



# Nutrición Hospitalaria



Pediatría

## Trabajo Original

### Association of body composition indices with insulin resistance in European adolescents: the HELENA study

*Asociación de índices de composición corporal con resistencia a la insulina en adolescentes europeos: el estudio HELENA*

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### Abstract

**Background:** The different body components may contribute to the development of insulin resistance and type 2 diabetes mellitus. The aim of the present study was to examine the association of fat mass and fat free mass indices with markers of insulin resistance, independently of each other and giving, at the same time, gender-specific information in a wide cohort of European adolescents.

**Methods:** A cross-sectional study in a school setting was conducted in 925 (430 males) adolescents ( $14.9 \pm 1.2$  years). Weight, height, anthropometric, bioimpedance and blood parameters were measured. Indices for fat mass and fat free mass, and homeostatic model assessment (HOMA) were calculated. Multiple regression analyses were performed adjusting for several confounders including fat free mass and fat mass when possible.

**Results:** Indices of fat mass were positively associated with HOMA (all  $p < 0.01$ ) after adjusting for all the confounders including fat free mass indices, in both sexes. Fat free mass indices were associated with HOMA, in both males and females, after adjusting for center, pubertal status, socioeconomic status and cardiorespiratory fitness, but the associations disappear when including fat mass indices in the adjustment's model.

**Conclusion:** Fat mass indices derived from different methods are positively associated with insulin resistance independently of several confounders including fat free mass indices. In addition, the relationship of fat free mass with insulin resistance is influenced by the amount of fat mass in European adolescents. Nevertheless, future studies should focus not only on the role of fat mass, but also on other body components such as fat free mass because its role could vary depending of the level and distribution of fat mass.

#### Key words:

Insulin resistance.  
Adolescent. Body fat.  
Obesity.

### Resumen

**Antecedentes:** los diferentes componentes corporales pueden contribuir al desarrollo de resistencia a la insulina y diabetes mellitus de tipo 2. El objetivo del presente estudio fue examinar la asociación entre la masa grasa y la masa libre de grasa con marcadores de resistencia a la insulina, valorando de forma independiente cada uno de ellos y aportando información específica por sexo, en una amplia cohorte de adolescentes europeos.

**Métodos:** se realizó un estudio transversal en el ámbito escolar en 925 (430 varones) adolescentes ( $14,9 \pm 1,2$  años). Se midieron peso, talla, pliegues cutáneos e impedancia bioeléctrica. Se calcularon índices de masa grasa y libre de grasa y se calculó el índice HOMA. Se realizaron análisis de regresión múltiple ajustándolos según varios factores de confusión, incluyendo la masa libre de grasa y masa grasa cuando fue posible.

**Resultados:** los índices de masa grasa se asociaron positivamente con HOMA (todos  $p < 0,01$ ) después de ajustarlos según todos los factores de confusión, incluidos los marcadores de masa libre de grasa, en ambos sexos. Los índices de masa libre de grasa se asociaron con HOMA, tanto en hombres como en mujeres, después de ajustar por país, estadio puberal, nivel socio-económico y capacidad cardiorrespiratoria, pero las asociaciones desaparecieron al incluir marcadores de masa grasa en el modelo de ajuste.

**Conclusión:** índices de masa grasa obtenidos según diferentes métodos se asocian con la resistencia a la insulina, independientemente de varios factores de confusión, incluyendo los índices de masa libre de grasa. Además, la cantidad de masa grasa influye en la relación de la masa libre de grasa con resistencia a la insulina en adolescentes europeos. Los estudios futuros deberían centrarse no solo en el papel de la masa grasa, sino también en el de otros componentes corporales como la masa libre de grasa, ya que su papel podría variar dependiendo del nivel y distribución de masa grasa.

#### Palabras clave:

Resistencia a la insulina. Adolescente. Grasa corporal. Obesidad.

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## ABBREVIATIONS

BIA: Bioelectrical impedance or bioimpedance.  
 BMI: Body mass index.  
 CRF: Cardiorespiratory fitness.  
 HELENA-CSS: Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study.  
 HOMA: Homeostatic model assessment.  
 FAS: Family affluence scale.  
 FM: Fat mass.  
 FMS: Fat mass calculated by Slaughter.  
 FMSI: Fat mass calculated by Slaughter index.  
 FFM: Fat-free mass.  
 FFMB: Fat-free mass estimated from BIA.  
 FFMBI: Fat-free mass estimated from BIA index.  
 FFMS: Fat-free mass calculated by Slaughter.  
 FFMSI: Fat-free mass calculated by Slaughter index.  
 QUICKI: Quantitative insulin sensitivity check index.  
 T2DM: Type 2 diabetes mellitus.

## INTRODUCTION

Insulin resistance is considered as a precursor of Type 2 diabetes mellitus (T2DM), being an emerging problem among children and adolescents (1), and leading to long-term serious health consequences (2). The main risk factor for insulin resistance and T2DM is obesity, as defined by an excess of body fat (3). In adolescents, the most frequently identified features are diabetes precursors, as impaired glucose tolerance, and especially insulin resistance.

In the literature, a positive association between adiposity and insulin resistance in adolescents has been frequently described. However, in most cases simple methods to assess total body fat, such as the body mass index (BMI), have been used (4-6). Other studies used different anthropometric measurements/indices like sum of skinfold thickness (4,7), waist circumference (8) or waist-hip ratio (3). However, in adolescents, there is limited information on the role of different body components (fat and fat free mass) on insulin resistance as these indices require specific measurement methods, such as bioelectrical impedance analysis (BIA) (5,9).

The majority of the studies observed a positive association between total and abdominal body fat with insulin resistance (10). However, there are limited studies assessing the effect of other body composition compartments as fat-free mass. Fat-free mass is an indicator of muscle amount which is an important organ in insulin metabolism and action (11).

The purpose of this study was to examine the association between different body components, assessed through anthropometric and BIA methods, and insulin resistance after adjusting for several confounders, in a cohort of European adolescents.

## METHODS

The HELENA-cross sectional study (HELENA-CSS) is a multi-centre study performed in ten European cities from nine countries: Herak-

lion and Athens (Greece), Dortmund (Germany), Ghent (Belgium), Lille (France), Pécs (Hungary), Rome (Italy), Stockholm (Sweden), Vienna (Austria) and Zaragoza (Spain). This study was designed to obtain reliable and comparable data on the nutritional status of urban European adolescents (12.5-17.5 years). The total sample of the HELENA-CSS fulfilling all the inclusion criteria was 3,528 adolescents. It was decided to obtain blood samples in one third of the total HELENA-CSS sample. The total sample size in which we obtained blood samples was 1,089. From the total number of adolescents with blood samples, 925 (430 males) had valid data for BMI, fat mass (FM)/fat-free mass (FFM) indices and homeostatic model assessment (HOMA) index, constituting the overall sample included in the current study. The sample size varied through the different variables used for the analysis (the specific sample sizes are defined in the corresponding tables). Data collection took place from 2006 to 2007 at school setting. Detailed description of the HELENA-CSS sampling and recruitment approaches, standardization and harmonization processes, data collection, analysis strategies, quality control activities, and inclusion criteria have been published elsewhere, with a complete description of ethical issues and good clinical practice (12,13).

## ETHICS STATEMENT

The study protocol was approved by the Ethics Committee at each study centre following the ethical guidelines of the Declaration of Helsinki 1964, the Good Clinical Practice, and the legislation about clinical research in humans. The original names of the ten ethics committees/institutional review boards were: 1) Ethics Committee of the Harokopio University from Athens; 2) Ethics Committee of the Medicine's University from Dortmund; 3) Ethics Committee from Ghent University Hospital; 4) Ethics Committee of the University of Crete School of Medicine from Heraklion; 5) Protection committees people from Lille; 6) A Pecs Orvostudományi és Egészségtudományi Központ Regionális Kutatás-Etikai Bizottsága from Pécs; 7) Ethics Committee of Medical Activities of the University of Naples Federico II, Naples; 8) Regional Ethics Committee from Stockholm; 9) Ethics Committee of the Medicine's University from Vienne, and 10) Ethics Committee of clinic research of Aragón from Zaragoza. Written informed consent was obtained from the parents (or guardian) and adolescents participating in the study.

## COVARIATES

Socioeconomic status was established by using the Family Affluence Scale (FAS), which is based on the concept of material conditions in the family to base the selection of items. Currie et al. (14) chose a set of items which reflected family expenditure and consumption that were relevant to family circumstances. Possessing these items was considered to reflect affluence and their lack, on the other hand, material deprivation. The FAS has been previously used in the HELENA-CSS as an index of socioeconomic status (15). Pubertal status was evaluated by experienced physicians according to the criteria of Tanner and Whitehouse (16).

Cardiorespiratory fitness (CRF) was measured by the progressive 20 m shuttle run test. This test required the subjects to run back and forth between two lines set 20 m apart following a running pace determined by audio signals and with an initial speed of 8.5 km/h, increasing by 0.5 km/h every minute (1 minute equals one stage). The test was finished when the adolescent failed to reach the end lines concurrent with the audio signals on two consecutive occasions and the final score was computed as the number of stages completed (precision of 0.5 stages).

## BODY COMPOSITION

### Anthropometry

The anthropometric method followed in the HELENA-CSS has been described in detail by Nagy et al. (17). In brief, body height was measured to the nearest 0.1 cm with a stadiometer (SECA 225; SECA, Hamburg, Germany) while adolescents were standing barefoot. Body mass was determined to the nearest 0.05 kg using a balance scale (SECA 861; SECA, Hamburg, Germany) with the subject in their underwear. BMI was calculated as body mass (kg) divided by height (m) squared. A set of six skinfold thicknesses (biceps, triceps, subscapular, suprailliac, thigh and medial calf) were measured three consecutive times on the left side of the body, with a Holtain caliper (Holtain, Ltd., Wales, UK) to the nearest 0.2 mm. The waist circumference was measured using a non-elastic tape (SECA 200; SECA, Hamburg, Germany) to the nearest 0.1 cm, according to Lohman's anthropometric standardization reference manual (18). In every city, the same trained investigator made all skinfold thickness measurements. For all the skinfold thickness measurements, intra-observer technical errors of measurement were smaller than 1 mm and reliability, greater than 95%. Inter-observer reliability for skinfolds was higher than 90% (17).

We calculated FM percentage (% FM) from skinfold thickness measurements using the Slaughter's equations (19) which have shown to be a valid equation in adolescents (20). FFM Slaughter (FFMS) in kilograms was derived by subtracting fat mass from total body weight.

### Bioelectrical impedance analysis

For BIA measurements, a classical tetra-polar bioelectrical device was used by means of a 50 KHz BIA 101 AKERN (Akern Srl., Firenze, Italy). Standard instructions for BIA measurements were followed (21). FFM was estimated from BIA (FFMB) as marker of muscle mass using validated formulas and, consequently, FM from BIA (FMB) was calculated by subtraction.

### Fat mass and fat-free mass indices

FM and FFM indices were calculated by dividing FM or FFM by the square of height in meters as proposed in previous studies (22).

## BLOOD SAMPLES

Detailed blood handling procedures have been described elsewhere (23). Serum concentrations of glucose and insulin were measured after an overnight fast. The HOMA index was calculated as: (fasting insulin [pmol/l]/6.945) x (fasting glucose [mmol/l]/22.5) (24). A quantitative insulin sensitivity check index (QUICKI) was calculated as  $QUICKI = 1/(\log \text{insulin } [\mu\text{U/mL}] + \log \text{glucose } [\text{mg/dl}])$  (25).

## STATISTICAL ANALYSIS

The data are presented as mean  $\pm$  standard deviation, unless otherwise stated. To achieve normality in the residuals, sum of six skinfold thickness, % FM, FM Slaughter (FMS), FMS index (FMSI) and FMB index (FMBI) were transformed to the natural logarithm and HOMA was raised to the power of  $1/3$ .

Multiple linear regression models were used to study the associations of both indices of FM and FFM with HOMA (outcome), after adjusting for pubertal status, socioeconomic status, country, indices of FM or FFM and cardiorespiratory fitness. Regression analysis was performed for different models in table II. Model I included pubertal status, socioeconomic status and country (entered as dummy variable) as confounders. Model II included model I plus the corresponding FFM index. Model III included model II plus cardiorespiratory fitness. In table III model I was similar to table II and model II included model I plus the corresponding FM index. Model III included model I plus cardiorespiratory fitness and model IV included model III plus the corresponding FM index. Sensitivity analyses were performed using QUICKI instead of HOMA and the results did not change (data not shown).

The analyses were performed using the Predictive Analytics Software (PASW, version 20; SPSS Inc., Chicago, IL, USA) and the level of significance was set to 0.05.

## RESULTS

Table I shows the descriptive characteristics of the study sample. All fat mass indicators were significantly higher in females than in males. At the opposite all fat-free mass indicators were higher in males than in females.

The results of the multiple linear regression models showing the association of indices of body fat with HOMA after adjusting for country, pubertal status and socioeconomic status (model I), FFM indicators (model II: model I plus corresponding FFM indicators) and cardiorespiratory fitness (model III: model II plus cardiorespiratory fitness) are presented in table II for males and females. In males, all indicators of FM were positively associated with HOMA (all  $p < 0.01$ ) in model I, II and III. In females, all indicators of FM were positively associated with HOMA in model I and II (all  $p < 0.001$ ) but only FMS and FMSI were associated with HOMA in model III ( $p < 0.01$ ).

Table III shows the association of indicators of FFM with HOMA after adjusting for country, pubertal status and socioeconomic

**Table I.** Descriptive characteristics of the studied sample of European adolescents

	All (n = 925)			Males (n = 430)			Females (n = 495)			p value
Age (years)	14.9	±	1.2	14.9	±	1.3	14.9	±	1.2	0.672
Puberal status (I/II/III/IV/V) <sup>a</sup>	1/5/19/44/31			1/7/19/42/30			0/4/19/46/31			
Weight (kg)	59.3	±	12.7	62.7	±	14.3	56.2	±	10.2	< 0.001
Height (m)	1.66	±	0.09	1.70	±	0.10	1.62	±	0.07	< 0.001
BMI (kg/m <sup>2</sup> )	21.4	±	3.7	21.5	±	4.0	21.4	±	3.4	0.677
Cardiorespiratory fitness (stages) <sup>b</sup>	4.8	±	2.8	6.3	±	2.8	3.4	±	1.8	< 0.001
<i>Indices of insulin resistance</i>										
Insulin (μIU/mL)	10.1	±	7.6	10.1	±	8.7	10.2	±	6.5	0.750
HOMA	2.3	±	1.9	2.3	±	2.2	2.3	±	1.6	0.572
QUICKI	0.151	±	0.013	0.152	±	0.014	0.151	±	0.012	0.096
<i>Body composition; fat mass indices</i>										
% Fat mass S (%) <sup>a,b,c</sup>	23.5	±	9.4	20.1	±	10.8	26.4	±	6.9	< 0.001
Fat mass S (kg) <sup>b,c</sup>	14.5	±	8.4	13.5	±	10.0	15.3	±	6.6	< 0.001
Fat mass index S (kg/ht <sup>2</sup> ) <sup>b,c</sup>	5.3	±	3.0	4.6	±	3.5	5.8	±	2.5	< 0.001
Fat mass B (kg) <sup>b,d</sup>	12.5	±	7.2	10.3	±	6.9	14.5	±	6.8	< 0.001
Fat mass index B (kg/ht <sup>2</sup> ) <sup>b,d</sup>	4.6	±	2.7	3.5	±	2.4	5.5	±	2.5	< 0.001
<i>Body composition; fat-free mass indices</i>										
Fat-free mass S (kg) <sup>c</sup>	44.6	±	8.2	49.2	±	8.9	40.9	±	5.2	< 0.001
Fat-free mass index S (kg/ht <sup>2</sup> ) <sup>c</sup>	16.1	±	1.7	16.8	±	1.7	15.6	±	1.4	< 0.001
Fat-free mass B (kg) <sup>d</sup>	46.6	±	8.7	52.2	±	8.9	41.7	±	4.6	< 0.001
Fat-free mass index B (kg/ht <sup>2</sup> ) <sup>d</sup>	16.8	±	1.8	17.9	±	1.8	15.9	±	1.1	< 0.001

All values are mean ± standard deviation or <sup>a</sup>percentages. B: Bioimpedance; BMI: Body mass index; HOMA: Homeostasis Model Assessment; S: Slaughter. Non-transformed data are presented in this table, but analyses were performed on <sup>b</sup>log-transformed data, <sup>c</sup>calculated from Slaughter (19) and <sup>d</sup> derived from bioimpedance.

status (model I), FM indicators (model II: model I plus corresponding FM indicator) or cardiorespiratory fitness (model III: model I plus cardiorespiratory fitness) and FM indicator and cardiorespiratory fitness simultaneously (model IV: model III plus the corresponding FM indicator) for males and females. In males, FFMB was positively associated with HOMA in models I and III (both  $p < 0.001$ ), but not in models including adjustments by FM indicators (models II and IV). FFMBI was significantly associated with HOMA in all the models of adjustment (all  $p < 0.001$ ). In females, all the FFM indices were significantly associated with HOMA in model I (all  $p < 0.05$ ); while FFMB and FFMBI were associated with HOMA in model III. No FFM indicator was associated with HOMA in models including adjustment by FM indicators (models II and IV).

## DISCUSSION

The main finding of our study indicates that all the FM indices derived from different methods are positively associated with indices of insulin resistance independently of several confounders

including FFM as index of muscle mass in both genders. Moreover, FFM seem to be related with insulin resistance in some cases but this association is mainly mediated by FM component, and when the relationship is adjusted by FM significance disappeared and the direction of this turned on negative (without significance).

Our results concur with recent published data in which the relationship between adiposity (triceps and subscapular skinfold thickness) and total body fat from dual energy X-ray absorptiometry in their associations with insulin resistance (HOMA) was analyzed for a large sample of US adolescents (26). They found that triceps and subscapular skinfold thicknesses estimate insulin resistance and identify those at highest risk for insulin resistance. Skinfold thickness provides an inexpensive and widely applicable measure of fatness that is appropriate for studies of insulin resistance and perhaps other metabolic variables in adolescents. Moreover, Carneiro et al. (2014) showed in 148 adolescents how several anthropometric indices were statistically and positively correlated to HOMA, however, any index of FFM was considered in this study (27). Weding VK et al. described in 1,298 adolescents that one of the best predictor of HOMA was percentage of body fat (BIA) suggesting that this can be attributed partly to the ability

**Table II.** Linear regression models showing the association of indices of body fat with HOMA

	N	Model I			Model II			N	Model III		
		$\beta$	R <sup>2</sup>	p	$\beta$	R <sup>2</sup>	p		$\beta$	R <sup>2</sup>	p
<i>Males</i>											
Fat mass S (kg) <sup>a,b</sup>	430	0.401	0.202	< 0.001	0.430	0.203	< 0.001	352	0.438	0.202	< 0.001
Fat mass S Index (kg/ht <sup>2</sup> ) <sup>a,b</sup>		0.417	0.206	< 0.001	0.416	0.204	< 0.001		0.423	0.201	< 0.001
Fat mass B (kg) <sup>a,c</sup>		0.353	0.161	< 0.001	0.299	0.164	< 0.001		0.258	0.163	< 0.001
Fat mass B index (kg/ht <sup>2</sup> ) <sup>a,c</sup>		0.354	0.164	< 0.001	0.218	0.19	< 0.001		0.189	0.187	< 0.01
<i>Females</i>											
Fat mass S (kg) <sup>a,b</sup>	495	0.295	0.149	< 0.001	0.327	0.149	< 0.001	365	0.200	0.166	< 0.01
Fat mass S index (kg/ht <sup>2</sup> ) <sup>a,b</sup>		0.294	0.149	< 0.001	0.325	0.149	< 0.001		0.205	0.164	< 0.01
Fat mass B (kg) <sup>a,c</sup>		0.237	0.126	< 0.001	0.215	0.126	< 0.001		0.091	0.16	0.231
Fat mass B index (kg/ht <sup>2</sup> ) <sup>a,c</sup>		0.236	0.127	< 0.001	0.201	0.128	< 0.001		0.083	0.16	0.283

Model I includes country, pubertal status, socioeconomic status. Model II includes model I plus the corresponding fat free mass marker. Model III includes II plus cardiorespiratory fitness.  $\beta$ : Standardized regression coefficients; R<sup>2</sup>: Coefficients of determination; B: bioimpedance; HOMA: Homeostasis Model Assessment; ht: Height; S: Slaughter. <sup>a</sup>: Log-transformed data; <sup>b</sup>: Calculated from Slaughter (19); <sup>c</sup>: Derived from bioimpedance. Fat mass indexes calculated by dividing fat mass by the square of height in meters.

**Table III.** Linear regression models showing the association of indices of fat free mass with HOMA

	Model I (n = 430)			Model II (n = 430)			Model III (n = 352)			Model IV (n = 352)		
	$\beta$	R <sup>2</sup>	p	$\beta$	R <sup>2</sup>	p	$\beta$	R <sup>2</sup>	p	$\beta$	R <sup>2</sup>	p
<i>Males</i>												
Fat free mass S (kg) <sup>a</sup>	0.102	0.06	0.098	-0.074	0.203	0.223	0.098	0.08	0.16	-0.096	0.202	0.174
Fat free mass S Index (kg/ht <sup>2</sup> ) <sup>a</sup>	0.096	0.061	0.078	0.005	0.204	0.916	0.099	0.082	0.101	-0.014	0.201	0.814
Fat free mass B (kg) <sup>b</sup>	0.31	0.117	< 0.001	0.105	0.164	0.114	0.297	0.134	< 0.001	0.127	0.163	0.090
Fat free mass B index (kg/ht <sup>2</sup> ) <sup>b</sup>	0.367	0.168	< 0.001	0.227	0.190	< 0.001	0.343	0.175	< 0.001	0.232	0.187	< 0.001
<i>Females</i>												
Fat free mass S (kg) <sup>a</sup>	0.112	0.089	< 0.05	-0.06	0.149	0.269	0.103	0.149	0.057	0.001	0.166	0.991
Fat free mass S Index (kg/ht <sup>2</sup> ) <sup>a</sup>	0.010	0.089	< 0.05	-0.058	0.149	0.282	0.081	0.146	0.134	-0.018	0.164	0.779
Fat free mass B (kg) <sup>b</sup>	0.146	0.099	< 0.01	0.042	0.126	0.428	0.148	0.159	< 0.01	0.094	0.160	0.179
Fat free mass B index (kg/ht <sup>2</sup> ) <sup>b</sup>	0.163	0.105	< 0.001	0.064	1.280	0.219	0.146	0.160	< 0.01	0.099	0.160	0.136

Model I includes country, pubertal status, socioeconomic status. Model II includes model I plus the corresponding fat mass marker. Model III includes I plus cardiorespiratory fitness. Model IV includes III plus the corresponding fat mass marker.  $\beta$ : standardized regression coefficients; R<sup>2</sup>: coefficients of determination; B: bioimpedance; HOMA: Homeostasis Model Assessment; ht: Height; S: Slaughter; <sup>a</sup>: Calculated from Slaughter (19); <sup>b</sup>: Derived from bioimpedance. Fat Mass Indexes calculated by dividing fat mass by the square of height in meters.

of percentage body fat to model HOMA among leaner participants (28). However, it is of importance to highlight that none of the cited studies studied the role of muscle mass neither accounted in their analyses for indices of muscle mass derived from Slaughter or BIA, which in some cases could affect these kinds of relationships. In this regard, our findings indicate the positive association between indices of fat mass and insulin resistance remains significant after adjusting for several confounders including muscle components in both genders.

On the other hand, the specific role of muscle components on insulin resistance has been little studied. Lee et al. (2013) studied the relationship of insulin sensitivity with intermuscular adipose

tissue, skeletal muscle mass (whole-body magnetic resonance imaging) and quality in 40 obese adolescents boys (29). They reported intermuscular adipose tissue and skeletal muscle quality, but not muscle mass, were associated with insulin sensitivity in obese adolescent boys after adjusting for pubertal status and race. However, Unni et al. (2009) analyzed the association of muscle mass (apendicular lean soft tissue) and body fat percentage (dual-energy X-ray absorptiometry) with insulin sensitivity in 51 young Indian men. They showed body fat percentage, BMI and muscle mass correlated with insulin resistance. Moreover, in the stepwise multiple linear regression model only BMI and muscle mass enter in the model and explained 49% of the variance in insulin sensi-

tivity (30). Despite using precise methods to assess muscle mass, these studies did not conclude in the same direction regarding the role of muscle mass on insulin resistance/sensitivity. Some aspects that could contribute to the controversy results between studies are the lack of a strong statistical adjustment model and the sample size of the studies. However, our study showed in an initial model that muscle mass was positively associated with insulin resistance in 925 European adolescents after adjusting for pubertal status, socioeconomic status, country and cardiorespiratory fitness. Moreover, when FM was included in the adjustment's model the association disappeared and the direction of association was inverted. These findings indicated that FM is a mediation factor in the role of muscle mass on insulin resistance in European adolescents.

Despite the fact that muscle is an important location for glucose disposal and insulin sensitivity, it is plausible that a low muscle mass and reduced muscle oxidation capacity (31) may act as determinant of whole-body fat oxidation and therefore, fat balance (32), thereby creating an interaction between fat and muscle mass as underlying mechanism related with insulin resistance.

The overall findings from this research could be useful for future studies in considering the different body components and adjustments when studying the relationship of body composition with insulin resistance. Although the study is not representative enough to recommend the generalization of its results to the population, it is plausible to suggest that the interaction between body components and insulin resistance could have a similar pattern in other adolescent populations, making these findings more interesting.

The present study has several limitations. Due to its cross-sectional design, the observed associations cannot be interpreted to reflect causal relationships. In addition, body composition has been measured with indirect methods (Slaughter equation and an estimating formula from BIA). However, several studies considered these methods as valid and accurate tools (18,19,33). Although FFM may be considered as an index of muscle mass, the way it was measured could include some bias as FFM also includes bone and residual mass. An important strength is the large and heterogeneous sample with gender-specific information, to control for several confounders including indices of fat mass and muscle.

Fat mass indices derived from different methods are positively associated with insulin resistance independently of several confounders including fat free mass indices. In addition, the relationship of fat-free mass with insulin resistance is mediated by the amount of fat mass in European adolescents. Nevertheless, future studies should focus not only on the role of fat mass, but also in other body components such as fat free mass because its role could vary depending of the level and distribution of fat mass in adolescents.

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