The incretin effect in obese adolescents with and without type 2 diabetes: impaired or intact?

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[1]*Department of Medicine, University of Cincinnati, Cincinnati, Ohio;* [2]*Department of Internal Medicine II, Ludwig-Maximillian's University, Munich, Germany;* [3]*Department of Medicine, Columbia University, New York, New York;* [4]*Baltimore Veterans Affairs Medical Center, Baltimore, Maryland;* [5]*Cincinnati Veterans Administration Medical Center, Cincinnati, Ohio; and* [6]*Department of Pediatrics, Cincinnati Children's Hospital, Cincinnati, Ohio*

Aulinger BA, Vahl TP, Prigeon RL, D'Alessio DA, Elder DA. The incretin effect in obese adolescents with and without type 2 diabetes: impaired or intact?. *Am J Physiol Endocrinol Metab* 310: E774–E781, 2016. First published March 15, 2016; doi:10.1152/ajpendo.00496.2015.—The incretin effect reflects the actions of enteral stimuli to promote prandial insulin secretion. Impairment of this measure has been proposed as an early marker of β-cell dysfunction and described in T2D, IGT, and even obesity without IGT. We sought to determine the effects of obesity and diabetes on the incretin effect in young subjects with short exposures to metabolic abnormalities and a few other confounding medical conditions. Subjects with T2D ($n = 10$; 18.0 ± 0.4 yr) or NGT, either obese ($n = 11$; 17.7 ± 0.4 yr) or lean ($n = 8$; 26.5 ± 2.3 yr), had OGTT and iso-iv. The incretin effect was calculated as the difference in insulin secretion during these tests and was decreased ~50% in both the NGT-Ob and T2D subjects relative to the NGT-Ln group. The T2D group had impaired glucose tolerance and insulin secretion during the OGTT, whereas the lean and obese NGT subjects had comparable glucose excursions and β-cell function. During the iso-iv test, the NGT-Ob subjects had significantly greater insulin secretion than the NGT-Ln and T2D groups. These findings demonstrate that in young subjects with early, well-controlled T2D the incretin effect is reduced, similar to what has been described in diabetic adults. The lower incretin effect calculated for the obese subjects with NGT is driven by a disproportionately greater insulin response to iv glucose and does not affect postprandial glucose regulation. These findings confirm that the incretin effect is an early marker of impaired insulin secretion in persons with abnormal glucose tolerance but suggest that in obese subjects with NGT the incretin effect calculation can be confounded by exaggerated insulin secretion to iv glucose.

insulin secretion; glucose tolerance; incretin effect; glucagon-like peptide-1; glucose-dependent insulinotropic polypeptide

THE PHYSIOLOGICAL REGULATION OF INSULIN SECRETION is adapted to connect meal absorption with nutrient assimilation. Healthy humans can ingest a wide range of carbohydrate loads with only modest variability in the excursion of blood glucose in great part because larger meals evoke more insulin secretion (4, 6, 31). Thus, the ability to match insulin responses with insulin needs is not a linear function of glycemic stimulation of the β-cell since blood glucose does not vary substantially between meals of different sizes. Rather, stimuli emanating from the gut in proportion to meal size adjust the gain on glucose-activated islets, allowing appropriate insulin secretion for a given nutrient load. Experimentally, this amplification by gut factors is best demonstrated as the incretin effect, the substantially greater insulin response observed when glucose is ingested relative to an isoglycemic delivery of intravenous glucose (7, 31). A large proportion of the incretin effect is accounted for by two intestinal peptides, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (5), both of which are essential for normal glucose tolerance (8, 9, 26, 36). The effects of the incretins on insulin secretion increase in proportion to the amount of glucose consumed (4, 31) and allow for normal glucose tolerance across a wide range of carbohydrate intakes.

Importantly, the incretin effect appears to be a component of the pathological insulin secretion characteristic of diabetic persons. In a now classic paper, Nauck et al. (29) demonstrated that in subjects with type 2 diabetes (T2D) the normal augmentation of insulin secretion with oral compared with intravenous (iv) glucose was nearly absent. This finding has been replicated by other investigators (4, 22, 25, 27) and is generally attributed to functional insensitivity of the diabetic β-cell to GIP and GLP-1 (21, 23, 30). More recently, defects in the incretin system have been extended to individuals with impaired glucose tolerance and suggested to be an early marker of β-cell dysfunction (16, 22, 27, 28). Indeed, healthy subjects given a 1- to 2-wk intervention of caloric excess and glucocorticoids become glucose intolerant with a reduced incretin effect (13, 17).

Based on the current literature, a case can be made that abnormal oral glucose tolerance is invariably associated with impairment of the incretin effect (16), which is not surprising given the centrality of gut signaling for postprandial insulin secretion. However, several groups have suggested that abnormalities of the incretin effect are present in obese subjects with normal glucose tolerance (22, 27), citing the possibility that metabolic, endocrine, or inflammatory consequences of increased adiposity interfere with the gut-islet connection. These studies have been conducted in middle-aged adults, some of whom may be at risk for diabetes, but beg the question of whether and how normal oral glucose tolerance can be maintained with abnormal meal-enhanced insulin secretion.

To examine the effects of obesity on the incretin effect, we studied lean and obese adolescents and young adults with T2D and normal glucose tolerance (NGT). The rationale for selecting young and relatively healthy subjects was to test the role of adiposity on incretin function at an early stage not confounded by other chronic diseases. We hypothesized that subjects with

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normal oral glucose tolerance would have a normal incretin effect.

RESEARCH DESIGN AND METHODS

Subjects. Three different cohorts of adolescent and young adult men and women participated in these studies: *1)* 10 obese subjects with T2D; *2)* 11 obese subjects with normal glucose tolerance (NGT-Ob); and *3)* eight nonobese subjects with normal glucose tolerance (NGT-Ln). Adolescent patients with T2D or obesity were recruited from the endocrinology clinic at the Cincinnati Children's Hospital Medical Center (CCHMC), whereas young adults were recruited by advertisement. All study procedures were approved by the Institutional Review Boards of the CCHMC and University of Cincinnati, and all subjects gave written informed consent. For minors under the age of 18 yr, both the subjects and their legal representatives were required to give written informed assent/consent.

The diabetic subjects were 16–20 yr old (18.0 ± 0.4 yr), with established T2D as defined by World Health Organization criteria. At the time of diagnosis, subjects presented with either hyperglycemia (>11.1 mmol/l; range 9.0–32.9 mmol/l), an Hb A_{1c} of 6.5% or higher (range 6.5–11.8%), or both (Table 1). Average age at the time of diagnosis was 14 ± 0.6 yr, duration of diabetes was 4 yr (48 ± 8 mo), and subjects were treated with metformin (7), insulin (2), or a combination of both (1). All adolescent diabetic patients were negative for autoantibodies indicative of type 1 diabetes, had elevated fasting C-peptide levels, and were obese (BMI 37.7 ± 2.0 kg/m²). At the time of the study, diabetic subjects presented with excellent glycemic control, as reflected in a mean Hb A_{1c} of 5.9 ± 0.2%, and had withheld treatment with metformin for 3 days, long-acting insulin doses for ≥24 h, and short-acting insulin for 12 h before the studies. The obese subjects did not differ in age or body weight from the T2DM cohort (17.7 ± 0.4 yr, BMI 42.1 ± 2.4 kg/m²; Table 1) and had normal fasting glucose and 75-g oral glucose tolerance tests. Because of concerns over recruiting healthy children for moderately invasive research by our institution's review board, we recruited lean control subjects >18 yr old (26.5 ± 2.3 yr, BMI 24.8 ± 1.3 kg/m²) who were able to consent independently; all had normal fasting glycemia and oral glucose tolerance and a negative family history for diabetes. Although age and BMI did not differ (*P* = 0.34) between subjects with T2D and obesity (NGT-Ob), the lean group (NGT-Ln) was significantly older (*P* < 0.001) and weighed less (*P* < 0.001) than the other two cohorts. All of the adolescents who participated in this study were Tanner IV for puberty as assessed by a pediatric endocrinologist.

Experimental procedures. All subjects were studied on two occasions in the morning after an overnight fast. They were asked to maintain their standard diet with ≥200 g/day of carbohydrates and to refrain from unusual physical activity for ≥3 days prior to each visit. T2D and NGT-Ob subjects were studied in the Clinical Research Unit (CRU) at the CCHMC and NGT-Ln subjects in the CRU at the

Cincinnati Veterans Affairs Medical Center by the same investigators (B. A. Aulinger and T. P. Vahl) starting at 0730. The first visit was an oral glucose tolerance test (OGTT). A plastic cannula was placed in the antecubital vein of one arm for withdrawal of blood samples, and a heating pad wrapped around the hand and arm to maintain good blood flow and arterialize venous blood, and subjects rested in a reclining position for the remainder of the study. Three fasting blood samples were taken starting at −10 min, and subjects were asked to drink 75 g of a liquid dextrose solution made ≤400 ml in tap water over 5 min at *time 0 min*. Frequent blood samples were taken at scheduled intervals over 180 min to determine blood glucose and plasma concentrations of insulin, C-peptide, GLP-1, and GIP.

On the morning of the second visit [isoglycemic intravenous infusion (iso-iv)], iv cannulae were placed in antecubital veins of both arms, one for withdrawal of samples and one for dextrose infusion. After withdrawal of baseline samples, a variable glucose infusion was started to mimic the glucose excursion measured during the OGTT. Blood glucose was determined in 5-min intervals using a bedside instrument (Yellow Springs Instruments, Yellow Springs, OH) and the glucose infusion adjusted to match the glycemic excursion from the OGTT. The two studies were separated by ≥5 days.

Analytical procedures. Whole blood samples were placed in prechilled blood containers containing either 50 mM EDTA (for measurement of glucose and insulin) or EDTA/500 kiu/ml aprotinin (for assay of C-peptide, GLP-1, and GIP). All tubes were immediately placed in an ice bath and centrifuged within 60 min to obtain plasma. The plasma was frozen and stored at −80°C until further analysis was performed. Insulin was measured using a previously described radioimmunoassay (10). C-peptide was measured using a commercial RIA (Millipore, St. Charles, MO). Total GLP-1 immunoreactivity was determined from ethanol extracts of plasma using a commercial RIA (Millipore), as reported previously (39). Plasma total GIP concentrations were measured using an ELISA according to the manufacturer's protocol (Millipore). Hb A_{1c} was determined using a standard method based on high-performance liquid chromatography (10).

Calculations and statistical analysis. Insulin secretion rates were derived from plasma C-peptide concentrations, using deconvolution with population estimates of C-peptide clearance (35, 40), and used to calculate insulin clearance (37). Incremental areas under the curve (AUC) over baseline for glucose, insulin, and C-peptide concentrations were calculated using the trapezoidal rule. The incretin effect was calculated as the relative insulin or C-peptide response of oral compared with iv glucose: $AUC_{(OGTT)} - AUC_{(Iso-iv)}/AUC_{(OGTT)} \times 100\%$ (29).

Homeostatic model assessment of insulin resistance 2 (HOMA2-IR) was calculated using the online HOMA calculator (available at http://www.dtu.ox.ac.uk/homacalculator/index.php) (41) based on fasting C-peptide and glucose concentrations. The insulinogenic index (IGI) was calculated as the increment above basal insulin over the first 30 min of the OGTT and iso-iv tests divided by the increment in 30-min

Table 1. *Clinical characteristics of the research subjects*

	T2D	NGT Obese	NGT Lean	P Value
n (Males/females)	10 (2/8)	11 (3/8)	8 (5/3)	
Age, yr	18.0 ± 0.4 (16–20)	17.7 ± 0.4 (16–20)	26.5 ± 2.3 (18–37)	<0.001
Body weight, kg	104.3 ± 3.9	119.5 ± 6.8	77.6 ± 6.2	<0.001
BMI	37.7 ± 2.0	42.1 ± 2.4	24.8 ± 1.3	<0.001
Hb A_{1c}, %	5.9 ± 0.2 (5.0–7.1)			
Glucose at diagnosis, mM	17.8 ± 2.7			
Hb A_{1c} at diagnosis, %	8.4 ± 0.7			
Diabetes duration, mo	48.0 ± 7.8			
Metformin treatment	8/10			
Insulin treatment	3/10			

Data are means ± SE (range). T2D, type 2 diabetes; NGT, normal glucose tolerance.

glucose (38). The disposition index (DI), a measurement of insulin secretion relative to insulin sensitivity, was calculated by multiplication of IGI × 1/HOMA2-IR; these measurements of insulin secretion and sensitivity have been validated as fulfilling the hyperbolic relationship that is the basis for DI (38). C-peptide AUC was corrected for glucose AUC across the 180-min studies as an additional index of insulin secretion.

All data are expressed as means ± SE. Statistical analysis was performed using GraphPad Prism version 5.01 (GraphPad Software, La Jolla, CA). Single variables were compared among the three groups, using analysis of variance with post hoc comparisons for significant group differences. Comparisons of variables from the oral and iv studies among the three groups were made with two-way analysis of variance and post hoc testing when significant interactions or main effects were obtained. Relationships between the incretin effect and insulin secretion were sought using linear regression.

RESULTS

Fasting blood glucose did not differ between the 2 study days within any of the three subject groups (Table 2). Diabetic subjects had significantly higher fasting blood glucose than the members of either NGT group, all of whom had fasting glucose levels of <5.6 mmol/l. In response to 75 g of glucose, the T2D subjects had relative hyperglycemia compared with both obese and lean NGT subjects (Fig. 1A). The glucose AUC was comparable between the OGTT and iso-iv studies in each group (Fig. 1B), with values for each subject along the time course of the OGTT and iso-iv studies that overlapped and relatively small deviations in the matching for each subject (mean coefficient of variation of the paired glucose excursions: T2D 4.1%, NGT-Ob 2.5%, and

NGT-Ln 4.9%). The incremental AUC for glucose was significantly higher in T2D than in NGT-Ob and NGT-Ln ($P < 0.05$; Fig. 1C), whereas glucose excursions were comparable in the lean and obese NGT subjects with glucose AUC that was not significantly different (270 ± 57 and 309 ± 44 mmol·l^{-1}·min^{-1}, $P = 0.60$; Fig. 1C). The glucose infusion rates followed a similar pattern among the three groups (Fig. 1D), and the amount of iv glucose given to match the glucose profiles did not differ among the three cohorts (NGT-Ln 42.6 ± 6.55 g, NGT-Ob 52.4 ± 4.56 g, and T2D 48.4 ± 6.40 g, $P = 0.41$).

Incretin effect. The incretin effect (IE) was computed for each individual using the AUC of either plasma insulin or C-peptide as the index of β-cell function (Fig. 2, A and B). For each group, the IE was greatest when computed from plasma insulin concentrations. IE was significantly higher in the NGT-Ln subjects ($P < 0.05$; Table 2) and did not differ between the T2D and NGT-Ob groups, results that were similar whether insulin or C-peptide was used in the computation.

Insulin secretion, sensitivity, and clearance. In the fasting state, insulin and C-peptide were comparable in the T2D and NGT-Ob groups despite significantly lower fasting glucose in the latter (Table 2). Fasting insulin and C-peptide were significantly lower in the NGT-Ln group compared with the two cohorts of heavier subjects ($P < 0.05$; Table 2). HOMA2-IR, a reflection of insulin resistance at the initiation of the glucose tolerance tests, did not differ within subjects on the days of the OGTT and iso-iv studies for any of the groups (Table 2). The T2D and NGT-Ob subjects were insulin resistant relative to the NGT-Ln group (Table 2 and Fig. 3B).

Table 2. *Fasting insulin and glucose and measurements of insulin sensitivity, secretion, and clearance in the diabetic, obese, and lean subjects*

Parameter	T2D	NGT-Ob	NGT-Ln	P Value
Fasting glucose, mM				
OGTT	5.94 ± 0.35†	4.81 ± 0.09	4.43 ± 0.07	0.281
iv-Iso	6.24 ± 0.53†	4.74 ± 0.05	4.35 ± 0.06	
Fasting insulin, pM				
OGTT	197.60 ± 21.05	231.47 ± 33.95	45.78 ± 9.68†	0.784
iv-Iso	228.40 ± 27.99	249.26 ± 41.65	43.46 ± 6.41†	
HOMA2-IR				
OGTT	2.93 ± 0.37	3.26 ± 0.33	1.11 ± 0.12†	0.955
iv-Iso	2.89 ± 0.32	3.31 ± 0.50	1.05 ± 0.12†	
IGI, pM/mM				
OGTT	67.81 ± 27.06	402.19 ± 103.59†	131.17 ± 27.70*	0.0033
iv-Iso	28.08 ± 8.78	193.85 ± 46.13†	30.44 ± 4.99	0.0004
DI (IGI/HOMA2-IR), pM·mM^{-1}·%S^{-1}				
OGTT	41.99 ± 10.05†	96.32 ± 13.74	120.85 ± 26.45	0.029
iv-Iso	19.73 ± 4.48	48.51 ± 6.55*†	28.18 ± 3.50*	
C-peptide AUC/glucose, ng·ml^{-1}·mM^{-1}				
OGTT	2.21 ± 0.48#	4.56 ± 0.63	3.52 ± 0.48	0.001
iv-Iso	1.74 ± 0.47	2.91 ± 0.40*	1.32 ± 0.17*#	
Insulin clearance, l/min				
OGTT	1.35 ± 0.16	1.25 ± 0.14	1.90 ± 0.19	0.094
iv-Iso	1.84 ± 0.18*	1.50 ± 0.21	2.76 ± 0.25*	
Incretin effect, %				
Insulin	43 ± 8	41 ± 8	69 ± 4†	0.025
C-peptide	26 ± 6	29 ± 7	53 ± 4†	0.014

Data are means ± SE. NGT-Ob, obese subjects with normal glucose tolerance; NGT-Ln, nonobese subjects with normal glucose tolerance; OGTT, oral glucose tolerance test; iv-iso, isoglycemic infusions of glucose; HOMA2-IR, homeostatic model assessment of insulin resistance 2; AUC, area under the curve; IGI, insulinogenic index, incretin effect calculated from insulin or C-peptide AUC; DI, disposition index. *P* values in column are for overall ANOVA (incretin effect comparisons) or the interaction of oral/iv and group (all other comparisons). *Within-subjects difference between OGTT and iv-iso, $P < 0.05$; †difference with the other 2 groups by post hoc comparison, $P < 0.05$; #difference with NGT-Ob, $P < 0.05$.
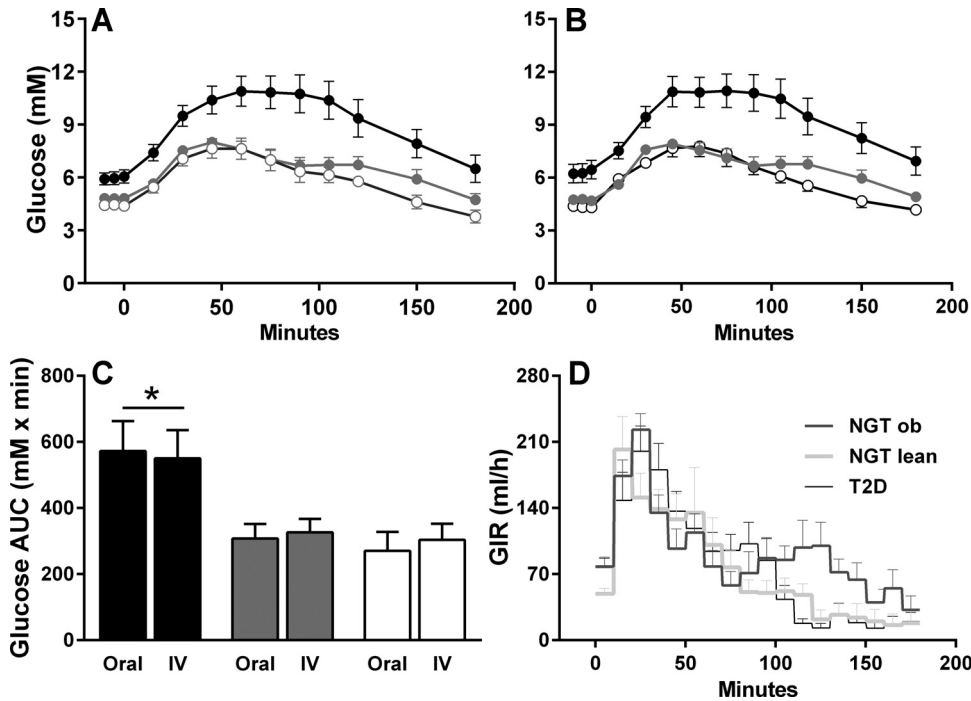
Fig. 1. Blood glucose and glucose infusion rates (GIR) during oral glucose tolerance and isoglycemic intravenous infusion (iv-iso). *A* and *B*: arterialized blood glucose levels before and after oral glucose ingestions (*A*) and intravenous (iv) glucose infusion (*B*) in subjects with type 2 diabetes (T2D; ●), obese subjects with normal glucose tolerance (NGT-Ob; gray circles), and nonobese subjects with normal glucose tolerance (NGT-Ln; ○). *C*: glucose area under the curve (AUC) for oral glucose tolerance tests (OGTT) and iso-iv tests in T2D (black bars), NGT-Ob (gray bars), and NGT-Ln (open bars) subjects. *D*: GIR for T2D (black lines), NGT-Ob (gray lines), and NGT-Ln (light gray lines) subjects. Data are plotted as means ± SE. *Significant difference from NGT groups at <0.05.

Similar to the fasting state, NGT-Ln had lower absolute measurements of insulin (Fig. 2*C*) and C-peptide (Fig. 2, *D–F*) than NGT-Ob in response to both oral and iv glucose. However, when corrected for insulin resistance as DI, the IGI was similar in the two NGT groups during the OGTT and signifi-

cantly higher than that of the T2D subjects (Table 2; Fig. 3*C*). In contrast, the DI in response to iv glucose did not differ between the T2D and NGT-Ln cohorts but was significantly elevated in the NTG-Ob individuals (Fig. 3*D*). DI during the OGTT was significantly higher than during the iso-iv study in
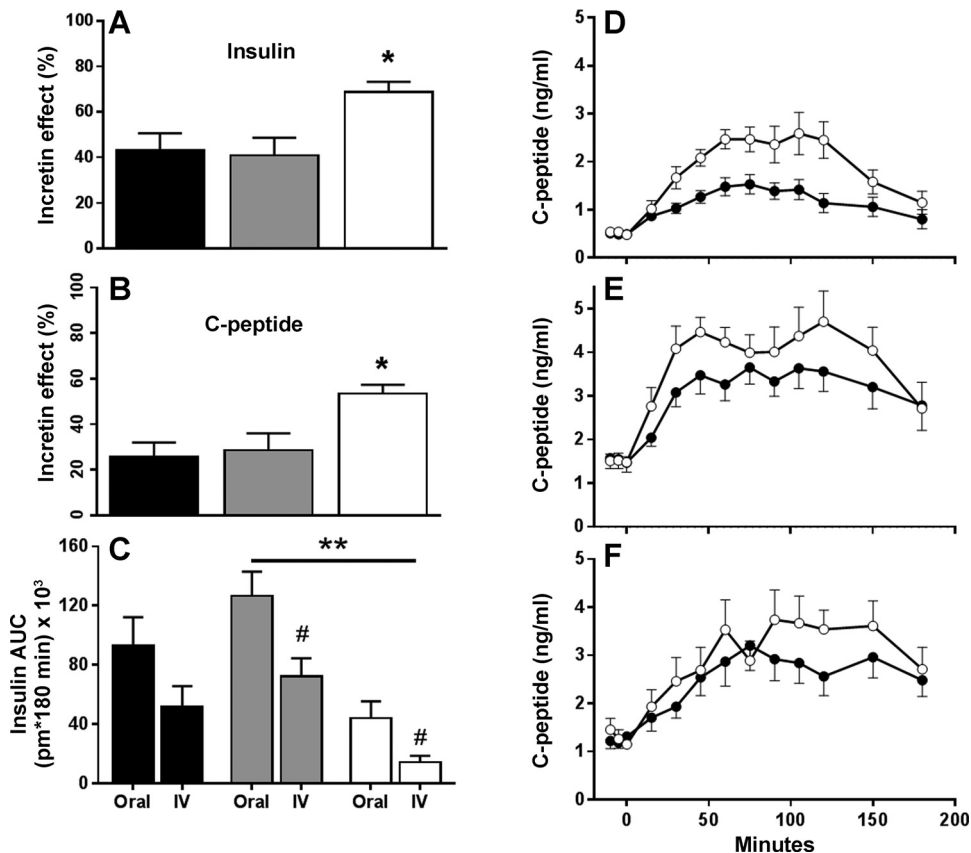

Fig. 2. *A* and *B*: incretin effect in T2D (black bars), NGT-Ob (gray bars), and NGT-Ln (open bars) subjects computed from insulin (*A*) and C-peptide values (*B*). *C*: insulin AUC during the oral and iso-iv tests. *D–F*: plasma C-peptide concentrations before and after oral (○) and iv glucose (●) in NTG-Ln (*D*), NTG-obese (*E*), and T2DM subjects (*F*). Data are plotted as means ± SE. *Significant difference from NGT groups at <0.05; #*P* < 0.05 compared with OGTT; **P* < 0.05, NGT-Ob compared with NGT-Ln.

Fig. 3. Insulin resistance and adjusted insulin secretion during OGTT and iso-iv tests. A: C-peptide AUC corrected for glucose AUC during oral and iv glucose tolerance tests in T2D (black bars), NGT-Ob (gray bars), and NGT-Ln subjects (open bars). B: insulin resistance in T2D (●), NGT-Ob (■), and NGT-Ln subjects (▲). C and D: insulinogenic index adjusted for HOMA2-IR during OGTT (C) and iso-iv (D) in T2D (black bars), NGT-Ob (gray bars), and NGT-Ln subjects (open bars); note the difference in y-axes. Data plotted as means ± SE. *Significance of difference vs. the other 2 groups at <0.05.
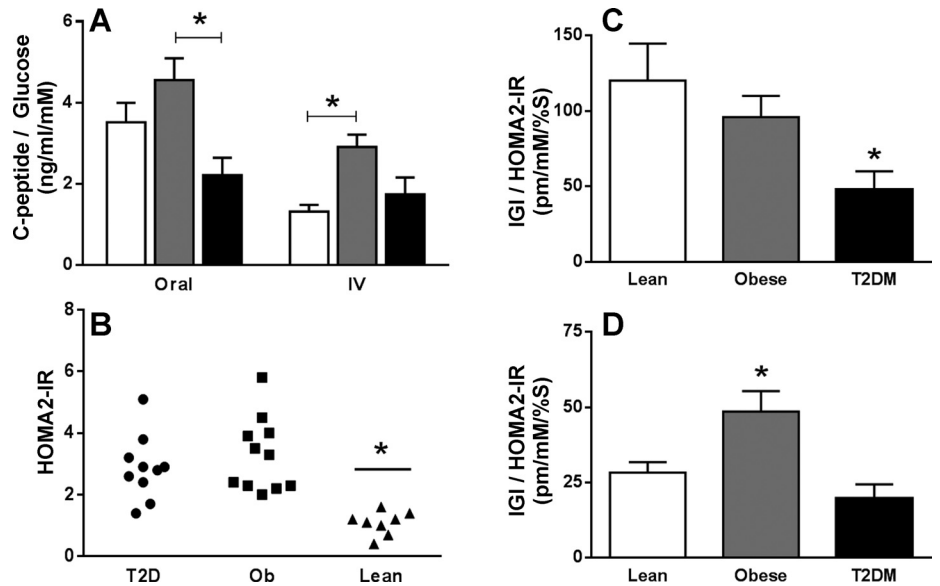
the NGT subjects but not the T2D group (Table 2). The findings with DI, which incorporates insulin secretion in the early phases of the glucose tolerance tests, were paralleled by the C-peptide responses across the 180-min studies corrected for glucose AUC (Fig. 3A and Table 2).

Despite comparable oral glucose tolerance and postprandial insulin secretion, the NGT-Ob subjects had significantly lower IE than the NGT-Ln group. Based on the DI computed for the OGTT and iso-iv studies, this result appeared to be driven by disproportionately high insulin responses to iv glucose in the NGT-Ob subjects. When the NGT subjects were considered in total, there was a significant inverse correlation between IE, calculated from plasma insulin, and corrected C-peptide during the iso-iv studies ($r = -0.53$, $P = 0.002$; Fig. 4, left). This relationship between IE and C-peptide was not present during the OGTT in the NGT subjects ($r = -0.01$; Fig. 4, right). Thus, in the nondiabetic subjects the β-cell response to iv glucose accounted for 28% of the variance in IE, whereas insulin secretion in response to oral glucose did not have any predictive effect. Similar relationships were found when IE calculated from C-peptide AUC was regressed against IGI/HOMA (data not shown).

Total body insulin clearance did not differ among the three groups but was greater during the iso-iv than OGTT in the NGT-Ln and T2D subjects (Table 2).

Plasma GLP-1 and GIP. Plasma concentrations of the incretins were generally comparable among the three groups (Fig. 5A). The T2D subjects had a higher mean fasting GLP-1 concentration and slightly elevated AUC in response to oral glucose. Plasma GIP was similar among the T2D, NGT-Ob, and NGT-Ln subjects (Fig. 5B).

DISCUSSION

The incretins are essential for NGT (8, 9, 26, 36), and defects in the incretin effect have been reported consistently in persons with diabetes and prediabetes (4, 22, 28, 29). These findings support the view that an impaired incretin effect is an early marker of β-cell dysfunction. This study was designed to test the effects of obesity and early diabetes on incretin function using a population of young and generally healthy research subjects with a minimum of confounding medical conditions. In these subjects, we observed that the incretin effect was comparable in subjects with well-controlled T2D and obese controls with NGT but significantly reduced compared with lean subjects. Despite differences in the incretin effect, the lean and obese NGT subjects had very similar insulin responses to oral glucose when normalized to insulin sensitivity. However, the obese group had a greater relative response to iv glucose, with this shift in the ratio of oral/iv insulin secretion causing an
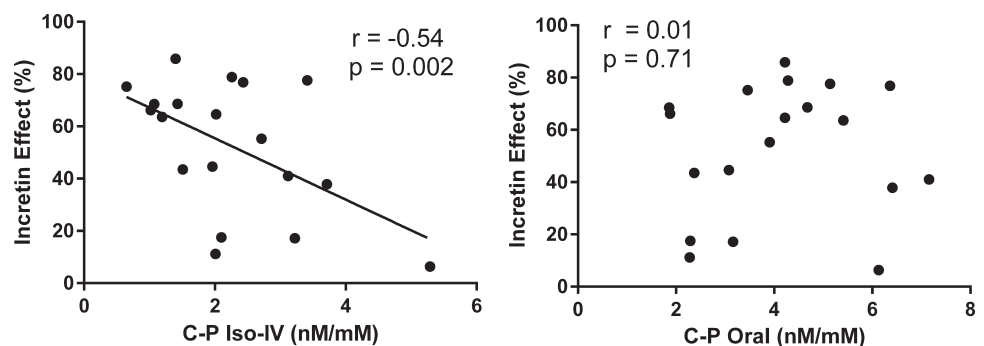

Fig. 4. Relationship between insulin secretion estimated from plasma C-peptide and the incretin effect computed from plasma insulin during the iso-iv (left) and oral glucose tolerance tests (right) in NGT subjects. ●, Individual subjects.
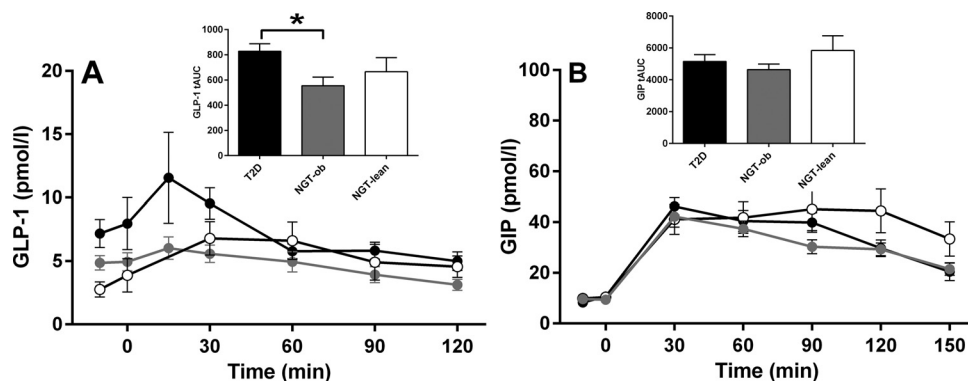
Fig. 5. Concentrations of glucagon-like peptide-1 (GLP-1; *A*) and glucose-dependent insulinotropic polypeptide (GIP; *B*) during the OGTT in T2D (●), NGT-Ob (gray circles), and NGT-Ln (○) subjects. AUCs for each incretin are plotted in the *insets* for T2D (black bars), NGT-Ob (gray bars), and NGT-Ln subjects (open bars). Data plotted as means ± SE. *Significance of difference at <0.05.

apparent incretin abnormality not associated with glucose intolerance. In contrast, the T2D subjects had reduced β-cell responses associated with abnormal oral glucose tolerance as the primary cause of reduced oral/iv insulin secretion. These findings extend previous work to show that even early and well-treated T2D is associated with abnormal incretin function but raises doubt as to whether the same association exists in obese people with NGT.

The key feature of this study was the young age and relative good health of the subjects, which allowed evaluation of the effects of diabetes and obesity on the incretin effect at a very early stage. We studied young people with definite but well-controlled T2D of a relatively short duration. In fact, at the time of the studies the mean Hb A_{1c} of these subjects was well below the values used to diagnose diabetes, and the group's OGTT was more consistent with impaired glucose tolerance than diabetes (1). Despite their excellent diabetic control, these subjects had obvious differences in their ability to handle a glucose load compared with age- and weight-matched subjects. And although their absolute values of insulin and C-peptide during the OGTT were not substantially different from the obese controls, when adjusted for prevailing glycemia these subjects had clearly diminished β-cell function. Our NGT-Ob cohort had uniformly normal fasting glucose levels and glycemic responses to 75 g of oral glucose that were very similar to the lean controls. The NGT-Ln group was slightly older than the other two but was comprised entirely of young adults and overlapped in age with the other cohorts. Despite significant differences in the calculated incretin effect, the two groups of NGT subjects had similar β-cell responses to oral glucose, using independent measurements of insulin secretion, DI based on the insulinogenic index, or C-peptide corrected for glycemia.

The incretin effect has been reported to be abnormal due to obesity per se (22, 27), and at first glance our results seem to confirm this; the NGT-Ob subjects had calculated incretin effects that were about half those in the NGT-Ln group. However, it is difficult to call this response impaired in the face of normal glycemic regulation after glucose ingestion and measurements of β-cell function during the OGTT that were comparable to the NGT-Ln subjects. In fact, the primary factor accounting for the lower incretin effect in the obese control group was a disproportionately greater β-cell response to iv glucose. Insulin hypersecretion in obese subjects is typically attributed to a compensatory response to insulin resistance (19), but in our subjects relative hyperinsulinemia persisted

even when secretion was adjusted for HOMA2-IR. Hyperinsulinemia in obese humans that is independent of compensation for insulin resistance has been reported previously (12, 20, 33), and on scrutiny other studies of the incretin effect in obese subjects with NGT also demonstrate relative insulin hypersecretion during isoglycemic glucose infusions (14, 22, 27). Similar to our results, Mari et al. (23) recently described an inverse relationship between the incretin effect and insulin secretion in response to isoglycemic glucose infusions in a group of overweight and obese subjects with NGT. Although glucose tolerance is a continuous variable and insulin secretion varies even within the NGT range (11), the difference in the glucose AUC between our lean and obese subjects was small, with considerable overlap; that this difference would be associated with a 50% difference in incretin function seems unlikely. Rather, it appears that calculation of the incretin effect can give artifactual results as a result of exuberant insulin responses to iv glucose; across our NGT subjects, the response to iv glucose, but not glucose ingestion, predicted the incretin effect. Thus, it is difficult to ascribe impairment of the incretin effect solely to obesity in otherwise healthy young people. In line with this, GLP-1-mediated insulin secretion is comparable in normal glucose-tolerant lean and obese subjects, similar to the cohorts reported here (3).

The T2D subjects also had reduced incretin effects, results that are in keeping with previous studies of adult subjects with well-controlled T2D (32, 35). In this case, the incretin abnormality was associated with glucose intolerance and a significantly reduced insulin response in the OGTT. Thus, the ~50% decreased incretin effect compared with NGT-Ln subjects reflects defective postprandial insulin secretion and would seem to be a robust indicator of β-cell dysfunction in what otherwise might be taken as mild disease. The T2D subjects described here have higher incretin effects than diabetic cohorts reported by other groups (22, 29), indicating some retention of enteral augmentation of insulin secretion. In all likelihood this was due to their tight glucose control, as recent studies have shown that effective treatment improves the response to incretins (2, 15). It is unclear why there was a relative preservation of DI in response to iv glucose in this group, as it has been demonstrated previously that adolescents with T2D have marked impairment to bolus injection of glucose (10). However, the relatively slow glucose infusion in an isoglycemic study has not been compared directly with other measurements of insulin secretion in diabetic subjects and may not be as sensitive a marker of β-cell impairment. Overall, our find-

ings are consistent with previous studies in diabetic subjects and indicate that even in early T2D there is a defective incretin effect.

The mechanism whereby the incretin effect was diminished in our diabetic cohort with relatively good glucose tolerance cannot be discerned from our data. The typical explanation for this abnormality, reduced effectiveness of GIP in T2D (24, 30), is open to question given that some of this response is restored with good glycemic control (15), a feature of our diabetic subjects. Moreover, the incretin effect in diabetic subjects cannot be attributed solely to GLP-1, since it is not completely abolished by blockade of the GLP-1 receptor (35), suggesting actions of another incretin. In our study and others (4, 24), plasma levels of the incretins did not differ meaningfully between diabetic and nondiabetic subjects. Nor does the magnitude of the incretin effect appear to be a function of differences in blood glucose (34). A previous suggestion that reduction of the incretin effect is due to the limited maximal capacity of diabetic β-cells to respond to the potent challenge of an OGTT seems unlikely in that the defect is more pronounced in response to lower, not higher, glucose loads (23). A more plausible explanation is that the decreased incretin effect in type 2 diabetes may be yet another manifestation of abnormal glucose augmentation of other β-cell stimuli (42). Impaired glucose potentiation is an early defect in diabetic insulin secretion (18) and may be manifested physiologically as insensitivity of the β-cells to GIP and GLP-1.

There are several limitations to this study that merit comment. First, the sample size in each group was relatively small and thus may not represent the diversity of responses in the wider population. This is particularly relevant in considering our finding of an enhanced insulin response to iv glucose in the NGT-Ob subjects, a finding reported in some (14, 20, 33) but not all papers (10). It is worth noting that our inclusion criteria were broad rather than selective and included groups that are typical of young people with diabetes and obesity. Second, our diabetic subjects were particularly well controlled at the time they were studied and may not be reflective of the usual adolescent or adult with T2D. Although this may limit the generalizability of our results, this group was useful in demonstrating that a defective incretin effect is a component of even early and mild diabetes. Third, the use of HOMA2-IR in small samples has been criticized because it is not as accurate as other more direct measurements of insulin sensitivity. However, insulin resistance was only a secondary measurement in this study and was used primarily to correct insulin secretion for each subject on their 2 study days; it seems likely that our within-subjects use of the HOMA index is sufficiently precise for this purpose (41). Finally, our lean controls were older than the T2D and obese subjects. Although parameters of glucose metabolism have not been well studied in healthy young adults, there is little reason to believe that they differ substantially from postpubertal adolescents.

In summary, we report here that the incretin effect is reduced even in very early diabetes, specifically adolescents with excellent glycemic control. This defect was not as severe as what has been reported previously in diabetic subjects with more advanced disease and was apparent only by comparison with lean controls. Based on comparisons of insulin secretion with the lean group during the OGTT, the reduced incretin effect in the NGT-Ob subjects was likely an artifact and suggests

caution when interpreting the relative effects of oral and iv glucose stimulation of insulin secretion. These findings add to a growing literature that supports reduction of the incretin effect as an early marker of β-cell dysfunction. However, we believe that at present it is not possible to dissociate normal glucose tolerance and normal incretin action.

AUTHOR CONTRIBUTIONS

B.A.A., T.P.V., R.L.P., D.A.D., and D.A.E. conception and design of research; B.A.A., T.P.V., and D.A.E. performed experiments; B.A.A., T.P.V., R.L.P., and D.A.D. analyzed data; B.A.A., T.P.V., R.L.P., and D.A.D. interpreted results of experiments; B.A.A. and D.A.D. prepared figures; B.A.A. drafted manuscript; B.A.A., T.P.V., R.L.P., D.A.D., and D.A.E. approved final version of manuscript; D.A.D. edited and revised manuscript.

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