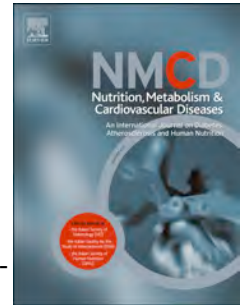# Accepted Manuscript

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

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1 **Ideal cardiovascular health and inflammation in European adolescents: the**

2 **HELENA study.**

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52 **ABSTRACT**

53

54 **Background and aims:** Inflammation plays a key role in atherosclerosis and this

55 process seems to appear in childhood. The ideal cardiovascular health index (ICHI) has

56 been inversely related to atherosclerotic plaque in adults. However, evidence regarding

57 inflammation and ICHI in adolescents is scarce. The aim is to assess the association

58 between the ICHI and inflammation in European adolescents.

59 **Methods and results:** 543 adolescents (251 boys and 292 girls) from the Healthy

60 Lifestyle in Europe by Nutrition in Adolescence (HELENA) study, a cross-sectional

61 multi-center study including 9 European countries, were measured. C-reactive protein

62 (CRP), complement factors C3 and C4, leptin and white blood cell counts were used to

63 compute an inflammatory score. Multilevel linear models and multilevel logistic

64 regression were used to assess the association between ICHI and inflammation

65 controlling by covariates. Higher ICHI was associated with a lower inflammatory score,

66 as well as with several individual components, both in boys and girls (p<0.01). In

67 addition, adolescents with at least 4 ideal components of the ICHI had significantly

68 lower inflammatory score and lower levels of the study biomarkers, except CRP.

69 Finally, the multilevel logistic regression showed that for every unit increase in the

70 ICHI, the probability of having an inflammatory profile decreased by 28.1% in girls.

71 **Conclusion:** Results from this study suggest that a better ICHI is associated with a

72 lower inflammatory profile already in adolescence. Improving these health behaviors,

73 and health factors included in the ICHI, could play an important role in CVD

74 prevention.

75

76 **Keywords:** Cardiovascular health; inflammation; European adolescents.

77

3

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.

110 The aims of the present study were to to assess the association between ICHI and

111 inflammatory markers in European adolescents and to examine the use of an

112 inflammatory score to assess the inflammatory status in adolescents (14).

113 **METHODS**

114

115 **Study design**

116 The HELENA study is a cross-sectional multi-center study (n=3528) conducted in 10

117 European cities: Athens and Heraklion in Greece, Dortmund in Germany, Ghent in

118 Belgium, Lille in France, Pecs in Hungary, Rome in Italy, Stockholm in Sweden,

119 Vienna in Austria and Zaragoza in Spain. HELENA study has been previously

120 described(16).

121 The study was performed according to the ethical guidelines of the Edinburgh revision

122 of the 1964 Declaration of Helsinki (2000). The local Ethics Committees of each center

123 approved the protocol and written informed consent was obtained.

124

125 **Study population**

126 Out of the total HELENA sample, one third from the 10 cities was chosen to provide

127 blood samples (n=1089, 31%). Therefore, around 100 adolescents in each city were

128 selected by means of the immunological parameters which were those with the highest

129 variability within the blood measurements that were included in the study (16). Overall,

130 543 participants (251 boys and 292 girls) met the inclusion criteria for the present

131 analysis: having data on the variables included in the ICH index and having measured

132 the CRP, C3 and C4 complement factors, leptin and WBC. (Supplementary Figure 1)

133

134 **Physical examinations**

135 Weight and height were measured in underwear and barefoot with a SECA 861 (Seca

136 Ltd) and with a stadiometer SECA 225 (Seca Ltd). In addition, body mass index (BMI)

137 was calculated as body weight in kilograms divided by the square of height in meters.

138 Pubertal maduration was examined by a clinician and was assessed according Tanner

139 (5-point-scale). Systolic and diastolic blood pressure was measured with an automatic

140 oscillometric device (Omron M6). Participants were seated in a quiet room for ten

141 minutes with their backs supported and feet on the ground. The lowest value of the two

142 measurements, taken with a difference of 5 minutes, was recorded and the mean was

143 used in data analysis. All anthropometric measures were taken following a standardized

144 protocol.

145

146 **Socioeconomic status**

6

147 A modified version of the family affluence scale (FAS) was used as a proxy of

148 socioeconomic status (SES). The adolescents completed a questionnaire asking about

149 the numbers of cars and computers at home, having internet and whether the adolescent

150 had his or her own room. In the HELENA study, the FAS was modified by replacing

151 'frequency of family holidays' by 'Internet availability at home'. Adolescents were

152 scored from 0 (very low SES) to 8 (very high SES).

153

154 **Blood analysis**

155 Blood withdrawal was performed in fasting status. WBC counts and percentages were

156 determined with automated blood cell counters. C-reactive protein (CRP) levels were

157 quantified by immunoturbidimetry (AU 2700, Olimpus, Rungis, France). Serum C3 and

158 C4 complement factors were analyzed by nephelometry (Behring Diagnostics, CA,

159 USA). The coefficient of variation (inter-assay precision) was 1.9% for CRP, 1.4% for

160 C3, and 1.2% for C4. Detection limits (sensitivity) were 0.007 mg/L for CRP, 0.01 g/L

161 for C3, and 0.002 g/L for C4. Serum leptin (ng/mL) was measured using the RayBio

162 Human Leptin ELISA (Enzyme-Linked Immunosorbent Assay; RayBiotech, Norcross,

163 GA, USA) kit. The sensitivity of the leptin assay was <6 pg/mL, with intra-assay and

164 interassay coefficients of variation of <10% and <12%.

165

166 **Ideal cardiovascular health index**

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

168

169 *Health behaviors*

170 Four health behaviors were considered for the ICH index: smoking behavior, physical

171 activity, BMI and diet.

172 Smoking status was categorized considering those who had never smoked as having an

173 ideal smoking behavior. Adolescents who performed more than 60 min of moderate to

174 vigorous self-reported exercise every day were classified as having an ideal physical

175 activity level. BMI z-score and BMI categories were derived using the British 1990

176 Growth Reference Data from the Child Growth.(17, 18)

177 To assess dietary intake the HELENA-Dietary Assessment Tool (HELENA-DIAT)(19),

178 a self-report dietary recall based on six meal occasions, was used. The dietary indicators

179 used to assess ideality of the diet were: consumption of fruit and vegetables (more than

180 400 g per day), fish and fish products (at least 28 g per day), fiber (at least 1.1 grams per

7

181 10 g of carbohydrates per day), sodium (less than 1500 mg per day), and soft drinks

182 (less than 145 mL per day). Having at least 4 of these indicators classified as 'ideal' was

183 considered as ideal healthy diet.

184

185

186 *Health factors*

187 The cut-off for the biomarkers assessed to consider them ideal was <170mg/dL for

188 plasma total cholesterol and <100mg/dL for glucose.

189 The lower value of the diastolic blood pressure and systolic blood pressure was used in

190 the analysis to classify blood pressure status as ideal when lower than the 90th centile

191 for the blood pressure (12).

192

193 **Inflammatory score**

194 A continuous score was computed from some inflammatory biomarkers: CRP, C3, C4,

195 WBC and leptin. The selection of these biomarkers was based on a preliminary analysis

196 with fatness and traditional cardio-metabolic risk factors as previously assessed within

197 the HELENA study (11) (Supplementary material table 1).

198 Standardized values of the biomarkers were calculated for boys and girls and by 1-year

199 age groups with the following formula: standardize value= (value – mean) / standard

200 deviation (SD), as has been done elsewhere.(11) Z-scores from biomarkers were

201 summed up to create a score of inflammation.

202

203 **Statistical analysis**

204 Analyses were stratified by sex. Normality assumption was checked and transformation

205 was performed if required. Partial correlations, adjusted for age, sex, pubertal stage and

206 center, between traditional and nontraditional cardio metabolic biomarkers were

207 performed for the selection of the inflammatory biomarkers for the inflammatory score.

208

209 Student t test and chi-squared test were performed for the differences between the study

210 participants by sex. Additionally, ANCOVA was performed to assess mean value of the

211 inflammatory score by the ideal category and non-ideal category of each component of

212 the ICHI, adjusting by tanner as covariate and center as random factor.

213

214 Multilevel linear models (level: center) were used to assess the associations between the

215 inflammatory score (dependent variable) and the ICHI. Two different models were

216 carried out. In the first model, the covariates used were Tanner and SES while in the

217 second model the cardiorespiratory fitness was included. Frequencies between number

218 of components of the ICHI and inflammatory score were assessed and the p for trend

219 was calculated.

220 Finally, a multilevel logistic regression (level: center) was performed. The

221 inflammatory index was transformed into a categorical variable using the median value

222 in order to split the sample into two groups (I: > -0.737; II: ≤ -0.737 for boys and I: > -

223 0.268; II: ≤-0.268 for girls) and the ideal cardiovascular health index was considered as

224 independent variable. Two different models were performed. In the first model, the

225 covariates used were Tanner and SES while in the second model the cardiorespiratory

226 fitness was included. Interactions between covariates and dependent variable were

227 assessed before calculating the multivariate regression model using Wald test in both

228 multilevel models: linear and logistic, and no statistical significance was observed in

229 any. Also, multicollinearity was assessed by means of variance inflation factor values

230 calculation for covariates in each multilevel linear model, and all were < 10.

231 Data were managed and analyzed with SPSS Statistics v.19 and R software with lme4

232 package for multilevel regression models and AED package to test for multicollinearity.

233 **RESULTS**

234

235 Baseline characteristics are shown in Table 1. There were significant differences by sex

236 in some of the ICHI components and some biomarkers. None of the boys and only 9%

237 of the girls followed a healthy diet, almost 47% of the girls had high total cholesterol

238 levels and 40% of the girls did not comply with PA guidelines. Results for the selection

239 of the inflammatory biomarkers are found in Supplementary table 1. Differences in

240 mean concentration of the inflammatory score by the categories of each ICHI

241 component are presented in Supplementary table 2. Significant sex differences were

242 found in BMI, physical activity and blood pressure. Plasma glucose showed significant

243 differences by category of ICHI component in boys.

244

245 Results for the multilevel linear models of the ICHI are presented in Table 2, for boys,

246 and Table 3, for girls. In model 1, the ICHI was significantly and inversely related to the

247 inflammatory score and its components: inflammatory score ($p<0.001$ for boys and

248 girls), C3 ($p=0.001$ for boys and $p<0.001$ for girls), C4 ($p=0.002$ for boys and $p=0.001$

249 for girls), WBC ($p=0.017$ for girls) and log-leptin ($p<0.001$ for boys and girls). In model

250 2, the biomarkers significantly and inversely associated with the ICH index were:

251 inflammatory score ($p=0.005$ for boys and $p=0.005$ for girls), C3 ($p=0.001$ in girls), C4

252 ($p=0.004$ in boys and $p=0.039$ in girls) and log-leptin ($p<0.001$ in boys and $p=0.006$ in

253 girls). Also, lower levels of inflammation were associated with a higher number of

254 components of the ICH index in boys ($p<0.001$) and girls ($p<0.001$) (Figure 1).

255

256 Finally, the multilevel logistic regression (Table 4) showed the probability of having a

257 higher or lower inflammatory state when increasing one unit of the ICHI. For boys,

258 when increasing the ICHI with one unit, the probability of having a higher

259 inflammatory status decreased 30.7% (OR=0.693, 95%CI: 0.544-0.883, $p=0.003$) in the

260 model 1, while this probability decreased 26.5% (OR=0.735, 95%CI: 0.533-1.014

261 $p=0.061$) in model 2. In girls, when increasing the ICHI with one unit the probability of

262 having a higher inflammatory status decreased 22.3% (OR=0.677, 95%CI: 0.539-0.850,

263 $p<0.001$) in the first model and 28.1% (OR=0.719, 95%CI: 0.534-0.969, $p=0.031$) for

264 model 2.

265

DISCUSSION

267

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

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

278 However, none of the European adolescents included in our study sample met the 7
279 components of the ICHI. This result is in line with previous studies reporting the same
280 outcome in adolescents (14, 20, 22). Maybe these results are due to the low scores of the
281 ideal diet score component; this component includes at least four ideal diet criteria out
282 of five, and was also the component least often met in our sample, 1.7%. In studies
283 performed in adults, the ideal diet score was also the less frequent component;
284 prevalence being <1%(23) and 0.4%(24). In our sample, among the diet components,
285 the optimum level of sodium intake was achieved only by 8.7% of the adolescents,
286 being the most difficult criteria to meet, but also one of the most challenging criteria to
287 measure accurately. In contrast, having <100mg/dL for glucose was the most commonly
288 achieved component of the ICHI since 91.2% of our sample met this criteria.
289

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

298 In the current study, the observed associations were found using the ICHI as a 7-
299 component variable and the inflammatory score, independently of sex. However, the
300 ICHI was associated with all the individual biomarkers of the inflammatory score
301 except CRP. This protein is the most widely clinical biomarker of inflammation because
302 it is easily and reliably measured and it has been related to adiposity and cardiovascular
303 risk factors in healthy children.(26) Moreover, CRP has been related to the prediction of
304 coronary heart disease (27) and atherosclerosis in adults (28). However, based on our
305 findings, it would be recommended to investigate other biomarkers related to traditional
306 metabolic risk factors, in addition to CRP, to evaluate the inflammatory status.
307
308 Cardiorespiratory fitness can be considered as a marker of cardiovascular health in
309 children and adolescents (29) and has been related to an increased prevalence of CVD
310 risk factors in adolescents and adults (30). A previous study with HELENA data
311 showed that higher levels of cardiorespiratory fitness were positively associated with
312 the ICHI in adolescents.(14) Our results show that the ICHI was associated with
313 inflammation independently of cardiorespiratory fitness in girls.
314
315 There were several limitations to our findings. First, the cross-sectional nature of the
316 study is a limitation. The inflammatory score is sample specific and each biomarker
317 weighted equally for the prediction of cardio-metabolic risk. Blood samples only reflect
318 inflammation at this specific time point. However, this study has many strengths
319 including the use of an inflammatory score that sums up several inflammatory
320 biomarkers, related to cardio-metabolic risk to assess an overall cardio metabolic status
321 as well as the use of standardized and harmonized information from 9 European
322 countries.
323
324 In conclusion, results from the current study show that there is an association between
325 the ideal cardiovascular health in adolescence and inflammatory status. Despite not
326 being significant for CRP, results were strongly associated with a composite index of
327 inflammation including CRP, WBC, C3, C4 and leptin, in both gender, and, in girls,
328 independently of the cardiorespiratory. Since the most difficult ICHI criteria to achieve
329 was ideal diet, we should concentrate efforts to improve consumption of those food
330 items included in the index, especially emphasizing the reduction of salt intake. These
331 results provide further insight to better understand the association between lifestyle and

332 cardiovascular risk. Longitudinal studies in adolescent populations measuring the

333 association between inflammation and cardiovascular risk are needed to confirm these

334 results and to prevent future related diseases.

335

336

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344

345

CONFLICT OF INTEREST: None declared

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470
471

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579

580 **TABLES**
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583 **Table 1.** Characteristics of the study participants.

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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**

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

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

600

BOYS	Model 1			Model 2		
	Inflammatory score			Inflammatory score		
	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**
Ideal cardiovascular health index	-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

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609 95% CI: Confidence Interval. CRP: C-reactive protein. C: Complement factor. WBC: Whole blood cells count.

610 *CRP and Leptin are log-transformed.

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

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

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613

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

615

GIRLS	Model 1			Model 2		
	Inflammatory score			**Inflammatory score**		616
	Beta	95% CI	**P**	Beta	95% CI	**P**
	-0.646	-0.973, -0.319	<0.001	-0.52	-0.885, -0.155	0.005 617
	CRP*			**CRP***		
	Beta	95% CI	**P**	Beta	95% CI	**P**
	-0.102	-0.240, 0.035	0.145	-0.017	-0.180, 0.145	0.83 618
	C3			**C3**		
	Beta	95% CI	**P**	Beta	95% CI	**P** 619
Ideal cardiovascular health index	-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** 620
	-0.012	-0.019, -0.005	0.001	-0.008	-0.016, -0.0004	0.039
	WBC			**WBC**		621
	Beta	95% CI	**P**	Beta	95% CI	**P**
	-0.202	-0.366, -0.037	0.017	-0.157	-0.358, 0.043	0.123 622
	Leptin*			**Leptin***		
	Beta	95% CI	**P**	Beta	95% CI	**P**
	-0.170	-0.257, -0.082	<0.001	-0.143	-5.469, -1.963	0.006 623

624 95% CI: Confidence Interval. CRP: C-reactive protein. C: Complement factor. WBC: Whole blood cells count.

625 *CRP and Leptin are log-transformed.

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

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

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

629

	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

630

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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640 **FIGURE LEGENDS**

641

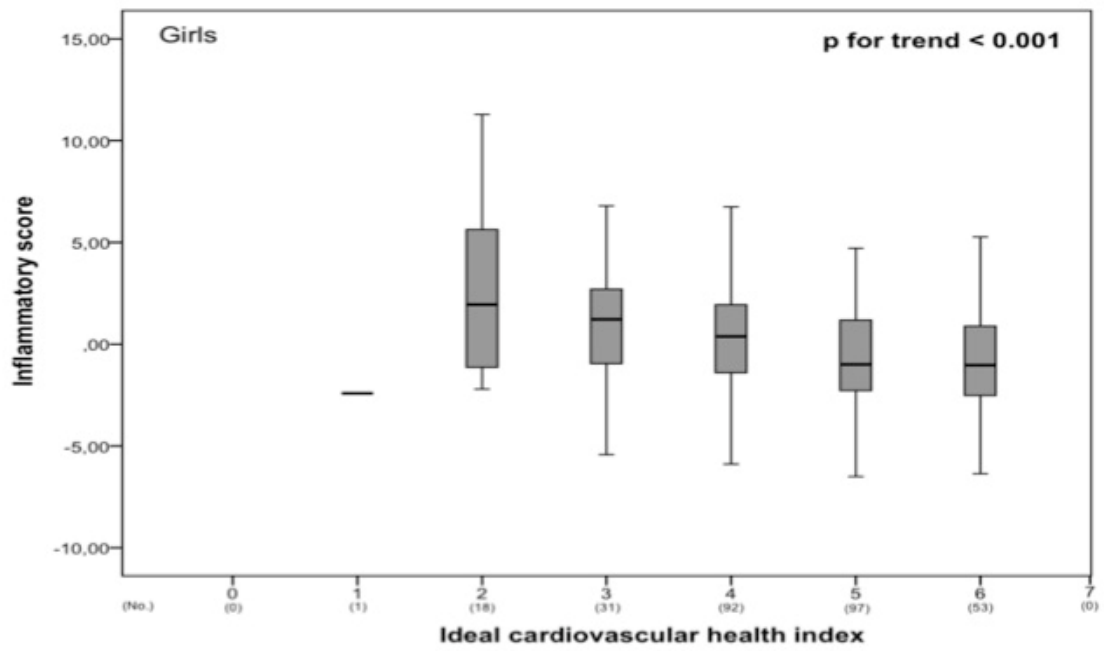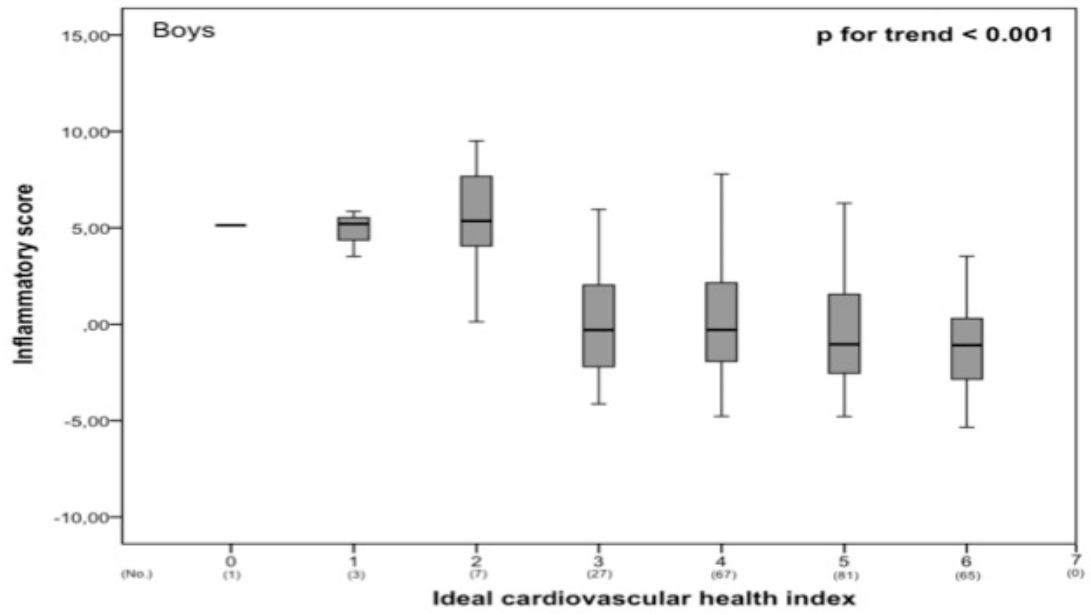642 **Figure 1.** Association between inflammatory score and Ideal Cardiovascular Health

643 index.

644

645 **Supplementary Figure 1**. Flow diagram of the study population.

646

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