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Longitudinal effects of *FTO* gene polymorphism on body composition, cardiorespiratory fitness, physical activity, inflammatory markers, and cardiovascular risk in children and adolescents. "The UP & DOWN study"

Julián Mier-Mota[1] | **Jesús G. Ponce-González**[2,3] | **Alejandro Perez-Bey**[3,4] | **Verónica Cabanas-Sánchez**[5,6,7] | **Oscar Veiga-Núñez**[5] | **Catalina Santiago-Dorrego**[8] | **Félix Gómez-Gallego**[9] | **José Castro-Piñero**[3,4]

[1]Salus Infirmorum, University of Cádiz, Cadiz, Spain

[2]ExPhy Research Group, Department of Physical Education, Faculty of Education Sciences, University of Cadiz, Cádiz, Spain

[3]Research Unit, Biomedical Research and Innovation Institute of Cadiz (INiBICA), Puerta Del Mar University Hospital, University of Cadiz, Cádiz, Spain

[4]GALENO Research Group, Department of Physical Education, School of Education, University of Cádiz, Puerto Real, Spain

[5]Department of Preventive Medicine and Public Health. School of Medicine, Universidad Autónoma de Madrid, Madrid, Spain

[6]CIBERESP (CIBER of Epidemiology and Public Health), Madrid, Spain

[7]IMDEA-Food Institute. CEI UAM+CSIC, Madrid, Spain

[8]Faculty of Physical Activity, Sport Sciences and Physiotherapy, Universidad Europea de Madrid, Madrid, Spain

[9]Department of Basic Health Sciences, Universidad Rey Juan Carlos, Madrid, Spain

Correspondence
Jesús G. Ponce-González, ExPhy Research Group, Department of Physical Education, Faculty of Education Sciences, University of Cadiz, Cádiz, Spain.
Email: jesusgustavo.ponce@uca.es

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Abstract

The role of polymorphism rs9939609 of the *FTO* gene has been related with fat mass and cardiovascular risk in adults, but it remains unclear in children and adolescents. Hence, the main aim of this study was to determine the *FTO* polymorphism effects on body composition, cardiorespiratory fitness (CRF), physical activity (PA), inflammatory markers, and cardiovascular risk both in cross-sectional analysis and after two-years of follow-up in children and adolescents. A total of 2129 participants were included in this study. The rs9939609 polymorphism was genotyped. Body composition measurements, CRF, and moderate-to-vigorous PA (MVPA) were determined at baseline and after two-year of follow-up. Moreover, plasma leptin and adiponectin were also determined as inflammatory markers. Furthermore, an index of cardiovascular disease risk factors (CVDRF-I) was calculated. Codominant (TT vs. TA vs. AA) and dominant (AA+AT vs. TT) models were applied for statistical analysis. The results showed a main effect of the *FTO* genotype on body composition measures in both first and third year ($p < 0.05$), with lower adiposity in TT compared with AA

or AA+AT group. These differences were maintained after accounting for pubertal maturity, sex, age, VO_2max, and MVPA. Moreover, lower leptin level was observed in TT compared to AA+AT group in the third year. An interaction in Gene*Time*Sex was found in height and neck circumference in dominant model ($p = 0.047$; $p = 0.020$, respectively). No differences were found in CRF, MVPA nor CVDRF-I between groups. Hence, homozygous TT allele could be a protective factor against weight gain from early childhood.

KEYWORDS

cardiovascular disease, childhood obesity, *FTO*, genotype

1 | INTRODUCTION

The accumulation of fat in childhood is related to health complications (i.e., increased blood pressure, hyperinsulinism, dyslipidaemia, and insulin resistance) that may persist into adulthood.[1] The lack of physical activity (PA) and an excess in energy intake are the main factors that increase the prevalence of obesity and overweight in children and adolescents which is associated with lower levels of cardiorespiratory fitness (CRF) and increasing inflammatory markers, such as leptin and adiponectin, and cardiovascular disease (CVD) risk factors.[2,3] However, there are many children who despite having a similar diet and PA, have different degrees of these parameters. In fact, there are more than 120 genes that affect the degree of fat accumulation, inflammatory markers, CRF, or CVD risk during life.[4]

A meta-analysis suggests that approximately 50%–70% of variation in the body mass index (BMI) is attributable to genetic differences.[5] The fat mass and obesity-associated (*FTO*) gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase, which catalyzes the demethylation of 3-methylthymine in single-stranded DNA with the production of succinate, formaldehyde, and carbon dioxide. This chemical and dynamic modification in RNA soon made it a target of study as an epitranscriptomic marker, playing an important role in the regulation of RNA processing.

FTO is ubiquitously expressed in human tissues but is highest in the hypothalamic nuclei that regulate energy balance. Indeed, *FTO* mRNA levels is also regulated by processes such as feeding and fasting.[6] Genetic variants in the *FTO* gene are associated not only with human adiposity but also with metabolic syndrome and CVD.[7] However, the relationship between *FTO* gene with CRF and PA remains unclear.[8] Despite that, it has been shown that the possible genetic susceptibility by *FTO* variants on adiposity could be blunted through PA,[9] hence this variable should be taken into account in the analysis.

Among the different variants of *FTO* gene, the rs9939609 polymorphism (A/T) in intron one of *FTO* gene is one of the most important genetic factor for obesity susceptibility to date, with subjects carrying the A allele showing a higher BMI compared to the TT genotype at adult age.[10]

It is important to note that most of these published studies on polymorphisms and adiposity have focused mainly on adult populations with contradictory results in children and adolescents' population. In addition, there are currently no longitudinal studies that determine the influence of the polymorphism of the *FTO* gene on the change of body composition, inflammatory markers, and CVD risk in childhood and adolescence taken into account CRF and PA. It has been shown that *FTO* gene may influence the CRF levels,[11] while it has little or any effect on physical activity between groups.[12]

Hence, the main aim of this study was to determine cross-sectionally and longitudinally (2-year follow-up) the influence of the *FTO* A/T polymorphism (rs9939609) on body composition, CRF, PA, inflammatory markers, and CVD risk factors in children and adolescents.

We defend the hypotheses that (i) subjects carrying at least one A allele of the polymorphism rs9939609 of the *FTO* gene will show worse levels of body composition, CRF, inflammatory markers, and CVD risk factors in children and adolescents, and (ii) participants with the AA and AT genotypes in the rs9939609 polymorphism of the *FTO* gene will accumulate greater amount of body fat throughout the two-years of follow-up in children and adolescents with a concomitant rise of inflammatory markers and CVD and a lowered CRF.

2 | MATERIALS AND METHODS

2.1 | Participants

We analyzed both cross-sectional and longitudinal data from the UP & DOWN study.[13] Baseline data (September 2011–June 2012) were collected from a sample of 2225

healthy Spanish children and adolescents (Figure 1) attending schools in the Cádiz and Madrid regions belonging to grades 1st/4th (6–7 and 9–10 years, respectively) for children, and 7th/10th (12–13 and 15–17 years old, respectively) for adolescents (baseline characteristics in Table 1). Two years later (September 2013–June 2014), 2129 participants completed the follow-up measurements of the studied variables (4.31% drop out).

Parents of the participants were informed about the study aims and written informed consents were provided. In accordance with the Helsinki Declaration, the study protocol was accepted by the Ethics Committee of the Puerta del Hierro Hospital (Madrid, Spain), the Bioethics Committee of the Research Council (Madrid, Spain) and the Research Committee on Human Subjects of the University of Cádiz (Cádiz, Spain).

2.2 | Pubertal maturity

Self-reported assessment of pubertal development of children and adolescents was determined by classifying them into one of the five stages of pubertal maturity defined by Tanner and Whitehouse.[14] For this purpose, participants were shown images with different levels of development (levels ranging from 1 to 5), so that the participant had to indicate which of all of them represented his or her current state of development.

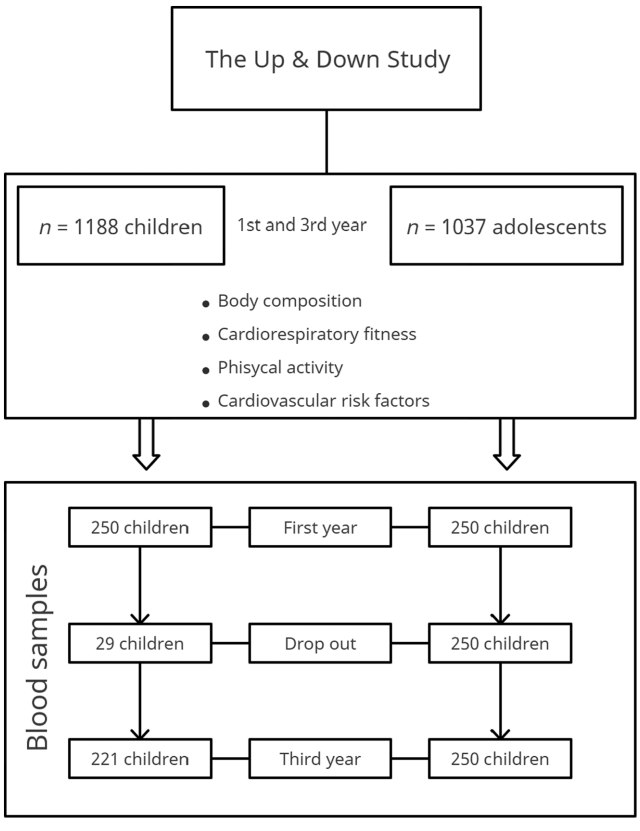

FIGURE 1 Flowchart for participants selection.

2.3 | Body composition measurements

Height and weight were measured using a telescopic stature-measuring instrument (Type SECA 225; range, 60–200 cm; precision, 1 mm; Hamburg, Germany) and an electronic scale (Type SECA 861; range, 0.05–130 kg; precision, 0.05 kg; Hamburg, Germany), respectively. BMI was calculated as weight/height squared (kg/m^2). Triceps and subscapular skinfold thickness were measured on the nondominant side of the body with a Holtain caliper (range, 0–40 mm; precision, 0.2 mm), and percentage of fat mass was estimated using Slaughter equations.[15] Waist circumference and neck circumference were measured with a nonelastic tape (SECA 200; range, 0–150 cm; precision, 1 mm). Two nonconsecutive measurements were carried out in all assessment and average was used in rearward analyses.

The waist-to-height ratio (WHtR) was calculated from the original body composition index (waist circumference/height). Fat mass and fat-free mass index were derived as body fat (kg) and fat-free mass (kg), respectively, and divided by squared height (m^2). Using fat mass %, and body mass, the fat mass index was calculated. The validity and reliability of these measures have been previously determined.[16] In addition, adiposity according to BMI and Slaughter's formulas have been found to correlate well with DXA and are therefore considered an acceptable method for initial estimation of body fat.[17]

2.4 | Cardiorespiratory fitness

In order to assess CRF, the 20-m shuttle run test was used. It is a valid and reliable test in children and adolescents[16] and is included in the ALPHA health-related physical fitness test battery.[18] In summary, the participants must run between two lines 20-m apart at a pace set by a prerecorded sound signal. The initial speed was 8.5 km/h, and it increased by 0.5 km/h in each section. The test is concluded when the participant fails to reach the line on time twice in succession. Maximal oxygen consumption (VO_2max [Y, ml kg^{-1} min^{-1}]) was estimate using the Léger equation[19] from the maximal aerobic shuttle running speed (X_1, km/h) and age (X_2, year as the lower rounded integer) through:

$$Y = 31.025 + 3.238\,X_1 - 3.248X_2 + 0.1536X_1X_2.$$

The regression equation of Leger et al. has been validated and can be used to estimate VO_2max within an acceptable margin of error in this age group, and it is

TABLE 1 Baseline characteristics divided by sex.

Parameter	All	Boys	Girls	Sig
N	2106	1095	1011	
Age (years)	10.75 ± 3.34	10.72 ± 3.37	10.79 ± 3.32	
TANNER STAGES	2.45 ± 1.18	2.53 ± 1.35	2.37 ± 1.21	*
Height (cm)	143.15 ± 18.56	144.08 ± 19.76	142.15 ± 17.10	*
Body mass (kg)	41.59 ± 16.05	42.34 ± 17.27	40.78 ± 14.59	*
BMI (kg/m^2)	19.47 ± 3.66	19.48 ± 3.70	19.46 ± 3.63	
Triceps skinfold (mm)	14.53 ± 6.52	13.17 ± 6.58	16.00 ± 6.13	*
Subscapular skinfold (mm)	11.04 ± 6.82	10.14 ± 6.98	12.01 ± 6.50	*
Waist circumference (cm)	63.33 ± 9.41	64.50 ± 9.89	62.06 ± 8.69	*
Neck circumference (cm)	29.16 ± 3.32	29.86 ± 3.62	28.41 ± 2.78	*
WtHR (cm)	0.44 ± 0.05	0.45 ± 0.05	0.44 ± 0.05	*
Fat mass (%)	21.81 ± 9.35	20.03 ± 10.35	23.74 ± 7.67	*
Fat mass index	4.51 ± 2.88	4.19 ± 3.17	4.86 ± 2.48	*
Fat-free mass index	9.67 ± 4.50	10.24 ± 5.26	9.06 ± 3.38	*
VO_2max (ml/kg/min)	45.16 ± 5.21	47.15 ± 4.63	42.98 ± 4.93	*
Total MVPA (min/day)	69.97 ± 34.07	78.80 ± 34.09	60.39 ± 31.38	*
Leptin (pg/ml)	9099.56 ± 8321.52	6771.78 ± 6694.49	11593.61 ± 9145.88	*
Adiponectin (pg/ml)	13.97 ± 7.38	13.27 ± 7.20	14.74 ± 7.53	*
CVDRFI (*z*-score)	49.20 ± 8.99	49.75 ± 9.91	48.58 ± 7.81	
CVDRFII (*z*-score)	45.56 ± 9.95	45.91 ± 10.98	45.16 ± 8.68	

Note: All data mean ± SD

Abbreviations: BMI, body mass index; CVDRF, cardiovascular disease risk factor index; CVDRF-II, cardiovascular disease risk factor index (excluding waist circumference); MVPA, moderate-to-vigorous physical activity.

*$p < 0.05$ significant difference.

recommended to consider the 20-m shuttle run test to include in schoolchildren's physical fitness batteries.[20] The continuous form of the variable was used for the main analyses.

2.5 | Physical activity

Moderate-to-vigorous physical activity (MVPA) was assessed by accelerometers (GT1M, GT3X, and GT3X+ models, Actigraph TM, LLC, Fort Walton Beach, FL, USA), which have previously been shown to be technically reliable instruments for this purpose.[21]

Different studies have shown strong agreement between GT1M, GT3X, and GT3X+measurements,[22] so additional calibration was not considered necessary.

Participants wore the accelerometer for 7 days, which was firmly attached to the back of the hip with an elastic band, positioned as close as possible to the centre of gravity, and participants were instructed to wear it only during waking hours, removing it during water activities according to previously established procedures.[23] The epoch was set at 10 s. To be considered a valid day, participants had to wear it for at least 10 h per day. Only participants whose accelerometer recorded at least three valid days were included in the analysis (14.30% failed).

Data were downloaded and analyzed using Actilife software (v.6.6.2 Actigraph TM, Pensacola, FL, United States). PA was expressed as average intensity expressed in counts per minute (cpm) and amount of time (minutes/day) spent in MVPA. The following cutoff points were used to define the different activity intensities: light between 100 and 2000 cpm; moderate between 2001 and 4000 cpm; and vigorous >4000 cpm. These cutoff points for defining the intensity categories were selected in relation to other previous studies conducted with European children and adolescents.[24] Time without wearing the accelerometer was defined as a 60-min period of zero counts and had an allocation for a maximum of two consecutive minutes (<100 cpm). In the case of sedentary habits, they were quantified using the accelerometer under the same conditions that we explained for the assessment of physical activity. Taking into account the cutoff points of the HELENA study, sedentary behaviors were considered to be those whose values were between 0 and 99 cpm.[24]

2.6 | Blood sampling

Inflammatory markers, leptin, and adiponectin were obtained in plasma after the extraction of a fasting blood sample from the cubital vein (Immunoassay (xMAP Techonology) using a kit plex: YB0000002Y Bio-Plex Human Diabetes 3-Plex Assay and 171-A7003M Bio-plex Pro Human Diabetes Adiponectin Assay), in the morning at the schools attended by the participants. Glucose, triglycerides, and high-density lipoprotein cholesterol (HDL-c) were determined through colorimetric assay (AU2700 Olympus analyser).

2.7 | Systolic blood pressure

Blood pressure was measured using a validated digital automatic blood pressure monitor (OMRON M6, OMRON HEALTH CARE Co., Ltd., Kyoto, Japan). The measurement was performed with the participants sitting quietly for 5 min before taking the measurements, with the left arm supported and in an extended position. Two measurements were taken 1–2 min apart. If the first two readings differed by >5 mm Hg, an additional third measurement was performed, eliminating the higher value. Mean values for systolic blood pressure and diastolic blood pressure (mm Hg) were calculated separately.

2.8 | Clustered cardiovascular disease risk factors

An index of CVD risk factors (CVDRF-I) was calculated from the mean of the standardized values (by population and sex) of systolic blood pressure, waist circumference, glucose, triglycerides, and HDL-c (CVD risk factors). Due to those higher levels of HDL-c represent a lower risk of CVD, the standardized value of HDL-c was multiplied by (−1). Considering to the close relationship between BMI and waist circumference, another index of CVD risk factors (CVDRF-II) was calculated excluding waist circumference. The calculations were done separately by population (children and adolescents) and sex (boys and girls).[25]

2.9 | Genetic sampling

Buccal cells were collected on swabs or FTA® cards and genomic deoxyribonucleic acid was extracted and purified according to standard phenol/chloroform procedures followed by alcohol precipitation. The allelic discrimination analysis was performed by predesigned Life Technologies TaqMan® SNP Genotyping Assays on demand for the fat mass and obesity associated (*FTO*) rs9939609 polymorphism (ID: C_30090620_10). Subsequently, a polymerase chain reaction (PCR) amplification was performed using a StepOne™ Real-Time PCR System (Life Technologies, Foster City, CA) with a denaturation stage at 95°C for 10 min, 50 cycles of denaturation at 92°C for 15 s, annealing/extension at 60°C for 1 min, and a final extension stage of 30 s at 60°C.

2.10 | Statistical analyses

Descriptive statistics are presented as mean and SD A chi-squared test was used to test the Hardy Weinberg equilibrium (HWE). Kolmogorov–Smirnov test was used to examine for normal distribution. Cross-sectional analysis was, respectively, performed at 1st and 3rd year to avoid the loss of sample size with one-way ANOVA to compare main effects between groups: TT versus TA versus AA (homozygotes and heterozygote); or with T-student to compare the difference between dominant model groups: AA+AT versus TT. Pubertal maturity, sex, age, VO_2max, and MVPA were used, individually and jointly, as covariates due to its relationship with body composition. Moreover, MVPA was analyzed after adjusting for wear time.

After adjusting for age firstly and all covariates jointly afterwards, a longitudinal analysis was performed using a 3 × 2 and 2 × 2 generalized linear mixed effect model for repeated measure analysis comparing two main effects: (a) the genotype: (3 groups) TT versus TA versus AA (homozygotes and heterozygote) or (2 groups) AA+AT versus. TT (dominant model), and (b) time: (2 moments) 1st-3rd Year with a genotype*time interaction. Moreover, time*gene*sex interactions were also analyzed. Bonferroni post hoc analysis was performed to identify differences. Statistical analyses were performed with SPSS version 21.0. for Windows (IBM Corp, Armonk, New York), with significance set at $p < 0.05$.

3 | RESULTS

Mean participants' characteristics in our population divided by genotype (TT, TA, and AA) are presented in Table 2. The HWE test showed that $\chi 2 = 0.778$, d.f. = 2, $p = 0.377$, so the population is consistent with HWE, and confirming that the allele types were randomly sampled. The expected frequencies were TT ($p = 0.3481$) $n = 742$, TA ($p = 0.484$) $n = 1029$, and AA ($p = 0.168$) $n = 357$. The distribution of rs9939609 genotype frequencies were 35.32% for TT, 47.44% for TA, and 17.24% for AA. Because age and sex covary with many features of body composition, it

TABLE 2 Differences of FTO genotypes divided by codominat model (TT vs. TA vs. AA) on body composition, cardiorespiratory fitness, physical activity, inflammatory markers and cardiovascular risk with the cross-sectional and longitudinal data.

	TT (752)					TA (1010)					AA (367)					ANOVA 1st Year	ANOVA 3rd Year	Generalized linear mixed effect model for repeated measures 1st-3rd Year		
Parameter	N	1st Year	sig	N	3rd Year	sig	N	1st Year	sig	N	3rd Year	sig	N	1st Year	sig	N	3rd Year	sig	Gene (p)	Gene (p)	Time (p)	Time*Gene (p)	Time*Gene*Sex (p)
Sex (n) F/M	752	364 / 388		752	364 / 388		1010	486 / 524		1010	486 / 524		367	170 / 197		367	170 / 197						
Age (years)	739	10.94 ± 3.4		650	12.53 ± 3.02		994	10.67 ± 3.3		887	12.53 ± 3.02		365	10.63 ± 3.35		330	12.53 ± 3.02		0.165	0.547			
TANNER STAGES	737	2.53 ± 1.3		648	3.06 ± 1.26		990	2.4 ± 1.26		885	3.02 ± 1.21		364	2.43 ± 1.32		328	3.08 ± 1.27		0.088	0.674	0.000	0.641	0.711
Height (cm)	739	143.89 ± 18.77		650	151.87 ± 16.21		994	142.53 ± 18.26		886	151.43 ± 15.56		365	143.34 ± 18.9		329	152.63 ± 16.29		0.315	0.499	0.000	0.712	0.120
Body mass (kg)	739	41.49 ± 15.84	*	650	47.5 ± 16.03	*	995	41.18 ± 15.77	*	886	47.84 ± 15.62		365	42.9 ± 17.19		329	49.98 ± 17.01		0.214	0.058	0.000	0.792	0.677
BMI (kg/m2)	739	19.23 ± 3.6	#*†‡§¶¤&¥	650	19.99 ± 3.81	#*†‡§¶¤	994	19.47 ± 3.63		886	20.32 ± 3.92		365	19.96 ± 3.88		329	20.83 ± 4.05		0.008	0.005	0.000	0.864	0.918
Triceps skinfold (mm)	737	14.07 ± 6.33	#&	646	14.71 ± 7.54	#*†‡§¶¤	994	14.68 ± 6.52		885	15.44 ± 7.46		362	15.08 ± 6.85		329	16.01 ± 8.01		0.003	0.030	0.000	0.929	0.481
Subscapular skinfold (mm)	737	10.72 ± 7.1	#*†‡§¶¤	644	11.28 ± 7.18	#*†‡§¶¤	994	10.94 ± 6.41	#†‡§¶¤	883	11.92 ± 6.99		362	11.97 ± 7.24		327	13.05 ± 8.4		0.013	0.002	0.000	0.973	0.296
Waist circumference (cm)	739	63.02 ± 9.34	*†‡§¶¤	650	65.22 ± 9.44	#*†‡§¶¤	994	63.15 ± 9.15	*	886	65.89 ± 9.43		365	64.45 ± 10.18		329	66.99 ± 10.82		0.042	0.027	0.000	0.141	0.897
Neck circumference (cm)	739	29.16 ± 3.37	*	650	30.11 ± 3.74		994	29.07 ± 3.22		886	30.01 ± 3.21		365	29.4 ± 3.49		329	30.32 ± 3.84		0.276	0.408	0.000	0.170	0.057
WtHR (cm)	738	0.44 ± 0.05	#*†‡§¶¤	650	0.43 ± 0.048	#*†‡§¶¤	993	0.444 ± 0.048		886	0.436 ± 0.051		365	0.45 ± 0.05		329	0.439 ± 0.058		0.001	0.014	0.000	0.235	0.833
Fat mass (%)	737	21.22 ± 9.48	#*†‡§¶¤	643	21.69 ± 10.33	#*†‡§¶¤	994	21.83 ± 8.95		882	22.76 ± 10.09		362	22.94 ± 10.03		327	239,480 ± 11.4		0.016	0.005	0.000	0.077	0.609
Fat mass index	735	4.34 ± 2.97	#*†‡§¶¤	643	4.6 ± 3.1	#*†‡§¶¤	993	4.5 ± 2.66		882	4.91 ± 3.07		362	4.89 ± 3.21		327	53,290 ± 3.61		0.012	0.003	0.000	0.830	0.701
Fat free mass index	735	9.71 ± 4.75	§	643	8.98 ± 5.38	#§¶	993	9.83 ± 3.88	§¶	882	8.78 ± 4.6	¶	362	9.19 ± 5.42		327	80,257 ± 6.34		0.065	0.024	0.000	0.637	0.780
VO_2max (ml/kg/min)	725	45.02 ± 5.22		633	44.34 ± 6.2		973	45.23 ± 5.22		869	44.29 ± 5.91		354	45.23 ± 5.13		318	441,031 ± 6.41		0.706	0.846	0.007	0.534	0.362
Total MVPA (min/day)	624	68.03 ± 34.1		571	62.92 ± 24.39		828	71.22 ± 34.23		778	63.53 ± 24.69		307	70.53 ± 33.54		299	631,871 ± 25.74		0.200	0.904	0.000	0.782	0.286
Leptin (pg/ml)	171	8783.7 ± 8524.3		152	4232 ± 4390		244	8939.9 ± 7828.3		208	4951.9 ± 4864.3		78	10291.6 ± 9321.9		71	5466.5 ± 6307.2		0.380	0.180	0.000	0.652	0.629
Adiponectin (pg/ml)	171	13.46 ± 7.48		156	13.03 ± 8.22		247	14.04 ± 7.13		213	12.97 ± 7.74		82	14.79 ± 7.93		71	12.21 ± 7.77		0.395	0.741	0.002	0.430	0.536
CVDRFI (z-score)	174	49.67 ± 8.6		155	52.29 ± 8.71		251	48.58 ± 9		213	52.16 ± 9.05		82	50.07 ± 9.69		72	540,286 ± 8.78		0.298	0.284	0.008	0.425	0.600
CVDRFII (z-score)	174	46.24 ± 9.46		155	48.83 ± 9.36		251	44.98 ± 10.23		213	48.53 ± 9.92		82	45.86 ± 10.13		72	502,170 ± 9.38		0.421	0.437	0.047	0.463	0.633

All data mean ± S.D.

 # p<0.05 significant difference with AA group

*** p<0.05 significant difference with AA group when was adjusted for Tanner, sex, age, VO_2max and MVPA**

† p<0.05 significant difference with AA group when was adjusted for Tanner

‡ p<0.05 significant difference with AA group when was adjusted for sex

§ p<0.05 significant difference with AA group when was adjusted for age

¶ p<0.05 significant difference with AA group when was adjusted for VO_2max

¤ p<0.05 significant difference with AA group when was adjusted for MVPA

& p<0.05 significant difference with TA group when was adjusted for Tanner, sex, age, VO_2max and MVPA

¥ p<0.05 significant difference with TA group when was adjusted for Tanner

ł p<0.05 significant difference with TA group when was adjusted for age

⊠ p<0.05 significant difference with TA group when was adjusted for VO_2max

was important that the genotype distribution for *FTO* was not significantly biased with respect to age both the first year ($F=1.802$, $p=0.165$) and the third year ($F=0.604$, $p=0.547$), while there were no bias in genotype frequency with respect to sex ($\chi 2=0.462$, d.f. $=2$, $p=0.794$).

3.1 | Differences by *FTO* genotype (TT vs. TA vs. AA)

Descriptive statistics are shown in Table 2 as mean ± S.D. There was a main effect of *FTO* genotype (TT vs. TA vs. AA) on different body composition measurements, both in the first and third year of evaluation ($p<0.05$). However, there was not any interaction of time*gene or time*gene*sex. No significant differences were observed in CRF, PA, inflammatory markers, or CVD risk clusters between FTO groups.

3.2 | TT versus AA

In terms of body composition measurements, Bonferroni post hoc comparison showed that in both, first and third year. TT genotype compared to AA group had lower BMI (point estimate – 0.724, CI −1.284/−0.163, $p=0.006$; point estimate −0.836, CI −1.470/−0.203, $p=0.005$), triceps skinfold (point estimate −1.014, CI −2.015/−0.013, $p=0.043$; point estimate −1.298, CI −2.529/−0.066, $p=0.035$), subscapular skinfold (point estimate −1.258, CI −2.305/−0.212, $p=0.012$; point estimate −1.769, CI −2.961/−0.578, $p=0.001$), WtHR (point estimate −0.011, CI −0.019/−0.004 $p=0.001$; point estimate −0.009, CI −0.018/−0.001 $p=0.020$), fat mass (point estimate −1.717, CI −3.152/−0.282, $p=0.013$; point estimate −2.262, CI −3.957/−0.567, $p=0.004$) and fat mass index (point estimate −0.548, CI −0.990/−0.106, $p=0.009$; point estimate −0.733, CI −1.251/−0.215, $p=0.002$). Waist circumference only showed differences in the third year (point estimate −1763 CI −3.335/−0.192 $p=0.027$). Related to lean mass parameters, TT showed higher fat-free mass index in third year compared to AA group (point estimate 0.954, CI 0.105/1.804, $p=0.021$), but not in the first year ($p=0.211$). Similar results about fat-free mass index were obtained after adjusting for age in the first year (point estimate 0.675, CI 0.028/1.322, $p=0.038$) and for age or VO_2max in the third year (point estimate 0.958, CI 0.119/1.797, $p=0.019$; point estimate 0.952, CI 0.204/1.701, $p=0.007$, respectively). There was no significant effect of *FTO* genotype neither, first nor third year, on body mass, neck circumference, VO_2max, MVPA, leptin, adiponectin, CVDRFI, and CVDRFII (all $p>0.05$).

After accounting by cofounding factors, all differences, except triceps skinfold in the first year ($p=0.082$), were maintained even when were adjusted for Tanner, sex, age, VO_2max, MVPA, individually, or jointly ($p<0.05$). Moreover, TT genotype showed lower values in body mass than AA genotype only after adjusting by Tanner, age, sex, VO_2max, and MVPA, both first and third year (point estimate −2.520, CI −3.951/−1.090, $p<0.001$; point estimate −1.879, CI −3.575/−0.184, $p=0.024$) and neck circumference in the first year (point estimate −0.442, CI −0.763/−0.121, $p=0.003$).

3.3 | TA versus AA

Related to body composition measurements, post hoc comparison only showed that TA had lower subscapular skinfold than AA group in the first year (point estimate −1.035, CI −2.036/−0.034, $p=0.040$) but this difference disappeared after adjusting for Tanner, sex, age, VO_2max, and MVPA jointly ($p>0.05$) but not individually ($p<0.05$). Moreover, after adjusting for these variables, TA showed lower values than AA group in body mass and waist circumference in the first year (point estimate −1.537, CI −2.907/−0.168, $p=0.022$; point estimate −1.182, CI −2.296/−0.068, $p=0.033$; respectively).

TA also showed higher fat-free mass index in first year compared to AA group after adjusting by age (point estimate 0.667, CI 0.048/1.285, $p=0.030$), and similar results were obtained after adjusting for VO_2max in the first year (point estimate 0.644, CI 0.069/1.220, $p=0.022$) and in the third year (point estimate 0.717, CI 0.004/1.429, $p=0.048$). However, these differences were disappeared after adjusting for Tanner, sex, age, VO_2max, and MVPA jointly.

3.4 | TT versus TA

TA group had in the first-year higher values compared to TT group in BMI, only when was adjusted both for Tanner (point estimate −0.417, CI −0.788/−0.046, $p=0.021$) and age (point estimate −0.382, CI −0.758/−0.007, $p=0.045$); and higher triceps skinfold, when was adjusted for VO_2max (point estimate −0.732, CI −1.397/−0.068, $p<0.025$). However, no differences were found in the third year.

3.5 | Differences between *FTO* dominant model groups (AA + AT vs. TT)

Differences on body composition, cardiorespiratory fitness, physical activity, inflammatory markers, and cardiovascular risk by *FTO* dominant model groups are shown in Table 3 as mean ± SD The distribution of rs9939609 genotype frequencies were 64.68% AA+AT groups and 35.32% for TT. Comparing all individuals with allele A (AA+AT) with TT group, significant differences appear in Tanner in the first year (point estimate 0.128, CI 0.013/0.244, $p=0.029$), while TT had lower subscapular skinfold and waist circumference in the third year (point estimate −0.943, CI −1.644/−0.241, $p=0.008$; point estimate −0.964, CI −1.888/−0.039, $p=0.041$). Moreover, BMI, triceps skinfold, fat mass, and fat mass index were also lower in TT group compared to AA+AT, both first and third year, even when was adjusted for Tanner, sex, age, VO_2max, and MVPA ($p<0.05$). TT group compared with AA+AT group showed also lower body mass, subscapular skinfold and waist circumference, both first and third year, only when was adjusted for Tanner, sex, age, VO_2max, and MVPA (all $p<0.05$). While there were differences in height and neck circumference, only in the first year (point estimate −0.668, CI −1.335/−0.002 $p=0.049$; point estimate −0.230, CI −0.418/−0.042, $p=0.016$), and leptin levels, only in the third year (point estimate −1332.099, CI −2207.738/−456.459, $p=0.003$).

No interaction was found when time*gene was analyzed. However, there was an interaction in time*gene*sex in height and neck circumference ($p=0.047$, $\eta p^2=0.002$; $p=0.020$, $\eta p^2=0.003$, respectively) after adjusting for age (Figure 2). However, these differences disappeared after adjusting for Tanner, age, VO_2max, and MVPA jointly ($p>0.05$).

4 | DISCUSSION

The main findings of this study indicate that within Spanish schoolchildren, the presence of the A allele, homozygous or heterozygous for rs9939609 polymorphism (*FTO* gene), was associated with higher levels of adiposity, and these differences were maintained after two-years of follow-up, even when were adjusted for Tanner, sex, age, VO_2max, and MVPA. In concordance, leptin level was lower in TT group compared to the group with the presence of the A allele (AA+AT) in the third year. Moreover, a sexual dimorphism is also observed in the influence of rs9939609 polymorphism during two-years of follow-up on height and neck circumference. However, this genetic variant has little or any effect on CRF, MVPA, or CVD clusters in our sample.

According to our results, some longitudinal and cross-sectional studies agree that carrying the A allele during infancy or childhood influences different obesity states.[26–28] In this line, some authors have found a positive association between additional minor alleles (A) and BMI from 5.5 years of age onwards,[29] other authors have observed

TABLE 3 Differences of FTO genotypes by dominant model (AA+AT vs. TT) on body composition, cardiorespiratory fitness, physical activity, inflammatory markers, and cardiovascular risk with the cross-sectional and longitudinal data.

	TT (752)			AA+AT (1377)					Year 1		Year 3		Generalized linear mixed effect model for repeated measures 1st-3rd Year		
Parameter	N	Year 1	sig	N	Year 3	sig	N	Year 1	N	Year 3	p t-student	D-Cohen	p t-student	D-Cohen	Time (p)	Time*Gene (p)	Time*Gene*Sex (p)
Sex (n) F/M	752	364 / 388		752	364 / 388		1377	656 / 721	1377	656 / 721							
Age (years)	744	10.94 ± 3.40		650	12.53 ± 3.21		1362	10.65 ± 3.31	1217	12.40 ± 3.13	0.058	0.087	0.392	0.042			
TANNER CONJUNTO	737	2.54 ± 1.30	#	648	3.06 ± 1.26		1354	2.41 ± 1.27	1213	3.03 ± 1.22	0.027	0.102	0.641	0.023	0.000	0.383	0.558
Height (cm)	739	143.89 ± 18.77	*	650	151.87 ± 16.21		1359	142.75 ± 18.43	1215	151.75 ± 15.77	0.179	0.061	0.883	0.007	0.000	0.973	**0.047**
Body mass (kg)	739	41.49 ± 15.84	*	650	47.50 ± 16.03	*	1359	41.64 ± 16.17	1215	48.42 ± 16.03	0.838	-0.009	0.238	-0.057	0.000	0.526	0.377
BMI (kg/m2)	739	19.23 ± 3.58	#*	650	19.99 ± 3.81	#*	1359	19.60 ± 3.70	1215	20.46 ± 3.96	0.027	-0.101	0.015	-0.119	0.000	0.681	0.932
Triceps skinfold (mm)	737	14.07 ± 6.33	#*	646	14.71 ± 7.54	#*	1356	14.79 ± 6.61	1214	15.59 ± 7.61	0.016	-0.111	0.017	-0.116	0.000	0.843	0.84
Subscapular skinfold (mm)	737	10.72 ± 7.10	*	644	11.28 ± 7.18	#*	1356	11.21 ± 6.65	1210	12.22 ± 7.41	0.116	-0.072	0.008	-0.129	0.000	0.823	0.271
Waist circumference (cm)	739	63.02 ± 9.34	*	650	65.22 ± 9.44	#*	1359	63.50 ± 9.45	1215	66.19 ± 9.83	0.265	-0.051	0.041	-0.099	0.000	0.286	0.942
Neck circumference (cm)	739	29.16 ± 3.37	*	650	30.11 ± 3.74		1359	29.16 ± 3.30	1215	30.10 ± 3.39	1.000	0.000	0.926	0.005	0.000	0.117	**0.020**
WtHR (cm)	738	0.44 ± 0.05	#*	650	0.43 ± 0.05	#*	1358	0.45 ± 0.05	1215	0.44 ± 0.05	0.002	0.200	0.007	0.20	0.000	0.370	0.585
Fat mass (%)	737	21.22 ± 9.48	#*	643	21.69 ± 10.33	#*	1356	22.13 ± 9.26	1209	23.08 ± 10.47	0.033	-0.097	0.006	-0.134	0.000	0.711	0.941
Adiposity index	735	4.34 ± 2.97	#*	643	4.60 ± 3.10	#*	1355	4.60 ± 2.82	1209	5.02 ± 3.23	0.048	-0.090	0.006	-0.135	0.000	0.555	0.842
Fat free mass index	735	9.71 ± 4.75		643	8.98 ± 5.38		1355	9.66 ± 4.35	1209	8.58 ± 5.14	0.808	0.011	0.115	0.077	0.000	0.362	0.654
VO_2max (ml/kg/min)	725	45.03 ± 5.22		633	44.34 ± 6.20		1327	45.23 ± 5.20	1187	44.24 ± 6.05	0.918	-0.039	0.743	0.016	0.007	0.331	0.410
Total MVPA (min/day)	624	68.03 ± 34.10		571	62.92 ± 24.39		1135	71.03 ± 34.03	1077	63.43 ± 24.98	0.077	-0.088	0.688	-0.021	0.000	0.544	0.984
Leptin (pg/ml)	171	8783.72 ± 8524.32		152	4231.99 ± 4389.97	*	322	9267.29 ± 8220.26	279	5082.85 ± 5261.72	0.540	-0.058	0.090	-0.171	0.000	0.364	0.377
Adiponectin (pg/ml)	171	13.46 ± 7.48		156	13.03 ± 8.22		329	14.23 ± 7.33	284	12.78 ± 7.74	0.263	-0.106	0.752	0.031	0.000	0.446	0.961
CVDRFI (z-score)	174	49.67 ± 8.60		155	52.29 ± 8.71		333	48.95 ± 9.19	285	52.63 ± 9.00	0.392	0.080	0.696	-0.039	0.008	0.193	0.683
CVDRFII (z-score)	174	46.24 ± 9.46		155	48.83 ± 9.36		333	45.20 ± 10.20	285	48.96 ± 9.80	0.264	0.104	0.889	-0.014	0.559	0.213	0.754

All data mean ± S.D.

Significant interaction is in bold (P< 0.05).

 # p<0.05 significant difference with AA+AT group

*** p<0.05 significant difference with AA+AT group when was adjusted by Tanner, sex, age, VO2max and MVPA**

Abbreviations: BMI=body mass index; MVPA= Moderate to Vigorous Physical Activity; CVDRF= Cardiovascular disease risk factor index; CVDRF-II Cardiovascular disease risk factor index (excluding waist circumference)

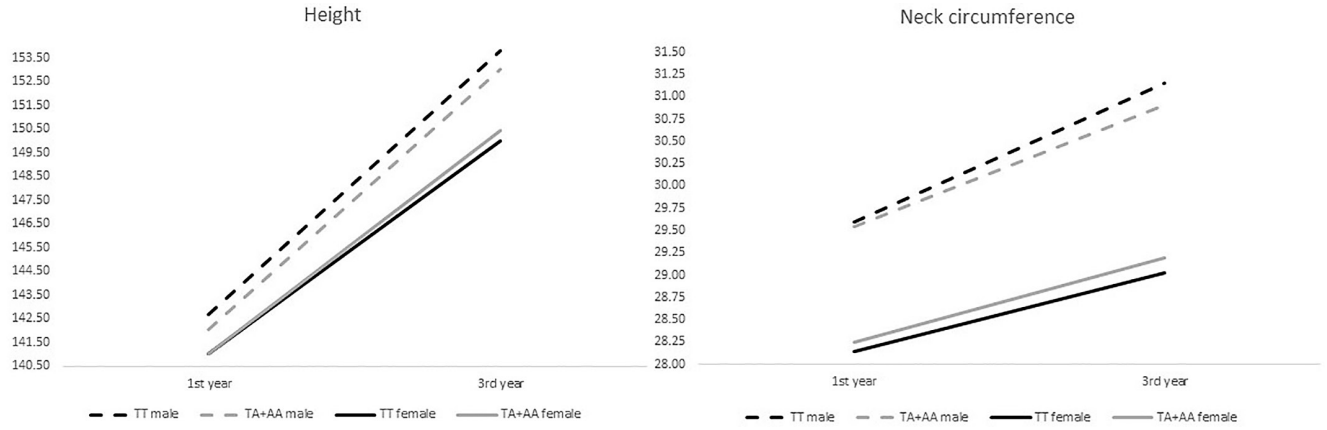

FIGURE 2 Significative differences in time*gene*sex interaction in height and neck circumference after adjusting for age.

an influence of the gene starting at 12 years of age,[30] while Rutters et al. added to this date a decrease at 13–14 years that intensifies later at 17.[31] However, in another longitudinal study of 32 years of follow-up, it was observed that neither the A nor the T allele at any age was associated with a greater increase or decrease in BMI, suggesting that the variant studied does not seem to affect changes in BMI levels at any stage of life.[32]

It is important to consider the role that PA may play in the effect of the polymorphism on obesity. Andreasen et al. showed that physically inactive *FTO* rs9939609 homozygous A allele carriers had higher BMI compared to inactive homozygous T allele carriers.[33] However, this difference between alleles in BMI was not found in physically active subjects. Hence, the influence of *FTO* rs9939609 polymorphism on BMI could be hidden by PA levels as described in a meta-analysis.[34] However, our results showed similar PA among groups, hence the differences found in the *FTO* group is not due to physical activity or inactivity.

Consistent with PA levels, all groups showed similar CRF levels in our study. This fact is important because a low CRF level is frequently associated with adiposity and contributes as an individual risk factor for many obesity-related comorbidities.[35] Although other authors have determined that CRF does not modify the effect of FTO variation on BMI or waist circumference.[36]

The mechanism by which FTO polymorphisms might affect fat accumulation is unclear. Although several studies support the association between *FTO* and food intake,[37] a meta-analysis published with 177 330 participants found no significant interactions between the *FTO* variant and total dietary energy intake but did find a positive relationship between BMI-increasing allele of the *FTO* variant and higher dietary protein intake, providing insights into the potential link between *FTO*, dietary protein intake, and adiposity.[38]

Leptin also plays an important role in metabolism stimulating fat oxidation in the cells and in the regulation of appetite in the hypothalamus.[39] As with obesity, chronically elevated leptin levels affect its resistance, which is characterized by reduced satiety, excessive nutrient intake, and impaired fat oxidation capacity and consequently an increase in total body mass.[39] In our study, the TT group showed lower leptin levels, which may mean a higher sensitivity to leptin and therefore an increase in satiety levels and an improvement in fat oxidation, contributing to explain the differences in adiposity observed in our results. In concordance, it has been shown that TT group had higher maximal fat oxidation during exercise compared with AA and AT groups.[40]

Although at younger ages we found no differences in CVD risk, a meta-analysis in adults (45–67 years) confirmed that the rs9939609 variant of the FTO gene is significantly associated with an increased risk of CVD and that the genetic effect is not mediated by changes in BMI and other conventional CVD risk factors,[41] suggesting that the presence of the A allele influences CVD risk. Although it is not known at what time of life it appears, according to our findings, this polymorphism could affect CVD risk in adulthood due to the influence on adiposity and leptin levels from early ages.

It also seems unclear whether sex may affect the influence of the gene over time. Although our results showed no sexual dimorphism in the influence of the FTO polymorphism in the 2 years of follow-up, an influence of the gene has been previously observed from the age of 12 years mainly in girls,[30] probably due to the variation of the endocrinological changes that occur at puberty and the influence of the different sex hormones.[42] However, in adults, some studies seem to relate the polymorphism to a greater influence in boys, which may be due to a different inheritance pattern in boys than in girls.[43] This reveals the need to consider sex as an important factor modulating

the genetic association of FTO with overweight and obesity, although we do not know at what age it occurs.

Given the above, longer-term follow-up studies are needed to examine at what ages and by what mechanisms the FTO genotype begins to influence fat mass and body composition. Longitudinal studies can shed light on the effect of the polymorphism on individuals over time. This would allow the study of this genetic marker in children and adolescents and its relationship with various phenotypic characteristics over time, while allowing a better understanding of the pathogenic mechanisms involved in obesity-associated diseases, being useful as a predictive element to buffer health problems related to these diseases.

Therefore, the TT genotype of the FTO gene could be a protective factor against weight gain from early childhood and could reduce the risk of obesity-associated comorbidities in individuals genetically predisposed to obesity.

4.1 | Perspective

Although all groups showed similar levels of CRF and MVPA, the presence of the A allele, homozygous or heterozygous for the rs9939609 polymorphism (FTO gene), was associated with higher levels of adiposity from baseline to 2 years of follow-up in schoolchildren. In concordance, the TT group showed lower leptin levels, which may mean a better sensitivity to leptin, increasing both satiety and fat oxidation.

One of the keys to avoid morbidities associated with childhood obesity both at this stage and in the adult phase is early detection. School age and adolescence are crucial stages for the configuration of eating habits and other lifestyles that will persist into adulthood.

These findings would make it possible in the future to predict individuals genetically predisposed to suffer from overweight/obesity and, therefore, to design intervention plans in critical periods of childhood and adolescent development and thus mitigate the problems derived from overweight in adulthood.

5 | LIMITATIONS AND STRENGTHS

The main limitations of our work are as follows (i) the lack of the evaluation of energy intake as important variable of fat gain, (ii) we do not have much ethnic variability, a factor that can have an important influence, and (iii) body composition measurements such as fat mass have been estimated from anthropometric measurements, without a more accurate method such as dual-energy X-ray absorptiometry (DXA). However, it has important strengths such as having many body composition variables, combined with CRF and MVPA, as well as blood and CVD measurements in a large sample. In addition, all these measurements have been studied longitudinally after two years of follow-up, providing novel results with several measurements taken into account objective MVPA and CRF as cofounders.

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CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Jesús G. Ponce-González https://orcid.org/0000-0002-5982-7761
Alejandro Perez-Bey https://orcid.org/0000-0002-9849-5544
Catalina Santiago-Dorrego https://orcid.org/0000-0003-3852-8217
José Castro-Piñero https://orcid.org/0000-0002-7353-0382

REFERENCES

1. Weihrauch-Blüher S, Schwarz P, Klusmann JH. Childhood obesity: increased risk for cardiometabolic disease and cancer in adulthood. *Metabolism*. 2019;92:147-152. doi:10.1016/j.metabol.2018.12.001
2. Wade KH, Chiesa ST, Hughes AD, et al. Assessing the causal role of body mass index on cardiovascular health in young adults: Mendelian randomization and recall-by-genotype analyses. *Circulation*. 2018;138(20):2187-2201. doi:10.1161/CIRCULATIONAHA.117.033278
3. Agbaje AO, Barker AR, Tuomainen TP. Cardiorespiratory fitness, fat mass, and Cardiometabolic health with endothelial function, arterial elasticity, and stiffness. *Med Sci Sports Exerc*. 2022;54(1):141-152. doi:10.1249/MSS.0000000000002757
4. Yang W, Kelly T, He J. Genetic epidemiology of obesity. *Epidemiol Rev*. 2007;29(1):49-61. doi:10.1093/epirev/mxm004
5. Allison DB, Kaprio J, Korkeila M, Koskenvuo M, Neale MC, Hayakawa K. The heritability of body mass index among an international sample of monozygotic twins reared apart. *Int J Obes Relat Metab Disord*. 1996;20(6):501-506.

6. Gerken T, Girard CA, Tung Y-CL, et al. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. *Science*. 2007;318(5855):1469-1472. doi:10.1126/science.1151710
7. Ganeff IMM, Bos MM, Van Heemst D, Noordam R. BMI-associated gene variants in FTO and cardiometabolic and brain disease: obesity or pleiotropy? *Physiol Genomics*. 2019;51(8):311-322. doi:10.1152/physiolgenomics.00040.2019
8. Martorell M, Mardones L, Petermann-Rocha F, et al. The FTO rs17817449 polymorphism is not associated with sedentary time, physical activity, or cardiorespiratory fitness: findings from the GENADIO cross-sectional study. *J Phys Act Health*. 2021;18(11):1352-1357. doi:10.1123/jpah.2021-0076
9. Rampersaud E, Mitchell BD, Pollin TI, et al. Physical activity and the association of common FTO gene variants with body mass index and obesity. *Arch Intern Med*. 2008;168(16):1791-1797.
10. Frayling TM, Timpson NJ, Weedon MN, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science*. 2007;316(5826):889-894. doi:10.1126/science.1141634
11. Schnurr TM, Gjesing AP, Sandholt CH, et al. Genetic correlation between body fat percentage and cardiorespiratory fitness suggests common genetic etiology. *PLoS One*. 2016;11(11):1-14. doi:10.1371/journal.pone.0166738
12. Loos RJF, Yeo GSH. The bigger picture of FTO: the first GWAS-identified obesity gene. *Nat Rev Endocrinol*. 2014;10(1):51-61. doi:10.1038/nrendo.2013.227
13. Castro-Piñero J, Carbonell-Baeza A, Martinez-Gomez D, et al. Follow-up in healthy schoolchildren and in adolescents with DOWN syndrome: psycho-environmental and genetic determinants of physical activity and its impact on fitness, cardiovascular diseases, inflammatory biomarkers and mental health;the UP&DOWN Study. *BMC Public Health*. 2014;14(1):1-12. doi:10.1186/1471-2458-14-400
14. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child*. 1976;51(3):170-179. doi:10.1136/adc.51.3.170
15. Slaughter MH, Lohman TG, Boileau RA, et al. Skinfold equations for estimation of body fatness in children and youth. *Hum Biol*. 1988;60(5):709-723.
16. Castro-Piñero J, Artero EG, España-Romero V, et al. Criterion-related validity of field-based fitness tests in youth: a systematic review. *Br J Sports Med*. 2010;44(13):934-943. doi:10.1136/bjsm.2009.058321
17. Steinberger J, Jacobs DR, Raatz S, Moran A, Hong C-P, Sinaiko AR. Comparison of body fatness measurements by BMI and skinfolds vs dual energy X-ray absorptiometry and their relation to cardiovascular risk factors in adolescents. *Int J Obes*. 2005;29(11):1346-1352. doi:10.1038/sj.ijo.0803026
18. Ruiz JR, Castro-Piñero J, España-Romero V, et al. Field-based fitness assessment in young people: the ALPHA health-related fitness test battery for children and adolescents. *Br J Sports Med*. 2011;45(6):518-524. doi:10.1136/bjsm.2010.075341
19. Léger LA, Mercier D, Gadoury C, Lambert J. The multistage 20 metre shuttle run test for aerobic fitness. *J Sports Sci*. 1988;6(2):93-101. doi:10.1080/02640418808729800
20. Liu NYS, Plowman SA, Looney MA. The reliability and validity of the 20-meter shuttle test in american students 12 to 15 years old. *Res Q Exerc Sport*. 1992;63(4):360-365. doi:10.1080/02701367.1992.10608757
21. Rothney MP, Apker GA, Song Y, Chen KY. Comparing the performance of three generations of ActiGraph accelerometers. *J Appl Physiol*. 2008;105(4):1091-1097. doi:10.1152/japplphysiol.90641.2008
22. Robusto KM, Trost SG. Comparison of three generations of ActiGraph™ activity monitors in children and adolescents. *J Sports Sci*. 2012;30(13):1429-1435. doi:10.1080/02640414.2012.710761
23. Ward DS, Evenson KR, Vaughn A, Rodgers AB, Troiano RP. Accelerometer use in physical activity: best practices and research recommendations. *Med Sci Sports Exerc*. 2005;37(11 Suppl):S582-S588. doi:10.1249/01.mss.0000185292.71933.91
24. Martinez-Gomez D, Ruiz JR, Ortega FB, et al. Recommended levels of physical activity to avoid an excess of body fat in European adolescents: the HELENA study. *Am J Prev Med*. 2010;39(3):203-211. doi:10.1016/j.amepre.2010.05.003
25. Pérez-Bey A, Segura-Jiménez V, Fernández-Santos JDR, et al. The role of adiposity in the association between muscular fitness and cardiovascular disease. *J Pediatr*. 2018;199:178-185.e4. doi:10.1016/j.jpeds.2018.03.071
26. Quan LL, Wang H, Tian Y, Mu X, Zhang Y, Tao K. Association of fat-mass and obesity-associated gene FTO rs9939609 polymorphism with the risk of obesity among children and adolescents: a meta-analysis. *Eur Rev Med Pharmacol Sci*. 2015;19(4):614-623.
27. Reuter ÉM, Reuter CP, de Castro Silveira JF, et al. FTO gene polymorphism and longitudinal changes in nutritional/obesity status in children and adolescents: Schoolchildren's health cohort study. *Eur J Pediatr*. 2021;180(11):3325-3333. doi:10.1007/s00431-021-04120-0
28. Lourenço BH, Qi L, Willett WC, Cardoso MA. FTO genotype, vitamin D status, and weight gain during childhood. *Diabetes*. 2014;63(2):808-814. doi:10.2337/db13-1290
29. Sovio U, Mook-Kanamori DO, Warrington NM, et al. Association between common variation at the FTO locus and changes in body mass index from infancy to late childhood: the complex nature of genetic association through growth and development. *PLoS Genet*. 2011;7(2):e1001307. doi:10.1371/journal.pgen.1001307
30. Zhang M, Zhao X, Cheng H, et al. Age- and sex-dependent association between FTO rs9939609 and obesity-related traits in Chinese children and adolescents. *PLoS One*. 2014;9(5):1-7. doi:10.1371/journal.pone.0097545
31. Rutters F, Nieuwenhuizen AG, Bouwman F, Mariman E, Westerterp-Plantenga MS. Associations between a single nucleotide polymorphism of the FTO gene (rs9939609) and obesity-related characteristics over time during puberty in a Dutch children cohort. *J Clin Endocrinol Metab*. 2011;96(6):E939-E942. doi:10.1210/jc.2010-2413
32. Jacobsson JA, Risérus U, Axelsson T, Lannfelt L, Schiöth HB, Fredriksson R. The common FTO variant rs9939609 is not associated with BMI in a longitudinal study on a cohort of Swedish men born 1920-1924. *BMC Med Genet*. 2009;10:4-11. doi:10.1186/1471-2350-10-131
33. Andreasen CH, Stender-Petersen KL, Mogensen MS, et al. Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body fat accumulation. *Diabetes*. 2008;57(1):95-101. doi:10.2337/db07-0910

34. Kilpeläinen TO, Qi L, Brage S, et al. Physical activity attenuates the influence of FTO variants on obesity risk: a meta-analysis of 218,166 adults and 19,268 children. *PLoS Med*. 2011;8(11):e1001116. doi:10.1371/journal.pmed.1001116
35. Sui X, Lee D-C, Matthews CE, et al. Influence of cardiorespiratory fitness on lung cancer mortality. *Med Sci Sports Exerc*. 2010;42(5):872-878. doi:10.1249/MSS.0b013e3181c47b65
36. Huuskonen A, Lappalainen J, Oksala N, et al. Determinants of cardiorespiratory fitness in men aged 42 to 60 years with and without cardiovascular disease. *PLoS One*. 2012;7(12):1-8. doi:10.1371/journal.pone.0051635
37. Melhorn SJ, Askren MK, Chung WK, et al. FTO genotype impacts food intake and corticolimbic activation. *Am J Clin Nutr*. 2018;107(2):145-154. doi:10.1093/ajcn/nqx029
38. Qi Q, Kilpeläinen TO, Downer MK, et al. FTO genetic variants, dietary intake and body mass index: insights from 177,330 individuals. *Hum Mol Genet*. 2014;23(25):6961-6972. doi:10.1093/hmg/ddu411
39. Verdich C, Toubro S, Buemann B, et al. Leptin levels are associated with fat oxidation and dietary-induced weight loss in obesity. *Obes Res*. 2001;9(8):452-461. doi:10.1038/oby.2001.59
40. Ponce-Gonzalez JG, Martínez-Ávila Á, Velázquez-Díaz D, et al. Impact of the FTO gene variation on appetite and fat oxidation in young adults. *Nutrients*. 2023;15(9):2037. doi:10.3390/nu15092037
41. Liu C, Mou S, Pan C. The FTO gene rs9939609 polymorphism predicts risk of cardiovascular disease: a systematic review and meta-analysis. *PLoS One*. 2013;8(8):e71901. doi:10.1371/journal.pone.0071901
42. Reiter EO, Root AW. Hormonal changes of adolescence. *Med Clin North Am*. 1975;59(6):1289-1304. doi:10.1016/s0025-7125(16)31930-7
43. Saldaña-Alvarez Y, Salas-Martínez MG, García-Ortiz H, et al. Gender-dependent association of FTO polymorphisms with body mass index in Mexicans. *PLoS One*. 2016;11(1):e0145984. doi:10.1371/journal.pone.0145984

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