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Research Paper

Independent effect of body fat content on inflammatory biomarkers in children and adolescents: The GENOBOX study

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ABSTRACT

Background and aims: To assess the relationship between body composition indicators and inflammatory biomarkers in children and adolescents of the GENOBOX study.
Methods and results: Anthropometry data from 264 subjects from the subsample of Zaragoza (Spain) included: weight, height, waist circumference, body mass index and triponderal index. Body composition was determined by Dual-energy X-ray Absorptiometry (DXA), obtaining visceral adipose tissue, fat mass index and lean mass index. Age and sex specific z-scores were computed. Simple linear regression models were performed with inflammatory biomarkers (hsCRP, IL8, TNF-α, adiponectin, leptin and resistin) as dependent variables, and each of the body composition indices as independent variables.
Prepubertal boys had higher IL8 and resistin values and pubertal girls had higher HOMA-IR and leptin values. hsCPR and leptin were associated with fat mass, both in prepubertals and pubertals, independently of lean mass, and regardless of how body composition was measured. All body composition indices were inversely associated with adiponectin, except for fat mass index in pubertals, but none of them were statistically significant.
Conclusion: A positive association between hsCRP and leptin with all body fat composition parameters, measured by standard nutritional indicators and DXA, was observed in both sexual stages.

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1. Introduction

Inflammation is a natural response of the body to repair damage caused by infection or injury. The inflammatory process and repair can be harmful and detrimental if it becomes chronic [1]. Some environmental and lifestyle factors can promote systemic chronic inflammation that may lead to several diseases such as cardiovascular disease (CVD), cancer and diabetes [2]. Body composition plays an important role in systemic chronic inflammation. Obesity is a condition in which the adipose tissue mass is altered and the endocrine function of this tissue becomes impaired, contributing to an inflammatory state [3]. The emerging tri-ponderal mass index (TMI), obtained as weight (kg)/height (m) [3] has been recently suggested to predict body fat percentage [4]. Body mass index (BMI), computed as weight (kg)/height(m) [2], in relation to age, is the most widely used indicator to diagnose obesity in children and adolescents, as it has a good correlation with total body fat [5]. These conventional indicators based on anthropometry are weakly associated with body components such as fat mass (FM) and lean body mass (LBM): muscle, extracellular water, and other non-adipocyte cells [6]. Dual-energy X-ray absorptiometry (DXA) allows scanning the whole body to measure regional body composition [7]. Rather than total fat mass, body fat distribution is a key factor in the development of obesity, related insulin resistance and cardiometabolic diseases, since it better predicts disease risk at the individual level [8]. Adipose tissue is not only a reservoir for energy storage but also an active secretory organ that releases to the bloodstream a variety of bioactive peptides, known as adipokines [9]. White adipose tissue excess, particularly in the visceral compartment, may become severely dysfunctional, increasing the production of pro-inflammatory cytokines, such as interleukin 6 (IL6), interleukin 8 (IL8) and tumor necrosis factor alpha (TNF-α) [10]. Cytokines stimulate the hepatic production of C-reactive protein (hsCRP), an independent predictor of CVD [11]. Adipocytes also secrete hormones such as leptin and resistin that contribute to inflammatory processes [9]. Adiponectin is another hormone secreted by adipose tissue, however, unlike the aforementioned inflammatory biomarkers, it has an inverse association with inflammatory states [12]. Numerous experimental and clinical observations suggest that adiponectin has anti-atherogenic and insulin-sensitizing properties [13]. Furthermore, LBM is metabolically involved in active processes such as resting energy expenditure and myokine secretion, which improve insulin sensitivity, reducing low-grade systemic inflammation and improving muscle glucose uptake [14]. Although chronic inflammation is more prevalent in older individuals, children and adolescents also present a chronic inflammatory state associated with obesity, as well as metabolic complications, which increase morbidity and mortality in adulthood [15]. Hence, one of the key elements to better understand the etiology and course of inflammation process is to perform studies focusing on younger populations, especially in early ages of development. In addition, the use of DXA for the intended body composition measurements allows to discriminate between fat and lean mass, facilitating more precise analysis which could determine the independent effect of each body compartment, in relation to inflammation biomarkers. In addition, the measures of lean and fat mass, among other anthropometric indicators used, were treated in the form of normalized indexes by the squared height instead of percentages, as well as being standardized by the use of z-scores. Therefore, the aim of this study is to assess the relationships between different indicators of body composition and inflammatory biomarkers in Spanish children and adolescents of the GENOBOX study.

2. Methods

2.1. Study design and population

The present study was performed multicentric, cross-sectional case–control GENOBOX study [16]. The total sample included 1175 children and adolescents recruited from three Spanish locations: Reina Sofía University Hospital (Córdoba), Santiago de Compostela Clinic University Hospital (Santiago de Compostela), and Lozano Blesa Clinic University Hospital (Zaragoza). All individuals who participated were selected from those already present to either confirm a diagnosis of overweight or obesity or to investigate minor disorders that were inconclusive following clinical and laboratory examinations. For the purpose of this analysis, we utilized a subset of data from Zaragoza (Fig. 1), applying the following inclusion criteria: children aged >5 years, without endogenous obesity (resulting from hormonal or metabolic dysfunction) or any other metabolic or hormonal disorders. Exclusion criteria included illness or malnutrition, use of medications affecting blood pressure, glucose, or lipid metabolism, or failure to meet the inclusion criteria.

2.2. Anthropometry, body composition and sexual maturation

Participants were instructed to remove their shoes and outer clothing, leaving them in underwear for measurement purposes. Body weight was assessed using an electronic scale (SECA 861; range: 0.05–130 kg; precision: 0.05 kg), while height was measured in the Frankfurt plane, using a standing stadiometer (SECA® 225 model; range: 60–200 cm; precision: 1 mm). Then, BMI was calculated as body weight (kg) divided by the height (m) squared, and TMI was calculated as body weight (kg) divided by the height (m) cubed. WC was measured in fasting state at the midpoint between the lowest rib cage and the top of the iliac crest standing after expiration with a non-elastic tape (Cescorf Equipamentos para Esportes, Porto Alegre, Brazil) to the nearest 0.1 cm. Children's weight categories, including normal weight, overweight, and obesity, were determined using the age- and sex-specific BMI cut-off points established by the International Obesity Task Force (IOTF). These cut-off points align with adult BMI values of 25 kg/m^2 for overweight and 30 kg/m^2 for obesity [17]. Body composition was determined with a whole-body scan using a DXA QDR-Explorer TM 4500 (Hologic Inc., Bedford, MA). The DXA equipment was calibrated at the start of each testing day by using a LS phantom as recommended by the manufacturer. The positioning of the participants and the analyses of the results were undertaken following recommendations from the International Society of Clinical Densitometry [18]. The total body scan was used to obtain total FM, LBM and abdominal fat (VAT). The Fat Mass Index (FMI) was computed by dividing the fat mass (in kilograms) by the square of the individual's height (in meters), while the Lean Body Mass Index (LMI) was determined by dividing the lean body mass (in kilograms) by the square of the individual's height (in meters). Additionally, a medical history and physical examination were conducted, which included assessing sexual maturity and classifying puberty stages based on Tanner's five-stage scale [19].

2.3. Biochemical analysis

Blood samples were obtained from the antecubital vein following an overnight fast. Standard blood tests were conducted at the hospital's laboratory of the participating centers. Plasma insulin levels were measured using radioimmunoassay (with a coefficient of variation of 2.6 %), employing an automated microparticle analyzer (AxSYM; Abbott Laboratories, Abbott Park, IL, USA). Insulin resistance (IR) was assessed using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) formula: insulin (μU/mL) × glucose (mmol/L)/22.5. High-sensitivity C-reactive protein levels were determined using immunoturbidimetry (Dade Behring Inc., Deerfield, IL, USA). Other inflammatory biomarkers were analyzed using X-Map technology and LINCOplex™ human monoclonal antibody kits on a Luminex® 200™ device, following the manufacturer's operating protocols.

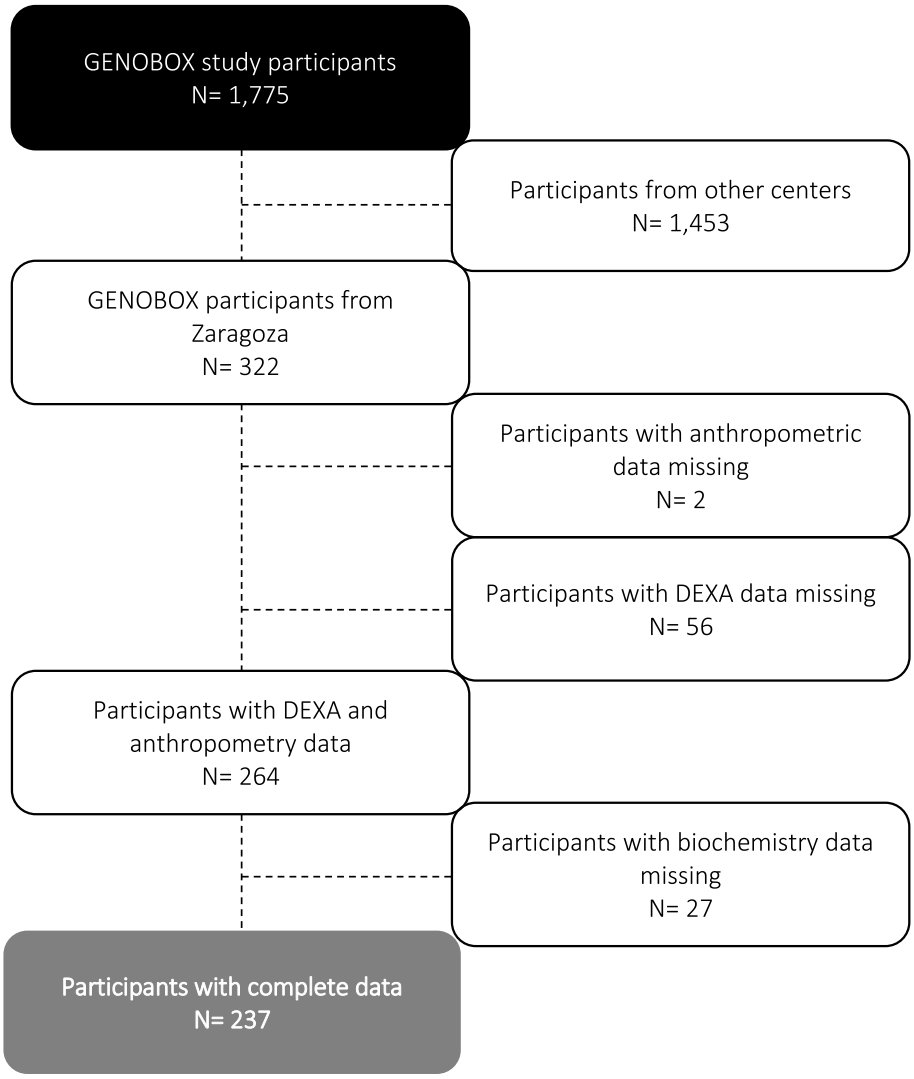

Fig. 1. Flow chart of the sample selection process. The final number of the study participants may vary depending on the inflammatory biomarker studied (see Table 1). The final sample of 237 participants considered discarding all missing values of all included variables simultaneously. HOMA-IR contributed with 8 missing values.

2.4. Statistical analysis

In order to obtain the variables' normality, the Shapiro–Wilk test was conducted. Since not all the variables used follow a normal distribution, descriptive data are displayed as median and interquartile range (IQR). To compare differences by sex, the Mann–Whitney–Wilcoxon test was considered for continuous variables. We computed age and sex specific z-scores for the body composition variables (TMI, LMI, FMI, WC and VAT) using the own sample of participants, except for the BMI, which the Orbegozo et al. z-score cut-off points were considered [20]. Simple linear regression models were performed with each of the inflammation biomarkers (hsCRP, IL8, TNF-α, adiponectin, leptin and resistin) as dependent variables, and each of the body composition indices (z-score) as independent variables, in separate subsamples according to prepubertal vs. pubertal status. Since body fat and lean mass have strong collinearity, the linear regression models assessing the effect of LMI on inflammation biomarkers considered the residuals of a previous linear regression of the FMI's effect on LMI. Similarly, in order to analyze the association of FMI independently of LMI on inflammation biomarkers, residuals of previous linear regression of LMI's effect on FMI were used. We used R version 3.6.1 for the statistical analysis conducted in the present manuscript. The significance level was set to 5 %, i.e., p-values <0.05 were considered statistically significant.

3. Results

Table 1 shows the main characteristics of the sample (median and IQR p25-p75). Boys were older than girls in both sexual maturation stages. Regarding body composition, boys had more lean mass in the prepubertal stage and girls had more fat mass in the pubertal stage. Regarding inflammatory biomarkers, boys had higher IL8 and resistin values in the prepubertal stage. Girls had higher HOMA-IR and higher leptin values in the pubertal stage. No differences were found regarding the other body composition indicators and biochemical markers in both sexual maturation stages.

Table 2 shows how inflammatory biomarkers are associated with body composition indices. All body composition indices, except the lean mass, were positively associated with hsCPR (prepubertals, adjusted R2 from 0.144 to 0.236 and pubertals, adjusted R2 from 0.059 to 0.131). Fig. 2 shows the regression coefficients of hsPCR according to fat mass and lean mass indicators in prepubertal and pubertal subjects. Body composition indices did not show association with IL8 neither in prepubertal nor in pubertals and in the case of TNF-α there was a positive association with body fat indicators TMI and WC both in prepubertals

Table 1
Demographic, anthropometric and biochemical markers in the studied population sample.

Total (n = 264)		Prepubertal (n = 126)			Pubertal (n = 138)			
	Total	**Boys** (n = 67)	**Girls** (n = 59)	**p**	**Total**	**Boys** (n = 58)	**Girls** (n = 80)	**p**
Age (years)	8.4 (7.6–9.4)	9.0 (7.9–9.7)	8.0 (7.2–8.8)	**<0.001**	11.9 (10.7–13.3)	12.7 (11.4–13.9)	11.5 (9.8–12.8)	**<0.001**
Weight (kg)	31.8 (26.0–44.7)	33.0 (26.2–48.9)	30.8 (25.8–38.9)	0.101	53.5 (42.9–63.7)	54.3 (47.8-63-8)	51.6 (40.6–63.6)	0.224
Height (cm)	133 (126–139)	136 (127–142)	131 (124–136)	**0.025**	153 (145–161)	156 (148–163)	150 (143–158)	**0.001**
WC (cm)	61.8 (56.2–73.7)	61.5 (56.3–78.4)	62.1 (55.7–69.4)	0.157	74.1 (65.5–84.8)	75.5 (67.3–88.5)	73.1 (64.3-82-5)	0.095
BMI (kg/m^2)	18.0 (15.6–23.8)	18.0 (15.8–25.0)	18.0 (15.5–21.7)	0.284	22.2 (19.2–27.3)	22.1 (18.9–26.8)	22.4 (19.2–27.5)	0.621
BMI categories (N,%)	66 (100.0)	36 (53.7)	30 (50.8)	0.885	56 (100.0)	27 (46.6)	29 (36.7)	0.326
Normo-weight OW/OB	60 (100.0)	31 (46.3)	29 (49.2)		82 (100.0)	31 (53.4)	51 (63.3)	
TMI (kg/m^2)	13.8 (12.2–17.4)	13.7 (12.1–17.6)	14.1 (12.2-16-7)	0.743	14.7 (12.1–17.8)	13.7 (12.1–17.4)	15.1 (12.5–18.1)	0.113
LMI (kg/m^2)	12.3 (11.4–14.3)	13.0 (11.9–14.6)	11.9 (11.2–13.1)	**0.002**	14.5 (13.2–16.3)	14.7 (13.4–16.3)	14.2 (13.1–16.1)	0.170
FMI (kg/m^2)	5.2 (3.5–8.6)	4.4 (3.1–8.8)	5.4 (3.8–8.2)	0.355	7.5 (4.6–10.8)	7.1 (4.1–9.2)	8.5 (5.6–11.0)	**0.043**
VAT (kg)	1.0 (0.5–2.1)	0.9 (0.5–2.3)	1.0 (0.5–1.7)	0.791	2.0 (0.8–3.3)	1.8 (0.8–3.0)	2.1 (1.0–3.3)	0.414
Total (n = 237)		**Prepubertal** (n = 107)			**Pubertal** (n = 130)			
	Total	**Boys** (n = 57)	**Girls** (n = 50)	**p**	**Total**	**Boys** (n = 55)	**Girls** (n = 75)	**p**
HOMA-IR (units)	1.5 (0.9–3.0)	1.6 (1.0–3.0)	1.6 (0.8–2.9)	0.565	3.0 (2.0–3.7)	2.6 (1.8–3.3)	3.3 (2.4–4.3)	**0.003**
hsCPR (mg/L)	0.8 (0.3–1.9)	0.5 (0.2–2.1)	0.9 (0.4–1.8)	0.210	0.8 (0.2–2.4)	0.5 (0.2–1.7)	1.2 (0.2–2.5)	0.087
IL8 (ng/L)	1.2 (1.0–1.7)	1.3 (1.0–2.0)	1.1 (0.9–1.3)	0.005	1.1 (0.8–1.7)	1.1 (0.8–5.4)	1.1 (0.8–5.2)	0.926
TNF (ng/L)	1.9 (1.5–3.1)	2.0 (1.5–3.1)	1.9 (1.5–3.0)	0.890	2.0 (1.3–2.8)	2.2 (1.6–3.1)	1.7 (1.3–2.7)	0.890
Adiponectin (mg/L)	14.6 (11.1–18.3)	15.9 (10.0–17.8)	14.1 (11.8–19.6)	0.819	11.9 (7.8–16.7)	11.7 (7.1–16.8)	12.1 (8.3–16.5)	0.534
Leptin (µg/L)	4.0 (1.3–13.49)	2.8 (1.0–15.7)	6.4 (2.0–11.9)	0.319	9.1 (3.8–17.2)	7.1 (1.9–16.6)	12.0 (4.7–17.4)	**0.026**
Resistin (µg/L)	23.6 (13.3–33.2)	26.1 (17.6–40.7)	20.3 (11.7–29.0)	**0.037**	28.5 (18.9–37.7)	27.2 (15.4–35.6)	30.0 (20.4–38.5)	0.125

Legend: Results shown as median and interquartile range (p25-p75). Significant results in the ANOVA between sex groups shown in bold ($p < 0.05$). Abbreviations: BMI (body mass index); FMI (fat mass index); HOMA-IR (homeostatic model assessment - insulin resistance); hsCRP (high sensitivity C reactive protein); IL8 (interleukin 8); LMI (lean mass index); OB (obesity); OW (overweight); TMI (triponderal mass index); TNF (tumor necrosis factor); VAT (visceral adipose tissue) and WC (waist circumference).

Table 2
Linear regression analysis of CRP, IL8 and TFN according to body composition indices stratified by pubertal stages.

	Prepubertal			Pubertal		
	β	Adjusted R^2	p	β	Adjusted R^2	p
hsCPR (mg/L)[a] (n = 256)		(n = 120)	(n = 136)			
z-BMI	0.815	0.235	**<0.001**	0.632	0.069	**0.001**
z-TMI	1.327	0.144	**<0.001**	0.957	0.064	**0.001**
z-LMI	−0.815	0.002	0.25	−0.434	<0.001	0.312
z-FMI	2.833	0.151	**<0.001**	1.297	0.059	**0.002**
z-WC	1.523	0.179	**<0.001**	1.066	0.085	**<0.001**
z-VAT	1.731	0.236	**<0.001**	1.300	0.131	**<0.001**
IL8 (ng/L)[a] (n = 254)		(n = 123)			(n = 131)	
z-BMI	0.031	−0.006	0.632	−0.003	−0.007	0.953
z-TMI	0.091	−0.004	0.502	0.028	−0.007	0.776
z-LMI	0.135	−0.006	0.639	0.066	−0.006	0.637
z-FMI	−0.033	−0.008	0.908	−0.036	−0.007	0.794
z-WC	0.008	−0.008	0.952	0.047	−0.005	0.627
z-VAT	0.007	−0.008	0.956	0.014	−0.007	0.885
TNF (ng/L)[a] (n = 253)		(n = 122)			(n = 131)	
z-BMI	0.120	0.020	0.062	0.141	0.019	0.060
z-TMI	0.290	0.031	**0.029**	0.242	0.024	**0.040**
z-LMI	0.526	0.020	0.061	0.055	0.742	0.742
z-FMI	0.025	−0.008	0.929	0.112	−0.004	0.504
z-WC	0.293	0.029	**0.031**	0.323	0.052	**0.004**
z-VAT	0.226	0.013	0.104	0.174	0.009	0.132

Legend: Due to strong collinearity, the z-LMI and z-FMI are represented by the residuals of a previous association of the LMI's effect on FMI and vice versa. Abbreviations: hsCRP (high sensitivity C reactive protein); IL8 (interleukin 8); TNF (tumor necrosis factor); z-BMI (body mass index z score); z-FMI (fat mass index z-score); z-LMI (lean mass index z-score); z-TMI (triponderal mass index z-score); z-VAT (visceral adipose tissue z-score) and z-WC (waist circumference z-score).

[a] Considered as dependent variable in separate models for each independent variable (i.e., z-scores of BMI, TMI, LMI, FMI, WC and VAT).

and pubertals.

Table 3 shows the relationship between body composition indices and the hormones leptin, resistin and adiponectin. All body fat composition indices were positively associated with leptin both in prepubertals (adjusted R2 from 0.233 to 0.457) and pubertals (adjusted R2 from 0.2 to 0.537). Regression coefficients of leptin according to fat mass and lean mass indicators in both pubertal stages are shown in Fig. 3. Fat composition indices WC, FMI and TMI were negatively associated with resistin only in the pubertal stage (adjusted R2 from 0.023 to 0.029). All body composition indices were inversely associated with adiponectin, except FMI in pubertals, but none of them was statistically significant.

4. Discussion

We studied a cohort of Spanish children and adolescents, homogeneously distributed in terms of sex and pubertal status. We determined the clinical nutritional indicators commonly used, such as BMI and TMI, and also as novelty of the study, further body composition indicators were accurately assessed by the independent effect of LBM, FM and VAT measures, performed with DXA techniques. Furthermore, based on the DXA measurements, LMI and FMI were determined. This study design allowed us to analyze certain biomarkers' behaviors, independently for fat mass and lean mass. We were able to verify the connection between some of the biochemical markers (hsCPR and leptin) specifically with the presence of fat mass, both in prepubertals and pubertals, independently of the lean mass present, and regardless of how body composition was measured. We also observed that those pubertal individuals with higher TMI and WC had higher TNF-α levels. Finally, higher TMI, FMI and WC were observed to have lower resistin levels, in pubertal individuals.

Nutritional status according to BMI was similar in both genders in the prepubertal stage, with about half of the sample being normo-weight and the other half overweight or obese. However, at the pubertal stage, girls showed a major difference, with almost two thirds of them having overweight or obesity. It could be assumed that the majority of girls at this pubertal stage are already in a Tanner stage II and III, something that can be reflected in their increased body fat (significative in the FMI) and in the HOMA-IR. These parameters were higher

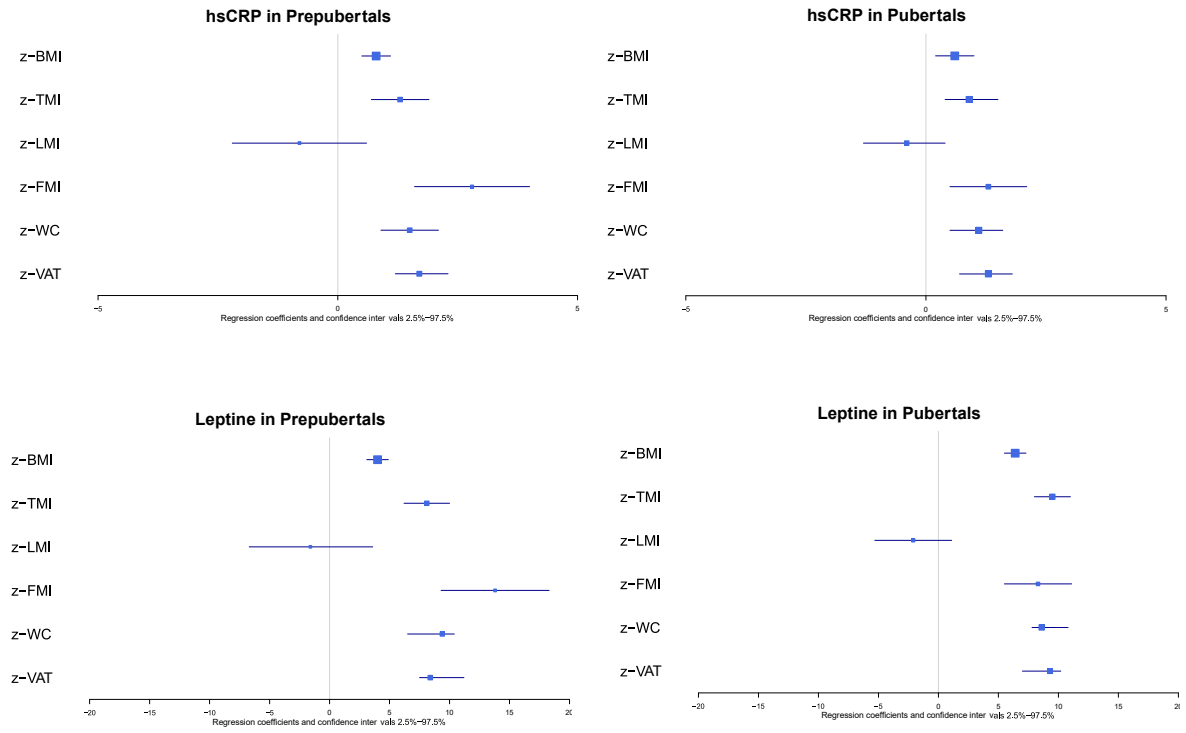

Fig. 2. Matrix of forest plots of the clinical and DEXA adiposity indicators in association with hsCRP and Leptine blood biomarkers, according to pubertal stage. Regression coefficients and confidence interval displayed. For the hsCRP plots, x-axis [-5,5] confidence interval range displayed; for the Leptin plots, [-25,25]. Confidence intervals [2.5%–97.5 %] range displayed. Abbreviations: z-BMI (z-score body mass index); z-TMI (z-score triponderal mass index); z-LMI (z-score lean mass index); z-FMI (z-score fat mass index); z-WC (z-score waist circumference) and z-VAT (z-score visceral abdominal fat).

Table 3
Linear regression analysis of leptin, resistin and adiponectin, according to body composition indices stratified by pubertal stages.

	Prepubertal			Pubertal		
	β	Adjusted R^2	p	β	Adjusted R^2	p
Adiponectin (mg/L)[a] (n = 254)		(n = 123)	(n = 131)			
z-BMI	−0.483	0.007	0.171	−0.480	0.002	0.242
z-TMI	−1.099	0.010	0.13	−0.955	0.009	0.139
z-LMI	−0.796	−0.006	0.606	−1.097	0.003	0.228
z-FMI	−0.361	−0.007	0.814	0.282	−0.007	0.757
z-WC	−1.118	0.010	0.133	−0.894	0.007	0.157
z-VAT	−1.098	0.009	0.145	−0.750	0.003	0.235
Leptin (μg/L)[a] (n = 249)		(n = 118)			(n = 131)	
z-BMI	3.986	0.379	**<0.001**	6.412	0.601	**<0.001**
z-TMI	8.109	0.374	**<0.001**	9.525	0.535	**<0.001**
z-LMI	−1.572	−0.005	0.551	−2.099	0.005	0.191
z-FMI	13.811	0.233	**<0.001**	8.296	0.200	**<0.001**
z-WC	8.436	0.377	**<0.001**	9.340	0.537	**<0.001**
z-VAT	9.381	0.457	**<0.001**	8.615	0.457	**<0.001**
Resistin (μg/L)[a] (n = 253)		(n = 123)			(n = 130)	
z-BMI	0.977	0.006	0.19	−2.100	0.019	0.059
z-TMI	0.998	−0.004	0.516	−3.769	0.028	**0.029**
z-LMI	−1.521	−0.006	0.641	3.425	0.007	0.161
z-FMI	5.133	0.012	0.112	−4.873	0.023	**0.045**
z-WC	0.006	−0.008	0.997	−3.743	0.029	**0.028**
z-VAT	1.752	0.001	0.272	−2.180	0.005	0.201

Legend: Due to strong collinearity, the z-LMI and z-FMI are represented by the residuals of a previous association of the LMI's effect on FMI and vice versa. Abbreviations: z-BMI (body mass index z score); z-FMI (fat mass index z-score); z-LMI (lean mass index z-score); z-TMI (triponderal mass index z-score); z-VAT (visceral adipose tissue z-score) and z-WC (waist circumference z-score).

[a] Considered as dependent variable in separate models for each independent variable (i.e., z-scores of BMI, TMI, LMI, FMI, WC and VAT).

compared to boys, as at this age it is usual for boys to be at Tanner stage I or II.

The results obtained are in accordance with what is described in the scientific literature. Gender differences in body composition prior to puberty are modest compared with post-pubertal differences [21]. Nevertheless, while lean mass shows similar patterns between genders during mid-childhood, boys tend to accumulate approximately 1 kg more absolute Fat-Free Mass (FFM) than girls before entering puberty [22]. During adolescence, girls typically experience an annual increase in absolute Fat Mass (FM) of about 1.14 kg, whereas boys tend to

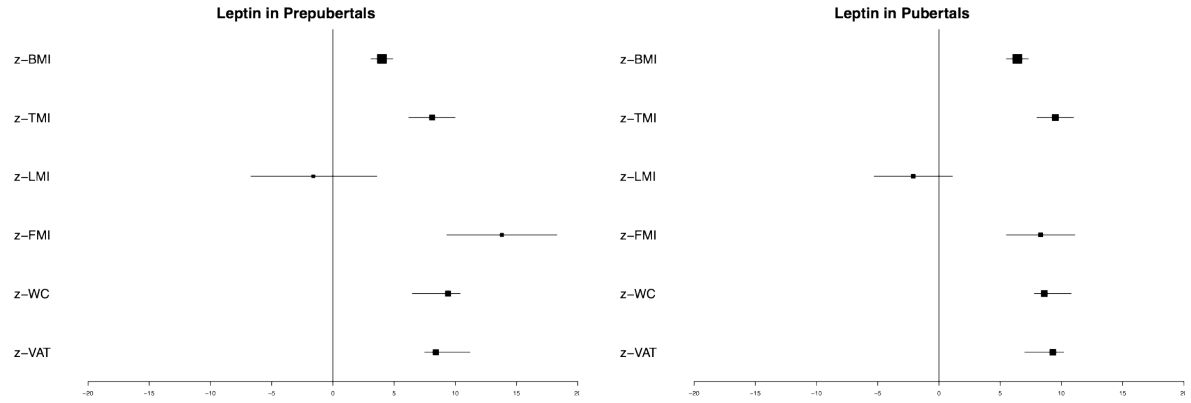

Fig. 3. Forest plots of the clinical and DXA adiposity indicators in association with leptin, according to pubertal stage. Regression coefficients and confidence interval 2.5%–97.5 %. Displayed (x-axis, [-20,20] confidence interval range). Abbreviations: z-BMI (z-score body mass index); z-TMI (z-score triponderal mass index); z-LMI (z-score lean mass index); z-FMI (z-score fat mass index); z-WC (z-score waist circumference) and z-VAT (z-score visceral adipose tissue z-score.

maintain a relatively stable absolute FM. With the onset of puberty, males typically exhibit an accelerated rate of lean mass gain [21]. However, our sample of pubescent males shows no significant difference in lean mass compared to girls in terms of body composition.

HOMA-IR is a well-established marker for insulin resistance, which has high specificity and sensitivity in pubertal adolescents with obesity [23]. During puberty there is a physiologic decline in insulin sensitivity and in our study the fat excess present in pubertal girls, may be also contributing to the higher insulin resistance. In the HELENA Study, the fat mass was strongly associated with the HOMA-IR values in European adolescents both normal weight or overweight [24].

All body fat composition indicators were positively associated with the inflammatory protein hsCRP both in prepuberty and puberty. Many large scale population studies have reported significant associations for hsCRP and body fat in both healthy and overweight and obese children and adolescents [24,25]. Also, in a large sample of young Italian subjects (18–21 years), the healthy overweight/obese group presented an increased fat mass and higher hsCRP circulating compared to the healthy lean group [26]. In a study conducted by Skinner et al. [27], that analyzed data from the NHANES, years 1999–2006 in children and adolescents (1–17 years old), hsCRP was strongly and positively associated with increasing weight status, and this relationship started as young as age 3. Similar to our study, Christaki et al. performed a study in Greece, with pediatric patients between 5 and 15 years old. They found that hsCRP strongly correlated with fat mass percentage [28]. In our study, the strongest association of hsCRP was with the abdominal fat (VAT) determined by DXA in both age groups, which was stronger than abdominal fat measured by WC. DXA has shown to be one of the most reliable tools to assess VAT in children. High deposition of VAT is associated with metabolic syndrome and cardiometabolic risk factors from early stages in an individual's life course [8]. On the other hand, in our study VAT did not show association with TNF-α values, but fat determined by WC and by the TMI indicator did show association with this inflammatory biomarker in both stages of sexual maturation. Other studies also showed a positive relationship between TNF-α and overweight or obesity [25]. However, the AVENA study did not find a significant correlation between TNF-α and body composition variables [29]. IL-8 is a proinflammatory cytokine that did not show association with fat mass in our study. IL-8 was found elevated in adults with obesity [30]; however, in children and adolescents the results are controversial. Herder et al. found no association between IL-8 and BMI and a weak inverse association with WC in German adolescents [31]. Tam et al. did not find significant differences in IL-8 levels between normal weight and overweight children at 8 years old; however, at the age of 15, girls with overweight and obesity (who were normal weight at 8) had higher levels of this proinflammatory cytokine [32].

Adiponectin is one of the main cytokines produced by adipose tissue. A reduced level of adiponectin has been reported in children with obesity, compared to normal weight control subjects. It showed an inverse correlation with WC, suggesting that visceral adipose tissue had a central role in reducing circulating levels of adiponectin [33]. In our study, we found an inverse correlation between adiponectin serum levels and body compartments, both fat and lean. However, the association was not statistically significant.

Leptin, an adipokine which is increased with obesity, was higher in the pubertal females of our sample, compared to the pubertal males. In prepubertal children, leptin concentrations gradually increase with age and show no sexual dimorphism. Later in puberty, leptin concentrations decrease in males and increase greatly in females leading to a striking sexual dimorphism [21]. However, Lahlou et al. [34] found pubertal females had increased serum leptin in both normo-weight and obese adolescents. This observation is also consistent with the mayor presence of overweight and obesity in the pubertal girls of our sample. All body fat composition indicators were associated with leptin values, both in prepuberty and puberty. The strongest correlation was in the pubertal stage. A study carried out in Brazil [35], with adolescents, showed that a greater proportion of variance in leptin concentration was explained by BMI, fat mass percentage and gender. Mean serum leptin concentration had a positive correlation with the pubertal Tanner stage in girls while the circulating leptin levels decreased from Tanner I to V in boys. Similar results were found in Portuguese children and adolescents [36], where being girls and having greater BMI were significantly and independently associated with increased serum leptin. The differences in leptin levels between the pubertal stages and sex might be explained by the higher testosterone levels in boys, which have a negative effect on leptin concentrations [37].

Resistin, another hormone derived from adipocytes, has a debated role in metabolic disorders. It is thought to be involved in inflammatory processes and has been associated with obesity and insulin resistance. Current knowledge suggests that macrophages present in visceral white adipose tissue primarily contribute to the circulating concentration of resistin in adults with obesity [38]. Resistin-related studies in pediatric populations are scarce and the results shown are controversial. However, our study showed a negative association between fat indicators and resistin serum levels, being significant in the pubertal stage for FMI, TMI and WC. A cross sectional study [39] showed lower concentrations of resistin in central precocious puberty girls than in prepubertal and pubertal girls with overweight and obesity. These resistin levels were independent of BMI and body fat percentage. In a Spanish population [40], no significant differences in serum resistin concentrations were observed between children with obesity, overweight, and normal weight at any age, and no significant correlations were observed between

resistin concentrations and weight or BMI. In another study, carried out in Germany, resistin levels showed a close correlation with pubertal stage ($p < 0.05$) and age ($p < 0.01$) [41]. Further biomarkers relevant to the topic, such as circulating fibroblast growth factor-21 (FGF21), and other behavioral determinants not included in the present analysis have also been studied in similar age populations, showing strong associations with metabolic related disturbances during early development [42].

One of the strengths of this study is that as we had data of body composition measured by DXA, we were able to specifically differentiate fat mass from lean mass and attribute to each of them the corresponding association with biochemical markers. The strong collinearity between fat and lean mass lead us to use the residuals of the previous associations between FMI and LMI, allowing us to better represent the differences between one another more accurately in the regression models. Another strength of this study was to integrate this type of biochemical data in children, something not usually observed in the literature. Finally, the results obtained in the present analysis showed significant associations relating body composition DXA measurements and inflammation biomarkers in a multicentric cohort of Spanish youth, a sort of population scarcely studied in the literature. On the other hand, the study presents some limitations. The present analysis was carried out in a subsample of young individuals, not representative of the population participating in the GENOBOX study. This approach could partly modulate the results obtained due to the effect attributed to the sample size. Moreover, in the pubertal stage, the presence of overweight and obesity was higher in girls, something that may have conditioned the results. In this line, the known adiposity and hormonal changes during puberty, and the biological mechanisms underlying these sex-specific differences could have had a potential effect in the inflammatory status of the studied participants, thus, future research should be explored through longitudinal studies, incorporating participants until the end of their puberty and including body composition and inflammatory status. This way, we could provide a more comprehensive interpretation of the results obtained. Finally, we acknowledge the present analysis could benefit from other potential confounders representing environmental or molecular determinants which could influence in the results obtained.

In conclusion, the present study allowed us to confirm the positive association between the inflammatory markers hsCRP and leptin with all the body fat composition parameters, measured by both, standard nutritional indicators and DXA, in both sexual stages. In the case of TNF-α, the association was positive only for body fat measured by TMI and WC indicators, also in both sexual stages. Resistin showed a negative association with fat compartments in the pubertal stage, adding another result to the existing controversial research on the subject. The present study provides data on inflammatory biomarkers in prepubertal children, a population that has been scarcely studied. The association between the inflammatory biomarkers and fat mass in this study, showed the presence of risk factors for the development of cardiovascular and other metabolic diseases in their future life. In the light of these results, it would be important to deepen in further research.

Consent to participate

All participants and their families were informed about the purpose of the study before giving their written consent. Written informed consent was obtained from the parents.

Author contributions

Dr. Estela Skapino participated in the conceptualization and design, writing first draft of the manuscript and developing the final version.

Dr. Miguel Seral-Cortes contributed to the study design, methodology, data curation and analysis, writing first draft of the manuscript and building the final version.

Mr. Sergio Sabroso-Lasa performed the data curation and analysis.

Dr. Luis A. Moreno participated in the study design, methodology, supervision, funding acquisition and commenting and editing previous versions of the manuscript.

Ms. Laura Gonzalez-Gayan, Dr. Maria Teresa Llorente-Cereza, Dr. Rosaura Leis, Dr. Concepción Aguilera, Dr. Mercedes Gil-Campos and Dr. Gloria Bueno-Lozano contributed to funding acquisition, review and editing previous versions of the manuscript.

All authors read and approved the final manuscript.

Ethics approval

The study was developed following the Declaration of Helsinki recommendations (as well as the Edinburgh review) and was approved by the ethics committees of each participating center (Code IDs: Santiago 2011/198, Zaragoza 10/2010, Córdoba 01/2017).

Data availability statement

Data used in the present manuscript can be accessed upon reasonable request to the representative principal investigator of the Zaragoza research group within the GENOBOX study.

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Declaration of competing interests

The authors have no conflict of interests to declare that are relevant to the content of this article. The authors have no relevant financial or non-financial interests to disclose.

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References

[1] Bansal P, Verma D, Tamber S, Sharma R. An update on pathogenesis of inflammatory disorders with its management. RPS Pharmacy and Pharmacology Reports 2023 Apr 2023;2(2). https://doi.org/10.1093/rpsppr/rqad011.
[2] Furman D, Campisi J, Verdin E, et al. Chronic inflammation in the etiology of disease across the life span. Nat Med 2019 Dec 2019;25(12). https://doi.org/10.1038/s41591-019-0675-0.
[3] de Heredia F, Gómez-Martínez S, Marcos A. Obesity, inflammation and the immune system. Proc Nutr Soc 2012 May 2012;71(2). https://doi.org/10.1017/S0029665112000092.
[4] Peterson C, Su H, Thomas D, et al. Tri-ponderal mass index vs body mass index in estimating body fat during adolescence. JAMA Pediatr 2017 Jan 2017;171(7). https://doi.org/10.1001/jamapediatrics.2017.0460.
[5] Hampl S, Hassink S, Skinner A, et al. Clinical practice guideline for the evaluation and treatment of children and adolescents with obesity. Pediatrics 2023 Jan 2023;151(2). https://doi.org/10.1542/peds.2022-060640.

[6] Ceniccola G, Castro M, Piovacari S, et al. Current technologies in body composition assessment: advantages and disadvantages. Nutrition 2019 Jun 2019;62doi. https://doi.org/10.1016/j.nut.2018.11.028.
[7] Shepherd J, Ng B, Sommer M, Heymsfield S. Body composition by DXA. Bone 2017 Nov 2017;104doi. https://doi.org/10.1016/j.bone.2017.06.010.
[8] Jin B, Lin H, Yuan J, et al. Abdominal adiposity and total body fat as predictors of cardiometabolic health in children and adolescents with obesity. Front Endocrinol 2020 Apr 2020;11doi. https://doi.org/10.3389/fendo.2020.00579.
[9] Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol 2005 May 2005;115(5). https://doi.org/10.1016/j.jaci.2005.02.023.
[10] Pellegrinelli V, Carobbio S, Vidal-Puig A. Adipose tissue plasticity: how fat depots respond differently to pathophysiological cues. Diabetologia 2016 Jun 2016;59(6). https://doi.org/10.1007/s00125-016-3933-4.
[11] Sproston N, Ashworth J. Role of C-reactive protein at sites of inflammation and infection. Front Immunol 2018 Apr 2018;9doi. https://doi.org/10.3389/fimmu.2018.00754.
[12] Chandran M, Phillips S, Ciaraldi T, et al. Adiponectin: more than just another fat cell hormone? Diabetes Care 2003 Aug 2003;26(8). https://doi.org/10.2337/diacare.26.8.2442.
[13] Zachariah J, Hwang S, Hamburg N, et al. Circulating adipokines and vascular function: cross-sectional associations in a community-based cohort. Hypertension (Dallas, Tex : 1979) 2016 Feb 2016;67(2). https://doi.org/10.1161/HYPERTENSIONAHA.115.05949.
[14] Córdoba-Rodríguez D, Iglesia I, Gomez-Bruton A, et al. Fat-free/lean body mass in children with insulin resistance or metabolic syndrome: a systematic review and meta-analysis. BMC Pediatr 2022 Jan 2022;22(1). https://doi.org/10.1186/s12887-021-03041-z.
[15] Weihrauch-Blüher S, Schwarz P, Klusmann J. Childhood obesity: increased risk for cardiometabolic disease and cancer in adulthood. Metab, Clin Exp 2019 Mar 2019;92doi. https://doi.org/10.1016/j.metabol.2018.12.001.
[16] Latorre-Millán M, Rupérez A, González-Gil E, et al. Dietary patterns and their association with body composition and cardiometabolic markers in children and adolescents: genobox cohort. Nutrients 2020 Aug 2020;12(11). https://doi.org/10.3390/nu12113424.
[17] Cole T, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatric obesity 2012 Aug 2012;7(4). https://doi.org/10.1111/j.2047-6310.2012.00064.x.
[18] Crabtree N, Arabi A, Bachrach L, et al. Dual-energy X-ray absorptiometry interpretation and reporting in children and adolescents: the revised 2013 ISCD Pediatric Official Positions. J Clin Densitometry : the official journal of the International Society for Clinical Densitometry 2014 Jun 2014;17(2). https://doi.org/10.1016/j.jocd.2014.01.003.
[19] Tanner J, Whitehouse R. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 1976 Mar 1976;51(3). https://doi.org/10.1136/adc.51.3.170.
[20] Sobradillo B, Aguirre A, Aresti U, et al. Curvas y Tablas de Crecimiento. Estudios longitudinal y transversal. Bilbao: Fundación Faustino Orbegozo 2004:36.
[21] Loomba-Albrecht L, Styne D. Effect of puberty on body composition. Curr Opin Endocrinol Diabetes Obes 2009 Feb 2009;16(1). https://doi.org/10.1097/med.0b013e328320d54c.
[22] Veldhuis J, Roemmich J, Richmond E, et al. Endocrine control of body composition in infancy, childhood, and puberty. Endocr Rev 2005 Feb 2005;26(1). https://doi.org/10.1210/er.2003-0038.
[23] Mayerhofer E, Ratzinger F, Kienreich N, et al. A multidisciplinary intervention in childhood obesity acutely improves insulin resistance and inflammatory markers independent from body composition. Frontiers in pediatrics 2020 Feb 2020:8doi. https://doi.org/10.3389/fped.2020.00052.
[24] Kondaki K, Grammatikaki E, Pavón D, et al. Comparison of several anthropometric indices with insulin resistance proxy measures among European adolescents: the Helena Study. Eur J Pediatr 2011 Jun 2011;170(6). https://doi.org/10.1007/s00431-010-1322-4.
[25] Giordano P, Del Vecchio G, Cecinati V, et al. Metabolic, inflammatory, endothelial and haemostatic markers in a group of Italian obese children and adolescents. Eur J Pediatr 2011 Jul 2011;170(7). https://doi.org/10.1007/s00431-010-1356-7.
[26] Stefanaki C, Peppa M, Boschiero D, et al. Healthy overweight/obese youth: early osteosarcopenic obesity features. Eur J Clin Invest 2016 Sep 2016;46(9):767–78. https://doi.org/10.1111/eci.12659.
[27] Skinner A, Steiner M, Henderson F, et al. Multiple markers of inflammation and weight status: cross-sectional analyses throughout childhood. Pediatrics 2010 Apr 2010;125(4). https://doi.org/10.1542/peds.2009-2182.
[28] Christaki EV, Pervanidou P, Papassotiriou I, et al. Stress, inflammation and metabolic biomarkers are associated with body composition measures in lean, overweight, and obese children and adolescents. Children 2022 Feb 2022;9(2):291. https://doi.org/10.3390/children9020291.
[29] Wärnberg J, Nova E, Moreno L, et al. Inflammatory proteins are related to total and abdominal adiposity in a healthy adolescent population: the AVENA Study. Am J Clin Nutr 2006 Sep 2006;84(3). https://doi.org/10.1093/ajcn/84.3.505.
[30] Straczkowski M, Kowalska I, Nikolajuk A, et al. Plasma interleukin 8 concentrations in obese subjects with impaired glucose tolerance. Cardiovasc Diabetol 2003 May 2003;2. https://doi.org/10.1186/1475-2840-2-5.
[31] Herder C, Schneitler S, Rathmann W, et al. Low-grade inflammation, obesity, and insulin resistance in adolescents. J Clin Endocrinol Metab 2007 Dec 2007;92(12). https://doi.org/10.1210/jc.2007-0955.
[32] Tam C, Garnett S, Cowell C, et al. IL-6, IL-8 and IL-10 levels in healthy weight and overweight children. Horm Res Paediatr 2010 2010;73(2). https://doi.org/10.1159/000277632.
[33] Nigro E, Scudiero O, Ludovica M, et al. Adiponectin profile and Irisin expression in Italian obese children: association with insulin-resistance. Cytokine 2017 Jun 2017;94. https://doi.org/10.1016/j.cyto.2016.12.018.
[34] Lahlou N, Landais P, De Boissieu D, et al. Circulating leptin in normal children and during the dynamic phase of juvenile obesity: relation to body fatness, energy metabolism, caloric intake, and sexual dimorphism. Diabetes 1997 Jun 1997;46(6). https://doi.org/10.2337/diab.46.6.989.
[35] Brandão C, Lombardi M, Nishida S, et al. Serum leptin concentration during puberty in healthy nonobese adolescents. Braz J Med Biol Res 2003 Oct 2003;36(10). https://doi.org/10.1590/s0100-879x2003001000003.
[36] Antunes H, Santos C, Carvalho S. Serum leptin levels in overweight children and adolescents. Br J Nutr 2009 Apr 2009;101(8). https://doi.org/10.1017/S0007114508055682.
[37] Wabitsch M, Blum W, Muche R, et al. Contribution of androgens to the gender difference in leptin production in obese children and adolescents. J Clin Invest 1997 Aug 1997;100(4). https://doi.org/10.1172/JCI119595.
[38] Lausten-Thomsen U, Christiansen M, Hedley P, et al. Reference values for fasting serum resistin in healthy children and adolescents. Clinica chimica acta; international journal of clinical chemistry 2017 Jun 2017;469. https://doi.org/10.1016/j.cca.2017.04.002.
[39] Zurita-Cruz J, Medina-Bravo P, Manuel-Apolinar L, et al. Resistin levels are not associated with obesity in central precocious puberty. Peptides 2018 Nov 2018;109. https://doi.org/10.1016/j.peptides.2018.09.009.
[40] Ortega L, Riestra P, Navarro P, et al. Resistin levels are related to fat mass, but not to body mass index in children. Peptides 2013 Nov 2013;49. https://doi.org/10.1016/j.peptides.2013.08.019.
[41] Gerber M, Boettner A, Seidel B, et al. Serum resistin levels of obese and lean children and adolescents: biochemical analysis and clinical relevance. J Clin Endocrinol Metab 2005 Aug 2005;90(8). https://doi.org/10.1210/jc.2005-0437.
[42] Christaki EV, Pervanidou P, Papassotiriou I, et al. Circulating FGF21 vs. Stress markers in girls during childhood and adolescence, and in their caregivers: intriguing inter-relations between overweight/obesity, emotions, behavior, and the cared-caregiver relationship. Children Jun 2022;2;9(6):821. https://doi.org/10.3390/children9060821. 2022.