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Associations of hyperglycemia and insulin resistance with biomarkers of endothelial dysfunction in Hispanic/Latino youths: Results from the Hispanic Community Children's Health Study/ Study of Latino Youth (SOL Youth)

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Abstract

Aims: We hypothesized that Hispanic/Latino youth at high risk for diabetes would have elevated biomarkers of endothelial dysfunction.

Methods: Among 1,316 children 8–16 years old from the Study of Latino Youth (SOL Youth), we used Poisson regression to obtain prevalence ratios (PRs) and 95% CIs for the cross-sectional association of quartiles of fasting glucose, HbA1c, and insulin resistance with E-selectin and plasminogen activator inhibitor-1 (PAI-1) levels above the median (48.1 and 2.02 ng/mL, respectively).

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Author contributions: CMP designed the study, interpreted the data, and wrote the manuscript; SH analyzed and interpreted the data, and reviewed/edited the manuscript; MRC, LCG, BIH, RBG, AMD, and RCK interpreted the data and reviewed/edited the manuscript; CRI designed the study, interpreted the data, and reviewed/edited the manuscript. CMP is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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The authors have no relevant conflicts of interest to disclose.

Results: Levels of E-selectin and PAI-1 were higher in children who were obese or had higher levels of hs-CRP ($p < 0.05$). Insulin resistance was independently associated with higher levels of PAI-1 (adjusted PR and 95% CI for the highest versus lowest quartile (Q4 vs Q1): 2.25 [1.64, 3.09]). We found stronger evidence of associations of insulin resistance with higher levels of PAI-1 among boys as compared with girls (p -interaction = 0.10).

Conclusions: Insulin resistance was associated with endothelial dysfunction, as measured by higher levels of PAI-1, in Hispanic/Latino youth. These biomarkers may be useful in risk stratification and prediction of diabetes and cardiovascular disease in high-risk youth.

Keywords

adolescents/children; diabetes; E-selectin; endothelial dysfunction; Hispanics; Latinos; hyperglycemia; insulin resistance; plasminogen activator inhibitor-1

Introduction

Endothelial dysfunction is characterized by impaired vasoregulation, abnormal angiogenesis (either increased or decreased), impaired permeability of the endothelium, and increased inflammation (Eringa et al., 2013). There is a complex interplay of endothelial function with inflammation, hyperglycemia, and diabetes. In the setting of diabetes, hyperglycemia may contribute to endothelial dysfunction through the development of advanced glycation endproducts (Eringa et al., 2013). However, there may be a bidirectional relation between endothelial dysfunction and hyperglycemia. The endothelium may help regulate insulin secretion by directing lipid transport into beta cells, maintaining blood flow, and promoting cytokine activity, and may therefore contribute to the development of diabetes (Eringa et al., 2013; Hamilton & Watts, 2013). This suggests the hypothesis that among children and adolescents at high risk of future diabetes, an association may be detected between endothelial function and markers of hyperglycemia and insulin resistance.

Several biomarkers may be used as noninvasive measures of endothelial dysfunction. E-selectin is a cellular adhesion molecule that is produced in response to inflammation (signaled by interleukin [IL]-1 and tumor necrosis factor [TNF]-alpha) (Goldberg, 2009; Price & Loscalzo, 1999). Plasminogen activator inhibitor-1 (PAI-1) inhibits the degradation of blood clots (Price & Loscalzo, 1999), and is a biomarker of coagulation. Inflammation may lead to endothelial dysfunction and a procoagulant state, both of which may contribute to the development of insulin resistance and atherosclerosis (Goldberg, 2009; Price & Loscalzo, 1999). In adults, higher levels of these biomarkers have been associated with hyperglycemia, insulin resistance, and diabetes (Almeida-Pititto, Ribeiro-Filho, Lotufo, Bensenor, & Ferreira, 2015; Blüher, Unger, Rassoul, Richter, & Paschke, 2002; Goldberg, 2009; Kanaya et al., 2013; Meigs, Hu, Rifai, & Manson, 2004; Mendivil, Robles-Osorio, Horton, Hamdy, & Caballero, 2013; Thorand, Baumert, Chambless, et al., 2006; Thorand, Baumert, Döring, et al., 2006; Wang, Liu, Wang, Tong, & Zhang, 2010).

Since endothelial dysfunction may contribute to the long-term development of atherosclerosis and cardiovascular disease (CVD) in people with diabetes, a better understanding of the relationship between endothelial biomarkers and future diabetes risk

could facilitate primary prevention of diabetes and CVD. Few studies have investigated the correlates of biomarkers of endothelial dysfunction in youth (Caballero et al., 2008). This may be especially relevant in those of Hispanic/Latino background, who by virtue of their race/ethnic background are identified as a high risk group, relative to youths from other backgrounds, that should be considered for testing for childhood onset type 2 diabetes (“Screening for Diabetes,” 2002). Those studies that have been conducted in youth have reported positive, negative, and null associations of biomarkers of endothelial dysfunction in relation to hyperglycemia and insulin resistance, and have therefore been inconclusive as a whole (Burns et al., 2014; Caballero et al., 2008; Lee, Gungor, Bacha, & Arslanian, 2007; Rao, Gurumurthy, Gururajan, Saibabu, & Cherian, 2011).

In a population based, multi-center sample of Hispanic/Latino children 8 to 16 years of age, we aimed to characterize the associations of hyperglycemia and insulin resistance with biomarkers of endothelial dysfunction. We hypothesized that biomarkers reflecting hyperglycemia and insulin resistance would be positively associated with endothelial dysfunction. The large sample size also allowed us to examine whether these patterns were consistent across demographic subgroups defined by age, sex and body mass index.

Subjects, Materials and Methods

Study population

The Hispanic Community Children’s Health Study / Study of Latino (SOL) Youth recruited 1,466 boys and girls from four field centers: Bronx, New York; Chicago, Illinois; Miami, Florida; and San Diego, California. The design of the SOL Youth Study has been described previously (Isasi et al., 2014). Participants were eligible for the study if they had a parent/legal guardian in the parent study (the Hispanic Community Health Study / Study of Latinos [HCHS/SOL] (Sorlie et al., 2010)) and were living 5 days per week and 9 months per year with the parent/legal guardian; were 8–16 years of age by the time of the baseline examination; and were free of any known physical or cognitive condition that could interfere with completing the examination.

The current study excluded participants who were missing E-selectin or PAI-1 (n=80); missing glucose, HbA1c, or HOMA-IR (additional n=10); fasting <8 hours or had unknown fasting status (additional n=5); had incomplete data for key covariates (additional n=53); or reported use of diabetes medications (additional n=1). There were no participants who self-reported a diagnosis of diabetes. There were 326 participants missing physical activity and 66 missing pubertal stage, so we imputed these values (see description in the *Statistical Analysis* section). We additionally excluded one participant with extremely high values of fasting glucose (246 mg/dL), HbA1c (9.8%), and HOMA-IR (22.8), because inclusion of this individual substantially influenced the results.

SOL Youth was approved by the institutional review board at each field center. Written informed consent and assent were obtained from all parents/legal guardians and children, respectively.

Laboratory measurements

All blood specimens were drawn in the morning under fasting conditions, processed and separated on site and stored at -70°C . The University of Minnesota's Advanced Research and Diagnostic Laboratory performed all laboratory assays. E-selectin and PAI-1 were measured from serum and plasma, respectively, using a sandwich enzyme immunoassay (R & D Systems, Minneapolis, MN) with the Beckman Coulter Biomek NXp (Beckman Coulter, Inc, Fullerton, CA). Fasting glucose was measured from plasma using a hexokinase enzymatic method on the Roche/Modular P Chemistry Analyzer and the Roche COBAS 6000 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). HbA1c was measured from whole blood using high performance liquid chromatography (HPLC) using a Tosoh G8 Automated HPLC Analyzer (Tosoh Bioscience, Inc, South San Francisco, CA) and was standardized to the Diabetes Control and Complications Trial assay. Insulin was measured from plasma using a sandwich immunoassay method on the Roche COBAS 6000 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). Lipids were measured from serum using the Roche/Modular P Chemistry Analyzer and the Roche COBAS 6000 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). HDL-c was measured using a direct precipitation method (Roche Diagnostics, Indianapolis, IN). Triglycerides and cholesterol were measured using an enzymatic method (Roche Diagnostics, Indianapolis, IN). LDL-c was calculated using the Friedewald equation (Friedewald, Levy, & Fredrickson, 1972). High-sensitivity C-reactive protein (hs-CRP) was measured from serum using an immunoturbidimetric method on the Roche COBAS 6000 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). Laboratory inter-assay coefficients of variation were 6.2 to 11.6% for biomarkers of endothelial dysfunction; 1.3% to 3.1% for fasting glucose, HbA1c, and insulin; 1.3% to 5.2% for lipids; and 6.7% for hs-CRP.

Self-reported and measured covariates

Age, sex, Hispanic/Latino background, and pubertal stage were self-reported by the participant. Pubertal stage was determined using the Pubertal Development Scale self-assessment tool. We classified participants as either pubertal (reported menarche for girls or at least started getting facial hair for boys) or prepubertal (girls not having reached menarche or boys reporting no facial hair growth). The participant's parent or legal guardian reported annual household income and level of educational attainment. Height was measured to the nearest cm using a wall stadiometer (SECA 222, Germany). The mean of three height measurements was used for analysis. Weight was measured using a digital scale to the nearest .1 kg (Tanita Body Composition Analyzer, TBF 300, Japan). Standardized programs from the Centers for Disease Control were used to calculate age- and sex-specific body mass index (BMI) percentiles from the measured heights and weights (Kuczmarski et al., 2002). Blood pressure was measured after 5 minutes of sitting rest using an OMRON HEM-907XL sphygmomanometer (Omron Healthcare Co. Ltd., Kyoto, Japan). The mean of the second and third measurements were used for analysis. National Heart, Lung, and Blood Institute blood pressure level tables were used to calculate age-, sex-, and height-specific diastolic and systolic blood pressure percentiles (NHLBI/NHBPEP, 2004). Waist circumference was measured three times and the mean of all three measurements was used for analysis. Participants wore accelerometers (Actical model 198-0200-03; Respironics Co. Inc., Bend, Oregon) for 7 days to measure physical activity. Participants were considered

adherent if they wore the accelerometer and had valid data for 8 hours per day for at least 3 days.

Statistical analyses

We categorized biomarkers of hyperglycemia and insulin resistance into quartiles (fasting glucose quartiles were Q1: <88, Q2: 88 to <92, Q3: 92 to <96, and Q4: 96 mg/dL; HbA1c quartiles were Q1: <5.1, Q2: 5.1 to <5.3, Q3: 5.3 to <5.4, and Q4: 5.4 %; and HOMA-IR quartiles were Q1: <1.9, Q2: 1.9 to <2.8, Q3: 2.8 to <4.4, and Q4: 4.4). For the purposes of assessing study population characteristics, hyperglycemia was defined in two ways: either as fasting glucose 100 mg/dL or HbA1c 5.7% (American Association Diabetes, 2016). Only one participant met the criteria for diabetes, based on fasting glucose 126 mg/dL. None had HbA1c 6.5%. We calculated HOMA-IR as $[\text{glucose (in mg/dL)} \times \text{insulin (in pmol/L)}] / 6 / 405$ (Matthews et al., 1985). Again, for the purposes of assessing sociodemographic and clinical characteristics by level of insulin resistance, insulin resistance was defined as HOMA-IR 2.6, since HOMA-IR levels above this threshold have recently been shown to be associated with higher cardiometabolic risk in adolescents (Burrows, Reyes, Blanco, Albala, & Gahagan, 2015). We used the median values of e-selectin (48.1 ng/mL) and PAI-1 (2.02 ng/mL) as cut-points. Age- and sex-specific BMI percentiles were determined based on the Centers for Disease Control and Prevention Growth Charts (Kuczmarski et al., 2002). We categorized weight status as follows: underweight/normal weight (BMI <85th percentile), overweight (BMI 85th to <95th percentile), or obese (BMI 95th percentile). Elevated levels of systolic blood pressure were considered to be those 90th percentile (NHLBI/NHBPEP, 2004). Elevated waist circumference was defined as having an age- and sex-specific waist circumference 90th percentile (Fryar, Gu, & Ogden, 2012). Moderate/vigorous activity was defined as 441 counts/15 seconds (Romanzini, Petroski, Ohara, Dourado, & Reichert, 2014), and was included in multivariable analyses since it is a known predictor of cardiometabolic risk in children and adolescents (Ekelund et al., 2012). We natural log (ln)-transformed any non-normally distributed variables.

We used multiple imputation to impute data for physical activity and pubertal stage, since these were the variables missing by the largest number of participants (326 were missing physical activity and 66 were missing pubertal stage). The following variables were included in the imputation models: endothelial dysfunction variables (E-selectin and PAI-1), insulin resistance and glycemia variables (fasting glucose, HbA1c, HOMA-IR), and all variables that were included in subsequent multivariable analyses. Imputation models were specified as predictive mean matching for physical activity and logistic regression for pubertal status. Twenty imputation data sets were created, and point estimates from each data set were pooled to obtain final results.

We calculated medians and percentages of study population characteristics by level of fasting glucose, HbA1c, and HOMA-IR (below versus above 100 mg/dL, 5.7%, and 2.6, respectively). We then assessed the associations of quartiles of fasting glucose, HbA1c, and HOMA-IR with e-selectin 48.1 ng/mL, and PAI-1 2.02 ng/mL, using Poisson regression with robust variance to calculate prevalence ratios and 95% CIs. We ran separate models for

fasting glucose, HbA1c, and HOMA-IR, and then a model in which they were simultaneously included. Model 1 (minimally adjusted) was adjusted for age, sex, field center (Bronx, NY; San Diego, CA; Miami, FL; Chicago, IL), Hispanic/Latino background (Mexican, non-Mexican), annual household income (< \$20,000; \$21,000-\$40,000; > \$40,000), and parental education (less than high school, high school, more than high school). Model 2 (fully adjusted) was further adjusted for elevated waist circumference (<90th percentile, 90th percentile), BMI percentile, pubertal status, physical activity, systolic blood pressure percentile, LDL-c, HDL-c, triglycerides, and ln of hs-CRP. We conducted several sensitivity analyses. First, we assessed the associations of fasting glucose, HbA1c, and HOMA-IR with E-selectin and PAI-1 operationalized as continuous variables using linear regression. Second, we included Von Willebrand factor (vWF), a biomarker of hemostasis and endothelial dysfunction and damage, in the analysis in the subset of participants for whom this biomarker was measured. Third, we stratified analyses by age (8–12 versus 13–16 years) to account for potential differences in growth hormone levels by age.

We formally tested for effect modification of the associations of fasting glucose, HbA1c, and HOMA-IR with E-selectin and PAI-1 by age category (8–12, 13–14, or 15–16 years), sex (male or female), and BMI category (non-obese or obese) in each of the fully adjusted models using Wald tests for joint significance.

All analyses accounted for complex survey sampling design. P-values were two-sided with a significance level at 0.05. All analyses were performed using Stata 14.0 (StataCorp LP, College Station, TX).

RESULTS

We included 1,316 participants in our analytic study population (mean age 12 years, 51% male, 46% overweight or obese). Children and adolescents who were obese, had elevated waist circumference, or had higher levels of triglycerides were more likely to have hyperglycemia or insulin resistance; and those who had higher levels of hs-CRP were more likely to have insulin resistance (Table 1) (all $p < 0.05$). Compared to girls, boys were more likely to have higher levels of fasting glucose and were less likely to have insulin resistance ($p < 0.05$), but had similar levels of HbA1c (Table 1). Participants with higher levels of HOMA-IR, fasting glucose, and HbA1c had higher median levels of E-selectin and PAI-1 ($p < 0.05$) (Table 1).

In multivariable analyses, adjustment was performed for age, sex, field center, Hispanic/Latino background, annual household income, parental education, elevated waist circumference, BMI percentile, pubertal status, physical activity, systolic blood pressure percentile, LDL-c, HDL-c, triglycerides, and ln of hs-CRP. In fully adjusted models that examined fasting glucose, HbA1c, and HOMA-IR separately, higher levels of HOMA-IR were statistically significantly associated with E-selectin 48.1 ng/mL and PAI-1 2.02 ng/mL: PRs and 95% CIs for associations with E-selectin versus Q1 were Q4: 1.35 (1.02, 1.79), Q3: 1.23 (0.95, 1.58), and Q2: 0.98 (0.77, 1.24) and for associations with PAI-1 versus Q1 were Q4: 2.43 (1.80, 3.29), Q3: 1.98 (1.46, 2.68), and Q2: 1.61 (1.19, 2.16) (Table 2). We observed statistically significant associations of higher levels of fasting glucose, but

not HbA1c, with e-selectin 48.1 ng/mL and PAI-1 2.02 ng/mL. Participants with levels of fasting glucose 96 mg/dL (Q4) had a higher prevalence of E-selectin 48.1 ng/mL and PAI-1 2.02 ng/mL (PRs and 95% CIs for Q4 vs Q1 were 1.30 [1.06, 1.61] and 1.39 [1.14, 1.68], respectively) than those with levels <88 mg/dL (Q1) (Table 2).

In models that simultaneously included all three biomarkers of hyperglycemia and insulin resistance, magnitudes of associations for all biomarkers were relatively unchanged, although inferences were slightly different. Only the association of higher levels of HOMA-IR with higher levels of PAI-1 remained statistically significant (Table 2). Results and inferences were similar in analyses that included the dependent variables (biomarkers of endothelial dysfunction) as continuous variables (eTable 1). There were no clear associations of hyperglycemia or insulin resistance with vWF (eTable 2).

There was a marginally statistically significant suggestion of an interaction of insulin resistance, as measured by HOMA-IR, and PAI-1 with sex ($P = 0.10$). In models that simultaneously included E-selectin and PAI-1, higher levels of HOMA-IR were associated with PAI-1 2.02 ng/mL in boys at greater magnitudes of association and stronger statistical significance than for girls (PRs and 95% CIs for boys for Q4 vs Q1 were 2.85 [1.87, 4.33] and for girls were 1.79 [1.21, 2.65]) (Figure 1). We did not observe statistically significant interactions of fasting glucose, HbA1c, or HOMA-IR with BMI in relation to E-selectin or PAI-1. However, higher levels of fasting glucose were associated with E-selectin 48.1 ng/mL in older children, but not younger children (P -interaction <0.01); and although not statistically significant, the magnitude of association of insulin resistance with PAI-1 was greater in younger children than older children (eTable 3).

DISCUSSION

In this study of 8- to 16-year-old Hispanic/Latino youth, higher levels of HOMA-IR (independent of fasting glucose and HbA1c) were associated with higher levels of PAI-1, but not E-selectin. However, there was a suggestion that the magnitude of this association was greater in boys than girls. This association of insulin resistance with endothelial function biomarkers persisted after adjustment for covariates, several of which including obesity, central adiposity, high levels of triglycerides, and high levels of hs-CRP, were themselves associated with endothelial biomarkers. Of particular importance, this suggests that there may be an inflammation-independent association between insulin resistance and PAI-1.

This study supports the hypothesized role of insulin resistance contributing to endothelial dysfunction, while suggesting that prior findings of endothelial dysfunction among adults with frank diabetes may also be relevant to high-risk individuals without diabetes during the earliest decades of life. In cross-sectional studies of adults, higher levels of E-selectin and PAI-1 have been associated with impaired fasting glucose (IFG), impaired glucose tolerance, diabetes, and insulin resistance (Almeida-Pititto et al., 2015; Blüher et al., 2002; Kanaya et al., 2013; Thorand, Baumert, Döring, et al., 2006; Wang et al., 2010). In longitudinal studies, elevated levels of these biomarkers have been shown to precede the development of type 2 diabetes (Goldberg, 2009; Meigs et al., 2004; Thorand, Baumert, Chambless, et al., 2006) and its complications (Goldberg, 2009). We found here, in children and adolescents, that

insulin resistance (elevated HOMAIR) was associated with higher levels of PAI-1 but not E-selectin. This finding could reflect the fact that these biomarkers play different roles in regulating the endothelium. E-selectin is expressed in endothelial cells and binds to glycoprotein receptors in response to inflammatory cytokines (Nimrichter et al., 2008). PAI-1, a biomarker of altered coagulation, is secreted by both endothelial cells and adipocytes and inhibits the breakdown of blood clots, and can therefore lead to thrombosis if present in high levels (Cesari, Pahor, & Incalzi, 2010). It is possible that increases in PAI-1 may occur prior to elevations in E-selectin. Furthermore, HOMA-IR may be an earlier indicator of glucose dysregulation than either fasting glucose or HbA1c (Lillioja et al., 1988; Warram, Martin, Krolewski, Soeldner, & Kahn, 1990), and therefore hyperglycemia may not manifest until further along in the development of endothelial dysfunction. In combination, this may explain why we found that PAI-1 but not E-selectin was associated with insulin resistance. It may also be that given the narrow range of dysglycemia in this cohort, it is harder to demonstrate an association between hyperglycemia and E-selectin or PAI-1.

Whereas associations in the present study appeared to be magnified in boys as compared with girls, the reasons explaining the observed marginally statistically significant interaction by sex remain to be elucidated. We have previously reported in this cohort that compared to girls, boys had a more adverse cardiometabolic risk profile, including higher prevalence of obesity and pre-diabetes (Isasi et al., 2016). However, others have reported that girls were more likely to exhibit insulin resistance than boys (Moran et al., 1999; Tester, Sharma, Jasik, Mietus-Snyder, & Tinajero-Deck, 2013). A previous study reported that levels of insulin resistance during puberty were not fully explained by BMI and adiposity alone, and that increased production of sex steroids, growth hormone, and IGF-1 during this time could contribute to the transient increases in insulin resistance observed in adolescence (Moran et al., 1999). Potential hormonal differences in boys and girls during adolescence could contribute to sex differences in the development of insulin resistance, and may help explain why we observed the association of endothelial dysfunction with insulin resistance in boys but not girls. Future studies are needed to help understand the roles of sex hormones and other factors in explaining this interaction.

Levels of traditional CVD risk factors in childhood are important predictors of adult health (Berenson et al., 1998; Li et al., 2003; Urbina et al., 2009). The presence of nontraditional risk factors, such as elevated levels of biomarkers of endothelial dysfunction, at an early age may also have important implications for future health. In fact, endothelial dysfunction is reversible, and may provide an important point of intervention in youths (Bruyndonckx et al., 2015; Park et al., 2012).

Limitations of this study include a cross-sectional study design, and we are therefore unable to infer temporality of these associations. Nonetheless, this research question had not previously been adequately studied in a young population, and the Hispanic/Latino youth population is of particular importance for its strong predisposition to future diabetes. This was an observational study and there is always the potential for residual confounding, particularly with certain variables that tend to be difficult to measure without error, including physical activity. However, accelerometers were used to objectively measure physical activity in SOL Youth, an important covariate in this analysis. Growth hormone levels were

not measured in SOL Youth, and we were therefore not fully able to account for the potential influence of growth hormone levels in this age group. Lastly, additional biomarkers of endothelial dysfunction, such as intercellular adhesion molecule 1 and vascular cell adhesion molecule 1, may have provided additional insight into our research question, however these were not measured in SOL Youth. Important strengths of the study included the large sample size of Hispanic/Latino children and adolescents with self-reported, measured, and blood specimen data collected according to a standardized protocol.

Since the endothelium may help regulate insulin secretion, biomarkers of endothelial function including PAI-1 and E-selectin may be useful in risk stratification and prediction of diabetes and CVD in young people. Whereas longitudinal studies in the future will be important to understand the causal nature of this relationship, this analysis was an important first step in characterizing associations of hyperglycemia and insulin resistance with endothelial biomarkers in a population of young Hispanics/Latinos.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Associations of HOMA-IR with PAI-1 by sex.

Poisson regression models were used to obtain prevalence ratios and 95% CIs for the associations of HOMA-IR with PAI-1 ≥ 2.02 ng/mL. Model included HOMA-IR, fasting glucose and hemoglobin A1c and linear combinations were used to obtain results for boys and girls, separately. P-values for the interaction of PAI-1 with sex were calculated using Wald tests for joint significance for the interaction terms from models that included both boys and girls. HOMA-IR quartiles are Q1: <1.9 , Q2: 1.9 to <2.8 , Q3: 2.8 to <4.4 , and Q4: ≥ 4.4 . Results are from the fully adjusted model and were adjusted for age, center, Mexican background, household income, parental education, elevated waist circumference, BMI percentile, pubertal status, systolic blood pressure percentile, physical activity, LDL-c, HDL-c, triglycerides, and $\ln$ of hs-CRP.

Table 1.

Study population characteristics (n = 1316).

	Fasting glucose				HbA1c				HOMA-IR			
	<100 mg/dL (n = 1169)		100 mg/dL (n = 147)		<5.7% (n = 1218)		5.7% (n = 98)		<2.6 (n = 583)		2.6 (n = 733)	
	Median (25th–75th percentile) or %	12 (10–14)	Median (25th–75th percentile) or %	12 (10–15)	Median (25th–75th percentile) or %	12 (10–15)	Median (25th–75th percentile) or %	11 (10–14)	Median (25th–75th percentile) or %	12 (9–15)	Median (25th–75th percentile) or %	12 (10–14)
Age, years												
Male	49%		72%		51%		56%		58%		46%	
Mexican	46%		49%		47%		39%		45%		48%	
Field center												
Bronx	38%		20%		36%		48%		41%		33%	
Chicago	14%		17%		14%		12%		13%		15%	
Miami	13%		26%		14%		11%		11%		17%	
San Diego	35%		37%		36%		30%		35%		35%	
Household income												
\$20,000 per year	52%		59%		51%		71%		53%		53%	
\$21–40,000 per year	32%		29%		33%		20%		30%		33%	
>\$40,000 per year	16%		13%		16%		10%		17%		14%	
Parental education												
<High school	37%		44%		39%		27%		38%		38%	
High school	30%		29%		29%		41%		28%		31%	
>High school	33%		27%		33%		32%		34%		31%	
BMI category												
Under/normal weight	55%		47%		55%		43%		76%		34%	
Overweight	20%		18%		20%		14%		18%		21%	
Obese	25%		35%		25%		43%		6%		44%	
Waist circumference 90th percentile	11%		22%		12%		21%		2%		22%	
Systolic BP 90th percentile	4%		13%		5%		4%		4%		5%	
Pubertal, %	58%		59%		58%		52%		53%		62%	

	Fasting glucose			HbA1c			HOMA-IR		
	<100 mg/dL (n = 1169)	100 mg/dL (n = 147)	p-Value *	<5.7% (n = 1218)	5.7% (n = 98)	p-Value *	<2.6 (n = 583)	2.6 (n = 733)	p-Value *
	Median (25th–75th percentile) or %	Median (25th–75th percentile) or %		Median (25th–75th percentile) or %	Median (25th–75th percentile) or %		Median (25th–75th percentile) or %	Median (25th–75th percentile) or %	
Moderate to vigorous physical activity, min/day	32 (20–46)	32 (20–47)	0.90	32 (20–46)	34 (22–47)	0.56	37 (25–52)	27 (17–41)	<0.001
Total cholesterol, mg/dL	152 (136–170)	158 (136–179)	0.24	152 (136–170)	157 (138–174)	0.42	148 (134–168)	155 (137–173)	0.01
Triglycerides, mg/dL	68 (50–94)	70 (51–107)	0.70	67 (49–94)	80 (58–100)	0.003	54 (42–73)	81 (60–113)	<0.001
HDL cholesterol, mg/dL	51 (44–59)	50 (44–60)	0.32	51 (45–59)	49 (43–56)	0.20	53 (47–62)	49 (42–56)	<0.001
LDL cholesterol, mg/dL	84 (71–99)	91 (73–104)	0.11	83 (71–99)	90 (77–104)	0.01	81 (70–98)	87 (72–101)	0.002
Fasting glucose, mg/dL	91 (87–94)	103 (101–106)	<0.001	92 (88–96)	93 (90–98)	0.39	90 (85–94)	93 (90–97)	<0.001
HbA1c, %	5.3 (5.1–5.4)	5.3 (5.1–5.6)	1.00	5.2 (5.1–5.4)	5.7 (5.7–5.8)	<0.001	5.2 (5.1–5.4)	5.3 (5.1–5.5)	0.001
HOMA-IR	2.6 (1.7–4.0)	4.0 (2.8–6.0)	<0.001	2.7 (1.7–4.2)	3.0 (2.1–5.6)	0.35	1.7 (1.3–2.1)	4.1 (3.2–5.3)	<0.001
PAI-1, ng/mL	1.8 (1.2–3.3)	2.7 (1.5–4.8)	0.004	1.9 (1.2–3.3)	3.1 (1.8–4.7)	0.006	1.4 (0.9–2.1)	2.7 (1.6–4.5)	<0.001
E-selectin, ng/mL	46.4 (34.1–63.2)	52.4 (37.6–65.4)	0.02	46.3 (34.1–63.0)	55.6 (37.1–70.8)	0.05	43.6 (32.1–58.1)	51.5 (37.0–66.6)	<0.001
vWF, IU/mL [†]	1.1 (0.9–1.4)	1.0 (0.9–1.2)	0.05	1.1 (0.9–1.4)	1.1 (0.7–1.5)	0.85	1.0 (0.8–1.3)	1.1 (0.9–1.4)	0.13
hs-CRP, mg/L	0.4 (0.2–1.1)	0.6 (0.2–1.2)	0.34	0.4 (0.2–1.1)	0.6 (0.4–2.2)	0.32	0.3 (0.2–0.7)	0.6 (0.3–1.6)	<0.001

* p-Values were calculated using Wald tests (from quantile regression for medians and logistic regression for categorical variables).

[†] vWF was measured in a subset of 656 participants. p-Values were calculated using Wald tests (from quantile regression for medians and logistic regression for categorical variables).

Table 2. Associations of biomarkers of hyperglycemia and insulin resistance with endothelial dysfunction

	E-selectin 48.1 ng/mL		PAI-1 2.02 ng/mL	
	Model 1 PR (95%CI)	Model 2 PR (95%CI)	Model 1 PR (95%CI)	Model 2 PR (95%CI)
Fasting glucose				
Q4 (highest)	1.41 (1.14,1.74)	1.30 (1.06,1.61)	1.66 (1.33,2.07)	1.39 (1.14,1.68)
Q3	1.27 (1.03,1.55)	1.17 (0.95,1.44)	1.44 (1.16,1.80)	1.20 (0.98,1.48)
Q2	1.39 (1.12,1.71)	1.31 (1.07,1.61)	1.10 (0.87,1.38)	0.99 (0.80,1.22)
Q1 (lowest)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
Hemoglobin A1c				
Q4 (highest)	1.04 (0.86,1.26)	0.93 (0.77,1.12)	1.43 (1.19,1.73)	1.18 (0.97,1.44)
Q3	1.07 (0.84,1.35)	0.97 (0.78,1.21)	1.08 (0.82,1.43)	0.96 (0.74,1.23)
Q2	0.86 (0.71,1.03)	0.83 (0.69,0.99)	0.92 (0.74,1.14)	0.88 (0.72,1.09)
Q1 (lowest)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
HOMA-IR				
Q4 (highest)	1.59 (1.27,1.98)	1.35 (1.02,1.79)	3.90 (0.99,5.10)	2.43 (1.80,3.29)
Q3	1.34 (1.08,1.66)	1.23 (0.95,1.58)	2.50 (1.83,3.40)	1.98 (1.46,2.68)
Q2	1.01 (0.79,1.28)	0.98 (0.77,1.24)	1.76 (1.30,2.37)	1.61 (1.19,2.16)
Q1 (lowest)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
When included in the model simultaneously: Fasting glucose				
Q4 (highest)	1.21 (0.96,1.54)	1.22 (0.97,1.55)	1.01 (0.83,1.23)	1.08 (0.88,1.31)
Q3	1.16 (0.94,1.43)	1.14 (0.91,1.41)	1.02 (0.85,1.23)	1.01 (0.83,1.23)

	E-selectin		PAI-1	
	Model 1 PR (95%CI)	Model 2 PR (95%CI)	Model 1 PR (95%CI)	Model 2 PR (95%CI)
Q2	1.34 (1.07,1.67)	1.30 (1.05,1.61)	0.92 (0.76,1.11)	0.88 (0.72,1.08)
Q1 (lowest)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
Hemoglobin A1c				
Q4 (highest)	0.98 (0.81,1.18)	0.91 (0.75,1.09)	1.17 (0.97,1.42)	1.08 (0.89,1.32)
Q3	1.02 (0.82,1.27)	0.95 (0.76,1.18)	1.00 (0.79,1.27)	0.93 (0.73,1.19)
Q2	0.85 (0.71,1.02)	0.82 (0.69,0.98)	0.90 (0.74,1.10)	0.86 (0.70,1.06)
Q1 (lowest)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
HOMA-IR				
Q4 (highest)	1.50 (1.17,1.93)	1.27 (0.93,1.74)	3.65 (2.76,4.83)	2.25 (1.64,3.09)
Q3	1.26 (1.00,1.60)	1.16 (0.88,1.52)	2.42 (1.76,3.31)	1.89 (1.38,2.59)
Q2	0.96 (0.75,1.23)	0.94 (0.73,1.20)	1.70 (1.27,2.28)	1.57 (1.16,2.11)
Q1 (lowest)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)

Bolded results indicate $p < 0.05$

Fasting glucose quartiles are Q1: <88, Q2: 88 to <92, Q3: 92 to <96, and Q4: 96

HbA1c quartiles are Q1: <5.1, Q2: 5.1 to <5.3, Q3: 5.3 to <5.4, and Q4: 5.4

HOMA-IR quartiles are Q1: <1.9, Q2: 1.9 to <2.8, Q3: 2.8 to <4.4, and Q4: 4.4

* There were 147 participants with fasting glucose 100 mg/dL, 98 participants with HbA1c 5.7%, and 733 participants with HOMA-IR 2.6.

Model 1: adjusted for age, sex, center, Mexican background, household income, and parental education

Model 2: Includes adjustment variables in Model 1, with additional adjustment for elevated waist circumference, BMI percentile, pubertal status, systolic blood pressure percentile, physical activity, LDL-c, HDL-c, triglycerides, and ln of hs-CRP