Inflammation in metabolically healthy and metabolically abnormal adolescents: the HELENA study.

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Conflict of interest

No conflicts of interest were declared

ABSTRACT

Background and aims: Inflammation may influence the cardio-metabolic profile which relates with the risk of chronic diseases. To assess the inflammatory status by metabolic health/body mass index (BMI) category and to assess how inflammation can predict the cardio-metabolic profile in European adolescents, considering BMI.

Methods and results: 659 adolescents (295 boys) from a cross-sectional European study were included. Adolescents were classified by metabolic health based on age- and sex-specific cut-off points for glucose, blood pressure, triglycerides, high density cholesterol and BMI. C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), interleukin (IL-6), complement factors (C3, C4) and cell adhesion molecules were assessed.

Results: Metabolically abnormal (MA) adolescents had higher values of C3 ($p<0.001$) and C4 ($p=0.032$) compared to those metabolically healthy (MH). C3 concentrations significantly increased with the deterioration of the metabolic health and BMI ($p<0.001$). Adolescents with higher values of CRP had higher probability of being in the overweight/obese-MH group than those allocated in other categories. Finally, high C3 and C4 concentrations increased the probability of having an unfavorable metabolic/BMI status.

Conclusions: Metabolic/BMI status and inflammatory biomarkers are associated, being the CRP, C3 and C4 the most related inflammatory markers with this condition. C3 and C4 were associated with the cardio-metabolic health consistently.

Keywords: Inflammation, metabolic health, metabolic syndrome, inflammatory biomarkers, adolescents.

Introduction

Obesity is characterized by an increase of the adipose tissue and this condition is often related with cardio-metabolic risk factors and inflammation [1]. Body mass index (BMI), the most used anthropometric index to assess obesity, is a strong predictor of CVD mortality [2,3]. However, literature suggests that some subjects with obesity could present a normal or healthy metabolic profile: the metabolically healthy obesity (MHO), in contrast to the so-called metabolically abnormal obesity (MAO) [4,5]. Currently, there is a lack of consensus on the definition of the MHO; usually, it is based on the definition for the metabolic syndrome (MS). In adults, the prevalence of MHO ranges from 10 to 40% [6]. In youth, there is a lack of information on prevalence due to the different definitions used. Ortega et al. [5] recently proposed a definition for youth and adults, aiming to harmonize the MHO definition, the latter based on the one proposed by Jolliffe and Janssen [7].

A review on the characterization of the MHO individuals concludes that low concentrations of inflammatory markers, low visceral adipose tissue deposition and preserved insulin sensitivity contribute to the MHO phenotype [8]. Furthermore, the potential addition of some of these markers to the definition of the MHO has also been proposed [9]. Evidence suggest that inflammation has a key role in the origin and development of metabolic disorders [1] and atherosclerosis [10]. New biomarkers have been related with inflammation. For instance, complement factors C3 and C4, depends on pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) or interleukin 6 (IL-6) [11]. Also, cell adhesion molecules have been suggested as markers for atherosclerosis and their concentrations are elevated during inflammatory processes [12].

A previous study showed that MHO subjects with low CRP levels had a trend towards lower coronary heart disease risk than those MHO with high CRP levels and a similar risk to that of healthy non-obese subjects [13]. This study suggests that CRP could help to identify those MHO individuals who are at low coronary heart disease risk. However, one study showed similarly adverse cardio metabolic profile in MHO and MAO adults [14].

Adolescents usually have lower prevalence of obesity in comparison with adults. Specifically, the HELENA sample presented a 3.8 % of obesity in boys and 7.3% in girls [15]. Therefore, the study of the metabolically health status, or the metabolic health (MH) in overweight/obese adolescents, instead of the MHO seems to be a more feasible approach in this population, as it has been previously done in the same study[16].

To our knowledge there are no studies measuring inflammation in metabolically healthy and/or metabolically abnormal overweight/obese adolescents. The aims of this study are: to assess the inflammatory status by metabolic health/body mass index (BMI) category and, on the other hand, to assess how the increases of the inflammatory concentrations can predict the cardio-metabolic health profile in European adolescents, considering also the weight status.

Methods

Study design

A cross-sectional multi-center study (n=3528), the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) study, was conducted between 2006 and 2007 in 10 European cities: Athens and Heraklion, Dortmund, Ghent, Lille, Pecs, Rome, Stockholm, Vienna and Zaragoza. Design and general procedures of the study have been previously described[17].

This study was performed according to the ethical guidelines of the Edinburgh revision of Declaration of Helsinki (2000). In addition, the local Ethics Committees of each center approved the protocol. Written informed consents were obtained from the adolescents and their caregivers.

Study sample

Out of the total HELENA sample, blood collection was randomly performed in approximately a third of the total sample (n=1089, 31%). 659 participants (295 boys and 364 girls) met the inclusion criteria of having measured the metabolic biomarkers for the definition of the healthy/abnormal metabolic status and the inflammatory biomarkers.

Physical measurements

Weight was measured with an electronic scale (SECA 861, Seca Ltd) and height were measured with a stadiometer (SECA 225, Seca Ltd) [18]. In addition, body mass index (BMI) was calculated. Systolic and diastolic blood pressure was measured with an automatic oscillometric device (Omron M6). The lowest value of the two measurements, taken with a difference of 5 minutes, was recorded.

Physical activity

Levels of physical activity were self-reported using the International Physical Activity Questionnaire for Adolescents (IPAQ-A) [19]. School-related physical activity (including physical education classes and breaks), transportation, housework, and activities during leisure time were included in this questionnaire.

Blood analysis

Detailed description of blood sampling procedures has been published [20]. Blood withdrawal was performed after 10 hours overnight fast. Serum triglycerides, glucose and high density lipoprotein were measured enzymatically in fresh seru, on the Dimension RxL clinical chemistry syste (Dade Behring, Schwalbach, Germany) using the manufacturer's reagents and instructions. The assessment methods for the CRP, C3 and C4 have been previously described elsewhere [21]. 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 coefficients of variability (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 analysed through commercial ELISA kit (Diaclone, France), the sensitivities of this kit was less than 1 ng/mL for L-selectin, 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 following molecules sE-Selectin, sVCAM-1, sICAM-1, in serum. The samples were analysed by cytometry (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.

Definition of MHO and MAO

Ortega et al. [5], recently suggested an harmonization for the definition of MHO in youth, based on the one by Jolliffe and Janssen for metabolic syndrome [7]. In this study, age- and gender- specific cut-off points for each marker of metabolic syndrome were developed except for the glucose criteria, considered a marker when the value was higher than 5.6 mmol/l or 100mg/dl. MHO is defined as 1) being obese/overweight according to the BMI cut-off points for youth by Cole et al. [22] and 2) no criterion of the following for the metabolic syndrome: high serum triglycerides (≥150mg/dL), fasting glucose(≥100mg/dL), systolic or diastolic blood pressure (Systolic ≥ 130 and diastolic ≥85 mm Hg) and low high density lipoprotein cholesterol (<40mg/dL in men and <50mg/dL in women). MAO was defined when 1 or more of the previous criteria were met. Waist circumference was excluded as criterion since high waist circumference is expected in overweight/obese individuals.

In contrast, metabolically healthy (MH) are those who accomplish having no criterion of the metabolic syndrome while metabolically abnormal (MA) are those with 1 or more than 1 criterion, both independently of their BMI status.

Statistical analysis

Normality of distributions was assessed with the Kolmogorov–Smirnov test. CRP, IL-6, TNF-α, L-selectin, sE-selectin and sICAM were normalized by natural logarithm transformation. T-tests were used for comparisons of continuous variables and chi-square test was performed to test the differences between categories.

Two-way analysis of covariance (ANCOVA) with Bonferroni post-hoc correction was applied to compare mean differences of each biomarker between these categories of metabolic health/BMI status by sex. The confounders included in this analysis were age and the self-reported moderate-to-vigorous physical activity.

Finally, four categories combining BMI status and metabolic health were created: normal weight MH, normal weight MA, overweight/obese-MH and overweight/obese-MA, and then multinomial logistic regression was performed to assess the association between these categories of metabolic health (dependent) and each marker of inflammation (independent) adjusting by age, sex and moderate-to-vigorous physical activity. Results for the C3 and C4 were expressed in change for 0.1 g/L.

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 1**. Boys were more frequently allocated in the MA group (50.7%) than girls (49.3%). Also, those adolescents in the MA group had higher mean value of BMI ($p<0.001$). Differences between percentages of adolescents by BMI status were also found. Adolescents with a MA profile had higher values of all the metabolic markers included in the definition for metabolic health: blood pressure (systolic and diastolic), glucose, triglycerides and lower concentrations of high density lipoprotein cholesterol in comparison with those in the MH group (all $p<0.001$). Regarding the inflammatory biomarkers measured, MA adolescents presented higher concentrations of CRP ($p=0.002$), C3 ($p<0.001$) and C4 ($p=0.032$) than those classified as MH.

In the ANCOVA (**Table 2**), mean biomarkers concentrations were different according to the categories of BMI status/metabolic health. In boys (Table 2), significant mean differences were found in CRP ($p=00.001$), C3 ($p<0.001$) and C4 ($p<0.001$). C3 concentrations increased with the deterioration of the metabolic health and the BMI status, being higher in the last group, i.e: overweight/obese-MA adolescents. Overweight/obese-MH adolescents had the highest value of CRP and C4 in boys. In girls, significant mean differences were found for C3 ($p<0.001$) being the highest mean concentration value found in the group of overweight/obese-MA adolescents.

The **supplementary table 1** shows the results for the multinomial logistic regression. This table presents the probability of being in a more unfavorable group of BMI/metabolic health by the increase of the concentration of the inflammatory biomarkers. The probability of being in the normal weight-MA or overweight/obese-MH increased in a 17% and in a 58%, respectively, and in a 52% for the overweight/obese MA per each increase of mg/L of the CRP. Per each additional 0.1

g/L in C3, the probability of being in the normal weight-MA category was 23%, in the overweight/obese a 51% and in the overweight/obese-MA a 62%.

In relation to C4, per each 0.1 g/L increases, the probability of being in the normal weight-MA increased in a 23%; and in a 205% and 126% of being overweight/obese-MH and overweight/obese-MA, respectively. Additionally, per each mg/L increase in sVCAM-1, the probability of being overweight/obese-MH decreased in a 1%.

Finally, **Figure 1** present the significant results found in the multinomial logistic regression.

Discussion

Findings from this study suggest that differences in inflammatory biomarkers' concentrations were found between MH and MA adolescents. Also, the probability of being in an unfavorable metabolic/BMI status increased with increasing C3 concentrations.

The prevalence of adults with obesity categorized as MHO, based on 10 population-based cohort studies in 7 European countries has been estimated as 12.1% [5,23] . In youth, information on MHO is scarce, due to the lack of agreed definition in this population group. In our sample, 0.8% of the adolescents were classified as MHO while 6.8% were classified as MH/overweight-obese. This low prevalence of MHO could be due to the low sample size of this study and the different definitions used for MHO. In previous studies, prevalence of MH overweight/obese and obese adolescents ranged from 6 to 68% [24,25]. In our study, 2.7% of the adolescents were allocated in the MAO group. This highlights the importance of a common definition already in adolescence to identify those subjects with a favorable metabolic profile.

A previous study on adult population measured cardio-metabolic risk factors and inflammation in MHO and MAO individuals and found similar adverse inflammatory profile in both groups [20]. However, when we assessed some inflammatory marker concentrations by groups, combining cardio-metabolic health and BMI status by sex, differences in mean concentrations were found. In both sexes, MHO had significantly higher CRP concentrations than their counterparts. This result contrast with previous literature as MHO seems to present a favorable inflammatory profile [4,13,26]. Moreover, in our sample, adolescents with high values of CRP, C3, C4 and sVCAM-1 had higher probability of being in the overweight/obese-MH group in comparison with the normal weight-MH. Similar gene expression of visceral adipose tissue and liver has been found in a previous study, from MHO and MAO patients, with no differences in CRP levels [14]. Also, a comparative study stated that the associations between inflammatory markers and MHO depend on the definition used [27].

However, the most consistent results across all the analysis were found for the C3. Overweight/obese-MA subjects had the highest concentrations of the C3 complement factor. In addition, high C3 concentrations increased the probability of being in an unfavorable metabolic/BMI status. C3 is recognized as a cardiovascular risk factor as it has been related with an increased likelihood of future cardiovascular heart disease (CHD) [28]. It has also been associated with metabolic disorders, like adiposity, dyslipidemia, insulin resistance, liver dysfunction and diabetes [29]. Results from the present study suggest that this relationship between C3 and metabolic disorders is found already in adolescence, especially on those individuals with the worse metabolic/BMI status. A previous study in adult population found that C3 concentrations were consistently lower in the MH individuals [26]. Therefore, C3 could be an important marker for the characterization of the metabolic health. Also C4 was associated with the probability of being in an unfavorable metabolic/BMI status. C4, along with C3, has been related with metabolic syndrome [30] and this relationship could be due to their involvement in the development of visceral adiposity in children and adolescence.

This study has strengths as well as some limitations. First, the cross-sectional design does not allow establishing causality. Also, blood samples only reflect inflammatory status at one-time point. The low sample size could be also a limitation. In contrast, this study has some strengths: the use of traditional and non-traditional biomarkers to reflect the inflammatory status and the use of a definition for MH based on age- and sex-specific cut-off points as the basis to establish health risk in adolescents, which is more appropriate from a clinical perspective. Finally, the use of standardized and harmonized information of adolescents from 9 European countries is another strength.

In conclusion, these results show that there is an association between some inflammatory biomarkers and metabolic/BMI status. C3 and C4 seem to be emerging biomarker related to the cardio-metabolic health, already in adolescence. Likewise, the increase of some inflammatory markers increased the probability of being in an overweight/obese-MH status. A unique definition for metabolic health is necessary to corroborate these results. Further longitudinal studies are needed to understand how these inflammatory markers influence the development of future cardiovascular diseases.

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Table 1. Characteristics of the study sample by cardio-metabolic health status.

	MH (n=383)	MA (n=276)	
	Mean±SD	**Mean**±SD	**p-value**
Age (years)	14.59±1.12	14.96±1.20	**<0.001**
Sex	**N(%)**	**N(%)**	
Boys	155 (40.5)	140 (50.7)	**0.009**
Girls	228 (59.5)	136 (49.3)	
BMI (kg/m^2)	20.17±2.85	22.08±3.63	**<0.001**
BMI group by Cole et al.	**N(%)**	**N(%)**	
Normal weight	338 (88.3)	194 (70.3)	**<0.001**
Overweight/Obese	45 (11.7)	82 (29.7)	
MVPA	779.03±570.08	784.95±607.99	0.898
Blood pressure			
Systolic (mm Hg)	110.84±8.68	124.09±13.83	**<0.001**
Diastolic (mm Hg)	61.88±7.28	68.13±8.97	**<0.001**
Glucose (mg/dL)	89.20±5.84	92.50±8.18	**<0.001**
Triglycerides (mg/dL)	62.15±22.31	82.90±47.76	**<0.001**
HDL-cholesterol (mg/dL)	59.09±9.00	51.59±11.22	**<0.001**
CRP* (mg/L)	0.66±1.04	0.91±1.28	**0.002**
IL6* (pg/mL)	24.40±32.99	19.59±24.68	0.085
TNF-α * (pg/mL)	6.65±5.25	6.16±4.77	0.141
C3 (g/L)	1.09±0.15	1.15±0.17	**<0.001**
C4 (g/L)	0.19±0.06	0.21±0.06	**0.032**
L-Selectin* (ng/mL)	3624.85±1583.08	3505.61±1355.87	0.985
sE-Selectin* (ng/mL)	37.19±18.89	36.97±20.95	0.335
sVCAM-1 (ng/mL)	1293.41±388.97	1270.42±413.48	0.472
sICAM-1* (ng/mL)	155.43±75.20	173.22±185.95	0.197

MH: metabolically healthy (no criterion of the following: high serum triglycerides (≥150mg/dL), fasting glucose(≥100mg/dL), systolic or diastolic blood pressure (Systolic ≥ 130 and diastolic ≥85 mm Hg) and low high density lipoprotein cholesterol (<40mg/dL in men and <50mg/dL in women); MA: metabolically abnormal (1 or more criteria of the following: high serum triglycerides (≥150mg/dL), fasting glucose(≥100mg/dL), systolic or diastolic blood pressure (Systolic ≥ 130 and diastolic ≥85 mm Hg) and low high density lipoprotein cholesterol (<40mg/dL in men and <50mg/dL in women); SD: standard deviation; BMI: Body mass index; MVPA: moderate to vigorous physical activity; HDL-cholesterol: High density lipoprotein; 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. *Log-trangsformed.

Table 2. Mean differences of the inflammatory biomarkers by group of metabolic/BMI status in boys and girls. Age and moderate-to-vigorous activity were used as confounders.

	Normal weight		**Overweight/Obese**		
Boys	**MH (n=134)**	**MA (n=98)**	**MH (n=21)**	**MA (n=42)**	**p-value**
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	
CRP (mg/L)*	0.58±0.77	0.79±1.16	1.23±1.98[a]	1.01±1.16	**0.001**
IL6 (pg/mL)*	27.62±35.61	22.65±26.91	34.34±58.25	24.53±31.31	0.176
TNF-α (pg/mL)*	7.99±7.19	7.03±6.85	7.17±5.22	6.74±.3.44	0.381
C3 (g/L)	1.06±0.13	1.10±0.16	1.17±0.14[a]	1.20±0.15[a]	<**0.001**
C4 (g/L)	0.18±0.06	0.19±0.05	0.23±0.07[a]	0.22±0.07[a]	<**0.001**
L-Selectin (ng/mL)*	3785.64±1618.29	3607.07±1344.40	3782.98±2120.99	3369.90±1280.04	0.908
sE-selectin (ng/mL)*	39.39±18.51	38.39±19.12	43.45±23.52	47.45±31.24	0.649
sVCAM-1(ng/mL)	1432.63±370.18	1370.99±404.49	1227.95±309.48	1408.76±398.84	0.388
sICAM-1(ng/mL)*	169.70±69.40	174.10±97.17	167.71±75.09	239.21±416.15	0.886
Girls	**MH (n=204)**	**MA (n=96)**	**MH (n=24)**	**MA (n=40)**	**p-value**
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	
CRP (mg/L)*****	0.63±1.04	0.87±1.29	0.97±1.06	1.16±1.16	0.070
IL6 (pg/mL)*****	21.32±28.24	16.13±21.65	23.89±22.88	15.20±14.89	0.179
TNF-α (pg/mL)*****	5.78±3.52	5.64±3.00	5.65±2.44	4.66±2.06	0.897
C3 (g/L)	1.09±0.16	1.16±0.18[a]	1.17±0.13 [a]	1.21±0.18 [a]	**0.001**
C4 (g/L)	0.19±0.06	0.21±0.06	0.24±0.08	0.22±0.05	0.089
L-Selectin (ng/mL)*****	3454.23±1448.89	3401.14±1337.38	4039.05±1851.72	3647.63±1514.56	0.802
sE-selectin (ng/mL)*****	34.80±18.88	31.93±16.32	39.66±13.99	34.38±17.57	0.508
sVCAM-1(ng/mL)	1225.61±392.78	1155.70±387.10	1149.72±310.45	1150.92±292.92	0.168
sICAM-1(ng/mL)*****	144.37±77.22	153.61±104.71	158.95±78.20	147.91±58.67	0.233

MH: metabolically healthy (no criterion of the following: high serum triglycerides (≥150mg/dL), fasting glucose(≥100mg/dL), systolic or diastolic blood pressure (Systolic ≥ 130 and diastolic ≥85 mm Hg) and low high density lipoprotein cholesterol (<40mg/dL in men and <50mg/dL in women); MA: metabolically abnormal (1 or more criteria of the following: high serum triglycerides (≥150mg/dL), fasting glucose(≥100mg/dL), systolic or diastolic blood pressure (Systolic ≥ 130 and diastolic ≥85 mm Hg) and low high density lipoprotein cholesterol (<40mg/dL in men and <50mg/dL in women); MA: metabolically abnormal; SD: standard deviation; 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. *Log-transformed. Bonferroni: [a]p<0.05 ref. normal weight-MH.

Figure Legends

Figure 1: Significant results of the multinomial logistic regression to assess the association between inflammatory biomarkers and BMI/metabolic status adjusted by sex, tanner and moderate-to-vigorous physical activity. (**) C3 and C4 present changes per 0.1 g/L. *p<0.05. Normal weight-MH was set as reference group.