Circulating Osteonectin and Adipokine Profiles in Relation to Metabolically Healthy Obesity in Chinese Children: Findings From BCAMS

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Background—The role of adipokine dysregulation in determining the metabolic fate of obesity is not well studied. We aimed to examine whether the matricellular protein osteonectin and the profiles of certain adipokines could differentiate metabolically healthy obese (MHO) versus metabolically unhealthy obese phenotypes in childhood.

Methods and Results—This study included 1137 obese children and 982 normal-weight healthy (NWH) controls recruited from the BCAMS (Beijing Child and Adolescent Metabolic Syndrome) study. MHO was defined by the absence of insulin resistance and/or any metabolic syndrome components. Six adipokines—osteonectin, leptin, adiponectin, resistin, FGF21 (fibroblast growth factor 21), and RBP-4 (retinol binding protein 4)—were assessed. Approximately 20% of obese children displayed the MHO phenotype. MHO children had a more favorable adipokine profile than metabolically unhealthy obese children, with lower osteonectin, leptin, and RBP-4 and higher adiponectin (all P<0.05). Compared with normal-weight healthy controls, MHO children displayed increased leptin, resistin, and RBP-4 levels and reduced adiponectin concentrations (all P<0.05) but similar osteonectin and FGF21 levels. Among obese subjects, decreased osteonectin (odds ratio [OR]: 0.82; 95% confidence interval [CI] per standard deviation, 0.70–0.97), RBP-4 (OR: 0.77; 95% CI per standard deviation, 0.64–0.93), and leptin/adiponectin ratio (OR: 0.58; 95% CI per standard deviation, 0.43–0.77) were independent predictors of MHO. In addition, compared with children without abnormalities, those with any 3 adipokine abnormalities were 80% less likely to exhibit the MHO phenotype (OR: 0.20; 95% CI, 0.10–0.43) and 3 times more likely to have metabolic syndrome (OR: 2.77; 95% CI, 1.52–5.03).

Conclusions—These findings suggest that dysregulation of adipokines might govern the metabolic consequences of obesity in children. Low osteonectin levels, along with a healthy adipokine profile, might be used as an early marker of the MHO phenotype. (J Am Heart Assoc. 2018;7:e009169. DOI: 10.1161/JAHA.118.009169.)

Key Words: adipokine • children • metabolic syndrome • metabolism • obesity • osteonectin

The rapid increase in the prevalence and severity of obesity in children and adolescents is likely to increase the incidence of metabolic syndrome (MS) and lower the age of onset of cardiovascular disease (CVD) worldwide.1 However, the presence of these metabolic disturbances varies widely among obese subjects. A unique group of obese people who exhibit better insulin sensitivity than expected for their level of adiposity, labeled as insulin-sensitive obese or metabolically healthy obese (MHO), display a less deleterious metabolic profile.2–4 Several studies have suggested that MHO individuals are more resistant to the development of diabetes mellitus and CVD than metabolically unhealthy obese (MUO) individuals.5–9 However, several recent large studies in adult populations have called into

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Accompanying Tables S1 through S3 and Figures S1, S2 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.118.009169

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Clinical Perspective

What Is New?

• Serum levels of osteonectin, a matricellular protein recently identified as an adipokine, clearly distinguish metabolically healthy obese (MHO) individuals from their metabolically unhealthy obese counterparts and are comparable in MHO and normal-weight healthy people.
• The MHO (versus metabolically unhealthy obese) phenotype in children was also characterized by more favorable profiles of several other adipokines (leptin, adiponectin, and RBP-4 [retinol binding protein 4]) in this study.
• In this large study of Chinese youth, abnormal adipokine levels were associated with increased risk of the metabolic syndrome and reduced likelihood of exhibiting the MHO phenotype.

What Are the Clinical Implications?

• Osteonectin, combined with other adipokines, may be useful in distinguishing between the MHO and metabolically unhealthy phenotypes during childhood.
• Dysregulation of adipokines, including the matricellular protein osteonectin, may govern the metabolic consequences of obesity and provide novel therapeutic targets to combat its deleterious effects.

question the existence of an MHO versus MUO phenotype.10–16

To date, the preponderance of studies examining the existence of obesity phenotypes have focused on adults, with limited investigation into the MHO phenotype in childhood. Because childhood obesity and its metabolic consequences may track into adulthood, leading to increased CVD risk,17 investigating metabolic health and adiposity in childhood may have widespread implications. Better understanding of childhood obesity may uncover novel targets for prevention of insulin resistance and metabolic disorders in children and guide therapeutic decision-making and the allocation of limited medical resources.18

The potential mechanisms that may underlie the differing metabolic profiles in MHO versus MUO individuals are still poorly understood. However, preliminary evidence suggests that adipose tissue itself might be a vital determining factor in the metabolic fate of obesity.3,4 Adipose tissue is now recognized as an important endocrine organ that secretes a variety of bioactive proteins (adipokines), which can greatly influence a host of metabolic effects, insulin resistance, systemic inflammation, and abnormal cardiovascular function.19 Several adipokines such as leptin,20 adiponectin,20 resistin,21 RBP-4 (retinol binding protein 4),22 and FGF21 (fibroblast growth factor 21)23 are being considered as potential novel biomarkers for insulin resistance and CVD risk, and an imbalance in the production of these pro- and anti-inflammatory adipokines in the obese condition may result in multiple complications.1 Although the number of studies assessing adipokine profiles in the MHO phenotype is still limited, the observed associations between adiponectin,24 leptin/adiponectin ratio,25 resistin,26 RBP-4,27 and FGF2128 and the MHO phenotype provide evidence that differing adipokine profiles might contribute to the dissociation of the MHO and MUO phenotypes.3 However, systematic studies of these obesity phenotypes and their accompanying adipokine profiles are still lacking in the pediatric and young adult populations.

Another novel adipokine, osteonectin—alternatively named SPARC (secreted protein acidic and rich in cysteine)—has been recognized recently as a key component in the deleterious effects of obesity, insulin resistance, and diabetes mellitus.29 Osteonectin was known to be a regulator of the extracellular matrix linked to adipose tissue fibrosis. Based on the concept that adipose tissue’s capacity for expansion under caloric excess is essential for obese individuals to maintain metabolic health, we hypothesized that lower osteonectin levels might be a novel biomarker for the MHO phenotype. Using a large representative sample of Chinese children and adolescents from the BCAMS (Beijing Child and Adolescent Metabolic Syndrome) study, we examined the associations among 6 functionally prominent adipokines and various metabolic phenotypes. Our hypothesis was that a favorable adipokine profile, as the reflection of healthy adipose tissue function,4 may play a vital role in establishing the MHO phenotype. And, if so, then these adipokine profiles may be essential to the elucidation of factors underlying the development of metabolic imbalance and comorbidities associated with obesity, particularly in childhood.

Method

Population

Participants were recruited from the BCAMS study,21 an ongoing prospective population-based study of obesity and related metabolic disorders from childhood to adulthood, which is registered at ClinicalTrials.gov (identifier NCT03421444). A representative sample of 19 593 children (aged 6–18 years, 50% male) was recruited from the Beijing area (7 districts: 4 urban and 3 rural) between April and October 2004. Within this cohort, a total of 4500 participants were identified as being at risk for MS based on having ≥1 of the following conditions: overweight by body mass index (BMI) ≥85th percentile, elevated blood pressure (BP) (systolic and/or diastolic BP ≥90th percentile), increased total cholesterol ≥5.2 mmol/L, triglyceride ≥1.7 mmol/L, or fasting blood glucose (FBG) ≥5.6
mmol/L based on initial finger capillary blood tests. Furthermore, all children at risk for MS, together with a parallel reference population of 1095 schoolchildren, were invited to participate in a medical examination including venipuncture blood sample tests. In total, 3211 participants, including 2226 with MS risk, ultimately completed the examination. Of these, 1137 obese participants and 982 normal-weight healthy (NWH) controls with complete data are included in this analysis. A CONSORT (Consolidated Standards of Reporting Trials) diagram of the study sample is shown in Figure S1. Age- and sex-specific BMI percentiles, developed by the Working Group for Obesity in China,30 were used to classify participants as normal weight (<85th percentile for BMI), overweight (≥85th and <95th percentiles for BMI), or obese (≥95th percentile for BMI). Signed informed consent was obtained from participants and/or parents or guardians. The BCAMS study was approved by the ethics committee at the Capital Institute of Pediatrics and Peking Union Medical College Hospital in Beijing. All phases of the study complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki. The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure.

Clinical and Anthropometric Measurements and Questionnaires

Participants’ height, weight, waist circumference, and systolic and diastolic BPs were measured according to our standard protocol.21 BMI was calculated as weight (kg) divided by height in square meters (m²). Percentage of body fat was assessed by bioelectrical impedance analysis (Tanita TBF-300A). Pubertal development was assessed by a pediatrician of the same sex as the child, and pubertal status was scored by Tanner stage from breast development in girls and testicular volume in boys.31

In addition, customized questionnaires were used to collect physical activity and dietary information.27 Physical activity was scored as low (<3 times per week) or as moderate to vigorous (≥3 times per week), according to the frequency of participation in extracurricular physical activities (eg, cycling, hiking, running, swimming) for at least 30 minutes each time. Diet scores were calculated according to the consumption frequency of several items including breakfast, beans, seafood, milk, vegetables, fruits, red meat, sugar-sweetened beverages, snacks, and Western fast food in the past 2 weeks. The response options were presented as a 5-point Likert scale of the frequency of consumption, from seldom or never to every day. We assigned ascending values for more frequent consumption of favorable foods (seldom or never=1; 1 time every 2 weeks=2; 1–2 times per week=3; 3–5 times per week=4; >5 times per week=5) and descending values for more frequent consumption of unfavorable foods such as red meat, soft drinks, snacks, and Western fast food (seldom or never=5; >5 times per week=1), according to the presumed association of these food items with metabolic variables in the current study. Dietary scores were summed for each participant; a higher score indicated better dietary quality, and vice versa.32 Place of residence was collected as urban or rural area.

Laboratory Measurement

Venous blood samples were collected after an overnight (≥10-hour) fast. The samples were aliquoted and immediately frozen for future analysis. Blood lipids and glucose were assayed using the Hitachi 7060 C automatic biochemistry analysis system. Insulin, leptin, and adiponectin were measured by ELISA, which was developed in the Key Laboratory of Endocrinology, Peking Union Medical College Hospital. The insulin assay had an interassay coefficient of variation of <9.0% and no cross-reactivity to proinsulin (<0.05%).21 An insulin resistance index was calculated by homeostasis model assessment of insulin resistance (HOMA-IR) as (fasting insulin μIU/L)×(FBG mmol/L)/22.5. The intra- and interassay coefficients of variation for leptin were <7.4% and <9.3%, respectively,33 and <5.4% and <8.5% for adiponectin, respectively.34 Serum resistin and FGF21 were measured by an ELISA kit (Phoenix Pharmaceuticals). The intra- and interassay coefficients of variation for resistin were <5.2% and <10.1%, respectively,21 and <6.0% and <8.6% for FGF21, respectively.35,36 RBP-4 was measured by commercial ELISA kits (Dou set; R&D Systems), with intra- and interassay coefficients of variation of <6.2% and <8.5%, respectively. For measurement of osteonectin/SPARC, mouse anti-human SPARC monoclonal antibody ON1-1, biotinylated polyclonal goat anti-human SPARC antibody EYR01, and human SPARC HON-3030 were used to establish an ELISA system. The intra- and interassay coefficients of variation were <5.2% and <9.1%, respectively.35 All samples were tested in duplicate in a blinded manner.

Definitions

The pediatric MS was defined by the modified criteria of Adult Treatment Panel III, that is, the presence of ≥3 of the following 5 components: (1) central obesity, defined as waist circumference ≥90th percentile for age and sex (established based on the BCAMS study); (2) systolic and/or diastolic BP ≥90th percentile for age, sex, and height; (3) hypertriglyceridemia, defined as triglyceride ≥1.24 mmol/L; (4) low serum HDL-C (high-density lipoprotein cholesterol), defined as ≤1.03 mmol/L; and (5) impaired FBG, defined as ≥5.6 mmol/L.21
MHO children were defined as those with HOMA-IR <3.0 (95th percentile of our reference NWH children in BCAMS)37 and absence of elevated systolic and/or diastolic BP, triglyceride ≥1.24 mmol/L, HDL-C ≤1.03 mmol/L, or FBG ≥5.6 mmol/L. Obese participants with ≥1 of these abnormalities were classified as MUO. In addition, the NWH children in the control group did not have insulin resistance or any classical feature of MS.

The presence of an abnormal adipokine profile among obese participants for identifying MHO children was determined by the optimal cut point of the receiver operating characteristic curve for each adipokine.

Statistical Analysis
All statistical analyses were carried out using SPSS v19.0 for Windows (IBM Corp). Level of statistical significance was accepted at P<0.05. All skewed distributions were natural logarithmically transformed before analysis. ANOVA with Bonferroni post hoc comparison was utilized for continuous outcomes. We recognized that body fat distribution and adipokine levels may be influenced by sex and pubertal stage. Thus, we analyzed the levels of adipokines in relation to stage of pubertal development and sex in our study (Tables S1 and S2). We found that the selected 3 adipokines (ie, leptin/adiponectin ratio, RBP-4, and osteonectin) were not affected by pubertal development but yielded a difference by sex. Consequently, we utilized a sex-adjusted z score for adipokines in predicting the MHO phenotype, calculated as (observed value—sex-specific mean)/sex-specific standard deviation. Logistic regression models were applied to analyze the association between each adipokine and metabolic status among obese participants after controlling for sex, age, pubertal stage, residence, diet score, physical activity, and BMI. To facilitate comparisons between adipokines, odds ratios (ORs) and 95% confidence intervals (CIs) were expressed as units per standard deviation. The receiver operating characteristic curve for MUO was used to determine the optimal cut points for abnormality of adipokine levels among obese participants. The optimal cutoff points were defined based on obtaining the maximum Youden index, calculated as sensitivity+specificity–1.38 In considering the sexual dimorphism in the levels of leptin/adiponectin ratio, we explored sex-specific cutoff points for leptin/adiponectin ratio.

Results
Anthropometric and Metabolic Features of MHO
Of 1137 obese participants, 232 exhibited the MHO phenotype and 905 exhibited MUO, representing a prevalence of 20.4% for the MHO phenotype in this obese cohort. The basic features of the study groups are summarized in Table 1.

Compared with MUO, a higher proportion of the MHO participants were prepubertal, female, and urban residents. The MHO phenotype was associated with younger age, more frequent physical activity, and lower adiposity indices (BMI, waist circumference, and percentage of body fat by bioelectrical impedance analysis). After adjusting for BMI and other confounding factors, the MHO phenotype had a more favorable metabolic profile than MUO, with lower BPs, total cholesterol, triglyceride, LDL-C (low-density lipoprotein cholesterol), FBG, fasting insulin, and HOMA-IR but higher HDL-C levels. Compared with NWH controls, in addition to higher BMI, waist circumference, and percent body fat, the MHO phenotype was accompanied by higher BPs, triglyceride, fasting insulin, and HOMA-IR and lower HDL-C levels.

Comparisons of Adipokine Profiles
Figure 1 shows the different levels of various adipokines across the 3 groups (NWH, MHO, and MUO) after adjusting for age, pubertal status, residence, diet score, and physical activity. Serum leptin, leptin/adiponectin ratio, and RBP-4 levels increased gradually and adiponectin concentrations decreased gradually from NWH to MHO and MUO groups (all P<0.05; Figure 1A, 1C, 1F, and 1B). No consistent pattern was observed between MHO and MUO groups with respect to resistin and FGF21 levels, whereas the NWH phenotype had lower concentrations of resistin than either obese group and higher concentrations of FGF21 than the MUO group (P<0.05; Figure 1D and 1E). Interestingly, the levels of osteonectin showed no difference between the MHO and NWH groups but were significantly higher in the MUO group (P<0.01; Figure 1G). Notably, the differences in leptin, adiponectin, leptin/adiponectin ratio, RBP-4, and osteonectin between the MHO and MUO groups remained significant even after adjusting for BMI or waist circumference (Figure S2).

Adipokines as the Independent Predictor of MHO Phenotype in Multivariable Regression Analysis
Separate multivariable logistic regression analysis was performed to examine each adipokine (with sex-specific z score) as a predictor of the MHO phenotype among obese participants. As shown in Table 2A, after adjustment for age, pubertal stages, residence, diet score, physical activity, and BMI, decreased osteonectin (OR: 0.82; 95% CI, 0.70–0.96), RBP-4 (OR: 0.74; 95% CI, 0.62–0.88), leptin (OR: 0.65; 95% CI, 0.50–0.83), and leptin/adiponectin ratio (OR: 0.56; 95% CI, 0.43–0.73) and increased adiponectin (OR: 1.28; 95% CI, 1.07–1.54) were independent predictors of the MHO phenotype; however, FGF21 and resistin were not predictive (all P>0.10) of the MHO phenotype. When all significant adipokines were assessed together (Table 2B), after adjusting for...
Table 1. General Characteristics of Study Participants According to Study Groups

| Variables | NWH (n=982) | MHO (n=232) | MUO (n=905) | P Values |
|-----------|-------------|-------------|-------------|---------|
|           |             |             |             | P1      |
|           |             |             |             | P2      |
|           | MHO vs NWH  | MUO vs NWH  | MHO vs MUO |

Unadjusted

- Male, %
  - NWH: 42.1
  - MHO: 59.9
  - MUO: 65.1
  - P: <0.001*
- Age, y, mean±SD
  - NWH: 12.1±3.2
  - MHO: 10.8±2.9
  - MUO: 11.9±2.8
  - P: <0.001*<0.001*0.133<0.001*
- Pubertal stage, %
  - NWH: <0.001*<0.001*0.133<0.001*
  - MHO: ...
  - MUO: ...
- Residence, urban (%)
  - NWH: 74.8
  - MHO: 72.0
  - MUO: 61.2
  - P: <0.001*
- Diet score, mean±SD
  - NWH: 36.2±4.8
  - MHO: 36.2±5.0
  - MUO: 35.9±4.7
  - P: 0.292 0.969 0.138 0.333
- MVPA, %
  - NWH: 62.3
  - MHO: 60.1
  - MUO: 52.1
  - P: <0.001* ...

Adjusted (mean±SEM)*

- BMI, kg/m²
  - NWH: 17.4±0.1
  - MHO: 25.3±0.2
  - MUO: 26.8±0.1
  - P: <0.001*<0.001*<0.001*<0.001*
- BMI, Z score
  - NWH: −0.28±0.02
  - MHO: 1.89±0.04
  - MUO: 2.00±0.02
  - P: <0.001*<0.001*<0.001*0.018*
- Waist circumference, cm
  - NWH: 61.5±0.2
  - MHO: 79.7±0.4
  - MUO: 84.3±0.2
  - P: <0.001*<0.001*<0.001*<0.001*
- FAT%,
  - NWH: 16.9±0.2
  - MHO: 30.0±0.4
  - MUO: 31.8±0.2
  - P: <0.001*<0.001*<0.001*<0.001*
- SBP, mm Hg
  - NWH: 98±0.3
  - MHO: 105±0.7
  - MUO: 116±0.3
  - P: <0.001*<0.001*<0.001*<0.001*
- DBP, mm Hg
  - NWH: 61±0.3
  - MHO: 67±0.5
  - MUO: 73±0.3
  - P: <0.001*<0.001*<0.001*<0.001*
- Total cholesterol, mmol/L
  - NWH: 4.06±0.03
  - MHO: 3.93±0.05
  - MUO: 4.17±0.03
  - P: <0.001*<0.001*<0.001*<0.001*
- Triglyceride, mmol/L
  - NWH: 0.71±0.02
  - MHO: 0.86±0.03
  - MUO: 1.31±0.02
  - P: <0.001*0.022*0.003*0.001*
- LDL-C, mmol/L
  - NWH: 2.42±0.02
  - MHO: 2.48±0.05
  - MUO: 2.70±0.02
  - P: <0.001*0.296<0.001*0.001*
- HDL-C, mmol/L
  - NWH: 1.59±0.01
  - MHO: 1.38±0.02
  - MUO: 1.25±0.01
  - P: <0.001*<0.001*<0.001*<0.001*
- FBG, mmol/L
  - NWH: 4.9±0.02
  - MHO: 4.9±0.03
  - MUO: 5.2±0.02
  - P: <0.001*0.169<0.001*<0.001*
- Fasting insulin, mIU/L†
  - NWH: 1.46±0.02
  - MHO: 2.06±0.04
  - MUO: 2.62±0.02
  - P: <0.001*<0.001*<0.001*<0.001*
- HOMA-IR†
  - NWH: −0.07±0.02
  - MHO: 0.54±0.04
  - MUO: 1.15±0.02
  - P: <0.001*<0.001*<0.001*<0.001*

ANOVA (continuous variables) and the χ² test (categorical variables) were used in unadjusted analysis, and data were expressed as percentage or mean±SD. GLM was used in adjusted analysis, and data were expressed as mean±SEM. P₁ values were for ANOVA or χ² test of difference in variables including age, sex, pubertal stages, residence, diet score, and physical activity. P₂ was from a post hoc analysis between MHO and NWH, MUO and NWH, and MUO and MHO groups. BMI indicates body mass index; FAT%, percentage of body fat; DBP, diastolic blood pressure; FBG, fasting blood glucose; GLM, general linear model; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; LDL-C, low-density lipoprotein cholesterol; MUO, metabolically unhealthy obese; MHO, metabolically healthy obese; NWH, normal-weight healthy; MVPA, moderate-to-vigorous physical activity.

Comparisons of Metabolic Features Among Obese Participants With the Clustering of Adipokine Abnormalities

Because decreased osteonectin, RBP-4, and leptin/adiponectin ratio were each independent predictors of the MHO...
Figure 1. Comparison of adipokines levels across the 3 groups. Data were natural logarithmically (ln) transformed and expressed in standard deviations after adjusting for age, pubertal stages, residence, diet score, and physical activity. \( P^* \) was further adjusted for body mass index based on the former models. Comparisons are shown across the 3 groups: ln leptin z-score (A), ln adiponectin z-score (B), ln leptin/adiponectin z-score (C), ln resistin z-score (D), ln FGF21 z-score (E), ln RBP-4 z-score (F), and ln osteonectin z-score (G). FGF21 indicates fibroblast growth factor 21; MHO, metabolically healthy obese; MUO, metabolically unhealthy obese; NWH, normal-weight healthy; RBP-4, retinol binding protein 4.
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Table 2. Associations Between Individual Adipokine and MHO Status

| Adipokines       | β  | SE  | OR (95% CI)     | P Value |
|------------------|----|-----|-----------------|---------|
| (A) Adipokines modeled separately |
| Leptin*          | −0.44 | 0.13 | 0.65 (0.50–0.83) | 0.001† |
| Adiponectin*     | 0.25  | 0.09 | 1.28 (1.07–1.54) | 0.007† |
| Leptin/adiponectin* | −0.59 | 0.14 | 0.56 (0.43–0.73) | <0.001† |
| Resistin*        | 0.06  | 0.08 | 1.06 (0.91–1.23) | 0.482   |
| FGF21*           | 0.00  | 0.08 | 1.00 (0.85–1.18) | 0.988   |
| RBP-4*           | −0.30 | 0.09 | 0.74 (0.62–0.88) | 0.001† |
| Osteonectin*     | −0.21 | 0.08 | 0.82 (0.70–0.96) | 0.012† |
| (B) Adipokines modeled together |
| Leptin/adiponectin* | −0.55 | 0.15 | 0.58 (0.43–0.77) | <0.001† |
| RBP-4*           | −0.26 | 0.09 | 0.77 (0.64–0.93) | 0.005† |
| Osteonectin*     | −0.20 | 0.09 | 0.82 (0.70–0.97) | 0.021† |

Data were natural logarithmically transformed, and all ORs were expressed in standard deviations and adjusted for age, pubertal stages, residence, diet score, physical activity, and body mass index. CI indicates confidence interval; FGF21, fibroblast growth factor 21; OR, odds ratio; RBP-4, retinol binding protein 4.

*Per sex-specific standard deviation increment of each natural logarithmic unit of adipokine.
†P<0.05.

Discussion

To identify children with the MHO phenotype, we combined 2 commonly held definitions of MHO (the absence of insulin resistance or any classical MS components) and identified a 20.4% prevalence of the MHO phenotype among obese children in a large pediatric Chinese cohort. Given that adipose tissue secretes a number of adipokines, which can greatly influence metabolism,19 we selected 6 functionally prominent adipokines to examine their associations with this MHO phenotype. First, we found that pediatric participants with the MHO (compared with MUO) phenotype exhibited a more favorable adipokine profile, with lower levels of osteonectin, RBP-4, and leptin but higher levels of adiponectin. Second, we found that a lower leptin/adiponectin ratio and lower levels of RBP-4 and osteonectin independently predicted the MHO phenotype among obese participants, even after adjustment for confounding factors including BMI and waist circumference. In addition, those with a greater number of aberrant adipokines (high osteonectin, RBP-4, and leptin/adiponectin ratio) displayed a higher risk of having central obesity, elevated BP, dyslipidemia, insulin resistance, and MS, with a lower likelihood of the MHO phenotype. Although an association of adiponectin and leptin levels with the MHO phenotype has been reported previously in pediatric and adult African American, Hispanic, and European obese individuals,37,39–41 this is the first large study to examine a pediatric Chinese population. The role of adipokine profiles (including RBP-4 and osteonectin) in differentiating MHO from MUO needs to be confirmed with additional studies; however, our results point to a robust association between circulating adipokines and the metabolic profile in early onset obesity.

This article is the first report of the novel adipokine osteonectin in relation to MHO versus MUO phenotypes. Osteonectin, also called SPARC or BM40, is a predominantly subcutaneous, adipocyte-expressed, profibrotic protein with...
### Table 3. Relationships Between the Number of Adipokine Abnormalities and Metabolic Characters in Obese Participants

| Phenotype | Number of Adipokine Abnormalities | P Values |
|-----------|-----------------------------------|---------|
|           | 0 (n=170) | 1 (n=406) | 2 (n=415) | 3 (n=146) |
| **Unadjusted** |          |          |          |          |
| Male, %    | 35.8     | 40.8     | 32.4     | 32.9     | 0.044*   |
| Age, y, mean±SD | 10.1±2.5 | 11.5±2.9 | 12.3±2.7 | 12.6±2.9 | <0.001*  |
| Pubertal stages, % |          |          |          |          |
| 1          | 64.6     | 42.0     | 34.9     | 29.8     | <0.001*  |
| 2          | 12.2     | 15.2     | 19.6     | 19.1     |          |
| 3          | 10.4     | 14.2     | 15.3     | 20.6     |          |
| 4          | 7.9      | 13.9     | 19.6     | 18.4     |          |
| 5          | 4.9      | 13.9     | 9.7      | 12.1     |          |
| Residence, rural (%) | 37.6     | 42.4     | 32.8     | 30.1     | 0.011*   |
| Diet score, mean±SD | 36.5±4.7 | 35.8±4.9 | 35.7±4.8 | 36.4±4.7 | 0.161    |
| MVPA, %    | 61.0     | 52.6     | 52.1     | 52.8     | 0.251    |
| BMI, kg/m², mean±SD | 24.1±2.9 | 26.0±3.5 | 27.3±3.5 | 28.3±3.8 | <0.001*  |
| **Adjusted (mean±SEM)** |          |          |          |          |
| Waist circumference, cm | 76.1±0.70 | 81.7±0.5 | 86.3±0.5 | 88.9±0.9 | <0.001*  |
| SBP, mm Hg | 108±0.8  | 113±0.6  | 116±0.6  | 119±1.1  | 0.034*   |
| DBP, mm Hg | 69±0.7   | 71±0.4   | 73±0.4   | 74±0.8   | 0.054    |
| Triglyceride, mmol/L | 0.99±0.04 | 1.05±0.02 | 1.34±0.03 | 1.48±0.06 | <0.001*  |
| HDL-C, mmol/L | 1.35±0.02 | 1.28±0.01 | 1.25±0.01 | 1.23±0.02 | 0.772    |
| FBG, mmol/L | 5.10±0.03 | 5.17±0.02 | 5.15±0.03 | 5.15±0.04 | 0.656    |
| Fasting insulin, μU/mL | 2.07±0.04 | 2.38±0.03 | 2.63±0.03 | 2.79±0.06 | <0.001*  |
| HOMA-IR | 0.58±0.04 | 0.91±0.03 | 1.15±0.03 | 1.31±0.06 | <0.001*  |
| Adipokines |          |          |          |          |
| RBP-4, μg/mL | 3.35±0.02 | 3.46±0.02 | 3.61±0.02 | 3.85±0.02 | <0.001*  |
| Osteocalcin, μg/mL | –0.24±0.02 | –0.01±0.02 | 0.23±0.02 | 0.55±0.03 | <0.001*  |
| Leptin/adiponectin | 0.12±0.06 | 0.78±0.05 | 1.42±0.04 | 1.82±0.05 | <0.001*  |
| **MS components (%)** |          |          |          |          |
| Central obesity | 77.6     | 76.1     | 84.3     | 92.5     | 0.050*   |
| High BP | 32.4     | 46.3    | 52.5    | 56.2    | 0.043*   |
| High triglyceride | 21.8     | 26.6    | 42.9    | 55.5    | <0.001*  |
| Low HDL-C | 11.8     | 15.8    | 18.3    | 19.9    | 0.948    |
| Hyperglycemia | 12.4     | 16.0    | 15.4    | 15.1    | 0.846    |
| HOMA-IR ≥3 | 15.3     | 37.4    | 51.8    | 62.3    | <0.001*  |
| MS | 14.7     | 24.9    | 34.5    | 45.9    | 0.005*   |
| MHO | 38.8     | 25.1    | 12.8    | 7.5     | <0.001*  |

ANOVA (continuous variables) and χ² test (categorical variables) were used in unadjusted analysis, and data were expressed as percentage or mean±SD. GLM (continuous variables) and logistic regression analysis (categorical variables) were used in adjusted analysis, and data were expressed as mean±SEM or percentage. P values were for ANOVA and χ² test of difference in variables including age, sex, pubertal stages, residence, diet score, physical activity, and BMI or for GLM test and logistic regression analysis of other variables adjusted for age, sex, pubertal stages, residence, diet score, physical activity, and BMI. BMI indicates body mass index; BP, blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; GLM, general linear model; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; MHO, metabolically healthy obesity; MS, metabolic syndrome; MVPA, moderate-to-vigorous physical activity; RBP-4, retinol binding protein 4; SBP, systolic blood pressure.

*P<0.05.

†Statistical significance in a post hoc test after adjusting for age, sex, pubertal stages, residence, diet score, physical activity, and BMI, where differences from the nonadipokine abnormality group are indicated as P<0.05.

‡Statistical significance in a post hoc test after adjusting for age, sex, pubertal stages, residence, diet score, physical activity, and BMI, where differences vs the group with 1 adipokine abnormality are indicated as P<0.05.

§Statistical significance in a post hoc test after adjusting for age, sex, pubertal stages, residence, diet score, physical activity, and BMI, where differences vs the group with 2 adipokine abnormalities are indicated as P<0.05.

∥Adjusted for age, sex, pubertal stages, residence, diet score, physical activity, and BMI.

¶Skewed distributions were natural logarithmically transformed.
pleiotropic functions that has been recently proposed as playing a decisive role in the pathogenesis of insulin resistance, obesity, and type 2 diabetes mellitus. The influence of osteonectin in insulin resistance and associated metabolic dysfunction may arise from its role in obesity-induced adipose tissue fibrosis by regulating extracellular matrix composition and inhibiting adipogenesis. Fibrosis of adipose tissue may restrict accumulation of triglycerides in this type of tissue, which could lead to elevated circulating triglycerides, systemic hyperlipidemia, and ectopic lipid deposition in the liver and skeletal muscle, contributing to insulin resistance. Our previous study and others have revealed an association between increased osteonectin/SPARC levels and insulin resistance in gestational diabetes mellitus, type 2 diabetes mellitus, diabetic retinopathy, and nephropathy. In the present study, osteonectin levels were lower in MHO and, in fact, comparable to levels found in NWH people. Consistent with this finding, we found significantly lower in MHO than MUO children. However, another study including more participants failed to identify a significant difference between MUO and MHO groups. Previous studies evaluating circulating leptin levels in adults with obesity did not find a significant difference between MHO and MUO groups. In addition, a small study (n=28) showed similar leptin levels in insulin-resistant and insulin-sensitive adolescents using the hyperinsulinemic–euglycemic clamp. Aside from the different criteria used to define the MHO phenotype, these apparently conflicting results might be explained by the different race, age, sex, and sample size characteristics found in the present study.

Adiponectin is the most abundant peptide secreted by adipocytes. It is considered an insulin sensitization factor in lipid and glucose metabolism, with anti-diabetic and antiatherogenic, and anti-inflammatory properties. In contrast to leptin and most other adipokines, a decrease in circulating adiponectin levels, which contributes to the development of insulin resistance, diabetes mellitus, and MS, has been observed in patients with obesity. Given its important role in metabolism, adiponectin has gained a great deal of attention in recent clinical studies of the MHO phenotype. We also found a significant association between higher adiponectin levels and the MHO phenotype in our large pediatric Chinese cohort, which has heretofore never been examined. Because the combination of leptin and adiponectin (ie, leptin/adiponectin ratio) was previously reported to be a better indicator of insulin resistance and MS than any single adipokine, we compared the associations of leptin, adiponectin, and their ratio with the MHO phenotype. Not surprisingly, the leptin/adiponectin ratio was more likely to predict the phenotype than either leptin or adiponectin alone.

Unlike the above-mentioned adipokines, there are no significant differences in levels of FGF21 and resistin between MHO and MUO groups, although levels were increased in comparison to the NWH group. Resistin is secreted mainly by adipocytes in rodents and by mononuclear cells in humans but is hypothesized to play a role in obesity, insulin resistance, and CVD in humans by competing with lipopolysaccharides for binding to TLR4 (Toll-like receptor 4) to mediate some of its proinflammatory effects. Two studies found recently that circulating resistin concentrations were significantly lower in MHO versus MUO people. However, another study including 444 middle-aged obese participants failed to identify a significant correlation between resistin and the MHO phenotype, which was defined less strictly than in the present study. FGF21 is mainly produced by liver and is associated with beneficial metabolic characteristics including reduced body weight and hepatic steatosis, as well as improved insulin sensitivity, lipid profiles, and blood sugar. A small study (n=20) in morbidly obese adults recently showed 2-fold higher FGF21 levels in MUO than MHO participants. Nevertheless, there is a clear need for further studies to improve our understanding of the association between FGF21 and resistin levels and metabolic health.
In an attempt to better understand the role of adipokine expression in the MHO versus MUO phenotypes, we compared adipokine profiles within a group of well-characterized obese participants. As expected, we found that those with more aberrant adipokine levels were more likely to exhibit the MUO phenotype; however, MHO participants still displayed a metabolic profile predisposed to early CVD16 (including elevated BP, triglyceride, FBG, fasting insulin, HOMA-IR, leptin, leptin/adiponectin ratio, resistin, and RBP-4, as well as decreased HDL-C and adiponectin levels in our study) compared with their NWH counterparts. The continuum of metabolic profiles from NWH to MHO to MUO observed in our large pediatric cohort implies that the MHO phenotype may be a precursor to MUO, as increasing evidence in adults has begun to suggest.2 This observation supports the premise that the MHO phenotype should be identified early and preventive strategies used to avoid the subsequent development of the adverse metabolic profile characteristic of the MUO phenotype.

The strength of our study derives from its large, well-characterized cohort and the systematic analysis of multiple potential adipokines used to distinguish between the MHO and MUO phenotypes. Further impact is derived from our ability to control for pubertal status, dietary habits, and levels of physical activity, which were assessed in this cohort. In addition, insulin resistance as used in our study was defined based on HOMA-IR cutoff (<3.0) in our own population (95th percentile of the reference NWH children in BCAMS). However, several limitations remain. First, the cross-sectional nature of the present study cannot be taken as definitive proof of a longitudinal progression in metabolic profiles, nor can it determine whether a cause-and-effect relationship exists between adipokine levels and the MHO and MUO phenotypes. Second, we did not validate the optimal cut points of the 3 adipokines that were found to be predictive of MHO/MUO in an independent cohort. This is presently being examined in an ongoing follow-up study, which may provide a better understanding of how adipokines, particularly the novel adipokine osteonectin, contribute to achieving and sustaining good metabolic health from childhood into adulthood. Third, our findings were obtained from only Chinese participants and therefore cannot be generalized to other ethnic populations without further replication. Finally, although we included 6 functionally prominent adipokines, we are aware that many others have been implicated in obesity-related disorders.19 Nonetheless, these observations are powerful and contribute to a better understanding of adipokines and their function as mediators or markers of metabolic dysregulation. Unfortunately, the lack of consensus regarding the definitions of MHO versus MUO makes it difficult to compare results across studies. Such comparisons await the development of more uniform definitions for these obesity phenotypes, as has already occurred for diabetes mellitus and the MS.

Conclusions

This study assessed the MHO phenotype among Chinese children by applying a strict definition consisting of preserved insulin sensitivity and the absence of MS criteria. We found that MHO participants were characterized by a relatively favorable adipokine profile, more akin to NWH than the MUO phenotype. Our study indicates that osteonectin and RBP-4, in combination with more well-established adipokines (leptin and adiponectin), may serve as novel markers to distinguish MHO from MUO individuals during childhood. Because the alterations in adipokine profiles in obesity precede the presence of metabolic disturbances, more detailed analysis of our data may allow these levels to be assembled into an “adipokine score” that reflects metabolic risk, much the way that NMR LipoProfile (LipoScience, Lnc.) measures have been used to derive the lipoprotein insulin resistance score.56

Figure 2. Associations between adipokine abnormalities and MHO and MS in obese participants. Data were calculated by logistic regression with adjustment for age, sex, pubertal stages, residence, diet score, physical activity, and body mass index. Participants with no abnormality of these adipokines were considered as reference. ***P<0.001. **P<0.01. CI indicates confidence interval; MHO, metabolically healthy obesity; MS, metabolic syndrome; OR, odds ratio.
Nonetheless, our findings suggest that mechanisms affecting adipokine regulation and adipose tissue expansion, beyond simple caloric excess, may contribute to the metabolic consequence of obesity. Consequently, our study may help in the early identification of high-risk individuals and pave the way for optimization of prevention and treatment strategies to combat obesity.

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Disclosures
None.

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SUPPLEMENTAL MATERIAL
Table S1. The levels of adipokines according pubertal development.

| Adipokines               | Normal weight healthy | Metabolically healthy obese | Metabolically unhealthy obese |
|--------------------------|-----------------------|-----------------------------|-------------------------------|
|                          | Pre-puberty | Mid-puberty | Post-puberty | P  | Pre-puberty | Mid-puberty | Post-puberty | P  | Pre-puberty | Mid-puberty | Post-puberty | P  |
| Leptin (ng/ml)           | 0.26 ± 0.14  | 0.39 ± 0.05  | 0.97 ± 0.14* | <0.001 | 2.29 ± 0.20  | 2.25 ± 0.13  | 2.29 ± 0.44  | 0.964 | 2.63 ± 0.09  | 2.54 ± 0.05  | 2.69 ± 0.10  | 0.118 |
| Adiponectin (ug/ml)      | 1.84 ± 0.09  | 1.81 ± 0.03  | 2.06 ± 0.09* | 0.001 | 1.83 ± 0.14  | 1.48 ± 0.09  | 1.35 ± 0.31  | 0.129 | 1.65 ± 0.06  | 1.40 ± 0.03* | 1.34 ± 0.07* | <0.001 |
| Leptin/adiponectin       | -1.55 ± 0.18  | -1.40 ± 0.06  | -1.09 ± 0.17  | 0.243 | 0.46 ± 0.25  | 0.78 ± 0.16  | 0.94± 0.55  | 0.630 | 0.98 ± 0.10  | 1.14 ± 0.06  | 1.34 ± 0.12  | 0.207 |
| FGF21 (pg/ml)           | 6.84 ± 0.23  | 6.49 ± 0.08  | 6.22 ± 0.22  | 0.306 | 6.50 ± 0.29  | 6.23 ± 0.21  | 6.11 ± 0.67  | 0.785 | 6.33 ± 0.15  | 6.14 ± 0.08  | 6.47 ± 0.18  | 0.030 |
| Resistin (ng/ml)        | 2.76 ± 0.09  | 2.67 ± 0.03  | 2.66 ± 0.09  | 0.555 | 2.84 ± 0.14  | 2.88 ± 0.09  | 2.60 ± 0.31  | 0.287 | 2.72 ± 0.06  | 2.79 ± 0.03  | 2.84 ± 0.07  | 0.536 |
| RBP-4 (ug/ml)           | 3.41 ± 0.06  | 3.35 ± 0.02  | 3.28 ± 0.06  | 0.518 | 3.40 ± 0.07  | 3.52 ± 0.05  | 3.61 ± 0.161 | 0.538 | 3.56 ± 0.03  | 3.57 ± 0.02  | 3.58 ± 0.04  | 0.936 |
| Osteonectin (ug/ml)     | 0.01 ± 0.05  | 0.03 ± 0.03  | -0.06 ± 0.05 | 0.235 | -0.05 ± 0.06 | 0.09 ± 0.07  | 0.13 ± 0.12  | 0.364 | 0.17± 0.04  | 0.14 ± 0.03  | 0.10 ± 0.05  | 0.692 |

The puberty categories were defined as prepuberty (Tanner stage 1), midpuberty (Tanner stage 2-3), postpuberty (Tanner stage ≥ 4).

* Skewed distributions were natural logarithmically (ln) transformed.

Data were expressed as mean ± SEM. General linear model was used to assess the differences among the three groups after adjusting for age, sex, residence, diet score, and physical activity. Where differences versus prepuberty group were indicated as *P < 0.05, differences versus Midpuberty group were indicated as *P < 0.05.

Values in bold are significant at P < 0.05.

FGF21, Fibroblast growth factor 21; RBP-4, Retinol binding protein 4.
### Table S2. The levels of adipokines according to sex.

| Adipokines          | Normal weight healthy |          | Metabolically healthy obese |          | Metabolically unhealthy obese |          |
|---------------------|-----------------------|----------|----------------------------|----------|-------------------------------|----------|
|                     | Male                  | Female   | \( P \)                    | Male     | Female                        | \( P \)  |
| Leptin (ng/ml) \( ^\& \) | 0.034 ± 0.05          | 0.91 ± 0.04 | <0.001                  | 2.14 ± 0.08 | 2.49 ± 0.10 | \( 0.010 \) |
| Adiponectin (ug/ml) \( ^\& \) | 1.83 ± 0.03         | 1.95 ± 0.03 | 0.005                   | 1.57 ± 0.05 | 1.76 ± 0.07 | \( 0.048 \) |
| Leptin/adiponectin \( ^\& \) | -1.79 ± 0.06         | -1.03 ± 0.05 | <0.001                  | 0.57 ± 0.09 | 0.73 ± 0.12 | 0.329     |
| FGF21 (pg/ml) \( ^\& \) | 6.52 ± 0.08          | 6.50 ± 0.06 | 0.835                   | 6.43 ± 0.11 | 6.25 ± 0.15 | 0.378     |
| Resistin (ng/ml) \( ^\& \) | 2.65 ± 0.03          | 2.73 ± 0.03 | 0.099                   | 2.80 ± 0.05 | 2.80 ± 0.07 | 0.987     |
| RBP-4 (ug/ml) \( ^\& \) | 3.34 ± 0.02          | 3.35 ± 0.02 | 0.839                   | 3.44 ± 0.03 | 3.52 ± 0.04 | 0.090     |
| Osteonectin (ug/ml) \( ^\& \) | -0.003 ± 0.03       | -0.016 ± 0.02 | 0.738                  | 0.08 ± 0.04 | -0.06 ± 0.06 | 0.095     |

\( ^\& \) Skewed distributions were natural logarithmically (ln) transformed.

Data were expressed as mean ± SEM. General liner model was used to assess the differences between the two groups after adjusting for age, pubertal stages, residence, diet score, and physical activity.

Values in bold are significant at \( P < 0.05 \).

FGF21, Fibroblast growth factor 21; RBP-4, Retinol binding protein 4.
Table S3. Area under ROC curves and optimal cut-offs of adipokines in predicting MUO.

| Adipokines                  | AUC  | 95%CI     | Optimal cut-offs | Sensitivity | Specificity | Youden index |
|-----------------------------|------|-----------|------------------|-------------|-------------|--------------|
| RBP-4                       | 0.604| 0.564-0.643| 37.08            | 0.441       | 0.741       | 0.182        |
| Osteonectin                 | 0.573| 0.532-0.614| 1.11             | 0.527       | 0.595       | 0.122        |
| Leptin/adiponectin          |      |           |                  |             |             |              |
| Male                        | 0.653| 0.592-0.694| 2.44             | 0.594       | 0.633       | 0.227        |
| Female                      | 0.686| 0.626-0.746| 2.78             | 0.674       | 0.624       | 0.298        |

ROC, Receiver operating characteristic curve; MUO, Metabolically unhealthy obesity; RBP-4, Retinol binding protein 4.
A representative sample of 19,593 children (aged 6–18 years, 50% boys) was recruited from the Beijing area between April and October, 2004. Within this cohort, a total of 4,500 subjects were identified to be at risk for metabolic syndrome based on having one or more of the following conditions: overweight, elevated blood pressure (BP) (systolic and/or diastolic BP ≥ 90th percentile), increased total cholesterol ≥ 5.2 (mmol/L), triglyceride ≥ 1.7 (mmol/L) or fasting blood glucose ≥ 5.6 (mmol/L) based on initial finger capillary blood tests. Further, all children at risk for metabolic syndrome, together with a parallel reference population of 1,095 schoolchildren, were invited to participate in a medical examination including venepuncture blood sample tests. Finally, 3,211 subjects (2,226 from subjects with risk of metabolic syndrome and 985 from reference school children) completed the full examination. The current study includes 1,137 obese participants and 982 normal weight healthy controls who had complete data for analysis.

---: Indicating sample source.
Figure S2. Comparison of adipokines levels between MHO and MUO after adjusting for waist circumference.

Data were analysed after natural logarithmic (ln) transformation and expressed in each SD units.
after adjusting for age, pubertal stages, residence, diet score, physical activity and waist circumference. Comparisons between MHO and MUO for Ln Leptin Z-score (A), Ln Adiponectin Z-score (B), Ln Leptin/adiponectin Z-score (C), Ln RBP4 Z-score (D) and Ln Osteonectin Z-score (E). RBP-4, Retinol binding protein 4.