Hypo-adiponectinaemia in Overweight Children Contributes to a Negative Metabolic Risk Profile 6 Years Later

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ABSTRACT

Context: Prognostic biomarkers are needed to identify children at increased cardiometabolic risk. **Objective:** To study whether markers of metabolism and inflammation, e.g. circulating plasma adiponectin, leptin, IL-8, and hepatocyte growth factor (HGF) are associated with cardiometabolic risk factors in childhood and adolescence. **Design:** Cross-sectional and prospective study **Setting:** Danish part of the European Youth Heart Studies I and II. **Participants:** Randomly selected girls and boys 8–10 years of age with complete baseline data (n=256) and complete follow-up data 6 years later (n=169). **Measurements:** Cardiometabolic risk profile was calculated using a continuous composite score derived from summing of 6 factors standardized to the sample means (Z-scores): BMI, HOMA-IR, total serum cholesterol/ serum HDL-cholesterol, serum triglycerides, systolic blood pressure, and the reciprocal value of fitness (max watt/kg). Overweight was defined using international classification of BMI cut-off points for children. Plasma adiponectin, leptin, IL-8, and HGF were assessed using immunochemical assays. **Results:** Linear relationships were found between metabolic risk score and both plasma adiponectin (inverse; $p=0.02$) and plasma leptin ($p<0.0001$) at baseline after adjustment for several confounders. In overweight but not normal weight children, plasma adiponectin at baseline was inversely associated with metabolic risk score 6 years later ($p=0.04$). **Conclusion:** In childhood, both hypo-adiponectinaemia and hyper-leptinaemia accompany a negative metabolic risk profile. In addition, circulating plasma adiponectin may be a useful biomarker to identify overweight children at greater future risk of the cardiometabolic adverse effects of overweight.

INTRODUCTION

Studies in childhood are essential to understand the early development of clustered cardiometabolic risk factors and the role of systemic low grade inflammation. Studies have shown that increased systemic inflammation, as indicated by alterations in adipokines and chemokines, is present in obese prepubertal children and correlates with hyper-insulinaemia (1), dyslipidaemia (2), inverted cardio-respiratory fitness (3), and clustered factors of the metabolic syndrome (4,5). In 10–19-year-old Native Canadians, hypo-adiponectinaemia together with dyslipidaemia, and hyper-leptinaemia together with adiposity represented two of five core traits of clustered metabolic risk. This suggests that adipokines may play a role in early dysmetabolism among high-risk children.

Pro-inflammatory chemokines such a IL-8 and hepatocyte growth factor (HGF) may cause inflammation through an increased influx of leucocytes into inflammed tissues (6). Among adults, circulating IL-8 concentrations were higher in subjects with than without diabetes (7), and were directly correlated to insulin resistance in men with abdominal obesity (8). Also circulating HGF concentrations were observed to be higher in overweight compared to normal weight adults (9). The secretion of adipokines and chemokines is influenced by overweight (9-11) and circulating adipokines are affected differently by male and female sex steroids (12,13). Therefore, overweight status, sex, and sexual maturity should be considered simultaneously when studying markers of metabolism and inflammation.

Based on current knowledge, it is possible that low plasma concentrations of adiponectin and high plasma concentrations of leptin, IL-8, and HGF may be associated with a negative cardiometabolic risk profile among children. The purpose of our study was to examine associations between plasma cytokines and the early development of cardiometabolic risk factors in randomly selected Danish children, followed 6 years from childhood into adolescence.

MATERIALS AND METHODS

Data are based on the Danish part of the European Youth Heart Studies (EYHS) I and II in Odense, Denmark. EYHS is a longitudinal, multi-centre study of early development of cardiovascular risk among children followed up longitudinally every 6 years from childhood over adolescence to early adulthood. The Danish baseline study was done in 1997/98 among randomly selected children from third and ninth school grade. In 2003/04, the first follow-up study was done among a new cohort of third graders and ninth graders who also participated at baseline as well as newly invited ninth graders. The overall participation rate was 75%. Parents gave written consent for their child to participate, and children had the option to withdraw at any time. The study was approved by the scientific ethics committee of the local counties of Funen and Vejle, Denmark (VF 20030067) and followed the principles stipulated in the Helsinki declaration.

Design

Our study includes third grade children at baseline in cross-sectional analyses, and third grade children who were followed up 6 years later in prospective analyses. A cluster-sampling of 25

schools was used according to the socio-demographic characteristics in the local areas. At follow-up, all third graders from baseline were eligible for the follow-up study 6 years later, also if they moved to another school. Data were collected from September 1997 to June 1998 and again from September 2003 to June 2004 (14). At baseline, the study comprised 590 predominantly ethnic Danish third graders 8–10 years of age, 53% girls and 47% boys. Of these, 384 were re-examined 6 years later as ninth graders 14–16 years of age, 56% girls and 44% boys.

Measurements

All measurements were collected and undertaken by health professionals and trained personnel following international standardized procedures of electronic questionnaires, physical and clinical examinations, and blood sampling (14). Information about sex and birth date of the child as well as affiliation to school location was collected with electronic questionnaires for the child and both parents or guardians. Sexual maturity was assessed using the five-stage picture scale for the development of breast and pubic hair in girls and the development of genital and pubic hair in boys, according to Tanner (15). Children were categorized as prepubertal with Tanner stage 1, pubertal with Tanner stage 2, 3 and 4, and post-pubertal with Tanner stage 5. Body weight in light clothing was measured to the nearest 0.1 kg and height without shoes was measured to the nearest 0.5 cm. Children were classified as overweight if they had a BMI equivalent to an adult BMI $\geq$ 25 kg/m^2 according to international extrapolations for age- and sex matched child cohorts (15,16). Overweight corresponded to a BMI above 18.4–20.5 among girls and boys from 8–10 years old, with the highest value among 10-year-old boys and the lowest among 8-year-old girls (16). Habitual physical activity was measured with an accelerometer attached to the hip (MTI-

actigraph model 7164) (14). The average daily electronic counts per minute were obtained from recordings of frequency and intensity of the child's activity. Recordings were weighted by the percentage of week days and weekend days to correspond to a standard week (17). From an incremental ergometer cycle-test, where the workload was increased by 3-minute intervals until exhaustion, individual max watt was obtained (Monark 839 Ergo medic) (14). Cardio-respiratory fitness was expressed as max watt per kilo body weight of the child.

Clinical factors

Systolic blood pressure was measured with a Dinamap paediatric and adult neonatal vital signs monitor (model XL, Critikron, Inc, Tampa, FL, USA). Five measurements were taken at 2-min intervals with the mean of the final three measurements used in all analyses (14). Blood samples were obtained from the right *antecubital* vein after an overnight fast. Within 30 min, aliquots of plasma and serum were separated and immediately stored at -80 °C until further analysis. At WHO-certified laboratories (Bristol, England in 1998 and Cambridge, England in 2004), serum glucose was analyzed using the hexokinase method and total serum cholesterol, serum HDL-cholesterol, and serum triglycerides were measured using enzymatic colorimetry at both study years (Olympus AU600 auto-analyzer, Olympus Diagnostica, Hamburg, Germany). Serum insulin was analyzed using enzyme immunoassays with micro-titre plate format (Dako Diagnostics, Ely, UK) at baseline and by two-site immunometric assays with either 125I or alkaline phosphatase labels at follow-up. Between-laboratory correlations for 30 randomly selected samples analyzed at both laboratories were r=0.942 for glucose and r=0.931 for insulin. Insulin resistance was calculated using the homeostasis model assessment (HOMA-IR): Insulin [μU/ml] x glucose [mmol/l] x 22.5^{-1} (18). A strong correlation has been found between HOMA-

IR and frequently sampled intravenous glucose tolerance test among obese, prepubertal and pubertal children (19).

Plasma cytokine markers of metabolism and inflammation

In stored baseline EDTA-plasma, concentrations of adiponectin, leptin, IL-8 and HGF were analyzed in samples of third graders in January–October 2007. A solid-phase protein immunoassay was applied for analysis (Luminex type 100 IS, Ramcon A/S, Birkerød, Denmark). Total plasma adiponectin was analyzed in single-determination after double-determination in 30 samples, showing good agreement between duplicates (5.5 % CV). Interassay variation was 3.73 % CV and intraassay variation was 4.26 % CV (Electrabox Diagnostica ApS, Rødovre, Denmark). Plasma concentrations of leptin, IL-8 and HGF were analyzed in duplicates. For multi-plexed assays of plasma leptin, IL-8 and HGF, interassay variations were 1.4–7.9 % CV and intraassay variations were <2.1 % CV (Linco Research, Missouri, USA). Hyper-leptinaemia and hypo-adiponectinaemia were used as relative terms on the scale of continuously distributed plasma concentrations.

Statistical analyses

All statistical analyses were carried out in SAS version 9.1 (Statistical Analysis System Institute Inc., Cary, NC). We constructed a composite score of cardiometabolic risk defined by a continuously distributed variable. This variable was derived from 6 metabolic factors: BMI, the reciprocal value of cardio-respiratory fitness, HOMA-IR, the ratio of total serum cholesterol to serum HDL-cholesterol, serum triglycerides, and systolic blood pressure. Each risk factor was standardized to a z-score, which is the number of standard deviations a specific value differs

from the sample mean: (Observed value- mean/SD). The metabolic risk score (Z-score) was calculated as the sum of the 6 z-scores. Generalized linear models were used to study the relationship between each independent marker and the metabolic outcome. All cross-sectional and prospective analyses were adjusted for sex and sexual maturity of the children. Multi-adjusted models were additionally adjusted for significant confounding from age, physical activity, other markers of metabolism and inflammation, and school location to account for the cluster sampling design (random effect) using backwards stepwise reduction of the model. Interactions with sex, sexual maturity or overweight at baseline were tested for each independent marker in the multi-adjusted model. Coefficient and 95%-confidence limits (CL) in the linear regression model were standardized by multiplication with one standard deviation of the independent marker. Statistical significance was determined by a two-sided probability level equal to or below five per cents in all models. Characteristics of the study sample are presented as means (SD).

RESULTS

Two hundred and fifty six of the recruited 590 children had complete baseline data. The dropout was due to missing data on metabolic risk factors (19%), markers of metabolism and inflammation (29%), physical activity (35%), and, sexual maturity (2%). At follow-up, 169 of 256 children from baseline had complete follow-up data on BMI, cardio-respiratory fitness and all clinical risk factors. The dropout at follow-up was due to missing data on BMI (30%), cardio-respiratory fitness (33%), HOMA-IR (31%), total serum cholesterol or serum HDL-cholesterol (31%), serum triglycerides (31%), and systolic blood pressure (30%). Comparisons of baseline

characteristics between children with complete and incomplete data showed that children with complete data had a 0.2 watt/kg higher fitness than those with incomplete data but were otherwise similar (**table 1**). Further, children with complete data at follow-up were 0.1 year younger at baseline compared with children with incomplete follow-up data but were but were otherwise similar (table 1).

Baseline characteristics

Of 256 children at baseline, 52% were girls and 48% were boys. None of the boys had entered puberty, whereas 32% of girls were in puberty. Differences in biochemical markers were present between girls and boys and between levels of sexual maturity in girls (**table 2**). Compared with boys, girls had lower BMI, cardio-respiratory fitness, systolic blood pressure and higher circulating concentrations of serum triglycerides and plasma adiponectin. Compared with prepubertal girls, pubertal girls had higher BMI, systolic blood pressure, metabolic risk score, plasma leptin, and lower cardio-respiratory fitness (table 2). Therefore, all multivariate analyses were adjusted for sex and sexual maturity.

Plasma cytokine markers and cardiometabolic risk factors at baseline

Plasma adiponectin was inversely associated with systolic blood pressure and metabolic risk score but not with BMI, cardio-respiratory fitness, HOMA-IR, total serum cholesterol to serum HDL-cholesterol ratio, and serum triglyceride after adjusting for sex and sexual maturity (**table 3**). The association between plasma adiponectin and metabolic risk score remained significant after additional adjustment for physical activity, plasma leptin, and school location: standardized β [CL]: -0.42 [-0.76;-0.08], p=0.02.

Plasma leptin was directly associated with BMI, HOMA-IR, total serum cholesterol to serum HDL-cholesterol ratio, serum triglycerides, systolic blood pressure, and metabolic risk score, and inversely associated with cardio-respiratory fitness after adjusting for sex and sexual maturity (table 3). The linear relationship between plasma leptin and metabolic risk score remained significant when additionally adjusted for school location, physical activity, and plasma adiponectin: standardized β [CL]: 2.20 [1.85; 2.55], p<0.0001. Non-significant coefficients were found for both plasma IL-8 and plasma HGF in relation to all metabolic risk factors and metabolic risk score (table 3). When we additionally adjusted for school location, physical activity, plasma concentrations of leptin and adiponectin, the association with metabolic risk score remained non-significant for both IL-8: standardized β [CL]: -0.17 [-0.51;0.17], p=0.32 and plasma HGF: standardized β [CL]: -0.26 [-0.61;0.08], p=0.13. No baseline interactions were identified with sex, sexual maturity or overweight for any of the markers.

Follow-up characteristics

One hundred and sixty nine of 256 children from baseline were followed up as adolescents on average 6.1 years (0.1) later. Their cardiometabolic characteristics are shown in **table 4**. Between baseline and follow-up, children increased their BMI with 4.0 kg/m^2 (0.1), their cardio-respiratory fitness with 0.3 watt/kg (0.1), their HOMA-IR with 0.3 units (0.1), and their systolic blood pressure with 2.8 mm Hg (0.7). Further, they decreased their total serum cholesterol to serum HDL-cholesterol ratio with 0.3 (0.1) whereas serum triglyceride did not change significantly between baseline and follow-up remaining at -0.1 mmol/l (0.1). Among the 134 with intact physical activity data, the average daily activity decreased with 211.6 counts/min

(22.5). Of 169 children studied both years, 138 were normal weight at both study years (NW/NW), 12 children were normal weight at baseline and overweight at follow-up (NW/OW), 9 children were overweight at baseline and normal weight at follow-up (OW/NW), and 10 children were overweight at both study years (OW/OW). Between subgroups, sex- and maturity-independent differences were present in BMI, cardio-respiratory fitness, HOMA-IR, total serum cholesterol to serum HDL-cholesterol ratio, and metabolic risk score at baseline and at follow-up. No differences were found in serum triglycerides and systolic blood pressure at baseline and nor at follow-up (table 4). At baseline, plasma leptin concentrations were 4.4 ng/ml among NW/NW- children, 7.2 ng/ml among NW/OW-children, 14.1 ng/ml among OW/NW-children, and 13.5 ng/ml among OW/OW-children. Plasma leptin concentrations differed significantly between the 4 subgroups ($p<0.05$) but similar concentrations were found between the two subgroups being normal weight at baseline and between the two subgroups being overweight at baseline. No subgroup-differences were found for plasma concentrations of adiponectin, IL-8 and HGF (data not shown).

In order to study the consequences of overweight at baseline, regardless of overweight at follow-up, we divided children two groups: the 150 normal weight children at baseline and the 19 overweight children at baseline. Compared to normal weight children, overweight children at baseline had lower cardio-respiratory fitness ($p<0.0001$), as well as higher HOMA-IR ($p=0.050$), higher total serum cholesterol to serum HDL-cholesterol ratio ($p=0.006$), higher serum triglycerides ($p=0.003$), and higher metabolic risk score ($p<0.0001$) after adjustment for sex and sexual maturity. Only systolic blood pressure was similar in normal weight and overweight children. At follow-up, the overweight children at baseline still had higher BMI ($p<0.0001$),

lower cardio-respiratory fitness (p=0.005), and higher metabolic risk score (p=0.0006), than normal weight children at baseline.

Plasma cytokine markers at baseline and cardiometabolic risk profile 6 years later

The metabolic risk score in adolescents decreased linearly with baseline plasma adiponectin among overweight children at baseline: standardized β [CL]: -2.35 [-4.44; -0.17], p=0.04. No association was found among normal weight children at baseline: standardized β [CL]: -0.15 [-0.61; 0.32], p=0.53 or the overall group standardized β [CL]: -0.31 [-0.91; 0.16], p=0.18. The associations were all adjusted for confounding factors and illustrated in **figure 1**. A significant interaction between plasma adiponectin and overweight versus normal weight was identified (p=0.01). Plasma leptin at baseline was not linearly associated with metabolic risk score at follow-up: standardized β [CL]: -0.04 [-0.64; 0.56], p=0.90, and no association was found for plasma IL-8: standardized β [CL]: -0.06 [-0.53; 0.41], p=0.80 or HGF: standardized β [CL]: -0.07 [-0.52; 0.39], p=0.77; all adjusted for baseline metabolic risk score, school location, sex, and sexual maturity.

DISCUSSION

In healthy normal weight and overweight Danish children aged 8–10 years, both hypo-adiponectinaemia and hyper-leptinaemia were independently correlated with a negative cardiometabolic risk profile. In addition, hypo-adiponectinaemia associated with a negative cardiometabolic risk profile 6 years later but only among overweight children. This is the first

study to observe the potential long term consequences of hypo-adiponectinaemia in overweight but otherwise healthy children.

That hypo-adiponectinaemia contributed to a negative cardiometabolic risk score 6 years later in overweight but not in normal weight children suggest a role of plasma adiponectin in the early development of clustering of cardiometabolic risk factors. Consistently with our findings, cross-sectional studies in children have found a direct association between plasma adiponectin concentrations and HDL-cholesterol in multiadjusted analyses (20,21), and an inverse association between plasma adiponectin concentrations and plasma triglycerides among girls (20). These findings are in keeping with the current hypotheses that circulating plasma adiponectin, through its insulin sensitizing and anti-inflammatory effects, may increase insulin sensitivity and improve plasma lipid composition (22,23). It has been suggested that adiponectin activates adenosine mono-phosphate protein kinase (AMPK), which could lead directly to decreased hepatic gluconeogenesis (22). Our cross-sectional results indicate that systolic blood pressure may be the main cardiometabolic risk factor affecting the relationship between plasma adiponectin and cardiometabolic risk score. A modest correlation has previously been reported between plasma adiponectin and systolic blood pressure among obese children (24), but other studies in children found no significant association with or without adjustment for body fatness (5,20). Among the Danish EYHS-children, the association remained after additional adjustment for BMI (data not shown).

That the association between plasma adiponectin and metabolic risk profile was found only in overweight Danish children may be a result of the obesity-induced inflammation, worsening the

inflammatory milieu induced by hypo-adiponectinaemia (23). Adiponectin inhibits pro-inflammatory cytokine production and adhesion molecule expression and induces anti-inflammatory factors (23). These activities are present in macrophages, endothelial cells and cardiac cells and may be mediated in part by inhibiting the stress signaling pathways, e.g. nuclear factor-kappa B (NF-kB) and in part by the activation of AMPK (23). This may explain at least parts of our finding of the prospective association between plasma adiponectin and cardiometabolic risk score in overweight Danish children. Concentrations of C-reactive protein in native Canadian children were negatively correlated with plasma HDL-cholesterol and positively with BMI, insulin resistance, and plasma triglycerides among obese but not normal weight children (5).

Circulating plasma leptin was highly correlated with cardiometabolic risk factors, individually, and the cardiometabolic risk profile after we adjusted for several confounding factors. In agreement with the present findings, previous studies found linear correlations between plasma leptin concentrations and insulin resistance and BMI in predominantly prepubertal children (1) as well as plasma triglycerides and inverse plasma HDL-cholesterol among obese children (5,25). Although we found strong correlations in the cross-sectional analysis, no prospective relationship was identified between plasma leptin and cardiometabolic risk score, as the children progressed into puberty. To our knowledge, there is no evidence in the literature of such a prospective relationship in children. In addition, a study among obese children showed that increased adipose tissue content was associated with not only increased plasma leptin concentrations but also increased concentrations of pro-inflammatory plasma cytokines, such as IL-1 beta, IL-6, and TNF alpha (26). In our study, in contrast to plasma leptin, plasma IL-8 and HGF were not

associated with any of the cardiometabolic factors or the cardiometabolic risk score among Danish children.

Study design considerations

The present study comprises a sizeable sample of randomly selected healthy children with extensive information of each individual at two time points. Although the study sample size is substantially reduced from the total study population, no major differences in baseline characteristics were found between those included and those excluded in the present analyses. This indicates that selection bias is a minor issue in the present study. Cardiometabolic risk profile was assessed using a continuously distributed score, which previously has been applied successfully in EYHS-studies (27). When generating this score, we have the benefit of avoiding the use of arbitrary cut-offs for each cardiometabolic risk factor, the utility of which is considered controversial in paediatric studies (28). In addition, statistical power is improved with the use of a continuous variable, rather than discrete categories. It has been shown that BMI is highly correlated with percentage body fat, total fat mass, and abdominal fat mass assessed by DEXA in children (29). This indicates that variations in body fat content among children are captured with such a crude measure as BMI.

In conclusion, low circulating plasma adiponectin was associated with a negative cardiometabolic risk profile among overweight but not among normal weight children. Hyper-leptinaemia co-exists with adverse cardiometabolic factors and a negative cardiometabolic risk profile in Danish children, but there seems to be no long-term association between plasma leptin and the cardiometabolic risk profile. We propose that low plasma adiponectin may be considered

as an early biomarker of identifying individuals at greater risk of the long-term cardiometabolic consequences of overweight.

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Figure legend

Figure 1. Relationships between baseline plasma adiponectin and metabolic risk score 6 years later among 150 children who were normal weight at baseline (A) and 19 children who were overweight at baseline (B), participants from the European Youth Heart Studies in both 1997/98 and 2003/2004. The relationships are linear regressions with 95% confidence intervals, adjusted for baseline metabolic risk score, sex, sexual maturity, and school location

Table 1. Baseline characteristics of children included and excluded in the statistical analyses due to incomplete data in the Danish part of the European Youth Heart Studies in 1997/98 (EYHS I) and 2003/04 (EYHS II)

	EYHS I				EYHS II †		
	Incl. n=256	Excl.	n ‡	P-value*	Incl. n=169	Excl. n=87	P-value**
Girls (% of n)	51.6	53.6	334	0.62	50.9	52.9	0.76
Prepubertal (% of n) ‖	85.2	83.6	325	0.59	82.3	86.2	0.42
Age (years)	9.7 (0.4)	9.6 (0.4)	334	0.33	9.6 (0.4)	9.7 (0.4)	0.03
Activity (counts/min)	6.7 (2.4)	6.3 (2.0)	127	0.22	6.7 (2.4)	6.6 (2.4)	0.78
BMI (kg/m^2)	17.2 (2.2)	17.4 (2.6)	334	0.42	17.0 (7.9)	17.5 (2.7)	0.11
Fitness (max watt/kg)	3.1 (0.5)	2.9 (0.6)	283	0.01	3.1 (0.5)	3.0 (0.6)	0.15
HOMA (units)	1.8 (1.0)	1.9 (1.1)	263	0.35	1.9 (1.0)	1.8 (1.1)	0.56
S-total chol. /HDL (ratio)	3.1 (0.6)	3.2 (0.7)	269	0.09	3.1 (0.6)	3.2 (0.6)	0.29
S-triglycerides (mmol/l)	0.9 (0.4)	0.8 (0.3)	269	0.43	0.9 (0.4)	0.8 (0.3)	0.65
Systolic BP (mm hg)	105.2 (7.3)	104.8 (7.7)	333	0.54	105.0 (7.1)	105.4 (7.6)	0.69
P-leptin (ng/ml)	5.1 (5.7)	6.0 (7.1)	174	0.17	4.6 (5.4)	6.0 (6.3)	0.06
P-adiponectin (µg/ml)	12.0 (4.1)	12.1 (4.6)	173	0.85	12.1 (4.4)	11.8 (3.7)	0.67
P-interleukin-8 (pg/ ml)	1.7 (1.3)	2.0 (4.5)	174	0.32	1.6 (1.0)	1.9 (1.7)	0.12
P-HGF (ng/ml)	0.7 (0.5)	0.8 (1.6)	173	0.26	0.7 (0.5)	0.7 (0.4)	0.62

Data are means (SD) where nothing else is noted; HGF, hepatocyte growth factor; S, serum; P, plasma.† Also completers in EYHS I; ‡ number of children excluded in cross-sectional analyses with valid data on some variables, note that the number differs between variables; * differences between children included and excluded in EYHS I, ANOVA for continuous variables and Chi Square for categorical variables;

** differences between children included and excluded in EYHS II, ANOVA for continuous variables and Chi Square for categorical variables; ‖ Prepubertal versus pubertal children

Table 2. Baseline characteristics of cardiometabolic factors and markers of metabolism and inflammation of prepubertal boys and girls, and pubertal girls. Children participated in the European Youth Heart Studies 1997/98 (n=256)

	Prepubertal boys	Prepubertal girls	Pubertal girls
N	124	90	42
BMI (kg/m^2)	17.2 (2.3)	16.5 (1.9)* †††	18.5 (2.2)
Fitness (max watt/kg)	3.2 (0.5)	3.0 (0.5)*** ††	2.7 (0.4)
HOMA-IR (units)	1.7 (1.0)	1.9 (1.0)	2.1 (1.2)
total S-cholesterol/ HDL (ratio)	3.0 (0.6)	3.2 (0.6)	3.3 (0.5)
S-triglycerides (mmol/l)	0.8 (0.3)	0.9 (0.4)**	0.9 (0.3)
Systolic BP (mm hg)	105.6 (6.7)	103.5 (7.3)* ††	107.5 (8.2)
Metabolic risk score (SD)	-0.7 (3.4)	-0.1 (3.6) ††	2.2 (3.7)
P-adiponectin (µg/ml)	11.4 (3.8)	12.5 (4.5)*	12.6 (4.0)
P-leptin (ng/ml)	4.1 (4.8)	4.6 (5.0) †††	9.2 (7.8)
P-IL-8 (pg/ ml)	1.8 (1.2)	1.6 (1.4)	1.4 (1.2)
P-HGF (ng/ml)	0.7 (0.4)	0.8 (0.6)	0.7 (0.3)

Data are means (SD); S, serum; P, plasma

* Statistical difference between prepubertal girls and boys $p<0.05$; ** $p<0.01$; *** $p<0.001$

† Statistical difference between prepubertal and pubertal girls $p<0.05$; †† $p<0.01$; ††† $p<0.001$

Table 3. Baseline linear regression coefficients of the relationship between markers of metabolism and inflammation and cardiometabolic factors of 8–10-year-old children from the European Youth Heart Study in 1997/ 98 (n=256)

	P-adiponectin (µg/ml)	P-leptin (ng/ml)	P-IL-8 (pg/ ml)	P-HGF (ng/ml)
		β [CL]		
Z-BMI	-0.10 [-0.22;0.02]	0.70 [0.61;0.79]	-0.07 [-0.19;0.05]	-0.02 [-0.14;0.10]
(SD)	p=0.10	p<0.0001	p=0.26	p=0.77
Z-watt/kg	-0.07 [-0.18;0.05]	-0.57 [-0.67;-0.47]	0.04 [-0.08;0.15]	0.01 [-0.11;0.12]
(SD)	p=0.26	p<0.0001	p=0.53	p=0.90
Z-HOMA-IR	-0.06 [-0.18;0.07]	0.38 [0.26;0.51]	-0.07 [-0.20;0.05]	-0.02 [-0.15;0.10]
(SD)	p=0.36	p<0.0001	p=0.82	p=0.72
Z-total chol. /HDL	-0.07 [-0.19;0.05]	0.22 [0.09;0.35]	-0.06 [-0.19;0.06]	-0.10 [-0.22;0.02]
(SD)	p=0.25	p=0.0007	p=0.31	p=0.11
Z-triglyceride	-0.12 [-0.24;0.01]	0.23 [0.10;0.36]	-0.09 [-0.22;0.03]	-0.03 [-0.16;0.09]
(SD)	p=0.06	p=0.0004	p=0.13	p=0.58
Z-systolic BP	-0.19 [-0.31;-0.07]	0.16 [0.04;0.29]	-0.02 [-0.14;0.10]	0.02 [-0.11;0.14]
(SD)	p=0.002	p=0.01	p=0.76	p=0.80
Metabolic risk score	-0.47 [-0.91;-0.03]	2.27 [1.91;2.63]	-0.35 [-0.79;0.08]	-0.17 [-0.61;0.27]
(SD)	p=0.03	p<0.0001	p=0.11	p=0.45

Data are β-coefficients (SEE), adjusted for sex and sexual maturity and standardized to express one SD of the independent marker; Z, score standardized to the sample mean; P, plasma; HGF, hepatocyte growth factor; SD, standard deviations.

Table 4. Cardiometabolic characteristics of normal weight and overweight children at baseline and at follow-up. Children participated in the European Youth Heart Studies in both 1997/98 and 2003/04 (n=169)

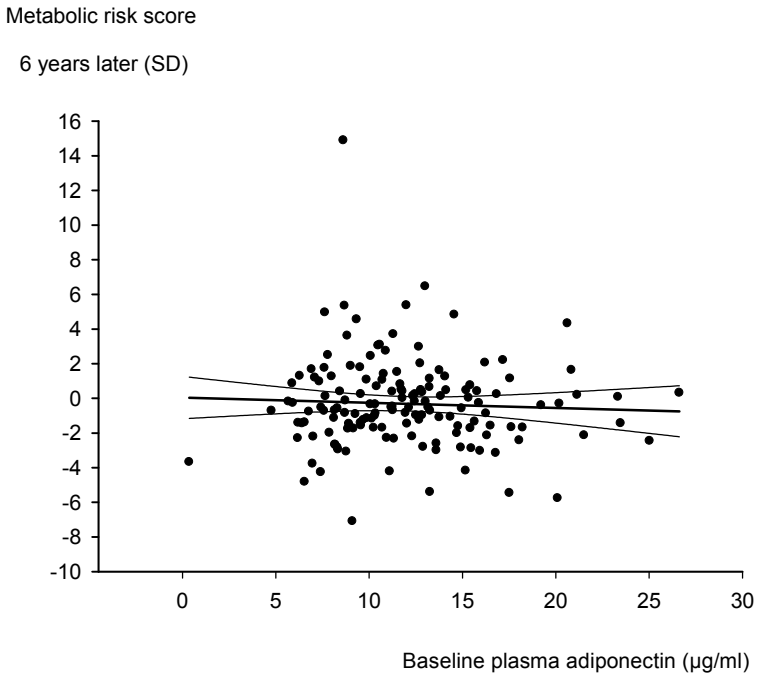
	NW/NW (n=138)	NW/OW (n=12)	OW/NW (n=9)	OW/OW (n=10)
BMI (kg/m^2) at baseline	16.4 (1.3) [A]	18.0 (0.9) [B]	20.2 (0.9) [C]	21.4 (2.0) [C]
BMI (kg/m^2) at follow-up	20.3 (1.8) [A]	25.3 (1.9) [B]	21.1 (1.6) [A]	26.7 (2.8) [B]
Fitness (max watt/kg) at baseline	3.2 (0.4) [A]	3.0 (0.5) [A]	2.5 (0.4) [B]	2.4 (0.4) [B]
Fitness (max watt/kg) at follow-up	3.5 (0.6) [A]	3.0 (0.4) [B]	3.1 (0.5) [B]	2.7 (0.3) [B]
HOMA-IR (units) at baseline	1.7 (0.9) [AC]	2.6 (1.7) [B]	2.3 (1.2) [BC]	2.3 (1.3) [BC]
HOMA-IR (units) at follow-up	2.0 (0.7) [A]	3.2 (2.0) [B]	1.8 (0.6) [A]	2.8 (1.8) [AB]
S-total chol./HDL (ratio) at baseline	3.0 (0.5) [A]	3.2 (0.7) [AB]	3.7 (0.6) [B]	3.2 (0.4) [AB]
S-total chol./HDL (ratio) at follow-up	2.7 (0.5) [A]	3.3 (0.1) [B]	2.9 (0.5) [AB]	2.9 (0.5) [AB]
S-triglycerides (mmol/l) at baseline	0.8 (0.3) [A]	0.8 (0.3) [A]	1.1 (0.5) [A]	1.1 (0.6) [A]
S-triglycerides (mmol/l) at follow-up	0.8 (0.4) [A]	0.8 (0.3) [A]	0.8 (0.3) [A]	0.9 (0.4) [A]
Systolic BP (mm hg) at baseline	104.9 (6.9) [A]	103.2 (8.8) [A]	105.6 (7.2) [A]	108.2 (7.9) [A]
Systolic BP (mm hg) at follow-up	107.9 (8.2) [A]	109.1 (8.2) [A]	105.1 (6.5) [A]	107.8 (9.3) [A]
Metabolic risk score (SD) at baseline	-0.8 (2.9) [A]	1.3 (3.5) [A]	5.1 (3.2) [B]	5.6 (4.3) [B]
Metabolic risk score (SD) at follow-up	-0.7 (2.5) [A]	4.4 (5.0) [B]	0.2 (2.4) [A]	4.5 (3.6) [B]

Data are means (SD); NW, normal weight; OW, overweight; P, plasma;

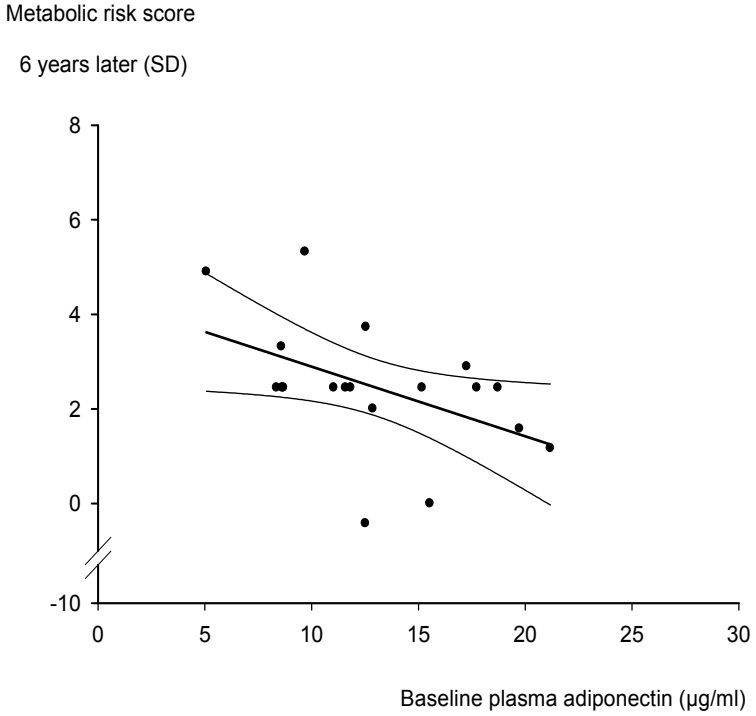
[A, B, C, D] Different letters define mean-differences between subgroups according to Bonferroni t-tests, adjusted for sex and baseline sexual maturity.

Figure 1.

A


Metabolic risk score
6 years later (SD)
16
14
12
10
8
6
4
2
0
-2
-4
-6
-8
-10
0
5
10
15
20
25
30
Baseline plasma adiponectin (µg/ml)

B


Metabolic risk score
6 years later (SD)
8
6
4
2
0
-10
0
5
10
15
20
25
30
Baseline plasma adiponectin (µg/ml)