The correlation of right 2D:4D finger length ratio to the low-grade inflammation marker IL-6 in children. The Healthy Growth Study

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Background: Second to fourth digit ratio (2D:4D) is associated with cardiometabolic risk in adults. Aim: To examine the association of right 2D:4D with cardiovascular disease risk factors in children. Study design: Cross-sectional study.

Subjects: A sample of 301 children (53.5% girls) aged 9–13 in Greece and their parents. Children who were sick during the previous week of examination (n = 44) were excluded from the analyses.

Outcome measures: Socio-demographic (gestational age, birth weight, age, gender, maternal education level), anthropometric (body weight and height, finger length), clinical (pubertal stage, sickness during the previous week of the examination), blood [serum high sensitivity C-reactive protein (CRP), serum high sensitivity interleukin-6 (IL-6), serum leptin], lifestyle (dietary intake, maternal smoking during pregnancy) and physical fitness (hand-grip strength) data were collected. CRP, IL-6 and leptin were measured with ELISA, using standard equipment and procedures, in accordance with manufacturers’ instructions.

Results: Full data were available for 257 children (52.1% girls). The rank values of right 2D:4D and IL-6 were included in the analyses. Right 2D:4D was correlated only with IL-6 at a bivariate level (r = 0.216, p = 0.012) in girls. At a linear multivariate level, this association remained significant, even after adjusting for several potential confounders such as age, Tanner stage, maternal education level, body mass index, maternal smoking during pregnancy, duration of pregnancy, protein-, carbohydrate-, fat-intake and physical fitness (β ± SE = 0.220 ± 0.066, p = 0.001).

Conclusions: Right 2D:4D was found to be associated with IL-6 in girls. Right 2D:4D may be a valuable, simple screening tool of low-grade inflammation in children.

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1. Introduction

The high prevalence of cardiovascular disease (CVD) [1] and myocardial infarction (MI) [2], observed over the last years, calls for urgent actions for their prevention. The origins of these diseases can be found already in the intrauterine environment [3–5], with the first clinical signs being observed in childhood and depicted as cardiovascular risk factors (e.g. blood lipids, blood pressure, markers of insulin resistance or low-grade inflammation). Previous studies have shown that CVD risk factors track from childhood to adulthood [6]. Moreover, CVD risk factors in childhood are associated with intima-media thickness in adulthood [7], and may predict adulthood CVD [8]. Therefore, the early identification of children with indices of CVD risk factors, i.e. the individuals who are potentially at high risk for CVD in later life, is essential for public health.

In this context, various anthropometric markers have been proposed over the last years, aiming to identify high risk populations [9–12]. The vast majority of these indices are depicting individuals’ risk at the time of the examination and are mainly reflecting the inverse effect of different body fat depots (e.g. visceral fat or upper body subcutaneous fat) on human metabolism, rather than an underlying pathogenic mechanism that may have exerted a negative effect on cardiovascular health during intrauterine life. Recently, Manning and Bundred [13] proposed the ratio of the second to the fourth finger length (2D:4D) as a new predictor of CVD predisposition. More specifically, it has been shown that 2D:4D reflects in utero exposure to the sex steroids, testosterone and estrogen, and there is a sexual dimorphism in the 2D:4D finger length ratio, with women having higher ratio than men [13,14]. Men with diagnosed MI were previously found to have higher 2D:4D in both hands compared to their age-matched healthy peers [15]. Moreover,
right 2D:4D was previously associated with the atherosclerotic plaque development in men [16].

Given the presumed stability of right 2D:4D over the life-cycle, this index could be a valuable anthropometric trait for identifying individuals at risk for CVD and MI. However, to the authors’ knowledge no previous study has examined the association between right 2D:4D and potential precursors of atherosclerosis and MI, such as indices of low-grade inflammation, in children. The aim of the present study was to examine the association between right 2D:4D and markers of low-grade inflammation, in a population of 9–13 year old children.

2. Subjects and methods

2.1. Study subjects

The current study is a sub-cohort of the Healthy Growth Study, a large scale cross-sectional epidemiological study initiated in May 2007 [17]. The study sample comprised of 301 apparently healthy children (53.5% girls) aged 9–13, recruited from primary schools in the wider regions of Attica and Thessaloniki, in Greece. The study protocol was approved by the Greek Ministry of National Education and the Ethical Committees of the National and Kapodistrian University of Athens and the Harokopio University of Athens. The parents and/or guardians of all subjects signed a written informed consent form before being enrolled in the study.

2.2. Data collection

All measurements of children were performed during school hours. During finger length measurement children were asked to sit comfortably on a chair, place their wrist on a standardized horizontal level, keep their fingers stretched and remain still until the completion of the measurement. Two researchers drew the outline of children’s hands, using the same type of pencil, which was placed perpendicularly onto the paper. The measurement of finger lengths was performed by one researcher, with a digital Vernier caliper recording to 0.01 mm. Finger length was measured between distal wrist crease and fingers’ tip, according to the method of Visnapuu and Jurimae [18]. 2D:4D was calculated for both hands.

The detailed protocol for blood sampling/analysis and clinical, anthropometric and dietary assessment is described elsewhere [19]. Children’s pubertal stage was assessed by one well-trained, experienced female pediatrician and children were classified according to the five Tanner stages [20]. The measurements of weight and height were taken twice by two trained members of the research team, with subjects having removed their shoes and heavy clothing prior to the measurements. Weight was measured to the nearest 0.1 g using a Seca digital scale (Seca Alpha, Model 770, Hamburg, Germany) and height to the nearest 0.1 cm using a commercially available stadiometer (Leicester Height Measure, Invicta Plastics Ltd., Oadby, Leicestershire, UK). For the measurement of height, subjects were asked to stand still and quite in an erect position, facing the researcher, hanging their arms freely and keeping their head aligned in the Frankfurt plane. Right handgrip strength was measured using an electronic handgrip dynamometer (TAKEI, Japan, Physical Fitness Test Grip-D). Two readings of this measurement were taken and an average value of these readings was calculated. Information regarding maternal education level (years of education), maternal smoking during pregnancy (status of smoking and number of cigarettes), duration of pregnancy (weeks) and children’s birth weight (kg) was collected via parental questionnaires.

Body mass index (BMI) was assessed from weight and height using the Quetelet’s equation (weight [kg] / height² [m²]). Children self-reported in questionnaires if they were sick during the previous week of the examination.

Serum C-reactive protein (CRP) levels were measured by ELISA (enzyme-linked immunosorbent assay) with CRP sandwich ELISA kit (R&D Systems Minneapolis USA), serum interleukin-6 (IL-6) levels by high-sensitivity IL-6 sandwich ELISA (Quantikine HS, R&D Systems Minneapolis, MN, USA) and serum leptin levels by human leptin ELISA, Clinical Range kit (BioVendor Research and Diagnostic products, Karasek, Czech Republic), according to manufacturers’ instructions.

2.3. Statistics

Normality of distribution was evaluated through the Kolmogorov–Smirnov test. Normally distributed data are expressed as mean values (standard deviation), whereas skewed variables as median (25th, 75th interquartiles). Differences between genders were analyzed with cross-tabs (χ²) for categorical data and Student’s t-test or the Mann–Whitney test for continuous data. The rank values of right 2D:4D and markers of low-grade inflammation were calculated and used in the bivariate and multivariate analyses. Correlations between 2D:4D and markers of low-grade inflammation were conducted with Pearson’s correlation. Multiple linear regression analysis was applied to further examine the associations. All analyses were performed using Statistical Package for Social Sciences (version 18, SPSS Institute, Chicago, IL, USA). p < 0.05 was set as level of significance.

3. Results

For the purposes of the current study, 44 children were excluded, because they were sick during the previous week of the examination. The characteristics of the study sample are presented in Table 1. The International Obesity Task Force (IOTF) criteria [21] were applied to calculate the prevalence of obesity in the study sample, which was higher among boys. 63.8% of the children (i.e. 82.1% of the boys and 47% of the girls) were categorized in Tanner stages I and II. At a bivariate level, right 2D:4D was positively correlated with IL-6 in girls (r = 0.216, p = 0.012), but not in boys. Right 2D:4D was correlated neither with CRP nor with leptin at a bivariate level, in both genders. In multivariate regression analyses (Table 2), right 2D:4D was associated with IL-6 in girls, even after adjusting for several potential confounders such as age, Tanner stage, maternal education level, BMI, maternal smoking during pregnancy, duration of pregnancy, protein-, carbohydrate-, fat-intake and physical fitness (β ± SE = 0.220 ± 0.067, p = 0.001). No similar findings were observed in boys. No associations were observed between right 2D:4D and CRP or leptin in both genders. Fig. 1 depicts the correlation of right 2D:4D with IL-6 in both genders.

4. Discussion

The present study aimed to examine the association between right 2D:4D and markers of low-grade inflammation in a population of 9–13 year old children in Greece. Right 2D:4D finger length ratio was found to be positively associated with IL-6 in girls both at bivariate and multivariate analyses, even after adjusting for various confounders. To the authors’ knowledge this is the first study to examine the relationship between right 2D:4D and low-grade inflammation in children, thus making comparisons with other studies difficult. Right 2D:4D finger length ratio has already been validated as a better indicator of prenatal sex androgens effect [22].

Our results in children contradict the findings of Kyriakidis and Papaioannidou [15], who found that 2D:4D in both hands was significantly higher in Greek hospitalized men with MI comparing to their age-matched healthy peers, but did not observe any statistically significant association in women. This inconsistency may be attributed to the different age-groups examined in each study since our study sample included only a limited number of adolescent boys. In a study comprising adolescents and young adults aged 12–30 years of both sexes, it has already been reported that the sex dimorphic 2D:4D ratio is age dependent and the sex difference in 2D:4D varied across the age groups in both hands [23]. Therefore the absence of significant percentage of
adolescent boys in our cohort may account for the lack of correlation between right 2D:4D and low-grade inflammation markers observed in the boys group included in our study. Moreover, the lack of association between right 2D:4D and other markers of low-grade inflammation, namely CRP and leptin, observed in this study cannot be compared against previous findings since such studies are currently lacking in the literature. Future studies are needed to elucidate these findings.

Right 2D:4D has been previously found to correlate negatively to the intrauterine exposure to prenatal testosterone [24]. Previous studies have also shown that right 2D:4D is associated with atherosclerotic plaque development in men [16] and that 2D:4D in both hands is associated with coronary heart disease [25] in men. In contrast, it is known that male sex steroids, such as testosterone and dihydrotestosterone, may impede the secretion of IL-6 via an androgen-specific c receptor [27]. Moreover, inflammatory cytokines, such as IL-6, are involved in the early formation of atherosclerotic plaque, whereas their prolonged expression is linked to increased plaque’s instability and proneness to falling-out, thus leading to unstable angina and MI clinical manifestations [28]. However, no previous study has ever examined the association of right 2D:4D with IL-6, a molecule whose levels are dependent on the intrauterine exposure to prenatal testosterone and which may be involved in the development of cardiovascular disease in adulthood. According to our findings, right 2D:4D may be proposed as an anthropometric trait, which could potentially serve as a useful, stable tool to indirectly assess CVD-risk across the lifespan. However, it remains to be studied in larger prospective studies investigating adolescent boys and young men whether a correlation between right 2D:4D ratio and IL-6 develops with advancing age.

The findings of the present study should be interpreted under the light of its limitations. Specifically, the relatively small and non-representative study sample and the cross-sectional design do not allow the establishment of causality regarding the association between right 2D:4D and IL-6 observed in the girls of our cohort. As already pointed out, the fact that significant results were observed only for girls and not for boys may be attributed to the limited number of pubertal male subjects compared to the number of pubertal female subjects, included in this study. Puberty is a period of life during which significant metabolic changes are observed [29], and the 2D:4D ratio in both hands still evolves [23], therefore further studies are needed to explore the utility of right 2D:4D in male adolescents. It is well known that children born either small or large for gestational age (SGA, LGA, respectively) are at higher risk for cardiovascular disease in later life [4]. However, our study sample did not include many small or large for gestational age born (SGA and LGA, respectively) children; therefore it was not possible to search for potential differences of these children with children born with birth weight appropriate for gestational age (AGA). In fact, a large study sample would enable the comparisons between SGA, LGA and AGA children, thus providing more insight regarding the association of right 2D:4D and IL-6 attributable to epigenetic intrauterine alterations and to clarify whether this association is more influenced by intra- or extra-uterine factors. Moreover, future large cohort studies should further explore the association between right 2D:4D and low-grade inflammation, including more pubertal boys, more children born SGA or LGA and even in populations other than Caucasians, while studies that would include more data of the intra-uterine environment (e.g. with the use of prenatal ultrasound for clear assessment

Table 1
Characteristics of study sample.

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 257)</th>
<th>Boys (n = 123)</th>
<th>Girls (n = 134)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)a</td>
<td>11.37 (10.83, 11.79)</td>
<td>11.16 (10.74, 11.70)</td>
<td>11.47 (10.91, 11.91)</td>
</tr>
<tr>
<td>BMI (kg/m²)a</td>
<td>19.61 (17.44, 22.5)</td>
<td>19.61 (17.40, 22.51)</td>
<td>19.55 (17.54, 22.52)</td>
</tr>
<tr>
<td>Overweightb</td>
<td>28.8</td>
<td>30.1</td>
<td>27.6</td>
</tr>
<tr>
<td>Obeseb</td>
<td>10.5</td>
<td>11.4</td>
<td>9.7</td>
</tr>
<tr>
<td>AGAb</td>
<td>74.7</td>
<td>71.6</td>
<td>77.6</td>
</tr>
<tr>
<td>SGAa</td>
<td>17.5</td>
<td>21.1</td>
<td>14.2</td>
</tr>
<tr>
<td>LGAa</td>
<td>7.8</td>
<td>7.3</td>
<td>8.2</td>
</tr>
<tr>
<td>Tanner stage Ib</td>
<td>31.1</td>
<td>45.5**</td>
<td>17.9</td>
</tr>
<tr>
<td>Tanner stage IIIb</td>
<td>32.7</td>
<td>36.8**</td>
<td>29.1</td>
</tr>
<tr>
<td>Tanner stage IVb</td>
<td>27.2</td>
<td>17.1</td>
<td>36.6**</td>
</tr>
<tr>
<td>Tanner stage Vb</td>
<td>7.0</td>
<td>0.8</td>
<td>12.7**</td>
</tr>
<tr>
<td>Tanner stage Vb</td>
<td>3.9</td>
<td>0.0</td>
<td>3.7**</td>
</tr>
<tr>
<td>Mothers smoking during pregnancyb</td>
<td>14.0</td>
<td>13.0</td>
<td>14.9</td>
</tr>
<tr>
<td>Protein intake (g/d)a</td>
<td>65.86 (50.78, 80.68)</td>
<td>69.40 (56.28, 82.50)</td>
<td>61.84 (47.68, 78.39)</td>
</tr>
<tr>
<td>Carbohydrate intake (g/d)a</td>
<td>200.68 (164.20, 243.47)</td>
<td>212.26 (170.09, 257.61)</td>
<td>191.9 (150.66, 229.34)</td>
</tr>
<tr>
<td>Fat intake (g/d)a</td>
<td>81.34 (62.30, 97.34)</td>
<td>85.76 (69.45, 101.13)</td>
<td>74.81 (59.47, 92.95)</td>
</tr>
<tr>
<td>Right grip strength</td>
<td>20.78 (5.13)</td>
<td>20.77 (4.99)</td>
<td>20.78 (5.28)</td>
</tr>
<tr>
<td>CRP (ng/ml)a</td>
<td>493.0 (177.5, 1736.5)</td>
<td>610.0 (218.0, 1732.0)</td>
<td>408.5 (155.2, 1138.5)</td>
</tr>
<tr>
<td>IL-6 (pg/ml)a</td>
<td>0.86 (0.58, 1.29)</td>
<td>0.889 (0.629, 1.360)</td>
<td>0.819 (0.543, 1.263)</td>
</tr>
<tr>
<td>Leptin (ng/ml)a</td>
<td>7.13 (3.25, 13.90)</td>
<td>7.47 (2.11, 11.00)</td>
<td>8.59 (3.13, 17.85)***</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.

BMI = Body Mass Index, AGA = appropriate for gestational age, SGA = small for gestational age, LGA = large for gestational age.

CRP: high sensitivity serum C-reactive protein; IL-6: high sensitivity serum interleukin-6.

The percentages of overweight and obesity were calculated using the IOTF-criteria.

a Data are presented as median (25th, 75th percentile).
b Data are presented as % percentages.

p < 0.05 for the comparisons between genders.

** p < 0.001 for the comparisons between genders.

Table 2
Multiple regression analysis model, exploring the association of right hand second to fourth finger ratio (right 2D:4D) with various markers of low-grade inflammation in boys and girls.

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
</tr>
<tr>
<td>CRP</td>
<td>0.004</td>
<td>0.039</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.050</td>
<td>0.075</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.001</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Abbreviations: CRP; C-reactive protein; IL-6: interleukin-6; Right 2D:4D: right hand finger length ratio; β: Standardized beta coefficient; SE: standard error.

Model adjusted for age, Tanner stage, body mass index, maternal smoking during pregnancy, maternal education level, duration of pregnancy, protein-, carbohydrate-, fat-intake and physical fitness.

Protein intake (g/d)a 65.86 (50.78, 80.68) 69.40 (56.28, 82.50) 61.84 (47.68, 78.39)

IL-6 (pg/ml)a 0.86 (0.58, 1.29) 0.889 (0.629, 1.360) 0.819 (0.543, 1.263)

Leptin (ng/ml)a 7.13 (3.25, 13.90) 7.47 (2.11, 11.00) 8.59 (3.13, 17.85)***
of fetal growth) would provide more insight regarding the association of right 2D:4D, prenatal exposure to androgens and IL-6.

In contrast, a major strength of this study is the inclusion of children of all weight categories, which broadens the applicability of the findings. The consideration of several confounders previously described as potential correlates of IL-6, i.e. age, Tanner stage, socio-economic status (assessed by maternal education level in this study), BMI, maternal smoking during pregnancy, duration of pregnancy, protein-, carbohydrate-, fat-intake and physical fitness, in the analyses comprises another strength of this study. In conclusion, the present study showed that right 2D:4D was associated with IL-6 in girls. Right 2D:4D may be a valuable, simple anthropometric tool for the identification of low grade inflammation in children and adolescents and early identification of individuals at high risk for MI. Large scale prospective studies comprising pubertal boys and girls may further elucidate the value of right 2D:4D in the identification of individuals at risk for MI.

Conflict of interest statement

None declared.

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Collaborators


References


Fig. 1. Correlation between the rank values of right hand second to fourth finger ratio (right 2D:4D) and rank values of high sensitivity serum IL-6 in 9–13 years old boys (n = 123) and girls (n = 134).


