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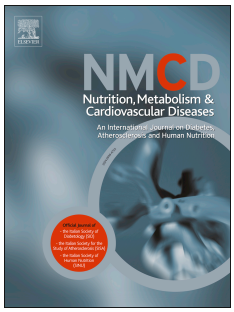

Ideal cardiovascular health and inflammation in European adolescents: the HELENA study

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Ideal cardiovascular health and inflammation in European adolescents: the HELENA study.

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ABSTRACT

Background and aims: Inflammation plays a key role in atherosclerosis and this process seems to appear in childhood. The ideal cardiovascular health index (ICHI) has been inversely related to atherosclerotic plaque in adults. However, evidence regarding inflammation and ICHI in adolescents is scarce. The aim is to assess the association between the ICHI and inflammation in European adolescents.

Methods and results: 543 adolescents (251 boys and 292 girls) from the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study, a cross-sectional multi-center study including 9 European countries, were measured. C-reactive protein (CRP), complement factors C3 and C4, leptin and white blood cell counts were used to compute an inflammatory score. Multilevel linear models and multilevel logistic regression were used to assess the association between ICHI and inflammation controlling by covariates. Higher ICHI was associated with a lower inflammatory score, as well as with several individual components, both in boys and girls ($p<0.01$). In addition, adolescents with at least 4 ideal components of the ICHI had significantly lower inflammatory score and lower levels of the study biomarkers, except CRP. Finally, the multilevel logistic regression showed that for every unit increase in the ICHI, the probability of having an inflammatory profile decreased by 28.1% in girls.

Conclusion: Results from this study suggest that a better ICHI is associated with a lower inflammatory profile already in adolescence. Improving these health behaviors, and health factors included in the ICHI, could play an important role in CVD prevention.

Keywords: Cardiovascular health; inflammation; European adolescents.

INTRODUCTION

Cardiovascular diseases (CVD), such as coronary artery disease, are the result of atherosclerosis progression (1). Evidence suggest that inflammation has a key role in the origin and development of atherosclerosis (2) as it triggers the formation of the fatty streak and its development into complex plaque (3). Atherosclerosis has its origins in childhood and is associated with early risk factors (4), yet symptoms may appear later in life (5). The relationship between inflammation and cardiovascular diseases is present already in childhood (6).

High concentrations of C-reactive protein (CRP) seem to track from childhood to adulthood (7). However, there are other biomarkers contributing to the characterization of the inflammatory process such as cytokines,(8) e.g. tumor necrosis factor alpha (TNF-alpha), or interleukins, e.g. interleukin 6 (IL-6). Nevertheless, other biomarkers have also been considered (9).

In addition, CRP is not always associated with atherosclerosis diagnosed by image techniques,(10) therefore, the use of a score that combines several inflammatory biomarkers could provide an overall estimation of the inflammatory status. A previous study (11) developed an inflammatory score, which included CRP, complement factors C3 and C4, leptin and white blood cells (WBC) being selected due to their high correlation with fatness and traditional cardio-metabolic risk factors.

In 2010, the American Heart Association (AHA) released the ideal cardiovascular health index (ICHI), (12) including four health behaviors and three health factors. The behavior-related criteria were: non-smoking, being physically active, having normal body mass index (BMI), and eating a healthy diet, while the health factors included were: normal blood pressure, plasma total cholesterol and glucose. The ICHI has been inversely related to the presence of atherosclerotic plaque in adults (13); therefore, it could represent a useful epidemiological tool to assess the cardiovascular profile.

Although there are some studies assessing the relationship between cardiovascular profile and metabolic risk factors in adolescents or young adults,(14, 15) there is not sufficient evidence on the association between inflammation and cardiovascular health in young populations.

The aims of the present study were to to assess the association between ICHI and inflammatory markers in European adolescents and to examine the use of an inflammatory score to assess the inflammatory status in adolescents (14).

METHODS

Study design

The HELENA study is a cross-sectional multi-center study (n=3528) conducted in 10 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. HELENA study has been previously described(16).

The study was performed according to the ethical guidelines of the Edinburgh revision of the 1964 Declaration of Helsinki (2000). The local Ethics Committees of each center approved the protocol and written informed consent was obtained.

Study population

Out of the total HELENA sample, one third from the 10 cities was chosen to provide blood samples (n=1089, 31%). Therefore, around 100 adolescents in each city were selected by means of the immunological parameters which were those with the highest variability within the blood measurements that were included in the study (16). Overall, 543 participants (251 boys and 292 girls) met the inclusion criteria for the present analysis: having data on the variables included in the ICH index and having measured the CRP, C3 and C4 complement factors, leptin and WBC. (Supplementary Figure 1)

Physical examinations

Weight and height were measured in underwear and barefoot with a SECA 861 (Seca Ltd) and with 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. Pubertal maduration was examined by a clinician and was assessed according Tanner (5-point-scale). Systolic and diastolic blood pressure was measured with an automatic oscillometric device (Omron M6). Participants were seated in a quiet room for ten minutes with their backs supported and feet on the ground. The lowest value of the two measurements, taken with a difference of 5 minutes, was recorded and the mean was used in data analysis. All anthropometric measures were taken following a standardized protocol.

Socioeconomic status

A modified version of the family affluence scale (FAS) was used as a proxy of socioeconomic status (SES). The adolescents completed a questionnaire asking about the numbers of cars and computers at home, having internet and whether the adolescent had his or her own room. In the HELENA study, the FAS was modified by replacing 'frequency of family holidays' by 'Internet availability at home'. Adolescents were scored from 0 (very low SES) to 8 (very high SES).

Blood analysis

Blood withdrawal was performed in fasting status. WBC counts and percentages were determined with automated blood cell counters. C-reactive protein (CRP) levels were quantified by immunoturbidimetry (AU 2700, Olimpus, Rungis, France). 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. Detection limits (sensitivity) were 0.007 mg/L for CRP, 0.01 g/L for C3, and 0.002 g/L for C4. Serum leptin (ng/mL) was measured using the RayBio Human Leptin ELISA (Enzyme-Linked Immunosorbent Assay; RayBiotech, Norcross, GA, USA) kit. The sensitivity of the leptin assay was <6 pg/mL, with intra-assay and interassay coefficients of variation of <10% and <12%.

Ideal cardiovascular health index

The AHA released the ICHI in 2010 (12) with the cut off values for adolescents.

Health behaviors

Four health behaviors were considered for the ICH index: smoking behavior, physical activity, BMI and diet.

Smoking status was categorized considering those who had never smoked as having an ideal smoking behavior. Adolescents who performed more than 60 min of moderate to vigorous self-reported exercise every day were classified as having an ideal physical activity level. BMI z-score and BMI categories were derived using the British 1990 Growth Reference Data from the Child Growth.(17, 18)

To assess dietary intake the HELENA-Dietary Assessment Tool (HELENA-DIAT)(19), a self-report dietary recall based on six meal occasions, was used. The dietary indicators used to assess ideality of the diet were: consumption of fruit and vegetables (more than 400 g per day), fish and fish products (at least 28 g per day), fiber (at least 1.1 grams per

10 g of carbohydrates per day), sodium (less than 1500 mg per day), and soft drinks (less than 145 mL per day). Having at least 4 of these indicators classified as 'ideal' was considered as ideal healthy diet.

Health factors

The cut-off for the biomarkers assessed to consider them ideal was <170mg/dL for plasma total cholesterol and <100mg/dL for glucose.

The lower value of the diastolic blood pressure and systolic blood pressure was used in the analysis to classify blood pressure status as ideal when lower than the 90th centile for the blood pressure (12).

Inflammatory score

A continuous score was computed from some inflammatory biomarkers: CRP, C3, C4, WBC and leptin. The selection of these biomarkers was based on a preliminary analysis with fatness and traditional cardio-metabolic risk factors as previously assessed within the HELENA study (11) (Supplementary material table 1).

Standardized values of the biomarkers were calculated for boys and girls and by 1-year age groups with the following formula: standardize value= (value – mean) / standard deviation (SD), as has been done elsewhere.(11) Z-scores from biomarkers were summed up to create a score of inflammation.

Statistical analysis

Analyses were stratified by sex. Normality assumption was checked and transformation was performed if required. Partial correlations, adjusted for age, sex, pubertal stage and center, between traditional and nontraditional cardio metabolic biomarkers were performed for the selection of the inflammatory biomarkers for the inflammatory score.

Student t test and chi-squared test were performed for the differences between the study participants by sex. Additionally, ANCOVA was performed to assess mean value of the inflammatory score by the ideal category and non-ideal category of each component of the ICHI, adjusting by tanner as covariate and center as random factor.

Multilevel linear models (level: center) were used to assess the associations between the inflammatory score (dependent variable) and the ICHI. Two different models were carried out. In the first model, the covariates used were Tanner and SES while in the second model the cardiorespiratory fitness was included. Frequencies between number of components of the ICHI and inflammatory score were assessed and the p for trend was calculated.

Finally, a multilevel logistic regression (level: center) was performed. The inflammatory index was transformed into a categorical variable using the median value in order to split the sample into two groups (I: > -0.737; II: ≤ -0.737 for boys and I: > -0.268; II: ≤-0.268 for girls) and the ideal cardiovascular health index was considered as independent variable. Two different models were performed. In the first model, the covariates used were Tanner and SES while in the second model the cardiorespiratory fitness was included. Interactions between covariates and dependent variable were assessed before calculating the multivariate regression model using Wald test in both multilevel models: linear and logistic, and no statistical significance was observed in any. Also, multicollinearity was assessed by means of variance inflation factor values calculation for covariates in each multilevel linear model, and all were < 10.

Data were managed and analyzed with SPSS Statistics v.19 and R software with lme4 package for multilevel regression models and AED package to test for multicollinearity.

RESULTS

Baseline characteristics are shown in Table 1. There were significant differences by sex in some of the ICHI components and some biomarkers. None of the boys and only 9% of the girls followed a healthy diet, almost 47% of the girls had high total cholesterol levels and 40% of the girls did not comply with PA guidelines. Results for the selection of the inflammatory biomarkers are found in Supplementary table 1. Differences in mean concentration of the inflammatory score by the categories of each ICHI component are presented in Supplementary table 2. Significant sex differences were found in BMI, physical activity and blood pressure. Plasma glucose showed significant differences by category of ICHI component in boys.

Results for the multilevel linear models of the ICHI are presented in Table 2, for boys, and Table 3, for girls. In model 1, the ICHI was significantly and inversely related to the inflammatory score and its components: inflammatory score ($p<0.001$ for boys and girls), C3 ($p=0.001$ for boys and $p<0.001$ for girls), C4 ($p=0.002$ for boys and $p=0.001$ for girls), WBC ($p=0.017$ for girls) and log-leptin ($p<0.001$ for boys and girls). In model 2, the biomarkers significantly and inversely associated with the ICH index were: inflammatory score ($p=0.005$ for boys and $p=0.005$ for girls), C3 ($p=0.001$ in girls), C4 ($p=0.004$ in boys and $p=0.039$ in girls) and log-leptin ($p<0.001$ in boys and $p=0.006$ in girls). Also, lower levels of inflammation were associated with a higher number of components of the ICH index in boys ($p<0.001$) and girls ($p<0.001$) (Figure 1).

Finally, the multilevel logistic regression (Table 4) showed the probability of having a higher or lower inflammatory state when increasing one unit of the ICHI. For boys, when increasing the ICHI with one unit, the probability of having a higher inflammatory status decreased 30.7% (OR=0.693, 95%CI: 0.544-0.883, $p=0.003$) in the model 1, while this probability decreased 26.5% (OR=0.735, 95%CI: 0.533-1.014 $p=0.061$) in model 2. In girls, when increasing the ICHI with one unit the probability of having a higher inflammatory status decreased 22.3% (OR=0.677, 95%CI: 0.539-0.850, $p<0.001$) in the first model and 28.1% (OR=0.719, 95%CI: 0.534-0.969, $p=0.031$) for model 2.

DISCUSSION

Findings from this study suggest that the ICHI proposed by the AHA is negatively associated with inflammation, measured by biomarkers and an inflammatory score, in a sample of European adolescents.

Less than optimal cardiovascular health during adolescence seems to be critical in the development of future CVD.(20) A very low prevalence of the ICHI has been shown in a U.S sample of adolescents, especially regarding both behavioral components, physical activity and diet.(21) Furthermore, in another study in adolescents, the ICHI was inversely associated with aortic intima-media and directly associated with aortic elasticity, already in adolescence, supporting the relevance of this tool as part of a primary prevention of future cardiovascular events.(22)

However, none of the European adolescents included in our study sample met the 7 components of the ICHI. This result is in line with previous studies reporting the same outcome in adolescents (14, 20, 22). Maybe these results are due to the low scores of the ideal diet score component; this component includes at least four ideal diet criteria out of five, and was also the component least often met in our sample, 1.7%. In studies performed in adults, the ideal diet score was also the less frequent component; prevalence being <1%(23) and 0.4%(24). In our sample, among the diet components, the optimum level of sodium intake was achieved only by 8.7% of the adolescents, being the most difficult criteria to meet, but also one of the most challenging criteria to measure accurately. In contrast, having <100mg/dL for glucose was the most commonly achieved component of the ICHI since 91.2% of our sample met this criteria.

In our sample, we observed a negative association between ICHI and the inflammatory score, suggesting that the higher the ICHI the lower the inflammatory score. To our knowledge, there are no previous studies assessing the relation between ICHI and inflammation in adolescence. However, a previous study observed that ICHI in adolescence was a good predictor of cardio-metabolic health in adulthood (20). As, individually, the components of the ICHI, such as cardiovascular risk factors, have been already related to biomarkers of inflammation(25). It seems that cardiovascular risk could be mediated through inflammation.

In the current study, the observed associations were found using the ICHI as a 7-component variable and the inflammatory score, independently of sex. However, the ICHI was associated with all the individual biomarkers of the inflammatory score except CRP. This protein is the most widely clinical biomarker of inflammation because it is easily and reliably measured and it has been related to adiposity and cardiovascular risk factors in healthy children.(26) Moreover, CRP has been related to the prediction of coronary heart disease (27) and atherosclerosis in adults (28). However, based on our findings, it would be recommended to investigate other biomarkers related to traditional metabolic risk factors, in addition to CRP, to evaluate the inflammatory status.

Cardiorespiratory fitness can be considered as a marker of cardiovascular health in children and adolescents (29) and has been related to an increased prevalence of CVD risk factors in adolescents and adults (30). A previous study with HELENA data showed that higher levels of cardiorespiratory fitness were positively associated with the ICHI in adolescents.(14) Our results show that the ICHI was associated with inflammation independently of cardiorespiratory fitness in girls.

There were several limitations to our findings. First, the cross-sectional nature of the study is a limitation. The inflammatory score is sample specific and each biomarker weighted equally for the prediction of cardio-metabolic risk. Blood samples only reflect inflammation at this specific time point. However, this study has many strengths including the use of an inflammatory score that sums up several inflammatory biomarkers, related to cardio-metabolic risk to assess an overall cardio metabolic status as well as the use of standardized and harmonized information from 9 European countries.

In conclusion, results from the current study show that there is an association between the ideal cardiovascular health in adolescence and inflammatory status. Despite not being significant for CRP, results were strongly associated with a composite index of inflammation including CRP, WBC, C3, C4 and leptin, in both gender, and, in girls, independently of the cardiorespiratory. Since the most difficult ICHI criteria to achieve was ideal diet, we should concentrate efforts to improve consumption of those food items included in the index, especially emphasizing the reduction of salt intake. These results provide further insight to better understand the association between lifestyle and

cardiovascular risk. Longitudinal studies in adolescent populations measuring the association between inflammation and cardiovascular risk are needed to confirm these results and to prevent future related diseases.

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CONFLICT OF INTEREST: None declared

REFERENCES

1. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005 Apr 21;352(16):1685-95.
2. Libby P. Inflammation in atherosclerosis. Nature. 2002 Dec 19-26;420(6917):868-74.
3. Libby P. Inflammation and cardiovascular disease mechanisms. Am J Clin Nutr. 2006 Feb;83(2):456S-60S.
4. Berenson GS, Srinivasan SR, Bao W, Newman WP, 3rd, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med. 1998 Jun 4;338(23):1650-6.
5. Kavey RE, Daniels SR, Lauer RM, Atkins DL, Hayman LL, Taubert K, American Heart A. American Heart Association guidelines for primary prevention of atherosclerotic cardiovascular disease beginning in childhood. Circulation. 2003 Mar 25;107(11):1562-6.
6. Ford ES, National H, Nutrition Examination S. C-reactive protein concentration and cardiovascular disease risk factors in children: findings from the National Health and Nutrition Examination Survey 1999-2000. Circulation. 2003 Sep 2;108(9):1053-8.
7. Juonala M, Viikari JS, Ronnemaa T, Taittonen L, Marniemi J, Raitakari OT. Childhood C-reactive protein in predicting CRP and carotid intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. Arterioscler Thromb Vasc Biol. 2006 Aug;26(8):1883-8.
8. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K, Curhan GC, Rifai N, Cannuscio CC, Stampfer MJ, Rimm EB. Inflammatory markers and the risk of coronary heart disease in men and women. N Engl J Med. 2004 Dec 16;351(25):2599-610.
9. van Holten TC, Waanders LF, de Groot PG, Vissers J, Hoefer IE, Pasterkamp G, Prins MW, Roest M. Circulating biomarkers for predicting cardiovascular disease risk; a systematic review and comprehensive overview of meta-analyses. PLoS One. 2013;8(4):e62080.
10. Lorenz MW, Karbstein P, Markus HS, Sitzer M. High-sensitivity C-reactive protein is not associated with carotid intima-media progression: the carotid atherosclerosis progression study. Stroke. 2007 Jun;38(6):1774-9.
11. Artero EG, Espana-Romero V, Jimenez-Pavon D, Martinez-Gomez D, Warnberg J, Gomez-Martinez S, Gonzalez-Gross M, Vanhelst J, Kafatos A, Molnar D, De Henauw S, Moreno LA, Marcos A, Castillo MJ, group Hs. Muscular fitness, fatness and inflammatory biomarkers in adolescents. Pediatr Obes. 2014 Oct;9(5):391-400.
12. Lloyd-Jones DM, Hong Y, Labarthe D, Mozaffarian D, Appel LJ, Van Horn L, Greenlund K, Daniels S, Nichol G, Tomaselli GF, Arnett DK, Fonarow GC, Ho PM, Lauer MS, Masoudi FA, Robertson RM, Roger V, Schwamm LH, Sorlie P, Yancy CW, Rosamond WD, American Heart Association Strategic Planning Task F, Statistics C. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. Circulation. 2010 Feb 2;121(4):586-613.

13. Robbins JM, Petrone AB, Carr JJ, Pankow JS, Hunt SC, Heiss G, Arnett DK, Ellison RC, Gaziano JM, Djousse L. Association of ideal cardiovascular health and calcified atherosclerotic plaque in the coronary arteries: the National Heart, Lung, and Blood Institute Family Heart Study. Am Heart J. 2015 Mar;169(3):371-8 e1.
14. Ruiz JR, Huybrechts I, Cuenca-Garcia M, Artero EG, Labayen I, Meirhaeghe A, Vicente-Rodriguez G, Polito A, Manios Y, Gonzalez-Gross M, Marcos A, Widhalm K, Molnar D, Kafatos A, Sjostrom M, Moreno LA, Castillo MJ, Ortega FB, on behalf of the Hsg. Cardiorespiratory fitness and ideal cardiovascular health in European adolescents. Heart. 2015 May 15;101:766-73.
15. Urbina EM, Srinivasan SR, Tang R, Bond MG, Kieltyka L, Berenson GS, Bogalusa Heart S. Impact of multiple coronary risk factors on the intima-media thickness of different segments of carotid artery in healthy young adults (The Bogalusa Heart Study). Am J Cardiol. 2002 Nov 1;90(9):953-8.
16. Moreno LA, De Henauw S, Gonzalez-Gross M, Kersting M, Molnar D, Gottrand F, Barrios L, Sjostrom M, Manios Y, Gilbert CC, Leclercq C, Widhalm K, Kafatos A, Marcos A, Group HS. Design and implementation of the Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. Int J Obes (Lond). 2008 Nov;32 Suppl 5:S4-11.
17. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ. 2000 May 6;320(7244):1240-3.
18. Cole TJ, Freeman JV, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. Stat Med. 1998 Feb 28;17(4):407-29.
19. Vereecken CA, Covents M, Sichert-Hellert W, Alvira JM, Le Donne C, De Henauw S, De Vriendt T, Phillipp MK, Beghin L, Manios Y, Hallstrom L, Poortvliet E, Matthys C, Plada M, Nagy E, Moreno LA, Group HS. Development and evaluation of a self-administered computerized 24-h dietary recall method for adolescents in Europe. Int J Obes (Lond). 2008 Nov;32 Suppl 5:S26-34.
20. Laitinen TT, Pahkala K, Magnussen CG, Viikari JS, Oikonen M, Taittonen L, Mikkila V, Jokinen E, Hutri-Kahonen N, Laitinen T, Kahonen M, Lehtimaki T, Raitakari OT, Juonala M. Ideal cardiovascular health in childhood and cardiometabolic outcomes in adulthood: the Cardiovascular Risk in Young Finns Study. Circulation. 2012 Apr 24;125(16):1971-8.
21. Shay CM, Ning H, Daniels SR, Rooks CR, Gidding SS, Lloyd-Jones DM. Status of cardiovascular health in US adolescents: prevalence estimates from the National Health and Nutrition Examination Surveys (NHANES) 2005-2010. Circulation. 2013 Apr 2;127(13):1369-76.
22. Pahkala K, Hietalampi H, Laitinen TT, Viikari JS, Ronnemaa T, Niinikoski H, Lagstrom H, Talvia S, Jula A, Heinonen OJ, Juonala M, Simell O, Raitakari OT. Ideal cardiovascular health in adolescence: effect of lifestyle intervention and association with vascular intima-media thickness and elasticity (the Special Turku Coronary Risk Factor Intervention Project for Children [STRIP] study). Circulation. 2013 May 28;127(21):2088-96.
23. Yang Q, Cogswell ME, Flanders WD, Hong Y, Zhang Z, Loustalot F, Gillespie C, Merritt R, Hu FB. Trends in cardiovascular health metrics and associations with all-cause and CVD mortality among US adults. JAMA. 2012 Mar 28;307(12):1273-83.

24. Dong C, Rundek T, Wright CB, Anwar Z, Elkind MS, Sacco RL. Ideal cardiovascular health predicts lower risks of myocardial infarction, stroke, and vascular death across whites, blacks, and hispanics: the northern Manhattan study. Circulation. 2012 Jun 19;125(24):2975-84.
25. Saito M, Ishimitsu T, Minami J, Ono H, Ohrui M, Matsuoka H. Relations of plasma high-sensitivity C-reactive protein to traditional cardiovascular risk factors. Atherosclerosis. 2003 Mar;167(1):73-9.
26. Cook DG, Mendall MA, Whincup PH, Carey IM, Ballam L, Morris JE, Miller GJ, Strachan DP. C-reactive protein concentration in children: relationship to adiposity and other cardiovascular risk factors. Atherosclerosis. 2000 Mar;149(1):139-50.
27. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, Lowe GD, Pepys MB, Gudnason V. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med. 2004 Apr 1;350(14):1387-97.
28. Libby P, Ridker PM. Inflammation and atherosclerosis: role of C-reactive protein in risk assessment. Am J Med. 2004 Mar 22;116 Suppl 6A:9S-16S.
29. Ortega FB, Ruiz JR, Castillo MJ, Sjostrom M. Physical fitness in childhood and adolescence: a powerful marker of health. Int J Obes (Lond). 2008 Jan;32(1):1-11.
30. Carnethon MR, Gulati M, Greenland P. Prevalence and cardiovascular disease correlates of low cardiorespiratory fitness in adolescents and adults. JAMA. 2005 Dec 21;294(23):2981-8.

APPENDIX

HELENA Study Group*

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22. Cárnicas Serrano SL (Spain)

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23. Cederroth International AB (Sweden)

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TABLES

Table 1. Characteristics of the study participants.

Mean ± SD	*Boys (n=251)*	*Girls (n=292)*	*p*
Age (years)	14.80±1.28	14.81±1.17	0.911
Tanner I % (n)	0.8 (2)	0 (0)	0.126
Tanner II % (n)	12.4 (31)	6.9 (20)	**0.028**
Tanner III % (n)	17.6 (44)	24.5 (71)	0.054
Tanner IV % (n)	46.0 (115)	45 (132)	0.887
Tanner V % (n)	23.2 (58)	23.1 (67)	0.964
Moderate-vigorous PA (min/day)	121.73±91.47	90.89±72.21	**<0.001**
BMI (kg/m^2)	21.04±3.96	21.14±3.38	0.763
Systolic blood pressure (mm Hg)	120.10±14.04	112.85±11.19	**<0.001**
Diastolic blood pressure (mm Hg)	64.02±8.61	65.04±8.72	0.174
Glucose (mg/dL)	92.21±6.93	88.43±5.99	**<0.001**
Total cholesterol (mg/dL)	152.60±26.06	167.67±27.49	**<0.001**
Inflammatory score	-0.02±3.23	0.14±3.06	0.517
CRP (mg/L)	0.82±1.18	0.85±1.27	0.781
C3 (g/L)	1.11±0.16	1.13±0.16	0.089
C4 (g/L)	0.20±0.06	0.21±0.06	0.271
Leptin (ng/mL)	9.17±14.93	29.1±25.06	**<0.001**
WBC (10x3/ µL)	6.06±1.34	6.45±1.55	**0.002**
Ideal health behaviours			
Smoking % (n)	61.8 (155)	59.2 (173)	0.552
Body mass index % (n)	78.5 (197)	82.9 (242)	0.195
Physical activity % (n)	70.5 (177)	59.9 (175)	**0.010**
Diet % (n)	0 (0)	9 (3.1)	-
Ideal health factors			
Total cholesterol % (n)	78.5 (197)	53.4 (156)	**<0.001**
Blood pressure % (n)	88.8 (223)	90.1 (263)	0.643
Plasma glucose % (n)	84.5 (212)	96.9 (283)	**<0.001**

SD: Standard deviation. PA: Physical activity. BMI: Body mass index. CRP: C-reactive protein. C: Complement factor. WBC: Whole blood cells count. ICHI: Ideal cardiovascular health index.

Table 2. Multilevel linear models of the ideal cardiovascular health index and inflammation in boys.

	Model 1			Model 2		
BOYS	**Inflammatory score**			**Inflammatory score**		
Ideal cardiovascular health index	Beta	95% CI	**P**	Beta	95% CI	**P**
	-0.794	-1.146, -0.442	<0.001	-0.597	-1.014, -0.181	0.005
	CRP*			**CRP***		
	Beta	95% CI	**P**	Beta	95% CI	**P**
	-0.040	-0.183, -0.096	0.540	0.029	-0.140, 0.199	0.732
	C3			**C3**		
	Beta	95% CI	**P**	Beta	95% CI	**P**
	-0.297	-0.047, -0.011	0.001	-0.017	-0.037, 0.003	0.110
	C4			**C4**		
	Beta	95% CI	**P**	Beta	95% CI	**P**
	-0.011	-0.018, -0.004	0.002	-0.012	-0.021, -0.004	0.004
	WBC			**WBC**		
	Beta	95% CI	**P**	Beta	95% CI	**P**
	-0.077	-0.229, 0.074	0.315	-0.045	-0.226, 0.136	0.625
	Leptin*			**Leptin***		
	Beta	95% CI	**P**	Beta	95% CI	**P**
	-0.393	-0.509, -0.227	<0.001	-0.298	-0.432, -0.164	<0.001

95% CI: Confidence Interval. CRP: C-reactive protein. C: Complement factor. WBC: Whole blood cells count.

*CRP and Leptin are log-transformed.

Model 1: Adjusted by tanner and socioeconomic status (SES)

Model 2: Adjusted by tanner, SES, and cardiorespiratory fitness.

Table 3. Multilevel linear models of the ideal cardiovascular health index and inflammation in girls.

	Model 1			Model 2		
GIRLS	**Inflammatory score**			**Inflammatory score**		
Ideal cardiovascular health index	Beta	95% CI	**P**	Beta	95% CI	**P**
	-0.646	-0.973, -0.319	<0.001	-0.52	-0.885, -0.155	0.005
	CRP*			**CRP***		
	Beta	95% CI	**P**	Beta	95% CI	**P**
	-0.102	-0.240, 0.035	0.145	-0.017	-0.180, 0.145	0.831
	C3			**C3**		
	Beta	95% CI	**P**	Beta	95% CI	**P**
	-0.036	-0.054, -0.019	<0.001	-0.034	-0.053, -0.015	0.001
	C4			**C4**		
	Beta	95% CI	**P**	Beta	95% CI	**P**
	-0.012	-0.019, -0.005	0.001	-0.008	-0.016, -0.0004	0.039
	WBC			**WBC**		
	Beta	95% CI	**P**	Beta	95% CI	**P**
	-0.202	-0.366, -0.037	0.017	-0.157	-0.358, 0.043	0.123
	Leptin*			**Leptin***		
	Beta	95% CI	**P**	Beta	95% CI	**P**
	-0.170	-0.257, -0.082	<0.001	-0.143	-5.469, -1.963	0.006

95% CI: Confidence Interval. CRP: C-reactive protein. C: Complement factor. WBC: Whole blood cells count.

*CRP and Leptin are log-transformed.

Model 1: Adjusted by tanner and socioeconomic status (SES)

Model 2: Adjusted by tanner, SES, and cardiorespiratory fitness.

628 **Table 4**. Multilevel logistic regression.

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	Model 1			Model 2		
BOYS	***OR***	***95% CI***	***p-value***	***OR***	***95% CI***	***p-value***
ICHI	0.693	0.544-0.883	0.003	0.735	0.533-1.014	0.061
GIRLS	***OR***	***95% CI***	***p-value***	***OR***	***95% CI***	***p-value***
ICHI	0.677	0.539-0.850	<0.001	0.719	0.534-0.969	0.031

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631 OR: Odds ratio

632 Model 1: Adjusted by tanner and socioeconomic status (SES)

633 Model 2: Adjusted by tanner, SES, and cardiorespiratory fitness.

634 CI: Confidence Interval.

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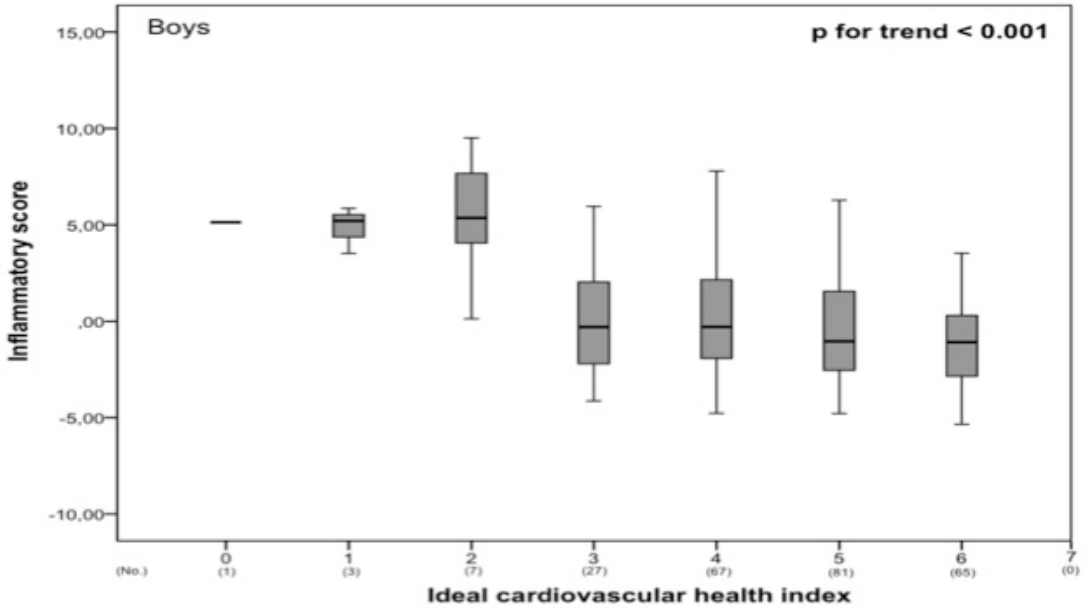
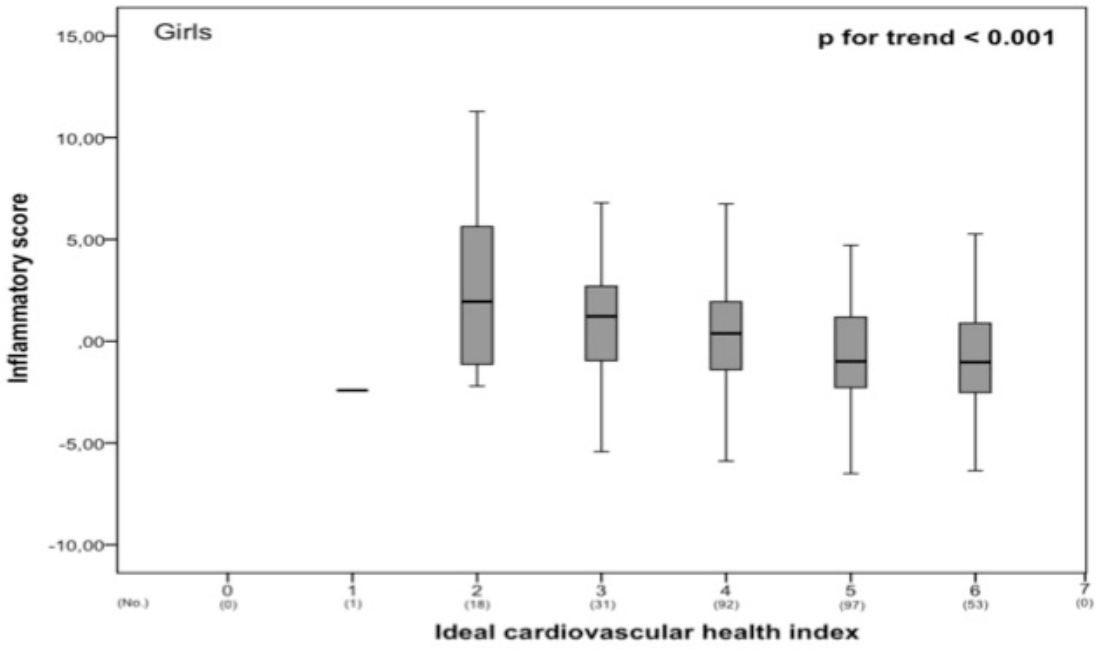
FIGURE LEGENDS

Figure 1. Association between inflammatory score and Ideal Cardiovascular Health index.

Supplementary Figure 1. Flow diagram of the study population.


Boys
p for trend < 0.001
Inflammatory score
15,00
10,00
5,00
,00
-5,00
-10,00
(No.)
0 (1)
1 (3)
2 (7)
3 (27)
4 (67)
5 (81)
6 (65)
7 (0)
Ideal cardiovascular health index


Girls
p for trend < 0.001
Inflammatory score
15,00
10,00
5,00
,00
-5,00
-10,00
(No.)
0 (0)
1 (1)
2 (18)
3 (31)
4 (92)
5 (97)
6 (53)
7 (0)
Ideal cardiovascular health index

HIGHLIGHTS

Less than ideal cardiovascular health is associated with inflammation in adolescence

C-reactive protein was not associated with cardiovascular health

Diet is the component of the cardiovascular index most difficult to achieve

Prevention should start early in life to avoid future cardiovascular diseases