Incretin effect determines glucose trajectory and insulin sensitivity in youths with obesity

Alfonso Galderisi, … , Nicola Santoro, Sonia Caprio

*JCI Insight*. 2023. [https://doi.org/10.1172/jci.insight.165709](https://doi.org/10.1172/jci.insight.165709).

**Graphical abstract**

Find the latest version:

[https://jci.me/165709/pdf](https://jci.me/165709/pdf)
Title: Incretin effect determines glucose trajectory and insulin sensitivity in youths with obesity.

Running title: Incretin effect and glucose tolerance in youths

Alfonso Galderisi¹, Domenico Trico², Jessica Lat¹, Stephanie Samuels¹, Ram Weiss¹, Michelle Van Name¹, Bridget Pierpont¹†, Nicola Santoro¹,⁵,⁶, Sonia Caprio¹

Affiliations
1 Yale University, Department of Pediatrics, New Haven, CT - USA
2 Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy
4 Department of Pediatrics, Ruth Rappaport Childrens' Hospital, Rambam Medical Center, Haifa, Israel
5 Department of Pediatrics Kansas University Medical Center, Kansas City, KS
6 Department of Medicine and Health Sciences University of Molise, Campobasso, Italy

Correspondence. Alfonso Galderisi, MD PhD , Department of Pediatrics, Division of Pediatric Endocrinology - 333 Cedar Street, 06520 – New Haven (CT), USA; Phone: +1-203.764.9199, Fax: +1-203-737-2829; email: alfonso.galderisi@yale.edu

Keywords: incretin effect, pediatric obesity, diabetes, prediabetes

Figures: 4
Tables: 1
Word Count: 3174

Conflict of interest : the authors have no conflict of interest to disclose.
Abstract

In youth with obesity, the gut hormone potentiation of insulin secretion - the *incretin effect* - is blunted. We explored the longitudinal impact of the incretin effect during pubertal transition on beta cell function and insulin sensitivity. Youths with obesity and 2-h glucose ≥ 120mg/dL underwent a 3-h OGTT and an isoglycemic intravenous glucose infusion to quantify the incretin effect. After 2 years, 30/39 participants had a repeated OGTT and were stratified into three tertiles according to the baseline incretin effect. Thirty participants completed the baseline and follow-up tests. The high-incretin effect group demonstrated a longitudinal increase in beta cell function (DI_{MM}) (p=0.034), with greater insulin sensitivity at follow-up (p=0.034) and stable insulin secretion (ϕ_{total}) (p=0.077). A lower incretin effect at baseline was associated with a higher 1-h and 2-h glucose at follow-up (r = -0.558, p=0.001 and r = -0.533, p=0.004). The high-incretin effect group displayed a greater increase of GLP-1_{7-36} than the moderate- and low-incretin group at baseline (p=0.008 and p=0.029), while such a difference did not persist after 2 years. Glucagon suppression was reduced at follow-up in those with low-baseline incretin respect to the high-incretin group (p=0.049).
Introduction

The gut produced incretins – glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) – are responsible for almost ~50% increase of insulin response to orally administered glucose in healthy adults (1), accounting for a large portion of the different insulin response between oral and intravenous (iv) glucose load. (1). This differential response to oral and iv glucose load – namely the \textit{incretin effect} – declines with progression of hyperglycemia to overt diabetes in both youths and adults (2-5). The temporal sequence of changes of hyperglycemia and incretin effect is still debated with conflictual results (6) (7).

We recently demonstrated that youths with obesity and low incretin effect exhibit q111dd reduced beta cell function as compared to their peers with preserved incretin function, in the absence of diabetes (8), however the long-term effect of incretin response on the trajectory of beta cell function and insulin sensitivity during pubertal transition is still unknown.

More than one third of US adolescents with obesity have a prediabetes glycemic profile (9, 10), with ~35% progressing to persistent dysglycemia or overt diabetes during adulthood (11). Unlike the gradual prolonged metabolic changes preceding adult-onset type 2 diabetes, a rapid progression of dysglycemia and beta cell failure in the context of insulin resistance occurs in youth onset type 2 diabetes (12-15) with at least one diabetes complication occurring before the age of 30 years (16). Nevertheless, there are no approved drugs to treat prediabetes in youths with a temporally limited window of intervention to prevent diabetes complications after the disease onset.

The identification of the metabolic phenotype of youths with obesity and prediabetes and its natural history with respect to beta cell function, insulin sensitivity and the incretin
effect is of pivotal relevance to customize diabetes-preventive strategies targeting the metabolic determinants of youth-onset diabetes to prevent this disease.

Herein we explore the longitudinal effect of the incretin effect on beta cell function in a contemporary cohort of adolescents with obesity and prediabetes evaluated during pubertal transition and 2 years later.

Results

Participant characteristics. Thirty out of 39 participants completed the follow-up OGTT after 2.2±0.4 years (12 of 13 from the high-incretin, 10 of 13 from the moderate-incretin, and 8 of 13 from the low-incretin groups). Participants had mean age at baseline of 16.3±2.2 years, 16 assigned female at birth, and identified as White (5), Black (11), and Hispanic (14). Participants’ characteristics are displayed in Table 1 according to the baseline incretin tertile. The three groups did not differ with respect to anthropometric and metabolic characteristics at baseline and exhibited a similar distribution of sex and ethnicity. Subjects who did not return for the follow-up OGTT did not differ from the analyzed cohort with respect to baseline metabolic and anthropometric characteristics (Supplemental Table 1).

While all participants (n=30) had a 2-hour glucose of ≥120mg/dL at baseline, seventeen of the thirty participants (57%) maintained a 2-h glucose ≥120mg/dL at the follow-up OGTT, with 10 out of the 17 exhibiting IGT and one overt diabetes.

The low-incretin group exhibited a higher BMI at follow-up than baseline (p<0.001) (Table 1), while the other two groups did not show any statistically significant changes in BMI (p=0.110 and p=0.410 for the high- and moderate-incretin groups, respectively). Participants were regularly followed up at the obesity clinic and the lifestyle educational interventions did
not differ across the three groups. None of the participants was on medications affecting insulin sensitivity during the study period.

**Glucose, insulin and C-peptide excursions over time.** At baseline, the 3-h glucose profile was greater in the low-incretin group (p=0.014) than the high incretin group, as quantified by the linear mixed model analysis, whereas the glucose response did not significantly differ between the moderate- and high-incretin groups (p=0.098) (**Figure 1A**). Conversely, insulin excursion was more pronounced in the high- than the low-incretin group (p=0.009), while there was no difference between the moderate- and high-incretin cohorts (p=0.831) (**Figure 1C**). C-peptide trajectory during the 3-h OGTT was not different among the groups at baseline (p=0.885 and 0.297 for the high-incretin vs the moderate- and low-incretin cohorts) (**Figure 1E**).

As displayed in the right panels of **Figure 1**, at follow up, the greater glucose excursion of the low-incretin group persisted after the oral glucose load (p<0.013) (**Figure 1B**) in the absence of a significant difference between the moderate- and high-incretin groups (p=0.098). The greater glucose increase over the 3-h OGTT was paralleled by higher insulin and C-peptide (**Figure 1D and 1F**) concentrations in the low-incretin group compared to the high-incretin cohort (p=0.003 and p=0.031 for C-peptide and insulin).

**Figure 2** describes the longitudinal changes of beta cell function (DI$_{MM}$), insulin sensitivity (SI) and beta cell responsiveness ($\phi_{\text{total}}$) in each incretin group as computed through the oral minimal model. Those with high- baseline incretin effect demonstrated a >5-times higher DI$_{MM}$ at follow-up compared to the baseline (p=0.034), while it remained unchanged in the medium- and low-incretin effect groups (p=0.734 and p=0.641) (**Figure 2A**). This difference was associated with an increase of SI over time (p=0.034) (**Figure 2B**) and a trend towards a raise of $\phi_{\text{total}}$ (p=0.077) (**Figure 2C**) in the high-incretin group.
The cross-group comparison of follow-up beta-cell function metrics displayed a greater DI_{MM} (Figure 2A) and SI (Figure 2B) in the high-incretin cohort compared to the low-incretin group at follow-up (p=0.044 and p=0.013) in the absence of difference for the beta-cell responsiveness (ϕ_{total}) (p=0.235) (Figure 2C).

**GLP-1\textsubscript{7-36} response.** At baseline, the high-incretin group displayed a greater fasting GLP-1\textsubscript{7-36} than the moderate- and low-incretin groups (Figure 3A) (p=0.077 and p=0.048), while this difference was not significant during the follow-up tests (Figure 3B) (p=0.899).

The dynamic GLP-1\textsubscript{7-36} response during the OGTT at baseline and follow-up is displayed in Figure 3E-G. The high-incretin effect group displayed a greater percentage increase than the moderate- and low-incretin group during the baseline test (p=0.008 and p=0.029, respectively). Such a difference did not persist at the follow-up OGTT with blunted excursion across the three groups.

**Glucagon response.** At baseline, fasting glucagon did not differ across the three groups (p=0.465) (Figure 3C) in spite of a trending higher glucagon of the low and moderate incretin respect to the high-incretin. The high-incretin group showed a lower fasting glucagon at follow-up than those with moderate- and low-incretin effect (p=0.025 and p=0.005, respectively).

While at baseline the glucagon excursion during the OGTT did not differ among the three groups (Figure 3C), at follow-up those in the low-incretin group demonstrated a reduced glucagon decrease than the high incretin group (p=0.049) (Figure 3D). This was mirrored by a reduced percentage suppression of glucagon at follow-up in those with low incretin respect to the baseline suppression (p=0.014) as described in Figure 3L, while we did not
observe significant changes in glucagon suppression respect to the baseline OGTT in those with high- and moderate- incretin effect (p=0.410 and 0.300) (Figure 3H and 3I).

**Determinants of glucose tolerance change over time.** In the whole study cohort, the baseline incretin effect was inversely associated with follow-up 1-h glucose (r=-0.558, p=0.001) and 2-h glucose (r=-0.533, p=0.004), but not with fasting glucose (Figure 4A-C). Using a multivariate regression analysis, the baseline incretin effect was a significant determinant of follow-up 2-h glucose after adjusting for baseline fasting and 2-h glucose, fasting glucagon, BMI, age and sex (β=-11.0; p=0.035). The baseline incretin effect of those with a 2-h glucose ≥120mg/dL at the follow-up OGTT was ~6 times lower than the group returning to 2-h glucose <120mg/dL (45.5%[18.3, 59.9] vs 7.4%[-1.1, 17.0], p=0.013) (Figure 4D), while glucose, C-peptide, insulin and glucagon profile did not differ between the two groups at baseline.

We estimated that a baseline incretin effect equal or lower than 16.8% would provide 92% sensitivity and 70% specificity to predict a 2-h glucose ≥120mg/dL at follow-up in this cohort with an area under the ROC of 0.890±0.071. Baseline fasting, 2-h glucose and BMI areas under the ROC for the same binary outcome were smaller than the baseline incretin effect (0.552±0.116, 0.648±0.111, and 0.530±0.121 respectively) (Figure 4E).

**Discussion**

In this study we followed-up, for two years, three groups of youths with obesity stratified according to the incretin effect measured during their pubertal transition by the use of a matched OGTT and iso-IVGTT (1). We demonstrated that a relatively high incretin effect acts as a protective factor toward the beta cell function over time. Youths with a low-incretin effect maintain a low insulin sensitive phenotype after ~2 years, while an high
incretin effect promote an increase of insulin sensitivity and, as a consequence, an
improvement of beta cell function. Secondly, we investigated the dynamic changes of the
active form of GLP-1 – GLP-17-36 – and glucagon at both baseline and follow-up during the
OGTT according to the baseline incetin effect. While GLP-1 excursion was preserved at
baseline in those with high incretin effect and more pronounced than the other two groups,
as expected, this difference was not detectable at follow-up (Figure 3E-F), with flattened
GLP-1 excursion in the three groups. This suggests that the protective role of incretins
during the pubertal transition acts as a priming effect for long-term beta cell function,
consistently with the higher disposition index and insulin sensitivity observed in the high-
incretin effeect group. This observation supports the need for therapeutic interventions
preserving the incretin effect during pubertal transition.

Glucagon is physiologically suppressed after the oral glucose load, however an
impaired glucagon suppression has been described in adults with obesity (17). Herein we
observed that a low incretin effect during pubertal transition is associated with a
progressive impairment of glucagon suppression over time. The follow-up measures of beta
cell function, active GLP-1 and glucagon, suggest that the glucagon suppression is primary
driven by the preserved beta cell function than by a contemporary high GLP-1 level.

Our findings are suggestive for the incretin effect to play a priming role during
pubertal transition at preserving beta cell function and insulin sensitivity over time and,
even in the absence of an adequate response of GLP-1 at later ages, this effect seems to
persist and impact both insulin sensitivity and glucagon suppression.

Lastly, the baseline incretin effect demonstrated an inverse linear association with
follow-up 1-h and 2-h glucose, supporting the concept of a continuous spectrum of glucose
tolerance in youths (18, 19). We adopted a cutoff of 120 mg/dL to explore a potential
treshold value for the incretin effect as a predictor of long-term glucose tolerance
impairment. This is based on previous reports demonstrating that youths with a 2-h glucose equal or greater than 120mg/dL exhibit a similar levels of both insulin resistance and beta-cell dysfunction seen in those with 2-h glucose ≥140mg/dL (20). Such a threshold has been associated to a 40% reduction of beta cell function in youths with obesity respect to their healthy peers (20, 21). In our cohort, a low baseline incretin effect was predictive for a 2-h glucose ≥120mg/dL after ~2 years, regardless the other anthropometric and metabolic baseline characteristics. This observation suggests that a low incretin effect might be associated with a higher risk for impaired glucose tolerance and overt diabetes in adolescents. By adopting the 120mg/dL cutoff, we identified a clinically significant threshold for the incretin effect in youths with obesity. Lean youths without dysglycemia have been shown to exhibit a ~50% higher insulin secretion during the oral glucose load respect to the intravenous administration (1, 22, 23), thus a 50% incretin effect is generally considered as “normal” in the absence of an evidence-based range of values. Obesity with normal glucose tolerance, in turn, is associated to an independent reduction of the incretin effect (~25% ) (3), while herein we describe that a lower threshold -16% - for the incretin effect would be able to identify >90% of subjects at risk for a 2-h glucose ≥120mg/dL. This threshold is therefore lower than the average incretin effect previously described in youths with obesity and normal glucose tolerance (3). As displayed by this cohort, the baseline metabolic characteristics of those with 2-h glucose <120mg/dL vs ≥120mg/dL did not differ with respect to glucose, insulin and C-peptide, but those with a persistent 2-h glucose ≥120mg/dL exhibited an early defect of incretin effect that therefore predates the change in glucose trajectory and the beta cell function longitudinal decline.

The main limitation of this study stands in the absence of a longitudinal assessment of incretin effect trajectory by the matched iso-IVGTT. However, the longitudinal measure of the active form of the GLP-1, supports the hypothesis that a priming effect of higher
peripubertal higher GLP-1, may provide a persistent metabolic benefit with respect to beta
cell function and insulin sensitivity. The absence of a measure of other incretin, as GIP, is
an additional limitation of this study. We did not record detailed dietary intakes of the three
groups over two years, however as they were regularly followed at the same obesity clinic
we do not expect additional confounders and we may infer that the low incretin effect, per
se, might have favored weight gain. The limited numerosity of the groups prevented
additional analyses to evaluate the role of other variables – including ethnicity and gender –
in the longitudinal progression. Existing evidence from our group and others suggest
ethnicity as a major determinant of disease progression and the incretin response in youths
with obesity (11, 24).

In conclusion we demonstrated that a low incretin effect predates worsening of glucose
trajectory over time and is longitudinally associated to a lower insulin sensitivity and higher
fasting glucagonemia in youths with obesity.

**Methods.**

We studied youths with obesity followed at the Yale Pediatric Obesity Clinic and
participants enrolled in the “Yale Study of the Pathophysiology of Prediabetes/T2D in Youth”
who had body mass index (BMI)>85th percentile for age and sex, age 8-21 years, 2-h glucose
≥120 -139mg/dL.

Exclusion criteria were the use of medications affecting glucose metabolism, a
diagnosis of syndromic obesity or the participation in clinical trials including a structured
dietary or exercise-based intervention.

At baseline, participants underwent an OGTT (25) and a matched iso-IVGTT, which
reproduced the same plasma glucose profile observed during the OGTT to quantify the
incretin effect. The differential insulin secretion between the OGTT and the iso-IVGTT represents the estimated incretin effect with a higher secretion during the OGTT vs the iso-
IVGTT being associated with a higher incretin effect.

After two years, the OGTT was repeated to evaluate beta cell responsiveness, insulin sensitivity, and glucose tolerance status. Tanner stage was determined by a pediatric endocrinologist based on breast development in girls and genitalia development in boys at baseline and follow-up (26, 27).

The baseline characteristics of the original cohort have been described previously (8).

Procedures and Calculations

Oral glucose tolerance test (OGTT). Subjects were admitted to the Yale Center for Clinical Investigation (YCCI) at 8 a.m. after a 12-hour overnight fast. After the local application of a topical anesthetic cream (Emla, Astra Zeneca, Wilmington, Del.), one antecubital intravenous catheter was inserted for blood sampling. Two baseline samples were then obtained for measurements of plasma glucose, insulin, C-peptide and glucagon. Thereafter, flavored glucose in a dose of 1.75 g per kilogram of body weight (up to a maximum of 75g) was given orally, and blood samples were obtained at 10, 20, 30, 60, 90, 120, 150 and 180 minutes for the measurement of plasma glucose, insulin, C-peptide and glucagon. Glucose samples were immediately processed at the bed side using a YSI2700-STAT-Analyzer (Yellow Springs Instruments, Yellow Springs, OH).

Iso-glycemic venous glucose tolerance test (iso-IVGTT) Detailed methods of the iso-
IVGTT have been reported previously (8). Briefly, participants were admitted within 1 week after the baseline OGTT for an intravenous infusion of dextrose (20%) used to reproduce the plasma glucose profile observed during the OGTT. Frequent adjustments of glucose infusion based on plasma glucose sampling every 5 minutes were adopted to match the
profile of the OGTT.

**Glycemic status.** In accordance with the American Diabetes Association criteria,(28) impaired glucose tolerance (IGT) as a 2-hr plasma glucose level between 140 and 199 mg/dl. Pre-IGT status was defined as a 2-hr glucose of 120-139 as previously described (20, 21). Individuals with type 2 diabetes (T2D) at baseline, were excluded from this study.

**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 Product (Los Angeles, CA). Plasma glucagon was measured with the Mercodia Glucagon ELISA (Winston- Salem, NC).

The active form of GLP-1 (GLP-1_{7-36}) was measured by radioimmunoassay (EMD Millipore Corporation, Billerica, MA) with 0.1% cross-reactivity with the unamidated forms GLP-1_{7-37} and GLP-1_{1-37} or other peptides such as human GLP-2, glucagon, human GIP, and vasoactive intestinal peptide.

**Beta cell function: Insulin secretion and insulin sensitivity.** β-Cell function and its components were reported by using both the minimal model estimates. The oral minimal model expresses beta cell function (disposition index, DI\textsubscript{MM}) as the product of the \(\varphi_{\text{total}}\) term – based on 9-point measures of plasma glucose and C-peptide during the 3-h OGTT – and the insulin sensitivity index (SI) quantified from plasma glucose and insulin during the 3-h OGTT (29, 30). The computational procedure to estimate the \(\varphi_{\text{total}}\) and SI terms has been previously described (8, 29), and was implemented in the SAAM-II 2.3 software (SAAM Institute, Seattle, WA).

The baseline incretin effect was calculated as the ratio of the difference between the area under the curve (AUC) of insulin secretion during the oral (AUC-SR\textsubscript{OGTT}) and the isoglycemic venous (AUC-SR\textsubscript{iso-IVGTT}) glucose tolerance test, over the AUC-SR\textsubscript{OGTT} and
expressed as percentage value (1, 8, 31). A higher value corresponds to a higher insulin secretion during the oral respect to the intravenous test, thus quantifying a higher incretin effect.

Statistical analysis.

Data were summarized using median (25th percentile, 75th percentile) for continuous variables and count (percent, %) for categorical variables.

The original cohort (n=39) was stratified by the baseline incretin effect into three tertiles: High- (>66th centile), Moderate- (33rd-66th centile) and Low- (<33rd centile) incretin effect tertile. The high-incretin effect group was adopted as the comparison term during the analyses (high- vs moderate-incretin and high- vs low-incretin effect group). Paired intra-group analyses were conducted to compare follow-up and baseline values.

Time-series from the OGTT measurements (glucose, C-peptide, insulin, glucagon and GLP17-36) were analyzed by linear mixed model effect. The GLP17-36 response was additionally evaluated with respect to the baseline value (at time 0) and expressed as percentage increase from baseline at each time point to normalize the distribution within the cohort.

A linear regression analysis was adopted to test the relationship between baseline incretin effect and fasting, 1-h and 2-h glucose at follow-up, after adjustment for age, BMI and sex (32, 33). Tanner stage was collinear with age.

Continuous variables were compared using the Kruskal-Wallis test, followed by post-hoc pair-wise Mann-Whitney test. Categorical variables were compared using the Chi-square test.
Receiver operating curve analysis (ROC) was also used to seek for the optimal cut point of incretin effect to predict a 2-h glucose equal or higher than 120mg/dL at follow-up. The choice of 120mg/dL as a threshold was based on available evidence for a decline of beta cell function starting above 120mg/dL in youths with obesity (10, 20).

The analyses have been conducted only for participants who returned for the follow-up assessment.

Analyses were performed using STATA.13 software (StataCorp, College Station, TX) and Prism 8.0 (GraphPad Software, San Diego, CA).

**Study approval.** 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.

**Data availability.** Anonymized data generated during the tests and the codes for the model identification adopted in SAAMII to compute the oral minimal model indices will be available upon request from the corresponding author. Supporting data values are provided.

**Author Contributions.** A.G., S.S., J.L. and S.C. performed the metabolic tests. A.G. ran the model analysis. A.G. and S.C. designed the study, collected and analyzed the data, and wrote the manuscript. B.P. enrolled participants and collected the data. D.T. and R.W. contributed to the data analysis and interpretation. N.S. genotyped the cohort. M.V.N., R.W., N.S. and S.C. critically revised the manuscript. All authors approved the manuscript in its final version. A.G. and S.C. are the guarantors of this work and, as such, had 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.
Acknowledgements. The authors thank all volunteers for their participation in the study and Rachel Goldberg, Cindy Guandalini, and Mary Savoye for their help in the Yale Pediatric Clinic.

Funding. This study was supported by the National Institutes of Health, National Institute of Child Health and Human Development (grants R01-HD-40787, R01DK111038, R01-HD-28016, S.C. and R01-MD015974 to N.S.), Clinical and Translational Science Award [grant UL1-RR-0249139] the American Diabetes Association (Distinguished Clinical Scientist Award to S.C.), the Robert Leet Patterson and Clara Guthrie Patterson Trust Mentored Research Award and the Juvenile Diabetes Research Foundation (to A.G.).

This article’s contents are solely the responsibility of the authors and do not necessarily represent the official view of the National Institutes of Health or of the other listed funding institutions.

Prior Presentation. Parts of this article were presented at the 81th Scientific Sessions of the American Diabetes Association, New Orleans, LA, 23–26 June 2022.
References


Figure 1. Glucose, insulin, C-peptide profile during the OGTT at baseline (left panels) and at follow-up (right panels) according to the baseline incretin tertile. Data are represented as median and interquartile ranges (25th, 75th centile). Linear mixed model effect analysis has been adopted for comparisons.
Figure 2. Baseline and follow-up beta cell function ($D_{IM}$), insulin sensitivity (SI) and beta cell responsiveness ($\phi_{total}$) at baseline and follow-up in those with high-, moderate- and low-incretin baseline effect. Data are represented as median and interquartile ranges (25th, 75th centile), in bold p<0.05. Krustal-Wallis test has been adopted for comparisons.
Figure 3. Baseline and follow-up GLP-$1_{7-36}$ (A, B) and glucagon (C, D) for the high-, moderate, and low-incretin groups. Percentage increase from basal for GLP-$1_{(7-36)}$ at baseline and follow-up visits for the high- (E), moderate- (F), and low-incretin effect (G) during the OGTT. Percentage increase from basal for glucagon at baseline and follow-up visits for the high- (H), moderate- (I), and low-incretin effect (L) during the OGTT. Data are represented as median and interquartile ranges (25th, 75th centile).
Figure 4. Linear regression analysis of incretin effect and fasting (A), 1-h (B) and 2-h (C) glucose. Data are represented as naturally log-transformed measures. Linear regression analysis $r$ and p-value are reported per each variable. (D) Baseline incretin effect by 2-h glucose at follow-up. Data are expressed as median and interquartile range (25th, 75th). Kruskal-Wallis test has been adopted for comparison. (E) ROC analyses for the binary outcome 2-h glucose $\geq 120$mg/dL at follow-up. The analysis includes baseline incretin effect, baseline fasting and 2-h glucose and baseline BMI as predictors.
Table 1. Participant characteristics by baseline incretin effect

<table>
<thead>
<tr>
<th></th>
<th>High Incretin Effect (n=12)</th>
<th>Moderate Incretin Effect (n=10)</th>
<th>Low Incretin Effect (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>Baseline</td>
</tr>
<tr>
<td>Incretin effect (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>55 (45, 62)</td>
<td>-</td>
<td>16 (7, 21)</td>
</tr>
<tr>
<td>Sex (F/M/Non-binary) (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9/3/0 (69/21/0)</td>
<td>3/7/0 (31/59/0)</td>
<td>4/4/0 (50/50/0)</td>
</tr>
<tr>
<td>Ethnicity NHW/NHB/Hispanic (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/5/6 (8/42/50)</td>
<td>1/5/4 (10/50/40)</td>
<td>3/1/4 (37/13/50)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>15.7 (14.5, 17.1)</td>
<td>17.5* (16.7, 19.6)</td>
<td>16.2 (15.0, 17.0)</td>
</tr>
<tr>
<td>Tanner Stage n(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-III</td>
<td>7 (58)</td>
<td>0 (0)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>IV-V</td>
<td>5 (42)</td>
<td>12 (100)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.5 (29.8, 40.3)</td>
<td>34.0 (33.4, 37.5)</td>
<td>37.2 (35.7, 40.8)</td>
</tr>
<tr>
<td>NGT/preIGT/IGT/T2D n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0/9/3/0 (67/33/0)</td>
<td>5/3/4/0 (42/25/33/0)</td>
<td>0/5/5/0 (0/50/50/0)</td>
</tr>
<tr>
<td>Fasting Glucose (mg/dl)</td>
<td>89 (86.5, 95.5)</td>
<td>89 (85, 97.5)</td>
<td>93 (88, 96)</td>
</tr>
<tr>
<td>2h-Glucose (mg/dl)</td>
<td>133.5 (122, 143.5)</td>
<td>118.5 (106.5, 143)</td>
<td>135.5 (128, 148)</td>
</tr>
<tr>
<td>Fasting Insulin (µU/mL)</td>
<td>26.5 (19.5, 41)</td>
<td>26 (14, 31.5)</td>
<td>40.5 (28.5, 49)</td>
</tr>
<tr>
<td>Fasting C-peptide (pmol/L)</td>
<td>1000 (850, 1297)</td>
<td>942.5 (882, 1275)</td>
<td>1315 (1060, 1575)</td>
</tr>
<tr>
<td>Fasting Glucagon (pmol/L)</td>
<td>3.89 (3.35, 6.12)</td>
<td>6.18 (3.97, 7.05)</td>
<td>6.72 (4.19, 12.69)</td>
</tr>
<tr>
<td>Fasting GLP-1 (pmol/L)</td>
<td>2.4 (1.1, 8.2)</td>
<td>12.2 (5.1, 18.2)</td>
<td>1.5 (1.0, 26.0)</td>
</tr>
</tbody>
</table>

NGT, normal glucose tolerance; pre-IGT, pre-impaired glucose tolerance; IGT, impaired glucose tolerance; BMI, body mass index; NHW, non-Hispanic White; NHB, non-Hispanic Black; H, Hispanic. *p<0.05 for paired comparison with baseline values. Only data of participants who completed both baseline and follow-up OGTTT are reported.