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Lack of association between apelin, insulin resistance, cardiovascular risk factors, and obesity in children: a longitudinal analysis

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ABSTRACT

Apelin has been proposed as a novel beneficial adipokine that is related to insulin resistance, cardiovascular risk factors, and obesity. However, findings in humans are controversial; and longitudinal analyses in childhood are still missing. We compared apelin levels between 80 obese and 40 lean children of the same age, sex, and pubertal stage. In addition, we analyzed the relationships between apelin levels and weight status (as standard deviation of body mass index [SDS-BMI]), body fat, insulin resistance (homeostasis model assessment [HOMA]), leptin, and cardiovascular risk factors associated with obesity (waist circumference, blood pressure, lipids, and adiponectin) in 80 obese children before and after participating in a 1-year lifestyle intervention. Apelin levels did not differ significantly ($P = .061$) between obese (1.50 ± 0.47 ng/mL, mean ± SD) and lean children (1.67 ± 0.49 ng/mL). Apelin concentrations were not significantly related to age, pubertal stage, SDS-BMI, body fat, leptin, or any cardiovascular risk factor. In longitudinal analyses, no significant correlations were found between changes of apelin and changes of SDS-BMI, body fat, leptin, HOMA, or any cardiovascular risk factor. Adiponectin, HOMA, blood pressure, waist circumference, and triglycerides improved significantly in 39 obese children with SDS-BMI reduction, whereas leptin decreased significantly and apelin did not change significantly in these children. In 41 children with increase of SDS-BMI, no significant changes were observed in 1-year follow-up period. This is the first study demonstrating that weight loss in obese children was not associated with a change of apelin concentrations. Our data do not support a significant relationship in childhood between apelin on one hand and leptin, HOMA, cardiovascular risk factors, or weight status on the other.

This study is registered at clinicaltrials.gov (NCT00435734).

Thomas Reinehr and Christian Roth developed the study design, Thomas Reinehr performed the anthropometric measurements, Christian Roth performed the laboratory measurements, Thomas Reinehr, Joachim Woelfle, and Christian Roth performed the discussion. Thomas Reinehr wrote the first draft of the paper.

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1. Introduction

Obesity is a complex disease involving a number of different peptides, transmitters, and their receptors controlling energy homeostasis [1,2]. Recent studies suggest that adipose tissue hormones (adipokines) are involved in body weight regulation and in the pathogenesis of various complications of obesity, including dyslipidemia, type 2 diabetes mellitus, arterial hypertension, atherosclerosis, and heart failure [1-3].

Recently, apelin, a 36–amino-acid peptide, has been described as a beneficial adipokine related to obesity, which is hypothesized to be one of the protective peptides of well-known obesity-related cardiovascular risk factors including type 2 diabetes mellitus [4-6]. Apelin exists in at least 3 bioactive forms, consisting of 13, 17, or 36 amino acids, all originating from a common 77–amino-acid precursor. Apelin is the endogenous ligand of the orphan G-protein–coupled receptor APJ [4]. Although synthesized in several tissues, apelin is expressed and secreted by human adipocytes [3,7,8]. Wei and colleagues [5] found that apelin messenger ribonucleic acids (mRNAs) were expressed in isolated mouse adipocytes and that apelin mRNA levels increased during the differentiation of 3T3-L1 cells to adipocytes.

The most documented functions of apelin/APJ concern the regulation of fluid homeostasis and the modification of cardiac contractility and blood pressure [4-6]. Recently, apelin has been suggested to be partly involved in the mechanism underlying the development of obesity-related hypertension [5,6]. Furthermore, animal models suggest that apelin negatively regulates lipolysis [9]. Very recently, it was shown that apelin inhibited insulin secretion in mice [4], suggesting a link between apelin and glucose homeostasis [10]. Apelin stimulates glucose utilization in normal and obese insulin-resistant mice [8]. In addition, insulin exerts a direct control on apelin gene expression in adipocytes [7]. Accordingly, apelin and APJ expression in mice has been reported to be regulated according to the severity of insulin resistance [4].

Apelin promotes angiogenesis in adipose tissue [11]. Therefore, a putative role for apelin in the etiology of obesity has been suggested [11]. In rat hypothalamus, apelin mRNA expression is detected in several areas including paraventricular and ventromedial nuclei, areas that are generally involved in control of feeding behavior [4]. Intracerebroventricular injection of apelin-13 decreased food intake in fed but not in fasted rats, whereas daytime administration of apelin-12 stimulated feeding [4]. Therefore, the physiological significance of these findings is unclear.

Moreover, the situation in humans, in particular in children, concerning apelin and its relation to obesity, insulin resistance, and cardiovascular risk factors is less clear. Some studies reported increased apelin levels in obese adults as compared with normal-weight humans [4,12-14], and a correlation between apelin and body mass index (BMI) [12,15]. Conversely, other studies did not find this relationship [16,17] or even lower apelin levels in obese vs nonobese pubertal children [17]. In children with type 1 diabetes mellitus, apelin levels were increased compared with healthy controls; but apelin was not related to BMI, lipids, or insulin sensitivity [18]. In adults, weight loss due to a hypocaloric diet was reported to be associated with a decrease of apelin [13], whereas other studies demonstrated no change of apelin in weight loss [16,19]. Some studies demonstrated a positive correlation between apelin, insulin [13,16], glucose [16], homeostasis model assessment (HOMA) index [4,15], and triglycerides [16]. Overall, the findings are controversial in humans [13,19].

We compared apelin between obese and normal-weight children and studied apelin levels in obese children participating in a lifestyle intervention as well as the relationship between apelin, insulin resistance, leptin, and cardiovascular risk factors such as blood pressure, waist circumference, lipids, and adiponectin in the course of 1 year. Longitudinal studies and studies in obese children seem preferable because cross-sectional studies cannot prove causality and are prone to many confounders, adverse patterns of insulin resistance itself begin in childhood, and studies in children have the advantage that there is no potential confusion with other diseases, medications, or active tobacco smoking. We hypothesized that apelin concentrations are increased in obese children as compared with normal-weight children and that changes of apelin were associated to changes of weight status and changes of leptin, cardiovascular risk factors, and insulin resistance. The novelty of our study is the longitudinal analysis of apelin concentrations in obese children participating in a lifestyle intervention.

2. Methods

2.1. Subjects

Written informed consent was obtained from all children and their parents. The study was approved by the local ethics committee of the University of Witten/Herdecke in Germany.

We examined 80 obese white children and 40 normal-weight children of similar age, sex, and pubertal stage. All 80 obese children participated in the lifestyle intervention "Obeldicks," which has been described in detail elsewhere [20,21]. Briefly, this outpatient intervention program for obese children is based on physical exercise, nutrition education, and behavior therapy including the individual psychological care of the child and his or her family. The nutritional course is based on a fat- and sugar-reduced diet as compared with the everyday nutrition of German children.

None of the children in the current study had endocrine disorders, premature adrenarche, or syndromic obesity.

2.2. Measurements

We analyzed apelin, leptin, and insulin resistance index HOMA in the 80 obese children and in the 40 normal-weight children. Furthermore, we determined anthropometric markers (waist circumference, body fat based on bioimpedance analyses and skinfold measurements), blood pressure, fasting serum apelin, adiponectin, leptin, lipids (high-density lipoprotein [HDL] cholesterol, low-density lipoprotein [LDL] cholesterol, triglycerides), and uric acid concentrations and, as parameters of insulin resistance, fasting glucose and insulin levels in the 80 obese children at baseline and 1 year later after participating in the lifestyle intervention "Obeldicks."

Height was measured to the nearest centimeter using a rigid stadiometer. Weight was measured unclothed to the

nearest 0.1 kg using a calibrated balance scale. Body mass index was calculated as weight in kilograms divided by the square of height in meters. The degree of overweight was quantified using Cole's least mean square method, which normalized the BMI skewed distribution and expressed BMI as a standard deviation score (SDS-BMI) [22]. Reference data for German children were used [23]. All children in the study were obese according to the definition of the International Obesity Task Force [24].

The pubertal developmental stage was determined according to Marshall and Tanner [25,26].

Triceps and subscapularis skinfold thickness was measured twice using a caliper and averaged to calculate the percentage of body fat using a skinfold thickness equation with the following formulas [27]: boys: body fat % = 0.783 × (subscapularis skinfold thickness + triceps skinfold thickness in millimeters) + 1.6; girls: body fat % = 0.546 × (subscapularis skinfold thickness + triceps skinfold thickness in millimeters) + 9.7.

Bioelectrical impedance was measured using leg-leg and hand-leg systems (BC418; TANITA, Uxbridge, United Kingdom). We used estimates of total body fat, lean body mass, and percentage body fat provided by the manufacturer's software based on age, sex, height, and weight. No information regarding the formulas used could be obtained from the manufacturer because of its commercially sensitive nature.

Blood sampling was performed in the fasting state at 8:00 AM. After clotting, blood samples were centrifuged for 10 minutes at 8000 rpm. Serum was stored at –81°C for later determination of apelin, leptin, adiponectin, and insulin. All probes were thawed only once. Apelin levels were measured with a commercially available enzyme-linked immunoassay (ELISA) kit (Phoenix Pharm, Burlingham, CA, USA). The sensitivity of the assay was 0.07 ng/mL, the intraassay coefficient of variation (CV) was less than 5%, and the interassay CV was less than 15%. The ELISA had a 100% cross-reactivity with human apelin-12, apelin-13, and apelin-36. Leptin and adiponectin concentrations were measured by a commercially available ELISA (Leptin [human] ELISA Kit; BioVendor, Alexis Biochemicals, Lausen, Switzerland, and [human] Adiponectin ELISA Kit; Linco Research, St Charles, MO), and insulin concentrations were measured by microparticle enhanced immunometric assay (MEIA; Abbott, Wiesbaden, Germany). Glucose levels were determined by colorimetric test using a Vitros analyzer (Ortho Clinical Diagnostics, Neckargmuend, Germany). Triglycerides, uric acids, LDL-cholesterol, and HDL-cholesterol were determined by commercially available test kits (Roche, Mannheim, Germany). Intra- and interassay CVs were less than 5% in all these methods. All variables were measured in duplicate and averaged. Homeostasis model assessment was used to detect the degree of insulin resistance using the following formula: resistance (HOMA) = [insulin (in microunits per liter) × glucose (in millimoles per liter)]/22.5 [28].

2.3. Statistics

Based on the published difference in apelin levels between obese (0.736 ± 0.050 ng/mL) and normal-weight adults (0.174 ± 0.014 ng/mL) [12], the required sample size of this study was estimated to compare apelin levels in obese and normal-weight children. Based on these results and to get a conservative sample size estimate, the mean difference was reduced by 50% and the standard deviation was increased by 50%, yielding 0.281 ng/mL as group difference in apelin levels. On an $\alpha = .05$ level, 2-sided tests, and 99% power, a sample size of 8 (4 per group) was estimated for this study. This study sample estimation was multiplied by 10 to account for probably lower apelin difference in children and to account for multiple comparisons.

Statistical analyses were performed using the Winstat software package (R Fitch Software, Bad Krozingen, Germany). All variables were normally distributed as tested by the Kolmogorov-Smirnov test. Apelin was correlated to leptin, adiponectin, anthropometric variables, blood pressure, lipids, uric acids, and insulin resistance index HOMA by Pearson correlation. Furthermore, a multiple stepwise linear regression was calculated with apelin as dependent variable and leptin and HOMA as independent variables adjusted for pubertal stage, age, sex, and BMI. Sex and pubertal stage were used as binary variables in this model. To compare variables at baseline or in the course of 1 year, Fisher exact test and Student *t* test for paired and unpaired observations were used as appropriate. Correlations between changes of apelin, adiponectin, leptin, anthropometric variables, and insulin resistance index HOMA in the course of 1 year were calculated by Pearson correlation. Changes were expressed as Δ variable calculated by variable at baseline minus variable measured 1 year later. A *P* value < .05 was considered as significant. Data are presented as mean and standard deviation.

3. Results

The leptin concentrations and the insulin resistance index HOMA were significantly higher in the obese children as compared with the normal-weight children, whereas the

Table 1 – Baseline characteristics (anthropometrics, apelin, leptin, and insulin resistance index HOMA) in 80 obese and 40 normal-weight children (data as percentage or mean and standard deviation)

	Obese children	Normal-weight children	*P* value
Age	10.9 ± 0.3	11.6 ± 0.4	.192
Sex	53% Female	42% Female	.397
Pubertal stage	47 (59%) Prepubertal 33 (41%) Early pubertal[a]	15 (37%) Prepubertal 25 (63%) Early pubertal[a]	.551
BMI (kg/m^2)	28.3 ± 0.5	17.0 ± 0.5	<.001
SDS-BMI	2.33 ± 0.48	–0.21 ± 0.14	<.001
Apelin (ng/mL)	1.50 ± 0.47	1.67 ± 0.49	.061
Leptin (ng/mL)	25.8 ± 1.7	2.1 ± 0.3	<.001
HOMA	3.5 ± 0.3	1.1 ± 0.1	<.001

[a] All these children were at early pubertal stage with PII-PIII and BII-BIII, or GII-GIII.

Table 2 – Changes of anthropometrics, cardiovascular risk factors, apelin, adiponectin, leptin, and insulin resistance HOMA in obese children in the course of 1 year separated to changes of SDS-BMI in the intervention period (data as percentage or mean and standard deviation)

	Obese children with decrease of SDS-BMI			Obese children with increase of SDS-BMI		
n	39			41		
Age (y)	10.9 ± 2.3			11.3 ± 3.9		
Sex	51% Female			42% Female		
Pubertal stage	20 (51%) Prepubertal 19 (49%) Early pubertal [a]			19 (46%) Prepubertal 22 (54%) Early pubertal [a]		
	Baseline	1 y later	*P* value	Baseline	1 y later	*P* value
BMI (kg/m²)	27.4 ± 3.3	24.6 ± 2.9	<.001	27.9 ± 4.5	29.2 ± 4.7	<.001
SDS-BMI	2.35 ± 0.44	1.67 ± 0.56	<.001	2.31 ± 0.51	2.44 ± 0.58	.027
Triceps skinfold thickness (cm)	31 ± 5	27 ± 5	<.001	31 ± 7	32 ± 11	.601
Subscapularis skinfold thickness (cm)	30 ± 6	25 ± 6	.005	30 ± 8	33 ± 8	.226
Body fat based on skinfold thickness (%)	45 ± 12	40 ± 12	.002	45 ± 13	47 ± 14	.685
Body fat based on BIA (%)	35 ± 4	28 ± 3	.002	36 ± 5	35 ± 6	.700
Waist circumference (cm)	83 ± 10	80 ± 10	.031	82 ± 12	83 ± 12	.581
Apelin (ng/mL)	1.42 ± 0.44	1.54 ± 0.56	.261	1.57 ± 0.49	1.59 ± 0.61	.856
Leptin (ng/mL)	21 ± 11	12 ± 6	<.001	28 ± 14	32 ± 13	.231
Adiponectin (μg/mL)	6.5 ± 2.8	8.7 ± 3.7	.042	6.7 ± 3.4	6.3 ± 3.1	.668
Systolic blood pressure (mm Hg)	118 ± 18	110 ± 16	.023	118 ± 14	114 ± 14	.091
Diastolic blood pressure (mm Hg)	68 ± 12	64 ± 8	.038	67 ± 10	66 ± 11	.542
LDL-cholesterol (mg/dL)	96 ± 28	91 ± 32	.171	102 ± 27	98 ± 27	.274
HDL-cholesterol (mg/dL)	54 ± 12	57 ± 15	.011	52 ± 11	49 ± 13	.159
Triglycerides (mg/dL)	89 ± 38	70 ± 24	.001	119 ± 38	120 ± 62	.960
Uric acid (mg/dL)	4.9 ± 1.0	4.5 ± 0.8	.011	4.9 ± 1.2	5.1 ± 1.1	.036
Glucose (mg/dL)	83 ± 7	85 ± 7	.226	86 ± 7	87 ± 7	.594
Insulin (mU/L)	16 ± 10	11 ± 8	.002	20 ± 16	23 ± 16	.249
HOMA	3.3 ± 2.3	2.4 ± 0.9	.031	4.3 ± 3.2	4.9 ± 3.7	.195

BIA indicates bioimpedance analysis.
[a] All these children were at early pubertal stage with PII-PIII and BII-BIII, or GII-GIII.

apelin concentrations did not differ significantly but showed a trend toward lower apelin levels in obese children (Table 1). Normal-weight and obese children did not differ significantly with respect to age, sex, or pubertal stage.

Apelin was not significantly correlated to BMI ($r = –0.02$, $P = .432$), SDS-BMI ($r = –0.05$, $P = .319$), body fat based on bioimpedance analyses ($r = 0.06$, $P = .353$), body fat based on skinfold measurements ($r = 0.01$, $P = .805$), age ($r = 0.12$, $P = .149$), leptin ($r = 0.03$, $P = .105$), adiponectin ($r = 0.05$, $P = .379$), insulin resistance index HOMA ($r = –0.14$, $P = .105$), waist circumference ($r = 0.09$, $P = .232$), systolic blood pressure ($r = –0.08$, $P = .232$), diastolic blood pressure ($r = –0.14$, $P = .100$), LDL-cholesterol ($r = –0.06$, $P = .289$), triglycerides ($r = –0.01$, $P = .458$), and uric acid ($r = –0.11$, $P = .168$). In a multiple stepwise linear regression ($r^2 = 0.18$) adjusted for pubertal stage, age, sex, and BMI, apelin was not significantly related to leptin ($P = .340$) and HOMA ($P = .590$).

Boys (1.49 ± 0.41 ng/mL) did not differ significantly ($P = .892$) with respect to their apelin levels as compared with girls (1.51 ± 0.52 ng/mL). The apelin concentrations did not differ significantly ($P = .854$) between prepubertal (1.51 ± 0.39 ng/mL) and pubertal children (1.49 ± 0.54 ng/mL). All pubertal children were at early pubertal stage (PII-PIII and BII-BIII, or GII-GIII).

In the 1-year follow-up period of the 80 obese children, the changes of apelin were not correlated significantly to changes of insulin resistance index HOMA ($r = –0.11$, $P = .168$), changes of SDS-BMI ($r = –0.06$, $P = .303$), changes of body fat based on bioimpedance analyses ($r = –0.16$, $P = .264$), changes of body fat based on skinfold measurements ($r = 0.06$, $P = .307$), changes of leptin ($r = –0.17$, $P = .114$), changes of adiponectin ($r = 0.07$, $P = .330$), changes of waist circumference ($r = 0.01$, $P = .471$), changes of systolic blood pressure ($r = –0.04$, $P = .362$), changes of diastolic blood pressure ($r = 0.06$, $P = .303$), changes of LDL-cholesterol ($r = 0.08$, $P = .266$), changes of triglycerides ($r = 0.101$, $P = .187$), and changes of uric acid ($r = 0.02$, $P = .233$).

In the 39 obese children with reduction of SDS-BMI in the intervention period, apelin did not change significantly; leptin and HOMA decreased significantly; adiponectin increased significantly; and the cardiovascular risk factors improved significantly (Table 2). In the 41 obese children with an increase of SDS-BMI in the intervention period, no significant changes could be observed.

The obese children with SDS-BMI increase and those with SDS-BMI decrease did not differ significantly at baseline according to age, sex, pubertal stage, any anthropometric parameter, cardiovascular risk factors, apelin, adiponectin, leptin, or HOMA levels (Table 2).

4. Discussion

To the best of our knowledge, this is the first study analyzing the longitudinal relationships between apelin, obesity, insulin resistance, leptin, and cardiovascular risk factors in obese children participating in a lifestyle intervention. Our hypothesis

was that circulating apelin levels would be increased in obese children and decrease in weight loss in a parallel manner with leptin, insulin resistance, and cardiovascular risk factors.

In contrast to our hypothesis, a reduction of overweight due to lifestyle intervention was not associated with a decrease of apelin levels. Accordingly, changes of body fat and changes of the adipocytokine leptin, which is strongly related to fat mass [1], were also not associated to changes of apelin. These findings are in concordance with most studies in adults demonstrating that weight loss due to hypocaloric diet or bariatric surgery is not associated with a decrease of apelin in obese adults [16,19]. Only one small study (n = 20) reported a decrease of apelin during short-term weight loss in obese women using a hypocaloric diet [13]. The discrepancies of the studies are unclear, but it has been suggested that only obese adults with disturbed glucose metabolism demonstrate a decrease of apelin in weight loss [16].

Our negative findings between apelin and weight status also in cross-sectional analysis are in concordance to some previous cross-sectional studies in adults demonstrating no correlations between apelin levels and BMI [7,16,29]. In addition, no difference in apelin concentrations was found between obese and normal-weight prepubertal children [17].

All these findings suggest that adipose tissue is not the main source of apelin serum levels in childhood. The missing relationship between apelin and weight status in our study might be explained by different sources of apelin secretion. Apelin is synthesized not only in the adipose tissue [3,8], but also in various other tissues such as, for example, central nervous system, heart, lung, testis, ovary, and mammary gland [13]. Therefore, even if weight loss is associated with decrease of adipose tissue expression of apelin as recently demonstrated [13], other sources of apelin may mask this effect in childhood. It is unlikely that a too low degree of weight loss is responsible for the unchanged apelin levels because the cardiovascular risk factors improved according to previous studies [30-32] and leptin decreased as well.

Interestingly, the apelin levels in our study were higher in both obese and normal-weight children as compared with those in studies in adults that are usually less than 1 ng/mL [12]. This difference as well as the contrary findings concerning the relationship between apelin and BMI in adults and children may be caused by age and effect of comorbidity. Apelin levels are changed in adults with heart failure [3,4,6], a disease usually not occurring in obese children. The blood vessels are one major source of apelin concentrations [3,4,6]. Therefore, it can be speculated that, in adult studies demonstrating a relationship between apelin and BMI [13,15,16], an obesity-associated heart disease (eg, as a consequence of hypertension) may be a driver of increased apelin levels. Accordingly, plasma apelin levels are not altered in obese nondiabetic and normotensive male subjects with nonalcoholic fatty liver disease [29]. However, further studies have to prove this hypothesis.

Our study also did not support a beneficial role of apelin on cardiovascular risk factors as suggested [3]. We did not find a significant relation between apelin, adiponectin, insulin, insulin resistance index HOMA, lipids, blood pressure, or waist circumference in both cross-sectional and longitudinal analyses, in accordance with some cross-sectional studies in adults [4,12-14]. In concordance, no relationship between apelin, adiponectin, BMI, lipids, and insulin sensitivity was reported in children with type 1 diabetes mellitus. These findings are in contrast to some other studies in adults demonstrating a link between apelin, triglycerides, insulin resistance, and hyperinsulinemia [4,12-16,19]. However, in contrast to our study, the analyzed adults were severely hyperinsulinemic, which might explain this difference.

Our study has a few potential limitations. First, BMI percentiles were used to classify overweight. Although BMI is a good measure for overweight, one needs to be aware of its limitation as an indirect measure of fat mass. Thus, we have also analyzed skinfold measurements and performed bioimpedance analyses to determine body fat. In addition, leptin levels were significantly higher in the obese children as compared with the normal-weight children, proving an increase of fat mass in the obese children. Second, the apelin levels in our study tended to be lower in obese children as compared with normal-weight children; and a larger study population may demonstrate significant differences. Indeed, a previous study by Tapan et al [17] reported significantly lower apelin concentration in 32 pubertal obese children as compared with pubertal normal-weight children. In the study of Tapan and colleagues [17], prepubertal obese and normal-weight children did not differ according to their apelin concentrations, in contrast to the pubertal children. This might point toward an influence of sex hormones on apelin levels similar to other adipokines such as leptin and adiponectin that are sex dependent in pubertal children [1]. We did not find an influence of early pubertal stage on apelin concentrations in our study. However, we studied only prepubertal children and early pubertal children, probably missing an effect of later pubertal stages on apelin levels. Third, the HOMA model is only an assessment of insulin resistance [29]. Clamp studies are actually the criterion standard for analyzing insulin resistance. Fourth, we were not able to differentiate the effect of diet, increased physical exercise, and weight status on apelin concentrations because of our study protocol. Finally, different test kits (measurement of all bioactive apelin forms or only in subgroups) or sources of apelin measurements (serum/plasma) may explain differences between our and previous studies. However, the ELISA used in this study had 100% cross-reactivity with human apelin-12, apelin-13, and apelin-36.

In summary, this is the first study in obese children demonstrating that overweight reduction due to lifestyle intervention was not associated with a decrease of apelin levels. Our data do not support a significant relationship between apelin, leptin, adiponectin, insulin resistance, cardiovascular risk factors, and weight status in childhood.

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