ARTICLE

Carotid Intima-Media Thickness and Serum Endothelial Marker Levels in Obese Children With Metabolic Syndrome

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Objective: To investigate carotid intima-media thickness (IMT) and serum endothelial marker levels in obese Chinese children.

Design: Observational and descriptive study.

Setting: Hangzhou, China.

Participants: A total 131 obese children, including 29 with at least 2 components of metabolic syndrome (MS) (MS group), 102 with less than 2 components of MS (obese group), and 31 nonobese children (control group) were enrolled.

Main Outcome Measures: Intima-media thickness, von Willebrand factor (vWF) level, and thrombomodulin level.

Results: Compared with the control group, the obese group had greater IMT and higher vWF level ($P < .05$ for all). The mean (SD) vWF levels in the obese, MS, and control groups were 2.08 (0.78), 2.42 (0.98), and 1.54 (0.48) IU/mL, respectively, which were significantly different ($P < .001$). Intima-media thickness in the obese and MS groups was significantly greater than that in the control group. Intima-media thickness in the MS group was greater than that in the obese group. Multiple regression analysis showed that ratio of waist to height, vWF level, and triglycerides level were independent determinants of IMT.

Conclusions: Our findings suggest endothelial injury in obese children. Intima-media thickness and vWF level might be useful to identify the degree of endothelial damage.

Arch Pediatr Adolesc Med. 2010;164(9):846-851

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THE WORLDWIDE PROBLEM OF obesity in children has become a crisis in public health.[1] Obesity used to manifest in adult life and is beginning to manifest in childhood.[2] Between 1980 and 2000, the incidence of overweight children aged 6 to 11 years more than doubled, while the incidence of overweight adolescents aged 12 to 19 years tripled.[3] Adulthood obesity is a significant independent predictor of cardiovascular risk and mortality.[4-6] Previous studies[7] showed that obese children, especially those with metabolic syndrome (MS), had biochemical and inflammatory factors that affect vascular physiologic function. However, the mechanisms of how a given cluster of risk factors influences the early development of pathologic vascular function in children are not completely understood.

Some variables, including von Willebrand factor (vWF) level, thrombomodulin (TM) level, circulating adhesion molecules (eg, ICAM-1 [(intercellular adhesion molecule 1)], VCAM-1 [vascular cell adhesion molecule-1], and E-selectin), and circulating endothelial progenitor cells,[7] are believed to have an important role in vascular disease. However, the exact mechanisms are unclear. von Willebrand factor is a multimeric high-molecular-weight glycoprotein synthesized by endothelial cells and megakaryocytes and stored in Weibel-Palade bodies of endothelial cells and α-granules of platelets. Atherosclerotic damage of endothelial cells is associated with increased levels of vWF[8] and is thought to be significantly related to peripheral vascular disease and coronary heart disease.[9,10] Thrombomodulin is a transmembrane anticoagulant glycoprotein synthesized by endothelial cells and secreted into the circulation. It is associated with atherosclerosis as an indicator of endothelial cell stress.[11] Previous studies[10,12,13] demonstrated that circulating serum TM levels positively correlate with coronary artery disease.

Noninvasive detection methods have recently been established to assess vascular function in children, which allow early detection of endothelial dysfunction before

clinical manifestations of atherosclerosis.[7] High-resolution B-mode ultrasonography was used to measure carotid intima-media thickness (IMT). It is a feasible, direct, and noninvasive method to evaluate and detect preclinical arterial wall lesions. Childhood studies[14-16] showed significantly greater IMT in children with type 1 diabetes mellitus, hypertension, and familial hypercholesterolemia. Moreover, studies[17,18] demonstrated greater IMT in obese adult patients than in lean control subjects. Some investigations in adults have shown that serum endothelial marker levels were indicators of vascular injury.[19] However, other investigators have concluded the opposite.[20]

Similar to their adult counterparts, children and adolescents with MS have demonstrated biochemical and inflammatory factors that affect endothelial and vascular physiologic function.[21] The underlying physiologic abnormality in patients with MS may be an increase in insulin resistance, and the impairment degree of insulin resistance confers premature atherogenicity and is linked to adult conventional cardiovascular risk factors.[22] However, few data about both IMT and serum endothelial marker levels in obese children have been reported, especially in Chinese children. Our study was designed to investigate IMT and serum endothelial marker levels in obese Chinese children.

METHODS

SUBJECTS

A total of 131 obese Chinese children were enrolled in the study from July 1, 2008, to February 28, 2009. These patients were recruited from our Department of Endocrinology. Children with other endocrine diseases, hereditary diseases, viral hepatitis, and kidney or infectious diseases were excluded. Obesity was defined as a body mass index (BMI) exceeding the 95th percentile for the Chinese pediatric population.

The subjects were divided into 2 groups (obese vs MS) according to features of MS defined by the International Diabetes Federation,[23] including increased waist circumference (>90th percentile) and at least 2 of the following 4 components: (1) impaired fasting blood glucose level (≥101 mg/dL on the suggested oral glucose tolerance test) or type 2 diabetes mellitus (to convert glucose to millimoles per liter, multiply by 0.0555), (2) increased blood pressure (≥130 mm Hg systolic or ≥85 mm Hg diastolic), (3) high serum triglycerides (TG) level (≥150 mg/dL) (to convert TG to millimoles per liter, multiply by 0.0113), and (4) elevated serum high-density lipoprotein cholesterol (>40 mg/dL) (to convert cholesterol to millimoles per liter, multiply by 0.0259). Defined as obese children with less than 2 components of MS, the obese group included 69 boys and 33 girls, with a mean (SD) age of 10.6 (1.8) years (age range, 6.1-14.8 years). Defined as obese children with at least 2 components of MS, the MS group included 21 boys and 8 girls, with a mean (SD) age of 10.6 (1.7) years (age range, 7.6-14.0 years). We used waist circumference data of Hong Kong Chinese children[24] because there were no waist circumference unified data of Chinese children and adolescents.

A control group consisted of 31 healthy nonobese children, with a mean (SD) age of 9.9 (1.5) years (age range, 7.0-13.0 years). They were recruited from the Department of Child Care for health examination.

No significant difference in age or sex was found between these 3 groups ($P > .05$ for all). A detailed medical and family history was obtained from all subjects. Informed consent was obtained from the parents. The study was approved by the ethical committee of The Children's Hospital of Zhejiang University School of Medicine, Hangzhou, China.

ANTHROPOMETRICS

Body weight was determined to the nearest 0.1 kg, and height was measured to the nearest 0.1 cm. Waist circumference was measured at the midlevel between the lower rib margin and the iliac crest, and hip circumference was measured at the level of the trochanter major. Systolic blood pressure and diastolic blood pressure were measured. Body mass index, BMI *z* score, and ratio of waist to height were calculated.

ORAL GLUCOSE TOLERANCE TEST AND BIOCHEMICAL MEASUREMENT

An oral glucose tolerance test was performed (1.75 g/kg of body weight; maximum, 75 g). Blood samples were obtained to determine glucose and insulin levels in the fasting state and 2 hours after the glucose load. Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR), which is calculated as insulin level (in micro–international units per milliliter) × glucose level (in milligrams per deciliter)/405.

Samples for fasting glucose, fasting insulin (FI), total cholesterol, TG, alanine aminotransferase (ALT), and aspartate aminotransferase levels were obtained in the morning after an overnight fast and were measured in the clinical laboratory of our unit. Serum glucose levels were measured using the glucose oxidase method (Beijing North Biotechnology Invest, Beijing, China), with intra-assay and interassay coefficients of variation of 2.1% and 4.4%, respectively. Serum insulin levels were determined by radioimmunoassay (Beijing North Biotechnology Invest). Apolipoprotein A1, apolipoprotein B, triglycerides, and total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol levels were measured by routine laboratory testing (Synchron Clinical System CX4; Beckman Instruments, Columbia, Maryland).

SERUM ENDOTHELIAL MARKER MEASUREMENT

Serum was collected and stored at –80°C until endothelial marker measurement. Serum vWF level (Assaypro LLC, Saint Charles, Missouri) and TM level (American Diagnostica Inc, Greenwich, Connecticut) were determined using commercially available enzyme-linked immunosorbent assay kits according to the manufacturers' protocols, with sensitivities of 0.01 IU/mL and 0.01 ng/mL, respectively.

VASCULAR MEASUREMENTS

In a quiet temperature-controlled room, children were examined in the supine position with the head turned 45° away from the side being imaged. High-resolution B-mode ultrasonography (HD7; Koninklijke Philips Electronics NV, Eindhoven, the Netherlands) of the right and left carotid arteries was performed using a linear 10-mHz transducer. Intima-media thickness was defined as the mean distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface of the far wall, approximately 1 cm proximal to the carotid bulb. The images were captured and stored in a computer and were then measured by another examiner. Three determinations of IMT were obtained and averaged. All measurements were performed by 2 examiners (X.Z.Y. and Chun Lin Wang, MD) who were blinded to the subjects' case status and risk factor level.

Table 1. Clinical Characteristics and Laboratory Data Among the 3 Study Groups

Variable	Control Group (n=31)	Obese Group (n=102)	MS Group (n=29)	*F* Score	*P* Value
Clinical Characteristics					
Age, mean (SD), y	9.9 (1.5)	10.6 (1.8)	10.6 (1.7)	2.001	.14
Sex				0.424	.66
Male	19	69	21	. . .	. . .
Female	12	33	8	. . .	. . .
Height, mean (SD), m	1.35 (0.01)	1.46 (0.12)[a]	1.52 (0.13[a,b]	16.557	<.001
Weight, mean (SD), kg	30.98 (7.67)	59.63 (14.39)[a]	69.48 (18.90)[a,b]	63.282	<.001
BMI, mean (SD)	16.78 (2.45)	27.64 (3.50)[a]	29.62 (4.48)[a,b]	131.845	<.001
BMI *z* score, mean (SD)	–0.09 (0.92)	3.28 (1.34)	3.74 (1.45)	91.344	<.001
Waist circumference, mean (SD), cm	58.64 (6.46)	87.98 (10.58)[a]	94.05 (9.86)[a,b]	127.053	<.001
Hip circumference, mean (SD), cm	78.92 (9.56)	92.09 (10.27)[a]	97.50 (10.16)[a,b]	28.498	<.001
Ratio of waist to height, mean (SD)	0.43 (0.03)	0.60 (0.05)[a]	0.62 (0.04)[a]	157.907	<.001
Systolic blood pressure, mean (SD), mm Hg	104.39 (7.05)	112.39 (11.86)[a]	119.28 (18.3)[a,b]	10.571	<.001
Diastolic blood pressure, mean (SD), mm Hg	63.65 (6.10)	66.76 (8.68)	69.52 (8.09)[a]	3.914	.02
Laboratory Data, Mean (SD)					
Total cholesterol level, mg/dL	149 (35)	175 (43)[a]	171 (35)[a]	5.203	.006
High-density lipoprotein cholesterol level, mg/dL	61 (14)	52 (11)[a]	38 (9)[a,b]	29.533	<.001
Low-density lipoprotein cholesterol level, mg/dL	80 (28)	107 (31)[a]	110 (27)[a]	9.115	<.001
log(TG)	–0.04 (0.21)	0.11 (0.25)[a]	0.36 (0.17)[a,b]	23.452	<.001
log(FI)	0.77 (0.31)	1.12 (0.32)[a]	1.34 (0.32)[a,b]	24.295	<.001
Ratio of apolipoprotein B level to apolipoprotein A1 level	0.52 (0.17)	0.65 (0.21)[a]	0.72 (0.20)[a]	7.089	.001
log(ALT)	1.16 (0.20)	1.58 (0.34)[a]	1.68 (0.37)[a]	25.299	<.001
log(HOMA-IR)	0.13 (0.31)	0.46 (0.33)[a]	0.71 (0.35)[a,b]	23.757	<.001

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); ellipses, not applicable; FI, fasting insulin; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triglycerides.

SI conversion factor: To convert total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol to millimoles per liter, multiply by 0.0259.

[a] $P<.05$ compared with the control group.

[b] $P<.05$ compared with the obese group.

STATISTICAL ANALYSIS

Statistical analysis was performed using commercially available software (SPSS version 16.0; SPSS Inc, Chicago, Illinois). Quantitative data with normal distribution are given as the mean (SD), and variables with skewed distribution are log transformed and presented as the mean (SD). Differences among the 3 groups were analyzed using 1-way analysis of variance, followed by least significant difference test multiple comparisons procedures. Pearson product moment correlation χ^2 test was used to compare the enumeration data among groups. Bivariate correlation analysis was used to examine the association between IMT and other variables. Stepwise multiple linear regression models were used to examine the determinants of IMT. The significance level was set at $P<.05$.

RESULTS

CLINICAL CHARACTERISTICS AND LABORATORY DATA

Characteristics of the study population are given in **Table 1**. Compared with the control group, the obese group and the MS group had significantly greater height, weight, BMI, waist circumference, hip circumference, systolic blood pressure, ratio of waist to height, ratio of apolipoprotein B level to apolipoprotein A1 level, log(HOMA-IR), log(TG), log(ALT), log(FI), and total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol levels. Compared with the obese group, the MS group had significantly greater height, weight, BMI, waist circumference, hip circumference, systolic blood pressure, log(HOMA-IR), log(TG), and log(FI) and had a significantly lower high-density lipoprotein cholesterol level. There was no significant difference in ratio of apolipoprotein B level to apolipoprotein A1 level, log (ALT), total cholesterol level, or low-density lipoprotein cholesterol level between the MS group and the obese group.

SERUM ENDOTHELIAL MARKER LEVELS AND VASCULAR MEASUREMENTS

The mean (SD) vWF levels in the obese, MS, and control groups were 2.08 (0.78), 2.42 (0.98), and 1.54 (0.48) IU/mL, respectively. The MS group had a higher vWF level than the other 2 groups, and the obese group had a higher vWF level than the control group ($P<.05$ for all). There was no significant difference in the TM level among the 3 groups, as summarized in **Figure**, A and B ($F=0.973$, $P=.38$).

The mean (SD) right IMT among the obese, MS, and control groups was 0.66 (0.12), 0.73 (0.13), and 0.37 (0.09) mm, respectively; the mean (SD) left IMT was 0.67 (0.14), 0.75 (0.15), and 0.36 (0.08) mm, respectively; and the mean (SD) IMT was 0.67 (0.12), 0.74 (0.17), and 0.37 (0.08) mm, respectively. Compared with the control group, the obese group and the MS group had significantly greater IMT in the left and right carotid arteries ($P<.001$ for both). Compared with the obese group, the MS group had significantly greater IMT ($P=.005$ for the

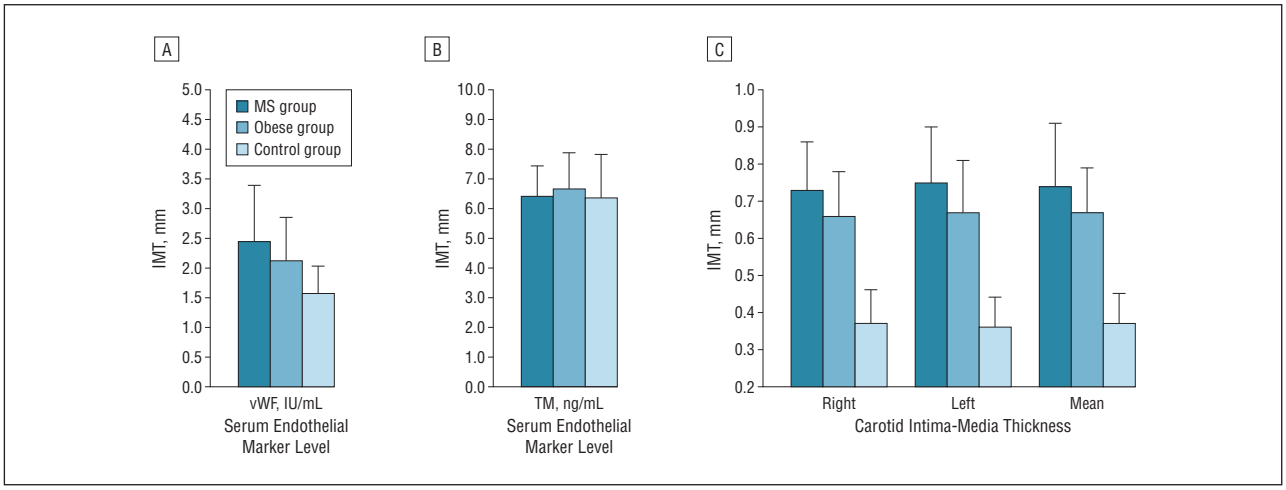

Figure. Carotid intima-media thickness (IMT) (A and B) among the 3 study groups (A) and endothelial markers (C). MS indicates metabolic syndrome; TM, thrombomodulin level; and vWF, von Willebrand factor level.

right carotid artery, $P = .01$ for the left carotid artery, and $P = .005$ for the mean), as shown in the Figure (panel C).

CORRELATION BETWEEN IMT AND OTHER VARIABLES

When analyzing the correlation between IMT and other variables, positive correlations were found between IMT and age, BMI, systolic blood pressure, ratio of waist to height, ratio of apolipoprotein B level to apolipoprotein A1 level, log(HOMA-IR), log(TG), log(ALT), log(FI), and vWF, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol levels ($P < .05$ for all). No significant correlation was found between IMT and TM level or diastolic blood pressure ($P > .05$ for both), as summarized in **Table 2**.

Stepwise multiple regression analysis of IMT included all variables for which bivariate correlation analysis resulted in $P < .10$. The ratio of waist to height, vWF level, and log(TG) were independent determinants of IMT. Intima-media thickness positively correlated with ratio of waist to height, log(TG), and vWF level ($P < .05$ for all) (**Table 3**). Excluded from the equations were age, systolic blood pressure, ratio of apolipoprotein B level to apolipoprotein A1 level, log(HOMA-IR), log(ALT), log(FI), and total cholesterol and high-density lipoprotein cholesterol levels.

COMMENT

Obesity, especially MS, represents a clustering of risk factors known to promote or increase subsequent cardiovascular disease. Recently, an 11-year-old obese child with MS complicated by ischemic stroke was seen in our unit.[25] Evidence has shown that MS is accompanied by a thrombotic and proinflammatory state[7]; however, the mechanistic effects of fat mass on vascular health are poorly understood.[7]

Childhood obesity has been associated with endothelial dysfunction. Several studies have investigated circulating vWF levels in obese children, with discrepant results. A 2007 study[26] showed that obese prepubertal children had elevated vWF levels that were significantly associated with several MS variables. However, another study[20] showed no significant difference in vWF levels between obese children and control subjects. In the present study, we noted higher vWF levels in obese Chinese children with MS. These findings support that vWF level is a good marker in measuring endothelial damage. Higher vWF levels suggest increased cardiovascular disease in obese children, especially those with MS.[27] Other studies[20,28] have shown that TM levels were significantly elevated in obese children, especially those with MS loads. However, no significant difference was observed in our study between obese children with and without MS. The discrepant results might be due to the small sample size in our study, different age groups studied, or racial/ethnic variation.

Increased IMT is regarded as one of the first signs of early atherosclerosis, and the measurement of IMT is considered a safe, inexpensive, precise, and reproducible method to predict future coronary disease and myocardial infarction at early stages of life. Most obese patients have greater IMT than lean control subjects. A recent study[29] showed that obese subjects with MS had greater mean IMT than those without MS in each racial/ethnic group investigated. In our study, IMT was greater in obese children, especially those with MS. This suggests that vascular lesions can be present in early childhood.

Some studies[30,31] have shown that ratio of waist to height is a simple and practical index for assessing central fat distribution and metabolic risk. Because this ratio takes into account children's height, a single cutoff point can likely be set for the ratio, without age and sex bias. Ratio of waist to height is widely used to assess central fat distribution. We found that the mean IMT was associated with ratio of waist to height and with vWF level. These results confirm that IMT is related to the severity of obesity, especially abdominal obesity, and suggest that endothelial cells in obese children may be injured. Moreover, the degree of endothelial cell injury increased with the accumulation of MS components. These pathologic changes can be assessed by measurement of IMT.

Table 2. Correlation Between Intima-Media Thickness and Other Variables

Variable	*r* Coefficient	*P* Value
Age	0.241	.002
BMI	0.665	<.001
BMI *z* score	0.579	<.001
Ratio of waist to height	0.645	<.001
Systolic blood pressure	0.188	.02
Diastolic blood pressure	0.131	.20
log(TG)	0.360	<.001
Total cholesterol level	0.226	.004
High-density lipoprotein cholesterol level	−0.337	<.001
Low-density lipoprotein cholesterol level	0.185	.02
log(ALT)	0.384	<.001
log(FI)	0.322	<.001
log(HOMA-IR)	0.312	<.001
Ratio of apolipoprotein B level to apolipoprotein A1 level	−0.150	.06
von Willebrand factor level	0.356	<.001
Thrombomodulin level	0.126	.11

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; FI, fasting insulin; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triglycerides.

Table 3. Stepwise Multiple Regression Analysis of Intima-Media Thickness

Variable	β Level (SE)	*t* Statistic	*P* Value	Adjusted R^2
Constant	−.044 (.071)	−0.622	.54	
Ratio of waist to height	1.037 (.126)	8.201	<.001	
log(TG)	.129 (.039)	3.321	.001	0.441
von Willebrand factor level	.029 (.012)	2.315	.02	

Abbreviation: TG, triglycerides.

Adipose tissue has become increasingly important in understanding the role of obesity in vascular disease. It produces several biologically active cytokine-like molecules that could mediate the increased risk of endothelial injury associated with obesity. Hypertriglyceridemia is associated with predominance of small, dense, low-density lipoprotein cholesterol particles, which are more toxic to endothelium. In our study, significant correlation was observed between IMT and TG level, suggesting that lipids and lipoproteins have an important role in the development of cardiac disease. Previous studies[32-35] indicated that ratio of apolipoprotein B level to apolipoprotein A1 level was an important risk factor for predicting atherosclerotic progression rate. In our study, we found no close relationship between IMT and this ratio in multiple regression analysis, although the ratio was associated with the mean IMT progression rate in bivariate correlation analysis. These differences also might be due to the small sample size in our study or samples of different age or racial/ethnic origin. Our findings suggest that TG level, but not ratio of apolipoprotein B level to apolipoprotein A1 level, correlated with endothelial cell injury. Whether controlling the TG level can protect obese children from cardiac disease requires further study.

Obesity is associated with increased risk of cardiovascular disease, which may persist from childhood and adolescence into young adulthood. Obese children, who more commonly show features of MS, exhibit a clustering of phenotypes associated with increased cardiovascular risk. Some studies[36-38] demonstrated that insulin resistance induced thrombocyte activation and aggregation, promoted smooth-muscle cell proliferation, increased monocyte adhesion molecule expression, and reduced the nitrous oxide bioavailability of endothelium. Recently, adipose tissue has become increasingly important in understanding the role of obesity in vascular disease.[35] Increased serum lipid invades the arterial wall and then stimulates smooth-muscle cell proliferation and mononuclear cell swallowing of lipid.[7,35] Meanwhile, vWF is released from endothelial Weibel-Palade bodies, and platelets adhere to damaged arterial walls and cause further vascular damage.[8] All these effects result in proatherosclerotic changes in the arterial wall. In fact, several biologically active cytokine-like molecules, chronic inflammation, and oxidative stress also have important roles in the development of endothelial dysfunction and atherosclerosis.[26] However, the mechanisms of action are still unclear.

This preliminary study has some limitations. First, the sample was too small to determine additional differences between groups that might exist, and other biochemical markers may not have been detected such as circulating adhesion molecules, circulating endothelial progenitor cells, inflammatory markers (C-reactive protein and cytokines), and adipokines (leptin and adiponectin). Second, we have no data on ABO system blood type phenotype. Because lower vWF levels have been found in subjects with type O blood,[39] this could have affected our findings. Third, no rigorous age limitations were applied in our study, which included prepubertal and postpubertal children. This is an important limitation, as puberty is a period of life characterized by hormonal changes that could influence precocious impairment of the arterial wall. Further study is required to investigate whether other variables affect IMT.

In summary, our findings that IMT and vWF level are increased in obese children with MS suggest endothelial injury in obese children. Intima-media thickness and vWF level might be useful to identify the degree of endothelial damage.

Accepted for Publication: March 8, 2010.
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Author Contributions: *Study concept and design:* Chen and Liang. *Acquisition of data:* Huang and Yang. *Analysis and interpretation of data:* Huang and Liang. *Drafting of the manuscript:* Huang, Yang, and Chen. *Critical revision of the manuscript for important intellectual content:* Zou and Liang. *Statistical analysis:* Huang and Zou. *Obtained funding:* Liang. *Administrative, technical, and material support:* Yang. *Study supervision:* Liang.
Financial Disclosure: None reported.
Funding/Support: This work was supported by the National Key Technology R&D Program of China (grant

2009BAI80B01) and in part by grant 2008C03002-1 from the Zhejiang Science and Technology Agency.
Additional Contributions: We thank the children and their parents for participating in this research project. Fang Hong, MD, Guan Ping Dong, MD, Chun Lin Wang, MD, and Xiu Min Wang, MD, PhD, provided exceptional patient care and organization.

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