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Genetic Obesity Risk and Attenuation Effect of Physical Fitness in Mexican-Mestizo Population: a Case-Control Study

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Summary

We analyzed commonly reported European and Asian obesity-related gene variants in a Mexican-Mestizo population through each single nucleotide polymorphism (SNP) and a genetic risk score (GRS) based on 23 selected SNPs. Study subjects were physically active Mexican-Mestizo adults ($n = 608$) with body mass index (BMI) values from 18 to 55 kg/m^2. For each SNP and for the GRS, logistic models were performed to test for simple SNP associations with BMI, fat mass percentage (FMP), waist circumference (WC), and the interaction with VO_{2max} and muscular endurance (ME). To further understand the SNP or GRS*physical fitness components, generalized linear models were performed. Obesity risk was significantly associated to 6 SNPs (ADRB2 rs1042713, *APOB* rs512535, *PPARA* rs1800206, *TNFA* rs361525, *TRHR* rs7832552 and rs16892496) after adjustment by gender, age, ancestry, VO_{2max}, and ME. ME attenuated the influence of *APOB* rs512535 and *TNFA* rs361525 on obesity risk in FMP. WC was significantly associated to GRS. Both ME and VO_{2max} attenuated GRS effect on WC. We report associations for 6 out of 23 SNPs and for the GRS, which confer obesity risk, a novel finding for Mexican-Mestizo physically active population. Also, the importance of including physical fitness components variables in obesity genetic risk studies is highlighted, with special regard to intervention purposes.

Keywords: Obesity, fitness, VO_{2max}, muscular endurance, SNP

Introduction

Obesity is currently one of the most prevalent metabolic diseases in both developed and developing countries. It is considered a risk factor for other chronic diseases such as cardiovascular disease, type 2 diabetes, hypertension, and atherosclerosis. Altogether, those conditions represent a first-order social and public health problem. In Mexico, the prevalence of overweight and obesity adds up to 71.3% (38.8% and 32.4%, respectively); thus, obesity and comorbidities are one of the first causes of premature and avoidable mortality in the country (Barquera et al., 2013). It is accepted that obesity risk depends mainly on (a) genetic variants conferring higher obesity genetic predisposition and (b) exposure to an obesogenic environment (i.e., poor nutritional habits and sedentary lifestyle, leading to a decrease in physical fitness) (Marti et al., 2008). These factors may interact, resulting in different levels of obesity and of the risk of obesity-related diseases.

The Human Obesity Gene Map reports 127 candidate genes for obesity. This large number of genes offers a glimpse

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on the complexity of identifying genes associated with susceptibility to obesity (Rankinen et al., 2006). In addition, several simulation and empirical studies have shown that the predictive value of a genetic variant could be improved by combining multiple loci simultaneously within a genetic risk score (GRS) model (Moonesinghe et al., 2010; Li et al., 2013, Goñi et al., 2015).

Although associations of many common genetic variants with obesity have been replicated in several European and Asian populations, there are only a few studies addressing the Mexican-Mestizo adult population (e.g. Canizales-Quinteros et al., 2007).

Exercise has been considered an important tool for prevention and treatment of obesity (Pietiläinen et al., 2008) and also for attenuating genetic-associated obesity risk (Li et al., 2010). Exercise refers to the physical activity that can be planned and follows an objective of improvement or maintenance of one or more components of physical fitness (Caspersen et al., 1985).

Health-related components of physical fitness include cardiorespiratory endurance, muscular endurance (ME), and body composition, which will not necessarily vary in concert (Caspersen et al., 1985). Cardiorespiratory fitness is traditionally considered best measured through the evaluation of maximal oxygen uptake consumed during 1 minute of exercise (VO_{2max}) (American College of Sport Medicine [ACSM], 2013). ME refers to the ability of a muscle or group of muscles to exert force to overcome resistance many times. Measuring ME requires the number of performed repetitions of a given exercise to be measured and depends specifically on the evaluation method employed (ACSM, 2013). Increases of both VO_{2max} and ME improve free-fat mass, glucose tolerance, and metabolic rate, which, in turn, are related with weight management (ACSM, 2013). For most of the reported common obesity genetic variants and with few exceptions, it is not known whether they interact with physical fitness components (Yu et al., 2014).

The goal for our study was to analyze associations between obesity and gene variants commonly adopted in European or Asian populations and their joint effect employing a GRS to assess obesity susceptibility in physically active Mexican-Mestizo population. The interactions of these parameters with physical fitness components were also evaluated.

Materials and Methods

Study Design, Data, and Sample Collection

Subjects voluntarily taking part of the study were members of a fitness club company (Sport City S.A. de C.V., Grupo Marti) in Mexico City, Mexico. They had to meet the following criteria to enroll in the study: they should be between 18 and 55 years old, of Mexican-Mestizo ancestry (with at least three grandparents born in Mexico), not related to other study participants, nonsmokers, and not taking any weight-loss medication. Regarding physical activity, with the aim of obtaining a homogenous sample, subjects were chosen among club members who had recently joined (3 months before starting the study) but were sedentary prior to becoming members. The training of the subjects occurred within a 3-month span before recording data, as this is the interval required for neuromuscular adaptation to occur as a result of training (Häkkinen, 1989). Highly trained people were not considered in this study. During those 3 months, participants followed the same exercise routine: three times a week, with the goal of losing weight or adopting a healthy life. The exercise protocol consisted of a moderated-intensity routine of 50–60 min, which included (1) 5 min of warm-up, (2) 20 min of ME exercising, doing three sets of 15–18 repetitions at 30%–50% of the maximum power and working only the main muscle groups (quadriceps, hamstrings, adductors, chest, back, abs), (3) 20 min of aerobic capacity training at 60%–85% of the maximum heart frequency, (4) 5–10 min of cool-down, and (5) 5 min of stretching. The exercise protocol was monitored by trainers.

Participants were selected so as to cover a range of body mass indexes (body mass index [BMI], calculated as weight over squared height in kg/m^2), including people meeting the criteria for obesity or not. In addition to BMI, other body composition measurements were taken: fat mass percentage (FMP) and waist circumference (WC). All anthropometric measures, height, and weight were determined while subjects were wearing light clothing and no shoes. FMP was calculated from seven skinfold measurements taken with a Lange caliper (chest, abdomen, axilla, subscapular, triceps, suprailiac, and thigh) following Jackson and Pollock (1985) protocols. WC was determined according to the National Institutes of Health (1998), by placing a measuring tape around the waist just above the uppermost lateral border of the iliac crest during normal minimal respiration. Fitness was measured using field tests following the ACSM (2013) guidelines. ME was assessed by recording the maximum number of abdominal crunches per minute (40). Indirect cardiorespiratory function measurement was obtained by VO_{2max} calculations after Cooper's test, a field test widely adopted also in Mexico, in which the person must run (or walk) the longest possible distance in 12 minutes (ACSM, 2013).

This project was approved by the ethics committee of Regional Hospital Lic. Adolfo López Mateos, from Institute for Social Security and Services for State Workers, México, and by the Institute of Biomedical Science, University of São Paulo, Brazil. All participants provided written informed consent prior to their inclusion in the study.

SNP Selection and Genotyping

A total of 23 SNPs in 18 genes were chosen for this study. Two criteria for selecting SNPs were used. According to the first, SNPs showed replications in at least five studies related to obesity, lipids metabolism, and body composition in European or Asian populations; *ADRB2* rs1042713 (A/G), *ADIPOQ* rs2241766 (G/T), *AGT* rs699 (C/T), *APOA4* rs675 (A/T), *APOB* rs512535 (A/G), *APOE* rs405509 (A/G), *FTO* rs1121980 (C/T), rs9939609 (A/T), *HNF4* rs1885088 (A/G), *LIPC* rs1800588 (C/T), LPL rs320 (G/T), *PPARA* rs1800206 (C/G), *PPARG* rs1801282 (C/G), *TNFA* rs361525 (A/G), *TRHR* rs7832552 (C/T), rs16892496 (T/G), and *SCARB1* rs10846744 (C/G) (Rankin*en et al.,* 2006, 2010). According to the second, SNPs were associated to obesity or obesity-related diseases in the sedentary Mexican population; *ABCA1* rs2230806 (T/C), rs9282541 (A/G), *CAPN10* rs2975760 (C/T), rs2975762 (A/G), rs3792267, and *TCF7L2* rs7903146 (C/T) (Canizales-Quinteros et al., 2007; Villarreal-Molina et al., 2007). Additionally, SNPs were chosen with a minimum allele frequency (MAF) equal to 0.10 in Mexican or Mexico-American populations. MAFs of the variants of interest were obtained from the 1000 Genomes Project (browser.1000genomes.org).

Additionally, 50 ancestry informative markers (AIMs) were included in our array design. These AIMs have been reported as the most informative unlinked markers (allele frequency differences of $d \geq 0.4$) to discriminate the Caucasian from the Native American population out of a panel of 260 AIMs previously validated for the Mexican population (Silva-Zolezzi et al., 2009).

Genomic DNA was obtained from a sample of 500 μL of whole blood using the automated system InviGenius® and DNA Mini Kit InviMag Blood (STRATEC Molecular GmbH, Germany). A nanofluidic Dynamic Array mounted on chips from the Fluidigm Corporation (South San Francisco, CA, USA) was used. Further description on such technology can be found elsewhere (Wang et al., 2009). End-point fluorescence values were measured on the EP1 system and its software, according to the Fluidigm genotyping user guide. Ten percent of samples were replicated to evaluate genotyping reproducibility. Data were edited in Fluidigm SNP Genotyping Analysis software, using an 85% confidence call rate threshold. Only those individuals with at least 85% of the genotyping rate were used in statistical analyses.

Statistical Analysis

Population stratification and individual admixed ancestry were analyzed through Bayesian clustering as implemented in Structure Software (Pritchard et al., 2000) and Principal Components Analysis in PLINK 1.9 software (Purcell et al., 2007). Data sets of AIMs of Native American and Caucasian European parental populations were obtained from Silva-Solezzi et al. (2009). Fisher exact tests were employed to assess the Hardy–Weinberg equilibrium, and Mendelian errors were estimated. Linkage disequilibrium among SNPs was tested using coefficient R^2. These tests were conducted separately for AIMs and for candidate SNPs, in PLINK 1.9 software (Purcell et al., 2007). For the 23 candidate SNPs, multiple comparisons were done using significance $P = 2.3 \times 10^{-5}$ after Bonferroni adjustment.

Three case-control groups were done based on BMI (obesity BMI $\geq 30\,kg/m^2$, nonobese otherwise), FMP (following ranges of cutoff values according to age and sex) (ACSM, 2013), and WC (abdominal obesity for men WC ≥ 102 cm, for women WC ≥ 88 cm, nonobese otherwise) following the International Diabetes Federation (Alberti et al., 2007).

Means and standard deviations were calculated for each variable in case and control groups, and for men and women separately. Pairwise comparisons were conducted between case and control groups, as well as among case groups for each variable, using Mann-Whitney test in R software (R Core Team, 2013).

Logistic regressions were used to test for associations among the 23 SNPs and the variables BMI, FMP, and WC. An obesity case-control analysis adjusted by ancestry, age, sex, VO_{2max}, and abdominal crunches was conducted, and also logistic regressions including the interaction terms SNP*VO_{2max} and SNP*ME. Associations were obtained under codominant, dominant, and recessive inheritance models. Results were considered statistically significant after Bonferroni adjustment ($P \leq 0.01$) for all tests. All association analyses were performed in PLINK 1.9 software (Purcell et al., 2007) .

Significant SNP*VO_{2max} or SNP*ME interactions resulting from logistic regression were further analyzed. A general linear model (GLM, (McCullagh and Nelder, 1989) with binomial distribution (loglink function) was adopted to examine the effect of the interactions on the probability of obesity. The explanatory variables included in the model were genotype, age, sex, VO_{2max} or ME, and their respective interactions. An initial model contained all single effects and all possible interactions of such explanatory variables, and model simplification was done by stepwise deletion of the least significant terms. We evaluated the relative models performance with the Akaike information criteria (AIC), where a difference of more than 2 units in AIC between models can be interpreted as an important difference in model fit (Burnham and Anderson, 2003). All GLMs were subjected to the customary residual analysis and performed in free software R (R Core Team, 2013).

The GRS was calculated employing 22 SNPs (for *TRHR,* only rs7832552 was employed because it was in linkage

disequilibrium with rs16892496) for the three obesity measurements (BMI, WC, and FMP). The scores were constructed assuming that each SNP acts independently and contributes equally to the risk of obesity under an additive inheritance model (He et al., 2010). Genotypes were coded as 0, 1, or 2 according to the number of risk alleles for each variant. The effect of GRS on obesity was analyzed adopting logistic regression adjusted by gender and age. The discriminative power of the GRS was tested by calculation of the area under the receiver operating characteristic (ROC) curves (area under the curve [AUC]). Significant GRS effect on obesity measurements was further explored for VO_{2max} and ME interaction, adopting GLM with binomial distribution (loglink function), as described above.

Power calculations were performed using a case-control outcome design and the disease prevalence reported in Barquera et al. (2013) using Quanto software version 1.2.4. (http://hydra.usc.edu/gxe/). Calculations were done for gene only and gene–environmental interactions analysis, under dominant, additive, and recessive inheritance models using the SNPs allele frequency reported in S1. Our study had 80% statistical power to detect OR ≥ 1.5 with an allele risk frequency ≥ 0.10 under an additive model, OR ≥ 1.5 with allele risk frequency ≥ 0.2 under a dominant model, and OR ≥ 2.0 with allele risk frequency ≥ 0.30 under a recessive model.

Results

The study included a total of 608 physically active adult Mexican-Mestizos (297 men and 311 women). When the total sample was divided into three case-control groups, the number of individuals differed among case groups. The highest number of individuals classified as people with obesity was found in FMP case groups ($n = 217$), followed by BMI ($n = 172$), and lastly by WC ($n = 160$). A description of the three case-control group data for BMI, FMP, and WC is shown in Table 1. All pairwise comparisons between case and control groups were significantly different ($P < 0.05$) for obesity as well as for physical fitness components.

None of the SNPs showed departure from Hardy–Weinberg equilibrium after Bonferroni correction, and no Mendelian errors were found. Out of the 23 candidate SNPs, 2 pairs from 2 genes showed linkage disequilibrium (*FTO*: rs1121980, rs9939609 $R^2 = 0.60$ and *TRHR*: rs16892496, rs7832552 $R^2 = 0.66$). Frequency information for those gene variants are shown in the Supporting Information (S1).

None of the AIMs was in linkage disequilibrium. The mean Caucasian component ancestry was 52% (range 40–62%). Bayesian clustering approaches and Principal Components Analysis strongly suggested that samples belonged to a single population ($K = 1$). Thus, the data set was not stratified by groups, but the result was used to adjust further analyses by individual ancestry.

Obesity Association Analysis for Each SNP among Case-Control Groups

Obesity risk was significantly associated to six SNPs in five genes (*ADRB2* rs1042713, *APOB* rs512535, *PPARA* rs1800206, *TNFA* rs361525, *TRHR* rs7832552 and rs16892496) after Bonferroni correction (Table 2). ME attenuated the influence of *APOB* rs512535 and *TNFA* rs361525 on obesity risk in FMP (Tables 2 and 3; only SNPs significantly associated to obesity are shown, unadjusted *P*-values are shown in Supporting Information S2).

Logistic regressions showed that BMI was associated with allele A of *ADRB2* rs1042713 under a recessive model (OR = 2.26, $P = 0.009$), WC was associated to allele G of *PPARA* rs1800206 (OR = 1.90, $P = 0.009$; OR = 2.75, $P = 0.003$ under codominant and dominant models, respectively), and FMP was associated to allele C of *TRHR* rs7832552 and allele T of rs16892496 (OR = 2.36, $P = 0.002$; OR = 2.24, $P = 0.002$, both under a recessive model). ME showed significant effect on FMP, for allele A of *APOB* rs512535 (OR = 1.04, $P = 0.005$; OR = 1.07, $P = 0.004$) and *TNFA* rs361525 (OR = 0.93 $P = 0.005$; OR = 0.92, $P = 0.007$, under codominant and dominant models, respectively) (Table 2).

Further exploration of such effect using GLMs showed that it is also affected by age (Table 3, Fig 1 for *APOB,* and Fig 2 for *TNFA*), but there were no significant gender-associated differences. A decrease in probability of obesity risk was related to significant *APOB* rs512535*ME interaction ($OR_{GG} = 7.91$, $P = 0.0193$; $OR_{GG*ME} = 0.94$, $P = 0.0381$). A significant attenuation on the probability of obesity risk associated to ME was found for *TNFA* rs361525 (OR = 0.92, $P < 0.0001$). Genetic risk of obesity increased with age for both SNPs.

Obesity Association Analysis for GRS among Case-Control Groups

The mean number of risk alleles per person was 18 (SD = 4.6) and ranged from 8 to 25. Logistic regression showed significant effect of GRS on WC (OR = 1.20, IC95% 1.07–1.30, $P = 0.001$), but not on BMI (OR = 1.09, IC95% 0.99–1.19, $P = 0.06$), nor on FMP (OR = 1.04, IC95% 0.95–1.14, $P = 0.41$). The GRS predicted obesity with a maximum discriminating ability for WC (AUC 0.62; 95% CI 0.58–0.67) followed by BMI (AUC 0.56; 95% CI 0.51–0.61) and FMP (AUC 0.55; 95 % CI 0.50–0.60).

Table 1 Description of the case-control data set used in physically active Mexican-Mestizos.

	BMI		FMP		WC	
	Case	Control	Case	Control	Case	Control
$N_{TOT} = 608$	172	436	217	391	160	448
Men	110	201	141	161	68	243
Women	62	235	76	230	91	206
Age	$39 \pm 8^{*}$	37 ± 8	$40 \pm 8^{*}$	37 ± 8	$40 \pm 8^{*}$	37 ± 8
BMI	$34.02 \pm 3.88^{**}$	24.89 ± 2.75	$31.23 \pm 5.80^{**}$	25.34 ± 4.28	$33.08 \pm 5.01^{**}$	25.42 ± 3.49
FMP_{MEN}	$0.24 \pm 0.06^{**}$	0.18 ± 0.05	$0.26 \pm 0.04^{**}$	0.16 ± 0.04	$0.27 \pm 0.05^{**}$	0.19 ± 0.05
FMP_{WOMEN}	$0.36 \pm 0.06^{**}$	0.26 ± 0.05	$0.38 \pm 0.03^{**}$	0.25 ± 0.05	$0.35 \pm 0.05^{**}$	0.25 ± 0.05
WC_{MEN}	$104.6 \pm 12.6^{**}$	87.3 ± 8.7	$101.3 \pm 12.3^{**}$	86.8 ± 9.0	$111.7 \pm 9.9^{**}$	88.1 ± 8.1
WC_{WOMEN}	$102.7 \pm 11.7^{**}$	76.5 ± 8.5	$98.6 \pm 13.9^{**}$	77.1 ± 10.1	$99.7 \pm 10.5^{**}$	74.2 ± 6.2
$VO_{2maxMEN}$	$30.2 \pm 8.9^{**}$	36.7 ± 9.6	$29.2 \pm 8.7^{**}$	38.4 ± 9.1	$25.6 \pm 8.4^{**}$	36.3 ± 9.3
$VO_{2max\ WOMEN}$	$20.0 \pm 6.4^{**}$	27.8 ± 8.1	$20.2 \pm 6.8^{**}$	27.6 ± 8.2	$20.8 \pm 7.7^{**}$	28.3 ± 8.2
$Crunches_{MEN}$	$29 \pm 14^{**}$	39 ± 13	$31 \pm 16^{**}$	40 ± 16	$23 \pm 12^{**}$	38 ± 12
$Crunches_{WOMEN}$	$23 \pm 10^{**}$	31 ± 11	$22 \pm 12^{**}$	30 ± 13	$24 \pm 12^{**}$	31 ± 11

P-value for case-control comparisons $^{*}P \leq 0.05$, $^{**}P \leq 0.001$.
Values are means ± standard deviation. Values are shown for Total sample size (N_{TOT}), Age (in years) body mass index (BMI in kg/m^2), fat mass percentage (FMP in decimals), waist circumference (WC in cm), and VO_{2max} = maximum oxygen consumption (mL/kg min). Subindices MEN and WOMEN indicate the data set used.

The GLMs showed that both fitness variables attenuated the effect of GRS on WC (effect of ME on WC: OR = 0.94, $P = 0.0001$, effect of VO_{2max} on WC: OR = 0.88, $P = 0.0001$), but age increased the effect on WC (Table 4). Gender influence was found to be not statistically significant in both models.

Discussion

Obesity risk was predicted by six genetic variants (*ADRB2* rs1042713, *APOB* rs512535, *PPARA* rs1800206, *TNFA* rs361525, *TRHR* rs7832552 and rs16892496) out of the 23 studied in this population. Obesity risk associated to the effect of *APOB* rs512535 and *TNFA* rs361525 was attenuated by higher levels of ME ability. The GRS resulted in a predictive tool on WC, and its effect was also attenuated by higher levels of ME and $VO_{2max.}$

Different numbers of individuals in the three case groups were expected because BMI, FMP, and WC are based on different anthropometric measurements and reflect different features of obesity (Janssen et al., 2004). The highest number of individuals classified as people with obesity was obtained for the FMP case group, which indicates that their condition relates to a heterogeneous distribution of body fat mass. If compared to BMI case group, the difference could be explained by some limitations of BMI as an obesity predictor. Subjects with high lean body or significant adiposity but adequate weight in relation to height could be misclassified as people with obesity or overlooked by BMI, respectively, but not by FMP (Lutoslawska et al., 2014, Garrido-Chamorro et al., 2009). Therefore, when studying SNP-obesity associations in a physically active population, our results suggest the adoption of complementary obesity assessment methods in order to recover as much variation as possible, as it relates to a heterogeneous process of gaining muscular mass or losing fat, with prevention and treatment purposes.

SNP-Obesity Associations

BMI was associated to allele A (or Arg16) for variant rs1042713 (commonly known as Arg16Gly) of B2-adrenergic receptor gene (*ADBR2*), such that obesity risk was twofold higher in AA carriers than in AG and GG carriers. Genetic predisposition to obesity could be explained, at least in part, by variation in *ADBR2* because it is the major lipolytic receptor gene associated to lipid mobilization (Zhang et al., 2014). Such association has been intensively studied, leading to inconclusive results probably due to differences in ancestry, gender, different obesity cut-off criteria and sample size (Zhang et al., 2014). Our result is in agreement with those for Korean (Lee et al., 2011) and Brazilian multiethnic population (Pereira et al., 2003), in studies with different sample sizes ($n = 356$ and $n = 1{,}576$, respectively, and $n = 608$ present data), which also adjusted analyses for age, sex, and ancestry. Our results suggest *ADBR2* rs1042713 genotypes may produce differences in the molecular pathway of lipid mobilization, being the AA genotype an obesity risk factor in the Mexican-Mestizo population.

Table 2 Associations *between obesity and SNPs, and their interaction (*) with* VO_{2max} *and muscular endurance (ME) in physically active Mexican-Mestizos.*

				BMI-obesityrisk, $n = 608$						WC-obesityrisk, $n = 608$						FMP-obesityrisk, $n = 608$					
						SNP*VO_{2max}		SNP*ME				SNP*VO_{2max}		SNP*ME				SNP*VO_{2max}		SNP*ME	
GENE	RS	A	INH	OR (CI 95%)	P	OR (CI 95%)	P	OR (CI 95%)	P	OR (CI 95%)	P	OR (CI 95%)	P	OR (CI 95%)	P	OR (CI 95%)	P	OR (CI 95%)	P	OR (CI 95%)	P
			COD	1.30 (1.00–3.53)	0.129	1.01 (0.98–4.13)	0.872	1.01 (0.98–2.12)	0.466	0.98 (0.95–4.32)	0.906	0.89 (0.71–2.12)	0.166	0.98 (0.67–3.08)	0.402	0.82 (0.72–1.06)	0.260	0.89 (0.71–1.02)	0.0672	1 (0.97–5.14)	0.995
ADRB2	rs1042713	A	DOM	1.05 (0.98–3.53)	0.832	0.99 (0.97–4.13)	0.954	1 (0.97–2.98)	0.994	0.97 (0.95–4.27)	0.902	0.9 (0.76–3.12)	0.343	0.99 (0.65–3.10)	0.618	0.87 (0.74–1.11)	0.562	0.89 (0.78–1.07)	0.1927	1.03 (0.99–2.14)	0.205
			REC	**2.26 (1.22–3.22)**	**0.009**	1.02 (0.98–4.13)	0.841	1.03 (0.98–2.11)	0.263	0.98 (0.89–5.09)	0.954	0.78 (0.61–2.02)	0.187	0.96 (0.67–3.08)	0.352	0.62 (0.55–1.04)	0.170	0.77 (0.69–1.06)	0.0733	0.92 (0.90–1.00)	0.055
			COD	1 (0.97–4.54)	0.980	0.91 (0.52–2.07)	0.134	1.03 (0.98–2.08)	0.060	0.73 (0.51–3.019	0.079	0.93 (0.72–3.07)	0.342	0.99 (0.67–3.09)	0.401	0.76 (0.70–1.06)	0.091	1.08 (0.99–3.17)	0.2417	**1.04 (1.01–2.10)**	**0.005**
APOB	rs512535	A	DOM	0.92 (0.90–4.54)	0.732	0.91 (0.52–2.08)	0.250	1.04 (0.99–2.09)	0.089	0.57 (0.34–0.72)	0.035	0.93 (0.72–3.11)	0.524	0.98 (0.66–3.10)	0.381	0.71 (0.60–1.06)	0.161	1.10 (0.99–3.22)	0.3293	**1.07 (1.02–2.12)**	**0.004**
			REC	1.11 (1.00–4.60)	0.722	0.85 (0.44–1.16)	0.208	1.03 (0.99–2.12)	0.175	0.81 (0.32–3.07)	0.520	0.86 (0.67–3.09)	0.399	0.99 (0.65–3.10)	0.802	0.67 (0.61–1.05)	0.174	1.11 (0.98–3.22)	0.3399	1.04 (1.00–2.10)	0.091
			COD	1.33 (0.99–3. 32)	0.231	0.88 (0.42–1.11)	0.234	1.02 (0.98–2.12)	0.344	**1.90 (1.42–2.26)**	**0.009**	0.87 (0.65–1.10)	0.330	1.02 (0.95–3.14)	0.281	1.03 (0.98–5.17)	0.908	0.97 (0.90–3.10)	0.7423	1.05 (1.02–2.11)	0.028
PPARA	rs1800206	G	DOM	1.57 (1.00–3.87)	0.180	0.86 (0.44–1.13)	0.302	1 (0.97–3.13)	0.927	**2.75 (1.80–3.20)**	**0.004**	0.78 (0.69–1.11)	0.205	1.03 (0.96–3.16)	0.265	1.06 (0.97–5.23)	0.878	0.96 (0.77–1.10)	0.7646	1.08 (1.02–2.21)	0.013
			REC	1.4 (0.99–3.82)	0.565	0.64 (0.34–1.05)	0.181	1.13 (1.04–3.87)	0.034	2.12 (0.99–3.42)	0.209	0.84 (0.71–1.08)	0.578	1.07 (0.98–3.23)	0.166	1.01 (0.94–5.17)	0.983	0.95 (0.77–1.11)	0.8057	1.06 (0.99–2.93)	0.237
			COD	0.78 (0.33–3.04)	0.287	1.15 (1.00–2.32)	0.107	0.98 (0.62–3.06)	0.437	0.81 (0.60–3.08)	0.406	1.07 (0.97–2.21)	0.471	0.99 (0.64–3.25)	0.675	0.95 (0.90–2.15)	0.825	1.00 (0.97–3.07)	0.9598	**0.93 (0.34–0.98)**	**0.005**
TNFA	rs361525	A	DOM	0.67 (0.34–3.04)	0.212	1.18 (0.99–2.37)	0.128	0.98 (0.60–3.07)	0.597	0.61 (0.46–3.03)	0.160	1.14 (0.98–2.23)	0.355	0.99 (0.63–4.27)	0.796	0.84 (0.71–2.11)	0.562	1.07 (0.98–2.23)	0.5081	**0.92 (0.33–0.97)**	**0.007**
			REC	0.82 (0.33–3.04)	0.727	1.3 (0.98–2.59)	0.202	0.95 (0.58–3.07)	0.382	1.31 (0.99–5.57)	0.636	1.08 (0.96–2.24)	0.721	0.97 (0.60–4.02)	0.577	1.28 (0.96–4.33)	0.633	0.8 (0.67–1.04)	0.2681	0.88 (0.79–1.00	0.050
			COD	0.93 (0.45–3.03)	0.691	1.09 (0.98–2.15)	0.199	1.02 (1.00–3.19)	0.339	0.99 (0.72–5.23)	0.957	1.01 (0.95–6.12)	0.937	0.99 (0.63–4.14)	0.585	1.30 (0.98–3.42)	0.147	0.98 (0.70–3.08)	0.8056	1.01 (0.99–4.13)	0.436
	rs7832552	C	DOM	0.66 (0.32–1.05)	0.131	1.19 (0.99–2.43)	0.138	1.02 (0.99–3.12)	0.432	0.65 (0.51–3.01)	0.131	1.09 (0.97–2.17)	0.556	0.96 (0.90–3.20)	0.097	0.8 (0.72–1.03)	0.416	0.99 (0.69–4.10)	0.9134	0.99 (0.70–4.06)	0.738
TRHR			REC	1.28 (0.99–1.39)	0.371	1.06 (0.98–2.17)	0.573	1.01 (0.98–2.98	0.582	1.57 (0.98–3.68)	0.133	0.96 (0.64–2.13)	0.747	1.02 (0.96–3.21)	0.445	**2.36 (1.67–3.84)**	**0.002**	0.97 (0.90–4.11)	0.7278	1.03 (0.98–3.32)	0.218
			COD	0.89 (0.35–3.12)	0.502	1.08 (0.98–2.18)	0.219	1.01 (0.99–3.43)	0.335	1.03 (0.97–6.17)	0.856	1.02 (0.91–3.08)	0.755	1 (0.89–5.24)	0.836	1.34 (1.00–1.51)	0.081	1.05 (0.99–3.12)	0.4472	1.02 (0.97–2.87)	0.280
	rs16892496	T	DOM	0.79 (0.33–1.11)	0.368	1.14 (0.99–1.27)	0.217	1.03 (0.99–3.11)	0.232	0.82 (0.69–3.09)	0.473	1 (0.05–3.10)	0.981	0.98 (0.78–3.09)	0.345	0.95 (0.84–4.11)	0.861	1.03 (0.98–3.45)	0.7705	1.02 (0.98–2.99)	0.374
			REC	0.95 (0.35–4.11)	0.859	1.08 (0.98–2.18)	0.411	1.01 (0.98–3.15)	0.721	1.35 (0.98–3.43)	0.297	1.08 (0.97–3.27)	0.524	1.02 (0.95–3.22)	0.558	**2.24 (1.54–3.60)**	**0.003**	1.09 (0.98–4.12)	0.3606	1.01 (0.98–3.14)	0.556

Obesity measures are body mass index (BMI), fat mass percentage (FMP) and waist circumference (WC). Odd ratio (OR) values from logistic models were obtained under codominant (COD), dominant (DOM) and recessive (REC) inheritance model (INH). RS = SNP reference sequence, A = reference allele, $P = P$-value. Statistically significant results after Bonferroni correction ($P \leq 0.01$) are shown in bolds.

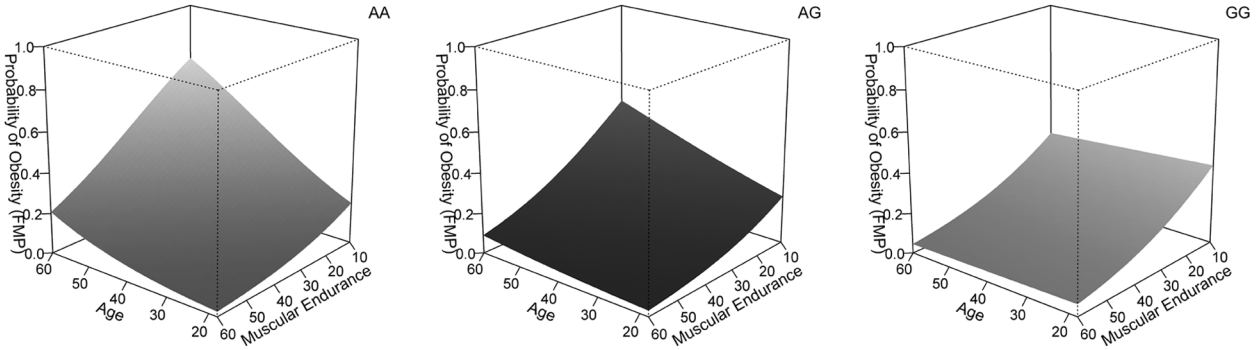

Figure 1 Effect of muscular endurance on obesity probability for *APOB* rs512535. The three-dimensional surfaces showing the interactions among the explanatory variables in the GLM for the Probability of Obesity (obesity measured as fat mass percentage, FMP) for *APOB* rs512535 genotypes (AA, AG, GG). Explanatory variables: Muscular endurance (measured as maximum crunches repetition during a minute), age, and genotype.

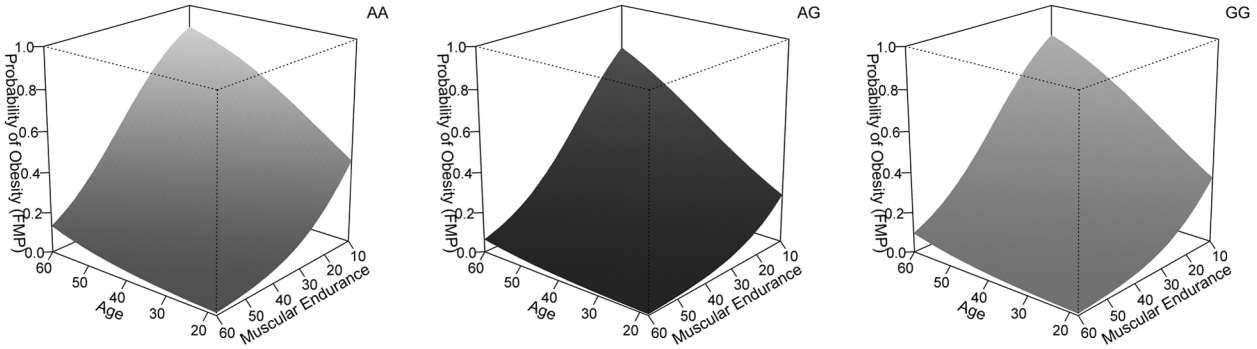

Figure 2 Effect of muscular endurance on obesity probability for *TNFA* rs361525. Three-dimensional surfaces showing the interactions among the explanatory variables in the GLM for the Probability of Obesity (obesity measured as fat mass percentage, FMP) for *TNFA* rs361525 genotypes (AA, AG, GG). Explanatory variables: Muscular endurance (measured as crunches), age, and genotype.

WC was associated to allele G (or 162Val) of *PPARA* rs1800206 (commonly known as Leu162Val), and obesity risk for GG carriers was twofold higher than for CC carriers. *PPARA* belongs to a gene family of nuclear receptors involved in lipid and carbohydrate metabolism, adipogenesis, and insulin sensitivity (Yong et al., 2008). Association analyses to cardiovascular disease and lipid content determined that allele G of *PPARA* rs1800206 is a risk factor for obesity in Caucasian (Tai et al., 2005), Chinese (Gu et al., 2014), and Latin populations (Chen et al., 2010). Our results are in agreement with previous studies of other ethnicities because G (162Val) allele was found associate to central obesity risk to Mexican-Mestizos. As central obesity is strong correlated with cardiovascular disease (Janssen et al., 2004), our results suggest that *PPARA* rs1800206 could be a predictor for both obesity and cardiovascular risk.

High FMP was associated to rs7832552 CC and rs16892496 TT genotypes in line with the reported link between the chromosomal region 8q23, which encodes *TRHR*, and BMI (Chagnon et al., 2001). Both SNPs of *TRHR*, rs7832552 and rs16892496, were in linkage disequilibrium ($R^2 = 0.66$), which agrees with previous results (Liu et al., 2009). *TRHR* encodes the thyrotropin-releasing hormone (TRH) receptor; it binds to TRH being the first step of the thyroid hormones cascade. It regulates crucial biological functions, including the increase of basal cell metabolism, growth, thermogenesis, total energy expenditure, lipid and carbohydrate catabolism, and myogenesis in skeletal muscle (Salvatore et al., 2014). Interestingly, a GWAS conducted on Caucasian and Asiatic populations showed a strong association of rs7832552 TT and rs16892496 GG genotypes to lean body mass (Liu et al., 2009). As rs7832552 TT and rs16892496 GG genotypes associated to lean body mass are complementary to rs7832552 CC and rs16892496 TT genotypes, which we found associated to FMP, our results are in the same line because the association with FMP may be linked

Table 3 SNP and muscular endurance interaction on obesity risk in physically active Mexican-Mestizos.

	Estimate	OR	*P*
Intercept	−1.92	0.15	0.029
APOB AG	0.69	2.00	0.376
APOB GG	2.07	7.91	0.019
Age	0.06	1.06	0.000
ME	−0.05	0.95	0.034
APOB AG*ME	−0.03	0.97	0.296
APOB GG*ME	−0.06	0.94	0.038
Intercept	−0.61	0.55	0.422
TNFA AG	−0.8	0.45	0.146
TNFA GG	−0.37	0.69	0.419
Age	0.06	1.06	0.000
ME	−0.08	0.92	0.000

Values are shown for parameter estimates, odd ratios (OR), and *P* values of the set of variables used to explain the probability of obesity (measured as fat mass percentage) for *APOB* rs512535 and *TNFA* rs361525, using generalized linear models with a binomial distribution (log link function). Explanatory variables were SNP, age, sex and muscular endurance (ME) measured as crunches.

Table 4 Genetic risk score and muscular endurance and VO_{2max} interaction on central obesity risk in physically active Mexican-Mestizos.

	GRS*ME			GRS*VO_{2max}		
	Estimate	OR	*P*	Estimate	OR	*P*
Intercept	−4.13	0.02	0.001	−2.38	0.09	0.05
GRS	0.18	1.20	0.001	0.18	1.20	0.001
Age	0.05	1.05	0.002	0.04	1.04	0.03
Sex	–	–	–	–	–	–
ME	−0.07	0.93	0.0001	–	–	–
VO_{2max}	–	–	–	−0.13	0.88	0.0001

Values are shown for parameter estimates, odd ratios (OR), and *P*-values of the set of variables used to explain the probability of central obesity (measured as waist circumference) using generalized linear models with a binomial distribution (log link function). Explanatory variables were GRS, age, sex and muscular endurance (ME) and VO_{2max} respectively.

to a decrease in lean body mass. In other words, rs7832552 CC and rs16892496 TT genotypes may decrease cellular basal metabolism, favoring fat accumulation.

Obesity risk was significantly affected by ME for *APOB* rs512535 and *TNFA* rs361525, though in a different manner. The probability of obesity decreased at higher levels of ME for *APOB*, but the interaction effect produced marked differences among genotypes and ages. The highest obesity probability was shown by older AA carriers (≥40 years old) with low levels of ME (<20), while the lowest probability of obesity was shown by younger subjects (<40 years old) of the same genotype and level of ME, as compared to AG and GG (Fig 1). Whereas an opposite pattern was found for GG carriers, the highest probability of obesity did not exceed 0.40. In summary, the stratum of the population with the highest probability of obesity is older AA carriers with low levels of ME. In this context, and in order to reduce the obesity risk, we can suggest that older AA carriers will require higher levels of ME so as to reach the same obesity probability as AG or GG carriers. Obesity risk effect associated to *APOB*, the main component of LDL and essential for triglyceride-rich lipoprotein secretion, is modified by nutrient intake (Phillips et al., 2011). To our knowledge, this is the first report that the obesity probability associated to *APOB* is attenuated by another environmental factor apart from nutrient intake such as ME ability.

Obesity probability was attenuated by ME for *TNFA* rs361525 (A/G also known as 238G/A) in elderly and young subjects. The highest obesity probability (0.90) was found in older AA carriers with low levels of ME; this probability decreases 5% for GG (0.85) and 10% for AG (0.80). Our results suggest that ME acts as a protector factor for AA, AG, and GG genotypes, but AA carriers require a higher level of ME to reach an attenuation effect similar to that of AG and GG carriers. *TNFA* has a critical role in the regulation of obesity-related pathways, being a pro-inflammatory cytokine whose expression and circulating levels are increased with obesity and decreased with weight loss (Maury & Brichard, 2010). Our results suggest that higher levels of ME could help to reduce circulating cytokine level, producing an attenuation effect of the probability of obesity, but this effect remains to be explored.

Central obesity probability was positively influenced by GRS and age, and attenuated by physical fitness level. GRS effect was higher than that of age and fitness variables, turning the score into the main variable determining the probability of obesity. Because the model presents a (not linear) logistic distribution, such probability does not increase steadily with equal increases in GRS, age, ME, and VO_{2max} (individual obesity probabilities can be obtained applying the model in Supporting Information S3). These results are in line with the finding that genetic predisposition to obesity is attenuated by the level of physical activity in European (Li et al., 2010) and Chinese Han populations (Zhu et al., 2014), evaluated with 12 SNP and 28 SNPs risk scores, respectively. In our case, the subjects were under the same physical activity intensity and volume through 3 months, but the level of ME and VO_{2max} achieved by the subjects varied. These differences (i.e., higher level of fitness capacity) produced attenuation genetic effects on obesity. The ROC curve estimates indicated that the

statistical discriminative ability to predict obesity through WC was similar to that previously estimated (ROC: 0.57–0.69) (Cheung et al., 2010; Li et al., 2010; Peterson et al., 2011; Belsky et al., 2013, Goñi et al., 2015).

A limitation of our study is that sample size was small to detect significant OR ≤ 1.50 at risk allele frequency ≤0.10. Previous studies in the sedentary Mexico-Mestizos have found an increase in obesity risk significantly associated to T allele of *FTO* rs9939609 (Villalobos-Comparán et al., 2008), G allele of *PPARG* rs1801282 (Canizales-Quinteros et al., 2007), and T allele of *ABCA1* rs9282541 (Villarreal-Molina et al., 2007). However, the associations we found were significant at 95% confidence level (*FTO*: OR = 1.40, $P = 0.042$ associated to FMP, *PPARG*: OR = 1.65, $P = 0.048$, OR = 1.70, $P = 0.038$ associated to BMI and FMP, respectively, *ABCA1*: OR = 5.25, $P = 0.012$ associated to WC) but did not pass Bonferroni correction. A not large enough sample size and obesity risk attenuation in a physically active population could be the underlying reason for the fact that we did not find strong associations, as those reported in previous studies. This was partly offset by combining SNPs, thus increasing the predictive value of the GRS for central obesity. Caution is needed when generalizing the results of this study. We do not recommend extrapolations to other groups, such as sedentary or highly trained people, as our results represent the consequence of neuromuscular adaptation occurring in fitness-aimed population who intend to improve general health or to lose weight under the specified conditions in this study.

In conclusion, we found association of obesity with six common European and Asian genetic variants and with GRS, for Mexican-Mestizos. To our knowledge, this is the first obesity genetic risk report for Mexican-Mestizos. Our results suggest that the level of ME necessary to attenuate obesity probability varies depending on genotype and age, and that, taking into account the general GRS, the probability of obesity is attenuated by higher levels of ME and aerobic capacity, which holds importance from the clinical point of view. These results help us to understand why the same exercise intensity or, even more so, the same ME or aerobic capacity produces different individual effects, which may be explained, at least partly, by individual genetic variation.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Gene variant frequency information. Minimum allele frequency (MAF) for 23 SNPs in Mexico-mestizo population. RA: reference allele.

Table S2. Unadjusted p-values (P) resulted from associations between obesity and SNPs, in physically active Mexican-mestizos.

S3.Obtain an individual obesity probability estimated under the logistic model by writing: Age, Genetic Risk Score (GRS), Muscular endurance (ME, assessed by recording the maximum number of abdominal crunches per minute) and VO2max (assessed as Indirect cardiorespiratory function measure, obtained by calculations after Cooper test, in which the person must run or walk the longest possible distance in 12 minutes).

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