Original Article

Abnormal Adipokines Associated with Various Types of Obesity in Chinese Children and Adolescents*

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Abstract

Objective To explore the role of adipokines including insulin, resistin, leptin, adiponectin, acylation stimulating protein (ASP) and complement C3 (C3) in various types of obesity (peripheral obesity, abdominal obesity and mixed obesity) in Chinese children and adolescents, and their relationships with body size and pubertal development.

Methods Children and adolescents (n=3 508) aged 6 to 18 years, with 1 788 boys and 1 720 girls were assessed for body mass index, waist circumference, pubertal development, blood insulin, resistin, leptin, adiponectin, ASP and C3 levels. Three types of obesity [peripheral obesity (n=43), abdominal obesity (n=473), mixed obesity (n=1 187)] and non-obese control (n=1 805) were defined with combined use of Chinese body mass index and waist circumference criteria.

Results Serum resistin, leptin and adiponectin levels were higher in girls than those in boys (all P<0.01). Insulin and leptin increased and adiponectin decreased across five Tanner stages in both girls and boys (all P<0.001), while ASP changed only in girls (P<0.001) and C3 only in boys (P<0.001). Insulin, leptin and ASP were higher, but adiponectin was lower in all three types of obesity vs. the non-obese control (all P<0.05). The greatest abnormalities of all six adipokines were found in the mixed obesity group. With inclusion of body mass index and waist circumference in simultaneous regression analyses, both body size indices were independently and significantly correlated with insulin, leptin and adiponectin after age and gender adjustment. Compared with waist circumference, the body mass index was stronger in interpreting insulin, leptin, adiponectin and ASP levels, whereas it was weaker in explaining variance of plasma C3.

Conclusions Obese children have a worse metabolic profile with high insulin, resistin, leptin, ASP and C3, and low adiponectin levels. The adipokine profile in mixed obesity is worse than that in peripheral or abdominal obesity. Identification of obese subjects with a malignant adipokine profile using a combination of body mass index and waist circumference is important for the prevention of obesity-related disease.

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INTRODUCTION

The striking increase in obesity constitutes a worldwide concern due to its associations with metabolic syndrome, insulin resistance, diabetes, dyslipidemia and cardiovascular disease[1-2]. This increase is notable even in developing countries, such as in China[3], where a more notable increase of obesity has been witnessed in the child and adolescent population[4]. There is now a growing body of evidence showing that childhood obesity not only tends to persist through life[5-6], but is also a risk factor for cardiometabolic syndrome and resultant cardiovascular risk in adulthood[7]. If current trends continue, the problem of pediatric obesity will account for an unmanageable proportion once these individuals reach adulthood. Thus, it is of great importance to identify predictive indices indicating early metabolic abnormalities in obese children. Timely identification of high-risk obese children and early interventions for them can reduce and delay the cardiometabolic complications.

Both the amount and distribution of body fat determine the extent of beneficial or harmful effects on health. The distribution of visceral adipose tissue in abdominal obesity, is related to higher susceptibility to cardiometabolic risk as compared to that in peripheral obesity, and the former is the most common type of obesity in the Chinese population. The International Diabetes Federation (IDF) has recommended a definition in which abdominal obesity, assessed by waist circumference, is an essential component of metabolic syndrome[8-9]. Many studies have confirmed that abdominal obesity is strongly associated with metabolic abnormalities[10], but no molecular mechanism explaining the relationship has yet been proposed.

Adipose tissue, now considered as an endocrine organ, secretes a variety of adipokines that influence body weight, glucose and lipid metabolism[11]. Resistin has been linked with obesity and hypothesized as a potential marker of insulin resistance in addition to being linked with acute inflammation. However, these links are still highly controversial in humans[12-14]. Leptin, a cytokine-like molecule secreted by adipose tissue, regulates adipose tissue mass and body weight by inhibiting food intake and stimulating energy expenditure[15-16]. Adiponectin is a collagen-like protein exclusively expressed in adipose tissue, with important anti-atherogenic, anti-diabetic and anti-inflamm atory properties[17-19]. Acylation stimulating protein (ASP) (aka C3adesArg) is produced through cleavage of its precursor complement C3 (C3) by interacting with adipsin and factor B. Both ASP and C3 have been shown, in a number of studies, to be associated with obesity, diabetes and cardiovascular disease in adults[20]. These adipokines are being examined for their potential links to obesity and other complications in adults such as diabetes and cardiovascular disease, and their dynamic interaction underlies the molecular pathophysiology of obesity-related disease.

However, to our knowledge, a systematic survey of the levels of these adipokines involved in Chinese children with different types of obesity has not yet been reported. Therefore, the present study was aimed to evaluate the adipokines such as insulin, resistin, leptin, adiponectin, ASP and C3 in terms of their association with anthropometric variables, pubertal development and changes in the non-obese, peripheral obese, abdominal obese and mixed obese children and adolescents in China.

SUBJECTS AND METHODS

Subjects

Subjects were recruited from a cross sectional population-based study: the Beijing Child and Adolescent Metabolic Syndrome Study (BCAMS)[21]. This study evaluated the presence of obesity and related metabolic abnormalities (hypertension, central obesity, type 2 diabetes, dyslipidemia) among a representative sample (n=19 593) of Beijing school children evaluated between April and October of 2004. The cohort included girls and boys aged 0 to 18 years. Evaluation included physical examination, blood pressure, fasting finger capillary blood test for glucose, total cholesterol, and triglyceride. Physical examination included body mass index (BMI), waist circumference, fat mass percentage

by bioelectrical impedance analysis, systolic, and diastolic blood pressure. Pubertal development was assessed by Tanner stage of breast development (girls) and testicular volume (boys).

Within this large group of children and adolescents, 4 500 were identified with risk factors defined as the presence of any one of the following: overweight based on BMI cutoffs, increased cholesterol (⩾ 5.2 mmol/L), triglyceride (⩾ 1.7 mmol/L) or glucose (⩾ 5.6 mmol/L). A parallel reference population of 1 045 school-age children was also identified. Within these two groups, 2 544 (BCAMS) and 981 (Reference) children/adolescents were recruited for blood samples[22]. Within this total group, the cohort analysis included 1 720 girls and 1 788 boys aged 6 to 18 years with valid BMI and waist circumference[23]. Signed informed consent was obtained from all participants and/or their parents or guardians throughout the study processes. The BCAMS was approved by the Ethics Committee at Capital Institute of Pediatrics in Beijing.

Definition and Grouping of Obesity

BMI categories: The age-, gender- specific BMI cutoff points recommended by the Working Group on Obesity in China were used to define normal weight (low BMI), overweight (middle BMI) and obesity (high BMI)[24]. **Waist circumference categories**: Those whose waist circumferences (WC) were equal to or greater than the 90th percentile for age and gender, a newly developed waist circumference references for Chinese school-age children, were defined as obese (high waist circumference), otherwise were normal (low waist circumference)[25]. Both BMI and waist circumference were used to define three types of obesity as follows: peripheral obesity (high BMI and low waist circumference), abdominal obesity (low or middle BMI and high waist circumference) and mixed obesity (high BMI and high waist circumference), and the non-obese (control) (low or middle BMI and low waist circumference).

Blood Samples

Simultaneously, two consecutive blood samples were drawn by direct venipuncture after an overnight (minimum 12 h) fast. One blood specimen was 3 milliliter with EDTA as anticoagulants, centrifuged at 3 000 r/min for 15 min, and plasma were divided into three aliquots, which were then stored at -80˚C until further analysis. The other was 4 milliliter of blood specimen without anticoagulant, left standing 30min, then centrifuged at 3 000 r/min for 15 min, and sera were divided into three aliquots, which were stored at -80˚C until further analysis.

Analytical Procedures

Plasma insulin was measured by monoclonal antibody-based sandwich enzyme-linked immunosorbent assays (ELISA)[26], which was developed in the Key Laboratory of Endocrinology, Peking Union Medical College Hospital. The assay had inter-assay CVs of <9.0% and had no cross-reactivity to proinsulin (<0.05%). Serum resistin was measured by ELISA as described in detail elsewhere with intra-assay and inter-assay coefficients of variation of <5.4% and <8.5%, respectively[22]. All antibodies, reagents and adiponectin standard were.

purchased from Phoenix Pharmac euticals Inc. (Belmont, CA, USA). Serum leptin and adiponectin were measured by ELISA as described elsewhere[27-28]. Plasma complement C3 concentration was determined by turbidimetric assay using a polyclonal anti-human antibody specific against complement C3 (Ling-Fei Co, P.R. China). Plasma ASP concentration was measured using a sandwich ELISA immunoassay method as previously described in detail[29]. For complement C3 and ASP assays, intra-assay coefficient of variations were <4% and inter-assay coefficient of variations were <8%.

Calculations and Statistical Analyses

BMI was calculated as weight divided by square of height (kg/m^2). Unless otherwise stated, all results are displayed as mean ± standard deviation (SD). Data analyses were performed with SPSS 13.0. One-way analysis of variance (ANOVA) and general linear model (GLM) were used to evaluate means differences among the groups. For contribution of multiple independent variables on one dependent variable, multiple linear regression analysis with enter method was used. To compare the influence of BMI and waist circumference on the adipokines, *Z*-scores of BMI and waist circumference were used to replace the original records, and calculated with the formula as follows: $Z\text{-score} = (x-\bar{x})/s$, where x indicates observed value, and $\bar{x}$ and s indicate the mean value and standard deviation (SD) of the study population. Age, gender and puberty were adjusted as covariables. All of the adipokines had skewed distributions and were logarithmically transformed for analysis. Statistical significance was set at P value <0.05 for all analyses.

RESULTS

Baseline Characteristics of Children and Adolescents

The baseline characteristics of the children and adolescents are summarized in Table 1. Subjects ranged from 6 to 18 in terms of their ages and were distributed across all Tanner stages, with a mean age of 12.4±3.1 years. Girls had a more advanced pubertal development than boys. There were no differences in levels of plasma insulin, ASP and C3 between girls and boys, while the groups differed significantly with respect to a number of other parameters, including leptin, adiponectin, and resistin levels, as well as body size indices.

Serum Adipokine Levels in Subjects with Pubertal Development

Children and adolescents were separa ted according to Tanner stage and gender (Table 2). In girls, ASP decreased at the first four Tanner stages, and then increased to pubertal maturation (Tanner stage V; insulin and leptin increased across all the tanner stages; while adiponectin decreased, and resistin and C3 were not changed significantly. In boys, the levels of leptin increased with the onset of puberty, and then decline in adulthood; the change in C3 not in ASP with Tanner stages were significant and the trend was like levels of leptin, while the changes in other adipokines were similar to those in girls. Overall, insulin, leptin, and adiponectin were related to the Tanner stages in both girls and boys, while resistin was not influenced by such stages.

Serum Adipokine Levels in Subjects with Different Types of Obesity

The prevalence of obesity in the study population was approximate 40%. In the obese group, regardless of definition, either by BMI or by waist circumference, adiponectin was lower and the other adipokines were higher than in the normal or overweight groups in both girls and boys except for C3 in boys (Table 3).

Table 1. Anthropometric and Metabolic Parameters in Girls and Boys

Variables	All	Girls	Boys
n	3 508	1 720	1 788
Age (years)	12.4±3.1	12.6±3.1**	12.2±3.0
Pubertal Development†	3 403	1 695	1 708
Tanner stage Ⅰ	1 002(29.4)	336(19.8)	666(39.0)
Tanner stage Ⅱ	490(14.4)	218(12.9)	272(15.9)
Tanner stage Ⅲ	469(13.8)	208(12.3)	261(15.3)
Tanner stage Ⅳ	816(24.0)	610(36.0)	206(12.1)
Tanner stage Ⅴ	626(18.4)	323(19.1)**	303(17.7)
BMI (kg/m^2)	21.9±4.9	21.0±4.5**	22.8±5.2
Waist Circumference (cm)	72.4±13.1	68.6±10.8**	76.0±14.0
Insulin (mU/L)#	7.92±2.13	7.90±2.07	7.94±2.19
Resistin (ng/mL)#	15.4±1.7	15.7±1.7*	15.0±1.7
Leptin (ng/mL)#	5.0±3.7	6.2±3.2**	4.1±4.1
Adiponectin (μg/mL)#	10.9±1.8	11.4±1.8**	10.4±1.8
ASP (nmol/L)#	52.82±2.23	54.38±2.19	51.56±2.26
C3 (g/L)#	1.38±1.62	1.37±1.64	1.38±1.59

Note. Data are expressed as $\bar{x} \pm s$. †Pubertal development was evaluated based on five tanner stages of testicle volume in boys and breast development in girls, and the valid number of participants was presented followed by proportion in parentheses. χ^2 test was used to compare the difference of proportion across tanner stages between girls and boys. #Data with skew distributions are expressed as geometric mean ± geometric standard deviation, data was logarithmically transformed for *t*-test. Differences between girls and boys are indicated as *P<0.01, **P<0.001. BMI, body mass index; ASP, acylation stimulating protein; C3, complement C3.

Table 2. Levels of Adipokines in Girls and Boys with Different Pubertal Developments

Gender	Pubertal Development	*n*	Age (years)	Insulin (mU/L)	Resistin (ng/mL)	Leptin (ng/mL)	Adiponectin (μg/mL)	ASP (nmol/L)	C3 (g/L)
Girls	Tanner stage Ⅰ	336	8.4±1.3	4.81±2.08	16.7±1.8	3.1±4.0	14.6±1.7	58.78±2.12	1.26±1.70
	Tanner stage Ⅱ	218	10.4±1.2	6.53±2.08	15.6±1.7	3.7±3.4	12.0±1.8	52.73±2.19	1.30±1.60
	Tanner stage Ⅲ	208	12.0±1.4	9.42±1.97	15.9±1.8	6.2±2.9	10.5±1.7	52.95±2.14	1.42±1.76
	Tanner stage Ⅳ	610	14.3±1.9	8.93±1.90	15.6±1.7	7.2±2.6	11.1±1.8	46.63±2.25	1.45±1.64
	Tanner stage Ⅴ	323	15.6±1.7	10.77±1.81	15.1±1.6	12.4±2.3	9.6±1.7	67.32±2.09	1.34±1.48
	F	/	1154.606	75.869	1.694	81.056	25.952	5.506	2.344
	P	/	<0.001	<0.001	0.149	<0.001	<0.001	<0.001	0.053
Boys	Tanner stage Ⅰ	666	9.3±1.6	5.89±2.22	15.6±1.8	4.2±4.5	13.5±1.8	53.22±2.26	1.23±1.73
	Tanner stage Ⅱ	272	11.7±1.3	8.85±2.15	14.8±1.7	5.5±4.2	9.6±1.8	47.63±2.47	1.52±1.56
	Tanner stage Ⅲ	261	13.8±1.7	9.85±2.19	14.9±1.8	3.5±3.7	8.6±1.7	48.08±2.05	1.48±1.49
	Tanner stage Ⅳ	206	15.1±1.8	8.33±1.96	14.3±1.7	2.8±3.8	8.6±1.8	52.59±2.11	1.44±1.54
	Tanner stage Ⅴ	303	15.6±1.7	10.25±1.94	14.3±1.6	3.7±3.4	8.4±1.8	53.87±2.37	1.43±1.44
	F	/	1090.189	41.503	1.852	7.701	60.323	0.801	7.199
	P	/	<0.001	<0.001	0.116	<0.001	<0.001	0.525	<0.001

Note. Age is expressed as $\bar{x}\pm s$. All the adipokines with skew distributions are expressed as geometric mean ± geometric standard deviation and logarithmically transformed for one way ANOVA. Pubertal development was evaluated based on five Tanner stages of testicle volume in boys and breast development in girls. ASP, acylation stimulating protein; C3, complement C3.

Based on BMI and waist circumference, four groups were defined as peripheral obesity, abdominal obesity, mixed obesity and non-obese groups. The proportions of peripheral obesity, abdominal obesity and mixed obesity in the study population were 1.2%, 13.5%, and 33.8%, respectively. Adipokines levels in the various types of obesity are presented in Table 4. Levels of adipokines including resistin, leptin, ASP and C3 as well as insulin were higher, while adiponectin was lower in all obese groups vs. the non-obese group, although there were no significant differences between the peripheral obese group and the abdominal obese group. In the mixed obese group, the changes in the adipokines were more pronounced versus peripheral obesity or abdominal obesity groups.

Combined Influence of BMI and Waist Circumference on Adipokines

The natural logarithms of adipokines were used as dependent variables, *Z*-scores of BMI (Z-BMI) and waist circumference (Z-waist) as the independent variables in multiple linear regression analyses with age and gender as covariables. The partial regression coefficients in the adipokines interpreted by Z-BMI and Z-waist, from multiple regression analyses with enter method, are listed in Table 5. Both waist circumference and BMI were independently related to insulin, leptin, and adiponectin levels. BMI was the only significant factor in interpreting plasma ASP, while waist circumference was associated with C3. BMI beyond waist circumference was the primary factor influencing insulin, leptin, adiponectin, and ASP levels. Neither BMI nor waist circumference was associated with serum resistin levels.

DISCUSSION

The consequences of obesity in children and adolescents have been given an increasingly high priority over the recent years. It is extremely important to precisely evaluate obesity. BMI is thought to be an indicator of overall adiposity, whereas waist circumference has been advocated as an indicator of abdominal fat content. Some studies have shown that abdominal obesity evaluated by waist circumference is related to the morbidity and mortality of metabolic abnormalities, cardiovascular disease and other chronic disease beyond peripheral

obesity based on BMI[30-33]. Recently a growing body of evidences in both adults and children have indicated that obesity assessed by the combined utilization of BMI and waist circumference is more closely associated with increased disease risk than that assessed by BMI or waist circumference alone, and it is recommended to assess waist circumference in parallel to, not just sequential to, the measurement of BMI in order to identify high-risk obese individuals[34-35]. Adipokines secreted by adipose tissue have been recognized as the linkers between obesity and cardiometabolic risk. However, information concerning childhood obesity and its relationship with adipokine levels remains limited.

In the present study, we investigated the potential of adipokines including resistin, leptin, adiponectin, ASP and C3, as well as insulin as biomarkers in more than 3 500 Beijing children and adolescents with varying types of obesity across the full range of puberty status. As with many other studies, insulin, resistin (boys), leptin, ASP and C3 (girls) were high and adiponectin was low in overweight and obese individuals[36]. With pubertal development, insulin increased and adiponectin

Table 3. Adipokine Levels in Girls and Boys Separated by BMI or Waist Circumference

Gender	Groups	*n* (%)	Insulin (mU/L)#	Resistin (ng/mL)#	Leptin (ng/mL)#	Adiponectin (μg/mL)#	ASP (nmol/L)#	C3 (g/L)#
	BMI							
	Normal	951(55.3)	6.00±1.02	15.3±1.0	3.3±1.0	12.9±1.0	43.59±1.04	1.27±1.03
	Overweight	327(19.0)	9.08±1.03■	16.4±1.0	9.4±1.0■	10.5±1.0■	60.84±1.06■	1.37±1.04
Girls	Obese	442(25.7)	13.10±1.03■,▲	16.3±1.0	17.1±1.0■,▲	9.2±1.0■,▽	67.35±1.05■	1.50±1.03■
	Waist Circumference							
	Normal	1033(60.1)	6.16±1.02	15.2±1.0	3.6±1.0	12.7±1.0	46.74±1.04	1.29±1.03
	Obese	687(39.9)	11.59±1.02■	16.6±1.0□	14.0±1.0■	9.6±1.0■	63.78±1.04■	1.45±1.03□
	BMI							
	Normal	673(37.6)	4.87±1.03	14.3±1.0	1.0±1.0	12.9±1.0	37.40±1.05	1.34±1.03
	Overweight	327(18.3)	8.00±1.04■	14.7±1.0	5.2±1.1■	10.4±1.0■	58.57±1.08■	1.39±1.05
Boys	Obese	788(44.1)	11.78±1.02■,▲	15.8±1.0□	11.4±1.0■,▲	8.6±1.0■,▲	61.09±1.04■	1.40±1.02
	Waist Circumference							
	Normal	815(45.6)	5.31±1.02	14.2±1.0	1.3±1.0	12.5±1.0	40.71±1.04	1.33±1.03
	Obese	973(54.4)	10.94±1.02■	15.7±1.0■	10.1±1.0■	8.9±1.0■	60.79±1.04■	1.41±1.02

Note. #Data were expressed as geometric mean ± geometric standard deviation error and logarithmically transformed before general linear model (GLM) with puberty and age adjustment. Differences versus the normal group are indicated as, □ $P<0.01$, ■ $P<0.001$, and versus the overweight group are indicated as ▽ $P<0.01$, ▲ $P<0.001$. BMI, body mass index; ASP, acylation stimulating protein; C3, complement C3.

Table 4. Comparison of Adipokine Levels in Children with Different Types of Obesity

Groups	*n* (%)	Insulin (mU/L)#	Resistin (ng/mL)#	Leptin (ng/mL)#	Adiponectin (μg/mL)#	ASP (nmol/L)#	C3 (g/L)#
Non-obese	1805(51.5)	5.62±1.02	14.7±1.0	2.1±1.0	12.8±1.0	42.79±1.03	1.31±1.02
Peripheral obesity	43(1.2)	9.11±1.10	15.2±1.1	8.2±1.2	10.0±1.1	61.82±1.13	1.29±1.08
Abdominal obesity	473(13.5)	8.81±1.03	16.1±1.0	7.7±1.0	10.1±1.0	56.89±1.06	1.38±1.04
Mixed obesity	1187(33.8)	12.69±1.02	16.1±1.0	15.1±1.0	8.8±1.0	63.88±1.03	1.45±1.02
F	/	374.625	7.421	1003.342	104.008	29.095	4.608
P	/	0.000	0.000	0.000	0.000	0.000	0.003

Note. #Data are expressed as geometric mean ± geometric standard deviation error and logarithmically transformed before general linear model (GLM) adjusted for gender, age and puberty. ASP, acylation

stimulating protein; C3, complement C3.

Table 5. Comparison of BMI with Waist Circumference in Relation to Adipokines with Multiple Linear Regression Analyses

Dependent Variables	Z-BMI†			Z-waist†		
	β	*se*(β)	*P* Value	*B*	*se*(β)	*P* Value
Insulin (mU/L)‡	0.249	0.031	0.000	0.160	0.031	0.000
Resistin (ng/mL)‡	0.029	0.027	0.288	0.025	0.027	0.352
Leptin (ng/mL)‡	0.562	0.043	0.000	0.425	0.043	0.000
Adiponectin (μg/mL)‡	-0.100	0.027	0.000	-0.085	0.027	0.002
ASP (nmol/L)‡	0.226	0.055	0.000	-0.050	0.056	0.371
C3 (g/L)‡	-0.063	0.034	0.064	0.104	0.034	0.002

Note. *Adjustment for gender and age as covariables; †Body mass index z-score (Z-BMI) and waist circumference z-score (Z-waist) were used to replace the original records; ‡Natural logarithmically transformation; ASP, acylation stimulating protein; C3, complement C3.

decreased in both genders. Serum leptin was different between girls and boys with pubertal development. The pubertal effect on these adipokines is consistent with German, American and Japanese studies in children[37-38], and has been suggested to be related to changes in sex hormones[39].

The present study demonstrated that levels of insulin and leptin were strongly associated with abdominal obesity defined by waist circumference within each of the BMI categories in both girls and boys, in contrast to adiponectin, resistin, ASP and C3, where the presence of high waist circumference was not additive to that of overweight or peripheral obesity (Supplemental table 1). To clarify which type of obesity was associated with the worst adipokine profile, four groups including peripheral obesity, abdominal obesity, mixed obesity and the non-obese control were divided by the combination of BMI and waist circumference. In this population, the prevalence of obesity was much higher than that in the general population (approximate 40% *versus* 8.9%), and the proportion of peripheral obesity alone was very low (1.2%). Levels of adipokines were then compared among the four groups with age, gender and puberty adjustment. The results showed that adiponectin was low while other adipokines including resistin, leptin, ASP, and C3, as well as insulin, were high in any type of obesity. Particularly in the mixed obese group, the changes in all adipokines were more pronounced versus peripheral obesity or abdominal obesity alone. These results suggested that individuals with mixed obesity were more likely to have a compromised adipokine profile than those with peripheral or abdominal obesity alone. Similar results supporting adipokine profiles as diagnostic markers were found in American[40-43], German[44], African[45], and Japanese[46] populations from childhood to adolescence and further to adulthood. Different types of obesity are associated with different adipokine profiles. In the multiple linear regression analyses, BMI and waist circumference values were transformed to z-scores in order to compare the effects of the two measurements on adipokines, and the results showed that there were combined effects of BMI and waist circumference on insulin, leptin and adiponectin levels. Therefore, it is important to identify obese subjects with a particularly malignant adipokine profile through the combination of BMI and waist circumference for the prevention of obesity-related disease.

Previous studies had also demonstrated a clustering of biomarkers in overweight/obese children[36,47-48], healthy children[49], and both[50], although these studies had only 300-600 subjects, in comparison to the large number of subjects in the present study, and did not necessarily cover the same age range. Interestingly, following lifestyle intervention in obese children, adiponectin proved to be the most significant predictor of improved metabolic profile, while changes in leptin were unrelated to positive metabolic outcomes[51]. Nonetheless, the clustering of multiple unfavourable biomarkers strongly supports the need for early intervention in childhood obesity, particularly in subjects that manifest adipokine profiles at risk.

Waist circumference is an indicator of fat distribution and has a strong positive correlation with the abdominal fat area detected by computer tomography (CT) or magnetic resonance imaging

(MRI). Furthermore, some adipokines were altered in obese children with non-alcoholic fatty liver disease[52]. However, waist circumference is a surrogate for abdominal obesity after all and does not exactly reflect the visceral and subcutaneous fat depots in the abdomen. And the definition of types of obesity is neither an international criterion nor a national standard. Utilization of the two body size indices could only preliminarily evaluate types of obesity. This is one shortcoming of this study. Therefore, further research on visceral and subcutaneous fat depots using precise measurements is needed to clarify the effects of visceral and subcutaneous adiposity on the adipokines and cardiometabolic risks.

In summary, in this large study of Chinese children and adolescents, we have demonstrated associations of the adipokine profile such as resistin, leptin, adiponectin, ASP and C3, as well as insulin, with various types of obesity. We have also identified that high insulin, resistin, leptin, ASP and low adiponectin levels exist even in the peripheral or abdominal obesity alone, and these changes are much more notable in the mixed obese children and adolescents, in both girls and boys. In future studies, the follow-up of these children may help to further demonstrate the usefulness of these biomarkers.

This study has some limitations. The study is a cross-sectional design for evaluating the prevalence of obesity and related metabolic abnormalities. And the results only suggest the presence of associations of the adipokines with various types of obesity in children and adolescents. In addition, the participants in the study were recruited for their high-risk of obesity-related metabolic abnormalities, which accounts for why the prevalence of obesity in this specific population is much higher than in the general population (approximate 40% *versus* 8.9%). Moreover, the proportion of peripheral obesity alone is very low, which may reduce the power of statistical analyses.

CONCLUSIONS

The adipokine profile in children and adolescents is related to gender, pubertal development and types of obesity. The obese children have a worse adipokine profile with high resistin, leptin, ASP, and C3, low adiponectin levels as well as increased. Insulin, which becomes aggravated in the presence of mixed obesity. It is important to identify the obese subjects with a malignant adipokine profile through the combination of BMI and waist circumference for the prevention of obesity-related diseases.

Supplemental Table 1. Adipokine Levels in Girls and Boys According to Body Mass Index (BMI) and Waist Circumference Category

Gender	BMI	Waist	*n*	Insulin	Resistin	Leptin	Adiponectin	ASP	C3
				(mU/L)[#]	(ng/mL)[#]	(ng/mL)[#]	(μg/mL)[#]	(nmol/L)[#]	(g/L)[#]
Girls	Normal Weight	Low	919	5.81±1.97	15.2±1.8	3.1±2.8	13.1±1.8	44.36±2.15	1.28±1.72
		High	32	9.27±1.72***	16.7±1.6	9.0±2.3***	10.7±1.7	44.10±2.42	1.17±1.64
	Overweight	Low	99	8.91±1.77	15.0±1.8	8.5±2.2	10.8±1.7	59.95±2.33	1.36±1.53
		High	228	10.52±1.74*	16.7±1.7	12.4±2.1***	9.7±1.7	60.39±2.15	1.39±1.49
	Obese	Low	15	6.39±1.79	16.4±1.4	6.8±2.5	10.9±1.8	70.26±2.00	1.31±1.68
		High	427	12.96±1.83**	16.4±1.6	16.2±2.1**	9.4±1.7	66.03±2.10	1.49±1.60
Boys	Normal Weight	Low	665	4.79±2.02	14.3±1.8	1.0±2.5	13.0±1.8	37.63±2.28	1.33±1.68
		High	8	10.19±1.78**	15.3±1.5	2.8±2.2**	11.7±3.6	25.16±2.39	1.31±2.01
	Overweight	Low	122	7.62±1.91	14.0±1.8	4.0±2.6	11.5±1.8	56.78±2.28	1.34±1.46
		High	205	9.33±1.88	14.8±1.6	6.0±2.3***	8.7±1.8	58.50±2.02	1.51±1.43
	Obese	Low	28	8.49±1.73	15.0±1.7	6.2±2.7	11.1±1.8	56.94±1.89	1.21±1.87
		High	760	11.61±1.98*	15.9±1.7	11.7±2.2***	8.8±1.8	61.58±2.19	1.41±1.56

Note. [#]Data with skew distributions are expressed as geometric mean ± geometric standard deviation and logarithmically transformed for general linear model (GLM) with age and puberty adjustment. Differences between low-waist and high-waist categories in each BMI category are indicated as *P<0.05, **P<0.01, ***P<0.001. ASP, acylation stimulating protein; C3, complement C3; BMI, body mass index; Waist, waist circumference.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTION

ZHANG MeiXian did adipokine assays, analyzed and interpreted data, drafted the manuscript; ZHAO XiaoYuan, CHENG Hong, HOU DongQing collected data and did adipokine assays; WEN Yu did adipokine assays; LI Ming and Katherine CIANFLONE designed and did adipokine assays, interpreted data and made critical revisions on this manuscript. MI Jie designed the study, collected, analyzed and interpreted the data, and made critical revisions on this manuscript.

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