CLINICAL STUDY

Acylation stimulating protein but not complement C3 associates with metabolic syndrome components in Chinese children and adolescents

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Abstract

Objective: Childhood obesity is increasing worldwide and is increasingly associated with metabolic syndrome (MetS). Our aim was to examine acylation stimulating protein (ASP) and its precursor complement C3, in normal, overweight, and obese Chinese children and adolescents, and the relationships with body size, blood parameters, pubertal development, family environment, and MetS. **Methods:** Children and adolescents ($n=1603$) from 6 to 18 years, boys ($n=873$) and girls ($n=730$), including normal weight ($n=603$), overweight ($n=291$) and obese ($n=709$) were assessed for body size parameters, pubertal development, blood lipids, glucose, insulin, ASP, and C3.

Results: ASP levels were increased in overweight and obese versus normal weight ($P<0.001$), while C3 showed little variation. This effect of overweight/obesity remained throughout early stages when boys and girls were separated by pubertal development or age, although age and pubertal status itself had no effect. Separation based on ASP quintiles demonstrated significant associations with blood cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-Chol), glucose, insulin, and homeostatic model assessment of insulin resistance in boys, and LDL-Chol, high-density lipoprotein cholesterol, and glucose in girls. A positive correlation with mother's body mass index in boys and girls ($P=0.002$ and $P=0.014$ respectively) as well as birth weight ($P<0.001$) was noted. MetS was strongly associated with increased ASP, the presence of a single MetS factor (especially hypertension, central obesity, or hyperglycemia) was associated with increased ASP.

Conclusion: Changes in the plasma adipokine ASP in early obesity are associated with blood lipid and glucose modifications, family environment, and distinct MetS risk factors.

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Introduction

Childhood obesity is increasing worldwide. Presently in the USA, 10.4% of children 2–5 years of age are overweight (1). Even in countries with traditionally low prevalence of obesity, the ratios are rising (2). A recent study in China, documented the changing rates of obesity over the last 20 years. The obesity prevalence of 0.2% for boys, and 0.1% for girls in 1985 rose to 6–8% for boys and 2–6% for girls in 1995, and is presently at a level of 12.9% for boys and 9.1% for girls (2, 3). Those numbers are even higher if the overweight subset of children is incorporated into the statistics. Overweight children are more likely to become overweight adolescents; 2.8 times more likely in one study on Chinese children (4). The numbers are worrisome, as childhood obesity is associated with

chronic disease risk factors such as high blood pressure, hyperlipidemia, and hyperinsulinemia (5). The incidence of type 2 diabetes, even in children and adolescents is increasing as well (6, 7). To develop effective prevention strategies, it is important to understand the causes and consequences of weight gain. Unfortunately the mechanisms that link adipose tissue with dyslipidemia and glucose dysfunction are still incompletely understood.

Adipose tissue, once considered to be a metabolically inert storage site, is now well recognized as an active endocrine tissue (review (8, 9)). Recent investigations have yielded a whole host of factors released from adipose tissue (collectively known as adipokines) that regulate a number of physiological processes including carbohydrate and fat metabolism (8). Many of these factors are being examined for their potential links to obesity and other

complications in adults such as diabetes and cardiovascular disease. The aim of the present study was to examine adipose secreted factors that are involved in fat metabolism, acylation stimulating protein (ASP; review (10)) and its precursor complement C3 in overweight, obese, and normal weight children and adolescents from 6 to 18 years old and the corresponding associations with body size, blood parameters, and environmental factors. These adipokines have been shown to be increased in very young obese children aged 2–6 years old, even in the absence of changes in blood lipids (11).

ASP (aka C3adesArg) is produced through cleavage of its precursor complement C3 by interaction with adipisin and factor B. All three factors are made by adipocytes in a differentiation-dependent manner that results in an increased production of ASP although the proportion of C3 which is converted to ASP is small ($< 1\%$) (10). ASP, in turn, increases triglyceride (TG) synthesis as well as glucose transport in adipocytes, therefore increasing overall fat storage. Both ASP and C3 have been shown in adults, in a number of studies, to be associated with obesity, diabetes, and cardiovascular disease (10).

Therefore, in the present study, our aim was to evaluate ASP and C3 in association with anthropometric variables, blood lipids and glucose parameters, pubertal development and family environment in normal weight, overweight and obese children, and adolescents. Furthermore, the impact of metabolic syndrome (MetS) factors on ASP and C3 was examined.

Methods

Subjects

Subjects were recruited from a cross-sectional population-based survey: the Beijing Child and Adolescent MetS (BCAMS) study (12). This study evaluated the presence of obesity and related metabolic abnormalities (hypertension, central obesity, type 2 diabetes, and dyslipidemia) among a representative sample that consisted of 19 593 Beijing school children aged 6–18 years, chosen with a stratified (urban versus rural) randomly clustered (with school as the cluster unit) sampling method evaluated between April and October 2004 in schools in Beijing (12). The cohort included boys and girls aged 6–18 years. Evaluation included physical examination, blood pressure, fasting finger capillary blood test for glucose, total cholesterol (TC), and TG. Physical examination included body mass index (BMI), waist circumference, fat mass percentage by bioimpedance analysis, systolic, and diastolic blood pressure. Pubertal development was assessed by Tanner stage of breast development (girls) and testicular volume (boys) (13). Data including birth weight (reported by parents), family history, and age at menarche were collected by questionnaire.

Within this large group of children and adolescents, 4500 were identified with risk factors defined as the presence of any one of the following: overweight based on BMI cutoffs (14), increased cholesterol (≥ 5.2 mM), TG (≥ 1.7 mM), or glucose (≥ 5.6 mM). In the absence of available guidelines for Chinese children, cut-offs for cholesterol and TG were used for screening based on the Guidelines for Prevention and Treatment of Dyslipidemia in Chinese Adults (15). The cutoff for fasting capillary blood glucose (≥ 5.6 mM) was used for screening based on the study by Borthey *et al.* (16) that was also cited by the WHO report issued in 2003. A parallel reference population of 1045 school-age children was also identified. Within these two groups, 2544 (BCAMS) and 981 (Reference) children/adolescents were recruited for blood samples. Within this total group, the cohort analyzed included 1603 children, boys (54.5%, $n = 873$) and girls (45.5%, $n = 730$), of normal weight ($n = 603$), overweight ($n = 291$), and obese ($n = 709$) as defined by age- and gender-specific BMI cutoffs (recommended by the Working Group on Obesity in China (14, 17). Signed informed consent was obtained from all participants and/or their parents or guardians through all the study processes. The BCAMS study was approved by the Ethics Committee at Capital Institute of Pediatrics.

A positive risk for pediatric metabolic abnormalities was defined by the presence of three or more of the following five components (18): i) central obesity defined as ≥ 90 th percentile for age and gender (established based on the BCAMS study), ii) elevated systolic and/or diastolic blood pressure > 90 th percentile for age, sex, and height (according to the BCAMS study), iii) hypertriglyceridemia defined as TG ≥ 1.24 mM, equal to the 90th percentile of the reference population, iv) low serum HDL cholesterol defined as ≤ 1.03 mM, equal to 5th percentile of the reference population, and v) impaired fasting glucose defined as ≥ 5.6 mM.

Fasting blood samples

Venous blood samples were collected by direct venipuncture after an overnight (minimum 12 h) fast. The samples were centrifuged, serum and plasma aliquoted and immediately frozen for future analysis of lipids and hormones.

Analytical procedures

Samples were analyzed for concentrations of blood glucose, insulin, nonesterified fatty acids (NEFAs), complement C3, ASP, TGs, TC, high-density lipoprotein cholesterol (HDL-Chol) and low-density lipoprotein cholesterol (LDL-Chol). Plasma glucose was determined by the glucose oxidase method. Serum TC and TG concentrations were determined using standard enzymatic methods. HDL-Chol and LDL-Chol were measured directly. The serum lipid levels and plasma glucose were assayed using the Hitachi 7060C automatic

Table 1 Clinical data in boys and girls.

N=	Boys (n=873)				Girls (n=730)			
	Normal 283	Overweight 124	Obese 466	ANOVA P value	Normal 320	Overweight 167	Obese 243	ANOVA P value
Age (years)	11.6±0.1	13.4±0.2 [‡]	11.6±0.1	<0.0001	11.9±0.1	12.6±0.2 [*]	11.1±0.1 [†]	<0.0001
BMI (kg/m ²)	17.6±0.1	24.0±0.2 [‡]	27.1±0.1 [‡]	<0.0001	17.9±0.1	23.2±0.1 [‡]	26.0±0.2 [‡]	<0.0001
%BF	15.6±0.2	25.8±0.4 [‡]	28.4±0.2 [‡]	<0.0001	19.2±0.3	30.3±0.4 [‡]	33.9±0.5 [‡]	<0.0001
Waist (cm)	63.0±0.4	79.7±0.7 [‡]	85.8±0.5 [‡]	<0.0001	61.8±0.3	73.5±0.4 [‡]	79.7±0.6 [‡]	<0.0001
WHR (cm/cm)	0.417±0.001	0.492±0.002 [‡]	0.549±0.002 [‡]	<0.0001	0.415±0.001	0.479±0.002 [‡]	0.534±0.003 [‡]	<0.0001
Systolic BP (mmHg)	102.7±0.8	119.0±1.1 [‡]	117.8±0.5 [‡]	<0.0001	101.4±0.7	108.9±0.8 [‡]	112.1±0.7 [‡]	<0.0001
Diastolic BP (mmHg)	64.8±0.6	74.0±0.8 [‡]	73.0±0.4 [‡]	<0.0001	64.5±0.6	69.3±0.6 [‡]	72.0±0.5 [‡]	<0.0001

All values are reported as mean ± S.E.M. %BF, % fat mass; BMI, body mass index (kg/m²); BP, blood pressure; WHtR, waist to height ratio. Significance was calculated by ANOVA where differences versus normal weight group are indicated as **P*<0.05, [†]*P*<0.01, and [‡]*P*<0.001.

biochemistry analysis system. Plasma NEFA was determined by colorimetric enzymatic assay (Wako Chemicals, Tokyo, Japan). Plasma complement C3 concentration was determined by turbidimetric assay using a polyclonal anti-human antibody specific against complement C3 (Lin-Fei Co., Wuhan P R China). Plasma ASP concentration was measured using a sandwich ELISA method as previously described in detail (19). For these last assays, (complement C3 and ASP) intra-assay coefficient of variations were <4% and interassay coefficient of variations were <8%. Plasma insulin was detected by sensitive and specific double-antibody sandwich ELISAs with intra- and inter-assay coefficient of variations <10% that was developed in the Laboratory of Peking Union Hospital.

Calculations and statistical analysis

BMI was calculated as weight divided by height (kg/m²). Insulin resistance index was calculated by homeostasis

model assessment of insulin resistance (HOMA-IR) as (fasting insulin UI/l) × (fasting glucose mmol/l) / 22.5 (20–22). Unless and otherwise stated, all results are displayed as mean ± S.E.M. ANOVA analyses were used to compare means among the groups. Correlations between two parameters were calculated using Pearson correlation coefficient. For contribution of multiple independent variables on one dependent variable, multiple regressions using forward stepwise regression analysis was used. Parameters not normally distributed were log transformed for analysis. A *P*<0.05 was considered statistically significant for all analyses.

Results

The BCAMS cross-sectional population-based study evaluated obesity status and related metabolic abnormalities (hypertension, central obesity, impaired glucose, and dyslipidemia) among a representative sample

Table 2 Lipid and glucose clinical parameters in boys and girls.

N=	Boys (n=873)				Girls (n=730)			
	Normal 283	Overweight 124	Obese 466	ANOVA P value	Normal 320	Overweight 167	Obese 243	ANOVA P value
Glucose (mmol/l)	5.22±0.05	5.35±0.04	5.26±0.02	NS	5.09±0.04	5.28±0.10	5.19±0.03	NS
Insulin (mU/l)	6.59±0.27	13.77±0.89 [‡]	16.31±0.77 [‡]	<0.0001	7.95±0.35	12.14±0.60 [‡]	15.96±0.93 [‡]	<0.0001
HOMA-IR	1.55±0.06	3.36±0.25 [‡]	3.95±0.21 [‡]	<0.0001	1.88±0.10	2.82±0.13 [‡]	3.79±0.25 [‡]	<0.0001
Total Chol (mmol/l)	4.00±0.05	3.97±0.07	4.07±0.03	NS	4.16±0.04	4.07±0.06	4.08±0.04	NS
TG (mmol/l)	0.81±0.02	1.33±0.05 [‡]	1.24±0.03 [‡]	<0.0001	1.01±0.02	1.11±0.04	1.30±0.04 [‡]	<0.0001
LDL-Chol (mmol/l)	2.38±0.04	2.51±0.06	2.63±0.03 [‡]	0.00003	2.53±0.03	2.64±0.05	2.62±0.04	NS
HDL-Chol (mmol/l)	1.51±0.02	1.20±0.02 [‡]	1.23±0.01 [‡]	<0.0001	1.49±0.01	1.26±0.02 [‡]	1.22±0.01 [‡]	<0.0001
NEFA (mmol/l)	0.67±0.02	0.62±0.02	0.68±0.01	NS	0.69±0.01	0.64±0.02	0.69±0.01	NS

All values are reported as mean ± S.E.M. Chol, Cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; NEFA, nonesterified fatty acid; TG, triglyceride. Significance was calculated by ANOVA where differences versus normal weight group are indicated as **P*<0.05, [†]*P*<0.01, [‡]*P*<0.001, and NS, not significant.

Figure 1 Plasma acylation stimulating protein and complement C3 in boys and girls. Plasma ASP (A) complement C3 (B) and the molar ratio of ASP to C3 (C) were measured in normal, overweight and obese boys (open bars) and girls (hatched bars). Results are expressed as mean $\pm$ S.E.M. Significance was calculated by ANOVA where differences versus normal weight group are indicated as * $P < 0.05$, ** $P < 0.001$.

($n = 19\,593$) of school children evaluated in 2004 in schools in Beijing, P R China. The age range was from 6 to 18 years and included both girls and boys. In the present study, we examined data from 1603 children and adolescent boys and girls (6–18 years). Obesity was defined based on Centre for Disease Control, US (CDC) BMI standards for 6-year-old children (23), and Chinese

BMI standards established for 7–18-year-old children and adolescents (14, 17), resulting in 603 normal weight, 291 overweight, and 709 obese girls and boys. The clinical and laboratory data of the 1603 subjects distributed according to their gender and body size are shown in Table 1. There was a significant difference in the average age between the normal, overweight, and obese groups for both boys and girls. Consistent with the BMI classification, % body fat (%BF), waist circumference and waist/height ratio were also significantly different between the different groups in boys and girls (Table 1). As shown in Table 1, systolic and diastolic blood pressures were significantly increased in the overweight and obese girls and boys (both $P < 0.0001$).

Glucose homeostasis and serum lipid profiles are shown in Table 2 for the boys and girls. Although no significant change was observed in fasting blood glucose levels, insulin and insulin-resistance index (HOMA-IR) were significantly increased in overweight and obese groups in both genders ($P < 0.0001$ by ANOVA). There was no difference in TC between groups, but significant differences in LDL-Chol (boys only) and in HDL-Chol (boys and girls) were detected. Similarly, although there was no difference in NEFAs levels, there were significant increases in serum TG in overweight and obese boys and girls.

Results for ASP, complement C3 and the % ASP/C3 ratio are shown in Fig. 1. There were significant increases in ASP ($P < 0.001$) in overweight and obese groups, both boys and girls, versus normal weight group. A comparison of ASP levels in boys versus girls indicated no overall difference between sexes in overweight and obese groups, but a significant increase in ASP in normal weight girls versus normal weight boys (Mann–Whitney Test, $P = 0.016$). By contrast, complement C3 showed no significant change in overweight and obese versus normal weight group in both genders, however, when pooled together, C3 was increased in overweight and obese subjects versus normal weight ($P = 0.025$). The % ASP/C3 ratio is an indication of the percentage of C3 converted to ASP that is usually $< 1\%$ (10). Conversion of C3 to ASP is tightly regulated, and leads to increases in ASP, the biologically active molecule that stimulates adipose TG storage. The % ASP/C3 was significantly increased only in obese and overweight boys ($P < 0.001$).

In order to evaluate the impact of age and/or pubertal development in combination with body size on ASP and C3 levels, boys and girls were further subdivided. Boys were separated in distinct pubertal development groups based on testicular size, as shown in Table 3. As shown in Fig. 2A, overall ASP was increased in overweight and obese boys at all levels of pubertal development (two-Way RM ANOVA, $P < 0.0001$). When each stage is examined individually, the increase in ASP in overweight and obese boys was most pronounced at the initial stages of development (Testicle V1, V2, and V3, $P < 0.0001$, $P = 0.0009$, and $P = 0.0004$ respectively).

Table 3 Evaluation of C3 in boys and girls based on pubertal development and body size.

Complement C3 (g/l)								
	(N)	Average age	Normal	(N)	Overweight	(N)	Obese	(N) P value
Testicle V1	(368)	9.2±1.7	1.34±0.05	(136)	1.48±0.09	(28)	1.52±0.05	(204) NS
Testicle V2	(100)	12.0±1.6	1.90±0.30	(25)	1.81±0.22	(14)	1.61±0.08	(61) NS
Testicle V3	(186)	14.0±1.8	1.68±0.10	(71)	1.66±0.09	(30)	1.53±0.06	(85) NS
Testicle V4	(136)	14.9±1.6	1.86±0.21	(38)	1.48±0.14	(38)	1.49±0.07	(60) NS
Testicle V5	(35)	15.4±1.7	1.58±0.16	(8)	1.61±0.11	(5)	1.53±0.09	(22) NS
Tanner 1	(137)	7.8±1.2	1.13±0.07	(59)	1.45±0.12	(18)	1.76±0.13 [‡]	(60) 0.0001
Tanner 2	(101)	9.9±1.1	1.31±0.08	(58)	1.46±0.09	(16)	1.90±0.23 [†]	(27) 0.009
Tanner 3	(96)	11.2±1.2	1.65±0.21	(43)	1.99±0.22	(19)	1.76±0.16	(34) NS
Tanner 4	(238)	13.4±1.8	1.78±0.09	(127)	1.46±0.11	(54)	1.65±0.08	(57) NS
Tanner 5	(144)	15.0±1.7	1.44±0.09	(28)	1.54±0.10	(60)	1.43±0.09	(56) NS

All values are reported as mean±s.e.m. Boys were separated based on testicular volume where V1=1–4 ml, V2=5–9 ml, V3=10–14 ml, V4=15–19 ml, V≥20 ml. Significance was calculated by ANOVA where differences versus normal weight group are indicated as [†] $P<0.01$, [‡] $P<0.001$, and NS, not significant.

While body size influenced ASP levels at all stages of pubertal development, on the other hand, pubertal development itself did not appear to influence plasma ASP levels (Pearson correlation coefficient of ASP with testicular volume $r=-0.013$, P NS), nor did age correlate with plasma ASP (Pearson $r=-0.060$, P NS). On the other hand, complement C3 showed no significant difference between overweight and obese groups versus normal weight at every pubertal development stage (Table 3), but there was correlation with pubertal development (Pearson correlation of C3 with testicular volume $r=0.070$, $P=0.04$) as well as age ($r=-0.081$, $P<0.02$).

As with boys, the girls were further separated in distinct pubertal development groups as assessed by Tanner stage of breast and pubic hair development (Table 3). Results for C3 and ASP are provided in Table 3 and Fig. 2B respectively. Although C3 increased in overweight and obese girls in the younger groups (Tanner stage 1 and 2), overall there was little significant change in C3. Furthermore, there was no correlation of C3 with either Tanner stage (Pearson $r=0.041$, P NS) or with age (Pearson $r=0.024$, P NS). The effect of overweight and obesity on plasma ASP during development in girls is shown in Fig. 2B. At all stages of development (Tanner 1 to Tanner 5) there was an overall increase in plasma ASP in overweight and obesity (two-Way RM ANOVA, $P<0.0001$). As with C3, there was no influence of Tanner development stage itself (Pearson $r=0.023$, P NS) or age (Pearson $r=0.042$, P NS) on plasma ASP.

To examine factors relating directly to plasma ASP, the data was separated based on ASP quintiles in boys and girls separately. As shown in Table 4, for boys, there was a marked increase in %BF, BMI, waist circumference and waist/height ratio with increasing quintile of ASP (all $P<0.0001$). There was also a strong association with both systolic and diastolic blood pressure (both $P<0.0001$), with cholesterol, TG, and LDL-C but not HDL-C. ASP was strongly associated with

glucose, insulin, and HOMA-IR. In all cases, the association was positive, with increasing lipids, glucose, and insulin in the higher ASP quintiles.

Results for ASP quintiles in girls are given in Table 5. As with boys, there were strong associations of plasma ASP with %BF, BMI, waist circumference, and waist/height ratio. Similar to boys, there was also a strong positive association of ASP quintile with systolic and diastolic blood pressure. Associations with lipids and glucose were less pronounced than with boys, with a weak correlation with glucose, and significant correlations of lipid parameters only with LDL-C (positive) and HDL-C (negative). Even at a very young age, ASP correlated with lipid parameters. In younger boys (age <9 years), ASP was positively correlated with TC ($r=0.098$, $P=0.003$), TG ($r=0.103$, $P=0.002$), and LDL-Chol ($r=0.12$, $P=0.0003$). At Tanner 1 for girls, positive correlations of ASP with TC ($r=0.141$, $P=0.025$), TG ($r=0.151$, $P=0.019$), and LDL-Chol ($r=0.195$, $P=0.002$) were noted, but negative correlation with HDL-Chol ($r=-0.128$, $P=0.046$).

The effect of family environment on plasma ASP levels was examined. As shown in Table 6, in both girls and boys there was no influence of father's BMI, although there was an influence of mother's BMI. In both boys and girls, higher quintile of ASP was associated with higher birth weight (ANOVA, $P<0.0001$ and $P=0.003$ respectively). There was no effect of twins or gestation stage (χ^2 , all P NS) in both boys and girls.

The data presented above demonstrate that the strongest effects on ASP profile are related to body size, blood pressure, lipids, and glucose homeostasis. As all of these are indices used to reflect the presence of MetS, we examined the relationship of these parameters to ASP. MetS in the present population is defined as three or more of the following indices: central obesity, high blood pressure, hypertriglyceridemia, low HDL-C, or impaired fasting blood glucose (using cut offs as defined in Methods). Boys and girls were stratified according to the absence of MetS indices (MetS 0) or the presence of 1–5

Figure 2 Plasma acylation stimulating protein in boys and girls according to pubertal development. Boys (A) were divided based on pubertal development according to testicular volume where V1=1–4 ml, V2=5–9 ml, V3=10–14 ml, V4=15–19 ml, V5≥20 ml. Girls (B) were separated based on Tanner stages 1–5. All values are reported as mean ± S.E.M. for normal (open bars), overweight (hatched bars) and obese (solid bars) groups where *n* values are provided in Table 3. Significance was calculated by two-way RM ANOVA versus normal weight group, $P < 0.0001$ for both boys (2A) and girls (2B) (see text for details).

MetS indices (MetS 1 to MetS 5). For boys, with increasing numbers of MetS indices, there were corresponding significant increases in average age, body size (%BF, BMI, and waist circumference), blood pressure, lipids (TG and LDL-C), glucose, insulin, and HOMA-IR, with decrease in TC or NEFA (results not shown). Similar results were found for girls (results not shown). As shown in Fig. 3A and B, average plasma ASP increased significantly in both boys and girls with increasing number of MetS indices. On the other hand, there was no significant change in complement C3. Interestingly, even in the presence of a single isolated MetS risk factor, average

plasma ASP was significantly increased. In order to identify the factor(s) that most influenced plasma ASP, this group ($n = 257$ for boys and $n = 222$ for girls) was further subdivided according to the individual MetS parameters. As shown in Fig. 3C (boys) and D (girls), impaired fasting glucose, central obesity, and high blood pressure had the greatest effect on plasma ASP, with isolated hypertriglyceridemia or low HDL-C having little or no influence.

The additive contributing influence of all the parameters examined (continuous and ranked) on plasma ASP was evaluated by forward stepwise regression. While many factors correlated significantly with plasma ASP, for boys the optimal combination was the following: number of MetS factors ($P < 0.001$), fasting plasma glucose ($P < 0.001$), presence of obesity ($P < 0.001$), presence of MetS ($P < 0.001$), birth weight ($P = 0.014$), and NEFA ($P = 0.036$; all positively) and complement C3 ($P < 0.001$; negatively), which best predicted the variation in ASP ($r = 0.410$). For girls, a similar analysis indicated that a combination of the following was the most significant: number of MetS factors ($P < 0.001$), presence of obesity ($P < 0.001$), presence of central obesity ($P = 0.009$), birth weight ($P = 0.033$), gestational index ($P < 0.001$), TG ($P = 0.004$), LDL-C ($P = 0.015$), and father's BMI ($P = 0.026$; all positively) and complement C3 ($P = 0.013$; negatively) best predicted the variation in ASP ($r = 0.402$).

Discussion

There has been an increasing focus recently on the consequences of overweight and obesity in children and adolescents. In the present study, the salient finding was that ASP was increased in both overweight and obese children and adolescents at all ages in both boys and girls. Increased ASP was associated not only with indices of body size, but also with increased glucose and lipid profiles in both boys and girls. Finally, there was an association with risk factors for MetS components, with increasing number of components associated with increasing ASP (Table 4), although the presence of even a single parameter (such as high blood pressure, central obesity, or high glucose) being sufficient to be associated with increased ASP (Fig. 3).

In the present study, there was a change in plasma C3 with overweight and obesity, although only when the data for boys and girls were pooled. Elevated C3 concentrations have been previously reported in adults with obesity, type 2 diabetes, hypertension, hyperlipidemia, and coronary artery disease, all of these known to be associated to obesity (review, (10)). Furthermore, increased C3 has been suggested to be a predictor of myocardial infarction (24, 25). These associations and predictive value of C3 have been reinforced over the last few years, with evaluation of C3 in additional studies as reviewed recently (26, 27), even demonstrating greater predictive value than insulin or C reactive protein.

Table 4 Variation in clinical parameters in boys according to acylation stimulating protein (ASP) quintile.

ASP (nmol/l)	Pearson <i>r</i> (<i>P</i>)	Q1 (<i>n</i> =174) 16.30±0.39	Q2 (<i>n</i> =175) 34.20±0.41	Q3 (<i>n</i> =175) 58.20±0.67	Q4 (<i>n</i> =175) 89.90±0.66	Q5 (<i>n</i> =174) 140.50±2.44	ANOVA <i>P</i> value
Age (years)	−0.060 (NS)	12.2±0.2 ^{a,b}	11.9±0.3 ^{a,b}	11.6±0.2 ^a	12.7±0.2 ^b	11.3±0.2 ^a	0.00039
%BF	0.178 (<0.0001)	21.9±0.6 ^a	22.5±0.6 ^a	23.7±0.6 ^{a,b}	25.7±0.6 ^b	25.8±0.5 ^b	<0.0001
BMI (kg/m ²)	0.173 (<0.0001)	22.2±0.4 ^a	22.7±0.4 ^a	23.1±0.4 ^a	25.1±0.4 ^b	24.9±0.3 ^b	<0.0001
Waist (cm)	0.151 (0.0001)	74.6±1.0 ^a	75.3±1.2 ^a	76.3±1.1 ^a	81.3±1.0 ^b	80.4±0.9 ^b	<0.0001
WHiR (cm/cm)	0.235 (0.0001)	0.475±0.005 ^a	0.484±0.005 ^a	0.5±0.005 ^{a,b}	0.509±0.004 ^{b,c}	0.527±0.004 ^c	<0.0001
Systolic BP (mmHg)	0.145 (<0.0001)	109.8±1.2 ^a	111.14±1.23 ^a	110.82±1.17 ^a	118.4±1.04 ^b	115.6±0.927 ^b	<0.0001
Diastolic BP (mmHg)	0.156 (<0.0001)	68.4±0.8 ^a	68.59±0.855 ^a	69.66±0.913 ^{a,b}	73.37±0.701 ^c	72.59±0.621 ^{b,c}	<0.0001
Total Chol (mmol/l)	0.099 (0.004)	4.04±0.07 ^{a,b}	3.91±0.05 ^a	3.95±0.06 ^a	4.12±0.07 ^{a,b}	4.18±0.06 ^b	0.00896
TG (mmol/l)	0.104 (0.002)	1.00±0.03 ^a	1.13±0.05 ^{a,b}	1.03±0.04 ^{a,b}	1.21±0.05 ^b	1.2±0.05 ^b	0.00229
LDL-Chol (mmol/l)	0.120 (0.003)	2.53±0.06 ^{a,b,c}	2.39±0.05 ^a	2.44±0.05 ^{a,b}	2.63±0.06 ^{b,c}	2.71±0.05 ^c	0.00018
HDL-Chol (mmol/l)	−0.054 (NS)	1.36±0.03	1.32±0.03	1.35±0.03	1.28±0.03	1.29±0.02	NS
NEFA (mmol/l)	−0.055 (NS)	0.71±0.02 ^{a,b}	0.63±0.02 ^a	0.75±0.04 ^b	0.61±0.02 ^a	0.65±0.02 ^a	0.0005
Glucose (mmol/l)	0.199 (<0.0001)	5.12±0.04 ^a	5.17±0.04 ^a	5.14±0.04 ^a	5.46±0.07 ^b	5.44±0.04 ^b	<0.0001
Insulin (mU/l)	0.104 (0.002)	9.92±0.66 ^a	11.77±0.81 ^{a,b}	12.38±1.11 ^{a,b}	15.81±1.21 ^c	14.12±1.22 ^{b,c}	0.00077
HOMA-IR	0.107 (0.002)	2.33±0.17 ^a	2.79±0.21 ^{a,b}	2.94±0.31 ^{a,b}	3.95±0.36 ^c	3.45±0.31 ^{b,c}	0.00064
Complement C3 (g/l)	−0.107 (0.0001)	1.72±0.08 ^a	1.56±0.06 ^a	1.54±0.06 ^{a,b}	1.49±0.04 ^b	1.46±0.06 ^b	0.01817
% ASP/C3 (mol/mol)	0.68 (<0.0001)	0.22±0.01 ^a	0.50±0.02 ^a	0.84±0.03 ^b	1.28±0.05 ^c	2.35±0.15 ^d	<0.0001
Number of MS components	0.225 (<0.0001)	1.2±0.1 ^a	1.4±0.1 ^a	1.4±0.1 ^a	1.8±0.1 ^b	2.0±0.1 ^b	<0.0001

All values are reported as mean±s.e.m. %BF, % body fat; BMI, body mass index; BP, blood pressure; Chol, Cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; NEFA, nonesterified fatty acid; TG, triglyceride; WHiR, waist to height ratio. Significance was calculated by ANOVA where a, b, and c are homogeneous subgroups (*P*<0.05).

Overall, this reflects an increasing recognition of the association of inflammatory markers with obesity, diabetes, and cardiovascular disease.

There are relatively few studies of C3 in children. Our study on very young children (age 2–6 years old) demonstrated a small but significant increase in plasma C3 in very young obese subjects (ideal body weight was on average 150%) (11). In subsets of the AVENA study, inflammatory markers and MetS were evaluated in groups of Spanish adolescents (400–500 subjects, aged 13–18.5 years (28–31). To date, results indicate that inflammatory markers, C reactive protein and especially C3 were associated with indices of fitness and fatness (27, 28). However, in these studies, while the increase in C3 is significant, the relative changes are small nonetheless (10–15% increase in C3) (10, 11). These results are consistent with the present study, although unfortunately no data for ASP are available in the AVENA study and the samples size and age ranges are smaller. Thus, the increase in C3 may become more pronounced in adults, and may not be as easily apparent in children and adolescents under all circumstances or in all ethnic groups.

The changes in plasma ASP, by contrast to C3, are much greater, with an average increase of ~50%. In adults, comparable increases in ASP are seen with obesity, diabetes, and cardiovascular disease (10). Overall, in spite

of the recent interest in inflammatory factors in metabolism, there are few studies that measure both C3 and ASP. Nonetheless, in the present study, the lack of marked changes in the ASP precursor, complement C3, suggests that the increased ASP is a consequence of increased conversion of C3 to ASP (as indicated by the increase in % ASP/C3 ratio) although the total amount of C3 cleaved to generate ASP is always small (1–2%). While factor B and adipsin are both required for conversion of C3 to ASP, the processes that regulate this in the adipocyte milieu remain undefined.

How early can the association between increased plasma ASP and obesity/altered lipid profiles actually be detected? In the present study, when taken as an overall group, there was an increase in plasma ASP in overweight and obesity, even in the absence of altered glucose, cholesterol or NEFA. Thus, the changes in glucose and cholesterol which are associated with obesity may only occur in adulthood. When separated according to age or pubertal stages, even in the youngest group, overweight and obesity were associated with increased ASP (both girls and boys). In the youngest children examined, the age range is situated just after the adiposity rebound which is known as a high risk factor (4, 32), and it could be suggested that the change in ASP precedes the altered lipid profile.

Table 5 Variation in clinical parameters in girls according to acylation stimulating protein (ASP) quintile.

ASP (nmol/l)	Pearson <i>r</i> (<i>P</i>)	Q1 (<i>n</i> =146) 18.30±0.54	Q2 (<i>n</i> =147) 37.20±0.42	Q3 (<i>n</i> =145) 59.50±0.71	Q4 (<i>n</i> =146) 93.70±0.78	Q5 (<i>n</i> =146) 143.30±2.42	ANOVA <i>P</i> value
Age (years)	0.042 (NS)	12.0±0.2	11.9±0.2	11.4±0.2	11.8±0.3	12.2±0.3	NS
%BF	0.172 (<0.0001)	25.1±0.8 ^a	24.5±0.8 ^a	25.9±0.8 ^{a,b}	28.3±0.8 ^{b,c}	29.6±0.8 ^c	<0.0001
BMI (kg/m ²)	0.195 (<0.0001)	20.8±0.4 ^a	20.9±0.4 ^a	21.3±0.4 ^a	22.9±0.4 ^b	23.5±0.4 ^b	<0.0001
Waist (cm)	0.161 (<0.0001)	68.4±0.9 ^a	68.5±0.9 ^a	69.2±1.0 ^a	72.9±0.9 ^b	73.4±0.9 ^b	<0.0001
WHR (cm/cm)	0.217 (<0.0001)	0.453±0.004 ^a	0.453±0.004 ^a	0.463±0.005 ^a	0.484±0.004 ^b	0.493±0.005 ^b	<0.0001
Systolic BP (mmHg)	0.242 (<0.0001)	102.5±1.1 ^a	104.2±1.1 ^a	104.8±1.2 ^a	111.2±0.9 ^b	111.0±0.9 ^b	<0.0001
Diastolic BP (mmHg)	0.208 (<0.0001)	65.3±0.8 ^a	66.3±0.9 ^a	67.1±0.9 ^a	71.4±0.7 ^b	70.8±0.7 ^b	<0.0001
Total Chol (mmol/l)	0.064 (NS)	4.09±0.07	4.08±0.06	4.11±0.06	4.10±0.07	4.21±0.07	NS
TG (mmol/l)	0.030 (NS)	1.15±0.07	1.12±0.05	1.04±0.04	1.15±0.05	1.21±0.05	NS
LDL-Chol (mmol/l)	0.122 (0.001)	2.53±0.06 ^a	2.48±0.06 ^{a,b}	2.58±0.05 ^{a,b}	2.63±0.06 ^{a,b}	2.73±0.07 ^b	0.03066
HDL-Chol (mmol/l)	-0.086 (0.02)	1.37±0.03 ^{a,b}	1.42±0.03 ^a	1.36±0.03 ^{a,b}	1.31±0.02 ^b	1.3±0.03 ^b	0.00666
NEFA (mmol/l)	-0.039 (NS)	0.70±0.02	0.68±0.03	0.72±0.03	0.64±0.02	0.68±0.02	NS
Glucose (mmol/l)	0.083 (0.030)	5.18±0.12 ^{a,b}	4.99±0.05 ^a	5.09±0.06 ^{a,b}	5.27±0.07 ^{a,b}	5.31±0.05 ^b	0.01958
Insulin (mU/l)	0.015 (NS)	11.04±0.83	11.43±0.83	11.38±1.35	12.55±0.64	11.51±0.55	NS
HOMA-IR	0.029 (NS)	2.58±0.20	2.58±0.19	2.71±0.39	3.05±0.19	2.75±0.14	NS
Complement C3 (g/l)	-0.085 (0.022)	1.88±0.12 ^a	1.5±0.06 ^c	1.54±0.06 ^b	1.43±0.05 ^b	1.58±0.08 ^{a,b}	0.0004
% ASP/C3 (mol/mol)	0.735 (<0.0001)	0.26±0.02 ^a	0.6±0.03 ^b	0.87±0.04 ^c	1.42±0.06 ^d	2.14±0.11 ^e	<0.0001
Number of MS components	0.225 (<0.0001)	1.1±0.1 ^a	1.10±0.10 ^a	1.26±0.10 ^a	1.70±0.09 ^b	1.84±0.10 ^b	<0.0001

All values are reported as mean±s.e.m. %BF, % body fat; BMI, body mass index; BP, blood pressure; Chol, Cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; NEFA, nonesterified fatty acid; TG, triglyceride; WHR, waist to height ratio. Significance was calculated by ANOVA where a, b, and c are homogeneous subgroups (*P*<0.05).

We have previously demonstrated that ASP is increased in obesity in even younger children (2–6 years old); even in the absence of a modified lipid profile (11). The present study examined children and adolescents aged 6–18 years, and is the first study to our knowledge, to examine ASP in the context of childhood and adolescence. It should be noted, however, that these results are in Chinese children, and, given the potential effects of ethnic background and diet on adipose tissue deposition, body weight, and metabolism, the results are not necessarily generalizable to all children in all populations.

Previous studies suggest that an individual's intrinsic regulatory process for control of body fatness can be altered permanently by early environmental modification and this may occur even *in utero*. Mother's body size and offspring birth weight were shown to be associated with ASP in both boys and girls. Birth weight was an independent contributing factor in predicting ASP and a high birth weight has been proposed as an independent risk factor for obesity (33, 34). An early association between ASP and obesity can be inferred from two previous studies. First, it has been demonstrated that cord

Table 6 Variation in family associations in boys and girls according to acylation stimulating protein (ASP) quintile.

	Q1	Q2	Q3	Q4	Q5	ANOVA	<i>r</i> (<i>P</i>)
Boys							
Father's BMI (kg/m ²)	24.6±0.3	25.0±0.2	25.6±0.3	24.9±0.3	25.1±0.3	NS	0.017 (NS)
Mother's BMI (kg/m ²)	23.3±0.3	23.2±0.3	23.3±0.3	23.5±0.2	23.9±0.3	NS	0.083 (0.020)
Birth weight (g)	2738±110	2987±91	3015±91	3165±72	3324±51	<0.0001	0.156 (<0.0001)
Gestation stage (P/N/O)	9/105/22	5/124/24	3/128/22	3/134/19	2/145/20	NS	
Twins (yes/no)	10/156	11/160	9/162	6/167	2/171	NS	
Girls							
Father's BMI (kg/m ²)	24.8±0.3 ^{a,b}	25.4±0.3 ^b	25.1±0.3 ^{a,b}	25.3±0.3 ^{a,b}	24.3±0.3 ^b	0.02229	-0.071 (0.05)
Mother's BMI (kg/m ²)	23.1±0.3 ^{a,b}	22.8±0.3 ^b	23.2±0.3 ^{a,b}	23.6±0.3 ^{a,b}	24.1±0.3 ^b	0.02331	0.104 (0.006)
Birth weight (g)	2776±113 ^a	2821±98 ^{a,b}	2910±108 ^{a,b}	3178±65 ^b	3147±67 ^b	0.00313	0.126 (0.001)
Gestation stage (P/N/O)	4/103/14	5/116/7	2/110/11	6/116/17	3/124/12	NS	
Twins (yes/no)	10/133	10/133	10/133	6/140	6/137	NS	

All values are reported as mean±s.e.m. Gestation stage is defined as premature (P), normal (N), or overdue (O). Significance was calculated by ANOVA where a and b are homogeneous subgroups (*P*<0.05). BMI, body mass index. Each ASP quintile for boys *n*=174, for girls *n*=146.

Figure 3 Plasma acylation stimulating protein in boys and girls according to presence of metabolic syndrome factors. Boys (A) and girls (B) were divided based on the absence or presence of 1–5 metabolic syndrome factors (MetS 0 to MetS 4&5). In addition, boys (C) and girls (D) with a single metabolic syndrome factor (MetS 1) were further subdivided based on the individual parameters of high blood pressure (Hi BP), central obesity (C Obes), hyperglycemia (Hi Glu), hypertriglyceridemia (Hi TG), or low HDL cholesterol (Lo HDL). Plasma ASP results are expressed as mean $\pm$ S.E.M. Significance was calculated by ANOVA where differences versus the group with no metabolic syndrome factors (MetS 0) are indicated as * P <0.05 and ** P <0.001, where NS, not significant.

blood ASP was predicted by maternal lipids and correlated with fetal birth weight (35). Second, increased maternal plasma ASP correlated with hyperlipidemia at late gestation (36). Thus, an intimate link between mother to child can be made for plasma ASP.

These associations between lipids, body weight and ASP, even at very early ages, raise the question of what is the signal for increased ASP and its potential role? First, since ASP is generated by adipose tissue, increased adipose tissue mass can lead to increased ASP (37, 38). However, this is not likely the only signal for production of ASP, as some nonobese subjects can also have increased ASP (19) but also the nonobese subjects with diabetic, thyroid, or polycystic ovary syndromes present with increased ASP (39–41). While we have no direct evidence, we hypothesize that increased ASP may be a compensatory response. In an early phase, increased ASP may be a positive factor, enhancing lipid storage and preventing alterations in circulating lipids and glucose. With chronic increases in plasma ASP, ASP resistance may evolve. This possibility has been raised in previous studies, in adults with increased ASP in insulin resistance, thyroid dysfunction, cardiovascular disease, and other studies (10, 39, 40). The hypothesis is further supported by biological studies in cultured adipocytes which suggest that metabolic factors such as high NEFA, steroid hormones and TNF α decrease levels of the ASP receptor C5L2 and decrease ASP responsiveness, inducing a state of ASP resistance (42, 43).

In summary, ASP is altered in overweight and obese children, and adolescents. Family environment is an important genetic and environment risk factor. These

changes initiate early on and are followed across pubertal development in boys and girls. Furthermore, these changes are associated with modification of lipid profiles, central obesity, and blood pressure, all linked to MetS that may lead to a chronic state at adult age.

Declaration of interest

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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