat percentage; BMI, body mass index; HC, hip circumference; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; MM, muscle mass; non-HDL-C, non-high-density lipoprotein cholesterol; TyG index, triglyceride-glucose index; VLDL-C, very low density lipoprotein-cholesterol; WC, waist circumference.

*Significance was tested on log-transformed values.

TABLE 2 Nutritional characteristics of the participants

Nutritional variables	Females RR/RX genotype ($n = 165$)	Females XX genotype ($n = 109$)	p	Males RR/RX genotype ($n = 70$)	Males XX genotype ($n = 53$)	p
Kilocalories	2068.1 ± 725.2	2145.3 ± 603.0	0.504	2515.1 ± 1041.9	2293.1 ± 697.2	0.351
CHO (%)	48.3 ± 10.1	50.3 ± 8.5	0.202	48.0 ± 8.2	50.7 ± 11.0	0.263
Protein (%)	17.2 ± 3.8	16.3 ± 3.6	0.148	17.3 ± 3.7	16.5 ± 3.8	0.420
Total fat (%)	35.1 ± 8.1	34.1 ± 7.9	0.483	34.7 ± 6.8	32.9 ± 9.3	0.370
SFA (%)	11.0 ± 3.9	10.2 ± 3.4	0.233	10.0 ± 3.5	10.2 ± 5.0	0.877
MUFA (%)	10.3 ± 4.2	10.6 ± 4.3	0.671	10.1 ± 3.8	10.3 ± 4.2	0.866
PUFA (%)	5.3 ± 2.4	5.4 ± 2.9	0.847	6.0 ± 2.8	5.1 ± 2.1	0.131
PUFA ω6 (%)	4.8 ± 2.3	4.7 ± 2.9	0.907	5.8 ± 2.5	4.8 ± 1.6	0.215
PUFA ω3 (%)	0.4 ± 0.2	0.5 ± 0.3	0.473	0.5 ± 0.2	0.4 ± 0.1	0.584
Cholesterol (mg)*	252.4 ± 237.2	291.8 ± 162.1	0.068	411.6 ± 284.2	318.9 ± 172.9	0.174

Abbreviations: CHO, carbohydrates; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

*Significance was tested on log-transformed values.

Metabolic alterations

As a result of the significant results described in Table 1, we evaluated the frequency of hypertriglyceridaemia, hyperglycaemia, insulin resistance (according to the TyG index) and high LDL-C by genotype. In women, a significantly higher frequency of hypertriglyceridaemia (40.7% vs. 24.4%) and insulin resistance (49.1% vs. 29.9%) was found in participants with the XX genotype compared to the RR/RX genotype (Figure 1a). In males, there was a significant trend towards a higher proportion of insulin resistance in those participants with the XX genotype than with the RR/RX genotype (66.0% vs. 48.6%) (Figure 1b). Based on these results, a binary logistic regression analysis was performed in females to test the association of the R577X polymorphism with hypertriglyceridaemia and insulin resistance; however, only the association between the polymorphism and hypertriglyceridaemia was significant (odds ratio = 2.684, 95% confidence interval = 1.203–5.986, $p = 0.016$) (Table 3).

Gene–diet interaction

Finally, we analysed the interaction of the R577X polymorphism with the carbohydrate intake on triglyceride levels in men and women. In women, those individuals with the XX genotype and with a carbohydrate intake greater than 50% of total energy intake had higher triglycerides levels than females with the RR/RX genotype; however, this result did not reach statistical significance ($p > 0.05$).

DISCUSSION

Mostly, *ACTN3* has been reported as a sports-related gene. Previous studies have associated the R allele of *ACTN3* with sprint/power performance, whereas the XX genotype has been related to the endurance performance given by the metabolic characteristics of muscle fibres.[12,28,29] Considering that the consequences of α-actinin-3 deficiency in non-athletes are poorly understood, in the present study, we have demonstrated new insights related to the R577X SNP and metabolic alterations in non-athletic women from western Mexico.

Women carrying the *ACTN3* 577XX genotype have higher values of triglycerides, glucose, VLDL-C and insulin resistance (TyG index), including a trend towards significance in WC and hip circumference, compared to those females with the RR/RX genotype. Nevertheless, these results were not found in men and we only observed a trend in insulin resistance. We used the TyG index for the diagnosis of insulin resistance because it is an accessible and inexpensive alternative test with high sensitivity and specificity compared to the euglycaemic-hyperinsulinaemic clamp test.[26,30]

These findings are similar to other populations where HIV+ patients carrying the *ACTN3* 577XX genotype had higher glucose levels and a tendency for higher serum triglyceride levels compared to patients carrying the RR or RX genotype.[20] Furthermore, Nirengi et al.[19] reported a close association of the *ACTN3* 577XX genotype with higher triglyceride levels compared to the RR or RX genotypes in rugby players, and Deschamps et al.[15] found differences in cardiometabolic parameters between XX and RX or RR genotype of healthy young adults and suggested a role for the *ACTN3* R577X polymorphism in human metabolism. Despite the aforementioned findings, Many et al.[31] did not show a significant association between *ACTN3* genotypes with cardiometabolic or fitness parameters in university students.

The prevalence of the *ACTN3* 577XX genotype has also been associated with type 2 diabetes mellitus patients from Sweden.[5] Therefore, we tested the hypothesis that insulin resistance is associated with the *ACTN3* R577X genotype. Indeed, we found a higher prevalence of insulin resistance and hypertriglyceridaemia in women with the 577XX genotype; however, in the adjusted analysis, the significance was maintained only in hypertriglyceridaemia. In males, there was a significant trend towards a higher proportion of insulin resistance in the 577XX genotype group.

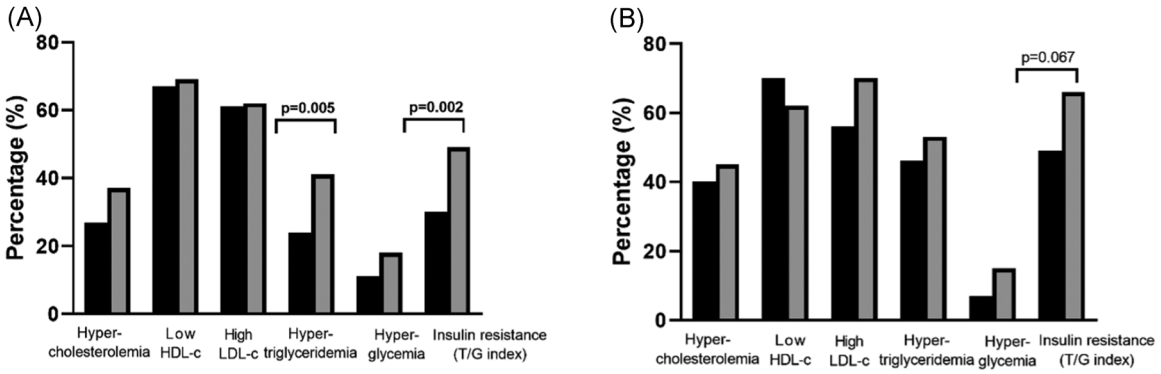

FIGURE 1 Frequencies of metabolic alterations according to the R577X polymorphism of the *ACTN3* gene. Frequencies of metabolic alterations according to the R577X polymorphism of the *ACTN3* gene in females (a) and males (b). Black bars indicate RR/RX genotype. Grey bars indicate XX genotype

TABLE 3 Association of metabolic alterations with the R577X polymorphism of the *ACTN3* gene in females

Variable	*B*	*p*	OR	95% CI
Hypertriglyceridaemia				
R^2 Nagelkerke = 28.1%				
Age	0.047	**0.004**	1.049	1.015–1.083
BMI normal weight = 0		0.638		
Overweight = 1	0.510	0.354	1.666	0.556–4.905
Obesity = 2	0.391	0.470	1.479	0.511–4.279
CHO intake (0 = ≤50%, 1 = >50% of kcal)	0.938	**0.023**	2.556	1.141–5.728
R577X polymorphism (0 = RR/RX, 1 = XX)	0.987	**0.016**	2.684	1.203–5.986
Insulin resistance (according to TyG index)				
R^2 Nagelkerke = 30.8%				
Age	0.055	**0.001**	1.056	1.022–1.092
BMI normal weight = 0		0.218		
Overweight = 1	0.903	0.087	2.467	0.877–6.939
Obesity = 2	0.388	0.465	1.474	0.520–4.174
CHO intake (0 = ≤50%, 1 = >50% of kcal)	1.024	**0.012**	2.784	1.256–6.171
R577X polymorphism (0 = RR/RX) 1 = XX)	0.715	0.076	2.044	0.927–4.508

Note: Bold values are statistically significant.

Abbreviations: BMI, body mass index; CHO, carbohydrates; kcal, kilocalories; TyG, triglyceride-glucose index.

The specific mechanism that explains how individuals carrying the *ACTN3* 577XX genotype have higher glucose or triglyceride concentrations than subjects carrying the *ACTN3* RR/RX genotype has not been elucidated. However, it is possible that differences in skeletal muscle metabolism are involved.[22] The evidence showed that several glycolytic enzymes, such as aldolase, phosphofructokinase and pyruvate kinase, bind to actin filaments. Furthermore, α-actinin plays an important role in mediating the binding of phosphorylase to actin filaments. This binding appears to regulate their catalytic activity[32]; therefore, the absolute absence of α-actinin-3 could be important in the regulation of glycolytic activity, and subsequently, in glucose homeostasis. However, more studies are needed to corroborate this metabolic regulation.

It was also reported that the absence of α-actinin-3 increases the enzymatic activity of calcineurin, a calcium-dependent protein expressed in fast muscle fibres with the ability to alter glucose metabolism, decrease oxidation and promote the storage of glycogen.[13] Furthermore, it has been shown that glycogen phosphorylase enzyme had significantly less enzymatic activity (26%) in knockout mice completely deficient for α-actinin-3 (Actn3KO mice) compared to controls (53%)[14] and the glycogen utilisation during sprint exercise was lower in type II muscle fibres of subjects with the XX genotype.[33] The decreased glycogen phosphorylase enzymatic activity appears to contribute to glycogen storage and could explain, in part, the glucose metabolism alteration through a compensatory mechanism in response to the limited breakdown of glycogen by skeletal muscle.[34,35] Additionally, the previous findings regarding α-actinin-3 protein deficiency, which confirm a metabolic shift in muscle cell profile characterised by increased oxidative pathways in fast fibres, appear to be indicate energy demands response when glycogen is limited.[8,36,37]

On the other hand, ACTN3 may change contractile and morphological muscle fibres properties, as well as muscle mass and diameter, and consequentially the characteristics of the whole muscle.[38,39] Furthermore, the strong sexual dimorphism reported in mitochondrial bioenergetics and muscle metabolism could explain our sex-specific results. Several studies have reported sex differences in mitochondrial bioenergetics,[40] intrinsic mitochondrial respiration,[41] mitochondrial volume density and function,[22] and human energy and substrate metabolism.[42] In this context, women oxidise more fatty acids and less carbohydrates than men during exercise,[43] potentially as a result of higher mitochondrial content,[22] differences in fat distribution,[44] lipid sources and higher glycerol.[45] In addition, Actn3KO mice showed an enhanced activity of mitochondrial enzymes; therefore, these results may explain the findings obtained in females.

Moreover, the 577X allele has been associated with low fat-free mass in women[46,47] and less activation of hypertrophy signalling,[48] whereas no association with muscularity phenotypes was found in men[49]; thus, it has been proposed as a candidate metabolically 'thrifty' allele[28]; however, a limited number of studies have examined this in detail. Our results failed to find a significant association between *ACTN3* 577XX and measurements of body composition[50] or obesity.[51] Nevertheless, we observed a trend towards a higher BMI (kg m^{-2}), WC (cm) and hip circumference (cm) in women. Indeed, this finding was similar to other studies reporting that healthy fit women with XX genotype exhibited a significantly higher body fat percentage,[15] which could indicate a possible 'thrifty' effect of this polymorphism on metabolism. However, our findings were the opposite to those of Kim et al.[18] because they reported that subjects with the XX genotype had a significantly lower waist–hip ratio and higher blood HDL-C level compared to the opposite genotype, with the interaction of *ACTN3* RR and *ACE* DD genotypes demonstrating a significantly higher metabolic syndrome score compared to any other groups in Korean children.

These findings support the heterogeneous role of *ACTN3* R577X in the body composition of individuals. However, the present study highlights the role of the XX genotype in human metabolism because our results are consistent with other population with different age range. Therefore, we propose the risk genotype as a possible predictor for metabolic alteration, particularly in women,

because we found the association to be sex-dependent. Differences in ethnicity or population diversity could be a major contributor as a result of the SNP (rs1815739) evolving in association with the global latitudinal gradient[52] because the allelic frequency varies between different ethnic groups (0.55 in Europeans, 0.52 in Asians and 0.09 in African populations) in response to metabolic adaptation and cellular membrane efficiency in colder climates.[8,11]

Regarding the nutritional characteristics of the participants, no differences were found in either women or men by genotypes. However, the diet intake of protein, total fat, saturated fatty acids and cholesterol was excessive, and the monounsaturated fatty acids and polyunsaturated fatty acids showed a deficient intake compared to the recommended daily intake for the general population in all study groups. These diet analyses are consistent with previous studies in West Mexico and represent the classic westernised diet, which increases the risk of obesity and metabolic diseases.[53,54]

Finally, we analysed a possible gene–nutrient interaction and, despite not finding a significant value, we observed that subjects who carried the risk genotype *ACTN3* 577XX and also had a carbohydrate intake greater than 50% of the total energy intake demonstrated higher triglyceride levels. Therefore, these results do not exclude the potential role of *ACTN3* R577X on gene–nutrient or gene–gene interactions related to metabolic alterations and, in addition, other characteristics such as being elderly, physical performance, consuming certain types of carbohydrate, diet quality and type of dietary fats should also be considered.

The limitations of the present study include not recording other genetic variants related to metabolic alterations, including variables related to metabolic syndrome, such as blood pressure and the level of physical activity. Furthermore, it is possible that the small sample size for men did not permit observation of associations of the polymorphism with the variables of interest.

CONCLUSIONS

In conclusion, our results support the notion that the absence of α-actinin-3 protein is closely related to metabolic alterations in muscle fibres. In our population, the risk genotype was associated with high levels of glucose, triglycerides, VLDL-C and insulin resistance in women, as well as a possible interaction with carbohydrate intake, which are all related to the onset of chronic diseases, such as obesity, type 2 diabetes mellitus and cardiovascular disease. Future studies that consider other SNPs related to body composition or metabolic alterations are required to reinforce our findings, as well as to elucidate sex-specific results.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

EB-C and EM-L designed the research. EB-C and NT-C participated in the analysis and data interpretation. EAZ-C, RT-V, IH-C and EM-L contributed to the data collection and analysis. EB-C and KG-B performed the experimental assays. EB-C, NT-C, KG-B, EAZ-C, RT-V, IH-C and EM-L wrote the manuscript. All authors read and approved the final manuscript submitted for publication.

ETHICAL APPROVAL

This research was approved by Ethics and Biosafety Committee for Human Research of the University of Guadalajara and the Committee for Research and Ethics in Health Research from Tepic, Nayarit.

TRANSPARENCY DECLARATION

The lead author affirms that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted.

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