A Reduced Incretin Effect Mediated by the rs7903146 Variant in the *TCF7L2* Gene Is an Early Marker of β-Cell Dysfunction in Obese Youth

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OBJECTIVE

The risk genotype for the common variant rs7903146 of the transcription factor 7-like-2 (*TCF7L2*) gene has been found to affect the incretin response in healthy and obese adults; however, whether a similar functional defect is also present in obese adolescents remains unexplored. Herein, we examined the functional effect of the rs7903146 variant in the *TCF7L2* gene on the incretin effect and determined its translational metabolic manifestation by performing deep phenotyping of the incretin system, β-cell function relative to insulin sensitivity, the gastrointestinal-induced glucose disposal (GIGD) in obese youth with normal and impaired glucose tolerance.

RESEARCH DESIGN AND METHODS

Thirty-nine obese adolescents without diabetes (median age 15 [25th, 75th percentile 14, 18] years; BMI 37 [33, 43] kg/m^2) were genotyped for the rs7903146 variant of *TCF7L2* and underwent a 3-h oral glucose tolerance test (OGTT) followed by an isoglycemic intravenous glucose infusion (iso-intravenous glucose tolerance test [IVGTT]) to match the plasma glucose concentrations during the OGTT and a hyperglycemic clamp with arginine stimulation. The incretin effect was measured as 100 * (AUC-SR$_{OGTT}$ − AUC-SR$_{iso\text{-}IVGTT}$) / AUC-SR$_{OGTT}$, where AUC-SR = area under the curve of C-peptide secretion rate. Participants were grouped into tertiles according to the percentage incretin effect (high, moderate, and low) to describe their metabolic phenotype.

RESULTS

The presence of T risk allele for *TCF7L2* was associated with a markedly reduced incretin effect compared with the wild-type genotype (0.3% [−7.2, 14] vs. 37.8% [12.5, 52.4], $P < 0.002$). When the cohort was stratified by incretin effect, the high, moderate, and low incretin effect groups did not differ with respect to anthropometric features, while the low incretin effect group exhibited higher 1-h glucose ($P = 0.015$) and a reduced disposition index, insulin sensitivity, and insulin clearance compared with the high incretin effect group. GIGD was reduced in the low incretin effect group ($P = 0.001$). The three groups did not differ with respect to intravenous glucose-induced insulin secretion and arginine response during the hyperglycemic clamp.

CONCLUSIONS

A reduced incretin effect and its association with the *TCF7L2* variant rs7903146 identify an early metabolic phenotype in obese youth without diabetes, featuring a higher plasma glucose peak at 1 h; lower insulin secretion, sensitivity, and clearance; and GIGD.

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The greater insulin response to an oral versus intravenous glucose load—namely the incretin effect—results from the sensitivity of the β-cell to the gut-derived incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (1). A reduced incretin effect has been described as a consequence of the diabetic state in adults (2) and associated with worsening of glucose tolerance in youth with prediabetes and overt diabetes (3–6). However, a relative reduction of incretin response to glucose has also been demonstrated in healthy adults carrying the diabetes risk allele for the rs7903146 variant of the transcription factor 7-like 2 (*TCF7L2*) gene in the absence of impaired glucose intolerance (IGT) (7–9). In a previous study (7), we showed that the rs7903146 variant in the *TCF7L2* gene increases the risk of IGT and type 2 diabetes (T2D) in obese adolescents by impairing β-cell function; however, the role of the rs7903146 variant on the incretin effect in obese adolescents was not explored.

Assessing the metabolic phenotype of obese youth for potential alterations in the incretin effect during the prediabetic phase is not only critical to the understanding of the pathophysiology of youth-onset T2D but also of great clinical relevance because it may help to customize therapeutic interventions that target the incretin axis as a preventive measure of T2D in youth, thereby maximizing their cost effectiveness (8). A single pediatric cross-sectional study that was based on a surrogate measure of incretin effect showed that the decline of the incretin effect across the spectrum of glucose tolerance (6) is not paralleled by a decreased GLP-1, but the effect of the *TCF7L2* risk variant was not studied. It should be noted that participants in that study (6) exhibited only marginally significant differences in acute insulin response to intravenous glucose during the hyperglycemic clamp, suggesting a prevailing role of incretin response in glucose tolerance change in youth (9).

In the current study, we hypothesized that the functional effect of the rs7903146 variant in the *TCF7L2* gene might be linked to impairment in the incretin system in obese youth with normal glucose tolerance (NGT) or IGT (7,10,11). Therefore, we genotyped a cohort of obese youth for the *TCF7L2* rs7903146 variant and performed a detailed phenotyping of the incretin system to quantify the incretin effect by using measurements of insulin secretion in response to oral and matched isoglycemic intravenous glucose infusion (iso-intravenous glucose tolerance test [IVGTT]), the most robust and sensitive test to assess the incretin effect (4,12,13). Thereafter, to assess the impact of potential changes in the incretin system on the metabolic profiles of these obese youth, we stratified the cohort into high, moderate, and low incretin effect and characterized them with respect to *1*) β-cell response to oral glucose load (oral glucose tolerance test [OGTT]) and to intravenous hyperglycemia (hyperglycemic clamp with arginine stimulation), *2*) insulin sensitivity (SI), *3*) insulin clearance, and *4*) gastrointestinal-induced glucose disposal (GIGD).

RESEARCH DESIGN AND METHODS

From 2017 to 2019, 273 overweight/obese adolescents were recruited from the Yale Pediatric Obesity Clinic and participated in the Yale Study of the Pathophysiology of Prediabetes/T2D in Youth, an ongoing study aimed at deciphering the genetic and metabolic underpinnings of prediabetes. All adolescents from this multiethnic cohort were genotyped for the *TCF7L2* rs7903146 variant, and 39 agreed to participate in further metabolic studies as described below. Main inclusion criteria were a BMI >85th percentile for age and sex and an age between 8 and 21 years. Adolescents using medications affecting glucose metabolism, diagnosed with syndromic obesity, or participating in clinical trials that included a structured dietary or exercise-based intervention were excluded from the study. All participants underwent a complete physical examination, including the assessment of pubertal development (Tanner stage) (14,15).

The study protocol was approved by the human investigations committee of the Yale School of Medicine. Participants provided assent and parents provided written informed consent to participate in the study. Eligible participants underwent an OGTT, as previously described (16), and a matched iso-IVGTT, reproducing the same plasma glucose profile observed during the OGTT, and an hyperglycemic clamp.

Procedures and Calculations

OGTT

Before the OGTT, all participants followed a weight maintenance diet consisting of at least 250 g of carbohydrates per day for 7 days before the study. They were admitted to the Yale Center for Clinical Investigation at 8:00 A.M. after a 12-h overnight fast. After the local application of a topical anesthetic cream (Emla; AstraZeneca, Wilmington, DE), one antecubital intravenous catheter was inserted for blood sampling. Two baseline samples were then obtained for measurements of plasma glucose, insulin, C-peptide, glucagon, and GLP-1. Thereafter, flavored glucose in a dose of 1.75 g/kg body weight (up to a maximum of 75 g) was given orally, and blood samples were obtained every 30 min for 180 min for the measurement of plasma glucose, insulin, C-peptide, glucagon, and active GLP-1 [GLP-1(7-36)]. To reduce the intrasubject variability often associated with the OGTT (17), glucose samples were immediately spun and processed at the bedside using the a YSI 2700 Stat Analyzer (Yellow Springs Instruments, Yellow Springs, OH).

In accordance with American Diabetes Association criteria (17), NGT was defined as a fasting plasma glucose <100 mg/dL and a 2-h plasma glucose <140 mg/dL and IGT as a 2-h plasma glucose level between 140 and 199 mg/dL. Individuals with T2D were excluded from this study (12).

Iso-IVGTT

Within 1 week after the OGTT, participants were admitted to the Yale Center for Clinical Investigation at 8:00 A.M. after a 12-h overnight fast for an intravenous infusion of dextrose (20%) used to reproduce the plasma glucose profile observed during the OGTT. Matched glucose profiles were obtained by repeatedly measuring plasma glucose concentrations (every 5 min over 180-min test), with frequent adjustments of the dextrose infusion rate according to the OGTT glucose target values at each time point (10,18).

Hyperglycemic Clamp Plus Arginine

Hyperglycemic clamp was performed to assess the β-cell response to intravenous glucose and arginine stimulation in the absence of gut-derived incretin potentiation. The test was done within 2 months from the OGTT after an overnight fast and consisted of two steps. Two intravenous

catheters were inserted before the clamp studies: one in an antecubital vein for administration of dextrose and the other in a vein of the hand or distal forearm of the contralateral arm for blood sampling. During the first step, baseline glucose, insulin, and C-peptide were measured at −20 and 0 min before glucose infusion, and the average value was used to calculate baseline values ($t = 0$).

Arterialized blood for plasma glucose was drawn every 2 min during the first 10 min and then every 5 min and immediately centrifuged and analyzed using the glucose oxidase method (YSI 2300 Stat Analyzer). A standardized priming 20% dextrose infusion was administered during the first 10 min (200 mg/kg body weight), and then infusion rates were adjusted every 5 min to maintain plasma glucose at 11.1 mmol/L (200 mg/dL) for 120 min (19). Blood samples for subsequent assays were drawn at 2, 4, 6, 8, 10, 20, 30, 40, 60, 80, 100, and 120 min.

During the second step, the target blood glucose 25 mmol/L (450 mg/dL) was achieved using a second bolus of 20% dextrose administered over 60 s (volume in mL calculated as weight [kg] ∗ [450 − current blood glucose in mg/dL] ∗ 1.1 / 180) as previously described (20). The dextrose infusion rate was adjusted according to bedside blood glucose monitoring every 5 min. Once the target blood glucose was attained for a minimum of 30 min, a bolus of 5 g L-arginine was administered over 1 min. Blood samples for subsequent assays were drawn at −5, −1, 2, 3, 4, and 5 min relative to the arginine injection (20). The hand chosen for blood sampling during the three metabolic tests was placed in a heated box (~65°C) to facilitate blood sampling and arterialize blood (21).

Biochemical Analysis

Plasma insulin was measured by radioimmunoassay (Linco, St. Charles, MO) that has <1% cross-reactivity with C-peptide and proinsulin. Plasma C-peptide levels were determined with an assay from Diagnostic Products (Los Angeles, CA). GLP-1(7-36) was measured by radioimmunoassay (EMD Millipore Corporation, Billerica, MA). GLP-1(7-36) amide assay had <0.1% cross-reactivity with the unamidated forms GLP-1(7-37) and GLP-1(1-37) or other related peptides such as human GLP-2, glucagon, human GIP, and vasoactive intestinal peptide. Glucagon was measured by ELISA (Mercodia, Winston-Salem, NC).

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the guanidine HCl DNA extraction protocol. The rs7903146 variant is characterized by a C to T substitution in the *TCF7L2* gene on chromosome 10. C represents the major allele, and T represents the minor allele. To genotype the rs7903146 single nucleotide polymorphism, the following pair of primers was used: 5′AGCCGTCAGATGGTAATGCAGA3′ and 5′TAGCAGTGAAGTGCCCAAGCTTCT3′. PCR was performed using an annealing temperature of 65.0°C. PCR products were analyzed by automated sequencing through the Yale W.M. Keck facility.

Calculations of Insulin Secretion, SI, and the Incretin Effect

β-Cell secretion was estimated from the C-peptide levels measured during the 180-min OGTT using the oral C-peptide minimal model (22,23). The model assumes that C-peptide kinetics is described by a two-compartment model (Eaton):

$$\begin{cases} CP_1(t) = -[k_{01} + k_{21}] \cdot CP_1(t) + k_{12} \cdot CP_2(t) + SR(t) & CP_1(0) = CP_{1b} \\ CP_2(t) = k_{21} \cdot CP_1(t) - k_{12} \cdot CP_2(t) & CP_2(0) = CP_{2b} = CP_{1b}\frac{k_{21}}{k_{12}} \end{cases}$$

where CP_1 and CP_2 (pmol/L) are C-peptide concentrations in the accessible and peripheral compartments, respectively; SR(t) is C-peptide (and insulin) secretion rate; and k_{01}, k_{12}, and k_{21} (min^{-1}) are C-peptide kinetic parameters fixed to standard values (24) to ensure numerical identification of the overall model (25).

The model also assumes that glucose-stimulated insulin secretion is made up of two components: *1*) a dynamic component representing secretion of promptly releasable insulin and proportional to the rate of glucose increase through the dynamic responsivity index ϕ_d (10^{-9}) and *2*) a static component derived from provision of new insulin to the releasable pool and characterized by a static responsivity index, ϕ_s (10^{-9} min^{-1}) and by a delay time constant, T (min) (13). From ϕ_d and ϕ_s, a total responsivity index (ϕ_{total}) can be derived, which measures the ability of β-cells to respond to a glucose stimulus (19,22,26).

SI was estimated from plasma glucose and insulin concentrations measured during the 3-h OGTT using the oral glucose minimal model (27). The oral disposition index (oDI) was estimated as the product of $\phi_{total} \times$ SI.

The incretin effect was calculated as follows (13):

$$\text{Incretin effect}(\%) = 100 \cdot \frac{AUC\text{-}SR_{OGTT} - AUC\text{-}SR_{iso-IVGTT}}{AUC\text{-}SR_{OGTT}}$$

Where $AUC\text{-}SR_{OGTT}$ and $AUC\text{-}SR_{iso\text{-}IVGTT}$ are the area under the curve of the insulin SR computed from the C-peptide serial measurements during the OGTT and the iso-IVGTT.

GIGD was calculated as follows:

$$\text{GIGD}(\%) = 100 \cdot \frac{GI_{OGTT} - GI_{iso-IVGTT}}{GI_{OGTT}}$$

Where GI_{OGTT} and $GI_{iso\text{-}IVGTT}$ were, respectively, the glucose ingested during the OGTT and the glucose infused during iso-IVGTT (12,28).

Insulin clearance was calculated as:

$$\text{Insulin Clearance} = \frac{AUC\text{-}SR_{OGTT} +}{AUC_{insulin}} - \frac{V \cdot \Delta Insulin}{AUC_{insulin}}$$

with $AUC\text{-}SR_{OGTT}$ being the AUC of the insulin SR (based on C-peptide measurements) during the OGTT, $AUC_{insulin}$ the AUC of the insulin measurements during the OGTT, V the distribution volume for insulin (which we previously estimated to be 141 mL/kg) and $\Delta Insulin$ the plasma insulin concentration at 180 min of the OGTT minus the baseline value (29,30). Minimal model parameters were estimated by implementing the model of C-peptide secretion in SAAM-II

2.3 software (SAAM Institute, Seattle, WA).

Hyperglycemic Clamp–Derived Measures

Acute (first-phase) C-peptide ($ACPR_g$) and insulin (AIR_g) responses to glucose were calculated as the mean incremental response above baseline (average of −10 and −5 min) from samples drawn at 2, 4, 6, 8, and 10 min after intravenous dextrose administration (17). Steady-state (second-phase) C-peptide and insulin concentrations were calculated as the mean of the respective measurements at 100, 110, and 120 min of the hyperglycemic clamp (16). Acute C-peptide ($ACPR_{max}$) and insulin (AIR_{max}) responses to arginine at maximal glycemic potentiation (>25 mmol/L) were calculated as the mean concentrations in samples drawn 2, 3, 4, and 5 min after arginine injection minus the average concentration of the samples drawn 1 and 5 min before arginine (19) (Supplementary Fig. 1).

Power Analysis

Using data from a previously published study (24), we estimated that with 12 participants per group we would have had 80% power at the two-sided α-level of 0.05 to detect a standardized difference in the range of 1–1.4 (Cohen effect size) for the oDI, using a two-sided Mann-Whitney test. This was estimated using 2,000 Monte Carlo samples from two normal distributions (each with mean 125, SD 75) under the null and from the alternative normal distributions (with mean 125, SD 75 vs. mean 50, SD 30).

Statistical Analysis

The cohort was stratified by genotype into two groups carrying the wild type (CC) and risk genotype (presence of T allele) for the *TCF7L2* rs7903146 variant. Incretin effect estimated from the OGTT and iso-IVGTT as described above and β-cell function indices computed from the OGTT were compared between the two groups. Thereafter, the cohort was stratified by incretin effect into three tertiles: high (>66th centile), moderate (33rd–66th centile), and low (<33rd centile).

Values for C-peptide, insulin, and GLP-1(7-36) per each time point >95th centile were defined as outlier measurements of the cohort distribution and excluded from the final analysis. Results were analyzed with and without the inclusion of outlier values. Time series from OGTT and iso-IVGTT measurements were analyzed by linear mixed-model effect.

Kruskal-Wallis test, followed by post hoc pairwise Mann-Whitney test, was used to compare continuous variables, and categorical variables were compared using the χ^2 test. Data were summarized using median (25th, 75th percentile) for continuous variables and count (%) for categorical variables. The high incretin effect group was adopted as the comparison term during the paired analyses.

Determinants of incretin effect were estimated by the use of multivariable logistic regression models. The following adjustment variables were selected a priori on the basis of published literature (31): fasting glucose, *TCF7L2* genotype (wild-type CC vs. T risk allele), glucose tolerance (IGT vs. NGT), sex, age (years), BMI *z*-score, and ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic) (31,32). Statistical significance for the effect of adjustment variables was established with $\alpha = 0.05$. Results were summarized as β-regression coefficient and 95% CIs. Not-normally distributed variables were naturally log-transformed before multivariable analysis. Covariates were examined for multicollinearity and excluded with a variance inflation factor >5 (Tanner stage was excluded as well as 2-h glucose).

Individual correlation between baseline, 1-h, and 2-h glucose and the incretin effect (%) was assessed by linear regression analysis. Thus, to estimate the predictive value of baseline, 1-h, and 2-h glucose on the risk for low incretin tertile (vs. moderate and high incretin effect tertile), we computed the area under the receiver operating characteristic (ROC) curve of each variable. ROC analysis was also used to establish the optimal cut point of 1-h plasma glucose for predicting a low incretin effect and to compare it with other cut points that have been proposed in youth for the diagnosis of IGT (33) and risk of diabetes progression (5,16). Linear regression analysis was performed to estimate the correlation between GIGD and incretin effect. Analyses were performed using Stata 13 software (StataCorp, College Station, TX) and GraphPad Prism 8.0 (GraphPad Software, San Diego, CA).

RESULTS

Participant Anthropometric and Metabolic Characteristics

We enrolled 39 participants, including 21 with NGT and 18 with IGT. Eighteen (49%) participants carried the *TCF7L2* risk allele. Carriers of the wild-type genotype and the risk allele did not differ with respect to anthropometric and metabolic characteristics (Table 1).

The rs7903146 variant reduces the incretin effect (Fig. 1). The wild-type genotype exhibited a greater insulin SR during the OGTT than during the iso-IVGTT (Fig. 1*G*), with an incretin effect of 37.8% (12.5, 52.9) (Fig. 1*I*) compared with the markedly reduced incretin effect in the risk allele genotype group (0.3% [−7.2, 14], $P = 0.002$) (Fig. 1*H* and *I*).

Anthropometric and Metabolic Characteristics Across Tertiles of Incretin Effects

On the basis of the distribution of the measured incretin effect from the matched OGTT and iso-IVGTT, the cohort was stratified into three tertiles (high, moderate, low). The anthropometric and metabolic features of the participants by tertiles of incretin effect are described in Table 2 and Fig. 2.

As shown in Table 2, the three groups did not differ with respect to age, sex, BMI, and pubertal developmental stage. Fasting glucose levels were similar across the three groups, while 1-h glucose was higher in the low incretin effect tertile ($P = 0.015$). Two-hour glucose was trending higher from the low to the high incretin effect group in the absence of a statistically significant difference. The differential distribution of impaired fasting glucose (IFG) and IGT did not reach statistical significance among the three groups owing to the relatively small sample size; non-Hispanic Black and Hispanic ethnic background prevailed in the moderate and high incretin effect tertiles ($P = 0.018$) (Table 2).

Incretin Effect

Glucose profiles were rigorously matched during the OGTT and iso-IVGTT across the three groups, with a difference between oral and intravenous glucose AUCs <5% in each group (Fig. 2*A*–*C*). As expected, insulin levels (Fig. 2*D*–*F*) were consistently higher during the OGTT than the iso-IVGTT across the three groups (OGTT vs. iso-IVGTT AUCs $P < 0.01$).

Table 1—Participant characteristics by *TCF7L2* genotype

	Wild-type genotype *TCF7L2* (n = 21)	Risk genotype *TCF7L2* (n = 18)	P value
Incretin effect (%)	37.8 (12.5, 52.9)	0.3 (−7.2, 14)	**0.002**
Age (years)	16 (15, 18)	15 (14, 17)	0.336
Male sex	12 (57)	8 (44)	0.527
BMI (kg/m^2)	37.0 (32.7, 41.0)	40.6 (34.8, 44.8)	0.499
Tanner stage			0.306
II–III	9 (43)	10 (55)	
IV–V	12 (57)	8 (44)	
Ethnicity			0.087
Non-Hispanic White	2 (9)	7 (39)	
Non-Hispanic Black	9 (43)	4 (22)	
Hispanic	10 (48)	7 (39)	
IFG	1 (13)	2 (20)	0.855
IGT	4 (31)	6 (46)	0.350
Fasting glucose (mg/dL)	90 (86.5, 96.5)	94 (89, 100)	0.222
1-h glucose (mg/dL)	153 (134, 168)	163.5 (147, 189)	**0.015**
2-h glucose (mg/dL)	134 (127, 146)	135.5 (119, 173)	0.526
Fasting insulin (μU/mL)	41 (26.5, 45.5)	28.8 (20.5, 62.5)	0.307
Fasting C-peptide (pmol/L)	1,001 (901.5, 1,279)	1,019 (823.5, 1,121.5)	0.887
Fasting GLP-1(7-36) (pmol/L)	2.0 (1.06, 16.7)	2.9 (1.30, 11.2)	0.868
Fasting glucagon (pmol/L)	6.9 (4.9, 8.1)	9.45 (6.6, 10.5)	0.474

Data are median (25th, 75th percentile) or n (%). Boldface indicates significance at $P < 0.05$.

Conversely, C-peptide rose to higher levels during the OGTT than during the iso-IVGTT only in the high and moderate incretin effect groups (AUC for C-peptide [$AUC_{C\text{-}pep}$]: high incretin 733,322 ± 11,385 vs. 542,100 ± 120,239 pmol ∗ min/L, $P <$ 0.001; moderate incretin 637,260 ± 18.137 vs. 549,770 pmol ∗ min/L, $P <$ 0.001), whereas no significant differences were noted in the $AUC_{C\text{-}pep}$ during the OGTT and the iso-IVGTT in the low incretin effect group ($AUC_{C\text{-}pep}$ 793,815 ± 35,238 vs. 818,273 ± 36,507 pmol ∗ min/L, $P = 0.09$) (Fig. 2*G–I*).

The linear mixed-effects analysis for the insulin SR (SR_{OGTT} and $SR_{iso\text{-}IVGTT}$), estimated from C-peptide measurements, was consistent with raw measurements of C-peptide AUC differences, with a higher SR during the OGTT than during the iso-IVGTT for the high and moderate incretin effect groups ($P < 0.001$ and $P < 0.001$) (Fig. 2*J* and *K*) in the absence of a difference in the low incretin effect group ($P = 0.269$) (Fig. 2*L*).

Raw C-peptide levels did not differ across the three groups during the iso-IVGTT ($P = 0.097$) (Fig. 2*G–I*) on a linear mixed-model analysis in spite of a trending higher C-peptide in the low incretin effect group; thereby, $SR_{iso\text{-}IVGTT}$ was greater in the low incretin effect group than the other two groups ($P < 0.001$) in the absence of a difference in the SR_{OGTT} across the three groups ($P = 0.261$), as described in Fig. 2*J–L*.

Baseline GLP-1(7-36) levels were similar among the three groups ($P = 0.868$ and $P = 0.474$, respectively), as displayed in Table 1. As described in Fig. 2*M–O*, the GLP-1(7-36) percentage increase, from baseline, was significantly greater during the OGTT than during the iso-IVGTT only in the high and moderate incretin effect groups ($P = 0.012$ and $P =$ 0.032) in the absence of an increase in the low incretin effect group ($P =$ 0.434) (Fig. 2*O* and Supplementary Table 1).

Figure 3 illustrates key metabolic phenotypes of the cohort with respect to the incretin effect (Fig. 3*A*), oDI (Fig. 3*B*) and its components (insulin secretion—ϕ_{total} [Fig. 3*D*]—and SI [Fig. 3*E*]), insulin clearance (Fig. 3*C*), and GIGD (Fig. 3*F*). The incretin effect, estimated from the SR of C-peptide, described an ∼50% greater insulin secretion during the OGTT than the iso-IVGTT in the high incretin effect group (49.3% [41.8, 62.8]) compared with the ∼8% increase of the moderate (8.9% [5.2, 20.4], $P = 0.007$) and low (−7.2% [−18.4, −1.31]) incretin effect groups ($P < 0.001$) (Fig. 3*A*). Of note, a lower incretin effect was paralleled by a reduced oDI in both the moderate ($P =$ 0.007) and the low ($P < 0.001$) incretin effect groups compared with the high incretin effect group (Fig. 3*B*), whereas a lower insulin secretion (ϕ_{total}) featured the moderate and low incretin groups ($P = 0.045$ and $P = 0.033$, respectively) (Fig. 3*C*). A similar SI between the high and moderate incretin effect groups ($P = 0.147$) was seen, although the former showed higher SI than the low incretin effect group ($P = 0.017$) (Fig. 3*D*). While insulin clearance did not differ between the high and moderate incretin effect groups ($P = 0.116$), the lower incretin tertile exhibited reduced insulin clearance. GIGD was −43.2% (−31, −52) in the high incretin effect group, while it was markedly reduced in the moderate and low incretin effect groups (−23.7% [−11.7, −28.7] and −9% [3.9, −18]), $P = 0.014$ and $P <$ 0.001, respectively) (Fig. 3*F*). GIGD and incretin effect were inversely associated, with an increased GIGD (negative GIGD) linearly correlated with a higher incretin effect ($r = -0.62$, $P < 0.001$), as described in Fig. 3*I*.

Determinants of the Incretin Effect

As shown in Fig. 3*G* and Table 1, three of four participants from the low incretin effect group (10 of 13, ∼80%) carried the T risk allele for *TCF7L2* compared with fewer than one of four (2 of 13, ∼15%) in the high incretin effect group ($P =$ 0.008), with wild-type genotype clustering

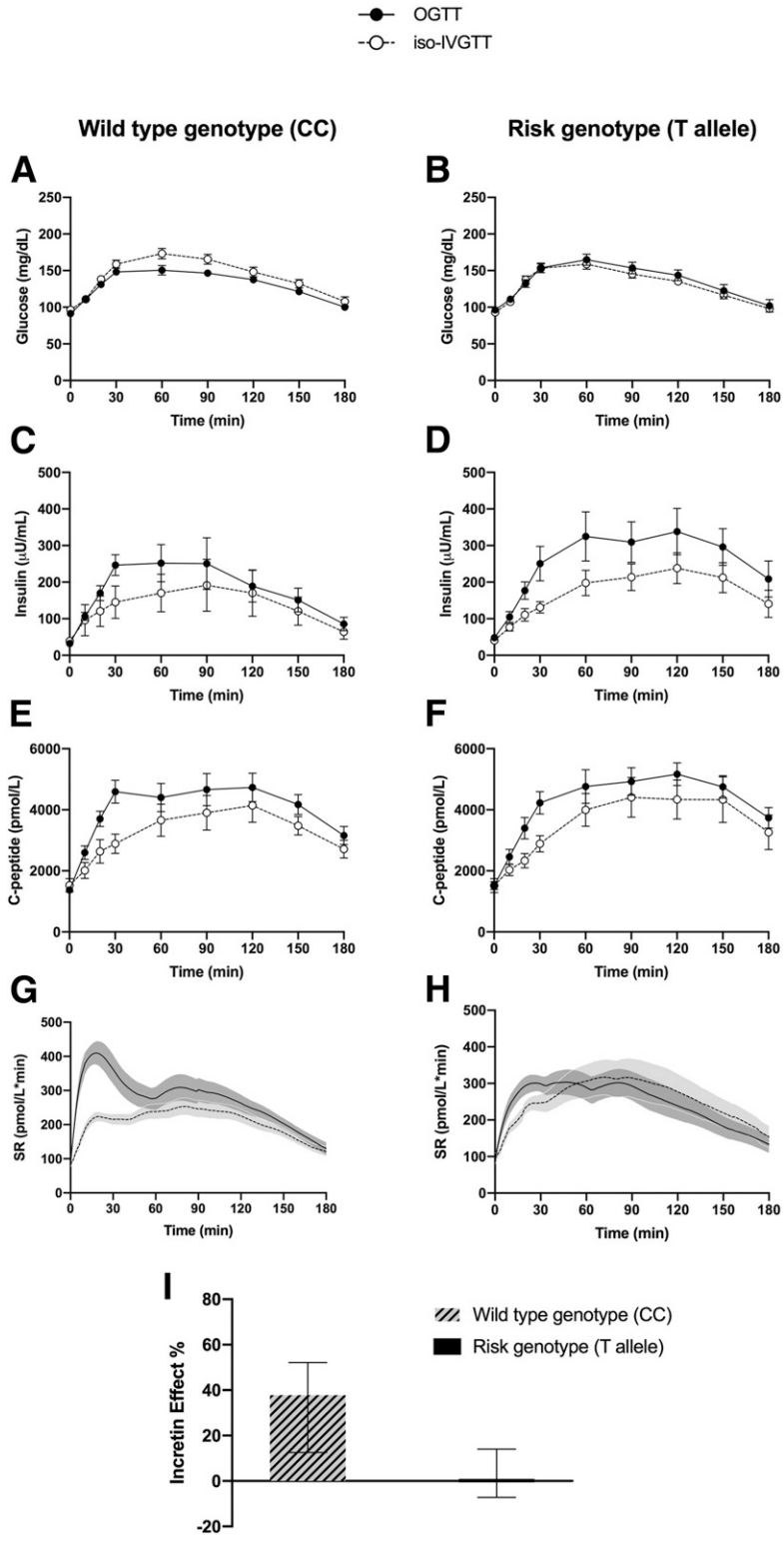

Figure 1—Glucose (*A* and *B*), insulin (*C* and *D*), and C-peptide (*E* and *F*) profiles and insulin SRs (*G* and *H*) during OGTT and iso-IVGTT in the wild-type (CC) and risk genotype (T allele) groups for the *TCF7L2* rs7903146 variant. Percentage incretin effect, estimated from the SR during OGTT and matched iso-IVGTT (*I*). Data are median (25th, 75th percentile).

in the high–incretin effect group. The presence of the T risk allele for the *TCF7L2* rs7903146 variant was associated, after multivariable regression analysis, with an ~20% reduction of the incretin effect ($\beta = -22.4 \pm 10.7$, $P = 0.045$) when the anthropometric and individual variables were held constant (Fig. 2*H* and Supplementary Table 2), confirming the observation from the two-group comparison between the wild-type and the risk genotype.

The Effect of the Low Incretin Effect on the Glucose Profile

The effect of incretin response on the 1-h and 2-h glucose levels during an OGTT was investigated in the whole cohort. Of note, the 1-h glucose increased across incretin tertiles, reaching significantly higher peak levels of 175 (153, 189) mg/dL in the low incretin effect group compared with 136 (120, 150) mg/dL in the high incretin effect group ($P < 0.015$) (Table 1). Furthermore, a lower 1-h glucose was associated with a higher incretin effect ($r = 0.40$, $P = 0.016$), while baseline and 2-h glucose did not exhibit a significant relationship with the incretin effect ($P = 0.101$ and $P = 0.179$). ROC analysis identified 173 mg/dL as the optimal cut point of 1-h plasma glucose levels for predicting a low incretin effect, with 62% sensitivity and 81% specificity. This cut point was similar between NGT (175 mg/dL) and IGT (173 mg/dL) in separate group analysis. Lowering the 1-h glucose threshold to 155 mg/dL or 133 mg/dL, as proposed to predict diabetes progression in youth (5,16), provided a higher sensitivity (69% and 92%, respectively) but a lower specificity (64% and 37%) compared with the 173 mg/dL cut point.

Hyperglycemic Clamp

The three groups were matched for glucose profiles during the hyperglycemic clamp, with similar glucose values at baseline and steady state. No statistically significant differences were observed between groups either in measure of β-cell function ($ACPR_g$, steady-state C-peptide, $ACPR_{max}$) or in insulin sensitivity (M/I) ($P = 0.106$) (Supplementary Fig. 1).

CONCLUSIONS

Our study provides a functional connection between the *TCF7L2* risk allele with changes in the incretin effects with the emerging translational manifestation of a number metabolic defects in obese youth. Specifically, we found that the presence of the T risk allele for the *TCF7L2* rs7903146 variant was associated with an ~20% reduction of the incretin effect in obese youth with early signs of glucose dysregulation. Having found that the functional defect largely

Table 2—Participant characteristics by incretin effect group

	High incretin effect (n = 13)	Moderate incretin effect (n = 13)	Low incretin effect (n = 13)	P value
Incretin effect (%)	49 (42, 63)	8.9 (5, 20)	−7 (−18, −1)	
Age (years)	15 (14, 16)	16 (15, 17)	15 (13, 18)	0.336
Male sex	9 (69)	4 (31)	7 (54)	0.179
BMI (kg/m^2)	37.0 (30.1, 41.9)	38.5 (35.8, 43.5)	37.0 (34, 41.9)	0.499
Tanner stage				0.774
II-III	7 (54)	6 (46)	6 (13)	
IV-V	4 (46)	7 (54)	5 (87)	
Ethnicity				**0.018**
Non-Hispanic White	1 (8)	1 (8)	7 (60)	
Non-Hispanic Black	5 (38)	7 (60)	1 (8)	
Hispanic	7 (54)	5 (38)	5 (38)	
TCF7L2 genotype				**0.008**
CC	11 (85)	7 (54)	3 (23)	
T allele	2 (15)	6 (46)	10 (77)	
IFG	1 (13)	2 (20)	3 (23)	0.855
IGT	4 (31)	6 (46)	8 (61)	0.350
Fasting glucose (mg/dL)	89 (86.5, 94.5)	94 (90, 99.5)	94 (89, 98.5)	0.222
1-h glucose (mg/dL)	136 (120, 150)	159 (150, 168)	175 (153, 189)	**0.015**
2-h glucose (mg/dL)	129 (121, 142)	134 (128, 152)	141 (124, 166)	0.526
Fasting insulin (μU/mL)	28 (20.5, 41.5)	45 (28.5, 50)	32.5 (21, 65.5)	0.307
Fasting C-peptide (pmol/L)	1,088 (948, 1,345)	1,292 (1,207, 1,532)	1,490 (1,235, 1,819)	0.887
Fasting GLP-1(7-36) (pmol/L)	3.09 (1.15, 11.19)	1.46 (0.98, 24.7)	2.55 (1.69, 5.95)	0.868
Fasting glucagon (pmol/L)	4.25 (3.5, 9.5)	7.4 (4.5, 13.2)	6.4 (3.2, 9.5)	0.474

Data are median (25th, 75th percentile) or n (%). Boldface indicates significance at $P < 0.05$.

affects the incretin system, we used deep metabolic phenotyping to better assess the translational manifestation of the low incretin effect.

Our findings provide the first evidence for an association between *TCF7L2* risk genotype and lower incretin effect in obese youth, in agreement with similar observations from adult cohorts (10,34–37). Intriguingly, carriers of the risk T allele of single nucleotide polymorphism rs7903146 showed a weaker response to oral than to intravenous glucose, suggesting a defective enteroinsular axis (Fig. 1). Through alteration of the *WNT* signaling pathway, polymorphisms in the *TCF7L2* gene directly affect β-cell growth, β-cell differentiation, and β-cell function (34). Furthermore, insulin secretion might also be affected indirectly through the enteroinsular axis either through an impaired overall GLP-1 secretion or through a defective or dysfunctional GLP-1–induced insulin secretion. Although the rs7903146 variant in the *TCF7L2* gene has been associated with T2D and impaired insulin response, the possibility exists that it might be in linkage disequilibrium with another gene variant that confers susceptibility to the phenotype. It has to be acknowledged, though, that several human, animal, and in vitro studies have confirmed the role of *TCF7L2* in the β-cell function and have shown that its impairment affects insulin secretion, predisposing to T2D (11,38–40).

The importance of a decreased incretin effect is reflected by significantly higher glucose peak at 1 h, suggesting that a low incretin effect primarily affects the early glucose excursion during an OGTT. It would be of great clinical relevance to identify the low incretin effect group because this metabolic phenotype is an early phenomenon that may predate youth-onset diabetes.

Changes in the glucose profiles are sustained by a reduced β-cell responsiveness to the oral glucose load (oDI), lower SI, and a reduced insulin clearance compared with high-incretin peers. Conversely, intravenous glucose-induced insulin secretion, as quantified during the hyperglycemic clamp at supraphysiologic glucose values (~200 mg/dL), was not significantly impaired in those with a lower incretin effect. While the hyperglycemic clamp describes the static β-cell responsiveness to intravenous glucose at a clamped hyperglycemic level, the iso-IVGTT depicts the dynamic response to more physiologic glucose change and was able to clearly bring out the functional defect of the risk allele of *TCF7L2* on the incretin system.

These findings were paralleled by similar C-peptide levels across the three groups during the iso-IVGTT (Fig. 2*G–I*), while a higher insulin SR was observed in those with a lower incretin effect (Fig. 2*L*). Raw C-peptide measurements and insulin SR describe, respectively, the static and the dynamic response to intravenous glucose. Insulin SR, as estimated by the deconvolution method (19,41), is influenced by the rate of change of insulin secretion during intravenous glucose infusion.

The low incretin effect in our cohort resulted from two components: a higher insulin secretion during the iso-IVGTT in the absence of a corresponding rise of insulin secretion during the matched OGTT (Fig. 2*C* and *E*) and a lower circulating active GLP-1. The paradoxical increase in intravenously induced insulin SR after glucose infusion at physiologic postprandial glucose levels resulted in the observed reduced incretin effect for the low incretin effect group. These findings are consistent with similar observations in adults (2). Indeed, an ~60%

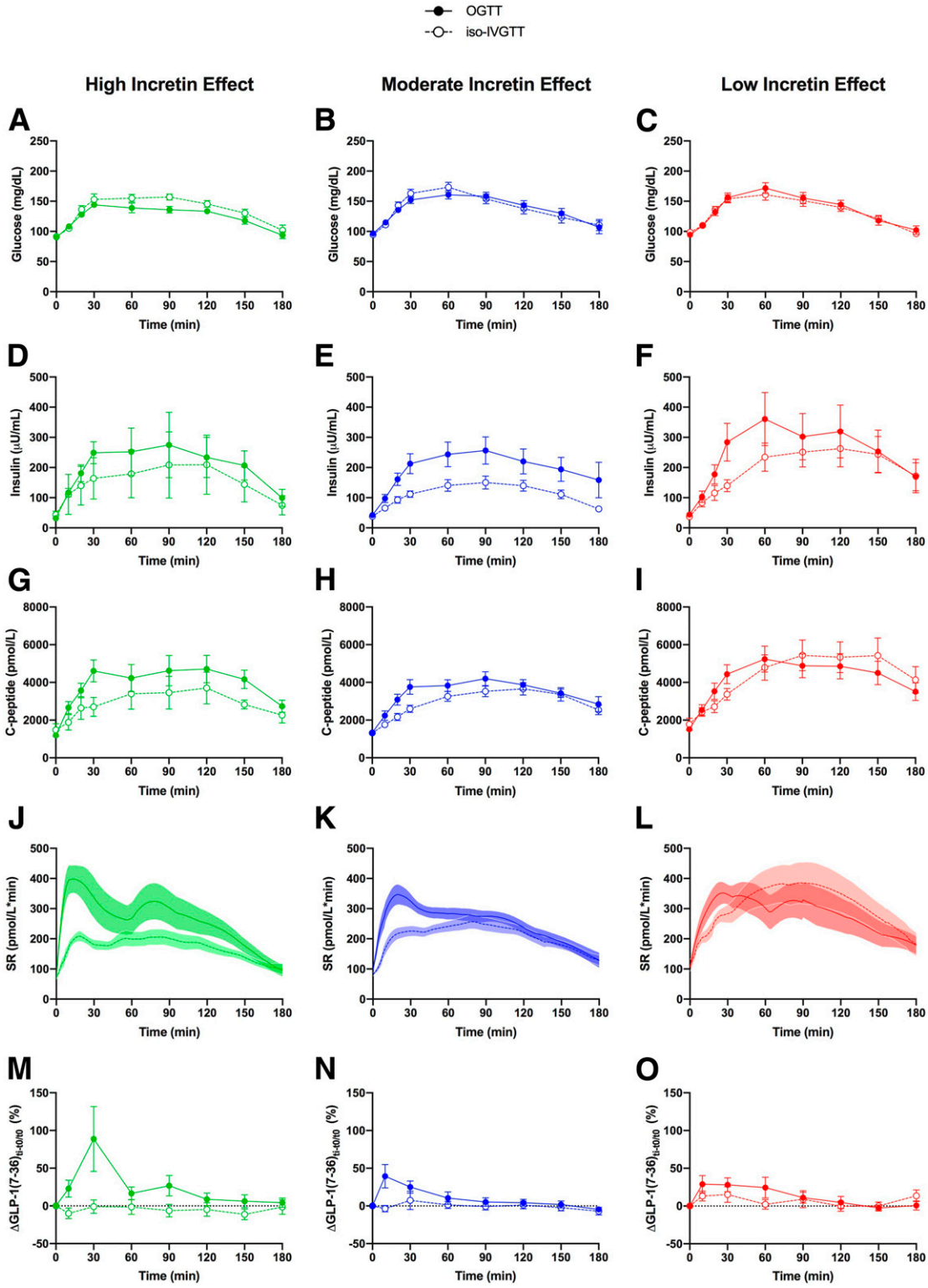

Figure 2—Glucose (*A–C*), insulin (*D–F*), and C-peptide (*G–I*) profiles and insulin SRs (*J–L*) during OGTT and iso-IVGTT in the high, moderate, and low incretin effect groups. *M–O*: Percentage incremental GLP-1(7-36) for the low, moderate, and high incretin effect groups during the OGTT. Data are median (25th, 75th percentile). ti-t0/t0, change from baseline value (t0) over baseline value (t0) at each time point (ti).

greater insulin SR during the iso-IVGTT vs. the OGTT was described in adults with T2D when compared with healthy subjects, with a consequent lower incretin effect in the former group (2). Therefore, the decline in the incretin effect can be in part due to a hyperresponsiveness to intravenous glucose during physiologic hyperglycemia in obese youth.

The apparent discrepancy among the hyperglycemic clamp results (intravenous glucose), iso-IVGTT, and the OGTT-based secretion suggests that a reduced incretin effect could predate the decline of

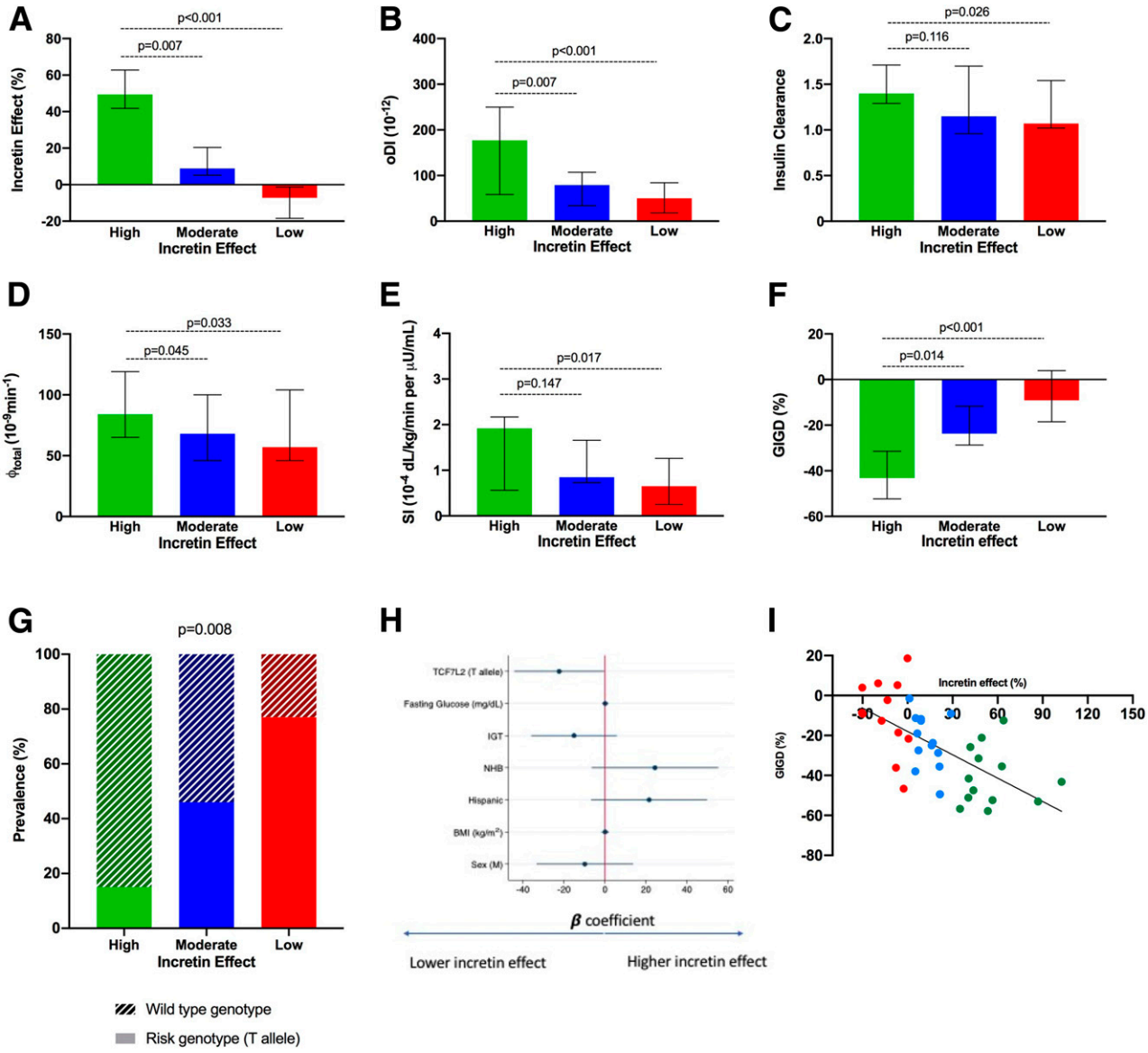

Figure 3—Incretin effect (%) (*A*), oDI (*B*), insulin clearance (*C*), Φ_{total} (*D*), SI (*E*), and GIGD (*F*).*G*: Percentage of wild-type/risk genotypes per incretin tertiles (low, moderate, and high). *H*: Multivariable regression model for the continuous outcome incretin effect (%). Data are β-coefficient (25th, 75th percentile). *I*: Linear regression analysis for GIGD and incretin effect. NHB, non-Hispanic Black.

glucose-induced β-cell response, and it is detectable by the use of matched OGTT and iso-IVGTT. Thereby, the blunted increase in the active GLP-1(7-36) during the OGTT for the low incretin effect group compared with the high incretin effect group (Fig. 2*M–O*) supports a role for GLP-1 by itself as a determinant of the low incretin effect phenotype. Nevertheless, GLP-1 titrated infusion studies are warranted to properly explore the role of incretin resistance in this context (42) as well as to assess the role of GLP-1 clearance. A reduced GIGD along with a lower SI were the metabolic hallmarks for the low incretin effect phenotype group, confirming findings from the adults (12,28,43,44).

Our group recently reported a marked decline in insulin clearance in obese youth and adults with insulin resistance (45) associated with a longitudinal failure in β-cell function (oDI) over time (45). In this cohort, we found a lower insulin clearance in those with reduced incretin effect. The estimate of insulin clearance relied on C-peptide and insulin secretion during the OGTT, as previously validated in healthy adults (29).

Despite the robustness of the matched iso-IVGTT approach used to estimate the incretin effect, this method cannot be extended to larger cohorts. Therefore, the correlation analysis between 1-h glucose and the incretin effect could provide a potential, minimally invasive marker of the incretin status to customize therapeutic options in youth with prediabetes. In this study, we identified a higher 1-h glucose threshold (173 mg/dL) as the optimal cut point with the best combination of sensitivity and specificity, without significant differences across groups of glucose tolerance.

Our study has several limitations. Lack of direct assessment of gastric emptying is acknowledged as a weakness that may have had a major determinant effect on

1-h plasma glucose (16). Its relationship with incretin hormone secretion has been recently studied and is likely bidirectional (given that GLP-1 reduces the gastric emptying rate). Duodenal infusion of glucose determines an increase in plasma concentrations of GIP or GLP-1 according to the rate of glucose infusion (46,47). Although the heated hand technique has been proven to provide blood closer to arterial blood than venous sampling, differences in glucose concentrations remain (21). Nevertheless, the effect of such a difference was minimized by the use of the same technique during OGTT, iso-IVGTT, and hyperglycemic clamp.

The lack of plasma measurements of GIP is a limitation of the current study. However, the use of matched iso-IVGTT and OGTT allowed us to estimate the incretin effect independently from GLP-1 and GIP measurements. The absence of euglycemic-hyperinsulinemic clamp to quantify insulin clearance has been counterbalanced by the use of a validated measure on the basis of the OGTT insulin and C-peptide measures.

The use of matched iso-IVGTT and OGTT to quantify the incretin effect is a major strength for this study because it provides, for the first time in a pediatric cohort, an unbiased estimate of the incretin action. Our results do not contradict previous findings of a reduced incretin effect across the spectrum of glucose tolerance in obese youth as obtained by the combined use of OGTT and hyperglycemic clamp (6) but do identify *TCF7L2* as an independent risk determinant for a lower incretin effect.

In summary, we describe a low-incretin phenotype in obese youth as the functional defect of a risk allele of *TCF7L2* manifested by the contemporary reduction of β-cell function adjusted to a low SI and insulin clearance. This is associated with a higher 1-h glucose and a lower gastrointestinal glucose extraction.

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