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PEDIATRIC HIGHLIGHT

The increase of fatty acid-binding protein aP2 in overweight and obese children: interactions with dietary fat and impact on measures of subclinical inflammation

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Background: In adults, circulating aP2 may link obesity, inflammation and the metabolic syndrome, but there are few data in children. Experimental models support that dietary factors, particularly dietary fat, may be major determinants of phenotype.
Objective: The aim of this study was to investigate, in normal, overweight and obese children, the relationships among aP2, the metabolic syndrome, inflammation and diet.
Design: This was a cross-sectional study conducted in Northern Switzerland.
Subjects: Subjects for this study were 6- to 14-year-old, prepubertal and early pubertal, normal weight, overweight and obese children ($n = 124$).
Main outcome measures: Body mass index (BMI), body fat percent, waist-to-hip ratio, blood pressure, circulating aP2, fasting insulin, C-reactive protein (CRP), plasma lipids and dietary intakes of macro- and micronutrients were determined.
Results: Circulating aP2 markedly increased with increasing central and total adiposity, and predicted measures of insulin resistance. Independent of BMI standard deviation scores and puberty, aP2 correlated with intake of the antioxidant vitamins A, C and E as well as circulating concentrations of CRP, leptin and low-density lipoprotein cholesterol. Children with lower aP2 concentrations consuming high-fat diets did not show an increase in fasting insulin or CRP, whereas those with higher aP2 concentrations showed marked increases in these measures with high intakes of fat or saturated fat.
Conclusions: Increased central and overall adiposity in children are associated with higher circulating aP2 concentrations. In children with high dietary intakes of total fat and saturated fat, but not those with low intakes, higher aP2 concentrations are associated with measures of insulin resistance and inflammation.
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Keywords: aP2; diet; childhood obesity; CRP; insulin resistance; fat intake; inflammation

Introduction

The adipocyte, fatty acid-binding protein aP2 (FABP4) is a ≈15 kDa protein abundantly expressed by adipocytes and also present in macrophages.[1–3] In these tissues, it is involved in fatty acid, cholesterol and phospholipid metabolism. The function of circulating aP2 is unclear, but it may link obesity, inflammation and the metabolic syndrome.[3] In adult studies, circulating aP2 concentrations correlate with insulin resistance and dyslipidemia[4–6] and predict development of the metabolic syndrome.[7] aP2 has been linked to inflammation in atherosclerotic lesions[2,8,9] and in allergic airway disease.[10]

aP2-deficient mice given normal diets have a normal phenotype, but when fed high-fat diets are protected against insulin resistance and dyslipidemia.[11,12] In apolipoprotein E-deficient mice, deletion of the *aP2* gene reduces atherosclerosis and increases survival in the face of a high-fat diet.[2] Thus, in $aP2^{-/-}$ animals, dietary factors, particularly dietary fat, are the major determinant of phenotype.

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The prevalence of childhood obesity is increasing and is closely linked to the metabolic syndrome and subclinical inflammation. There are few published data on aP2 in children and, although dietary factors are central determinants of phenotype in aP2-deficient animals, no studies have examined the relationship between dietary intakes and the effects of circulating aP2 in humans. Therefore, the aim of this study was to investigate, in normal and overweight children, the relationships among aP2, the metabolic syndrome, inflammation and diet.

Participants and methods

The participants for this study were 6- to 14-year-old children ($n=124$), living in northern Switzerland. The children were a convenience sample recruited in two steps (first step $n=72$, second step $n=52$) through letters to both primary schools and pediatricians. Data collection started in spring 2005 and was finished in fall 2007. The children recruited through pediatricians were otherwise healthy overweight and obese children not yet enrolled in a weight loss program. Data on subclinical inflammation, retinol-binding protein 4 and low-density lipoprotein (LDL) particle size from a subgroup of these children ($n=79$) have been published previously.[13–15] Informed written consent was obtained from the parents and informed oral assent from the children. Ethical approval for the study was obtained from the ethics committee of the Swiss Federal Institute of Technology in Zürich.

Dietary assessment was done using two 24-h recalls and a 1-day weighed food record in the first group of children ($n=72$) and two 24-h recalls combined with one 3-day weighed food record in the second group of children ($n=52$). All interviews were carried out by well-trained female interviewers in the family home. Each child was visited twice by the same interviewer within 3 weeks. The 24-h recalls were done at each visit and, at the first visit, the interviewer gave instructions and guidelines for the weighed food record. At the second visit, the weighed food record was carefully reviewed. Volumes and portion sizes for the 24-h recalls were estimated using photographs of different portions of a variety of foods and meals. Combining 24-h recalls and a food record provides a good overview of a child's habitual diet; this approach has been validated in children as young as 8 years of age.[16] An appointment was scheduled at the hospital clinic, at the parents' convenience, usually within 1–2 weeks of the dietary assessment.

The children presented to the hospital clinic in the morning after a 12-h overnight fast. Blood (12 ml) was taken by venipuncture. Height was measured to the nearest 0.5 cm and weight to the nearest 100 g using a digital balance (Beurer BF 18, Ulm, Germany). Pubertal staging was done by presenting drawings of the different Tanner stages to the child and the parents. Waist and hip circumferences were measured using a nonstretchable measuring tape. Skinfold thicknesses were measured at the biceps, triceps, suprailiacal and subscapular sites using a Harpenden Skinfold Caliper (HSK-BI; British Indicators, West Sussex, UK) with a constant spring pressure of $10\,g\,mm^{-2}$ and a resolution of 0.2 mm. After a 15-min rest, resting blood pressure was measured manually by auscultation with the children sitting in the examination chair.

Data analysis

Dietary data obtained from the three records were thoroughly checked and entered into a nutrition software system (EBISpro for Windows 4.0, Dr J Erhardt, University of Hohenheim, Germany). This system translates the amount of food eaten into individual nutrients and assigns consumed foods into 22 food groups. The program is based on the German Food and Nutrition Data Base BLS 2.3 (Federal Health Department, Berlin, Germany), and, for foods specific to Switzerland, incorporates values from the Swiss Food Composition Database.[17] Energy and nutrient data were averaged across the 3 or 5 days to obtain a mean daily energy and nutrient intake for each child. The reference values for nutrient intake for Germany, Austria and Switzerland, the D-A-CH references,[18] were used for comparison of the actual intake to the recommendations for the respective age groups.

Body mass index (BMI) of the children was calculated as weight (kg)/height $(m)^2$. To scale the data for comparison across ages and sex, BMI standard deviation scores (BMI-SDS: individual BMI value−reference mean BMI value/SD) were calculated using the program Epi Info (version 3.4.1) and the reference values proposed by the US Centers for Disease Control and Prevention (CDC) in 2000. Age and gender specific criteria also from CDC[19] were used to classify children as normal weight, overweight (between the 85th and the 95th percentile) or obese (above the 95th percentile). These criteria have been previously validated in Swiss children at this age.[20] Body density (D) and body fat percentage (%BF) were calculated according to the following equations by Deurenberg *et al*.[21] using the four measured skinfold thicknesses:

$$D\,(boys)(g/ml) = 1.1690 - 0.0788 \cdot log(sum\,of\,four\,SFT)$$

$$D\,(girls)(g/ml) = 1.2063 - 0.0999 \cdot log(sum\,of\,four\,SFT)$$

$$body\,fat\,(\%) = \left(\frac{562 - 4.2 \cdot (age - 2)}{body\,density}\right) - (525 - 4.7 \cdot (age - 2))$$

Laboratory analysis

Glucose was measured immediately using reflection photometry (Reflotron Sprint, Roche, Switzerland). On fresh serum high-density lipoprotein (HDL) and LDL cholesterol and triglycerides were measured on Hitachi 917 (Triglyceride-GPO-PAP and HDL-C/LDL-C plus 2nd generation; Roche). Serum was stored at $-20\,^{\circ}C$ for later determination of aP2

(Human Adipocyte FABP ELISA; BioVendor GmbH, Heidelberg, Germany), insulin (radioimmunoassay; Schering (Schweiz) AG, Baar, Switzerland), high-sensitivity C-reactive protein (CRP; chemiluminescent immunometry, IMMULITE; Bühlmann Laboratories AG, Switzerland), Interleukin 6 (IL-6) (high-sensitivity enzyme-linked immunosorbent assay (ELISA), Quantikine HS Human IL-6 Immunoassay; R&D Systems, MN, USA), Leptin (Leptin (human) ELISA Kit, BioVender, Alexis Biochemicals, Lausen, Switzerland) and free fatty acids (FFAs, NEFA C Assay Kit; Wako Chemicals, Neuss, Germany). IL-6, leptin and FFAs were done on a subgroup of 72 children. For all children, the quantitative insulin sensitivity check index (QUICKI) was calculated as follows: QUICKI = 1/(log(fasting insulin mU/l) + log(fasting glucose mg per 100 ml)).[22]

Statistical analysis

Statistical analysis was performed using the statistical package SPSS 13.0 for Windows (SPSS, Chicago, IL, USA). Nonnormally distributed variables were log-transformed for comparisons. Normally distributed data are presented as means ± s.d. and nonnormally distributed data as medians (range). One-way analysis of variance (ANOVA) with a *post hoc* Bonferroni test was used to compare means. Multiple regression models were used to study the effect of nutrients, metabolic and personal covariates on metabolic variables, focusing on aP2. All equations were checked for confounding factors like adiposity, age, puberty and gender and they were added as covariates if they were significant predictors.

Results

In Table 1 the anthropometric and metabolic data of the children are shown by weight classification. aP2 concentrations were significantly higher in overweight and obese children, as well as in those children with greater waist-to-hip ratio (W/H ratio) (Figure 1). There were highly significant correlations between aP2 and all three measures of adiposity in univariate correlations (BMI-SDS $r = 0.669$, $P < 0.001$; W/H ratio $r = 0.499$, $P = < 0.001$ and %BF $r = 0.750$, $P = < 0.001$) as well as after controlling for age, gender and puberty (BMI-SDS (controlled for puberty only) $\beta = 0.629$, $P < 0.001$; W/H ratio $\beta = 0.532$, $P < 0.001$ and %BF $\beta = 0.752$, $P < 0.001$).

Dietary intake data of the three groups of children by weight classification are listed in Table 2. Although there were no significant differences in energy intake by weight classification, there were significant differences between groups in intakes of saturated fatty acids (SFAs), percentage energy as protein, meat products and total vitamin A ($P < 0.05$).

In univariate correlations, aP2 was associated with pubertal stage ($r = 0.325$, $P < 0.001$) and multiple components of the

Table 1 Anthropometric and metabolic data of 6- to 14-year-old Swiss children by weight classification

	Normal weight[1]	*Overweight*[1]	*Obese*[1]
n	38	36	50
Age (years)	10.35 ± 1.89[2,a]	10.70 ± 1.75[a]	9.93 ± 1.84[a]
Gender ratio (M/F)	15/23	19/17	25/25
BMI (kg/m^2)	16.6 ± 2.0[a]	21.8 ± 2.2[b]	25.7 ± 3.9[c]
BMI-SDS	−0.39 ± 1.01[a]	1.35 ± 0.20[b]	2.05 ± 0.26[c]
Body fat (%)	19.9 ± 5.0[a]	32.7 ± 6.1[b]	38.5 ± 5.8[c]
Waist-to-hip ratio	0.80 ± 0.04[a]	0.83 ± 0.06[b]	0.89 ± 0.05[c]
Tanner stage (no. of children)			
Stage I	21	12	22
Stage II	13	16	19
Stage III	2	2	5
Stage IV	2	6	4
aP2 (ng ml^{-1})	8.12 (4.63–28.41)[3,a]	15.60 (6.43–64.08)[b]	20.49 (7.80–52.66)[c]
Fasting insulin (pmol l^{-1})	82.5 (25.0–451.0)[a]	110.0 (52.0–230.0)[b]	161.0 (68.0–778.0)[c]
QUICKI	0.34 ± 0.02[a]	0.32 ± 0.02[b]	0.30 ± 0.02[c]
CRP (mg/100 ml)	0.03 (0.01–0.42)[a]	0.09 (0.03–0.99)[b]	0.15 (0.03–2.25)[c]
Triglycerides (mmol l^{-1})	0.5 (0.3–2.1)[a]	0.8 (0.3–1.5)[b]	0.9 (0.3–2.2)[c]
HDL cholesterol (mmol l^{-1})	1.59 ± 0.25[a]	1.51 ± 0.30[a,b]	1.40 ± 0.34[b]
LDL cholesterol (mmol l^{-1})	2.38 ± 0.58[a]	2.83 ± 0.68[b]	2.75 ± 0.77[b]
Free fatty acids (imol l^{-1})[4]	754.0 ± 265.6[a]	686.8 ± 226.7[a]	644.2 ± 301.0[a]
IL-6 (pg ml^{-1})[4]	0.3 (0.1–1.8)[a]	0.4 (0.1–2.0)[a,b]	0.8 (0.1–2.1)[b]
Leptin (ng ml^{-1})[4]	1.88 (0.11–15.52)[a]	9.79 (1.78–62.87)[b]	22.38 (8.98–62.12)[c]
Systolic blood pressure (mm Hg)	98.1 ± 9.0[a]	105.4 ± 12.2[b]	110.8 ± 12.6[b]
Diastolic blood pressure (mm Hg)	64.6 ± 8.4[a]	64.4 ± 12.2[a]	70.4 ± 8.6[b]

Abbreviations: BMI, body mass index; CRP, C-reactive protein; HDL, high-density lipoprotein; IL, Interleukin; LDL, low-density lipoprotein; QUICKI, quantitative insulin sensitivity check index; SDS, standard deviation scores. Means not sharing a common superscript letter are significantly different from each other at $P < 0.05$ (ANOVA with *post hoc* Bonferroni correction). [1]Normal weight, overweight and obesity were defined according to the age- and sex-specific criteria defined by Ogden *et al.*[19] [2]Mean ± s.d. (all such values). [3]Median (range) all such values. [4]Parameters only measured in 72 children (29 NW, 17 OW, 26 OB).

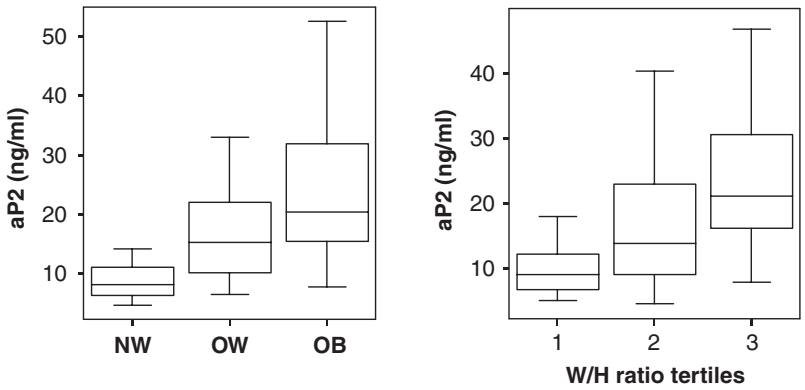

Figure 1 Circulating aP2 concentrations by weight classification and by tertiles of waist-to-hip ratio (W/H) ratio in 6- to 14-year-old children. For both, differences between all groups are significant ($P<0.05$). Normal weight (NW) $n=38$, overweight (OW) $n=36$, obese (OB) $n=50$.

Table 2 Dietary intake of 6- to 14-year-old Swiss children by weight classification

	Normal weight	*Overweight*	*Obese*
n	38	36	50
Energy (kcal)	1842 ± 444[1]	1773 ± 227	1809 ± 374
Total fat (g)	76.1 ± 20.7	69.8 ± 11.6	72.9 ± 19.4
Energy as fat (%)	36.4 ± 4.7	34.7 ± 4.1	35.5 ± 4.1
SFA (g)	31.8 ± 9.6	27.9 ± 6.5	28.2 ± 7.9
Energy as SFA (%)	15.3 ± 2.8[a]	14.0 ± 2.4[a,b]	13.9 ± 2.5[b]
PUFA (g)	9.8 ± 3.5	10.1 ± 4.7	10.6 ± 5.7
Energy as PUFA (%)	4.7 ± 1.2	5.1 ± 1.4	5.2 ± 2.0
MUFA (g)	27.2 ± 8.0	24.8 ± 4.8	27.4 ± 8.8
Energy as MUFA (%)	13.1 ± 2.3	12.5 ± 2.0	13.5 ± 3.5
Total carbohydrates (g)	228.9 ± 62.9	223.0 ± 38.0	222.6 ± 55.6
Energy as carbohydrates (%)	50.1 ± 4.9	50.8 ± 4.0	49.8 ± 6.1
Glucose (g)	12.1 ± 5.7	11.9 ± 5.9	9.8 ± 4.3
Fructose (g)	15.6 ± 7.6	16.0 ± 8.5	12.4 ± 8.1
Total protein (g)	59.9 ± 13.2	62.8 ± 9.0	65.5 ± 13.7
Energy as protein (%)	13.3 ± 2.1[a]	14.5 ± 2.2[a,b]	14.8 ± 2.3[b]
Vitamin C (mg)	102.1 ± 49.0	99.2 ± 49.1	92.6 ± 51.0
Vitamin E (mg)	4.5 ± 3.3	6.5 ± 4.1	5.8 ± 4.1
Total vitamin A (mg RAE)	0.82 ± 0.31[a]	0.65 ± 0.23[b]	0.62 ± 0.30[b]
Meatproducts (g)	63.0 ± 41.4[a]	82.3 ± 41.4[a,b]	94.8 ± 47.4[b]
Dairy products (g)	306.6 ± 154.3	268.3 ± 136.1	255.4 ± 156.3
Fruits and vegetables (g)	381.5 ± 182.0	391.2 ± 193.2	321.9 ± 202.9

Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; RAE, retinol activity equivalents; SFA, saturated fatty acids. Means not sharing a common superscript letter are significantly different from each other at $P<0.05$ based on ANOVA and *post hoc* Bonferroni correction. [1]Mean ± s.d. (all such values).

metabolic syndrome: fasting insulin ($r=0.444$, $P<0.001$), QUICKI ($r=-0.436$, $P<0.001$), systolic blood pressure (SBP, $r=0.354$, $P<0.001$), diastolic blood pressure ($r=0.266$, $P=0.005$), triglycerides ($r=0.380$, $P<0.001$), HDL cholesterol ($r=-0.170$, $P=0.059$) and LDL cholesterol ($r=0.338$, $P<0.001$). It was also associated with inflammatory markers (CRP ($r=0.413$, $P<0.001$), IL-6 ($r=0.352$, $P=0.002$), leptin ($r=0.753$, $P<0.001$)). Although there was no association between FFA and aP2 concentrations, higher FFAs were correlated with two measures of insulin sensitivity: FFAs were directly correlated with QUICKI ($r=0.311$, $P=0.008$) and indirectly correlated with fasting insulin ($r=-0.282$, $P=0.016$). Tables 3 and 4 show the univariate and multivariate (controlled for BMI-SDS and puberty) associations between aP2 and components of the metabolic syndrome and inflammatory markers. After controlling for adiposity and puberty, aP2 remained significantly associated with LDL cholesterol, CRP and leptin.

Circulating aP2 correlated with several dietary factors: glucose ($r=-0.196$, $P=0.030$), fructose ($r=-0.184$, $P=0.041$), fruits and vegetables ($r=-0.183$, $P=0.042$), vitamin C ($r=-0.215$, $P=0.017$), vitamin A ($r=-0.271$, $P=0.002$) and vitamin E ($r=0.439$, $P<0.001$). After controlling for BMI-SDS and pubertal stage, the antioxidant vitamins A ($\beta=-0.137$, $P=0.040$), C ($\beta=-0.156$, $P=0.017$) and E ($\beta=0.249$, $P<0.001$) remained associated with aP2.

The interactions among aP2, dietary fat and saturated fat intake and measures of insulin resistance (fasting insulin) and inflammation (CRP) are shown in Figure 2. In children with low circulating aP2 concentrations, there was no significant increase in fasting insulin or CRP at higher intakes of fat and saturated fat. In contrast, in children with higher circulating aP2, fasting insulin and CRP increased sharply with increasing fat or saturated fat intake. The tertiles of aP2 as displayed in Figure 2 were (median (range)) 7.69 (4.63–10.64), 14.29 (10.79–20.45) and 27.54 ng ml^{-1} (20.53–64.08 ng ml^{-1}). For the intake of fat in percentage of energy the tertiles were 31 (25–33), 36 (33–37) and 40% (37–53%) and for the intake of saturated fat as percentage of energy they were 11.8 (8.0–13.3), 14.4 (13.3–15.4) and 16.6% (15.4–22.3%). For example, Figure 2b shows that children in the lower tertile for aP2 show a small nonsignificant decrease in fasting insulin concentrations at higher saturated fat intakes; in contrast, those in the middle and upper tertiles for aP2 show a 60–150% increase in fasting insulin at higher intakes. This interaction between fat intake, aP2 and inflammation was also evident in regression analysis: aP2 was a significantly associated with CRP ($r=0.413$) and adding dietary fat or saturated fat to the regression significantly improved the model (percentage energy as

Table 3 Univariate and multivariate associations between aP2 and components of the metabolic syndrome in 6- to 14-year-old children ($n=124$)

	Fasting insulin		*QUICKI*		*Triglycerides*		*LDL cholesterol*		*Systolic blood pressure*		*Diastolic blood pressure*	
	P	r[a]	P	r	P	r	P	r	P	r	P	r
Univariate	<0.001	0.444	<0.001	−0.436	<0.001	0.380	<0.001	0.338	<0.001	0.354	0.005	0.266
	P	β[b]	P	β	P	β	P	β	*P*	β	*P*	β
Multivariate[c]	0.617	0.043	0.856	-0.016	0.242	0.088	0.007	0.182	0.799	0.020	0.385	0.062

Abbreviations: LDL, low-density lipoprotein; QUICKI, quantitative insulin sensitivity check index. [a]Pearson's correlation coefficient. [b]Standardized coefficient β. [c]Multivariate regression models with log aP2 as the dependent variable and controlled for BMI-SDS and pubertal stage.

Table 4 Univariate and multivariate associations between aP2 and inflammatory parameters in 6- to 14-year-old children ($n=124$)

	CRP		*IL-6*		*Leptin*	
	P	r[a]	P	r	P	r
Univariate	<0.001	0.413	0.002	0.352	<0.001	0.753
	P	β[b]	P	β	P	β
Multivariate[c]	0.023	0.179	0.431	0.077	<0.001	0.671

Abbreviations: CRP, C-reactive protein; IL, Interleukin. [a]Pearson's correlation coefficient. [b]Standardized coefficient β. [c]Multivariate regression models with log aP2 as the dependent variable and controlled for BMI-SDS and pubertal stage.

fat: $P=0.001$, $\beta=0.281$, $r=0.500$; percentage energy as SFA: $P=0.049$, $\beta=0.171$, $r=0.447$), as did addition of vitamins A and C ($r=0.443$).

Discussion

Our findings demonstrate circulating aP2 concentrations are markedly elevated in overweight and obese 6- to 14-year-old children. BMI, %BF and W/H ratio, independent of age, gender or puberty, were all positively associated with higher aP2 concentrations, suggesting both total and central adiposity are linked to circulating aP2. Higher aP2 concentrations were also significantly correlated with most other components of the metabolic syndrome, including higher blood pressure, higher triglycerides, measures of insulin resistance, as well as multiple markers of subclinical inflammation.

These findings are generally consistent with findings of previous adult studies. In adults with type 2 diabetes, aP2 was positively correlated with adiposity, SBP, CRP, triglycerides and diabetes duration, but not with circulating insulin or glucose.[6] In adults with the metabolic syndrome, aP2 serum level correlated positively with fasting levels of insulin, glucose, triglycerides, BMI and waist circumference, but negatively with HDL cholesterol and QUICKI.[4] Women who carry a functional genetic variant of the *aP2* gene that reduces adipose tissue *aP2* gene expression have a reduced risk for type 2 diabetes and coronary artery disease.[23] In a 5-yr prospective study in Chinese adults, controlling for insulin resistance and BMI, baseline aP2 was a highly significant independent predictor of the development of the metabolic syndrome.[7]

Our findings differ from those of a previous study in 30 obese German children,[24] where aP2 correlated significantly to BMI-SDS and leptin, but not age, sex, pubertal stage, lipids, blood pressure, fasting glucose, insulin or CRP and tumor-necrosis factor-α. These differences may be explained in part by differences in adiposity (our sample included both normal and overweight children), age and pubertal development (our sample was slightly younger and mainly prepubertal), or may be due to the small sample size and a possible β-error in the study by Reinehr *et al*.[24]

aP2 in macrophages have been suggested to mediate the inflammation of atherosclerosis and allergic airway disease.[2,8–10] This effect may be due to regulation of PPARγ and nuclear factor (NF)-κB in macrophages[25,26] and/or through binding to and reducing turnover of long-chain fatty acids, such as arachidonic acid and leukotriene A_4.[27,28] In adults with type 2 diabetes, aP2 is directly correlated with CRP concentrations.[6] The present study is the first to link aP2 to inflammation in children: independent of adiposity, aP2 concentration was significantly correlated with CRP, a downstream marker of subclinical inflammation. It was also associated with circulating levels of the proinflammatory adipokines leptin and IL-6. Although none of the children reported allergic airway disease, obese children have higher risk of asthma than normal weight children; this effect could be due to differences in aP2-mediated inflammation.[10]

Variations in dietary fat are the major determinant of phenotype in aP2-deficient animals.[2,11] aP2-deficient mice eating normal rat chow grow and develop normally.[11] However, when fed a high-fat Western diet that is diabetogenic and atherogenic in control animals, they have lower cholesterol and triglyceride levels, and are protected against insulin resistance and atherosclerosis.[2,11] The present data show a remarkably similar pattern. In children with lower circulating aP2 levels, higher intakes of saturated and total fat were not associated with fasting insulin or CRP (Figure 2). In contrast, children with higher aP2 concentrations display markedly elevated insulin and CRP concentrations at higher fat intakes. Human studies also suggest genetic factors are involved in determining aP2 phenotype and that individuals

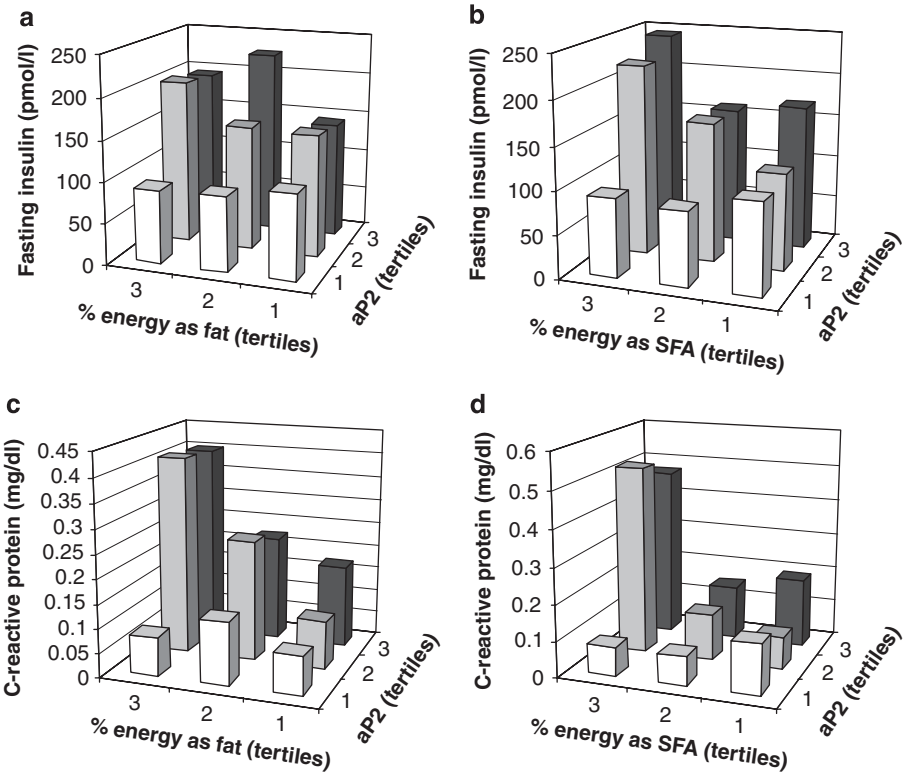

Figure 2 The interactions of circulating aP2 concentrations and dietary intake of total fat (**a**, **c**) and saturated fat (SFA) (**b**, **d**) on concentrations of fasting insulin (**a**, **b**) and C-reactive protein (**c**, **d**) in 6- to 14-year-old normal, overweight and obese children ($n = 124$). Data are presented as tertiles for aP2 and dietary fat intakes.

expressing low levels of aP2 are to some degree protected from the development of insulin resistance and coronary heart disease.[23]

Consumption of high amounts of fat increases the pool of circulating FFAs. Increased circulating FFAs in obesity are thought to contribute to insulin resistance and inflammation.[29–31] Paradoxically, *aP2*$^{-/-}$ mouse models are more insulin sensitive despite higher levels of circulating FFAs.[11,32,33] Similarly, in our data higher FFAs were associated with two measures of insulin sensitivity (QUICKI and fasting insulin), but there was no correlation between circulating FFA and aP2 concentrations. Although circulating FFA concentrations rise after the ingestion of meals high in fat or rapidly digestible starches,[34,35] in our data, we found no correlations of fasting FFA concentrations and any measured dietary factors, including total fat or carbohydrate type. However, such an effect might be visible postprandially.

Dietary intakes of the antioxidant vitamins A and C were negatively correlated with aP2, after controlling for adiposity and puberty. Expression of aP2 in cholesterol-loaded THP-1 macrophages is stimulated by oxidized LDL, an effect inhibited by treatment of the cells with an antioxidant inhibitor of NF-κB.[36,37] Dietary antioxidants can reduce oxidation of LDL cholesterol,[38] and this mechanism may explain why circulating aP2 was lower in children with higher intakes of vitamins A and C. The present data also suggest an interaction between antioxidant vitamin intake, aP2 and inflammation. In regression analysis, aP2 was significantly positively associated with CRP and the model including vitamins A and C suggested that when aP2 concentrations are high, increasing vitamin A and C intakes is associated with lower CRP concentrations.

We found no differences in mean energy intakes by weight classification, despite the fact that if energy requirements were based only on body size, we would have expected higher energy intakes in the overweight and obese children to maintain their body weight. Similar findings have also been reported in previous studies[39,40] and could be due to several factors. It is possible that the results were confounded by underreporting, which may be more common in overweight and obese individuals.[41] However, we tried to minimize significant underreporting by excluding nine children who reported energy intakes less than their resting metabolic rate multiplied by a minimal physical activity level of 1.2.[42] Alternatively, the overweight and obese children may have been limiting their energy intakes in an attempt to control their weight. Another explanation may be that physical activity levels were higher in the normal weight children, as has been previously shown in Swiss children at this age[43] and this may result in higher energy requirements to maintain body weight.

In summary, increased central and overall adiposity in 6- to 14-year-old children were associated with higher circulating aP2 concentrations. In children with high dietary intakes of total and saturated fat, but not those with low intakes, higher aP2 concentrations were associated with measures of insulin resistance and inflammation. Because childhood obesity increases risk for diabetes and atherosclerosis in late adolescence and adulthood, our findings argue that weight loss interventions at this age, particularly in children with higher aP2 concentrations, should emphasize reductions in dietary intakes of total fat and saturated fat.

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