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Hypoadiponectinemia in overweight children contributes to a negative metabolic risk profile 6 years later

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Abstract

Prognostic biomarkers are needed to identify children at increased cardiometabolic risk. The objective was to study whether markers of metabolism and inflammation, for example, circulating plasma adiponectin, leptin, interleukin-8, and hepatocyte growth factor, are associated with cardiometabolic risk factors in childhood and adolescence. This was a cross-sectional and prospective study, and the setting was the Danish part of the European Youth Heart Studies I and II. Participants were randomly selected girls and boys 8 to 10 years of age with complete baseline data (n = 256) and complete follow-up data 6 years later (n = 169). Cardiometabolic risk profile was calculated using a continuous composite score derived from summing of 6 factors standardized to the sample means (*Z* scores): body mass index, homeostasis model assessment of insulin resistance, total serum cholesterol to serum high-density lipoprotein cholesterol ratio, serum triglycerides, systolic blood pressure, and the reciprocal value of fitness (maximum watts per kilogram). *Overweight* was defined using international classification of body mass index cutoff points for children. Plasma adiponectin, leptin, interleukin-8, and hepatocyte growth factor were assessed using immunochemical assays. Linear relationships were found between metabolic risk score and both plasma adiponectin (inverse, $P = .02$) and plasma leptin ($P < .0001$) 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 ($P = .04$). In childhood, both hypoadiponectinemia and hyperleptinemia 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.

1. 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 hyperinsulinemia [1], dyslipidemia [2], inverted cardiorespiratory fitness [3], and clustered factors of the metabolic syndrome [4,5]. In 10- to 19-year-old Native Canadians, hypoadiponectinemia together with dyslipidemia, and hyperleptinemia together with adiposity represented 2 of 5 core traits of clustered metabolic risk. This suggests that adipokines may play a role in early dysmetabolism among high-risk children.

Proinflammatory chemokines such as interleukin (IL)-8 and hepatocyte growth factor (HGF) may cause inflammation through an increased influx of leukocytes into inflamed 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]. In addition, circulating HGF concentrations were observed to be higher in overweight compared with 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.

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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 Danish children, followed 6 years from childhood into adolescence.

2. Materials and methods

Data are based on the Danish part of the European Youth Heart Studies (EYHS) I and II in Odense, Denmark. The EYHS is a longitudinal multicenter 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 1997/1998 among children from third and ninth school grade. In 2003/2004, 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 Declaration of Helsinki.

2.1. 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, ninth graders, including third graders from baseline, were eligible for the follow-up study 6 years later. 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 to 10 years of age, 53% girls and 47% boys. Of these, 384 were reexamined 6 years later as ninth graders 14 to 16 years of age, 56% girls and 44% boys.

2.2. Measurements

Physical and clinical examinations, and blood sampling were collected and undertaken by health professionals and trained personnel following international standard procedures [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 5-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 and Whitehouse [15]. Children were categorized as prepubertal with Tanner stage 1; pubertal with Tanner stages 2, 3, and 4; and postpubertal 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 body mass index (BMI) equivalent to an adult BMI of at least 25 kg/m^2 according to international extrapolations for age- and sex-matched child cohorts [15,16]. Overweight corresponded to a BMI greater than 18.4 to 20.5 among girls and boys from 8 to 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, Manufacturing Technology, Fort Walton Beach, FL) [14]. The average daily electronic counts per minute corresponds to amount of activity per time unit for each child. Recordings were weighted by the percentage of week days and weekend days to correspond to a standard week. From an incremental ergometer cycle test, where the workload was increased by 3-minute intervals until exhaustion, individual maximum watt was obtained (Monark 839 Ergo medic, VPS International, Merchtem, Belgium) [14]. Cardiorespiratory fitness was expressed as maximum watt per kilogram body weight of the child.

2.2.1. Clinical factors

Systolic blood pressure was measured with a Dinamap pediatric and adult neonatal vital signs monitor (model XL; Critikron, Tampa, FL). Five measurements were taken at 2-minute intervals, with the mean of the final 3 measurements used in all analyses [14]. Blood samples were obtained from the right antecubital vein after an overnight fast. Within 30 minutes, aliquots of plasma and serum were separated and immediately stored at −80°C until further analysis. At World Health Organization–certified laboratories (Bristol, England, in 1998 and Cambridge, England, in 2004), serum glucose was analyzed using the hexokinase method; and total serum cholesterol, serum high-density lipoprotein (HDL) cholesterol, and serum triglycerides were measured using enzymatic colorimetry at both study years (Olympus AU600 autoanalyzer; Olympus Diagnostica, Hamburg, Germany). Serum insulin was analyzed using enzyme immunoassays with microtiter plate format (Dako Diagnostics, Ely, United Kingdom) at baseline and by 2-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 (in microunits per milliliter) × glucose (in millimoles per liter) × 22.5^{-1} [17]. A strong correlation has been found between HOMA-IR and frequently sampled intravenous glucose tolerance test among obese, prepubertal, and pubertal children [18].

2.2.2. 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 to October 2007. A solid-phase protein immunoassay was applied for analysis (Luminex type 100 IS; Ramcon, Birkerød, Denmark). Total plasma adiponectin was analyzed in single determination after double determination in 30 samples, showing good agreement between duplicates (5.5% coefficient of variation [CV]). Interassay variation was 3.73% CV, and intraassay variation was 4.26% CV (Electrabox Diagnostica, Rødovre, Denmark). Plasma concentrations of leptin, IL-8, and HGF were analyzed in duplicates. For multiplexed assays of plasma leptin, IL-8, and HGF, interassay variations were 1.4% to 7.9% CV; and intraassay variations were less than 2.1% CV (Linco Research, St Charles, MO). Hyperleptinemia and hypoadiponectinemia were used as relative terms on the scale of continuously distributed plasma concentrations.

2.3. Statistical analyses

All statistical analyses were carried out in SAS version 9.1 (Statistical Analysis System Institute, 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 cardiorespiratory 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 deviation (SDs) 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. Multiadjusted 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 backward stepwise reduction of the model. Interactions with sex, sexual maturity, or overweight at baseline were tested for each independent marker in the multiadjusted model. Coefficient and 95% confidence limits (CLs) in the linear regression model were standardized by multiplication with 1 SD of the independent marker. Statistical significance was determined by a 2-sided probability level of 5% or less in all models. Characteristics of the study sample are presented as means (SD).

3. Results

Two hundred 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

Table 1
Baseline characteristics of children included and excluded in the statistical analyses because of incomplete data in the Danish part of the EYHS in 1997/1998 (EYHS I) and 2003/2004 (EYHS II)

	EYHS I				EYHS II[a]		
	Incl n = 256	Excl	n[b]	P value[c]	Incl n = 169	Excl n = 87	P value[d]
Girls (% of n)	51.6	53.6	334	.62	50.9	52.9	.76
Prepubertal (% of n)[e]	85.2	83.6	325	.59	82.3	86.2	.42
Age (y)	9.7 (0.4)	9.6 (0.4)	334	.33	9.6 (0.4)	9.7 (0.4)	.03
Activity (counts/1000* min)	6.7 (2.4)	6.3 (2.0)	127	.22	6.7 (2.4)	6.6 (2.4)	.78
BMI (kg/m^2)	17.2 (2.2)	17.4 (2.6)	334	.42	17.0 (7.9)	17.5 (2.7)	.11
Fitness (max W/kg)	3.1 (0.5)	2.9 (0.6)	283	.01	3.1 (0.5)	3.0 (0.6)	.15
HOMA (U)	1.8 (1.0)	1.9 (1.1)	263	.35	1.9 (1.0)	1.8 (1.1)	.56
S total cholesterol/HDL (ratio)	3.1 (0.6)	3.2 (0.7)	269	.09	3.1 (0.6)	3.2 (0.6)	.29
S triglycerides (mmol/L)	0.9 (0.4)	0.8 (0.3)	269	.43	0.9 (0.4)	0.8 (0.3)	.65
Systolic BP (mm hg)	105.2 (7.3)	104.8 (7.7)	333	.54	105.0 (7.1)	105.4 (7.6)	.69
P leptin (ng/mL)	5.1 (5.7)	6.0 (7.1)	174	.17	4.6 (5.4)	6.0 (6.3)	.06
P adiponectin (μg/mL)	12.0 (4.1)	12.1 (4.6)	173	.85	12.1 (4.4)	11.8 (3.7)	.67
P IL-8 (pg/mL)	1.7 (1.3)	2.0 (4.5)	174	.32	1.6 (1.0)	1.9 (1.7)	.12
P HGF (ng/mL)	0.7 (0.5)	0.8 (1.6)	173	.26	0.7 (0.5)	0.7 (0.4)	.62

Data are means (SD) where nothing else is noted. U indicates units; S, serum; P, plasma; BP, blood pressure.

[a] Also completers in EYHS I.

[b] Number of children excluded in cross-sectional analyses with valid data on some variables; note that the number differs between variables.

[c] Differences between children included and excluded in EYHS I; analysis of variance for continuous variables and χ^2 for categorical variables.

[d] Differences between children included and excluded in EYHS II; analysis of variance for continuous variables and χ^2 for categorical variables.

[e] Prepubertal vs pubertal children.

Table 2
Baseline characteristics of cardiometabolic factors and markers of metabolism and inflammation of prepubertal boys and girls, and pubertal girls

	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 W/kg)	3.2 (0.5)	3.0 (0.5)‡‖	2.7 (0.4)
HOMA-IR (U)	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)

Children participated in the EYHS 1997/1998 (n = 256). Data are means (SD).
Statistical difference between prepubertal girls and boys: *$P < .05$; †$P < .01$; ‡$P < .001$.
Statistical difference between prepubertal and pubertal girls: ‖$P < .01$; ¶$P < .001$.

baseline had complete follow-up data on BMI, cardiorespiratory fitness, and all clinical risk factors. The dropout at follow-up was due to missing data on BMI (30%), cardiorespiratory 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-W/kg higher fitness than those with incomplete data but were otherwise similar (Table 1). Furthermore, children with complete data at follow-up were 0.1 year younger at baseline compared with children with incomplete follow-up data but were otherwise similar (Table 1).

3.1. 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, cardiorespiratory fitness, and 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, and plasma leptin and lower cardiorespiratory fitness (Table 2). Therefore, all multivariate analyses were adjusted for sex and sexual maturity.

3.2. 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, cardiorespiratory 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]; $P = .02$).

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 was inversely associated with cardiorespiratory 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], $P <$ .0001). Nonsignificant 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, and plasma concentrations of leptin and adiponectin, the association with metabolic risk score remained nonsignificant for both IL-8 (standardized β [CL], −0.17 [−0.51, 0.17], $P = .32$) and plasma HGF (standardized β [CL], −0.26

Table 3
Baseline linear regression coefficients of the relationship between markers of metabolism and inflammation and cardiometabolic factors of 8- to 10-year-old children from the EYHS in 1997/1998 (n = 256)

	P adiponectin (μg/mL)	P leptin (ng/mL)	P IL-8 (pg/mL)	P HGF (ng/mL)
	β (CL)			
Z BMI (SD)	−0.10 (−0.22; 0.02), $P = .10$	0.70 (0.61, 0.79), $P < .0001$	−0.07 (−0.19, 0.05), $P = .26$	−0.02 (−0.14, 0.10), $P = .77$
Z Max W/kg (SD)	−0.07 (−0.18, 0.05), $P = .26$	−0.57 (−0.67, −0.47), $P < .0001$	0.04 (−0.08, 0.15), $P = .53$	0.01 (−0.11, 0.12), $P = .90$
Z HOMA-IR (SD)	−0.06 (−0.18, 0.07), $P = .36$	0.38 (0.26, 0.51), $P < .0001$	−0.07 (−0.20, 0.05), $P = .82$	−0.02 (−0.15, 0.10), $P = .72$
Z total cholesterol/HDL (SD)	−0.07 (−0.19, 0.05), $P = .25$	0.22 (0.09, 0.35), $P = .0007$	−0.06 (−0.19, 0.06), $P = .31$	−0.10 (−0.22, 0.02), $P = .11$
Z triglyceride (SD)	−0.12 (−0.24, 0.01), $P = .06$	0.23 (0.10, 0.36), $P = .0004$	−0.09 (−0.22, 0.03), $P = .13$	−0.03 (−0.16, 0.09), $P = .58$
Z systolic BP (SD)	−0.19 (−0.31, −0.07), $P = .002$	0.16 (0.04, 0.29), $P = .01$	−0.02 (−0.14, 0.10), $P = .76$	0.02 (−0.11, 0.14), $P = .80$
Metabolic risk score (SD)	−0.47 (−0.91, −0.03), $P = .03$	2.27 (1.91, 2.63), $P < .0001$	−0.35 (−0.79, 0.08), $P = .11$	−0.17 (−0.61, 0.27), $P = .45$

Data are β coefficients (CL), adjusted for sex and sexual maturity and standardized to express 1 SD of the independent marker. Z indicates score standardized to the sample mean.

Table 4
Cardiometabolic characteristics of normal-weight and overweight children at baseline and at follow-up

	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 W/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 W/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 (U) at baseline	1.7 (0.9)^{AC}	2.6 (1.7)^{B}	2.3 (1.2)^{BC}	2.3 (1.3)^{BC}
HOMA-IR (U) 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 cholesterol/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 cholesterol/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}

Children participated in the EYHS in both 1997/1998 and 2003/2004 (n = 169). Data are means (SD). Different letters ($^{A, B, C, D}$) define mean differences between subgroups according to Bonferroni *t* tests, adjusted for sex and baseline sexual maturity.

[−0.61, 0.08], P = .13). No baseline interactions were identified with sex, sexual maturity, or overweight for any of the cytokine markers.

3.3. Follow-up characteristics

One hundred 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 by 4.0 kg/m^2 (0.1), their cardiorespiratory fitness by 0.3 W/kg (0.1), their HOMA-IR by 0.3 U (0.1), and their systolic blood pressure by 2.8 mm Hg (0.7). Furthermore, they decreased their total serum cholesterol to serum HDL cholesterol ratio by 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 by 211.6 counts per minute (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, cardiorespiratory 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 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 < .05), but similar concentrations were found between the 2 subgroups being normal weight at baseline and between the 2 subgroups being overweight at baseline. No subgroup differences were found for plasma concentrations of adiponectin, IL-8, and HGF (data not shown).

To study the consequences of overweight at baseline, regardless of overweight at follow-up, we divided children into 2 groups: the 150 normal-weight children at baseline and the 19 overweight children at baseline. Compared with normal-weight children, overweight children at baseline had lower cardiorespiratory fitness (P < .0001), as well as higher HOMA-IR (P = .050), higher total serum cholesterol to serum HDL cholesterol ratio (P = .006), higher serum triglycerides (P = .003), and higher metabolic risk score (P < .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 < .0001), lower cardiorespiratory fitness (P = .005), and higher metabolic risk score (P = .0006) than normal-weight children at baseline.

3.4. 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 = .04). No association was found among normal-weight children at baseline (standardized β [CL], −0.15 [−0.61, 0.32], P = .53) or the overall group (standardized β [CL], −0.31 [−0.91, 0.16], P = .18). The associations were all adjusted for confounding factors and Fig. 1 illustrates the associations for normal weight and overweight children. A significant interaction between plasma adiponectin and overweight vs normal weight was identified (P = .01). Baseline plasma leptin was not linearly associated with metabolic risk score at follow-up (standardized β [CL], −0.04 [−0.64, 0.56], P = .90); and no association was found

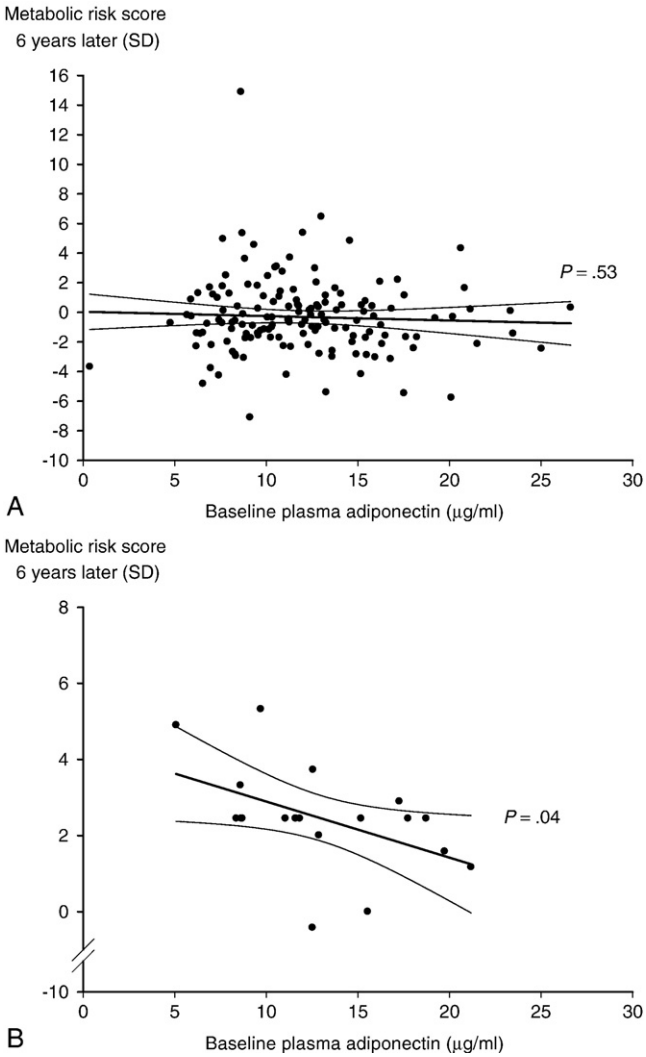

Fig. 1. Relationships between baseline plasma adiponectin and metabolic risk score 6 years later among 150 children who were normal weight at baseline (A) and 19 children who were overweight at baseline (B) (participants from the EYHS in both 1997/1998 and 2003/2004). The relationships are linear regressions with 95% confidence intervals, adjusted for baseline metabolic risk score, sex, sexual maturity, and school location.

for plasma IL-8 (standardized β [CL], −0.06 [−0.53, 0.41], $P = .80$) or HGF (standardized β [CL], −0.07 [−0.52, 0.39], $P = .77$), all adjusted for baseline metabolic risk score, school location, sex, and sexual maturity.

4. Discussion

In healthy normal-weight and overweight Danish children aged 8 to 10 years, both hypoadiponectinemia and hyperleptinemia were independently correlated with a negative cardiometabolic risk profile. In addition, hypoadiponectinemia 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 hypoadiponectinemia in overweight but otherwise healthy children.

That hypoadiponectinemia contributed to a negative cardiometabolic risk score 6 years later in overweight but not in normal-weight children suggests 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 [19,20] and an inverse association between plasma adiponectin concentrations and plasma triglycerides among girls [19]. 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 [21,22]. It has been suggested that adiponectin activates adenosine monophosphate protein kinase, which could lead directly to decreased hepatic gluconeogenesis [21]. 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 [23], but other studies in children found no significant association with or without adjustment for body fatness [5,19]. 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 hypoadiponectinemia [22]. Adiponectin inhibits proinflammatory cytokine production and adhesion molecule expression and induces anti-inflammatory factors [22]. These activities are present in macrophages, endothelial cells, and cardiac cells and may be mediated in part by inhibiting the stress signaling pathways, for example, nuclear factor–κB, and in part by the activation of adenosine monophosphate protein kinase [22]. 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 correlated 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 [5,24]. 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 proinflammatory plasma cytokines, such as IL-1β, IL-6, and tumor necrosis factor α [25]. 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.

4.1. Study design considerations

The present study comprises a sizeable sample of healthy children with extensive information of each individual at 2 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 [26]. When generating this score, we have the benefit of avoiding the use of arbitrary cutoffs for each cardiometabolic risk factor, the utility of which is considered controversial in pediatric studies [27]. 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 dual-energy x-ray absorptiometry in children [28]. 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 6 years later among overweight but not among normal-weight children. Hyperleptinemia coexists 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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