

## Original Article

## Relation of circulating oxidized LDL to obesity and insulin resistance in children

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**Introduction:** Circulating oxidized low-density lipoprotein (LDL), a marker of oxidative stress, is associated with obesity, insulin resistance, metabolic syndrome, and cardiovascular disease in adults. However, little is known about its relation to insulin resistance and cardiovascular risk factors in children. The purpose of this study was to assess the relation of oxidative stress, measured by circulating oxidized LDL, with measures of adiposity and insulin resistance in children.

**Methods:** Oxidized LDL, measures of body fatness (body mass index: BMI, percent body fat, waist circumference, percent trunk fat, abdominal visceral and subcutaneous fat), insulin resistance with euglycemic insulin clamp (Mlbm), blood pressure, and blood lipids were obtained in 78 children.

Oxidized LDL was compared between normal weight children (BMI < 85th percentile) and overweight/obese children (BMI ≥ 85th percentile) and levels were evaluated for associations with body fatness and insulin resistance.

**Results:** Oxidized LDL levels were significantly higher in overweight/obese vs. normal weight children ( $p < 0.0001$ ). Oxidized LDL was significantly correlated with BMI, percent body fat, waist circumference, percent trunk fat, abdominal visceral fat, and abdominal subcutaneous fat (all  $p$ -values < 0.0001). Moreover, oxidized LDL was negatively correlated with Mlbm, even after adjustment for adiposity ( $p < 0.01$ ).

**Conclusions:** Oxidized LDL is significantly associated with adiposity and with insulin resistance, independent of body fatness, in children. Oxidative stress may be independently related to the development of insulin resistance early in life, especially in obese youth.

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Increased oxidative stress, reflected by elevated levels of oxidized low-density lipoprotein (LDL), may precede the development of insulin resistance (1) and therefore be important in the early pathophysiology of type 2 diabetes mellitus. Moreover, oxidative modification of LDL is thought to be a seminal event in the initiation and progression of atherosclerosis. Circulating oxidized LDL facilitates the maturation of macrophages and subsequent conversion to foam cells in the arterial wall (2). In adults, circulating oxidized LDL is associated with obesity (3, 4), insulin resistance (5), metabolic syndrome (6–9), and cardiovascular disease (10–12) but little is known about its relation to insulin resistance and cardiovascular risk

factors in children. The purpose of this study was to assess the relation of oxidative stress, as measured by circulating oxidized LDL, with measures of adiposity and insulin resistance in children and adolescents.

### Methods

This study included all pediatric participants (N = 78; age 6–18 yr) who were consecutively enrolled from June to December 2008 in an ongoing study investigating the early development of obesity, insulin resistance, and other cardiovascular risk factors. The children are offspring of parents who have been participating in a longitudinal study in which initial testing was performed when the parents were children. The

protocol was approved by the University of Minnesota Institutional Review Board and consent/assent was obtained from parents/participants. Measures were obtained at the University of Minnesota General Clinical Research Center after participants had been fasting  $\geq 10$  h.

Height and weight were obtained using a standard stadiometer and electronic scale, respectively. Waist and hip circumferences were measured to the nearest 0.5 cm. Seated blood pressure was obtained after 5 min of quiet rest, on the right arm using an automatic sphygmomanometer. Tanner stage was determined by trained pediatricians. Body fat percentage was obtained using dual energy X-ray absorptiometry (DXA) (Prodigy, 3M, Madison, WI, USA). Abdominal visceral and subcutaneous fat were measured with computed tomography (Somatom Sensation, Siemens Medical Solutions, Malvern, PA, USA). Insulin sensitivity was determined by euglycemic insulin clamp as previously described (13). Insulin was infused at a constant rate of 1 mU/kg/min for 3 h, and glucose was infused at a variable rate to maintain euglycemia. Insulin sensitivity (M) was expressed as the glucose infusion rate (mg/kg/min of glucose), with adjustment for lean body mass (MIbm). Low MIbm represents insulin resistance.

Fasting lipid profile, glucose, and insulin assays were conducted with standard procedures at the Fairview Diagnostic Laboratories, Fairview-University Medical Center (Minneapolis, MN), a Center for Disease Control and Prevention–certified laboratory. Plasma oxidized LDL levels (sensitivity  $\leq 0.3$  U/L) were measured in the University of Minnesota Cytokine Reference Laboratory with competitive enzyme-linked immunosorbant assay (ELISA) (Merckodia, Inc., Winston-Salem, NC, USA).

Participants were classified as normal weight [body mass index (BMI)  $< 85$ th percentile] and/or overweight/obese (BMI  $\geq 85$ th percentile) based on age- and gender-specific pediatric BMI criteria. In the initial analysis, linear regression (GLM procedure, SPSS version 16.0—SPSS, Inc., Chicago, IL, USA), adjusting for age, gender, and race, was used to compare oxidized LDL levels between the normal weight and overweight/obese groups. Next, the entire cohort of 78 was used for correlation analyses to evaluate the relationship between oxidized LDL and measures of body fatness (adjusted for age, gender, and race) and MIbm (adjusted for age, gender, race, and percent body fat). Additional adjustments were made and reported separately for LDL cholesterol levels (because LDL is the substrate for oxidized LDL) in an effort to see whether any finding for oxidized LDL was simply a consequence of having higher LDL cholesterol rather than the propensity of LDL to oxidize. Tanner stage data were missing

in 17 of the participants. Therefore, we performed a separate analysis with adjustments for Tanner stage with the reduced sample size ( $N = 61$ ) and results were not significantly different from those unadjusted for Tanner. Other studies from our group have shown that Tanner stage does not affect the relation between BMI and insulin resistance (14). Data are presented as mean  $\pm$  standard deviation.

## Results

The comparison of the clinical variables between the normal weight and overweight/obese groups are shown in the Table 1. There were no significant differences between the normal weight vs. overweight/obese groups for age or gender. Compared with normal weight participants, overweight/obese children had significantly higher BMI, percent body fat, waist circumference, percent trunk fat, abdominal visceral and subcutaneous fat, diastolic blood pressure, LDL cholesterol, fasting insulin, and significantly lower high-density lipoprotein (HDL) cholesterol. Oxidized LDL levels were significantly higher in overweight/obese vs. normal weight children ( $p < 0.0001$ ) (Fig. 1) and remained significant, even after adjustment for LDL cholesterol (adjusted  $p < 0.0001$ ). To evaluate possible interactions of oxidized LDL and weight status by age (children vs. adolescents), we compared means between two different age groups. Although not statistically significant ( $p = 0.21$ ), mean oxidized LDL was higher in the overweight/obese ( $N = 12$ ;  $67.1 \pm 14.4$  U/L) vs. normal weight ( $N = 20$ ;  $61.3 \pm 11.2$  U/L) in the younger (age 6–11) children. Oxidized LDL levels were significantly higher ( $p < 0.0001$ ) in the overweight/obese ( $N = 26$ ;  $70.1 \pm 14.2$  U/L) vs. normal weight ( $N = 20$ ;  $51.9 \pm 12.1$  U/L) in the older (age 12–18) children.

Oxidized LDL was significantly and positively correlated with LDL cholesterol ( $r = 0.71$ ,  $p < 0.0001$ ), BMI ( $r = 0.50$ ,  $p < 0.0001$ ), percent body fat ( $r = 0.52$ ,  $p < 0.0001$ ), waist circumference ( $r = 0.48$ ,  $p < 0.0001$ ), percent trunk fat ( $r = 0.52$ ,  $p < 0.0001$ ), abdominal visceral fat ( $r = 0.42$ ,  $p < 0.0001$ ), and abdominal subcutaneous fat ( $r = 0.49$ ,  $p < 0.0001$ ). Oxidized LDL was significantly and negatively associated with MIbm ( $r = -0.40$ ,  $p = 0.006$ ), independent of body fatness (adjusted for total body fat percentage). Oxidized LDL was not significantly related to fasting insulin ( $r = 0.14$ ,  $p = 0.32$ ).

Correlations remained significant after additional adjustment for LDL cholesterol levels: BMI ( $r = 0.43$ ,  $p < 0.0001$ ), percent body fat ( $r = 0.44$ ,  $p < 0.0001$ ), waist circumference ( $r = 0.43$ ,  $p < 0.0001$ ), percent trunk fat ( $r = 0.44$ ,  $p < 0.0001$ ), abdominal visceral fat ( $r = 0.45$ ,  $p < 0.0001$ ), abdominal subcutaneous fat ( $r = 0.42$ ,  $p < 0.0001$ ), and MIbm ( $r = -0.30$ ,

Table 1. Clinical characteristics of normal weight vs. overweight/obese children

Variable	Normal weight (N = 40)	Overweight/obese (N = 38)	p-Value
Age (yr)	11.7 ± 3.5	12.4 ± 3.3	0.318
Gender (male/female)	25/15	26/12	0.639
BMI (kg/m <sup>2</sup> )	18.3 ± 2.4	27.7 ± 6.6	<0.0001
Body fat (%)	19.1 ± 7.2	37.5 ± 10.0	<0.0001
Waist circumference (cm)	66.2 ± 7.8	87.3 ± 16.0	<0.0001
Trunk fat (%)	17.6 ± 7.8	38.9 ± 11.1	<0.0001
Visceral fat (cm <sup>3</sup> )	12.8 ± 5.7	26.5 ± 11.9	<0.0001
Subcutaneous fat (cm <sup>3</sup> )	37.2 ± 28.1	155.3 ± 94.5	<0.0001
SBP (mmHg)	104.4 ± 9.6	106.6 ± 8.9	0.470
DBP (mmHg)	55.7 ± 8.8	60.4 ± 6.0	0.008
Total cholesterol (mg/dL)	149.1 ± 26.7	155.7 ± 28.9	0.136
LDL cholesterol (mg/dL)	83.0 ± 23.0	91.4 ± 24.1	0.047
HDL cholesterol (mg/dL)	52.8 ± 12.0	47.0 ± 11.4	0.049
Triglycerides (mg/dL)	66.6 ± 29.0	85.8 ± 57.7	0.076
Glucose (mg/dL)	86.8 ± 6.1	88.0 ± 8.0	0.498
Insulin (mU/L)	7.5 ± 4.4	11.9 ± 9.9	0.024
Mlbm (mg/kg/min)	13.4 ± 3.6	13.7 ± 6.6	0.474
Oxidized LDL (U/L)	56.6 ± 12.4	69.1 ± 14.1	<0.0001

Data are presented as mean ± standard deviation. All variables except age and gender are adjusted for age, gender, and race.

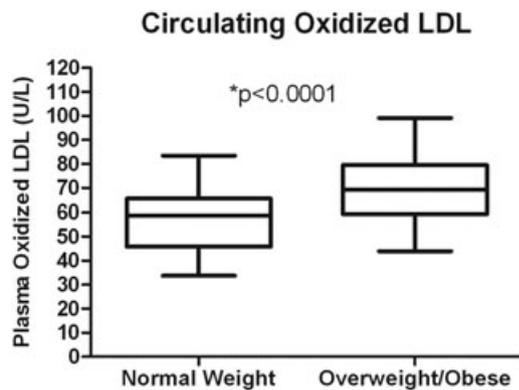


Fig. 1. Circulating oxidized LDL in normal weight vs. overweight/obese children.

$p < 0.05$ ). Results after adjustment for triglycerides were similar to the LDL adjustment. We concluded that the correlation analysis was a good fit to the data because means of body fatness, cardiovascular risk factors, and Mlbm were graded across quartiles of oxidized LDL.

### Discussion

The main finding of this study is that oxidative stress, as measured by oxidized LDL, is significantly related to overweight/obesity and insulin resistance (independent of adiposity) in children. Because these findings are independent of LDL cholesterol, they can be considered a property of the propensity of LDL particles to oxidize, rather than the amount of cholesterol these particles contain.

Only a few studies have examined the relation of oxidized LDL with obesity and associated risk factors in children and none have addressed its potential

association with insulin resistance. Data from the Cardiovascular Risk in Young Finns Study demonstrated a significant negative association between levels of oxidized LDL and brachial artery nitrate-mediated dilation, a measure of arterial health (15). In a study of First Nation youth, although children with type 2 diabetes mellitus had higher levels of oxidized LDL than controls, the levels were not significantly different in obese children without type 2 diabetes (16). In a study comparing obese and lean adolescents with polycystic ovary syndrome to healthy controls, there were no differences in oxidized LDL levels among the groups (17). Finally, another study reported that levels of oxidized LDL did not differ among groups of normal weight and overweight children with and without risk factors (18). In contrast to those studies, our data, despite using a similar technique for measuring oxidized LDL (ELISA), demonstrate that overweight and obese children have elevated levels of oxidized LDL compared with their normal weight counterparts. Although it is not clear why our results differ from prior studies, they may be related to differences in study design and sample size. To our knowledge, our study comparing oxidized LDL levels in a two-group design (normal weight vs. overweight/obese children and adolescents) is the largest to date. Alternatively, slightly different procedures for oxidized LDL measurement may detect different types of oxidized LDL (19), possibly explaining the discrepancy.

Oxidized LDL was significantly correlated with all measures of body fatness, with r-values ranging from 0.42 to 0.52. After additional adjustment for LDL cholesterol levels, the range of correlations became even narrower ( $r = 0.42-0.45$ ), suggesting that no

single measure of body fatness was superior in its relation to oxidized LDL. Oxidized LDL was significantly associated with insulin sensitivity, independent of body fatness. Although the correlation of oxidized LDL with Mlbm was relatively modest compared with the correlation with measures of body fatness, these findings support the concept that insulin resistance, independent of level of adiposity, is associated with higher levels of oxidative stress early in life. These findings, if confirmed by future investigations, may have implications toward the understanding of the development of insulin resistance in the context of pediatric obesity and risk of future type 2 diabetes mellitus. Recent data in adults suggest that oxidative stress precedes insulin resistance (1); however, data from longitudinal studies will be needed to address this issue in children.

In conclusion, circulating oxidized LDL levels are higher in overweight and obese, vs. normal weight, children and adolescents. Body fatness and Mlbm are independently correlated with oxidized LDL suggesting that adiposity and insulin resistance are associated with oxidative stress within the first two decades of life. Future research should seek to better characterize the nature of these associations in children and to determine whether elevated levels of oxidative stress are a cause, or consequence, of obesity and insulin resistance.

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### Conflict of interest

The authors have no relevant conflicts of interest.

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