Oxidative Stress and Cardiovascular Risk in Overweight Children in an Exercise Intervention Program

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Abstract

Background: This study aimed to determine whether oxidative stress was related to cardiovascular risk indices in children, and whether an exercise intervention would reduce oxidative stress.

Methods: A randomized trial of two different doses of exercise and a no-exercise control group included 112 overweight and obese children, 7–11 years old. Plasma isoprostane levels were obtained at baseline and after the intervention. Cross-sectional analysis of oxidative stress and metabolic markers at baseline was performed. The effect of the exercise training on oxidative stress was tested.

Results: Lower isoprostane levels were observed in blacks. At baseline, isoprostane was positively related to measures of fatness (BMI, waist circumference, percent body fat), insulin resistance and β -cell function (fasting insulin, insulin area under the curve, Matsuda index, disposition index, oral disposition index), and several lipid markers (low-density lipoprotein, triglycerides, total cholesterol), and inversely with fitness [peak oxygen consumption (VO_2)], independent of race, sex, and cohort. No relation was found with visceral fat, blood pressure, or glycemia. Independent of percent body fat, isoprostane predicted triglycerides, $\beta=0.23$, total cholesterol-to-high-density lipoprotein (TC/HDL) ratio, $\beta=0.23$, and insulin resistance (insulin area under the curve, $\beta=0.24$, Matsuda index, $\beta=-0.21$, oral disposition index, $\beta=0.33$). Exercise did not reduce oxidative stress levels, despite reduced fatness and improved fitness in these children.

Conclusions: Isoprostane levels were related to several markers of cardiovascular risk at baseline; however, despite reduced fatness and improved fitness, no effect of exercise was observed on isoprostane levels. To our knowledge, this is the first report in children to demonstrate a correlation of oxidative stress with disposition index, fitness, and TC/HDL ratio, the first to test the effect on oxidative stress of an exercise intervention that reduced body fat, and the first such exercise intervention study to include a substantial proportion of black children.

Introduction

Oxidative stress is the net imbalance between free radicals created from reactive oxygen species and multiple antioxidant mechanisms; these free radicals in turn attack various biological molecules. The F2-isoprostanes are one such group of oxidation products from the nonenzymatic peroxidation of arachidonic acid and are the best available marker of systemic oxidative stress¹ and an emerging biomarker for potential clinical application.² Increasing evidence suggests an association of oxidative stress

markers and human disease,³ and oxidative stress has been proposed as an underlying mechanism in cardiovascular disease.⁴ Free F2-isoprostane has been shown to predict cardiac events.⁵

Oxidative stress has been found to be elevated in children with obesity and features of the metabolic syndrome,^{6–8} and a cross-sectional relationship of isoprostane levels with BMI and insulin resistance has been demonstrated in adolescents.⁹ A positive correlation of visceral adipose tissue with isoprostane levels has been seen in children⁷ and adults.¹⁰ Data on the relationship of oxidative

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stress and lipid levels is limited, but a correlation between isoprostane levels and total cholesterol (TC) has been seen in adults with hypercholesterolemia.¹¹ Reduction in oxidative stress in adults has been reported after an exercise intervention that resulted in weight loss,¹² whereas oxidative stress levels were not shown to change in children with an exercise intervention that did not result in weight loss.¹³

We hypothesized that indices of cardiovascular risk would be correlated with oxidative stress levels in children at baseline. We also hypothesized that aerobic training would result in decreased fatness, increased fitness, and decreased oxidative stress compared to a control condition, and that this change in oxidative stress would be mediated by changes in fatness and fitness.

Methods

A total of 112 healthy overweight or obese ($\geq 85^{\text{th}}$ BMI percentile, 45% male, 63% black) children 7–11 years old underwent anthropometry, hemodynamics, dual-energy X-ray absorptiometry (DXA), abdominal magnetic resonance imaging (MRI), fasting blood draw, and oral glucose tolerance test (OGTT) at baseline in three cohorts, due to capacity and transportation considerations, for a trial of exercise dose on health outcomes. Cohorts varied in composition by race and sex (97%, 47%, 41% black; 64%, 56%, 46% female), with no differences in age or pubertal stage ($p > 0.2$). Only those children in whom isoprostane levels were obtained at baseline were included in this study. Measures were repeated at posttest, which occurred 1–3 days following their last exercise session to minimize the acute effects of exercise.

Anthropometrics were measured at least twice until consistent measures were obtained. Waist circumference was measured in centimeters with a Gulick fiberglass tape with tension gauge (M-22C, Creative Health Products, Plymouth, MI) at the narrowest point of the torso above the umbilicus and below the ribcage. Height and weight were measured by standard methods using a wall-mounted stadiometer and a scale, respectively. BMI percentiles and z-scores were determined based on CDC 2000 growth charts.¹⁴ Tanner staging was performed on each child by a pediatrician. Peak oxygen consumption (VO_2) was measured as previously described¹⁵ via a multistage treadmill test protocol modified from the protocol for poorly fit children proposed by Rowland¹⁶ and the American College of Sports Medicine (ACSM).¹⁷ Seated blood pressure was measured via Dinamap[®] at 1-minute intervals 5 times after 10 minutes of rest. The last three blood pressure measurements were averaged. Body composition was evaluated using DXA (QDR 4500W, Hologic Inc., Bedford, MA), which quantifies body fat; percent body fat adjusts for body mass. Abdominal visceral adipose tissue (VAT) was quantified in cm^3 via MR (five 1 cm transverse slices centered around the L4–L5 disk of the lumbar spine). The basic technique was described previously.¹⁸ Measurements

were performed for fasting serum glucose using the glucose oxidase method (Analox, London, UK), fasting serum insulin using radioimmunoassay (human specific insulin, Linco Research Inc., St. Charles, MO), and plasma lipid profile using a Sirrus analyzer (Boerne, TX). Glucose and insulin area under the curve (AUC) during the 2-hour OGTT (1.75 g/kg dextrose based on ideal body weight, samples q30') were calculated via the trapezoidal rule, as described previously.¹⁹ AUC values are reported in grams/dL ($\text{mg/dL} \times 10^{-3}$) and mU/mL ($\mu\text{U/mL} \times 10^{-2}$) due to large values. The Matsuda index of insulin resistance (higher values indicate less insulin resistance),^{20,21} the disposition index (Matsuda index $\times$ insulinogenic index, higher values indicate better beta cell function),²² and the oral disposition index (1/fasting insulin $\times$ insulinogenic index)²³ were calculated.

Plasma 8-iso prostaglandin F₂ (PGF₂) levels were measured as a batch using the STAT-8-isoprostane kit from Cayman Chemical (Ann Arbor, MI, cat. no. 500431) as recommended by the manufacturer. The assay relies on the detection of 8-iso PGF₂ α , which is generated from random oxidation of tissue phospholipids. Fasting plasma samples were centrifuged and then immediately stabilized with butylated hydroxytoluene. Samples were sent to the reference laboratory, and all 8-iso PGF₂ assays were performed in a blinded manner as a batch, with each sample assayed in duplicate. Each run had an equal mix of pre and post samples. The coefficients of variation (CV) were determined by the manufacturer at multiple points over the standard curve with standard concentrations most relevant to our study (187.5 and 375 pg/mL) having intraassay CV of 7.4% and 6.3%, respectively, and interassay CV of 7.3% and 8.2%, respectively.

The International Diabetes Federation (IDF) criteria were used to assess risk factors for the metabolic syndrome.²⁴ The five risk factors were waist circumference $\geq 90^{\text{th}}$ percentile for age,²⁵ triglycerides ≥ 150 mg/dL, high-density lipoprotein (HDL) < 40 mg/dL, systolic blood pressure (SBP) ≥ 130 or diastolic blood pressure (DBP) ≥ 85 mmHg, and fasting glucose ≥ 100 mg/dL. The total number of risk factors was tallied for each participant. For the purposes of our study, a child was said to have the metabolic syndrome if they met waist circumference criteria plus any two of the other four criteria for all ages, although we note a clinical diagnosis of metabolic syndrome is deferred by IDF criteria in children under the age of 10. Characteristics of the sample are presented in Table 1. Because of varied pediatric risk criteria, the prevalence of obesity,²⁶ impaired glucose tolerance,²⁷ elevated TC and low-density lipoprotein (LDL),²⁸ and elevated blood pressure values (according to the National High Blood Pressure Education Program; $\geq 90^{\text{th}}$ and $\geq 95^{\text{th}}$ percentile for age, gender, and height) are also shown.²⁹

Children were randomly assigned within race, sex, and cohort to one of three experimental conditions: A no-exercise control condition ($n = 42$), low-dose aerobic exercise (20 minutes per day, $n = 34$), or high-dose aerobic

Table 1. Characteristics of Sample at Baseline (N = 112)

Variable	M ± SD
Age (years)	9.3 ± 1.1
Female	55%
African American	63%
BMI (kg/m ²)	25.9 ± 4.9
BMI z-score	2.0 ± 0.5
Obese (≥ 95 th percentile BMI-for-age)	83%
Waist circumference (cm)	76.6 ± 10.5
% Body fat	39.3 ± 6.7
VAT (cm ²)	30.2 ± 17.0
Peak VO ₂ (mL/kg per minute)	28.0 ± 5.5
Fasting insulin (μU/mL)	20.8 ± 11.8
Insulin area under the curve (mU/mL)	14.7 ± 9.0
Fasting glucose (mg/dL)	95.0 ± 7.8
Glucose area under the curve (g/dL)	16.0 ± 2.1
Glucose 120 min (mg/dL)	121.5 ± 21.1
Impaired glucose tolerance (>140 mg/dL)	16%
Matsuda index	2.6 ± 1.6
Disposition index	5.51 ± 3.20
Oral disposition index	0.143 ± 0.085
Total cholesterol (TC, mg/dL)	157.3 ± 31.1
TC ≥200, 170–199 mg/dL	11%, 19%
HDL (mg/dL)	48.3 ± 12.4
LDL (mg/dL)	94.8 ± 28.3
LDL ≥130, 110–129 mg/dL	15%, 10%
Triglyceride (mg/dL)	73.8 ± 36.0
TC/HDL ratio	3.5 ± 1.2
Triglyceride/HDL ratio	1.8 ± 1.4
Systolic blood pressure (SBP, mmHg)	99.4 ± 9.3
SBP ≥95 th , 90–94 th percentile	2%, 2%
Diastolic blood pressure (mmHg)	54.2 ± 5.9
Metabolic syndrome	
Metabolic syndrome	5%
Number of risk factors (0, 1, 2, 3, 4)	32%, 41%, 21%, 4%, 1%
Waist >90 th percentile	51%
Triglyceride ≥150 mg/dL	4%
HDL <40 mg/dL	22%
Impaired fasting glucose (≥100 mg/dL)	22%
SBP >130	1%
F2-Isoprostane (pg/mL)	281.0 ± 85.7

SD, Standard deviation; VAT, visceral adipose tissue; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; SBP, systolic blood pressure.

exercise (40 minutes per day, $n=36$). The exercise programs were offered every school day afternoon for 13 ± 1.7 weeks, with excellent adherence. The average heart rate was 167 ± 7 beats per minute (bpm). Attendance was $86 \pm 14\%$ in the 20-minute group and $88 \pm 14\%$ in the 40-minute group, $p=0.66$. The low- and high-dose groups differed only on time and, therefore, volume of exercise. The intervention has been described previously.¹⁵

Statistical Analyses

Analysis of variance (ANOVA) and correlations assessed cross-sectional relationships of isoprostane levels at baseline with potential covariates (cohort, race, sex, age, pubertal stage). Analysis of covariance (ANCOVA) assessed cross-sectional relationships of isoprostane levels with cardiovascular risk variables at baseline, adjusting for covariates. Interactions with race and sex were tested. Where significant relations with isoprostane were observed, further regression models tested whether relations of isoprostane with cardiovascular risk variables were independent of percent body fat. Linear trend analysis was conducted on risk factor tally, adjusting for covariates, to assess the dose–response relationship with isoprostane level at baseline. Pairwise comparisons examined any significant trend.

Baseline differences between intervention groups were tested with ANOVA. ANCOVA tested group differences on posttest measures of isoprostane, BMI, BMI z-score, waist circumference, percent body fat, VAT, and peak VO₂, adjusting for cohort, race, sex, age, and baseline score. An *a priori* contrast testing linear trend and another comparing the control group to the two exercise groups were performed. Statistical significance was assessed at $\alpha=0.05$.

Results

Race differences were observed at baseline ($t=2.3$, $p=0.02$), with higher isoprostane levels for white [$M \pm$ standard deviation (SD) = 305 ± 81] than black children ($M \pm$ SD = 267 ± 86). There was no relationship with pubarche, gonadarche/thelarche, age, or sex; 75% of the sample was Tanner stage I for gonadarche (boys) or thelarche (girls). Race, sex, and cohort were included as covariates in further models.

Relationships of isoprostane with cardiovascular risk factors at baseline are presented in Table 2. There were no significant interactions with race or sex. Significant relations in the expected direction were found for most, but not all, of the cardiovascular risk factors, covaried for cohort, race, and sex (model 1). BMI, BMI z-score, percent body fat, and waist circumference were positively related to isoprostane, but visceral adiposity was not. Isoprostane was inversely related to fitness and the Matsuda index of insulin sensitivity. No relationships were observed with glycemia, blood pressure, TC, or HDL. Isoprostane was directly associated with measures of insulin resistance (fasting insulin, insulin AUC) and β -cell function

Table 2. Plasma F2-Isoprostane Multiple Regression Models^a

Dependent variable	Model 1	Model 2
BMI	0.30**	NS
BMI z-score	0.27**	NS
Waist circumference	0.33**	NS
% Body fat	0.26*	—
VAT	NS	—
Peak VO ₂	−0.23*	NS
Fasting insulin	0.25*	NS
Insulin area under the curve	0.31*	0.24*
Fasting glucose	NS	—
Glucose area under the curve	NS	—
Matsuda index	−0.32**	−0.21*
Disposition index	0.26*	NS
Oral disposition index	0.34**	0.33**
Total cholesterol	NS	—
HDL	NS	—
LDL	0.24*	NS
Triglyceride	0.29*	0.23*
TC/HDL	0.26*	0.23**
Triglyceride/HDL	0.22*	NS
SBP	NS	—

Model 1, Adjusted for race, sex, and cohort; model 2, adjusted for race, sex, cohort, and percent body fat.

^aStandardized regression coefficients of plasma F2-isoprostane are presented.

* $p < 0.05$.

** $p < 0.01$.

NS, Not significant; VO₂, oxygen consumption; VAT, visceral adipose tissue; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; SBP, systolic blood pressure.

(disposition index, oral disposition index), LDL, triglycerides, TC/HDL, and triglycerides/HDL ratio. Model 2 included the above covariates and was adjusted additionally for percent body fat. Independently of percent body fat, isoprostane was directly related to insulin AUC, oral disposition index, triglycerides, and TC/HDL ratio, and inversely related to the Matsuda index.

The 6 children with metabolic syndrome had similar isoprostane levels to those who did not meet criteria (284 ± 76 vs. 281 ± 87 , $p \geq 0.9$). A linear trend was detected on risk factor tally predicting isoprostane levels, independent of covariates ($p = 0.002$; Fig. 1). Pairwise comparisons showed that the group with zero risk factors had lower isoprostane levels than the group with two or more ($n = 36$,

Figure 1. Plasma F2 isoprostane levels by metabolic syndrome risk factor tally, demonstrating a dose-response relationship. Caption: Linear trend ($p = .002$) on plasma F2-isoprostane level with increasing metabolic syndrome risk factor tally at baseline, adjusting for cohort, race, and sex.

30; $M \pm SD = 181 \pm 6.8$, 212 ± 7.6 for zero and two or more risk factors, respectively). The group with one risk factor was intermediate ($n = 46$, 198 ± 5.9), but did not differ from the other groups ($p = 0.054$ vs. zero risk factors, $p = 0.14$ vs. 2 or more risk factors). No quadratic trend was detected.

There were no baseline differences between groups on BMI, BMI z-score, waist circumference, percent body fat, VAT, VO₂ peak, or isoprostane. No effects of intervention were observed on isoprostane levels. Significant reductions in BMI, BMI z-score, waist circumference, percent body fat, VAT, and an increase in VO₂ peak were observed in the exercise groups (Table 3). Additional exercise outcomes are reported elsewhere.¹⁵

Discussion

In this sample of overweight and obese children, oxidative stress (*i.e.*, plasma isoprostane level) was related to cardiovascular risk independent of cohort, race, and sex. Isoprostane was unrelated to age, sex, and pubertal stage. Children who were more aerobically fit had lower levels of oxidative stress. Isoprostane level was directly related to BMI, BMI z-score, waist circumference, percent body fat, insulin resistance, β -cell function, and lipid markers, but not visceral fat. There was no difference between black and white children in relationships of isoprostane with cardiovascular risk indices, although white children had higher isoprostane levels than black children.

Although isoprostane was related with fatness and fitness in sedentary overweight children, a 3-month aerobic training intervention did not reduce levels of oxidative stress, despite inducing relative weight loss, reducing general and visceral adiposity, and improving fitness in this randomized, controlled trial. However, the lack of a significant reduction in oxidative stress from this exercise intervention does not discount the benefits of exercise for reducing cardiovascular risk in children, and a benefit in reducing oxidative stress may still exist that cannot be seen in such a young, relatively healthy sample. A study in adolescents with more advanced cardiovascular risk

Table 3. Fatness, Fitness, and Plasma 8-Isoprostane at Post-Test by Group, Adjusted for Age, Race, Sex, Cohort, and Baseline Value (M ± SE)

Outcome variable	Control group	Low-dose group	High-dose group	p dose ^a	p exercise ^b
BMI (kg/m ²)	26.3 ± 0.16	26.0 ± 0.18	25.3 ± 0.18	<0.001	0.001
BMI z-score	2.07 ± 0.024	2.03 ± 0.026	1.93 ± 0.027	<0.001	0.004
Waist circumference (cm)	77.7 ± 0.59	76.9 ± 0.64	74.8 ± 0.65	0.001	0.02
Body fat (%)	38.8 ± 0.39	37.8 ± 0.42	36.5 ± 0.43	<0.001	0.001
VAT (cm ³)	31.1 ± 1.0	29.2 ± 1.1	26.7 ± 1.1	0.003	0.01
VO ₂ peak (mL/kg per minute)	25.5 ± 1.0	27.9 ± 1.1	29.3 ± 1.2	0.02	0.02
F2-Isoprostane (pg/mL)	252 ± 14	286 ± 15	261 ± 15	0.63	0.21

^ap dose tests the linear trend indicating dose–response.

^bp exercise compares control group to the two exercise groups.

SE, Standard error; VAT, visceral adipose tissue; VO₂, oxygen consumption.

using a similar intervention, or a combined diet and exercise intervention, might show a significant reduction in oxidative stress levels compared to a control condition. To our knowledge, no previous randomized trial has investigated whether an exercise intervention resulting in improved body composition is efficacious in ameliorating systemic oxidative stress. A small randomized trial of overweight children found that isoprostane levels did not change with an 8-week exercise intervention, which improved fitness with no change in weight or body fat.¹³ Roberts and colleagues have observed weight loss and reduced isoprostane in small uncontrolled studies of short-term diet and exercise interventions in overweight children and adolescents,³⁰ diabetic men,³¹ and obese men.¹²

The cross-sectional findings extend the prior literature with a multiethnic sample and detailed measures of fitness, body composition, and metabolism. In a study of Japanese youth, isoprostane was elevated in obese compared to lean children, and it was directly related with visceral fat and waist circumference, but not BMI z-score.⁷ Oxidative stress measured by oxidized LDL levels was higher in overweight than normal weight Minnesota children, and was positively related to BMI, BMI z-score, percent body fat, and visceral fat.³² Isoprostane was higher in a group of adolescents with higher BMI than their leaner peers.⁹ Positive relations of urinary isoprostane with BMI,³³ visceral fat (independent of BMI), and waist circumference¹⁰ have been reported in adults in the Framingham study. Further studies are needed, including representation of black American youths, to further elucidate the relationship of oxidative stress and visceral fat.

Insulin resistance measures were related to isoprostane levels, including positive relations with fasting insulin and insulin AUC, and an inverse relation with the Matsuda index. On the other hand, the disposition index, an excellent predictor of type 2 diabetes onset,²² and the oral disposition index, which has recently been validated in

children and adolescents,²³ were directly related to isoprostane. There is evidence that reactive oxygen species mediate insulin secretion,^{34,35} but more research would be needed to replicate and fully explain the relationship between disposition index and isoprostane seen in our study. Isoprostane was related to several of these indices independently of percent body fat. The relationship of insulin resistance with oxidative stress in the Framingham offspring study was attenuated with BMI.³⁶ Euglycemic clamp studies have demonstrated higher isoprostane in adolescents who were both heavier and more insulin resistant, as compared to leaner or heavy but insulin-sensitive peers.⁹ In a later study, another measure of oxidative stress (*i.e.*, oxidized LDL) was higher in overweight or obese children and adolescents than normal weight peers, and was correlated with BMI, percent fat, waist circumference, visceral fat, and insulin resistance measured via clamp.³² However, these studies provided no data assessing the adequacy of β -cell function. A positive correlation with fasting insulin and the homeostasis model assessment (HOMA) index has been seen in Japanese youth,⁷ but a multiethnic study of obese children detected no correlation of isoprostane with insulin or glucose measures from the OGTT, including the Matsuda index.⁸ The triglycerides/HDL ratio has proven to be a sensitive and specific marker of insulin resistance in nondiabetic adults,³⁷ but was not correlated with isoprostane independently of adiposity in our sample. Measures of glycemia were not correlated with isoprostane levels in our young, nondiabetic sample, despite the evidence for hyperglycemia underlying oxidative stress.⁴

We found that LDL, triglycerides, and the TC/HDL and triglycerides/HDL ratios were positively correlated with isoprostane levels. TC and HDL were unrelated with oxidative stress. Regression models confirmed that the relationships of isoprostane with triglycerides and the TC/HDL ratio were independent of adiposity. Triglycerides and HDL were related to isoprostanes in Japanese youth,⁷

but a multiethnic study of obese children detected no correlation of isoprostane with lipid profile.⁸ A previous study found no difference in oxidative stress between children with hypercholesterolemia and controls.³⁸ In contrast, other studies have found a difference in oxidative stress between children with hypercholesterolemia and age-matched controls.^{39,40} In adults, hypercholesterolemia is also associated with elevated oxidative stress,¹¹ but in a large cohort of predominantly white adults, both TC and TC/HDL ratio were inversely related with isoprostane.³³ Acute, postprandial elevations in triglycerides have been shown to be associated with elevated oxidative stress in a small cohort of adults.⁴¹

SBP was not correlated with oxidative stress levels in our sample, with similar results seen in the adult literature.³³ One study of obese children and adolescents detected a correlation of urinary isoprostane with ambulatory blood pressure (masked hypertension).⁸ It has been difficult to elucidate the relationship between hypertension and oxidative stress. Individual blood pressure measurements may be inadequate to assess the relationship of blood pressure and oxidative stress, because one study found oxidative stress levels to be higher in hypertensives but not correlated with blood pressure values themselves.⁴² Very few children in this study had high blood pressure, which limited the sensitivity of the study.

A dose-response relation was seen with the components of the metabolic syndrome, in agreement with the findings of Araki and colleagues.⁷ However, the metabolic syndrome itself was not significantly correlated with isoprostane levels, probably due to the low prevalence (5%) in our young sample. This is in contrast to the adult literature where such an association has been demonstrated.⁴³ The association with the number of risk factors appeared to be related to central obesity, in combination with dyslipidemia and impaired fasting glucose (IFG). Of the 30 children with two or more risk factors, 19 had low HDL, 14 had IFG, and only 2 had normal waist girth. All 5 children with high triglycerides had metabolic syndrome. Thus, although no relationship was detected in regression models with glycemia or HDL, oxidative stress appears to cluster with each of these metabolic syndrome factors in children.

Conclusions

To our knowledge, this is the first report to demonstrate a correlation of oxidative stress with fitness, β -cell function, and TC/HDL ratio in children, and the first exercise intervention study to include a substantial proportion of black children. This study adds to the emerging literature relating cardiovascular risk to oxidative stress in children at a time of increasing concern about children's health.

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