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## **Associations of Childhood Adiposity with Adult Intima Media Thickness and Inflammation: A 20-Year Longitudinal Population-based Cohort**

**Running title:** Childhood adiposity, adult carotid IMT and inflammation

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## **Abstract**

### **Background**

The associations between childhood adiposity and adult increased carotid intima thickness (cIMT) have been well-established, which might be corroborated by the association between adiposity in children and inflammation in adults. However, longitudinal data regarding biological pathways associated with childhood adiposity are lacking.

### **Methods**

The present study included participants from the STANISLAS cohort who had adiposity measurements at age 5-18 years (N=519, mean [SD] age, 13.0 [2.9] years; 46.4% male), and who were measured with cIMT, vascular- and metabolic-related proteins at a median follow-up of 19±2 years. Body mass index (BMI), waist-to-height ratio and waist circumference were converted to age- and sex-specific z-scores.

### **Results**

A minority of children were overweight/obese (16.2% overweight-BMI z-score>1; 1.3% obesity-z-score>2). Higher BMI, waist-height ratio and waist circumference in children were significantly associated with greater adult cIMT in univariable analysis, although not after adjusting for C-reactive protein (CRP). These associations were more pronounced in those with consistently high adiposity status from childhood to middle-adulthood. Participants with higher adiposity during childhood (BMI or waist-height ratio) had higher levels of insulin-like growth factor binding protein-1, and -2, matrix metalloproteinase-3, osteopontin, hemoglobin and CRP at adulthood. Network analysis showed that interleukin-6, insulin-like growth factor-1 and fibronectin were the key proteins associated with childhood adiposity.

### **Conclusions**

In a population-based cohort followed for 20 years, higher BMI or waist-to-height ratio in childhood was significantly associated with greater cIMT and enhanced levels of proteins reflective of inflammation, supporting the importance of inflammation as the progressive atherosclerosis in childhood adiposity.

**Keywords:** childhood adiposity, carotid intima media thickness, biomarkers, inflammation.

## Introduction

The prevalence of overweight and obesity has substantially increased in children over the last decades<sup>1</sup>. Large epidemiological studies have established that a high childhood adiposity status is associated with a risk of increased carotid intima media thickness (cIMT) in mid-adulthood<sup>2,3</sup>. However, given that childhood adiposity is highly related to adult adiposity, the attributable risk specific to childhood adiposity is difficult to quantify. Hence, the assessment of trajectories of child-to-adult adiposity may better discriminate the relative impact of childhood and/or adult adiposity status on adult increased cIMT.

The underlying mechanisms in adults associated with childhood adiposity might underpin the associations between childhood adiposity and adult increased cIMT. Inflammation is related to adiposity and atherosclerotic disease but the interplay between these 3 features (adiposity, atherosclerotic disease, and inflammation) were predominantly studied in cross-sectional studies<sup>4-6</sup>. Longitudinal data whether children with adiposity status were prone to have heightened inflammation status in adults are lacking. An approach to better understand biological pathways is to use knowledge-based network analysis on the results retrieved by circulating protein analysis. This approach may help better understand which circulating proteins may be pivotal of these protein-protein relationships<sup>7,8</sup>.

In the STANISLAS (Suivi Temporaire Annuel Non-Invasif de la Santé des Lorrains Assurés Sociaux) cohort followed over 20 years, circulating proteins of various pathophysiological pathways were collected<sup>9</sup>. In the present study, we aimed at exploring: 1) the association between childhood adiposity and adult cIMT as well as circulating proteins expressing vasculature and adiposity, 2) the associations of child-to-adult adiposity trajectories with adult cIMT, and 3) identifying the biological pathways underlying the association between childhood adiposity and adult vascular alterations.

## **Methods**

### **Study population**

The STANISLAS cohort is a single-center familial longitudinal cohort from the Nancy region in France, the features of which have been previously described <sup>10</sup>. Among 4,295 participants undergoing the 1<sup>st</sup> cohort visit from 1993 to 1995, 519 children aged 5 to 18 years with available data on vascular variables at the 4th visit (from 2011 to 2016) were included in the current analysis (*Supplemental Figure 1*). The study was approved by the Ethics Committee (Comité de Protection des Personnes Est III, Nancy, France), and all study participants gave their written informed consent to participate (ClinicalTrials.gov identifier NCT01391442).

All participants completed medical and socio-demographic questionnaires, and underwent a physical examination and vascular measurements. Collected variables included age, sex, height, weight, smoking status and physical activity. Standard biochemical blood parameters included triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, blood glucose, glycated hemoglobin and estimated glomerular filtration rate (eGFR, as calculated from the Schwartz formula in children <sup>11</sup>, and the Chronic Kidney Disease Epidemiology Collaboration formula in adults <sup>12</sup>). Metabolic syndrome in adults was defined according to Adult Treatment Panel III criteria <sup>13</sup>.

### **Body mass index, waist circumference and waist-to-height ratio**

BMI was calculated by weight in kilograms divided by the square of the height in meters. Waist circumference was measured at the narrowest level, and waist-to-height ratio was calculated from the ratio of waist circumference and height. Body mass index (BMI) was converted to age- and sex-specific z score based on the International Obesity Task Force (IOTF)<sup>14</sup>, while waist-to-height ratio and waist circumference were based on the Third National Health and Nutrition Examination Survey (NHANES III) <sup>15</sup>. Overweight and obesity

were respectively defined by a BMI z-score >1 and >2 as proposed by the IOTF<sup>16</sup>.

### **Vascular variables**

cIMT was measured by high-resolution echo-tracking in a controlled environment at 22±1°C after 10 min of rest in the supine position. Carotid diameter, distension and intima media thickness were measured on the right common carotid artery<sup>17</sup>. Examinations were successively performed with the wall track system (WTS, ESOATE, Maastricht, The Netherlands) and the ART.LAB (ESAOTE, Maastricht, The Netherlands). The detailed methodology and excellent reproducibility of cIMT measurements in this cohort has previously been reported<sup>18</sup>.

### **Circulating proteins**

For vascular-related biomarkers, extracellular matrix remodeling in vasculature, angiogenesis and endothelial cell dysfunction were supported by the expression of matrix metalloproteinase-2 (MMP-2), MMP-3, MMP-9, vascular endothelial growth factor A (VEGF-A), bone morphogenetic protein-9 (BMP-9), insulin-like growth factor binding protein-1 (IGFBP-1), IGFBP-2, osteopontin (OPN) and Cystatin C<sup>19-26</sup>. For adiposity-related biomarkers, fatty acid-binding protein-4 (FABP-4), leptin, resistin, cystatin C, kidney injury molecule-1 (KIM-1), hemoglobin, interferon-gamma (IFN- $\gamma$ ), C-reactive protein (CRP), triglycerides were also studied<sup>27-29</sup>. These selected biomarkers were measured using Olink Proseek® Multiplex cardiometabolic, cardiovascular II, III and Inflammation 96x96 kits in serum using proximity extension assay technology (<https://www.olink.com/products/complete-protein-biomarkers-list>)<sup>30</sup>.

### **Complex network analysis**

The Fight-HF graph knowledge box was used to build the complex network<sup>7</sup>. Direct interaction between studied biomarkers were extracted from the STRING database<sup>31</sup>. The other proteins known to directly interact with at least five biomarkers were added to the

network. Biomarkers belonging to the same REACTOME pathway were linked as indirect interaction<sup>32</sup>.

## **Statistical analysis**

Categorical variables are presented as frequencies (percentages) and continuous variables as means  $\pm$  standard deviation or median (25th and 75th percentiles) depending on variable distribution. Comparisons of demographic, clinical and biological variables according to tertiles of childhood adiposity status were analyzed using ANOVA, Kruskal-Wallis test and  $\chi^2$  test, as appropriate.

Linear regression analyses were used to study the associations of childhood adiposity as assessed by BMI, waist-height ratio, and waist-circumference with adult vascular variables. These models were adjusted for clinical variables specific for the childhood and adult periods, respectively: childhood model – age, sex, systolic blood pressure (BP), blood glucose, total cholesterol, triglycerides and natural log of C-reactive protein (CRP); adult model – age, sex, prevalence of metabolic syndrome<sup>33</sup>, hypertension, systolic BP, diastolic BP, total cholesterol and triglycerides.

To assess whether the biomarkers were expressed differently between children with higher (top tertile) and lower (bottom tertile) BMI or waist-height ratio, multinomial logistic regression analyses were performed after adjustment for respective BMI or waist-height ratio in adults as an independent variable plus age, a prevalence of metabolic syndrome, hypertension, diabetes mellitus, systolic BP and total cholesterol.

All analyses were performed using R version 4.0.1 (R Development Core Team, Vienna, Austria). A two-sided p-value  $<0.05$  was considered statistically significant.

## **Results**

### **Baseline characteristics according to childhood adiposity**

Among the 519 children included in this study, slightly less than a half were males



(46.4%), mean age (SD) was 13.0 (2.9) years, 38.7% (N=201) were adolescents ( $\geq 14$  years), mean BMI z-score was 0.04 (0.90) (16.2% overweight, 1.3% obesity) and the median follow-up period was 19.1 years (25th and 75th centiles 17.9 to 20.0).

Children with higher age- and sex-standardized BMI had higher CRP levels at childhood, and more frequent prevalence of metabolic syndrome at adulthood (all-p-values  $< 0.05$ ) (*Table 1*). Children with higher age- and sex-standardized waist-height ratio were male, had higher blood pressure, lower heart rates, greater inflammation status at childhood, higher BP and cIMT at adulthood (*Table 1*).

Baseline characteristics according to the tertiles of age- and sex-standardized waist circumference are presented in *Supplemental Table 1*. Baseline characteristics associated with waist circumference were similar to those associated with waist-to-height ratio.

#### **Associations between childhood adiposity and adult intima media thickness**

In univariable models, high childhood adiposity status as assessed by higher BMI, waist-height ratio and waist circumference was significantly and consistently associated with greater cIMT at adulthood. These associations persisted after adjustment for clinical covariates measured at both childhood and adult periods. However, after adjustment for covariates including CRP measured at childhood, these associations no longer remained significant (all p-values  $> 0.05$ ) (*Table 2*).

The observed associations between childhood adiposity status and adult cIMT were similar in adolescents ( $\geq 14$  years) and children ( $< 14$  years) (all-P for interaction  $> 0.10$ ), whereas the associations between childhood adiposity status and cIMT were prominent in females (all-P for interaction  $< 0.01$ ) (*Supplemental Table 2*).

The association of overweight or obesity in children with adult cIMT was not modified by CRP levels in children (P-value  $> 0.10$ ).

#### **Association between child-to-adult adiposity trajectories and adult intima media**

## **thickness**

Subjects were divided into 4 categories according to a median value of childhood and adult adiposity (*i.e.*, BMI, waist-to-height ratio and waist circumference). Subjects with consistently high BMI from childhood to middle adulthood were likely to have a greater cIMT than those who had low BMI in both periods, with these associations persisting after adjustment for potential confounders (*Figure 1 & Supplemental Table 3*). In addition, subjects who had a high waist-to-height ratio as adults, irrespective of their childhood waist-to-height ratio, were likely to have greater cIMT, with these associations also remaining significant even in multivariable models (*Figure 1 & Supplemental Table 3*).

## **Complex network analysis**

IGFBP-1, IGFBP-2, MMP-3, osteoponin, hemoglobin and CRP levels were significantly higher (up-regulated), whereas VEGF-A, FABP-4, leptin, and IFN-gamma were significantly lower (down-regulated) in individuals with higher childhood adiposity status (based on BMI or waist-height ratio) (*Supplemental Table 4*). Among these proteins associated with childhood adiposity, interleukin-6 (IL-6), insulin-like growth factor-1 (IGF-1) and fibronectin were the pivotal proteins linked to 5 proteins. *Figure 2*.

## **Discussion**

In the present French population-based cohort followed over a period of 20 years, a higher adiposity status as assessed by higher BMI, waist-height ratio or waist circumference in childhood was found to be consistently and independently associated with greater cIMT in adulthood, although these associations no longer remained significant after adjusting for inflammation (as assessed by CRP at childhood). In addition, subjects with consistently high BMI from childhood to adulthood were likely to have greater cIMT in adulthood. Inflammation, as expressed by IL-6 and IGF-1, may possibly represent central proteins associated with childhood

adiposity.

Taken together, these data point to the potential relevance of inflammatory-related pathways in explaining the associations between childhood adiposity and middle-age atherosclerosis.

### **Associations between childhood adiposity and adult cIMT**

In the present study, the proportions of overweight and obese children were similar to those documented in previous reports<sup>34,35</sup>. Childhood adiposity as assessed by higher BMI, waist-height-ratio or waist circumference was associated with greater cIMT in middle-aged adults<sup>2,3,36,37</sup>. Furthermore, subjects with consistently high BMI from childhood to adulthood were likely to have greater cIMT in adults, which are consistent with previous reports<sup>3,36</sup>. By contrast, higher waist-to-height ratio in adults rather than children was associated with greater adult cIMT. These findings suggest that waist circumference may less likely express adiposity status particularly in children, and that assessing childhood abdominal fat may be less reliable to predict the degree of adult cIMT. Interestingly, although physiological studies show overweight or obese individuals have higher or similar heart rate than lean ones, in the current analysis, higher waist circumference was associated with lower heart rate. As, in prior studies, body weight and height-based measurements (*i.e.*, BMI) were used to define overweight or obesity<sup>38,39</sup>. This may have an impact on the results as larger height has been shown to be associated with lower heart rate<sup>40</sup>, which may counterbalance the differences in heart rate among BMI tertile groups.

Of note, the association between childhood adiposity status and adult increased cIMT was more pronounced in females than in males, which may be partly explained by the substantial sex-related differences in the adipose distribution<sup>41,42</sup>. Women are likely to have greater peripheral fat depots and lower skeletal muscle mass, while men are likely to have greater central adiposity and higher skeletal muscle mass. Although prior longitudinal data was consistent with our results<sup>43</sup>, such sex-related differences are modified by several

environmental factors (i.e., diet, physical activity), and further data are required to rigorously evaluate these sex-related interactions. Of further interest, all observed associations between childhood adiposity status and adult cIMT lost their statistical significance when CRP was included in the multivariable models. Although, among middle-aged adults, our team reported that adiposity was an important predictor of long-term increases in pulse wave velocity, independent of inflammation status<sup>44</sup>, the current findings may highlight that adiposity status in children induces an inflammation state, which may be involved in atherosclerotic progression<sup>45, 46</sup>. Our observations, therefore, suggest that inflammation may be a key biological factor underlying the association between childhood adiposity status and adult cIMT.

### **Inflammation markers as central proteins associated with childhood adiposity**

A growing number of papers use many circulating proteins resulting in many relevant associations. Filtration and/or integration of these associations, therefore, should be required to better interpret the results. In this regard, network analysis is becoming a relevant step of omics-based analyses to better demonstrate protein, disease, and pathway relations.

Our network analysis demonstrated that childhood adiposity was associated with inflammatory response over 20 years, which was mainly mediated by IL-6 and IGF-1 in the network analysis. IL-6 is a well-known cytokine involved in the regulation of the inflammation and immune response<sup>47</sup>. IL-6 and interleukin-1 $\beta$  as a driver of IL-6, play important roles on progressive atherosclerosis such as the development of atherothrombotic plaque, the advancement of monocyte/leukocyte adhesion to vascular endothelial cells, and the progress of vascular smooth-muscle cells<sup>48,49</sup>. Prior reports showed higher IL-6 levels were associated with greater degrees of cIMT in population settings<sup>50, 51</sup>. IGF-1 is also known as a mediator of inflammation response, and its excess induces pro-inflammatory cytokine production, may promote endothelial dysfunction, and increase oxidative stress, possibly contributing to the

progression of atherosclerosis<sup>52,53</sup>. Fibronectin, another central protein in our network, may represent a link between inflammation and vascular remodeling: Budatha and colleagues reported that fibronectin and integrin  $\alpha 5$  signaling are key elements of pathological vascular remodeling in hypertension models<sup>54</sup>. Fibronectin in injured, remodeled, or inflamed vessels, could sensitize endothelial cells to inflammation through integrins upregulation<sup>54</sup>.

Taken together, these findings highlighted that childhood adiposity was associated with inflammatory-related process in adults, irrespective of adult adiposity status, and that inflammation, supported by fibronectin sensitization, may be a key determinant of progressing atherosclerosis as evaluated by cIMT.

### **Clinical perspectives**

Increased cIMT is associated with a high risk of future development of atherosclerotic diseases such as stroke and/or coronary artery disease<sup>55, 56</sup>. Our results consequently suggest that childhood adiposity is associated with deleterious long-term vascular evolution. From a prevention perspective, assessing childhood adiposity status and subsequently implementing interventions toward weight reduction should therefore be promoted, as already emphasized in several studies<sup>3, 36</sup>.

Our findings emphasize the importance of inflammation pathway in the link between childhood adiposity and adult atherosclerotic disease. Indeed, the conjunction of adiposity and inflammation, possibly related to visceral obesity, appears as a potent vector of long-term vascular impairment<sup>57</sup>.

Recently published data showed that, canakinumab, as anti-inflammatory agents for Il-6 cytokine, diminished atherosclerotic progression in an experimental animal study<sup>58</sup>. Moreover, this treatment significantly reduced rate of recurrent cardiovascular events (*i.e.*, myocardial infarction, stroke, or cardiovascular death), compared with placebo, independent of lipid-level lowering agents<sup>59</sup>. Further studies, therefore, should be required to test the effects of canakinumab on alterations in

intima media thickness, particularly in people who had a childhood adiposity status, in light of our results.

### **Limitations**

Our results should be interpreted in light of the following limitations. This study is limited by its inherent observational design; thus, causality cannot be inferred. The proportions of overweight and obese children were relatively small, although these proportions were similar to those in previous reports<sup>34, 35</sup>. Waist-to-height ratio and waist circumference were converted to age- and sex-specific z scores based on the NHANES III, which is often applied to children in the US<sup>15</sup>. Nevertheless, we observed similar associations of BMI, waist-to-height ratio and waist circumference, suggesting that different scales may have less of an impact on the observed associations between childhood adiposity and adult vascular alterations. In addition, no data were available regarding vascular-related proteins measured in the childhood period. Further study is warranted to externally validate our findings in other community-based cohorts.

### **Conclusions**

In a population-based cohort followed for 20 years, higher BMI or waist-to-height ratio in childhood was significantly associated with greater cIMT and enhanced levels of proteins reflective of inflammation, supporting the importance of inflammation as the progressive atherosclerosis in childhood adiposity.

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## Graphical abstract

BMI, body mass index; IMT, intima media thickness; MMP3, matrix metalloproteinase-3; OPN, osteopontin; IGFBP1; insulin-like growth factor-1; IGFBP2, insulin-like growth factor-2; IFNG, interferon-gamma; LEP, leptin; FABP4, fatty acid-binding protein-4; CRP, C-reactive protein; VEGF-A, vascular endothelial growth factor-A; IL-6, interleukin-6; IGF-1, insulin-like growth factor-1; FN, fibronectin.

### **Figure 1. Univariable model for the association of childhood-to-adult adiposity with adult cIMT**

Abbreviations are presented in Table 1.

### **Figure 2. Network analysis relating the top proteins associated with body mass index and/or waist-height-ratio in children**

Network analysis presents that the top proteins are associated with higher vs lower levels of body mass index and/or waist-height-ratio in children.

MMP3, matrix metalloproteinase-3; OPN, osteopontin; IGFBP-1; insulin-like growth factor-1; IGFBP-2, insulin-like growth factor-2; IFNG, interferon-gamma; LEP, leptin; FABP-4, fatty acid-binding protein-4; CRP, C-reactive protein; VEGF-A, vascular endothelial growth factor-A; IL-6, interleukin-6; IGF-1, insulin-like growth factor-1; FN, fibronectin.

## Graphical abstract

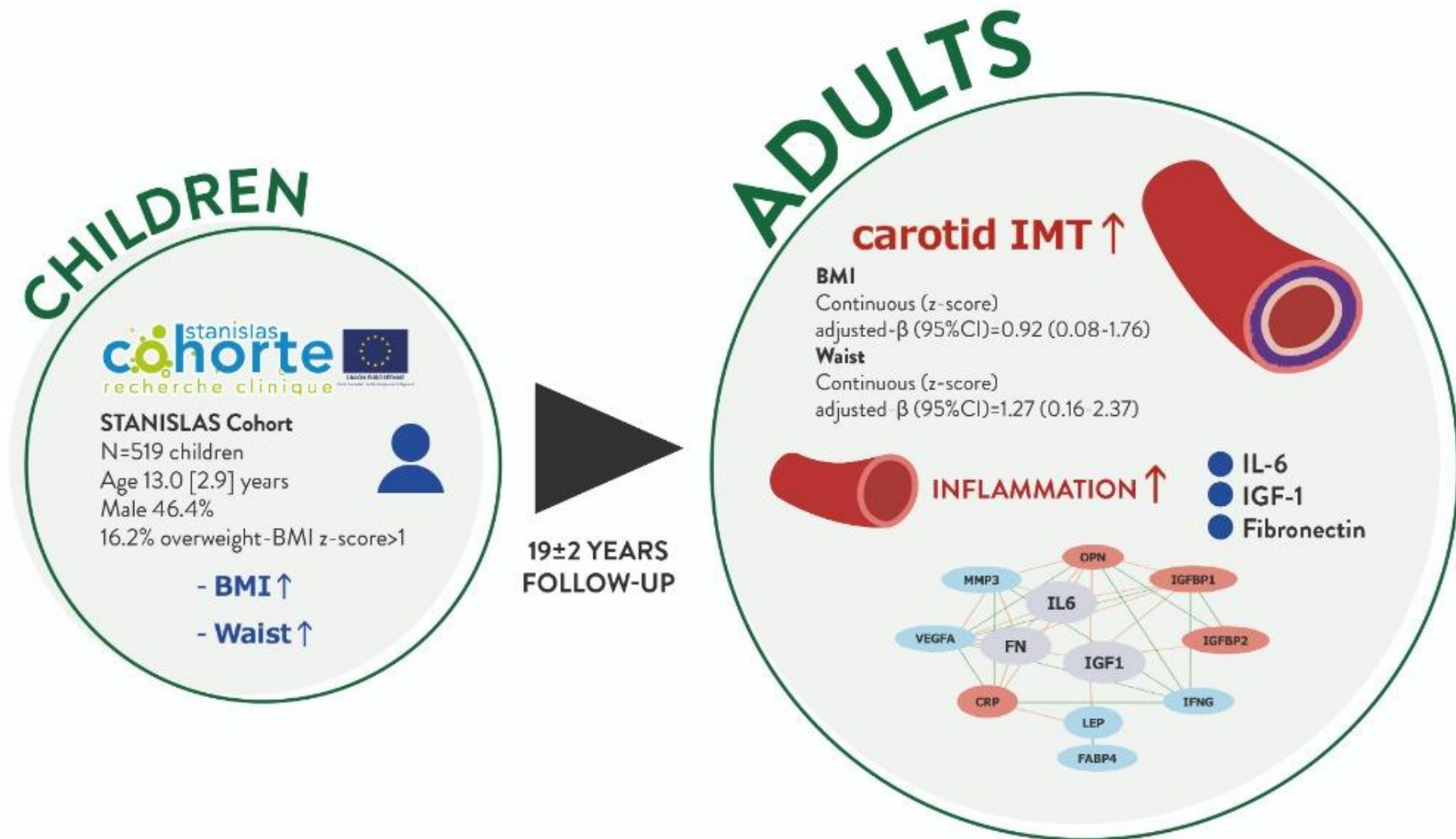
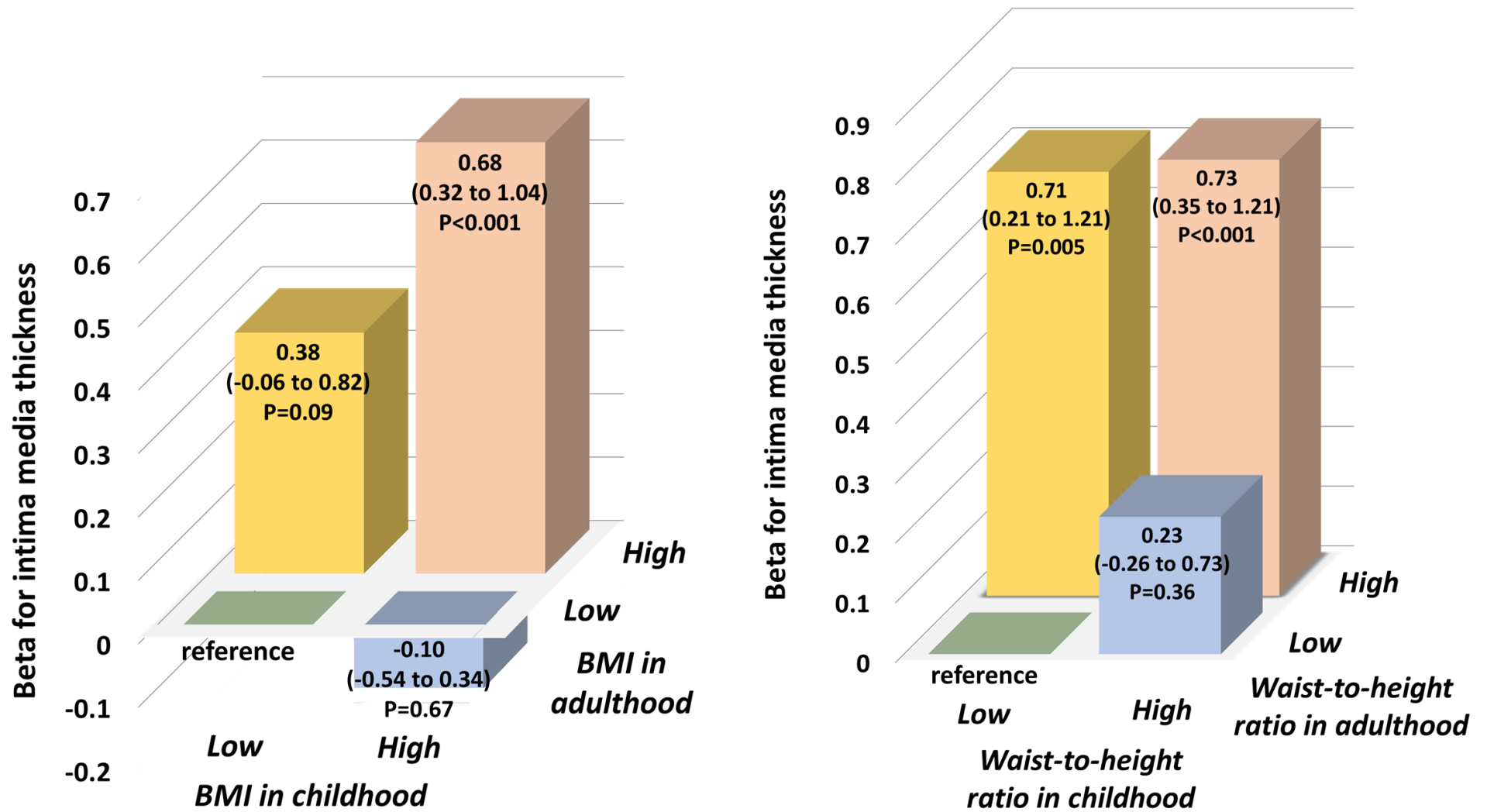
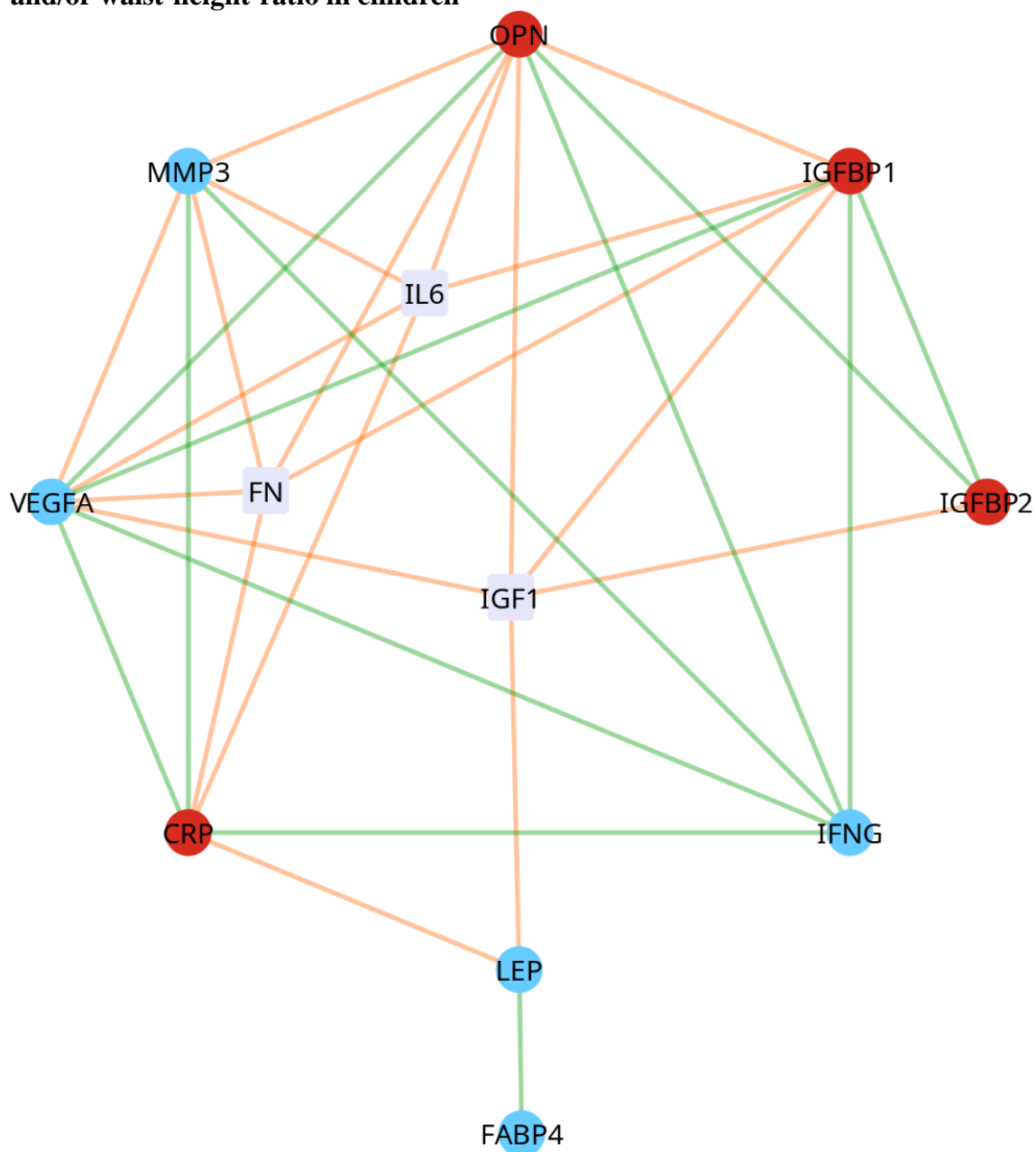




Figure 1. Univariable model for the association of childhood-to-adult adiposity with adult cIMT



**Figure 2. Network analysis relating the top proteins associated with body mass index and/or waist-height-ratio in children**



**Table 1. Baseline characteristics according to the tertiles of BMI and waist-to-height ratio z-scores in children**

Values are expressed as mean  $\pm$ SD, n (%) or median (25th to 75th percentile)

BMI was converted to age- and sex-specific z score based on the International Obesity Task Force (IOTF)<sup>1</sup>, while waist-to-height ratio was based on the Third National Health and Nutrition Examination Survey<sup>2</sup>.

BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate, CRP, C-reactive protein; MET, metabolic equivalent; IMT, intima media thickness.

**Table 2. Linear regression analyses of the association between childhood adiposity and adult cIMT**

A dependent variable was 10 $\mu$ m increase in adult carotid intima media thickness.

BMI was converted to age- and sex-specific z score based on the International Obesity Task Force (IOTF)<sup>1</sup>, while waist-to-height ratio and waist circumference were based on the Third National Health and Nutrition Examination Survey<sup>2</sup>. Overweight and obesity were respectively defined by a BMI z-score >1 and >2 as proposed by the IOTF<sup>3</sup>.

Model for the childhood period included age, sex, systolic blood pressure, blood glucose, total cholesterol, and triglycerides. Model for the adult period included age, sex, metabolic syndrome, hypertension, systolic blood pressure, blood glucose, total cholesterol, and triglycerides.

CI, confidential interval; remaining abbreviations are presented in Table 1.

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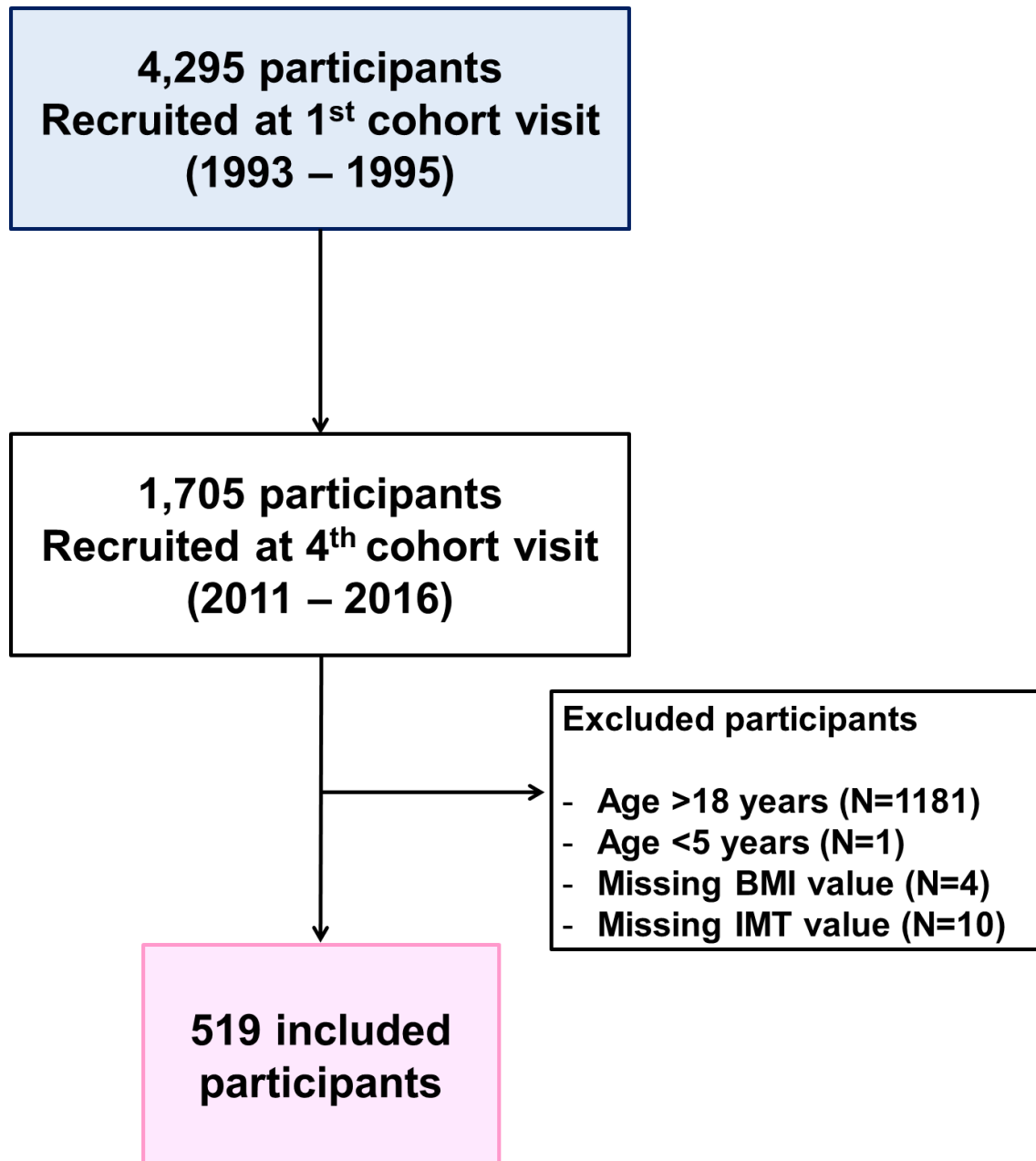
**Table 1. Baseline characteristics according to the tertiles of BMI and waist-to-height ratio z-scores in children**

	BMI z score tertile			p-value	Waist-to-height ratio z score tertile			p-value
	T1, < -0.35 (N=173)	T2, -0.35 - 0.40 (N=173)	T3, > 0.40 (N=173)		T1, < -1.20 (N=148)	T2, -1.20 - -0.53 (N=146)	T3, > -0.53 (N=146)	
<b>Variables for the childhood period</b>								
Age, years	12.5 ± 2.9	12.9 ± 2.9	13.1 ± 2.8	0.13	13.0 ± 2.8	12.9 ± 2.9	13.1 ± 2.8	0.84
Age ≥14 years, N (%)	57 (32.9 %)	73 (42.2 %)	71 (41.0 %)	0.16	56 (37.6 %)	66 (44.0 %)	58 (39.2 %)	0.50
Male, N (%)	83 (48.0 %)	81 (46.8 %)	77 (44.5 %)	0.81	35 (23.5 %)	73 (48.7 %)	98 (66.2 %)	<0.001
Height, cm	150 ± 16	154 ± 16	157 ± 15	<0.001	155 ± 14	154 ± 17	154 ± 15	0.83
Weight, kg	37 ± 11	44 ± 12	54 ± 15	<0.001	41 ± 11	44 ± 13	51 ± 16	<0.001
BMI (z score)	-0.91 ± 0.48	-0.02 ± 0.21	1.05 ± 0.49	<0.001	-0.63 ± 0.68	-0.01 ± 0.62	0.79 ± 0.79	<0.001
Waist circumference, cm	59 ± 6	63 ± 6	69 ± 8	<0.001	59 ± 5	63 ± 6	69 ± 8	<0.001
Waist/Height ratio	0.39 ± 0.02	0.41 ± 0.02	0.44 ± 0.03	<0.001	0.38 ± 0.01	0.41 ± 0.01	0.45 ± 0.03	<0.001
Regular sports activity	105 (60.7 %)	108 (62.4 %)	113 (65.3 %)	0.67	89 (59.7 %)	95 (63.3 %)	103 (69.6 %)	0.20
<b>Means for commuting to school</b>								
Walking	73 (42.2 %)	64 (37.0 %)	62 (35.8 %)	0.55	62 (41.6 %)	64 (42.7 %)	48 (32.4 %)	0.19
Bicycle	3 (1.7 %)	3 (1.7 %)	1 (0.6 %)		4 (2.7 %)	1 (0.7 %)	2 (1.4 %)	
Other	97 (56.1 %)	106 (61.3 %)	110 (63.6 %)		83 (55.7 %)	85 (56.7 %)	98 (66.2 %)	
Birth weight, g	3231 ± 481	3265 ± 452	3376 ± 414	0.06	3235 ± 394	3314 ± 484	3309 ± 518	0.44
Birth height, cm	55 ± 45	50 ± 2	50 ± 2	0.33	50 ± 2	50 ± 2	50 ± 2	0.37
Systolic BP, mmHg	118 ± 11	119 ± 10	120 ± 13	0.55	117 ± 9	119 ± 12	121 ± 12	0.03
Diastolic BP, mmHg	62 ± 11	65 ± 10	65 ± 10	0.053	64 ± 10	62 ± 12	66 ± 9	0.02
Heart rate, bpm	72 ± 12	70 ± 11	68 ± 11	0.08	72 ± 11	70 ± 12	68 ± 11	0.04
Total cholesterol, g/l	1.84 ± 0.30	1.80 ± 0.30	1.84 ± 0.32	0.38	1.83 ± 0.31	1.81 ± 0.31	1.84 ± 0.32	0.57
Triglyceride, g/l	0.65 ± 0.35	0.64 ± 0.29	0.68 ± 0.33	0.35	0.64 ± 0.28	0.66 ± 0.36	0.68 ± 0.32	0.55
eGFR, ml/min/1.73m <sup>2</sup>	126 ± 20	126 ± 21	126 ± 19	0.95	123 ± 19	128 ± 18	129 ± 23	0.02
CRP, mg/l	0.17 (0.16 - 0.43)	0.22 (0.16 - 0.52)	0.45 (0.21 - 0.95)	<0.001	0.17 (0.16 - 0.53)	0.27 (0.16 - 0.45)	0.38 (0.17 - 0.81)	<0.001
<b>Variables for the adult period</b>								
Age, yrs	31.3 ± 3.8	31.3 ± 4.1	31.9 ± 3.7	0.20	31.6 ± 3.7	31.6 ± 3.5	31.9 ± 3.7	0.66
Height, cm	171 ± 9	171 ± 9	171 ± 10	0.97	170 ± 8	171 ± 9	173 ± 9	0.018
BMI, kg/m <sup>2</sup>	22 ± 3	24 ± 3	27 ± 5	<0.001	22 ± 3	24 ± 4	27 ± 5	<0.001
Hypertension, N (%)	3 (1.7 %)	4 (2.3 %)	7 (4.0 %)	0.39	4 (2.7 %)	3 (2.0 %)	5 (3.4 %)	0.76
Diabetes mellitus, N (%)	1 (0.6 %)	1 (0.6 %)	2 (1.2 %)	0.78	2 (1.3 %)	0 (0.0 %)	1 (0.7 %)	0.36
Metabolic syndrome, N (%)	2 (1.2 %)	3 (1.7 %)	13 (7.5 %)	0.002	1 (0.7 %)	4 (2.7 %)	11 (7.4 %)	0.006
Total MET, MET/min/week	1578 (552 - 3930)	1288 (356 - 2816)	1580 (690 - 4044)	0.10	1212 (445 - 2945)	1438 (507 - 2868)	1427 (594 - 4158)	0.33
Systolic BP, mmHg	114 ± 13	114 ± 13	115 ± 14	0.65	110 ± 12	114 ± 13	117 ± 14	<0.001
Diastolic BP, mmHg	65 ± 8	65 ± 7	66 ± 9	0.19	64 ± 7	65 ± 8	67 ± 9	<0.001
Heart rate, bpm	66 ± 9	63 ± 9	63 ± 9	0.004	65 ± 8	63 ± 10	63 ± 9	0.06
Total cholesterol, g/l	1.94 ± 0.36	1.96 ± 0.34	1.93 ± 0.33	0.78	1.96 ± 0.32	1.91 ± 0.38	1.97 ± 0.32	0.30
Triglyceride, g/l	0.92 ± 0.59	0.91 ± 0.50	0.97 ± 0.52	0.61	0.90 ± 0.47	0.93 ± 0.63	1.01 ± 0.56	0.22
Carotid IMT, μm	517 (468 - 563)	522 (484 - 579)	528 (475 - 598)	0.15	518 (475 - 572)	514 (478 - 562)	540 (478 - 610)	0.038

Table 2. Linear regression analyses of the association between childhood adiposity and adult cIMT

	Models with covariates measured in the childhood period						Models with covariates measured at the adult period	
	Crude model		without CRP		with CRP		adjusted-beta (95%CI)	p
	beta (95% CI)	p	adjusted-beta (95%CI)	p	adjusted-beta (95%CI)	p		
<b>Body mass index</b>								
<b>Continuous</b>	0.55 (0.31 to 0.78)	<b>&lt;0.001</b>	0.36 (0.01 to 0.72)	<b>0.043</b>	0.28 (-0.09 to 0.65)	0.14	0.44 (0.16 to 0.73)	<b>0.002</b>
<b>Continuous (z-score)</b>	0.97 (0.16 to 1.79)	<b>0.02</b>	0.91 (-0.06 to 1.89)	0.07	0.74 (-0.27 to 1.74)	0.15	0.92 (0.08 to 1.76)	<b>0.03</b>
<b>Tertile, T1</b>	(reference)		(reference)		(reference)		(reference)	
<b>T2</b>	1.35 (-0.46 to 0.05)	0.14	1.67 (-0.50 to 3.85)	0.13	1.96 (-0.20 to 4.11)	0.08	1.48 (-0.40 to 3.35)	0.12
<b>T3</b>	1.86 (3.16 to 3.67)	<b>0.04</b>	1.67 (-0.52 to 3.85)	0.13	1.40 (-0.80 to 3.59)	0.21	1.69 (-0.18 to 3.56)	0.08
<b>Overweight</b>	1.52 (-0.50 to 3.53)	0.14	1.91 (-0.54 to 4.35)	0.17	0.82 (-1.79 to 3.44)	0.54	1.69 (-0.38 to 3.76)	0.11
<b>Obesity</b>	6.47 (0.11 to 12.82)	<b>0.046</b>	4.74 (-2.37 to 11.85)	0.19	1.80 (-5.77 to 9.37)	0.64	4.18 (-1.99 to 10.35)	0.18
<b>Waist/Height</b>								
<b>Continuous</b>	2.39 (-0.02 to 4.80)	0.052	2.09 (-0.95 to 5.13)	0.18	1.16 (-2.03 to 4.36)	0.47	1.53 (-1.05 to 4.10)	0.24
<b>Continuous (z-score)</b>	1.41 (0.36 to 2.46)	<b>0.009</b>	0.78 (-0.57 to 2.13)	0.26	0.36 (-1.03 to 1.75)	0.61	0.69 (-0.47 to 1.86)	0.24
<b>Tertile, T1</b>	(reference)		(reference)		(reference)		(reference)	
<b>T2</b>	0.05 (-1.93 to 2.02)	0.96	-0.57 (-2.90 to 1.76)	0.63	-0.41 (-2.74 to 1.91)	0.73	-0.96 (-2.98 to 1.06)	0.35
<b>T3</b>	2.77 (0.79 to 4.74)	<b>0.006</b>	0.86 (-1.64 to 3.37)	0.50	0.69 (-1.88 to 3.26)	0.60	0.97 (-1.17 to 3.12)	0.37
<b>Waist circumference</b>								
<b>Continuous</b>	0.31 (0.21 to 0.41)	<b>&lt;0.001</b>	0.20 (0.03 to 0.37)	<b>0.02</b>	0.14 (-0.03 to 0.31)	0.11	0.22 (0.09 to 0.36)	<b>0.001</b>
<b>Continuous (z-score)</b>	1.76 (0.75 to 2.77)	<b>0.001</b>	1.40 (0.15 to 2.65)	<b>0.03</b>	1.02 (-0.25 to 2.29)	0.12	1.27 (0.16 to 2.37)	<b>0.03</b>
<b>Tertile, T1</b>	(reference)		(reference)		(reference)		(reference)	
<b>T2</b>	1.52 (-0.45 to 3.50)	0.13	1.37 (-0.94 to 3.68)	0.24	1.69 (-0.61 to 3.98)	0.15	0.05 (-1.98 to 2.07)	0.96
<b>T3</b>	2.89 (0.91 to 4.87)	<b>0.004</b>	1.69 (-0.74 to 4.11)	0.17	0.78 (-1.65 to 3.20)	0.53	1.28 (-0.83 to 3.40)	0.23

Supplemental Figure 1. Study flow chart



**Supplemental Table 1. Baseline characteristics according to the tertiles of waist circumference z-score**

	Waist circumference z-score tertile			p-value
	T1, -4.60 - -0.99 (N=147)	T2, -0.99 - -0.32 (N=148)	T3, -0.32 - 1.79 (N=145)	
<b>Variables for the childhood period</b>				
Age, years	12.7 ± 2.8	13.1 ± 2.9	13.1 ± 2.8	0.40
Age ≥ 14 years, N (%)	53 (35.1 %)	63 (42.9 %)	64 (43.0 %)	0.28
Male, N (%)	42 (27.8 %)	73 (49.7 %)	91 (61.1 %)	<b>&lt;0.001</b>
Height, cm	149 ± 14	155 ± 15	159 ± 15	<b>&lt;0.001</b>
Weight, kg	38 ± 10	45 ± 12	54 ± 15	<b>&lt;0.001</b>
BMI (z score)	-0.66 ± 0.65	-0.05 ± 0.64	0.86 ± 0.69	<b>&lt;0.001</b>
Waist circumference, cm	58 ± 4	64 ± 5	71 ± 7	<b>&lt;0.001</b>
Waist/Height ratio	0.39 ± 0.02	0.41 ± 0.02	0.44 ± 0.03	<b>&lt;0.001</b>
Regular sports activity	89 (58.9 %)	101 (68.7 %)	97 (65.1 %)	0.20
<b>Means of commuting to school</b>				
Walking	61 (40.4 %)	59 (40.1 %)	54 (36.2 %)	0.31
Bicycle	4 (2.6 %)	3 (2.0 %)	0 (0.0 %)	
Other	86 (57.0 %)	85 (57.8 %)	95 (63.8 %)	
Commuting time (min/week)	100 (60 - 200)	120 (90 - 250)	130 (80 - 210)	0.57
Birth weight, g	3185 ± 448	3288 ± 429	3387 ± 506	<b>0.015</b>
Birth height, cm	50 ± 2	50 ± 2	50 ± 2	0.60
Systolic BP, mmHg	116 ± 10	119 ± 10	122 ± 13	<b>0.003</b>
Diastolic BP, mmHg	61 ± 11	64 ± 11	67 ± 9	<b>&lt;0.001</b>
Heart rate, bpm	72 ± 12	71 ± 10	67 ± 11	<b>0.003</b>
Total cholesterol, g/l	1.86 ± 0.30	1.83 ± 0.32	1.79 ± 0.31	0.18
Triglyceride, g/l	0.65 ± 0.26	0.65 ± 0.35	0.68 ± 0.34	0.66
eGFR, ml/min/1.73m <sup>2</sup>	121 ± 18	127 ± 21	131 ± 20	<b>&lt;0.001</b>
CRP, mg/l	0.17 (0.16 - 0.53)	0.26 (0.16 - 0.54)	0.35 (0.17 - 0.76)	<b>0.001</b>
<b>Variables for the adult period</b>				
Age, yrs	31.4 ± 3.7	32.0 ± 3.4	31.8 ± 3.9	0.36
Height, cm	167 ± 8	172 ± 8	175 ± 9	<b>&lt;0.001</b>
Body mass index, kg/m <sup>2</sup>	22 ± 3	24 ± 4	27 ± 5	<b>&lt;0.001</b>
Hypertension	3 (2.0 %)	4 (2.7 %)	5 (3.4 %)	0.76
Diabetes	2 (1.3 %)	0 (0.0 %)	1 (0.7 %)	0.38
Metabolic syndrome	0 (0.0 %)	5 (3.4 %)	11 (7.4 %)	<b>0.003</b>
Total MET, MET/min/week	1188 (432 - 3012)	1326 (463 - 2775)	1488 (720 - 4044)	0.14
Systolic BP, mmHg	110 ± 11	114 ± 14	117 ± 13	<b>&lt;0.001</b>
Diastolic BP, mmHg	63 ± 7	66 ± 8	67 ± 9	<b>&lt;0.001</b>
Heart rate, bpm	66 ± 8	63 ± 8	62 ± 9	<b>&lt;0.001</b>
Total cholesterol, g/l	1.93 ± 0.32	1.97 ± 0.38	1.94 ± 0.32	0.55
Triglyceride, g/l	0.89 ± 0.47	0.90 ± 0.55	1.05 ± 0.63	<b>0.018</b>
Carotid IMT, μm	518 (466 - 561)	526 (486 - 586)	532 (477 - 602)	0.08

Values are expressed as mean ±SD, n (%) or median (25th to 75th percentile)

Waist circumference was converted to age- and sex-specific z score based on the Third National Health and Nutrition Examination Survey <sup>1</sup>.

BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate, CRP, C-reactive protein; MET, metabolic equivalent; IMT, intima media thickness.

(reference)

1.Sharma AK, Metzger DL, Daymont C, Hadjiyannakis S and Rodd CJ. LMS tables for waist-circumference and waist-height ratio Z-scores in children aged 5-19 y in NHANES III: association with cardio-metabolic risks. *Pediatr Res.* 2015;78:723-9.

**Supplemental Table 2. Linear regression analyses for the association between childhood adiposity and adult IMT according to men and women**

	Women			Men			p-value for interaction with sex difference
	beta (95% CI)		p-value	beta (95% CI)		p-value	
<b>Body mass index</b>							
<b>Continuous (z-score)</b>	0.94	-0.03 to 1.90	0.06	1.08	-0.25 to 2.42	0.11	0.86
<b>Tertile, T1</b>		(reference)			(reference)		<b>0.004</b>
<b>T2</b>	2.05	-0.07 to 4.16	0.06	0.64	-2.33 to 3.60	0.67	
<b>T3</b>	2.03	-0.07 to 4.13	0.06	1.90	-1.10 to 4.89	0.21	
<b>Overweight</b>	0.98	-1.36 to 3.32	0.41	2.28	-1.06 to 5.62	0.18	0.52
<b>Obesity</b>	8.45	0.17 to 16.74	<b>0.046</b>	4.37	-5.06 to 13.8	0.36	0.53
<b>Waist/Height</b>							
<b>Continuous (z-score)</b>	1.15	-0.21 to 2.51	0.10	0.34	-1.41 to 2.08	0.71	0.46
<b>Tertile, T1</b>		(reference)			(reference)		<b>0.003</b>
<b>T2</b>	-0.12	-2.30 to 2.06	0.92	-2.40	-6.33 to 1.54	0.23	
<b>T3</b>	2.05	-0.46 to 4.55	0.11	-0.11	-3.88 to 3.66	0.95	
<b>Waist circumference</b>							
<b>Continuous (z-score)</b>	1.73	0.46 to 2.99	<b>0.008</b>	0.80	-0.85 to 2.46	0.34	0.38
<b>Tertile, T1</b>		(reference)			(reference)		<b>0.002</b>
<b>T2</b>	2.14	-0.08 to 4.35	0.06	-1.47	-5.21 to 2.27	0.44	
<b>T3</b>	1.93	-0.47 to 4.32	0.11	0.67	-3.00 to 4.31	0.72	

BMI was converted to age- and sex-specific z score based on the International Obesity Task Force (IOTF), while waist-to-height ratio and waist circumference were based on the Third National Health and Nutrition Examination Survey. Overweight and obesity were respectively defined by a BMI z-score >1 and >2 as proposed by the IOTF.



**Supplemental Table 3. Associations between childhood-to-adult adiposity and adult IMT in multivariable models**

	Univariable			Multivariable		
	beta	95%CI	p-value	beta	95%CI	p-value
<b>Body mass index</b>						
Low in children & Low in adults		(reference)			(reference)	
High in children & Low in adults	-0.10	-0.54 to 0.34	0.67	0.18	-0.43 to 0.80	0.56
Low in children & High in adults	0.38	-0.06 to 0.82	0.092	0.21	-0.37 to 0.79	0.48
High in children & High in adults	0.68	0.32 to 1.04	<b>&lt;0.001</b>	0.63	0.12 to 1.14	<b>0.015</b>
<b>Waist/Height ratio</b>						
Low in children & Low in adults		(reference)			(reference)	
High in children & Low in adults	0.23	-0.26 to 0.73	0.36	0.46	-0.17 to 1.08	0.15
Low in children & High in adults	0.71	0.21 to 1.21	<b>0.005</b>	0.70	0.06 to 1.34	<b>0.033</b>
High in children & High in adults	0.73	0.35 to 1.12	<b>&lt;0.001</b>	0.48	-0.05 to 1.01	0.074
<b>Waist circumference</b>						
Low in children & Low in adults		(reference)			(reference)	
High in children & Low in adults	0.35	-0.15 to 0.86	0.17	0.46	-0.21 to 1.13	0.18
Low in children & High in adults	0.47	-0.01 to 0.95	0.055	0.12	-0.56 to 0.80	0.73
High in children & High in adults	0.85	0.46 to 1.24	<b>&lt;0.001</b>	0.38	-0.21 to 0.97	0.20

The multivariable model included variables measured in the childhood period (i.e., birth weight, systolic BP, heart rate, total cholesterol, eGFR, natural log of C-reactive protein) and those measured in the adult period (i.e., height, metabolic syndrome, peripheral systolic BP and heart rate).

**Supplemental Table 4. Circulating proteins associated with body mass index and waist-to-height ratio z-scores**

	BMI z-scores >T3 vs <T1			Waist/Height z-scores >T3 vs <T1		
	Adjusted-OR (95%CI)	p-value	FDR	Adjusted-OR (95%CI)	p-value	FDR
<b>IGFBP-1</b>	1.43 (1.14 - 1.80)	<b>0.002</b>	<b>0.011</b>	1.29 (1.01 - 1.63)	0.11	0.19
<b>IGFBP-2</b>	1.86 (1.37 - 2.52)	<b>&lt;0.001</b>	<b>&lt;0.001</b>	2.58 (1.82 - 3.66)	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<b>MMP-2</b>	2.03 (1.09 - 3.79)	0.057	0.16	1.03 (0.48 - 2.21)	0.99	0.99
<b>MMP-3</b>	0.79 (0.55 - 1.15)	0.12	0.22	1.91 (1.30 - 2.80)	<b>0.003</b>	<b>0.009</b>
<b>MMP-9</b>	0.87 (0.64 - 1.19)	0.66	0.70	1.21 (0.89 - 1.66)	0.32	0.44
<b>Osteopontin</b>	1.38 (0.89 - 2.12)	<b>0.011</b>	<b>0.04</b>	3.09 (1.80 - 5.29)	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<b>VEGF-A</b>	0.40 (0.26 - 0.61)	<b>&lt;0.001</b>	<b>&lt;0.001</b>	1.25 (0.82 - 1.91)	0.034	0.071
<b>BMP-9</b>	0.77 (0.52 - 1.14)	0.42	0.50	0.53 (0.32 - 0.89)	<b>0.021</b>	0.05
<b>FABP-4</b>	1.25 (0.81 - 1.93)	0.60	0.67	0.42 (0.27 - 0.65)	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<b>Leptin</b>	0.64 (0.40 - 1.02)	0.16	0.28	0.43 (0.34 - 0.54)	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<b>Resistin</b>	0.82 (0.66 - 1.03)	0.20	0.31	1.70 (1.00 - 2.89)	0.12	0.19
<b>Cystatin-C</b>	1.25 (0.81 - 1.93)	0.091	0.20	1.51 (0.89 - 2.58)	0.14	0.21
<b>KIM-1</b>	1.32 (0.86 - 2.02)	0.28	0.38	1.20 (0.78 - 1.86)	0.71	0.80
<b>Hemoglobin</b>	0.96 (0.61 - 1.51)	0.91	0.91	1.44 (1.15 - 1.82)	<b>0.006</b>	<b>0.016</b>
<b>IFN-gamma</b>	0.68 (0.45 - 1.03)	0.094	0.20	0.00 (0.00 - 0.02)	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<b>C-reactive protein</b>	14.39 (2.09 - 99.26)	<b>0.004</b>	<b>0.021</b>	1.02 (0.96 - 1.09)	0.52	0.62
<b>Triglyceride</b>	0.95 (0.90 - 1.01)	0.30	0.38	1.00 (0.99 - 1.00)	0.52	0.62

OR, odd ratio; FDR, false discovery rate.

IGFBP-1, insulin-like growth factor-1; IGFBP-2, insulin-like growth factor-2; MMP-2, matrix metalloproteinase-2; MMP-3, matrix metalloproteinase-3; MMP-9, matrix metalloproteinase-9; VEGF-A, vascular endothelial growth factor-A; BMP-9, bone morphogenetic protein-9; FABP-4, Fatty Acid-Binding Protein 4; KIM-1, Kidney Injury Molecule-1; IFN, interferon.

The multivariable model included variables measured in the adult period (i.e., age, body mass index [or waist and height ratio], metabolic syndrome, hypertension, diabetes mellitus, systolic blood pressure and total cholesterol).