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Trabajo Original

Pediatría

Inflammation and insulin resistance according to body composition in European adolescents: the HELENA study

Inflamación y resistencia a la insulina según composición corporal en adolescentes europeos: el estudio HELENA

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Abstract

Introduction: Inflammation is related to insulin resistance in adults, especially on those individuals with high levels of body composition.

Objectives: The aim of this study is to assess the relationship between a set of inflammatory biomarkers and insulin resistance by levels of body composition in a sample of European adolescents.

Material and methods: Nine hundred and sixty-two adolescents (442 boys and 520 girls) from nine European countries met the inclusion criteria of having measurements for the homeostasis model assessment (HOMA) and a set of inflammation-related biomarkers: C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), interleukin (IL-6), complement factors C3 and C4 and selected cell adhesion molecules. Body mass index (BMI), fat mass index (FMI) and waist circumference (WC) were categorized using tertiles. To assess the associations stratifying by body composition indexes, ANOVA and linear regression models were performed.

Results: Mean biomarkers' concentrations differed across BMI, FMI and WC tertiles ($p < 0.05$) by sex. In both sexes, insulin, HOMA, CRP, C3 and C4 were significantly different between categories ($p < 0.001$), always showing the highest mean concentration in the upper category of BMI, FMI and WC. The most consistent finding was an association between insulin resistance and C3 concentrations ($p < 0.05$), in the adolescents in the highest tertile of BMI, FMI and WC, except in the case of FMI in girls.

Conclusion: Inflammatory and glucose metabolism markers differed by tertiles of body composition, being usually higher in the highest tertile. C3 complement factor was associated with insulin resistance in adolescents, especially those with high total and abdominal adiposity.

Key words:

Inflammation. Insulin resistance. Body composition. European adolescents.

Resumen

Introducción: la inflamación está relacionada con la resistencia a la insulina en adultos, especialmente en individuos con altos valores de composición corporal.

Objetivos: valorar la relación entre diferentes marcadores inflamatorios y la resistencia a la insulina según valores de composición corporal en adolescentes europeos.

Material y métodos: novecientos sesenta y dos adolescentes (442 chicos y 520 chicas) de nueve países europeos cumplían el criterio de inclusión de tener medidos la evaluación del modelo de homeostasis (HOMA) y diferentes marcadores inflamatorios: proteína C-reactiva (PCR), factor de necrosis tumoral alfa (TNF-α), interleukina (IL-6), factores de complemento C3 y C4 y moléculas de adhesión. El índice de masa corporal (IMC), el índice de masa grasa (IMG) y la circunferencia de cintura (CC) se categorizaron en tertiles. Para valorar las asociaciones por índices de composición corporal se realizó ANOVA y regresión.

Resultados: las concentraciones de los marcadores diferían entre los tertiles de IMC, IMG y CC ($p < 0,05$), por sexo. En ambos sexos, insulina, HOMA, PCR, C3 y C4 fueron significativamente diferentes entre categorías ($p < 0,001$), presentando la mayor concentración en la categoría superior de IMC, IMG y CC. El resultado más consistente para los adolescentes del tertil superior de IMC, IMG y CC fue la asociación entre resistencia a la insulina y concentraciones de C3 ($p < 0,05$), excepto para IMG en chicas.

Conclusión: los marcadores del metabolismo inflamatorio y de la glucosa diferían según tertiles de composición corporal, siendo mayores en el tertil superior. El C3 se asoció con resistencia a la insulina en adolescentes, especialmente en aquellos con adiposidad total y abdominal.

Palabras clave:

Inflamación. Resistencia a la insulina. Composición corporal. Adolescentes europeos.

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INTRODUCTION

Obesity is a condition in which the adipose tissue mass is increased due to a high adipocytes number and size (1). In obese individuals, the endocrine function of the adipose tissue is impaired and the adipocytes, as well as the pre-adipocytes, macrophages, and adipose stem cells, contribute to the production of pro-inflammatory cytokines (2). Both the hypertrophied adipocytes and other adipose tissue immune cells could lead to chronic inflammation through innate and adaptive immune responses (3). Additionally, the adipose tissue dysfunction is related to the development of co-morbidities such as insulin resistance, type 2 diabetes and cardiovascular diseases (4). The inflammatory state triggered by the impaired function of the adipose tissue also seems to be related with these comorbidities (5,6).

Among a large number of inflammatory-related biomarkers, C-reactive protein (CRP) has been the one most widely used. Hepatic synthesis of CRP, complement factors C3 and C4, depends on pro-inflammatory cytokines released by the adipose tissue such as tumor necrosis factor alpha (TNF-α) or interleukin 6 (IL-6) (7). Moreover, cell adhesion molecules are elevated during inflammatory conditions and have been suggested as markers for atherosclerosis (8).

Table I. Characteristics of the study participants

Center (country)	Boys (n = 442)		Girls (n = 520)		
	n	%	n	%	-
Athens (Greece)	29	6.6	39	7.5	-
Dortmund (Germany)	60	13.6	47	9.0	-
Gent (Belgium)	54	12.2	52	10.0	-
Heraklion (Greece)	44	10.0	42	8.1	-
Lille (France)	34	7.7	50	9.6	-
Pecs (Hungary)	44	10.0	79	15.2	-
Roma (Italy)	46	10.4	48	9.2	-
Stockholm (Sweden)	46	10.4	49	9.4	-
Vienna (Austria)	45	10.2	58	11.2	-
Zaragoza (Spain)	40	9.0	56	10.8	-
	Mean	**SD**	**Mean**	**SD**	**p**
Age (years)	14.77	1.21	14.73	1.16	0.616
Height (cm)	169.94	9.89	161.84	6.96	< 0.001
Weight (kg)	61.83	13.66	55.86	10.12	< 0.001
BMI	21.27	3.74	21.27	3.30	0.980
Fat mass index (kg/m^2)	13.33	10.01	15.09	6.37	0.001
Waist circumference (cm)	74.43	8.95	70.58	7.86	< 0.001
Glucose (mg/dl)	92.80	7.30	89.48	6.58	< 0.001
Insulin (µlU/ml)	10.16	9.19	10.38	6.77	0.666
HOMA	2.36	2.34	2.32	1.66	0.725
CRP (mg/l)	0.86	1.25	0.83	1.25	0.766
IL-6 (pg/ml)	27.19	37.07	19.89	25.53	0.001
TNF-α (pg/ml)	7.35	5.99	5.65	3.47	< 0.001
C3 (g/l)	1.12	0.16	1.14	0.17	0.134
C4 (g/l)	0.20	0.06	0.21	0.06	0.167
L-selectin (ng/ml)	3,836.56	1,603.85	3,700.91	1,510.49	0.180
sE-selectin (ng/ml)	41.76	21.07	35.89	18.79	< 0.001
sVCAM-1 (ng/ml)	1,419.78	406.28	1,221.47	378.49	< 0.001
sICAM-1 (ng/ml)	181.56	158.93	151.51	102.97	0.001

SD: Standard deviation; BMI: Body mass index; HOMA: Homeostasis model assessment; CRP: C-reactive protein; IL-6: interleukin 6; TNF-α: Tumor necrosis factor alpha; C3 and C4: Complement factors; sVCAM-1: Soluble vascular cell adhesion protein 1; sICAM-1: Soluble intercellular adhesion molecule.

Inflammation seems to be an important step in the pathogenesis of insulin resistance (IR) (9). In obese subjects, the inflammatory response can lead to altered insulin mediated signaling pathway by directly inhibiting insulin receptors (10). The relation between traditional inflammatory cells, cytokines, and chemokines and insulin resistance has been studied in adult populations (10). In children, obesity-related adipose tissue dysfunction develops early in childhood and is related to IR (11). In a previous study in adolescents, an association between IR and some inflammatory biomarkers was observed, and these relationships were stronger in obese subjects (12). However, another study performed in adolescents and young adults suggested that low grade inflammation did not appear to play a role in the development of IR (13). Due to these controversial findings, there is a need of further research in early stages of life, as it is a critical period for the development of future co-morbidities. Body composition, especially the body fat, could determine the associations between inflammatory markers and insulin resistance. Thus, the aim of this study is to assess the relationship between inflammatory markers and insulin resistance by body composition in a sample of European adolescents.

Table II. Mean differences and standard deviations of biomarkers in boys and girls by body mass index categories

	BMI I		BMI II		BMI III		
Boys	n = 146		n = 147		n = 149		
	Mean	SD	Mean	SD	Mean	SD	p
Glucose (mg/dl)†	91.69	6.70	92.94	8.09	93.75	6.94	0.056
Insulin (µIU/ml)†	7.34	4.33	10.21	8.24	12.89*†	12.31	< 0.001
HOMA†	1.67	1.01	2.35	2.02	3.05*†	3.22	< 0.001
CRP (mg/l)†	0.70	1.21	0.67	0.81	1.20*†	1.55	< 0.001
IL-6 (pg/ml)†	31.12	42.63	25.17	31.08	25.44	37.20	0.642
TNF-α (pg/ml)†	7.11	3.86	7.13	6.73	7.85	6.84	0.277
C3 (g/l)	1.07	0.14	1.09	0.14	1.20*†	0.17	< 0.001
C4 (g/l)	0.19	0.06	0.19	0.05	0.23*†	0.07	< 0.001
Lselectin (ng/ml)†	3,958.25	1,679.11	3,659.34	1,434.20	3,894.13	1,682.69	0.206
sE-selectin (ng/ml)†	42.43	19.08	37.58	18.48	45.40†	24.57	0.005
sVCAM-1 (ng/ml)	1,458.04	411.79	1,392.11	383.19	1,409.95	423.39	0.363
sICAM-1 (ng/ml)†	173.56	96.41	173.28	96.27	198.07	240.01	0.733
Girls	n = 169		n = 176		n = 175		
	Mean	SD	Mean	SD	Mean	SD	p
Glucose (mg/dl)†	89.84	7.16	89.28	6.24	89.34	6.33	0.733
Insulin (µIU/ml)†	9.63	8.03	9.18	4.16	12.33*†	7.14	< 0.001
HOMA†	2.18	2.08	2.03	0.96	2.74*†	1.69	< 0.001
CRP (mg/l)†	0.60	0.95	0.81	1.24	1.08*†	1.46	< 0.001
IL-6 (pg/ml)†	17.65	23.73	19.99	27.13	22.08*	25.55	0.045
TNF-α (pg/ml)†	5.61	2.79	5.76	4.03	5.56	3.50	0.997
C3 (g/l)	1.08	0.17	1.13	0.16	1.19*†	0.16	< 0.001
C4 (g/l)	0.19	0.06	0.20	0.06	0.23*†	0.06	< 0.001
L-selectin (ng/ml)†	3,656.23	1,355.57	3,566.20	1,600.20	3,878.23	1,552.44	0.190
sE-selectin (ng/ml)†	37.12	18.83	35.94	22.18	34.65	14.68	0.459
sVCAM-1 (ng/ml)	1,300.20	404.84	1,184.45*	352.42	1,181.63†	367.01	0.005
sICAM-1 (ng/ml)†	164.63	141.47	150.31	91.68	139.95	58.82	0.247

*BMI: body mass index; SD: Standard deviation; HOMA: Homeostasis model assessment; CRP: C-reactive protein; IL-6: interleukin 6; TNF-α: tumor necrosis factor alpha; C3 and C4: complement factors; sVCAM-1: soluble vascular cell adhesion protein 1; sICAM-1: soluble intercellular adhesion molecule 1. †Biomarkers log-transformed. Post-hoc test for multiple comparisons: Bonferroni. *p < 0.05 ref CRP I, †p < 0.05 ref CRP II.*

Table III. Mean differences and standard deviations of biomarkers in boys and girls by fat mass index categories

	FMI I		FMI II		FMI III		
Boys	n = 132		n = 137		n = 137		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p**
Glucose (mg/dl)†	91.78	7.51	92.06	7.49	94.22*†	6.92	0.010
Insulin (µlU/ml)†	7.95	5.40	9.27	7.67	13.61*†	12.78	< 0.001
HOMA†	1.79	1.15	2.13	1.93	3.24*†	3.35	< 0.001
CRP (mg/l)†	0.72	1.22	0.68	0.89	1.13*†	1.43	< 0.001
IL-6 (pg/ml)†	30.19	36.14	21.24	27.75	25.15	37.20	0.184
TNF-α (pg/ml)†	7.26	3.91	6.67	3.44	7.58	6.88	0.260
C3 (g/l)	1.05	0.13	1.10	0.14	1.20*†	0.17	< 0.001
C4 (g/l)	0.18	0.05	0.19	0.05	0.22*	0.07	< 0.001
L-selectin (ng/ml)†	3,910.89	1,604.22	3,622.45	1,524.49	3,833.36	1,561.94	0.163
sE-selectin (ng/ml)†	42.80	19.19	38.50	18.89	44.18	25.08	0.099
sVCAM-1 (ng/ml)	1,452.09	395.95	1,409.38	410.73	1,369.82	410.35	0.263
sICAM-1 (ng/ml)†	169.16	62.44	179.27	121.36	193.51	249.90	0.977
Girls	n = 166		n = 176		n = 172		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p**
Glucose (mg/dl)†	89.95	6.95	89.56	6.57	89.05	6.24	0.480
Insulin (µlU/ml)†	8.94	5.56	10.01	7.41	12.17*†	6.87	< 0.001
HOMA†	2.01	1.34	2.24	1.93	2.70*†	1.61	< 0.001
CRP (mg/l)†	0.62	0.96	0.78	1.23	1.10*†	1.47	< 0.001
IL-6 (pg/ml)†	18.66	23.20	20.17	28.13	21.28	25.18	0.157
TNF-α (pg/ml)†	5.38	2.70	6.11	4.70	5.43	2.47	0.625
C3 (g/l)	1.08	0.18	1.13*	0.16	1.19*†	0.17	< 0.001
C4 (g/l)	0.19	0.05	0.20	0.05	0.23*†	0.05	< 0.001
L-selectin (ng/ml)†	3,650.69	1,513.99	3,580.29	1,487.21	3,877.74	1,524.27	0.256
sE-selectin (ng/ml)†	37.29	18.54	34.51	19.27	36.06	18.70	0.181
SVCAM-1 (ng/ml)	1,315.54	390.64	1,166.80*	380.15	1,186.32*	352.50	< 0.001
SICAM-1 (ng/ml)†	170.01	144.46	138.62*	83.33	146.71	64.18	0.011

*FMI: Fat mass index; SD: Standard deviation; HOMA: Homeostasis model assessment; CRP: C-reactive protein; IL-6: Interleukin 6; TNF-α: Tumor necrosis factor alpha; C3 and C4: Complement factors; sVCAM-1: Soluble vascular cell adhesion protein 1; sICAM-1: Soluble intercellular adhesion molecule 1. †Biomarkers log-transformed. Post-hoc test for multiple comparisons: Bonferroni. *p < 0.05 ref CRP I; †p < 0.05 ref CRP II.*

MATERIAL AND METHODS

STUDY DESIGN

The HELENA study is a cross-sectional multi-center study (n = 3,528) conducted between 2006 and 2007 in ten European cities: Athens and Heraklion, in Greece; Dortmund, in Germany; Ghent, in Belgium; Lille, in France; Pecs, in Hungary; Rome, in Italy; Stockholm, in Sweden; Vienna, in Austria; and Zaragoza, in Spain. General procedures and methodology of the HELENA study have been previously described (14).

The study was performed according to the ethical guidelines of the Edinburgh revision of the 1964 Declaration of Helsinki (2000), the International Conferences on Harmonization for Good Clinical Practice and the legislation on clinical research from each of the participating countries. The local Ethics Committees of each center approved the protocol. Written informed consent was obtained from the adolescents and their parents.

Table IV. Mean differences and standard deviations of biomarkers in boys and girls by waist circumference categories

	WC I		WC II		WC III		
Boys	n = 145		n = 149		n = 148		
	Mean	SD	Mean	SD	Mean	SD	p
Glucose (mg/dl)†	92.33	8.22	92.41	7.10	93.65	6.46	0.174
Insulin (µlU/ml)†	8.25	7.17	9.14	6.18	13.08*†	12.33	< 0.001
HOMA†	1.90	1.82	2.09	1.40	3.09*†	3.22	< 0.001
CRP (mg/l)†	0.74	1.24	0.62	0.73	1.21*†	1.57	< 0.001
IL-6 (pg/ml)†	28.34	41.36	26.28	32.25	26.93	37.25	0.931
TNF-α (pg/ml)†	7.27	4.04	7.04	6.78	7.74	6.78	0.312
C3 (g/l)	1.07	0.14	1.10	0.13	1.20*†	0.18	< 0.001
C4 (g/l)	0.19	0.06	0.19	0.05	0.23*†	0.07	< 0.001
L-selectin (ng/ml)†	3,854.80	1,578.72	3,735.36	1,517.87	3,922.43	1,716.10	0.766
sE-selectin (ng/ml)†	40.69	17.46	41.57	21.15	43.04	24.17	0.870
sVCAM-1 (ng/ml)	1,449.98	389.41	1,433.69	404.64	1,375.16	423.25	0.261
sICAM-1 (ng/ml)†	179.79	102.81	168.36	87.53	196.82	240.74	0.471
Girls	n = 173		n = 167		n = 174		
	Mean	SD	Mean	SD	Mean	SD	p
Glucose (mg/dl)†	89.82	7.34	89.41	6.26	89.31	6.11	0.816
Insulin (µlU/ml)†	9.62	8.00	9.35	4.52	12.14*†	7.01	< 0.001
HOMA†	2.17	2.06	2.08	1.09	2.69*†	1.64	< 0.001
CRP (mg/l)†	0.59	0.85	0.88	1.29	1.04*†	1.51	< 0.001
IL-6 (pg/ml)†	22.06	28.53	20.09	26.89	17.91	20.59	0.791
TNF-α (pg/ml)†	5.81	3.71	5.82	3.60	5.33	3.12	0.644
C3 (g/l)	1.10	0.18	1.13	0.16	1.18*†	0.17	< 0.001
C4 (g/l)	0.20	0.06	0.20	0.06	0.22*†	0.06	0.001
L-selectin (ng/ml)†	3,734.12	1,570.48	3,588.98	1,497.37	3,779.13	1,464.04	0.434
sE-selectin (ng/ml)†	36.65	20.25	35.13	19.04	35970	17.16	0.699
SVCAM-1 (ng/ml)	1,290.41	382.20	1180.06	358.23	1193.12	389.84	0.014
SICAM-1 (ng/ml)†	170.01	144.46	138.62*	83.33	146.71	64.18	0.011

*WC: Waist circumference; SD: Standard deviation; HOMA: Homeostasis model assessment; CRP: C-reactive protein; IL-6: Interleukin 6; TNF-α: Tumor necrosis factor alpha; C3 and C4: Complement factors; sVCAM-1: Soluble vascular cell adhesion protein 1; sICAM-1: Soluble intercellular adhesion molecule 1. †Biomarkers log-transformed. Post-hoc test for multiple comparisons: Bonferroni. *p < 0.05 ref CRP I; †p < 0.05 ref CRP II.*

STUDY SAMPLE

Blood collection was randomly performed in approximately a third of the total sample (n = 1,089, 31%). Nine hundred and sixty-two participants (442 boys and 520 girls) met the inclusion criteria of having measured the homeostasis model assessment (HOMA) and the set of biomarkers related with inflammation: TNF-α, IL-6, CRP, complement factors C3 and C4 and cell adhesion molecules: vascular cell adhesion molecule-1 (sVCAM-1), intercellular adhesion molecule-1 (sICAM-1), sE-selectin and L-selectin.

PHYSICAL MEASUREMENTS

Weight and height were measured in underwear and barefoot with an electronic scale (SECA 861, Seca Ltd., Birmingham, UK) and a stadiometer (SECA 225, Seca Ltd.). In addition, body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters. From skinfolds, percentage of fat was calculated using the Slaughter formula and then the fat mass index was also calculated (kg/m^2) (15). Waist circumference was measured with an un-elastic tape. All anthropometric measures were taken following a standardized protocol. Inter-observer reli-

Table V. Linear regression with HOMA-IR as dependent variable and the markers of inflammation as independent variables for each category of body mass index (adjust: center)

		BMI I		BMI II		BMI III	
Boys		n = 146		n = 147		n = 149	
		ß	p	ß	p	ß	p
HOMA-IR†	CRP (mg/l)†	-0.115	0.185	-0.062	0.482	-0.002	0.983
	IL-6 (pg/ml)†	0.106	0.270	0.027	0.783	0.047	0.595
	TNF-α (pg/ml)†	0.017	0.863	-0.028	0.761	0.027	0.767
	C3 (g/l)	-0.058	0.518	0.004	0.968	0.245	0.004
	C4 (g/l)	-0.067	0.445	0.082	0.361	0.032	0.704
	Lselectin (ng/ml)†	-0.171	0.064	-0.048	0.583	-0.031	0.724
	sE-selectin (ng/ml)†	-0.056	0.531	-0.137	0.122	0.083	0.327
	SVCAM-1 (ng/ml)	-0.067	0.443	-0.069	0.424	-0.067	0.443
	SICAM-1 (ng/ml)†	-0.097	0.269	-0.107	0.238	-0.055	0.506
Girls		n = 169		n = 176		n = 175	
		ß	p	ß	p	ß	p
HOMA-IR†	CRP (mg/l)†	0.100	0.198	0.003	0.966	-0.046	0.550
	IL-6 (pg/ml)†	0.247	0.007	-0.048	0.579	-0.048	0.557
	TNF-α (pg/ml)†	0.208	0.014	0.112	0.191	-0.077	0.369
	C3 (g/l)	0.271	0.001	0.213	0.008	0.163	0.029
	C4 (g/l)	0.205	0.009	0.048	0.551	0.026	0.739
	Lselectin (ng/ml)†	0.012	0.883	-0.053	0.519	0.029	0.702
	sE-selectin (ng/ml)†	-0.159	0.062	-0.072	0.373	0.160	0.044
	sVCAM-1 (ng/ml)	0.100	0.200	-0.003	0.972	0.100	0.200
	sICAM-1 (ng/ml)†	-0.074	0.368	-0.161	0.048	0.201	0.015

BMI: Body mass index; HOMA: Homeostasis model assessment; CRP: C-reactive protein; IL-6: Interleukin 6; TNF-α: Tumor necrosis factor alpha; C3 and C4: Complement factors; sVCAM-1: Soluble vascular cell adhesion protein 1; sICAM-1: Soluble intercellular adhesion molecule 1. †Biomarkers log-transformed.

ability for skinfold thicknesses and circumferences measurements was always greater than 90% (16).

BLOOD ANALYSIS

Blood withdrawal was performed after 12 hours overnight fast. C-reactive protein (CRP) levels were quantified by immunoturbidimetry (AU 2700, Olimpus, Rungis, France). Adolescents with higher CRP concentrations than 10 mg/dl were excluded. Serum C3 and C4 complement factors were analyzed by nephelometry (Behring Diagnostics, CA, USA). The coefficient of variation (inter-assay precision) was 1.9% for CRP, 1.4% for C3, and 1.2% for C4. Serum cytokines IL-6 and TNF-α were determined using the High Sensitivity Human Cytokine MILLIPLEX™ MAP kit (Millipore Corp., Billerica, MA, USA) and collected by flow cytometry (Luminex-100 v.2.3, Luminex Corporation, Austin, TX, USA). The intra- and inter-assay precision CVs were: 3.5% and 4.5%, respectively, for IL-6; and 3.5% and 3.8%, respectively, for TNF-α. Detection limits (sensitivity) for all the analyses were 0.007 mg/l for CRP, 0.01 g/l for C3, 0.002 g/l for C4, 0.1 pg/ml for IL-6, and 0.05 pg/ml for TNF-α. Undetectable values were recorded as the specific detection limit. Children with values of 0.12 pg/ml for TNF-α and IL-6 were excluded as it was an assigned value for children with concentration values under the detection curve. The serum adhesion molecule sL-selectin was analyzed through commercial ELISA kits (Diaclone, France); the sensitivity of this kit was less than 1 ng/mL for L-selectin. The analyzed by Universal Microplate Spectophotometer (Power WaveTM XS, Biotek® Instruments, INC USA).

The multiplex assay kit was used to detect for the simultaneous quantification of the molecules sE-Selectin, sVCAM-1, sICAM-1, in serum. The samples were analyzed by citometry (Luminex® 100). The sensitivities of these assays were: Min DC 0.079 ng/ml for sE-Selectin, 0.016 ng/ml for sVCAM-1 and 0.009 ng/ml for sICAM-1. The intra-assay CVs were 11.2% for sE-Selectin, 4.5% for sVCAM-1 and 7.9% for sICAM-1.

Table VI. Linear regression with HOMA-IR as dependent variable and the markers of inflammation as independent variables for each category of fat mass index (adjust: center)

		FMI I		FMI II		FMI III	
Boys		n = 132		n = 137		n = 137	
		ß	p	ß	p	ß	p
HOMA-IR†	CRP (mg/l)†	-0.095	0.309	0.048	0.594	-0.074	0.419
	IL-6 (pg/ml)†	0.121	0.243	0.085	0.424	-0.024	0.795
	TNF-α (pg/ml)†	0.051	0.623	-0.059	0.557	0.108	0.231
	C3 (g/l)	-0.065	0.466	0.002	0.983	0.187	0.040
	C4 (g/l)	-0.057	0.527	-0.052	0.581	0.056	0.527
	Lselectin (ng/ml)†	-0.108	0.259	0.001	0.990	-0.108	0.259
	sE-selectin (ng/ml)†	-0.107	0.234	-0.038	0.685	0.076	0.395
	SVCAM-1 (ng/ml)	-0.055	0.549	-0.050	0.607	-0.053	0.552
	SICAM-1 (ng/ml)†	-0.127	0.170	-0.019	0.835	-0.079	0.375
Girls		n = 166		n = 176		n = 175	
		ß	p	ß	p	ß	p
HOMA-IR†	CRP (mg/l)†	0.058	0.455	-0.027	0.723	0.045	0.555
	IL-6 (pg/ml)†	0.190	0.035	0.013	0.885	0.014	0.861
	TNF-α (pg/ml)†	0.153	0.076	0.196	0.021	-0.056	0.503
	C3 (g/l)	0.274	0.001	0.147	0.073	0.130	0.090
	C4 (g/l)	0.146	0.062	0.039	0.629	0.054	0.499
	Lselectin (ng/ml)†	-0.061	0.438	0.018	0.822	-0.061	0.438
	sE-selectin (ng/ml)†	-0.102	0.246	-0.056	0.499	0.028	0.724
	sVCAM-1 (ng/ml)	-0.042	0.604	-0.019	0.816	0.079	0.323
	sICAM-1 (ng/ml)†	-0.058	0.485	-0.037	0.658	-0.016	0.853

FMI: Fat mass index; HOMA: Homeostasis model assessment; CRP: C-reactive protein; IL-6: Interleukin 6; TNF-α: Tumor necrosis factor alpha; C3 and C4: Complement factors; sVCAM-1: Soluble vascular cell adhesion protein 1; sICAM-1: Soluble intercellular adhesion molecule 1. †Biomarkers log-transformed.

STATISTICAL ANALYSIS

Analyses were performed separately for boys and girls. Normality of distributions was assessed with the Kolmogorov-Smirnov test. Glucose, insulin, HOMA, CRP, IL6, TNF-α, L-selectin, sE-selectin and sICAM were normalized by natural logarithm transformation. t-tests were used for comparisons of continuous variables by sex.

For BMI, FMI and WC, the standardized regression residuals by age were calculated and then categorized in tertiles.

Analysis of variance (ANOVA) with Bonferroni post-hoc correction was applied to compare mean differences of each biomarker between the categories of each indicator of body composition.

Finally, multiple linear regressions were performed to assess the association between HOMA-IR (dependent) and each marker of inflammation (independent) adjusted by center within body composition tertiles (BMI, FMI or WC). The effect of the city of residence was controlled in all regressions by using dummy variables. The dependent variable, HOMA-IR, was log-transformed. For markers of inflammation that were also log-transformed, results were expressed as percentage of change of the geometrical mean of the HOMA-IR for 10% increases of the corresponding biomarker. If the independent variable was normally distributed, results were expressed as percentage of change of the geometrical mean of HOMA per unit increase of the corresponding biomarker.

Data were managed and analyzed with the IBM SPSS Statistics v.21 (IBM Corp., New York, NY, USA, 2012).

RESULTS

Descriptive characteristics are presented in table I. Boys were significantly taller and heavier than girls and had significantly higher values of waist circumference, whereas girls had higher levels of FMI. Regarding the biomarkers measured, boys had significantly higher concentrations of glucose, HOMA, IL-6, TNF-α, L-selectin, sE-selectin, sVCAM-1 and sICAM-1 than girls, while girls had significantly higher levels of insulin and C3 than boys.

Table VII. Linear regression with HOMA-IR as dependent variable and the markers of inflammation as independent variables for each category of waist circumference (adjust: country)

		FMI I		FMI II		FMI III	
Boys		n = 145		n = 149		n = 148	
		ß	p	ß	p	ß	p
HOMA-IR†	CRP (mg/l)†	-0.083	0.348	0.003	0.976	-0.066	0.458
	IL-6 (pg/ml)†	0.140	0.149	-0.033	0.724	-0.001	0.993
	TNF-α (pg/ml)†	-0.071	0.454	0.013	0.889	0.073	0.418
	C3 (g/l)	-0.076	0.404	0.064	0.431	0.233	0.007
	C4 (g/l)	-0.084	0.345	0.107	0.207	0.007	0.936
	Lselectin (ng/ml)†	-0.086	0.326	-0.109	0.195	-0.075	0.404
	sE-selectin (ng/ml)†	-0.204	0.020	0.013	0.874	0.089	0.299
	SVCAM-1 (ng/ml)	-0.153	0.092	0.038	0.655	-0.056	0.520
	SICAM-1 (ng/ml)†	-0.145	0.103	-0.041	0.649	-0.067	0.426
Girls		n = 173		n = 167		n = 174	
		ß	p	ß	p	ß	p
HOMA-IR†	CRP (mg/l)†	0.043	0.564	-0.015	0.855	0.046	0.546
	IL-6 (pg/ml)†	0.227	0.007	-0.008	0.930	0.018	0.820
	TNF-α (pg/ml)†	0.142	0.087	0.203	0.023	-0.058	0.484
	C3 (g/l)	0.226	0.004	0.100	0.236	0.284	< 0.001
	C4 (g/l)	0.231	0.002	-0.062	0.459	0.091	0.243
	Lselectin (ng/ml)†	-0.036	0.633	0.030	0.725	0.027	0.740
	sE-selectin (ng/ml)†	-0.117	0.146	-0.036	0.675	0.035	0.662
	sVCAM-1 (ng/ml)	-0.099	0.196	0.056	0.494	-0.003	0.968
	sICAM-1 (ng/ml)†	-0.083	0.295	-0.072	0.369	0.020	0.822

WC: Waist circumference; HOMA: Homeostasis model assessment; CRP: C-reactive protein; IL-6: Interleukin 6; TNF-α: Tumor necrosis factor alpha; C3 and C4: Complement factors; sVCAM-1: Soluble vascular cell adhesion protein 1; sICAM1: Soluble intercellular adhesion molecule 1. †Biomarkers log-transformed.

Results for the ANOVA are presented in tables II, III and IV. Significant differences were found in mean values of the measured biomarkers across categories of each marker of body composition, by sex. When BMI increased, the mean concentrations of insulin and HOMA in boys, CRP in girls and C3 and C4 in both sexes significantly increased ($p < 0.001$). In addition, CRP and sE-selectin in boys, and insulin, HOMA and sVCAM-1 in girls presented also significantly different mean values across categories. Also, when FMI increased, the mean levels of glucose in boys, CRP in girls and insulin, HOMA, C3 and C4 in both sexes, significantly increased ($p < 0.001$). Additionally, CRP in boys and sVCAM-1 presented significant differences ($p < 0.001$) across categories. Finally, when WC increased, the mean level of insulin in boys and CRP in girls increased ($p < 0.001$); HOMA, CRP, C3 and C4 significantly increased ($p < 0.001$) in both sexes. Mean values of sVCAM-1 presented as well significant differences ($p = 0.024$) between waist circumference categories, but only in girls.

Tables V, VI and VII show results of the linear regression between HOMA-IR and the markers of inflammation for each body composition index. Table V shows the results of the linear regression by tertiles of BMI. In the lowest tertile, in girls, HOMA-IR increased in a 31.1% and 22.7% per each additional g/l of C3 and C4, respectively, and in 2.0% and in 2.4% when TNF-α and IL-6 increases by 10%, respectively. Also, in the second tertile of BMI, we expect about 23.7% increase in HOMA-IR per each additional g/l of C3 and about 14.8% decrease in HOMA-IR per each additional ng/ml of sICAM-1. Moreover, in the highest tertile of BMI, in girls, HOMA-IR increased by 22.3% and 17.7% per each additional ng/ml of sICAM-1 and 1.5% when sE-selectin increases by 10%. Significant associations between HOMA-IR and C3 in the highest tertile of BMI in both sexes were found: HOMA-IR increased in a 17.7% per each additional g/l of C3 in girls and 27.7% per each additional g/l of C3 in boys.

Table VI presents the results of the linear regression between HOMA-IR and the markers of inflammation by tertiles of FMI. In the lowest tertile, HOMA-IR increased in 1.8% when IL-6 increased 10% and in 31.5% per each additional g/l of C3 in girls. Furthermore, in the second tertile of FMI, we expect about 1.9% increase

in HOMA-IR when TNF-α increases by 10% in girls. Moreover, in the highest tertile of BMI, HOMA-IR increased by 20.5% per each additional g/l of C3 in boys.

Finally, table VII shows the results of the linear regression by tertiles category of WC. In the lowest tertile of WC, HOMA-IR decreased 1.9% when sE-selectin increased 10% for boys; while in girls, HOMA-IR increased 2.2% when IL-6 increased 10% and 25.3% and 25.9% per each additional g/l of C3 and C4, respectively. In the second tertile of waist circumference, HOMA-IR increases 1.9% when TNF-α increases 10% in girls. In the highest tertile of waist circumference, significant associations between C3 were observed in both sexes: HOMA-IR increased 24.9% and 32.8% per each additional g/l of C3 in boys and girls, respectively.

DISCUSSION

The main finding of this study is the consistent significant association between C3 complement factor and insulin resistance, irrespective of total and abdominal fat deposition. To our knowledge, this is the first study assessing the relationship between different inflammatory markers and insulin resistance in a relatively large sample of European adolescents from different cities.

In our sample, mean concentrations of glucose, insulin, HOMA and some inflammatory markers such as CRP or C3 and C4 were significantly higher in the highest tertile of each marker of body composition. Even in children, obesity has been related to low-grade inflammation (17). Adipocyte hypertrophy has been associated with HOMA- insulin resistance and inflammation in obese children (11). Results from our study also support the hypothesis that, even in adolescence, there is a link between adiposity, glucose metabolism and inflammation as some of these biomarkers were increased in the highest levels of total and abdominal adiposity.

Furthermore, in the present study there were linear associations between some inflammatory markers and HOMA as dependent variable, by categories of body composition indices. Previous studies suggest that inflammatory markers can interfere with insulin action by directly inhibiting insulin receptors (18). However, there are some discrepancies between studies regarding the relationship between inflammation and insulin resistance in adolescents (12,13). A recent study in obese adolescents failed to show a significant relation between obesity and IR mediated by low-grade inflammation using traditional inflammatory markers (19).

Although previous studies have associated some traditional inflammatory biomarkers with the development of diabetes or insulin resistance (20-22), we did not find any relationship between the traditional inflammatory markers and the HOMA for adolescents with the highest levels of BMI, FMI and WC. Out of all the inflammatory markers measured in the present study, only C3 was consistently related with insulin resistance, measured by HOMA, especially in the highest tertiles of total and abdominal adiposity, except FMI in girls. Our results are in line with those of some previous studies. Serum C3 was the strongest inflammatory marker related to insulin resistance in a study in an elderly population (23). This complement factor is an emerging cardio metabolic risk factor related to some comorbities such as type 2 diabetes (24). In a sample of Spanish adolescents, serum C3 levels were associated with body fat, especially with abdominal obesity (25), and were higher in adult subjects with insulin resistance (26). A previous study performed in adults showed that low-grade inflammation and insulin resistance might represent two independent pathways by which body fat leads to elevated C3 (27). However, it seems that changes in C3 levels over a 7-year follow-up period were associated with changes in several measures of insulin resistance and that baseline C3 was associated with the 7-year incidence of type 2 diabetes (28). Although the main production of the C3 is in the liver, C3 is also synthesized by activated macrophages (29) and adipocytes (30) as an inflammatory cytokine or an adipokine. Its hepatic production is induced by cytokines, such as interleukin-6 and TNF-α (31), which may interfere with insulin receptor functioning and cause insulin resistance (32). In addition, the complement system is a regulator of both the innate and adaptive system and, as a part of the inflammatory response, could also contribute to insulin resistance. We also found associations between insulin resistance and C4 complement factor in girls. Production of C4 depends, as production of C3, on proinflammatory cytokines released by the adipose tissue such as tumor necrosis factor alpha (TNF-α) or interleukin 6 (IL-6) (7). However, literature on the relationship between C4 and insulin resistance is scarce.

In our study, associations between TNF-α and insulin resistance were found in girls, in the lowest tertiles of body fat composition but not in the highest tertiles. TNF-α interferes negatively with the insulin signaling pathway, but also induces insulin resistance indirectly by altering adipocyte differentiation and adipocyte lipid metabolism (10).

This study has strengths as well as some limitations. First, its cross-sectional design, which does not allow drawing conclusions on causality; however, in adults it was observed that C3 was associated with the development of insulin resistance in a longitudinal study (28). Furthermore, the study is limited by the fact that blood samples only reflect inflammation, glucose and insulin concentrations at a given specific time point. On the other hand, the strengths of the study are: the use of traditional and non-traditional inflammatory markers that could be also involved in the pathogenesis of insulin resistance, and the use of standardized and harmonized information on body composition of adolescents from nine European countries.

CONCLUSIONS

In conclusion, results from the current study show that there is an association between C3 and HOMA in a multicenter sample of adolescents, especially in those with high levels of total and abdominal adiposity. To avoid chronic insulin resistance, efforts should be made to reduce deposition of total and abdominal fat in obese children and adolescents. This may impact on the reduction of serum C3 concentrations and prevent future insulin-related

diseases such as diabetes. Longitudinal studies assessing this relationship between C3 and insulin resistance are needed to confirm these results.

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FG, MGG, AK, KW, JD, YS, DM, AM and LAM designed and directed the study; SGM, LED and AM conducted and directed the laboratory analysis; EMGG performed the statistical analysis and wrote the manuscript. EMGG, LGM, JS, DM, FA, FG, AA, AK, KW, YM, AS, MGG, SGM, LED, CL, JD, AM and LAM critically reviewed the manuscript.

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