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Clinical Research Article

The Relationship Between Lipoproteins and Insulin Sensitivity in Youth With Obesity and Abnormal Glucose Tolerance

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Abstract

Context: Youth with obesity and abnormal glucose tolerance have an increased risk for atherosclerosis but the relative contributions of insulin resistance and hyperglycemia to dyslipidemia and the development of subclinical atherosclerosis are unknown.

Objective: This work aims to determine the association between insulin resistance, dyslipidemia, and carotid intimal thickness (cIMT) in adolescents with normal and abnormal glucose tolerance.

Methods: An observational cohort study in 155 youth: 44 obese insulin sensitive (OIS; fasting insulin ≤ 20 μM/mL, body mass index [BMI] ≥ 95th percentile), 35 obese insulin resistant (OIR; fasting insulin > 20 μM/mL, BMI ≥ 95th percentile), 34 obese abnormal glucose tolerant (AGT; BMI ≥ 95th percentile), and 42 Lean (BMI 5th-85th percentile). Lipids, lipoprotein particle size and concentration (-P), insulin sensitivity (S_I an intravenous glucose test), and CMIT were compared using linear models adjusted for age, race/ethnicity, biological sex, and Tanner stage. Lipid/lipoprotein profile and CMIT were reevaluated in a subset after 2 years.

Results: Compared to OIS and Lean, OIR and AGT had elevated triglycerides and low high-density lipoprotein cholesterol (HDL-C) but similar total cholesterol and low-density lipoprotein cholesterol (LDL-C). Among OIS, OIR, AGT, lower S_I was associated with atherogenic lipids (higher triglycerides, LDL-C, non–HDL-C, and lower HDL-C) and lipoproteins (higher total LDL-P and small HDL-P, and lower large HDL-P). There was a steeper decline in the association of S_I with HDL-C and large HDL-P in AGT compared with OIR and OIS. cIMT was comparable across groups and inversely correlated with S_I, with no change after 2 years.

Conclusion: Among youth with obesity, insulin resistance was associated with an atherogenic lipoprotein/lipid profile and cIMT, regardless of glucose tolerance status. Insulin resistance in AGT youth was associated with a shift to smaller HDL-P compared to normoglycemic youth with obesity. Alterations in HDL-P metabolism may be early adverse manifestations of hyperglycemia in youth with obesity.

Key Words: insulin sensitivity, dyslipidemia, pediatrics, type 2 diabetes, obesity, subclinical atherosclerosis

Abbreviations: AGT, abnormal glucose tolerant; ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index; CHOP, The Children's Hospital of Philadelphia; CMIT, carotid intima-media thickness; CTRC, Clinical Translational Research Center; HbA_{1c}, glycated hemoglobin A_{1c}; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particles; HOMA-IR, Homeostatic Model of Insulin Resistance; IM-FSIGT, insulin-modified frequently sampled intravenous glucose tolerance test; IV, intravenous; LDL-C, low-density lipoprotein cholesterol; LDL-P, low-density lipoprotein particles; Ln, natural logarithm; NIH, National Institutes of Health; NMR, nuclear magnetic resonance; OGTT, oral glucose tolerance test; OIR, obese insulin resistant; OIS, obese insulin sensitive; S_I, insulin sensitivity index; T2DM, type 2 diabetes mellitus; TRL-P, triglyceride-rich lipoprotein particles.

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Youth and young adults with obesity and type 2 diabetes mellitus (T2DM) have an increased risk of atherosclerotic cardiovascular disease (ASCVD) as adults (1-3). However, there is a dearth of scientific evidence as to which children are at the highest risk and burden. Obesity and T2DM are both pathologic determinants of ASCVD, but it is unclear whether hyperglycemia combined with insulin resistance during adolescence increases the ASCVD risk beyond the effects of obesity or insulin resistance alone. Obesity is linked to greater carotid intimal thickness (cIMT) and higher coronary artery calcium scores in adolescents (4, 5). This relationship with ASCVD is largely mediated by metabolic dyslipidemia—elevated triglycerides and low-density lipoprotein cholesterol (LDL-C) and low high-density lipoprotein cholesterol (HDL-C) (6, 7). Moreover, metabolic dyslipidemia is common and occurs in approximately 10% to 20% of youth with obesity and 20% to 25% in youth with newly diagnosed T2DM (8). Hyperglycemia may further accelerate the atherogenic risk via enhanced endothelial injury, oxidative stress, protein glycosylation of LDL particles, decreased nitric oxide production, and/ or increased coagulability (9). In addition, puberty is a state of physiologic insulin resistance that may exacerbate metabolic dyslipidemia and the development of subclinical atherosclerosis (10). Therefore, determining whether there is an additive effect of insulin resistance and hyperglycemia in the development of subclinical atherosclerosis during adolescence is critical for assessing the optimal timing and type of risk interventions.

To develop ASCVD risk-reduction strategies in youth, it is imperative to elucidate the relationship of both traditional lipid values as well as nuclear magnetic resonance (NMR)-derived lipoproteins with insulin resistance and subclinical atherosclerosis. We and others previously demonstrated insulin resistance was associated with a more atherogenic lipoprotein profile among adolescents with prediabetes compared to peers with obesity and normal glucose tolerance (7, 11, 12). However, past analyses have not examined the relationship of insulin resistance with atherogenic lipoprotein profile and cIMT to determine if hyperglycemia alters the strength of these associations. Therefore, we compared youth with obesity with and without insulin resistance (obese insulin sensitive [OIS] and obese insulin resistant [OIR]) to youth with obesity and abnormal glucose tolerance (AGT), and lean controls (Lean). We hypothesized that an atherogenic lipid/lipoprotein profile would be associated with lower insulin sensitivity, irrespective of dysglycemia. Our primary aims were to examine the relationships between lipid and lipoprotein profiles and insulin sensitivity among youth with obesity, determined during intravenous (IV) and oral glucose tolerance tests (OGTTs). Secondary aims were to compare subclinical atherosclerosis, measured by CMIT, and explore its relationships with metabolic dyslipidemia and insulin sensitivity at baseline and after 2 years.

Materials and Methods

This was an observational cohort study (cross-sectional primary aim and prospective exploratory aim) conducted between 2007 and 2012. Youth were recruited from 4 primary care clinics and the Endocrinology and Diabetes clinic of The Children's Hospital of Philadelphia (CHOP). Written informed consent and age-appropriate assent were obtained from all individuals before participation, and the study was approved by the CHOP Institutional Review Board. As previously mentioned, data in a subset of youth have been previously published (11, 13).

Patient Population

Supplementary Fig. S1 (14) details the participant flow diagram for the cross-sectional and longitudinal studies. A total of 215 youth were screened and 155 enrolled in the cross-sectional analysis and 56 in the follow-up study.

Cross-sectional Study

Pubertal (Tanner stage 2-5) youth, aged 12 to 17 years, were recruited into 2 body mass index (BMI) categories: Lean (BMI 5th-85th percentile for age and sex) and Obese (BMI ≥ 95th percentile for age and sex). As planned per study protocol—after study entry—youth with obesity were further stratified into 3 groups as outlined here (insulin sensitive: OIS, insulin resistant: OIR, and abnormal glucose tolerant: AGT). Exclusion criteria included major chronic illness (except T2DM), pregnancy, genetic syndromes known to affect glucose tolerance, familial hypercholesterolemia, medications known to affect lipids and insulin sensitivity (statins, high-dose vitamin A, systemic or high-dose inhaled steroids, except T2DM medications in AGT). AGT included those with prediabetes and diabetes. Prediabetes was defined as fasting glucose 100 to 125mg/dL and/or 2-hour glucose 140 to 199 mg/dL and diabetes defined as fasting glucose greater than or equal to 126 mg/dL and/or 2-hour glucose greater than or equal to 200 mg/dL or previous history of T2DM (15). Of note, glycated hemoglobin A_{1c} (HbA_{1c}) was not used as a diagnostic criterion because it was not a recommended diagnostic test at the time of the study (15). Additional inclusion criteria for the participants with T2DM were HbA_{1c} less than 8.5%, and 2 out of 3 negative diabetes autoantibodies (GAD-65, ICA-512, and insulin autoantibodies). Pubertal assessment was performed by a single pediatric endocrinologist and based on Tanner staging of breast development in girls and testicular volume in boys (< 4 cc: stage 1; 4-6 cc: stage 2; 7-10 cc: stage 3; 11-15 cc: stage 4; > 15 cc: stage 5).

Longitudinal Study

Between 2007 and 2009, participants were consecutively enrolled into the 2-year longitudinal study. In November 2009, recruitment to the prospective aim was discontinued because of budgetary limitations. Fifty-six participants completed the 2-year follow-up visit (n = 56: Lean: n = 20, OIS: n = 19, OIR: n = 10, AGT: n = 7; see Supplementary Fig. S1) (14) and underwent OGTT, lipid/lipoprotein, and CMIT analysis as described next.

Procedures

Study visits took place at the Clinical Translational Research Center (CTRC) at CHOP. Height (wall-mounted stadiometer, Holtain Inc) and weight (digital scale, ScaleTronix) were measured 3 times and average values were calculated. BMI and BMI percentiles were assessed using age- and sex-specific BMI reference data (16). Dietary histories were assessed using 3 24-hour dietary recalls (1 weekend day and 2 weekdays) and total kilocalories per kg weight reported. Physical activity history was collected using the 2-item PACE (Patient-centered Assessment and Counseling for Exercise) questionnaire, a validated measure of adolescent physical activity (17). All fasting measurements were performed after a 10- to 12-hour overnight fast.

Lean youth had a single blood draw to measure fasting glucose, insulin, HbA_{1c}, lipid panel, and lipoprotein subclass particle analysis. Youth with obesity were evaluated on consecutive days (day 1: OGTT and day 2: insulin-modified frequently sampled IV glucose tolerance test [IM-FSIGT]) as 2 outpatients visits (OIS and OIR) or a 2-day hospital stay (AGT).

Before the study visit, youth with T2DM (n = 11) discontinued oral diabetes medications (4 days for metformin and 2 weeks for thiazolidinediones) and blood sugars were monitored by home glucometer. If fasting blood glucoses were greater than 125 mg/dL, basal glargine and/or intermittent doses of short-acting subcutaneous insulin were administered. Participants on chronic insulin therapy continued their home insulin treatment until 36 hours before day 1 for glargine, 24 hours before day 1 for NPH insulin, and 8 pm the night before day 1 for short-acting insulin. Youth with T2DM were admitted to the CHOP inpatient CTRC the afternoon of day 1 for glucose monitoring and stabilization. They were given a standard dinner and then fasted overnight for 10 to 12 hours. Blood sugars were monitored by glucometer, and hyperglycemia after dinner (target blood glucose < 150 mg/dL) was treated between 9 pm and midnight with short-acting subcutaneous insulin.

On day 1, after a 12-hour overnight fast, fasting blood samples were collected for glucose, insulin, HbA_{1c}, lipid panel, and lipoprotein subclass particle analysis. Youth then underwent an OGTT (1.75 g/kg up to a maximum of 75 g) and glucose and insulin measured at 0, 30, 60, 90, and 120 minutes. Per protocol, youth with obesity and normal glucose tolerance were categorized based on fasting insulin level based on the upper range of normal assay for insulin (OIS: insulin ≤ 20 μM/mL and OIR: insulin > 20 μM/mL) (18). On day 2, all participants with obesity underwent an IM-FSIGT. A baseline sample was obtained for glucose and insulin, after which a bolus of IV dextrose (0.25 g/kg of 25% dextrose) was infused over 30 seconds at t = 0, and an IV bolus of regular human insulin (OIR and OIS: 0.015 U/kg and AGT: 0.05 U/kg) infused over 30 seconds at t = 20 minutes (19, 20). Blood samples were obtained for glucose and insulin at t = –5, 2, 4, 8, 19, 22, 30, 40, 50, 70, 100, and 180 minutes.

Fat mass was determined with dual x-ray absorptiometry scan (Hologic QDR2000). Dual x-ray absorptiometry scan data were unavailable for 8 youth (OIR: n = 3 and AGT: n = 5) because of exceeding the scanner weight limit of 300 pounds (136 kg; n = 6) and technical difficulties (n = 2).

Biochemical Analyses and Calculations

Lipid and lipoprotein analyses

All plasma samples were processed and stored at –80 °C and underwent one freeze-thaw cycle before batched analysis analysis. Lipoprotein particle size and subclass concentrations were measured by the amplitudes of the lipid-methyl group NMR signals and reported in particle concentration units (nmol/L) using a 400-Mhz proton NMR Profiler and Vantera Clinical Analyzer platforms (Supplementary Table S1) (14, 21). Partial least square regression was used to derive apolipoprotein B and A1 concentrations (22). Triglycerides, total cholesterol, and HDL-C were assayed on a Hitachi 912 using Roche reagents. LDL-C was calculated using the Freidewald equation [LDL-C = TC – HDL-C – (TG/5)]. Percentage high LDL-C was determined as percentage of youth with LDL-C greater than or equal to 130 mg/dL (23), and percentage high LDL-P was determined by a threshold used in adults: LDL-P greater than or equal to 1000 nmol/L (24).

Insulin and metabolites

Serum insulin concentrations were measured with standardized assays (reported coefficient variations ≤ 10%); enzyme-linked immunosorbent assay, ALPCO Diagnostics (catalog No. 80-INSHU-E01.1, RRID:AB_2801438). HbA_{1c} was measured with high-performance liquid chromatography. Data for HbA_{1c} (OIS: n = 1, OIR: n = 1), glucose (Lean: n = 1), and insulin (Lean: n = 2) were not available because of technical difficulties.

Calculations

Insulin sensitivity and β-cell function (sigma)

Insulin sensitivity (S_I) was calculated in 2 ways: insulin sensitivity index (IM-FSIGT) and insulin sensitivity (OGTT). The primary measure of S_I was by IM-FSIGT estimated using the MINMOD software program (19). Data for 35 youth (OIS: n = 1, OIR: n = 10, AGT: n = 24) were unavailable for S_I calculations because of initial protocol stipulations that excluded individuals found to have prediabetes on day 1 that were later modified, as well as technical issues.

OGTT-modeled S_I (10^{-4}/mU/ml/min) and β-cell function (sigma, unitless) were estimated to provide a secondary measure of insulin sensitivity at baseline, and a measurement at 2 years (when IM-FSIGT was not performed). We adapted a model (25, 26) for glucose and insulin homeostasis defined by the following equations for plasma glucose concentration (G) and serum insulin concentration (I):

$$\frac{dG}{dt} = OGTT + HGP - (E_{GO} + S_I I)\ G$$

$$\frac{dI}{dt} = \frac{\beta}{V} ISR(\sigma,\ G) - kI$$

Glucose concentration was determined by the balance of influx (OGTT flux, $OGTT$, plus hepatic glucose production, HGP) and uptake (insulin-independent uptake, E_{G0}, also known as glucose effectiveness) plus insulin-dependent uptake ($S_I I$). Insulin concentration was determined by the balance of secretion rate (ISR) and clearance rate (k). The parameters fitted were S_I(OGTT-derived S_I) and σ(OGTT-derived sigma), obtained using the least-squares minimization function fminsearch in MATLAB (version 9.5.0 (R2018b), The MathWorks Inc).

The Homeostatic Model of Insulin Resistance (HOMA-IR) was calculated as follows:

$$\frac{fasting\ \ blood\ \ glucose\ \ (mg/dL) * fasting\, insulin\ \ (mIU/L)}{405}.$$

Measurements of carotid intimal thickness

B-mode carotid artery images for IMT measurement were acquired with a Siemens Acuson Sequoia ultrasound system, using a linear array 8L5 or 6L3 transducer, and cIMT was measured using an automated computerized edge-detection program, the "Carotid Analyzer" (Medical Imaging Applications LLC). While supine, the proximal portion of the carotid bulb was included in all images as an anatomical

reference point and the posterior wall of the distal common carotid artery, 1 cm below the bifurcation, was the site selected for measurements. CMIT was measured as the distance between echoes arising from the blood-intima interface and the media-adventitia interface. The interrater correlation was 0.84 and intrarater correlation was 0.88 reliability (27).

Statistical Analyses

A priori sample size calculations were performed for the primary analysis—the association between S_I, as measured by IM-FSIGT, and HDL-C at baseline. Forty youth in each group provided 90% power to detect a correlation greater than or equal to 0.3 between HDL-C and S_I. Data are presented as mean ± SD, except where otherwise indicated. Continuous variables were compared across groups with one-way analysis of variance for parametric variables or the Kruskal-Wallis test for nonparametric variables. Spearman correlations (r) tested the relationship between cIMT and lipid/lipoproteins. Categorical variables were compared using Fisher exact test. Normality test was performed with Shapiro-Wilk W test, and logarithmic transformations (Ln) were used for nonparametric variables before regression analyses. Linear regression was used to measure associations between insulin sensitivity and lipids/lipoproteins and cIMT in the obese groups (Lean not included in models). Models were adjusted for age, biological sex, race, ethnicity, and Tanner stage. Group by lipid/lipoprotein interactions were included to identify associations differing between groups. The lincom command in STATA was used to identify associations differing between the AGT group and OIR or OIS. For the exploratory analysis, the changes in lipoproteins, insulin sensitivity, and CMIT over 2 years were compared between groups with one-way analysis of variance with Bonferroni corrections. Missing data were omitted from analyses and not imputed. Analyses were conducted with STATA (version 16.1; Stata Corp LLC). Statistical significance was inferred at a 2-tailed P value of less than .05.

Results

Cross-sectional Study

Descriptive and cardiometabolic variables for all youth are shown in Table 1. Groups were similar in age, sex, race, and Tanner stage; metabolic variables were different by design. Physical activity reports were not different by group. Self-reported mean total daily calorie intake per kg was highest in Lean youth. As expected, LnS_I, as well as OGTT-derived insulin sensitivity and sigma, differed by group (see Table 1 and Supplementary Fig. S2) (14).

Insulin sensitivity was negatively associated with an atherogenic lipid profile

Total cholesterol and LDL-C were similar among all youth. Compared to Lean, youth with obesity (OIS, OIR, and AGT) had lower HDL-C, higher triglycerides, and higher non–HDL-C compared to Lean (Table 1). Among youth with obesity, LnS_I was inversely associated with traditional lipid values and apolipoprotein B (Fig. 1). LnS_I was positively associated with HDL-C (Fig. 1). There was effect modification by group for the association of LnS_I with HDL-C, demonstrating steeper slope of LnS_I with HDL-C in AGT compared to OIS and to OIR (Fig. 1). OGTT-derived LnS_I was inversely related to triglycerides and positively related to HDL-C (Supplementary Fig. S3) (14).

Insulin sensitivity was negatively associated with an atherogenic lipoprotein profile

Compared to Lean, youth with obesity (OIS, OIR, and AGT) had an atherogenic lipoprotein profile characterized by higher total, remnant, and very small TRL-P, higher total and small LDL-P, higher small HDL-P and lower large HDL-P, and higher apolipoprotein B (Fig. 2, Table 1). TRL-P, LDL-P, and HDL-P size and concentrations varied modestly among OIS, OIR and AGT (see Table 1). Among adolescents with obesity, LnS_I was inversely associated with total LDL-P and small HDL-P (Fig. 3). LnS_I was positively associated with large HDL-P (see Fig. 3) and negatively associated with large TRL-P and (Supplementary Table S2) with effect modification by group present for both (14). There was a steeper positive association between LnS_I and large HDL-P (see Fig. 3) and a steeper negative association between LnS_I and large LnTRL-P in AGT vs OIR and AGT vs OIS (see Supplementary Table S2) (14). LnS_I was not associated with total or remnant TRL-P or small LDL-P. Analyses performed using OGTT-derived S_I revealed similar associations (Supplementary Fig. S4) (14).

Table 1. Participant demographic and metabolic characteristics

Variable	Lean	OIS	OIR	AGT	P	P
	N = 42	N = 44	N = 35	N = 34	Whole group	OIS-OIR-AGT
Demographic characteristics						
Age, y	14.7 ± 1.3	14.7 ± 1.4	14.2 ± 1.4	14.5 ± 1.4	.293	.245
Male sex	21 (50)	19 (43)	12 (34)	17 (50)	.497	.433
Black race	34 (81)	36 (82)	27 (77)	28 (82)	.662	.941
Tanner stage[c]						
2-3	2 (5)	5 (11)	5(15)	7 (21)	.041	.205
4-5	40 (95)	39 (89)	30 (85)	26 (79)		
Tobacco use	0 (0)	2 (5)	2 (6)	0 (0)	.307	.562
Activity score	3.5 ± 2.1	2.9 ± 2.2	3.1 ± 2.2	3.1 ± 2.2	.649	.839
Average daily calorie intake, Kcal/kg	34 ± 11	18 ± 6	15 ± 4	13 ± 5	< .001	.001
BMI, kg/m^2	20.0 ± 1.8	33.6 ± 5.2	36.2 ± 6.4	35.1 ± 6.4	< .001	.156
BMI z score	0.02 ± 0.7	2.2 ± 0.3	2.4 ± 0.3	2.3 ± 0.3	< .001	.041

Table 1. Continued

Variable	Lean	OIS	OIR	AGT	P	P
	N = 42	N = 44	N = 35	N = 34	Whole group	OIS-OIR-AGT
Fat mass, %[a]	18.6 ± 6.4	35.3 ± 7.4	37.0 ± 4.9	36.8 ± 5.4	**< .001**	.438
Visceral fat, cm²[b]	30.5 (25.0-35.8)	66.7(51.7-83.7)	75.1 (65.3-92.5)	85.3 (75.9-106.3)	**< .001**	**.006**
Metabolic characteristics						
Hemoglobin A_{1c}, %[c]	5.2 ± 0.3	5.3 ± 0.3	5.4 ± 0.3	5.7 ± 0.6	**< .001**	**< .001**
Fasting glucose, mg/dL[d]	86.0 ± 5.8	86.9 ± 5.8	89.1 ± 5.9	98.1 ± 10.3	**< .001**	**< .001**
Fasting insulin, µIU/mL[c]	8.3 (5.2-9.5)	14.1 (10.5-16.8)	27 (23.1-34.2)	25.1 (13.3-38.3)	**< .001**	**< .001**
HOMA-IR[c]	1.7 (1.1-2.0)	3.0 (2.2-3.6)	5.8 (5.1-8.1)	6.3 (3.5-9.0)	**< .001**	**< .001**
OGTT model sigma[f]	–	1.0 (0.7, 1.4)	1.7 (1.2-2.2)	0.8 (0.4-1.4)	–	**< .001**
OGTT model S_I, 10^{-4}/mU/ml/min	–	3.7 (2.4-4.8)	1.5 (1.3-1.8)	1.4 (0.6-2.4)	–	**< .001**
IM-FSIGT S_I, $(mU/L)^{-1}min^{-1}x10^{-4}$[e]	–	1.89 (1.21-3.55)	1.05 (0.80,-1.55)	0.84 (0.43-1.38)	–	**< .001**
Lipid panel, mg/dL						
Total cholesterol	151 ± 26	155 ± 31	153 ± 28	154 ± 30	.911	.935
HDL-C	55 ± 12	44 ± 8	43 ± 11	39 ± 9	< .001	.097
LDL-C	84 ± 22	98 ± 28	92 ± 24	97 ± 27	.059	.599
Non-HDL cholesterol	96 ± 22	111 ± 31	110 ± 25	114 ± 31	.014	.827
Triglycerides	57 (45-68)	59 (47-80)	88 (66-103)	80 (67-103)	< .001	.001
Triglycerides:HDL ratio	1.1 ± 0.5	1.6 ± 0.8	2.4 ± 1.3	2.4 ± 1.3	< .001	.003
High LDL-C, %	2 (5)	7 (11)	4 (9)	3 (7)	.690	.787
TRL-P, nmol/L						
Total TRL-P	60.6 (48.1, 91)	82.7 (52.8-105.2)	94 (74.1-138.3)	105.5 (69.1-140.9)	< .001	.051
Very large TRL-P	0.1 (0.1-0.1)	0.1 (0.1-0.2)	0.1 (0-0.2)	0.1 (0.1-0.1)	.474	.383
Large TRL-P	0.2 (0.1-0.3)	0.2 (0-0.6)	1.0 (0.2-3.1)	0.9 (0.4-3.1)	< .001	.001
Medium TRL-P	2.9 (1-5.4)	2.8 (0.7-8.6)	8.5 (3.7-16)	8.3 (4.2-14.2)	< .001	.001
Small TRL-P	31.5 (21.1-46.8)	31.3 (18.1-44.4)	38.3 (29.6-50.3)	36.2 (31.5-52.2)	.120	.074
Very small TRL-P	27.2 (16.7-40.4)	43.2 (32.3-57.2)	48 (28.8-68.8)	49.9 (29.4-74.9)	< .001	.692
Remnant TRL-P	55.85 (46-81)	79.3 (51.7-99.6)	80.5 (66.6-124.4)	97.3 (55.7-127.2)	< .001	.135
LDL-P, nmol/L						
Total LDL-P	781.5 (644-937)	1030 (787-1164.5)	933 (781-1147)	977.5 (729-1149)	< .003	.928
Large LDL-P	243 (156-331)	228 (136-347)	225 (140-320)	156 (127-237)	.103	.105
Medium LDL-P	31 (21-84)	109.5 (11-215)	81 (32-166)	56 (0-174)	.064	.446
Small LDL-P	457.5 (396-557)	568 (458.5-669.5)	556 (392-784)	639 (518-754)	< .001	.131
High LDL-P, %	6 (14)	23 (52)	15 (43)	17 (50)	.001	0.726
HDL-P, nmol/L						
Total HDL-P	17.6 (16.2-18.9)	17.2 (15.9-18.3)	16.7 (15.3-18.4)	17.4 (16.1-18.7)	.3471	.631
Large HDL-P	3.3 (2.5-4.7)	1.4 (1.0-2.3)	1.2 (0.8-2.4)	0.9 (0.7-1.3)	.0001	.013
Medium HDL-P	4.6 (3.4-5.5)	4.7 (3.3-5.4)	4 (3.4-5)	3.6 (2.8-4.9)	.2361	.226
Small HDL-P	9.6 (8.6-11.5)	11.3 (9.4-12.3)	11.2 (9.5-12.7)	12.2 (10.5-13.9)	.0003	.057
Lipoprotein size, nm[3]						
TRL-P	37.9 (36.1-39.8)	38.5 (36.1-39.8)	41.1 (38.2-45.5)	41.7 (39-44.6)	< .001	.002
LDL-P	21.1 (20.7-21.3)	21 (20.6-21.2)	21 (20.7- 21.2)	20.7 (20.5-20.9)	**.007**	**.018**
HDL-P	9.5 (9.3-9.7)	9.1 (8.9-9.3)	9 (8.8-9.2)	8.8 (8.7-9.1)	**< .001**	**.014**
Apolipoproteins, mg/dL						
Apolipoprotein A1	127 (115-140)	109 (101-121)	108 (96-118)	105 (99-120)	**< .001**	.502
Apolipoprotein B	44.5 (37-53)	58 (43.5-67.5)	56 (48-65)	60.5 (43-67)	**< .001**	.933

Data are mean ± SD or No. (%) or median (25th-75th percentile). Groups compared with one-way analysis of variance, Kruskal-Wallis test, or chi-square analysis. Lipoprotein particle diameter sizes are in provided in Supplementary Table S1 (14).
Abbreviations: AGT, abnormal glucose tolerant; BMI, body mass index; FSIGT, insulin-modified frequently sampled intravenous glucose tolerance test; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particles; HOMA-IR: homeostatic model of insulin resistance index; LDL-C, low-density lipoprotein cholesterol; LDL-P, low-density lipoprotein particles; *Ln*: natural logarithm; OGTT, oral glucose tolerance test; OIR, obese insulin resistant; OIS, obese insulin sensitive; S_I, insulin sensitivity index; TRL-P, triglyceride-rich lipoprotein particles.
[a]n = 147.
[b]n = 145.
[c]n = 153.
[d]n = 154.
[e]n = 93.
[f]Sigma is a marker of β-cell function.

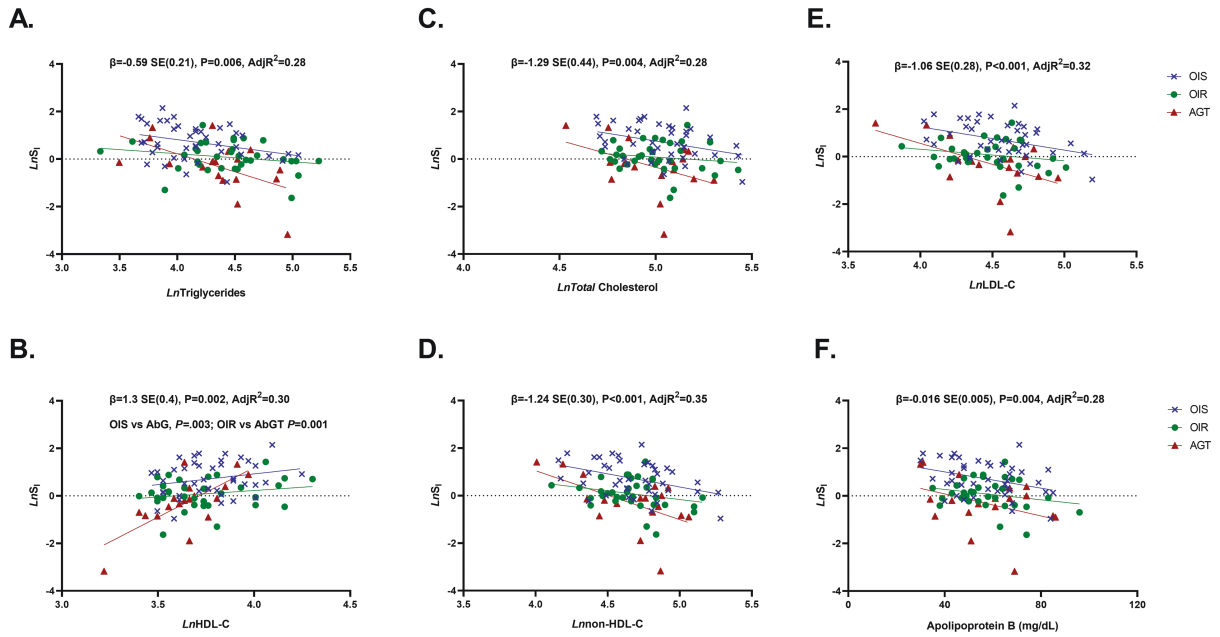

Figure 1. Association of model-derived insulin sensitivity (S_I) from FSIGT with standard lipid measures. Scatterplot of the association of LnS_I with A, *Ln*triglycerides; B, high-density lipoprotein cholesterol; C, *Ln*total cholesterol; D, *Ln*non–HDL-C; E, *Ln*LDL-C; and F, apolipoprotein B in obese insulin sensitive (OIS; blue X), obese insulin resistant (OIR; green circles), and abnormal glucose tolerant (AGT; red triangles). Linear regressions were performed to determine the association of LnS_I with each independent variable adjusted for age, biological sex, race, ethnicity, and Tanner stage. FSIGT, insulin-modified frequently sampled intravenous glucose tolerance test.

Insulin sensitivity was negatively related to carotid intimal thickness

CMIT (mm) was not different among the 4 groups (Lean: 0.56 [0.51-0.62], OIS: 0.56 [0.53-0.64], OIR: 0.56 [0.49-0.62], AGT: 0.58 [0.55-0.63], $P = .24$). Among all 4 groups, cIMT negatively correlated with LnS_I ($r = –0.36$, $P < .001$), weakly correlated with LDL-C ($r = 0.19$, $P = .03$), *Ln*TRL-P ($r = 0.19$, $P = .02$), and remnant TRL-P ($r = 0.18$, $P = .03$), but not with total or small LDL-P, apolipoprotein B, or total HDL-P. In regression analyses, CMIT was independently associated with LnS_I (β = –2.99 SE [0.97], adjusted $R^2 = 0.23$, $P = .001$) and there was no effect modification by group after adjustment for covariates.

Longitudinal cohort

The changes in metabolic characteristics, lipid panel, and lipoproteins were similar and the change in CMIT was negligible among the 4 groups (Table 2). Based on American Diabetes Association criteria, 1 of 19 OIS and 1 of 10 OIR youth became AGT, and 2 participants in the AGT group became OIR at the 2-year follow-up. OGTT-derived S_I and sigma did not significantly change over 2 years (see Table 2, Supplementary Fig. S5) (14). Because of the small sample size, we were unable to perform additional adjustment for demographic covariates, pubertal status, or other factors.

Discussion

T2DM and insulin resistance are critical pathological determinants of ASCVD but the differential roles of the components of metabolic dysregulation in the development of dyslipidemia and subclinical atherosclerosis during youth are not known. Specifically, it is unclear whether insulin resistance combined with hyperglycemia during adolescence, a period of physiologic insulin resistance, increases the ASCVD risk beyond the effects of insulin resistance or hyperglycemia alone. This study used a cohort design in youth across the spectrum of glucose tolerance to assess the relationships between individual risk components of metabolic dyslipidemia and insulin sensitivity. In keeping with our hypothesis, S_I was inversely related to atherogenic lipids/lipoproteins (triglycerides, LDL-C, non–HDL-C and total LDL-P) and CMIT across obesity groups regardless of glucose tolerance status. Notably, decreased large HDL-P and lower HDL-C were related to lower S_I, and this relationship differed by group, and was steepest in those with AGT. Within our cohort, there was also only a weak association between cIMT and dyslipidemia, positing that reduced S_I may be an early risk marker of ASCVD risk independent of changes in atherogenic lipoproteins. Prior studies demonstrated increased ASCVD risk in youth in AGT and OIR youth but did not investigate the relationship of insulin resistance with ASCVD (12). We now demonstrate that insulin resistance is an important correlate of cIMT—and ASCVD risk—in youth with and without T2DM. Together, these novel data support the mediating role of insulin resistance as an early risk marker of ASCVD risk among youth with obesity with and without abnormal glucose tolerance.

While the relationship between insulin sensitivity and metabolic dyslipidemia in healthy and overweight adults (28) and children (7) is appreciated, our present analysis adds new information about this relationship in youth with severe obesity who have insulin resistance with and without hyperglycemia. Diabetes is a well-recognized strong independent risk factor for ASCVD (29) and each 1% increase in HbA_{1c} increases rates of ASCVD by up to 15% in adults (30). Yet, these large analyses aggregated adults with obesity and diabetes—all

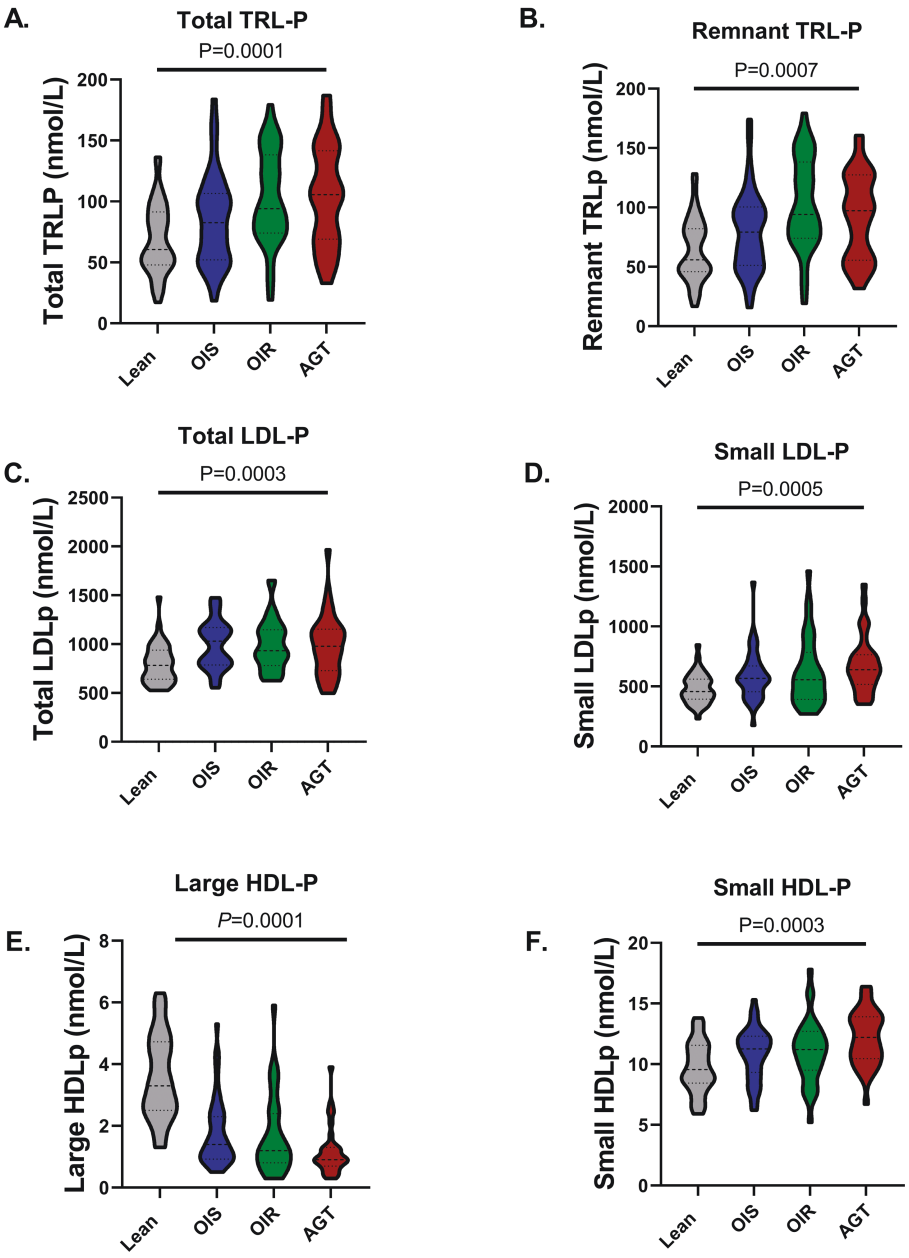

Figure 2. Lipoprotein particle concentrations in youth. Violin plots of A, total TRL-P; B, remnant TRL-P; C, total low-density lipoprotein (LDL)-P; D, small LDL-P; E, large high-density lipoprotein (HDL)-P; and F, small HDL-P in lean (gray), OIS (blue), OIR (green), and AGT (red). Groups were compared with Kruskal-Wallis tests. AGT, abnormal glucose tolerant, n = 34; Lean: n = 42, OIR, obese insulin resistant, n = 35; OIS, obese insulin sensitive, n = 44.

with profound insulin resistance—and it would be difficult to distinguish the contribution of hyperglycemia to ASCVD development. Other studies evaluated independent effects of obesity and hyperglycemia and found a similar relationship of insulin resistance with an atherogenic pattern of LDL and lipoproteins regardless of glucose tolerance status (31, 32). In youth, we previously observed an atherogenic lipoprotein profile among obese adolescents with prediabetes, compared to obese normoglycemic adolescents (11). Much of this difference seemed to be attributable to insulin resistance as measured by HOMA-IR. Now, we confirm our prior observations with 2 detailed and discriminate modeling measures of insulin sensitivity during IV and OGTTs. By systematically evaluating the independent and combined contributions of obesity and abnormal glucose tolerance, we extend prior observations to show that early dysglycemia is associated with marked shifts in HDL metabolism.

Interestingly, we also observed a discordant relationship between S_I and HDL-C and S_I and HDL-P (Fig. 3). Previous studies have shown that lower insensitivity was associated with small HDL-P (7, 28). This study replicates these associations in youth and provides additional data showing that the relationship between HDL-P subclass concentration with insulin sensitivity differs across the spectrum of glucose

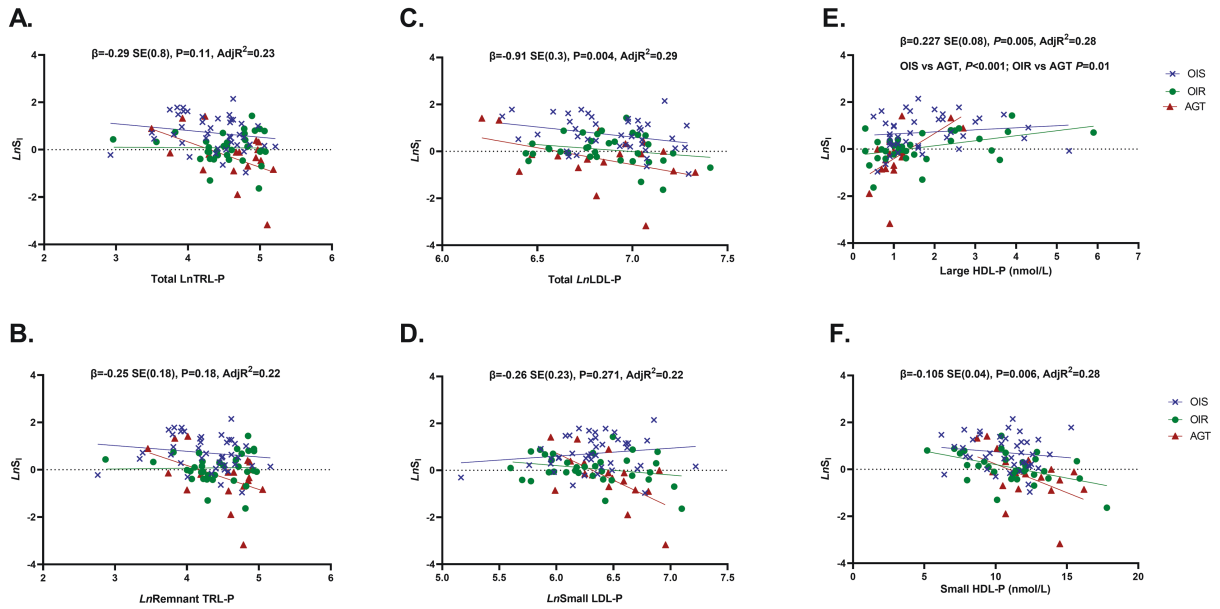

Figure 3. Association of model-derived insulin sensitivity (S_I) from FSIGT with lipoprotein particle concentrations. Scatterplot of the association of LnS_I with A, *Ln*TRL-P; B, *Ln*remnant TRL-P; C, total low-density lipoprotein (LDL)-P; D, small LDL-P; E, large high-density lipoprotein (HDL)-P; and F, small HDL-P in obese insulin sensitive (OIS; blue X), obese insulin resistant (OIR; green circles), and abnormal glucose tolerant (AGT; red triangles). Linear regressions were performed to determine the association of LnS_I with each independent variable adjusted for age, biological sex, race, ethnicity, and Tanner stage. FSIGT, insulin-modified frequently sampled intravenous glucose tolerance test.

tolerance. Compared to OIS and OIR, similar declines in insulin sensitivity in AGT youth were associated with steeper decreases in large HDL-P and HDL-C. In keeping with our findings in youth, adults with T2DM were found to have higher small HDL-P compared to healthy insulin-sensitive groups (31). However, in contrast to adults, we observed that AGT youth have a steeper decline in HDL-P compared to OIS and OIR, indicating that the alterations in HDL-P metabolism associated with insulin sensitivity were further modified and altered by the presence of hyperglycemia. The inverse relationship with insulin sensitivity and small HDL-P likely reflected reductions in large, buoyant, cholesterol-rich HDL-P and differential production of smaller triglyceride-rich HDL-P (33). HDL-P are heterogeneous in size and function and a full appreciation of its relationship with insulin resistance and diabetes is just coming into focus (34, 35). For example, even when HDL-C is within the normal range, greater concentrations of small, dense HDL-P were associated with chronic kidney disease and ASCVD (35). Our novel findings of HDL-P profiling in youth are consistent with observations in adults (34, 36), indicating distinct and opposing correlations of large and small HDL-P with S_I. The underlying mechanism by which hyperglycemia results in a shift toward smaller HDL-P remains to be elucidated but has been related to modifications in the proteome and lipidome (37) and/or increased HDL catabolism (38).

Importantly, the disparate observations with large and small HDL-P would have been missed if total HDL-P or HDL-C were examined alone. Therefore, the present study results add to the growing body of evidence indicating the superiority of NMR-derived lipoproteins over traditional lipid panels for risk assessment in youth (11, 39, 40). But the effect of this improved risk assessment on clinical care and clinical outcomes remains to be demonstrated. Exploring the use of sensitive early biomarkers will likely have a direct clinical effect because there is already evidence of subclinical atherosclerosis and endothelial dysfunction in youth with T2DM, even without frank elevations in LDL-C concentrations (12, 41). At present, standard of care guidelines recommend using a traditional lipid panel for pediatric hyperlipidemia screening, but current thresholds were primarily derived from studies in youth with familial hypercholesterolemia. In contrast to familial hypercholesterolemia, metabolic dyslipidemia is first characterized by elevated triglycerides and low HDL-C, before frank elevations in LDL-C (6, 42). Beyond a traditional lipid panel, if our findings are confirmed and clinical benefit demonstrated, a detailed analysis of lipoprotein size and number could improve cardiovascular discrimination of risk in youth. In adults, it is well recognized that the standard lipid panel may underestimate ASCVD risk and alternative markers, such as LDL-P and apolipoprotein B, are sometimes used to guide primary and secondary ASCVD risk strategies in individuals older than 40 years and those with T2DM (24, 43). But there are insufficient data in youth to include these lipoproteins in risk paradigms, and pediatric risk-reduction strategies—that rely on total and non–HDL-C—are conservative (44).

Our analyses addressed this knowledge gap for 3 potential biomarkers that are germane for developing cardiometabolic risk stratification paradigms in youth: (1) apolipoprotein B, (2) LDL-P, and (3) TRL-P remnant lipoproteins. First, the relationship of insulin sensitivity with apolipoprotein B concentrations—representing the total burden of atherogenic particles—did not differ between youth with obesity across the insulin sensitivity and glycemic spectrum, and therefore including apolipoprotein B in risk paradigms may reflect a cumulative burden associated with obesity. Second, in our cohort the number of youth who had LDL-C below 130 mg/

Table 2. Change in participant and metabolic characteristics over 2 years

Delta variables	Lean	OIS	OIR	AGT	*P*
	N = 20	N = 19	N = 10	N = 7	
Demographic and metabolic characteristics					
Age, y	2.2 ± 0.4	2.2 ± 0.2	2.3 ± 0.3	2.1 ± 0.2	.526
BMI, kg/m^2	1.6 ± 1.2	1.8 ± 3.5	1.5 ± 2.9	1.9 ± 1.9	.989
Fat mass, %	1.82 ± 2.85	0.03 ± 4.2	–1.64 ± 4.39	–1.2 ± 3.43	.072
Hemoglobin A_{1c}, %	0.2 ± 0.2	0.1 ± 0.2	0.2 ± 0.2	1.0 ± 2.3	.073
Fasting glucose, mg/dL	3.5 ± 9.8	0.45 ± 6.8	2 ± 9.9	29.9 ± 71.3	.682
Fasting insulin, µIU/mL	5 (0.9 to 7.9)	2.8 (–1.7 to 9.6)	–11.4 (–16.8 to 9.4)	–9.8 (–14.2 to 16.8)	.065
OGTT model sigma[a]		–0.1 (–0.3 to 0.3)	–0.4 (–0.8 to 0.4)	–0.1 (–0.7 to –0.01)	.504
OGTT model S_I	–	–0.4 (–1.3 to 1.0)	0.34 (–0.5 to 3.2)	–0.02 (–0.8 to 1.1)	.289
Lipid panel, mg/dL					
Total cholesterol	–4 (–25 to 2)	15 (–8 to 28)	12 (–3 to 32)	15 (–6 to 21)	**.032**
HDL-C	–2 (–6 to 5)	4 (–4 to 10)	1.5 (0 to 4)	4 (–4 to 10)	.396
LDL-C	–8 (–18 to –1)	3 (–11 to 14)	10 (2 to 19)	1 (–8 to 16)	**.047**
Triglycerides	6 (–13 to 22)	12 (–18 to 43)	16 (–5 to 63)	4 (–21 to 16)	.803
Lipoprotein panel, nmol/L					
Total TRL-P	3.1 (–15.2 to 19.9)	8.4 (–19 to 41)	10.7 (–1.1 to 29)	18.2 (–22.6 to 30.8)	.627
Remnant TRL-P	–0.4 (–14.7 to 15.7)	11.5 (–8.8 to 38.6)	11.1 (–2.7 to 18.2)	20 (–27.1 to 29.5)	.458
Total LDL-P	47 (–39 to 152.5)	164 (–52 to 342)	132.5 (0 to 351)	35 (–4 to 292)	.425
Small LDL-P	8 (–76 to 102)	–119 (–219 to 219)	145.5 (35 to 414)	27 (–137 to 314)	.173
Total HDL-P	–0.9 (–1.6 to 0.5)	0.3 (–0.7 to 1.8)	0.9 (–0.4 to 2.8)	1.6 (–0.9 to 2)	**.044**
Large HDL-P	–0.8 (–1.7 to 0.2)	0.1 (–0.4 to 0.5)	0 (–0.6 to 0.2)	0.2 (–0.1 to 0.4)	**.027**
Small HDL-P	–0.8 (–2 to 0.8)	0.2 (–0.7 to 1.4)	0.95 (–0.9 to 7.1)	0.6 (–1 to 3.8)	.294
Apolipoprotein A1, mg/dL	–6.5 (–13.5 to 0)	0 (–5 to 21)	4.5 (–5 to 7)	6 (–9 to 12)	**.043**
Apolipoprotein B, mg/dL	4 (–4.5 to 7)	6 (–2 to 17)	9.5 (–1 to 18)	5 (0 to 8)	.455
Marker of subclinical atherosclerosis					
cIMT, mm	–0.06 (–0.1 to 0.01)	–0.02 (–0.12 to 0.04)	0.04 (0.02 to 0.04)	–0.04 (–0.14 to 0)	.606

Data are mean ± SD or median (25th-75th percentile). Groups compared with one-way analysis of variance or Kruskal-Wallis test.
Abbreviations: AGT, abnormal glucose tolerant; BMI, body mass index; cIMT, carotid intimal thickness; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particles; LDL-C, low-density lipoprotein cholesterol; LDL-P, low-density lipoprotein particles; OGTT, oral glucose tolerance test; OIR, obese insulin resistant; OIS, obese insulin sensitive; S_I, insulin sensitivity index; TRL-P, triglyceride-rich lipoprotein particles.
[a]Sigma is a marker of β-cell function.

dL but met an adult threshold for increased risk of total LDL-P (≥ 1000 nmol/L) was high (40%), indicating an urgent need to address this problem. Discordance (low values of LDL-C despite elevated LDL-P) suggests a high proportion of small LDL-P that carry small amounts of cholesterol but still have high atherosclerotic burden. Discordance in LDL-C:LDL-P strongly tracked with acute coronary events and was more common in individuals with T2DM and insulin resistance (45). An atherogenic lipoprotein profile—characterized by high LDL-P and apolipoprotein B—has been related to certain measures of vascular structure and function in youth with obesity and diabetes (12, 39). Third, there were concomitant increases in TRL-P (large and remnant) in all youth with obesity irrespective of glycemic status that correlated with cIMT. Further research is needed to investigate the contribution(s) of remnant particles to development subclinical atherosclerosis in youth.

Some study limitations are noteworthy. The primary cross-sectional study design in predominantly African American youth precluded inferences of causality and limits generalizability to the broader population of multiethnic youth with T2DM. Of note, when this study was designed in 2007, there was no consensus on an appropriate fasting insulin concentration or HOMA-IR cutoff for youth. Since then, several studies have suggested different cutoffs based on age or pubertal status (46, 47). However, there is still no agreement on an optimal cutoff for fasting insulin or HOMA-IR in youth (48). Lipid concentrations may also be substantially influenced by diet and physical activity, and we conducted a broad assessment of nutritional habits and activity with self-reporting instruments. However, self-reporting questionnaires are subject to recall and social desirability biases (overreporting of activity [49] and underreporting of diet [50]). In addition, the longitudinal aim was limited by the small sample size, and 2 years may not have been enough time to detect a statistically significant progression in cIMT. Study strengths include the rigorous assessments of insulin sensitivity and detailed lipoprotein profiles in predominantly African American young people, who constitute the largest burden of T2DM in youth. Although limited in size, the longitudinal 2-year analysis provided estimates for prospective studies to evaluate temporal changes of lipoproteins with ASCVD risk markers in youth and young adults.

In summary, insulin sensitivity was inversely associated with an atherogenic lipid/lipoprotein profile and CMIT in youth with obesity. The presence of abnormal glucose tolerance modified the relationship of HDL-C and large HDL-P, with insulin sensitivity indicating that alterations in HDL-P metabolism may be an early adverse manifestation of hyperglycemia in youth with AGT. Further refinement and reevaluation of ASCVD risk-reduction paradigms are urgently needed in all youth with obesity.

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Conflict of Interest

The authors have nothing to disclose.

Data Availability

Some or all data sets generated during and/or analyzed during the present study are not publicly available but are available from the corresponding author on reasonable request.

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