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Triglyceride-rich very low density lipoproteins (VLDL) are independently associated with insulin secretion in a multiethnic cohort of adolescents

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Abstract

Excess insulin secretion and hyperinsulinemia contribute to the progression of type 2 diabetes. However, the mechanisms leading to insulin hypersecretion remain largely unknown. Based on our preliminary data, we examined whether triglycerides and very low-density lipoprotein (VLDL) are independently associated with insulin secretion, and whether the ethnicity/race modulates these associations. Fasting triglycerides and VLDL were measured in a multiethnic cohort of 630 non-diabetic adolescents. Insulin secretion, β -cell function parameters, insulin sensitivity and insulin clearance were estimated through a 3-h OGTT. Metabolic assessments were repeated after 2 years in 239 subjects. Triglycerides and triglyceride-rich VLDL (large and medium size fractions) were associated with both basal and glucose-stimulated insulin secretion, after adjustment for age, sex, ethnicity, BMI z-score, plasma glucose, and insulin sensitivity. Ethnicity *per se* had an impact on lipid profile and β -cell function, but did not modulate the effect of triglycerides/VLDL on insulin secretion. At follow-up, changes in triglyceride levels were proportional to changes in insulin secretion. These findings support the hypothesis that hypertriglyceridemia is an important stimulus for β -cell insulin release in youths under both fasting and fed conditions.

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Author contributions

DT: study design, mathematical modeling of insulin secretion and β -cell function parameters, analysis and interpretation of results, manuscript writing; AN and EF: analysis and interpretation of results, manuscript editing; AM: mathematical modeling of insulin secretion and β -cell function parameters, analysis and interpretation of results, manuscript editing; NS: funding, data collection, analysis and interpretation of results, manuscript editing; SC: funding, data collection, analysis and interpretation of results, manuscript editing, study supervision. DT and SC are the guarantors of this work and, as such, have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

The authors have no conflicts of interest pertinent to this study.

Introduction

Excess insulin secretion and chronic hyperinsulinemia have a pathogenic role in the progression of type 2 diabetes (T2D) [1–3]. However, the signals through which β -cells increase insulin secretion and their relative relevance are not fully understood.

Preliminary evidence suggests a primary role for plasma triglycerides (TG) in directly enhancing β -cell function. In fact, although chronic exposure to high TG may lead to β -cell dysfunction [4], an overnight infusion of lipids was able to markedly increase fasting insulin secretion in youths [5], and endogenous TG were directly associated with both fasting and glucose-stimulated insulin secretion in a large cohort of non-diabetic adults ($n=1,016$) [6].

Endogenous TG are transported from liver to peripheral tissues in the core of very low density lipoproteins (VLDL), whose size closely reflects their TG content. Circulating VLDL might directly interact with pancreatic β -cells [7], but their effect on insulin release has not been examined.

A greater sensitivity to the stimulatory effect of lipids on β -cells might explain higher insulin levels observed in African Americans compared with Caucasians and Hispanics [8]. However, African Americans typically show lower plasma TG and smaller VLDL, even in the presence of severe insulin resistance [9]. Thus, ethnic/racial differences in the relationship of TG/VLDL with insulin secretion could be hypothesized.

Based on these observations, in this study we sought to evaluate the relationship of plasma TG and specific VLDL fractions with insulin secretion in a multiethnic cohort of non-diabetic adolescents, independent of insulin resistance and other confounders. Furthermore, we examined whether these associations are modulated by the ethnic background in the three major race/ethnic groups in the United States.

Materials and Methods

Study design

We recruited 630 overweight/obese non-diabetic adolescents from the Yale Pathogenesis of Youth Onset Type 2 Diabetes study cohort, including 281 (44.6%) Caucasians, 166 (26.3%) African Americans, and 158 (25.1%) Hispanics (Table 1). A detailed medical and family history was obtained and a physical examination was performed, including Tanner staging. Subjects with medical conditions or taking medications were excluded. Metabolic studies were repeated after a 2-year follow-up in 239 participants who received standard nutritional and lifestyle recommendations (Table S1).

The study was conducted according to the principles expressed in the Declaration of Helsinki and approved by the Yale Human Investigation Committee. Written parental informed consent and written child assent were obtained from all participants.

Metabolic studies

Glucose tolerance was assessed by a 3-h oral glucose tolerance test (OGTT). Insulin secretion rate (ISR) was estimated by C-peptide deconvolution, and β -cell function

parameters were calculated by modeling ISR and glucose concentration [10]. Insulin clearance was calculated as the ISR/plasma insulin ratio [11]. Insulin sensitivity was estimated by the Whole-Body Insulin Sensitivity Index (WBISI), which has been validated against the euglycemic-hyperinsulinemic clamp in adolescents [12]. Further details are reported in Supplemental Methods.

Lipoprotein analysis

Fasting VLDL particle concentration and size were measured as reported in Supplemental Methods. VLDL were separated into 3 subclass categories according to their size: large (>60 nm), medium (35–60 nm), and small (27–35 nm) [13].

Statistical analysis

Non-normally distributed variables were log-transformed. Continuous and nominal variables were analyzed using Student's *t* tests or χ^2 tests, respectively. Correlations were tested using Pearson's correlations. Multivariable linear regression analyses for each lipid variable initially included age, sex, race, Tanner stage, BMI *z*-score, fasting/OGTT glucose, and WBISI (cross-sectional analysis) or follow-up duration and changes in BMI *z*-score, fasting/OGTT glucose, and Tanner stage (longitudinal analysis) as covariates. The Tanner stage was eventually removed as it showed no significant effect in any model. Participants were stratified by WBISI tertiles. To identify subjects with high fasting TG, a threshold value of 1.24 mmol/L (110 mg/dL) was taken as the 90th percentile in adolescents [14]. Associations were also described using quartiles of TG. The effect modification by race/ethnicity was evaluated by adding product terms (TG×race, VLDL particles×race, VLDL size×race) to linear regression models. Analyses were performed using JMP Pro 13.2.1 (SAS Institute, Cary, NC) at a two-sided α level of 0.05.

Results

Fasting triglycerides are independently associated with plasma insulin and insulin secretion

Within each WBISI tertile, adolescents with high fasting TG had significantly higher fasting insulin levels and insulin secretion rates (ISR) than adolescents with normal TG (Table 1). In the whole cohort, fasting TG were directly correlated with both fasting and OGTT insulin concentrations ($r=0.35$, $p<0.0001$ and $r=0.33$, $p<0.0001$, respectively), even after adjustments for age, sex, race, BMI *z*-score, plasma glucose, and WBISI (fasting insulin $St.\beta=0.07$, $p=0.04$ and OGTT insulin $St.\beta=0.09$, $p=0.01$). Consistently, fasting TG were associated with both fasting and glucose-stimulated ISR in univariate analysis ($r=0.43$, $p<0.0001$ and $r=0.35$, $p<0.0001$, respectively), in multivariate analysis ($St.\beta=0.14$, $p<0.0001$ and $St.\beta=0.08$, $p=0.03$, respectively) (Table S2), and in the analysis by quartiles of TG (Figure 1). Among single components of β -cell function, high TG were associated with higher ISR at 5 mmol/L glucose (ISR@5) in the analysis by WBISI tertiles (Table 1) and in the whole cohort ($r=0.28$, $p<0.0001$; $St.\beta=0.11$, $p=0.002$). No association was observed between TG and β -cell glucose sensitivity ($r=0.05$, $p=0.24$), rate sensitivity ($r=0.05$, $p=0.20$), and potentiation ($r=-0.04$, $p=0.37$).

VLDL particles have a differential impact on insulin secretion depending on their size

VLDL particle concentration was 48[32–64] nmol/L, being lower in African Americans (34–19–51 nmol/L) than in Caucasians and Hispanics (52[39–66] and 54[35–68] nmol/L, respectively, $p<0.0001$). VLDL particles correlated with TG levels ($r=0.69$, $p<0.0001$) and WBISI ($r=-0.20$, $p<0.0001$), and were associated with both fasting ISR ($r=0.28$, $p<0.0001$; St.β=0.09, $p=0.004$) and glucose-stimulated ISR ($r=0.22$, $p<0.0001$; St.β=0.06, $p=0.09$) (Table S3).

Average VLDL size was 52 ± 9 nm, being larger in boys than in girls (54 ± 9 vs 51 ± 8 nm, $p=0.0001$), and in Hispanics than in African Americans (54 ± 9 vs 50 ± 8 nm, $p=0.003$). VLDL size was associated with TG ($r=0.38$, $p<0.0001$), WBISI ($r=-0.35$, $p<0.0001$), age ($r=-0.11$, $p=0.007$), and BMI z-score ($r=0.17$, $p<0.0001$). VLDL size correlated positively with both fasting ISR ($r=0.34$, $p<0.0001$; St.β=0.08, $p=0.007$) and glucose-stimulated ISR ($r=0.27$, $p<0.0001$; St.β=0.07, $p=0.04$). Consistently, large and medium VLDL were directly proportional to ISR parameters (Figure 1), while small VLDL showed no correlations (absolute concentrations) or even negative correlations (relative percent concentrations) (Table S3).

Ethnicity does not modulate the stimulatory effect of lipids on β-cells

TG levels were lower in African Americans (65[49–98] mmol/L) than in Caucasians and Hispanics (94[69–138] and 95[69–130] mmol/L, respectively, $p<0.0001$). Similarly, fasting and OGTT ISR was lower in African Americans (140 ± 60 pmol/m²/min and 78 ± 31 nmol/m², respectively) than in Caucasians (158 ± 63 pmol/m²/min, $p=0.01$, and 91 ± 38 nmol/m², $p=0.001$, respectively) and Hispanics (164 ± 55 pmol/m²/min, $p=0.0001$, and 92 ± 32 nmol/m², $p<0.0001$, respectively) (Table S2).

We did not observe a significant effect of the interaction between TG and ethnicity on ISR either at fasting ($p=0.16$) or during the OGTT ($p=0.66$). In fact, the association between TG and ISR was similar between the three ethnic subgroups (Figure S1). Similarly, ethnicity did not modulate the effect on fasting/OGTT ISR of either VLDL particle ($p=0.11$ and $p=0.72$, respectively) or VLDL size ($p=0.46$ and $p=0.83$, respectively).

Changes in fasting triglycerides over time are independently associated with changes in insulin secretion

Changes in fasting TG levels over time were associated with changes in fasting ISR ($r=0.24$, $p=0.002$) and OGTT ISR ($r=0.31$, $p<0.0001$) in 239 adolescents with follow-up data (median follow-up 1.6 year, interquartile range 0.9–2.3 years) (Table S1). These associations remained statistically significant after adjustments (fasting ISR St.β=0.15, $p=0.04$; OGTT ISR St.β=0.18, $p=0.01$) and followed an asymmetric pattern, as an increase in plasma TG was associated with higher insulin secretion, while a decrease in TG had no effect (Figure S2).

Discussion

In this study, we examined whether plasma TG and VLDL are associated with insulin secretion and β -cell function in a multiethnic cohort of non-diabetic adolescents. We observed that: 1) plasma TG and VLDL are independently associated with basal and glucose-stimulated ISR; 2) the impact of VLDL particles on ISR depends on their size; 3) ethnicity *per se* has an impact on lipid profile and ISR, but does not influence the strength of the association of TG/VLDL with ISR; 4) increases in fasting TG over time are proportional to increases in ISR.

Excess insulin secretion and hyperinsulinemia can lead to T2D progression, independently of insulin resistance [1–3]. In this view, gaining a deeper knowledge of the signals by which β -cells can be stimulated to release insulin would be clinically relevant. In agreement with previous observations [4–6], in this study we found that adolescents with high fasting TG have 34% higher fasting insulin levels and 35% higher ISR than subjects with normal TG, despite similar insulin sensitivity and insulin clearance (Table 1). This translated into a much higher basal insulin daily output (139 [179–107] vs 101 [70–142] U/24h, $p<0.0001$). The association between TG and ISR is supported by our follow-up data and seems physiologically relevant, as its strength is similar or even greater to that of glucose and BMI in multivariable models (Table S2). The effect of TG might depend on their extra- or intra-cellular metabolism to free fatty acids (FFA) [15]. However, fasting plasma FFA were not correlated with insulin secretion in multivariable models accounting for insulin sensitivity and BMI [16].

Besides a direct effect of TG on pancreatic β -cells, other components of VLDL might explain the relationship between VLDL and ISR. Our current findings do not support this hypothesis, as only VLDL fractions with greater TG concentrations (*i.e.* larger VLDL) were correlated with increased ISR (Table S3).

Racial differences in insulin levels have been proposed to be driven by different β -cell sensitivity to the stimulatory effect of lipids [8, 15]. However, we found that the correlation between lipids and ISR was similar among the three major race/ethnic groups in the United States (Figure S1), thereby not supporting this hypothesis.

Strengths of this study include the accurate estimation of ISR and β -cell function parameters and the longitudinal assessment. Our cohort included mostly obese, insulin resistant adolescents, thus results might not extend to the general population. We acknowledge the absence of an independent measure of insulin sensitivity and the lack of information on the phase of menstrual cycle in girls. The asymmetric shape of dose–response curves in follow-up analyses – also described in adults [6] – could depend on type 2 errors due to small sample size or indicate non-causal relationships. Regression analyses do not allow the definition of causality, which would need intervention studies to be ascertained.

These data support the role of circulating TG and triglyceride-rich VLDL as main determinants of insulin secretion independent of insulin sensitivity, and highlight their physiological relevance in comparison with well-established modulators of β -cell function, such as adiposity and glucose levels. Since a pathogenic role of primary insulin

hypersecretion has been proposed, our results might expose a mechanism of disease progression and suggest novel approaches to the prevention and early treatment of T2D, as TG are highly susceptible to lifestyle and pharmacological interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Fasting and glucose-stimulated insulin secretion rates across quartiles of fasting triglycerides, large VLDL (>60 nm), and medium VLDL (35–60 nm) in a multiethnic cohort of non-diabetic adolescents ($n = 630$). Bars indicate medians and interquartile ranges.

* P values from multivariable regression analyses including age, sex, race, BMI z -score, plasma glucose, and insulin sensitivity as covariates.

Table 1. Clinical and metabolic characteristics of study subjects stratified by tertiles of whole-body insulin sensitivity index (WBISI).

	WBISI First Tertile (least IS)			WBISI Second Tertile			WBISI Third Tertile (most IS)		
	Triglycerides < 1.24 mM	<i>p</i>	Triglycerides 1.24 mM	Triglycerides < 1.24 mM	<i>p</i>	Triglycerides 1.24 mM	Triglycerides < 1.24 mM	<i>p</i>	Triglycerides 1.24 mM
CLINICAL FEATURES									
Number	112	-	88	122	-	167	30	-	-
Age (years)	13.0 ± 2.6	0.32	13.4 ± 3.2	13.1 ± 2.9	0.80	14.3 ± 3.8	13.8 ± 2.7	0.34	0.34
Sex (M/F, %)	39.3/60.7	0.38	45.4/54.6	44.3/55.7	0.27	41.3/58.7	53.3/46.7	0.22	0.22
Race (C/AA/H/O, %)	33/38/25/4	0.06	20/47/30/3	41/32/23/4	0.004	46/29/21/4	57/10/30/3	0.17	0.17
Tanner Stage (1/2/3/4/5, %)	5/15/23/38/19	0.65	8/13/19/34/26	6/19/11/38/26	0.60	6/13/15/31/35	0/23/10/37/30	0.36	0.36
Body Mass Index (kg/m ²)	36.9 ± 7.8	0.05	34.7 ± 6.9	33.1 ± 6.7	0.85	27.8 ± 7.2	30.1 ± 6.1	0.04	0.04
Body Mass Index z-score	2.47 ± 0.39	0.07	2.36 ± 0.39	2.22 ± 0.55	0.20	1.51 ± 1.04	1.90 ± 0.77	0.025	0.025
LIPID PROFILE									
Triglycerides (mmol/L)	0.9 [0.7–1.1]	<0.0001	1.8 [1.4–2.4]	0.8 [0.6–1.0]	<0.0001	0.7 [0.6–0.9]	1.5 [1.4–2.2]	<0.0001	<0.0001
VLDL Particles (nmol/L)	43 [31–56]	<0.0001	65 [54–80]	40 [28–55]	<0.0001	36 [24–52]	79 [59–87]	<0.0001	<0.0001
VLDL Size (nm)	52 ± 7	<0.0001	58 ± 9	51 ± 7	<0.0001	48 ± 8	53 ± 7	0.005	0.005
Total Cholesterol (mmol/L)	3.8 ± 0.7	<0.0001	4.4 ± 1.1	3.8 ± 0.8	0.0003	3.8 ± 0.8	4.8 ± 1.4	0.001	0.001
HDL Cholesterol (mmol/L)	1.1 ± 0.2	<0.0001	0.96 ± 0.21	1.2 ± 0.3	<0.0001	1.3 ± 0.3	1.1 ± 0.2	<0.0001	<0.0001
LDL Cholesterol (mmol/L)	2.3 ± 0.7	0.13	2.47 ± 0.77	2.3 ± 0.7	0.39	2.2 ± 0.7	1.1 ± 0.2	0.005	0.005
GLUCOSE METABOLISM									
Whole-Body Insulin Sensitivity Index	0.9 [0.8–1.1]	0.14	0.9 [0.7–1.1]	1.6 [1.4–1.8]	0.72	3.1 [2.4–4.1]	2.3 [2.1–2.9]	<0.0001	<0.0001
Fasting Glucose (mmol/L)	5.3 ± 0.5	0.86	5.3 ± 0.4	5.1 ± 0.5	0.46	5.0 ± 0.4	4.9 ± 0.4	0.99	0.99
OGTT Glucose (mmol/L)	6.9 ± 0.9	0.08	7.2 ± 0.9	6.5 ± 0.9	0.99	6.0 ± 0.9	6.3 ± 0.8	0.11	0.11
Fasting Insulin (pmol/L)	326 [269–389]	0.04	354 [301–420]	196 [172–238]	0.04	112 [88–140]	149 [117–175]	0.0007	0.0007
OGTT Insulin (nmol/L)	1.6 [1.3–2.1]	0.08	1.8 [1.2–2.2]	0.9 [0.8–1.1]	0.07	0.5 [0.3–0.6]	0.6 [0.5–0.7]	0.02	0.02
Fasting Insulin Secretion (pmol m ⁻² min ⁻¹)	189 [158–219]	0.01	202 [173–260]	145 [123–168]	0.0002	100 [82–123]	139 [113–155]	<0.0001	<0.0001
OGTT Insulin Secretion (nmol/m ²)	104 [82–128]	0.08	114 [90–143]	84 [69–96]	0.39	61 [49–76]	70 [55–84]	0.11	0.11
ISR@5 (pmol min ⁻¹ m ⁻²) ***	160 [116–206]	0.01	173 [143–238]	138 [100–181]	0.55	106 [77–144]	144 [120–171]	0.0002	0.0002

	WBISI First Tertile (least IS)			WBISI Second Tertile			WBISI Third Tertile (most IS)		
	Triglycerides < 1.24 mM	Triglycerides 1.24 mM	<i>p</i>	Triglycerides < 1.24 mM	Triglycerides 1.24 mM		Triglycerides < 1.24 mM	Triglycerides 1.24 mM	<i>p</i>
Glucose Sensitivity (pmol min ⁻¹ m ⁻² mM ⁻¹)	148 [112–218]	150 [113–191]	0.58	146 [104–191]	144 [108–216]		127 [89–178]	116 [83–153]	0.12
Rate Sensitivity (nmol m ⁻² mM ⁻¹)	2.3 [1.6–3.8]	2.4 [1.4–4.3]	0.89	2.1 [1.3–3.2]	2.2 [1.1–3.3]		1.5 [1.0–2.4]	1.7 [1.0–2.7]	0.44
Potentiation Factor (ratio)	1.0 [0.8–1.3]	1.0 [0.7–1.3]	0.87	1.0 [0.8–1.3]	1.0 [0.8–1.2]		1.1 [0.9–1.4]	1.1 [0.7–1.4]	0.33
Fasting Insulin Clearance (L min ⁻¹ m ⁻²)	0.68 ± 0.18	0.71 ± 0.20	0.34	0.86 ± 0.21	0.95 ± 0.27		1.09 ± 0.42	1.08 ± 0.32	0.67
OGTT Insulin Clearance (L min ⁻¹ m ⁻²)	0.38 ± 0.11	0.40 ± 0.13	0.57	0.53 ± 0.14	0.59 ± 0.15		0.84 ± 0.29	0.76 ± 0.21	0.22
LIVER ENZYMES									
Alanine Transaminase (U/L)	21 [15–30]	23 [16–39]	0.09	18 [12–24]	21 [14–26]		15 [11–20]	15 [13–27]	0.09
Aspartate Transaminase (U/L)	22 [18–26]	23 [20–28]	0.12	20 [18–26]	22 [18–26]		20 [17–25]	21 [18–24]	0.76

Data are reported as either mean ± standard deviation, median [interquartile range], or percentage.

* *p* value < 0.05 after correction for race (second tertile) or ISI and BMI *z*-score (third tertile).

** C, Caucasians; AA, African Americans; H, Hispanics; O, Others.

*** ISR@5, Insulin Secretion Rate at 5 mmol/L glucose.