Association between Oxidative Stress and Masked Hypertension in a Multi-Ethnic Population of Obese Children and Adolescents

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Objective To evaluate whether oxidative stress is correlated with adiposity, obesity-related metabolic abnormalities, and ambulatory blood pressure (ABP) in a multi-ethnic pediatric population.

Study design We conducted a prospective study enrolling 42 obese children (age, 12.8 ± 2.4 years) and 34 non-obese children (age, 11.8 ± 3.4 years). We measured urine 8-isoprostane and hydrogen peroxide (markers of oxidative stress) in both obese and non-obese groups. In the obese group, we measured the 24-hour ABP and obtained an oral glucose tolerance test, lipid panel, interleukin-6, and tumor necrosis factor-α.

Results 8-isoprostane and hydrogen peroxide were correlated with body mass index standard deviation score and waist circumference. The mean 8-isoprostane and hydrogen peroxide levels of the obese group were higher than those of the non-obese group. In the subset of obese subjects who underwent ABP monitoring, 8-isoprostane was correlated with mean 24-hour systolic blood pressure: within the obese group, 8-isoprostane was higher in obese children with elevated mean 24-hour systolic blood pressure.

Conclusions Our findings provide evidence of a significant correlation between oxidative stress, adiposity, and blood pressure in children. Longitudinal studies in a larger population sample are needed to validate the association between elevated urine 8-isoprostane level and cardiovascular risk factors in an obese pediatric population. *(J Pediatr 2011;158:628-33)*.

In recent years, several disease states in childhood have implicated a causative role for reactive oxygen species (ROS). Oxidative stress describes a condition in which intracellular production of ROS challenges the buffer capacity of cellular antioxidant defense systems, potentially leading to cellular dysfunction or damage.[1] When present at elevated levels or in inappropriate locations, ROS can directly damage vascular endothelium through oxidative modification of lipids, proteins, and DNA and can indirectly enhance vascular inflammation.

Obesity correlates with oxidative stress in humans and mice[2]: excess adiposity leads to increased activation of pro-oxidant enzymes and decreased expression of anti-oxidant enzymes. The resultant increased production of ROS in the adipose tissue induces the synthesis of pro-inflammatory cytokines, which further exacerbate the oxidative stress-mediated endothelial dysfunction and vascular inflammation.[3]

Oxidative stress has been linked with an important cardiovascular risk factor, hypertension.[4,5] Although it has been shown that increased oxidative stress is associated with overt hypertension in obese children, there is no evidence of its association with early, pre-clinical abnormalities of blood pressure. In addition, little is known about the correlation between oxidative stress and obesity-related dyslipidemia in the pediatric population.

In our study, we compared urine 8-isoprostane and hydrogen peroxide, two markers of oxidative stress, in two groups of obese children and age-matched, normal-weight children. In addition, we have evaluated the correlation among these two markers, 24-hour ambulatory blood pressure, and dyslipidemia in obese children.

Methods

Obese (body mass index [BMI] >95th percentile for age and sex) children (6-18 years old) evaluated at the Section of Endocrinology and Diabetes at St. Christopher's Hospital for Children were invited to participate in the study. A control group of

ABP	Ambulatory blood pressure
BMI	Body mass index
HDL	High-density lipoprotein
HOMA-IR	Homeostasis model assessment of insulin resistance
IL	Interleukin
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SDS	Standard deviation score
TNF-α	Tumor necrosis factor-alpha
WBISI	Whole-body insulin sensitivity index

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Supported by a Creight Center Grant provided by Drexel University College of Medicine. The authors declare no conflicts of interest.

 10.1016/j.jpeds.2010.09.081

age-matched, non-obese (BMI ≤95th percentile for age and sex) children was recruited at the ambulatory clinic of St. Christopher's Hospital for Children. Exclusion criteria included: (1) diagnosis of diabetes mellitus (type 1 or type 2) or hypertension; (2) clinic (office) blood pressure ≥95th percentile for sex, age, and height during the initial examination;[6] and (3) use of medications known to affect insulin sensitivity, glucose metabolism, or both (metformin, thiazolidones, steroids, beta-adrenergic agonists or blockers, diuretics). The institutional regulatory board of Drexel University College of Medicine approved the study. Informed consent was obtained from parents and participants on enrollment.

Enrolled subjects underwent an extensive outpatient evaluation. Body weight was measured with a balance scale, and height was measured with a wall-mounted stadiometer. BMI was calculated as weight in kilograms divided by height in squared meters and expressed as a standard deviation score (SDS) by using the Centers for Disease Control and Prevention 2000 program.[7] Waist circumference was measured at the midpoint between the lower edge of the ribcage and the iliac crests. Three blood pressure measurements in a seated position were obtained at least 1 minute apart after approximately 5 minutes of rest, with a random-zero sphygmomanometer and an appropriate cuff size that covered 80% to 100% of the circumference of the non-dominant arm. The average of the 3 measurements (systolic and fifth phase Korotkoff diastolic) was calculated.[6] Detailed medical history and family history were also obtained.

In our obese cohort, we performed an oral glucose tolerance test and obtained a fasting lipid panel (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein [HDL] cholesterol, and triglyceride levels). Insulin resistance was estimated by using two measurements: the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), calculated as fasting plasma glucose (mg/dL) x fasting insulin (μU/mL)/405[8]; and the whole-body insulin sensitivity index (WBISI), calculated according to the formula:

$$\frac{10\,000}{\sqrt{(\text{fasting glucose} \times \text{fasting insulin})(\text{mean glucose} \times \text{mean insulin})}}$$

Serum interleukin (IL)-6 and tumor necrosis factor alpha (TNF-α) levels were measured with enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, Minnesota). Dyslipidemia was defined as the presence of a triglyceride level ≥90th percentile for age and sex,[9] an HDL cholesterol level ≤10th percentile for age and sex,[9] or both. All enrolled children had a BMI >95th percentile for age and sex and normal clinic (office) blood pressure (systolic and diastolic blood pressure <90th percentile adjusted for height, age, and sex).

To evaluate oxidative stress, we collected a random urine sample in both the obese and normal-weight children and measured 8-isoprostane (BIOMOL International, Plymouth Meeting, Pennsylvania) and hydrogen peroxide (Cayman Chemical, Ann Arbor, Michigan) with enzyme-linked immunosorbent assay. Hydrogen peroxide belongs to the ROS and is a ubiquitous, toxic metabolic by-product of oxidative stress and oxidative injury; 8-isoprostane is a product of lipid peroxidation, which is an effect of the increased activity of ROS. Although several markers of oxidative stress have been proposed, the measurement of 8-isoprostane and hydrogen peroxide in the urine are now considered accurate and non-invasive methods for evaluating oxidative stress.[10-13] 8-isoprostane levels were expressed in pg/mg creatinine, and hydrogen peroxide levels were expressed in μmol/L. Both the intra-assay and the interassay coefficient of variation for 8-isoprostane were 11%, and the intra-assay and the interassay coefficients of variation for hydrogen peroxide were 5.5% and 4.6%, respectively. Microalbumin and creatinine levels were measured in the urine sample of obese children with high performance liquid chromatography. IL-6, TNF-α, 8-isoprostane, and hydrogen peroxide level measurements were performed in our research laboratory.

Ambulatory blood pressure (ABP) measurement was performed by using an oscillometric recorder (SpaceLabs model 90207; Spacelabs HealthCare, Issaquah, Washington). The accuracy of the devices was determined by measuring the blood pressure of 15 subjects with both an oscillometric recorder and a mercury sphygmomanometer. Because the difference of the mean values of 3 oscillometric recorder and sphygmomanometer measurements was within 10 mm Hg in all subjects (and within 5 mm Hg in 14 of 15 subjects), we considered the oscillometric recorders adequately calibrated.[14] We used an appropriate-size cuff that covered 80% to 100% of the circumference of the non-dominant arm.[6] ABP measurements were performed for a 24-hour period. Subjects were instructed not to engage in vigorous physical exercise or contact sports and to record their activities during the 24-hour blood pressure monitoring. Recording frequency was programmed for every 20 minutes between 8 AM and 10 PM and every 30 minutes from 10 PM until 8 AM. The data were downloaded and analyzed by using the SpaceLab medical ABP report management system program (Spacelabs HealthCare). Subjects with <65% successful readings were not included in the study.

To be consistent with earlier pediatric studies on ABP,[14-17] we considered a mean ABP value to be elevated when it was ≥95th percentile.[14] On the basis of the exclusion criteria of the study, all enrolled subjects had a normal clinic (office) blood pressure. As a result, subjects with an elevated 24-hour ABP value (≥95th percentile) were diagnosed with "masked hypertension."

Data were analyzed with SPSS software version 16.0 for Windows (SPSS, Chicago, Illinois). All data were expressed as the mean plus or minus SD or range. A P value <.05 was considered to be statistically significant. Differences in the mean values in the groups were evaluated by using a Student t test or analysis of variance. Bivariate correlation among 8-isoprostane, hydrogen peroxide, and selected variables (mean ABP values, HOMA index, WBISI, BMI, waist circumference, fasting glucose, 2-hour glucose, peak insulin, triglycerides, low-density lipoprotein cholesterol, and HDL