International Journal of Pediatric Obesity, 2011; Early Online, 1–8

informa
healthcare

ORIGINAL ARTICLE

Fitness and fatness are independently associated with markers of insulin resistance in European adolescents; The HELENA Study

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Abstract

Objective. To examine the independent association of total and central body fat and cardiorespiratory fitness with markers of insulin resistance after controlling for several potential confounders in European adolescents participating in the HELENA-CSS (Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional) study. *Methods*. We conducted a cross sectional study (the HELENA-CSS) which comprised 1053 (12.5–17.5 years) adolescents from 10 European cities. Weight, height, waist circumference and skinfold thickness were measured, and body mass index (BMI) was calculated. Cardiorespiratory fitness was measured by the 20-m shuttle run test. Markers of insulin resistance were fasting insulin and glucose, and homeostasis model assessment (HOMA). *Results*. HOMA and insulin were positively associated with BMI, skinfolds and waist circumference after controlling for center, age, pubertal status and cardiorespiratory fitness (all $P \leq 0.01$). HOMA and insulin were negatively associated with cardiorespiratory fitness in adolescents with moderate to high levels of total and central body fat (all $P \leq 0.01$). *Conclusions*. HOMA and insulin were associated with total and central body fat in European adolescents. Moreover, cardiorespiratory fitness explained a part of the HOMA and insulin variance in those adolescents with moderate to high levels of total and central body fat, and also, to some extent, in those with low to middle fat mass.

Key words: *Insulin, glucose, HOMA, cardiorespiratory fitness, total and central body fat*

Introduction

The prevalence of type 2 diabetes has increased among children and adolescents (1). One of the causes might be the increased prevalence of obesity, which is one of the primary risk factors for type 2 diabetes (2). Insulin resistance is strongly associated with obesity in adults as well as in children and adolescents (3). Likewise, both total and central adiposity are negatively associated with cardiorespiratory fitness in young people (4,5).

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(Received 15 September 2010; final version received 25 February 2011)

ISSN Print 1747-7166 ISSN Online 1747-7174
DOI: 10.3109/17477166.2011.575158

We observed that cardiorespiratory fitness is inversely associated with insulin resistance in children aged 9–10 years with high levels (third tertile) of total and central body fat (6). Similarly, others have shown negative association of cardiorespiratory fitness with insulin resistance in samples of adolescents with different characteristic; obese (7,8) and non-obese children (9), adolescents from the US (10,11) or those from different European countries (12,13). Recent reviews concluded that there is strong evidence indicating that higher levels of cardiorespiratory fitness at childhood and adolescence are associated with healthier cardiovascular profile later in life (14) and with better insulin sensitivity in adolescents (15).

Given the global increase in the prevalence of obesity, type 2 diabetes, and the metabolic syndrome (16) there is a need to further understand how markers of insulin resistance are influenced by potentially modifiable factors, such as fatness and fitness in culturally different adolescents from a European population. To our knowledge, there are no studies that examined the influences of fatness and cardiorespiratory fitness on markers of insulin resistance in a specific cohort of European adolescents using standardised methodology.

The purpose of the present study was to examine the independent association of total and central body fat and cardiorespiratory fitness with markers of insulin resistance after controlling for several potential confounders, in adolescents from 10 European cities participating in the HELENA-CSS (Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional study).

Methods

The HELENA-CSS is a multi-centre study aiming to obtain reliable data from European adolescents aged 12.5–17.5 years about nutritional habits and patterns, body composition and levels of physical activity and fitness (17,18). The total sample of the HELENA-CSS was 3528 adolescents with a subset of 1089 in which a blood sample was obtained. The present work is confined to a sample of 1053 adolescents (554 females and 499 males), with complete data on at least glucose, insulin, body weight and height.

Ten cities in nine different European countries were chosen due to an existing network of research groups and a rough geographical balance across Europe (see Appendix 1) (17). Data collection took place between October 2006 and December 2007. The study was performed following the ethical guidelines of the Declaration of Helsinki 1961 (revision of Edinburgh 2000), the Good Clinical Practice, and the legislation about clinical research in humans in each of the participating countries. The protocol was approved by the Human Research Review Committees of the involved centres (19) (see Appendix 1).

Physical examination

The anthropometric methods followed in the HELENA-CSS were described in detail by Nagy et al. (20). Weight was measured in underwear and without shoes with an electronic scale (Type SECA 861) to the nearest 0.05 kg, and height was measured barefoot in the Frankfort plane with a telescopic height measuring instrument (Type SECA 225) to the nearest 0.1 cm. Body mass index was calculated as body weight (kg) divided by the height (m) squared (kg/m^2). Skinfold thickness was measured to the nearest 0.2 mm in triplicate in the right side at biceps, triceps, subscapular, suprailiac, thigh, and medial calf with a Holtain Caliper (Crymmych, UK) (21). The sum of 6 skinfold thickness was used as an indicator of total body fat. Waist circumference was measured in triplicate at the midpoint between the lowest rib and the iliac crest with an anthropometric tape SECA 200 (21), and was used as a surrogate marker of central body fat. Regarding skinfold thickness and waist circumference, in every city the same trained investigator made all measurements, and reliability was greater than 95%. Inter-observer reliability for both were higher than 90% (20). Pubertal stage was assessed by a medical doctor according to Tanner and Whitehouse (22).

Blood samples

A detailed description of the blood analysis has been reported elsewhere (23). Serum concentrations of glucose and insulin were measured after an overnight fast. The homeostasis model assessment (HOMA) was calculated (24) as fasting insulin (μlU/ml) × fasting glucose (mmol/l)/22.5 (to convert insulin values in μlU/ml to pmol/l multiply by 6.945).

Cardiorespiratory fitness

Cardiorespiratory fitness was measured by the progressive 20-m shuttle run test (25). This test required subjects to run back and forth between two lines set 20 m apart. Running pace was determined by audio signals, emitted from a pre-recorded CD. The initial speed was 8.5 $km \cdot h^{-1}$, increasing by 0.5 $km \cdot h^{-1}$ every minute (1 min equals 1 stage). The test was finished when the subject failed to reach the end lines concurrent with the audio signals on two consecutive

occasions. The test was done once and the final score was computed as the number of stages completed (precision of 0.5 stages). VO_{2max} (ml min^{-1} kg^{-1}) was estimated using the equation reported by Léger (25).

Statistical analysis

The data are presented as mean ± SD, unless otherwise stated. To achieve normality in the residuals, insulin, waist circumference, sum of skinfold thickness and cardiorespiratory fitness were transformed to the natural logarithm, and HOMA was raised to the power of 1/3. Sex differences were assessed by one-way analysis of variance.

Bivariate correlation analyses were performed to examine the association between the outcome and exposure variables. The association between markers of insulin resistance and total (BMI categories and sum of skinfold thickness) and central (waist circumference) body fat was assessed by one-way analysis of covariance with BMI, skinfold thickness, and waist circumference as fixed factors, and HOMA and insulin as dependent variables. Center, age, pubertal status and cardiorespiratory fitness, were entered as covariates. Both skinfold thickness and waist circumference were recorded into thirds to be entered into the models, whereas BMI was categorized into four categories according to the BMI international cut-off values: underweight, normal weight, overweight and obese (26,27).

We used multiple regressions to study the association between markers of insulin resistance and cardiorespiratory fitness after controlling for age, pubertal status and center. Regression analysis was performed separately by BMI categories (underweight, normal weight, overweight and obese) (26,27), and thirds of skinfold thickness and waist circumferences (low, middle and high equals first, second and third, respectively).

The association between cardiorespiratory fitness and HOMA was assessed by one-way analysis of covariance with HOMA as fixed factor, and cardiorespiratory fitness as dependent variable. Center, age, pubertal status and markers of body fat, were entered as covariates. Transformed values of HOMA were recorded into thirds to be entered into the models. The analyses were performed using the Statistical Package for Social Science (SPSS, v. 15.0 for Windows; SPSS Inc., Chicago, IL, USA) and the level of significance was set to 0.05.

Results

A set of valid data for cardiorespiratory fitness, skinfold thickness, waist circumference and BMI were available in 68% ($n = 718/1053$), 83% ($n = 873/1053$), 88% ($n = 926/1053$) and 89% ($n = 937/1053$) of the adolescents, respectively. Table I shows the descriptive characteristics of the study sample. Bivariate correlations between HOMA, insulin, glucose, fatness parameters and cardiorespiratory fitness are displayed in Table II.

The associations of HOMA and fasting insulin with BMI, skinfold thickness and waist circumference are shown in Table III. HOMA and fasting insulin were positively associated (all $P \leq 0.01$) with BMI, skinfold thickness and waist circumference in both females and males after controlling for center, age, pubertal status and cardiorespiratory fitness. Glucose was not significantly associated with any of the studied markers of total and central body fat (all $P > 0.1$) (data not shown).

Table I. Descriptive characteristics of European adolescents.

	All ($n = 1053$)	Females ($n = 554$)	Males ($n = 499$)	*P* value
Age (years)	14.9 ± 1.2	14.9 ± 1.2	14.9 ± 1.3	0.716
Puberal status (I/II/III/IV/V)[a]	1/6/20/44/29	0/5/21/44/30	2/8/20/42/28	
Weight (kg)	58.9 ± 12.4	56.0 ± 10.2	62.1 ± 14.0	<0.001
Height (m)	1.7 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	<0.001
BMI (kg/m^2)	21.4 ± 3.6	21.3 ± 3.4	21.4 ± 3.8	0.845
BMI categories (U/N/OV/O)[a,b]	5/72/17/6	6/75/15/4	5/69/18/8	
Skinfold thickness (mm)[c]	90.2 ± 39.6	102.8 ± 35.7	76.0 ± 39.2	<0.001
Waist circumference (cm)[c]	72.4 ± 8.8	70.6 ± 8.0	74.5 ± 9.1	<0.001
HOMA[d]	2.3 ± 1.9	2.3 ± 1.6	2.3 ± 2.2	0.786
HOMA (median and range)	1.92 (0.36–26.06)	2.01 (0.39–22.77)	1.35 (0.36–26.06)	
Insulin (μlU/ml)[c]	10.1 ± 7.6	10.2 ± 6.4	10.1 ± 8.7	0.019
Glucose (mmol/l)	5.1 ± 0.4	5.0 ± 0.4	5.2 ± 0.4	<0.001
Cardiorespiratory fitness by Leger (ml min^{-1} kg^{-1})[c]	40.1 ± 7.8	36.2 ± 5.5	44.3 ± 7.8	<0.001
Cardiorespiratory fitness (stage)	4.8 ± 2.8	3.4 ± 1.8	6.3 ± 2.8	<0.001

All values are means ± SD, or [a]percentages. [b]BMI categories: underweight (U); normal weight (N); overweight (OV); obese (O). BMI indicates body mass index; HOMA, homeostasis model assessment. Non-transformed data are presented in this table, but analyses were performed on [c]log-transformed data or [d]data transformed to the power of 1/3.

Table II. Person correlations between markers of insulin resistance, body composition and cardiorespiratory fitness in female and male adolescents.

	Insulin[a]		HOMA[b]		Glucose		Cardiorespiratory fitness[a,c]	
	Females	Males	Females	Males	Females	Males	Females	Males
Body mass index	0.28†	0.44†	0.23†	0.42†	−0.06	0.12*	−0.40†	−0.45†
Skinfold thickness[a]	0.29†	0.44†	0.23†	0.42†	−0.00	0.15†	−0.34†	−0.42†
Waist circumference[a]	0.27†	0.39†	0.23†	0.37†	−0.04	0.10*	−0.32†	−0.42†
Cardiorespiratory fitness[a,c]	−0.21†	−0.23†	−0.18†	−0.23†	−0.02	−0.13†		

Analyses were performed on [a]log-transformed data or [b]data transformed to the power of 1/3. Values are bivariate correlation (Pearson coefficient). [c]by Leger (ml min^{-1} kg^{-1}).
*$P < 0.05$.
†$P < 0.01$.

The results of the linear regression models showing the association of HOMA, fasting insulin and glucose with cardiorespiratory fitness stratified by BMI categories, and thirds of skinfolds and waist circumference are presented in Table IV. HOMA and fasting insulin were negatively associated with cardiorespiratory fitness in normal weight females and in those with middle to high levels of skinfold thickness (all $P \leq 0.001$). In addition, both HOMA and insulin were negatively associated with cardiorespiratory fitness for all levels of waist circumference but the highest percentage of variance was explained in females with middle to high waist circumference. Likewise, fasting glucose was negatively associated with cardiorespiratory fitness in normal weight and overweight females, as well as in those with high levels of skinfold thickness and waist circumference (all $P \leq 0.05$).

In males, HOMA and fasting insulin were negatively associated with cardiorespiratory fitness in those who were overweight ($P \leq 0.01$), those with high levels of skinfold thickness ($P < 0.01$), or those with high levels of waist circumference ($P < 0.001$). Glucose was negatively associated with fitness in those males classified as underweight and normal weight, with low skinfold thickness or with low waist circumference (first third). For the relationships with waist circumference, additional analyses controlling for height or skinfold thickness did not modify the results.

The analyses were repeated using the number of stages completed or the equation reported by Ruiz et al. (28) instead of using the equation reported by Léger et al. (25) and the results did not materially change (data not shown).

The associations of cardiorespiratory fitness with insulin resistance (thirds of HOMA) are shown in Figure 1. Cardiorespiratory fitness was negatively associated in both genders (Females, $P < 0.001$; Males, $P < 0.05$) with HOMA after controlling

Table III. Markers of insulin resistance and total and central body fat adjusting for center, age, pubertal status and cardiorespiratory fitness.

	HOMA[a]		Insulin[b]	
	Females ($n = 554$)	Males ($n = 499$)	Females ($n = 554$)	Males ($n = 499$)
By BMI categories				
Underweight	1.22 ± 0.20	1.17 ± 0.21	2.07 ± 0.50	1.91 ± 0.55
Normal weight	1.28 ± 0.21	1.23 ± 0.24	2.21 ± 0.47	2.04 ± 0.54
Overweight	1.36 ± 0.23	1.34 ± 0.34	2.40 ± 0.46	2.27 ± 0.62
Obese	1.50 ± 0.34	1.62 ± 0.39	2.65 ± 0.54	2.84 ± 0.60
P value for trend	<0.001	<0.001	<0.001	<0.001
By thirds of skinfold thickness				
Low	1.27 ± 0.22	1.20 ± 0.25	2.17 ± 0.49	1.95 ± 0.60
Middle	1.27 ± 0.20	1.25 ± 0.25	2.19 ± 0.47	2.09 ± 0.51
High	1.34 ± 0.24	1.37 ± 0.34	2.37 ± 0.47	2.35 ± 0.61
P value for trend	0.01	<0.001	<0.01	<0.001
By thirds of waist circumference				
Low	1.26 ± 0.22	1.19 ± 0.26	2.15 ± 0.48	1.94 ± 0.58
Middle	1.29 ± 0.22	1.26 ± 0.24	2.23 ± 0.49	2.10 ± 0.54
High	1.35 ± 0.24	1.38 ± 0.34	2.38 ± 0.49	2.38 ± 0.61
P value for trend	<0.01	<0.001	<0.001	<0.001

All values are means ± SD. BMI, Body mass index; HOMA, homeostasis model assessment. [b]log-transformed data or [a]data transformed to the power of 1/3.

Table IV. Multiple regression coefficients (β) and coefficient of determination (R^2), examining the association between markers of insulin resistance and cardiorespiratory fitness separately by body mass index (BMI) categories, and thirds of skinfold thickness and circumference in female and male adolescents.

		Females			Males		
Outcome		β	*P*	R^2	β	*P*	R^2
By BMI categories							
HOMA	Underweight	−0.317	0.321	0.094	−0.319	0.192	0.224
	Normal weight	−0.319	<0.001	0.089	−0.085	0.191	0.040
	Overweight	−0.081	0.541	0.251	−0.761	<0.01	0.254
	Obese	−0.426	0.436	0.280	−0.105	0.629	0.117
Insulin	Underweight	−0.305	0.335	0.106	−0.264	0.228	0.193
	Normal weight	−0.341	<0.001	0.106	−0.079	0.220	0.043
	Overweight	−0.054	0.689	0.219	−0.439	<0.01	0.272
	Obese	−0.517	0.352	0.266	−0.143	0.510	0.118
Glucose	Underweight	−0.040	0.894	0.180	−0.567	<0.05	0.448
	Normal weight	−0.156	<0.05	0.047	−0.138	<0.05	0.021
	Overweight	−0.283	<0.05	0.372	−0.059	0.721	0.047
	Obese	−0.037	0.942	0.357	−0.015	0.944	0.112
By thirds of skinfold thickness							
HOMA	Low	−0.171	0.328	0.034	−0.040	0.594	0.025
	Middle	−0.522	0.001	0.109	−0.244	0.112	0.178
	High	−0.521	<0.001	0.182	−0.628	<0.01	0.157
Insulin	Low	−0.135	0.442	0.016	−0.031	0.683	0.035
	Middle	−0.529	<0.001	0.141	−0.263	0.234	0.199
	High	−0.401	0.001	0.173	−0.714	<0.01	0.173
Glucose	Low	−0.116	0.482	0.135	−0.355	<0.05	0.018
	Middle	−0.245	0.123	0.067	−0.151	0.756	0.027
	High	−0.205	<0.05	0.150	−0.060	0.656	0.054
By thirds of waist circumference							
HOMA	Low	−0.280	<0.01	0.078	−0.183	0.102	0.066
	Middle	−0.360	0.001	0.209	−0.021	0.823	0.049
	High	−0.333	0.001	0.235	−0.335	<0.001	0.171
Insulin	Low	−0.304	0.001	0.088	−0.156	0.164	0.052
	Middle	−0.356	0.001	0.195	−0.027	0.772	0.060
	High	−0.312	<0.01	0.233	−0.335	<0.001	0.183
Glucose	Low	−0.188	<0.05	0.125	−0.281	<0.05	0.078
	Middle	−0.087	0.442	0.110	−0.093	0.337	0.030
	High	−0.283	<0.01	0.244	−0.112	0.212	0.039

Analysis controlled for age, center and pubertal status.

for center, age, pubertal status and markers of body fat, except in males after controlling for skinfold thickness.

Discussion

The results of the present study indicate that HOMA and fasting insulin are positively associated with total and central body fat in European adolescents. In addition, cardiorespiratory fitness explained a part of the HOMA and fasting insulin variance in those adolescents with middle to high levels of total and central body fat, which suggests that high levels of cardiorespiratory fitness may have a protective effect against the deleterious consequences ascribed to high fatness. Especially in those adolescents with middle to high levels of fatness but also, to some extent, in those with low to middle. Overall, these findings suggest that intervention studies oriented to prevent the development of insulin resistance, related to obesity in youth, should focus not only on decreasing fatness but also on enhancing cardiorespiratory fitness (29), even in adolescents with relatively low levels of fatness.

Our results show that BMI, skinfold thickness (total adiposity) and waist circumference (central adiposity) were significantly associated with HOMA and fasting insulin variance after adjusting for potential confounders including cardiorespiratory fitness, which concurs with other studies (30,31). Raman et al. (31) in 8- to 10-year-old African-American children with BMI greater than the 85th percentile showed that children with higher waist circumference had higher insulin resistance after adjusting for age, sex, pubertal stage, socioeconomic index, and family history of diabetes. Likewise, Bacha et al. (30) reported that adolescents with severe insulin resistance had higher visceral adiposity

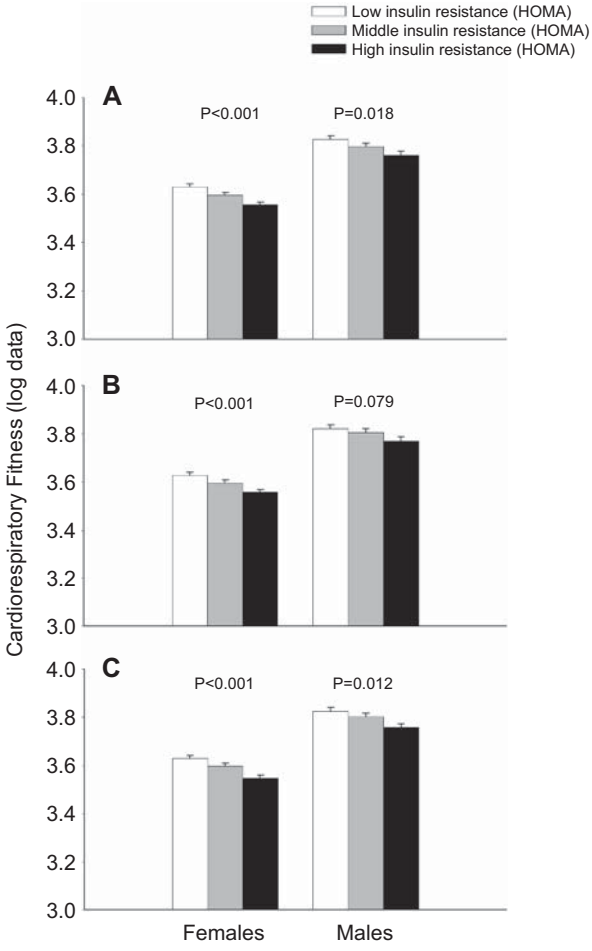

Figure 1. Associations of cardiorespiratory fitness with insulin resistance (HOMA) adjusting for center, age, pubertal status and markers of body fat. (A) Associations of cardiorespiratory fitness with HOMA adjusting for center, age, pubertal status and body mass index. (B) Associations of cardiorespiratory fitness with HOMA adjusting for center, age, pubertal status and skinfold thickness. (C) Associations of cardiorespiratory fitness with HOMA adjusting for center, age, pubertal status and waist circumference. Data are mean and standard error of the mean.

than pair-matched control subjects being moderately insulin-resistant (30).

We observed in a previous study on younger European populations that cardiorespiratory fitness explained a significant proportion of the HOMA and fasting insulin variance in both girls and boys with high levels of body fat and waist circumference (6). Likewise, authors showed an inverse association between cardiorespiratory fitness and a clustering of cardiovascular risk factors including insulin resistance in adolescents from three European countries. In contrast, findings from the Study of Latino Adolescents at Risk for Diabetes revealed that insulin sensitivity or secretion was not independently associated with cardiorespiratory fitness in a relatively small sample of overweight Hispanic children aged 8–13 years with a family history of type 2 diabetes (32).

In the present study, HOMA and fasting insulin were significantly associated with cardiorespiratory fitness in those females who were normal weight and with middle to high levels of skinfold thickness or waist circumference. In males, HOMA and fasting insulin were negatively associated with fitness in those with moderate to high levels of total and central body fat. Additionally, glucose was negatively associated with fitness in those males with low total or central body fat and those females with moderate to high total or central body fat.

Our results confirm those found in European populations (12,13) but using a heterogeneous sample from nine countries. However, differences in subject characteristics, methodologies and maturity of some metabolic systems such as hormonal regulation, may partially explain the different glucose behaviour between gender and studies (32). Moreover, the HELENA-CSS involved only adolescents who were apparently healthy and without any diagnosed lifestyle-related diseases. Pubertal status may partially explain the sex differences in glucose metabolism (33–35). Pubertal status is known to be related with changes in body fat, insulin resistance, and other hormones (33–36). To note is that the pubertal maturation process is different between genders.

In a recent systematic review of longitudinal studies, Ruiz et al. (14) concluded that due to a limited number of studies, there is inconclusive evidence indicating that changes in cardiorespiratory fitness from adolescence to adulthood are associated with diabetes. Exercise seems to induce alterations in skeletal muscle substrate metabolism dealing to some specific adaptations into the skeletal muscle such as an increased mitochondrial volume, density, oxidative enzyme capacity and an increased GLUT-4 concentration which in turn lead to improvements in glucose metabolism as well as increases in VO_{2max} (37). School-based intervention studies focusing on enhancing fitness levels have shown an improvement in body composition and insulin sensitivity not only in obese (7,8) but in non-obese children (9).

The present study has several limitations. Owing to its cross-sectional design, we cannot infer that our observed associations reflect causal relationships. However, this study was performed in a heterogeneous sample of European adolescents from nine countries and using harmonized and standardized methodology.

In conclusion, the present study showed that HOMA and fasting insulin were significantly associated with total and central body fat in European adolescents. In addition, cardiorespiratory fitness explained a part of the HOMA and fasting insulin variance, mainly, in those adolescents with moderate

to high levels of total and central body fat but also in those with low to middle levels of body fat. Collectively, the findings suggest that the deleterious consequences ascribed to high fatness could be counteracted to some degree by having high levels of cardiorespiratory fitness. The assessment of the effect of an exercise intervention focusing on reducing fatness and improving fitness is warranted. Programs aimed at improving childhood and adolescence cardiorespiratory fitness, through moderate and vigorous physical activity, may have the potential to prevent insulin resistance and should be considered in obese and non-obese adolescents.

Acknowledgements and funding

We gratefully acknowledge all participating children and adolescents, and their parents and teachers for their collaboration. We also acknowledge all the members involved in the field work for their efforts and great enthusiasm as well as to John J. Reilly for his help as external advisor (in Appendix the list of the HELENA researchers). The HELENA study took place with the financial support of the European Community Sixth RTD Framework Programme (Contract FOOD-CT-2005-007034). This study was also partially supported by grants from the Spanish Ministry of Education (AP-2005-3827), the Swedish Council for Working Life and Social Research (FAS), the Swedish Heart-Lung Foundation (20090635), and the Spanish Ministry of Science and Innovation (RYC-2010-05957).

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. The results of the present study do not constitute endorsement by *International Journal of Pediatric Obesity*.

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Supplementary material available online

Supplementary Appendix 1.
Supplementary Appendix 2.