

# Increased Rates of Meal Absorption Do Not Explain Elevated 1-Hour Glucose in Subjects With Normal Glucose Tolerance

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**Context:** In subjects with normal fasting glucose (NFG) and normal glucose tolerance (NGT), glucose concentrations >155 mg/dL 1 hour after 75 g of oral glucose predict increased risk of progression to diabetes. Recently, it has been suggested that the mechanism underlying this abnormality is increased gut absorption of glucose.

**Objective:** We sought to determine the rate of systemic appearance of meal-derived glucose in subjects classified by their 1-hour glucose after a 75-g oral glucose challenge.

**Design:** This was a cross-sectional study. Participating subjects underwent a 75-g oral glucose challenge and a labeled mixed meal test.

**Setting:** An inpatient clinical research unit at an academic medical center.

**Participants:** Thirty-six subjects with NFG/NGT participated in this study.

**Interventions:** Subjects underwent an oral glucose tolerance test. Subsequently, they underwent a labeled mixed meal to measure fasting and postprandial glucose metabolism.

**Main Outcome Measures:** We examined  $\beta$ -cell function and the rate of meal appearance (Meal  $R_a$ ) in NFG/NGT subjects. Subsequently, we examined the relationship of peak postchallenge glucose with Meal  $R_a$  and indices of  $\beta$ -cell function.

**Results:** Peak glucose concentrations correlated inversely with  $\beta$ -cell function. No relationship of Meal  $R_a$  with peak postchallenge glucose concentrations was observed.

**Conclusion:** In subjects with NFG/NGT, elevated 1-hour peak postchallenge glucose concentrations reflect impaired  $\beta$ -cell function rather than increased systemic meal appearance.

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**Freeform/Key Words:** splanchnic glucose uptake, disposition index, 60-min glucose

Abbreviations:  $\phi$ ,  $\beta$ -cell responsiveness to glucose; DI, disposition index; EGP, endogenous glucose production; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; Meal  $R_a$ , rate of meal appearance; NFG, normal fasting glucose; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test;  $R_d$ , rate of glucose disappearance; SGLT-1, sodium-dependent glucose cotransporter 1;  $S_i$ , insulin sensitivity index.

The transition from normal fasting glucose (NFG) and normal glucose tolerance (NGT) to type 2 diabetes is characterized by impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). This transition is variable [1] and influenced by lifestyle [2] and heredity [3]. Another contributing factor to this variability is a subset of people with prediabetes who have isolated IFG with NGT (*i.e.*, IFG/NGT). Despite elevated fasting glucose concentrations,  $\beta$ -cell function in this group does not differ from that in subjects with NFG/NGT [4, 5] as measured using the oral minimal model [6]. However, a recent longitudinal study [7] reported that the rate of conversion to type 2 diabetes is similar in IFG/NGT to people with NFG and IGT (NFG/IGT), although it is still higher in people with IFG/IGT.

Longitudinal studies have also demonstrated that glucose concentrations 60 minutes after an oral challenge  $>155$  mg/dL are better predictors of progression to type 2 diabetes [8, 9] than are glucose concentrations measured 120 minutes after a 75-g oral glucose tolerance test (OGTT). Indeed, in people with NFG/NGT, there is a small, but significant, rate of progression to type 2 diabetes [1]. This could be explained by the subset of subjects with a 60-minute glucose  $>155$  mg/dL [10]. Previous studies intended to characterize the pathophysiological abnormalities underlying postprandial hyperglycemia have reported that individuals with a 60-minute glucose concentration  $\geq 155$  mg/dL exhibit reduced insulin sensitivity, decreased insulin clearance, and impaired  $\beta$ -cell function compared with NGT individuals with a 60-minute glucose value  $<155$  mg/dL [10–13].

Another potential contributor to postprandial hyperglycemia is enhanced systemic appearance of ingested glucose as a result of increased intestinal absorption of glucose alone or in combination with decreased hepatic extraction of glucose [14]. Animal models of type 2 diabetes exhibit enhanced activity and abundance of intestinal sodium-dependent glucose cotransporter 1 (SGLT-1) and solute carrier family 2 member 2 (SLC2A2 or GLUT-2), thereby increasing intestinal glucose absorption and postchallenge glucose concentrations [15–18]. The abundance of SGLT-1 is also increased significantly in the duodenal brush border of people with type 2 diabetes [19]. Mice overexpressing SGLT-1 also develop visceral obesity [20, 21].

Recently, Fiorentino *et al.* [10] examined duodenal expression of SGLT-1 in individuals with NGT categorized on the basis of “high” ( $\geq 155$  mg/dL) or “low” ( $<155$  mg/dL) 60-minute glucose concentrations, as well as in people with IGT or type 2 diabetes. They report that SGLT-1 expression is increased in NGT individuals with an elevated 60-minute glucose concentration to the degree observed in subjects with IGT or type 2 diabetes, suggesting that increased proximal gut glucose absorption may contribute to the elevated postchallenge glucose concentrations observed in these subjects. However, the actual absorption of oral glucose [22, 23] or the systemic rate of appearance of ingested glucose [24] was not measured in this series of experiments.

We therefore used a labeled mixed meal [24, 25] to simultaneously measure  $\beta$ -cell function using the oral minimal model together with insulin action and postprandial glucose metabolism in subjects with NFG/NGT categorized on the basis of their 60-minute glucose concentrations (after an OGTT). We report that in this cohort, the appropriateness of insulin secretion for the prevailing insulin action, as assessed by the disposition index (DI) [26], is decreased in individuals with elevated 1-hour glucose concentrations. In contrast, the rate of systemic meal glucose appearance and, by implication, the rate of intestinal glucose absorption did not differ between groups. Additionally, net insulin action, suppression of endogenous glucose production (EGP), glucose disposal, and postprandial glucagon concentrations also did not differ. Taken together, these data indicate that in humans with NFG/NGT, elevated 1-hour postchallenge glucose concentrations are more likely to be due to impaired  $\beta$ -cell function.

## 1. Materials and Methods

After approval from the Mayo Clinic Institutional Review Board, 36 subjects gave informed written consent to participate in the study. All subjects were white, in good health, at a stable weight, and did not engage in regular vigorous physical exercise. At the time of the study, subjects were on no medications other than stable hormone replacement (*i.e.*, thyroid hormone). Subjects then underwent a 4-hour 75-g OGTT to characterize their glucose tolerance

status. The subjects recruited had NFG (fasting glucose <100 mg/dL) and NGT (2-hour glucose <140 mg/dL). These subjects were then categorized into two groups according to their 60-minute glucose after the OGTT [8] as either high ( $\geq 155$  mg/dL) or low (<155 mg/dL).

All were then instructed to follow a weight maintenance diet (~55% carbohydrate, 30% fat, and 15% protein) for at least 3 days prior to admission to the clinical research unit at least 2 weeks, but not more than a month, after the OGTT. Body composition was measured at the time of screening with dual-energy X-ray absorptiometry (iDXA scanner; GE Healthcare, Wauwatosa, WI).

On the day prior to study, subjects were admitted to the clinical research unit at 1700 hours, and after consuming a standard 10 kcal/kg meal (55% carbohydrate, 30% fat, and 15% protein), fasted overnight. The following morning, an 18-gauge cannula was inserted at 0600 hours into a forearm vein to allow for tracer infusion. Another 18-gauge cannula was inserted in a retrograde fashion into a dorsal vein of the contralateral hand that was then placed in a heated box (~55°C) to enable sampling of arterialized venous blood. A primed (12 mg/kg), continuous (0.12 mg/kg/min) infusion of [6,6-<sup>2</sup>H<sub>2</sub>]glucose (MassTrace, Woburn, MA) commenced at 0700 hours (–180 minutes).

At 1000 hours (0 minutes), subjects ingested a mixed meal consisting of three scrambled eggs, 55 g of Canadian bacon, and Jell-O containing 75 g of glucose that was enriched (to ~4%) with [1-<sup>13</sup>C]glucose, as previously described [27]. An IV infusion of [6-<sup>3</sup>H]glucose was also started at 0 minutes and infused in a pattern that mimicked the anticipated pattern of systemic appearance of meal [1-<sup>13</sup>C]glucose. Also, at 1000 hours (0 minutes), the [6,6-<sup>2</sup>H<sub>2</sub>]glucose infusion was varied in a manner that mimics the anticipated pattern of change of EGP. Meal ingestion is standardized in a manner similar to that described previously [28] and the meal was consumed within 10 minutes. The beaker containing the Jell-O was rinsed twice with 20 mL of water, and the rinse solution was consumed. The rationale for this design (to minimize the change in specific activity; *i.e.*, the tracer/tracee ratio) has been described in detail [29].

#### A. Analytical Techniques

Plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at –20°C until assay. Glucose concentrations were measured using a glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured using a chemiluminescence assay with reagents obtained from Beckman (Access Assay; Beckman, Chaska, MN). Plasma glucagon and C-peptide were measured by radioimmunoassay using reagents supplied by Linco Research (St. Louis, MO). Plasma [6,6-<sup>2</sup>H<sub>2</sub>]glucose and [1-<sup>13</sup>C]glucose enrichments were measured using gas chromatography–mass spectrometry (Thermoquest, San Jose, CA) to simultaneously monitor the C-1, C-2, and C-3 to C-6 fragments, as described by Beylot *et al.* [30]. [6-<sup>3</sup>H]glucose specific activity was measured by liquid scintillation counting following deproteinization and passage over anion- and cation-exchange columns.

#### B. Calculations

The systemic rates of meal appearance (Meal  $R_a$ ), EGP, and glucose disappearance ( $R_d$ ) are calculated using Steele *et al.*'s model [31]. Meal  $R_a$  is calculated by multiplying the  $R_a$  of [1-<sup>13</sup>C]glucose (obtained by dividing the infusion rate of [6-<sup>3</sup>H]glucose with the ratio of [6-<sup>3</sup>H]glucose and [1-<sup>13</sup>C]glucose) by the meal enrichment. EGP is calculated from the infusion rate of [6,6-<sup>2</sup>H<sub>2</sub>]glucose and the ratio of [6,6-<sup>2</sup>H<sub>2</sub>]glucose to endogenous glucose concentration.  $R_d$  is calculated by subtracting the change in glucose mass from the overall rate of glucose appearance (*i.e.*, Meal  $R_a$  plus EGP). Values from –30 to 0 minutes are averaged and considered as basal. Area above basal is calculated using the trapezoidal rule.

Net postprandial insulin action [insulin sensitivity index ( $S_i$ )] is measured by the oral minimal model [26].  $\beta$ -Cell responsivity indices are estimated using the oral C-peptide minimal model [26], incorporating age-associated changes in C-peptide kinetics [32]. The model assumes that insulin secretion comprises a static and a dynamic component with an index of total  $\beta$ -cell responsivity to glucose ( $\Phi$ ) derived from these two components. The DI is subsequently calculated by multiplying  $\Phi$  by  $S_i$ .

### C. Statistical Analysis

All data are presented as means  $\pm$  SEM. Rates of glucose turnover are expressed per kilogram of lean body mass. The primary analyses used an unpaired, two-tailed *t* test (parametric values) or a Wilcoxon matched-pairs signed rank test (nonparametric values). The statistical analysis was undertaken in GraphPad Prism 5 (GraphPad Software, San Diego, CA). Regression analysis adjusting for the effects of age, sex, and weight (see below) was performed in JMP Pro 11 (SAS Institute, Cary, NC) and in GraphPad Prism 5 (GraphPad Software). Residuals for the conditional logistic regression of a particular parameter with the covariates were used to confirm or refute the contribution of that parameter to variation in glucose concentrations. A *P* value  $<0.05$  was considered statistically significant.

## 2. Results

### A. Subject Characteristics

Age, body mass index, lean body mass, fasting plasma glucose, and 2-hour plasma glucose at the time of screening did not differ between the two groups (Table 1).

### B. Plasma Glucose, Insulin, C-Peptide, and Glucagon Concentrations During OGTT

Fasting glucose concentrations (Fig. 1A) did not differ between groups. However, by definition, 60-minute ( $10.0 \pm 0.3$  vs  $7.8 \pm 0.2$  mmol/L) and, subsequently, peak ( $10.3 \pm 0.3$  vs  $8.6 \pm 0.2$  mmol/L) and integrated ( $450 \pm 28$  vs  $358 \pm 39$  mol per 4 hours) postchallenge glucose concentrations at screening were higher ( $P < 0.001$ ) in the high group compared with the low group.

Fasting insulin (Fig. 1B) did not differ between groups. In response to the oral glucose challenge, peak ( $389 \pm 47$  vs  $313 \pm 36$  pmol/L,  $P < 0.05$ ) but not integrated ( $6014 \pm 831$  vs  $4449 \pm 417$  pmol per 4 hours,  $P > 0.05$ ) insulin concentrations were increased in the high group compared with the low group.

Fasting, peak, and integrated C-peptide (Fig. 1C) concentrations before and after the oral glucose challenge did not differ ( $P > 0.05$ ) significantly between groups.

Fasting ( $68.5 \pm 3.6$  vs  $71.4 \pm 6.1$  ng/L), nadir ( $51.6 \pm 4.1$  vs  $52.2 \pm 7.0$  ng/L), and postprandial integrated glucagon (Fig. 1D) concentrations did not differ ( $P > 0.05$ ) between groups.

### C. Plasma Glucose, Insulin, C-Peptide, and Glucagon Concentrations During the Mixed Meal Test

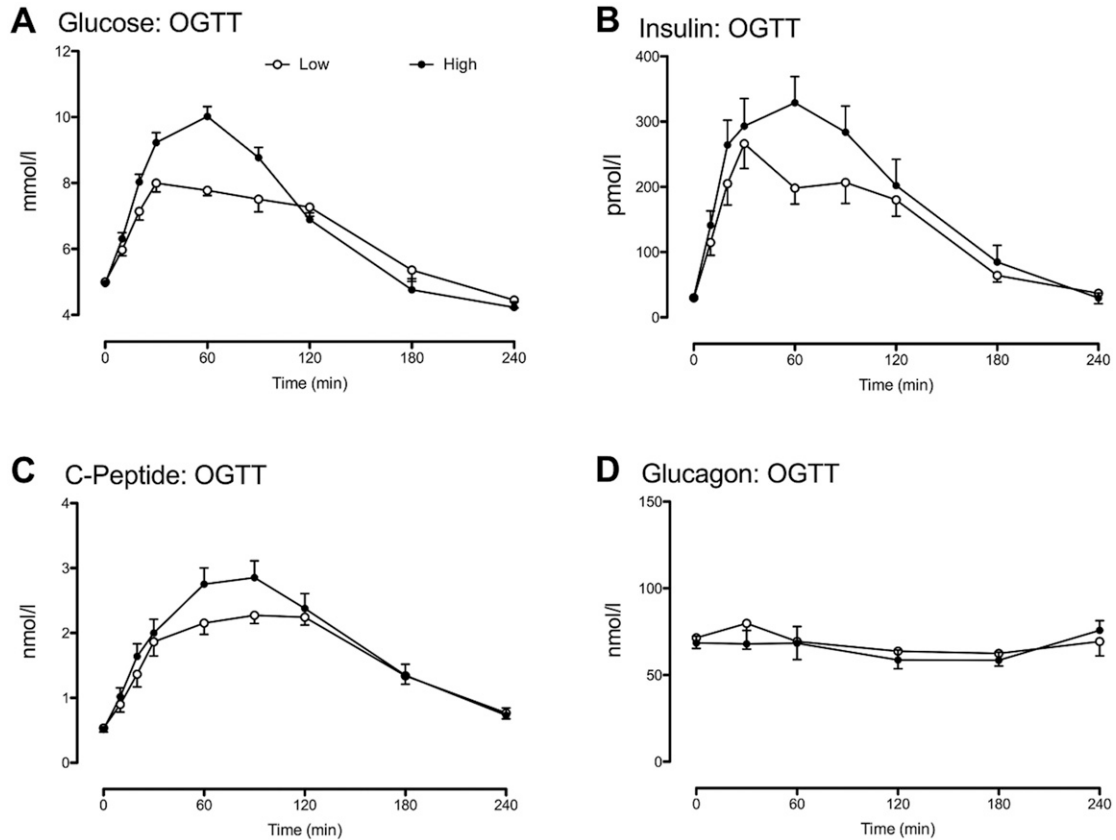
Fasting glucose concentrations ( $5.0 \pm 0.1$  vs  $4.9 \pm 0.1$  mmol,  $P > 0.05$ ; Fig. 2A) did not differ prior to meal ingestion. Following meal ingestion, peak glucose was higher in the high group compared

**Table 1. Demographic Characteristics of Study Subjects**

	All Subjects	Low	High
n	36	14	22
Age, y	$49.1 \pm 1.8$	$47.6 \pm 3.6$	$50.1 \pm 1.8$
Body mass index, kg/m <sup>2</sup>	$28.0 \pm 0.7$	$28.5 \pm 1.6$	$27.7 \pm 0.7$
Lean body mass, kg	$47.5 \pm 1.7$	$48.7 \pm 2.9$	$46.8 \pm 2.2$
Fasting glucose, mmol/L	$5.0 \pm 0.1$	$5.0 \pm 0.1$	$5.0 \pm 0.1$
60-min glucose, mmol/L	$9.1 \pm 0.3$	$7.8 \pm 0.2^a$	$10.0 \pm 0.3$
2-h glucose, mmol/L	$7.0 \pm 0.2$	$7.3 \pm 0.3$	$6.9 \pm 0.2$

Data are presented as mean  $\pm$  SEM unless otherwise indicated.

<sup>a</sup> $P < 0.001$ .



**Figure 1.** Glucose (A), insulin (B), C-peptide (C), and glucagon (D) in response to a 75-g oral glucose challenge in subjects with low (○) and high (●) 60-min glucose. Values plotted are means  $\pm$  SEM.

with the low group ( $10.6 \pm 0.3$  vs  $9.5 \pm 0.3$  mmol/L,  $P < 0.05$ ). Integrated glucose concentrations ( $433 \pm 43$  vs  $344 \pm 25$  mmol per 6 hours,  $P > 0.05$ ) did not differ between groups.

Fasting insulin concentrations were similar ( $30 \pm 4$  vs  $32 \pm 5$  pmol/L,  $P > 0.05$ ; Fig. 2B) between groups and rose to a comparable peak following meal ingestion. Integrated postprandial insulin concentrations did not differ between groups. Similarly, C-peptide concentrations (Fig. 2C) did not differ between groups (all  $P > 0.05$ ). Fasting and postprandial glucagon concentrations (Fig. 2D) also did not differ (all  $P > 0.05$ ).

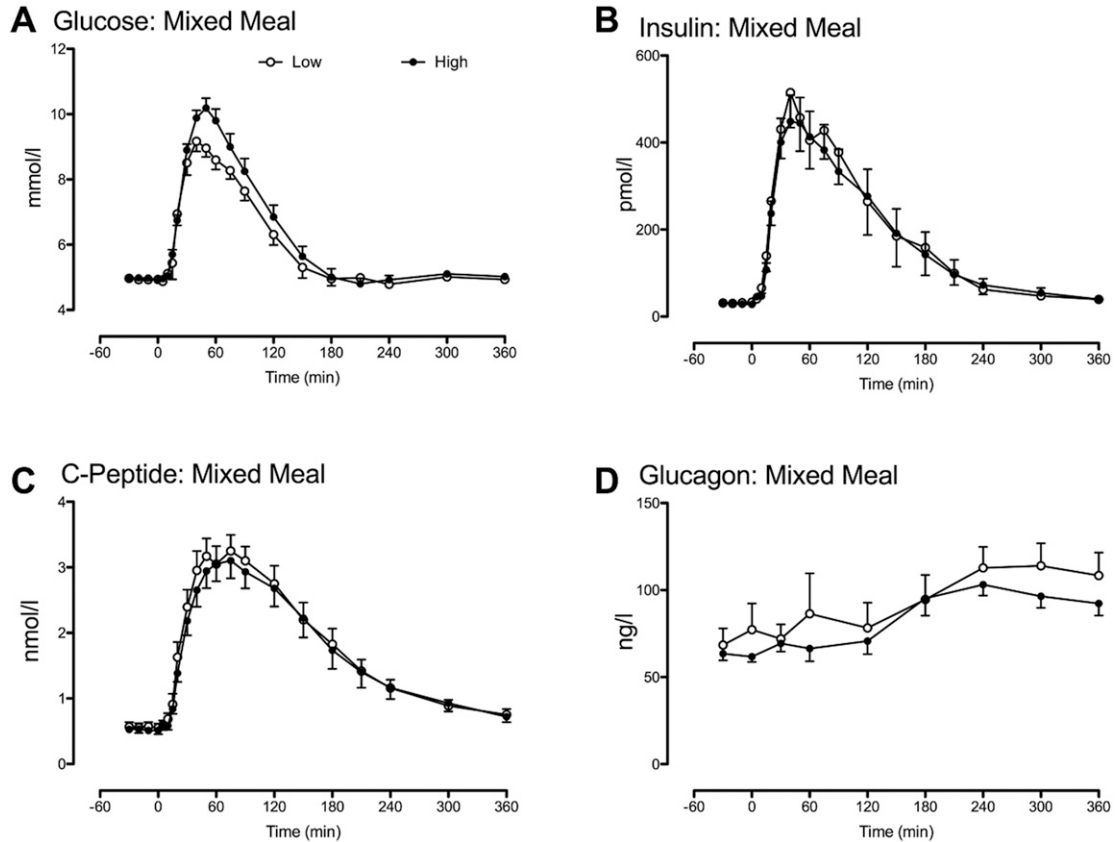
#### D. Insulin Action, $\beta$ -Cell Responsivity, and DI During the OGTT and Mixed Meal Test

During the OGTT, insulin action ( $S_i$ ) did not differ between the high group and low group ( $13 \pm 2$  vs  $17 \pm 3$   $10^{-4}$  dL/kg/min per  $\mu$ U/mL,  $P > 0.05$ ; Fig. 3A). Total  $\Phi$  ( $42 \pm 4$  vs  $48 \pm 4$   $10^{-9}$  min $^{-1}$ ; Fig. 3B) also did not differ ( $P > 0.05$ ). However, when  $\Phi$  is expressed as a function of  $S_i$ , the resulting DI was impaired ( $647 \pm 67$  vs  $1196 \pm 291$   $10^{-14}$  dL/kg/min $^2$  per pmol/L,  $P < 0.05$ ; Fig. 3C) in the high group compared with the low group.

Following mixed meal ingestion,  $S_i$  ( $11 \pm 2$  vs  $10 \pm 2$   $10^{-4}$  dL/kg/min per  $\mu$ U/mL,  $P > 0.05$ ; Fig. 3D) did not differ between groups. Additionally, total  $\Phi$  ( $47 \pm 4$  vs  $58 \pm 4$   $10^{-9}$  min $^{-1}$ ; Fig. 3E) did not differ ( $P > 0.05$ ). When  $\Phi$  was expressed as a function of  $S_i$ , the resulting DI also did not differ between groups ( $613 \pm 81$  vs  $706 \pm 117$  dL/kg/min $^2$  per pmol/L,  $P > 0.05$ ; Fig. 3F).

#### E. EGP, Meal Glucose Appearance, and $R_d$ During the Mixed Meal Test

Fasting EGP did not differ in subjects in the two groups ( $13.7 \pm 0.5$  vs  $13.4 \pm 0.7$   $\mu$ mol/kg/min,  $P > 0.05$ ; Fig. 4A). Additionally, nadir EGP also was similar between groups ( $3.2 \pm 0.2$



**Figure 2.** Glucose (A), insulin (B), C-peptide (C), and glucagon (D) in response to a mixed meal test in subjects with low (○) and high (●) 60-min glucose in response to a 75-g oral glucose challenge. Values plotted are means  $\pm$  SEM.

vs  $3.3 \pm 0.3 \mu\text{mol/kg/min}$ ,  $P > 0.05$ ). The systemic rate of appearance of meal-derived glucose reached a comparable peak ( $94.3 \pm 10.3$  vs  $82.8 \pm 11.6 \mu\text{mol/kg/min}$ ,  $P > 0.05$ ; Fig. 4B) in both groups. Integrated rates of meal appearance also did not differ between groups. Fasting ( $14.3 \pm 0.7$  vs  $14.0 \pm 0.7 \mu\text{mol/kg/min}$ ; Fig. 4C) and  $R_d$  ( $94.6 \pm 5.5$  vs  $80.6 \pm 7.4 \mu\text{mol/kg/min}$ ) also did not differ between groups (both  $P > 0.05$ ).

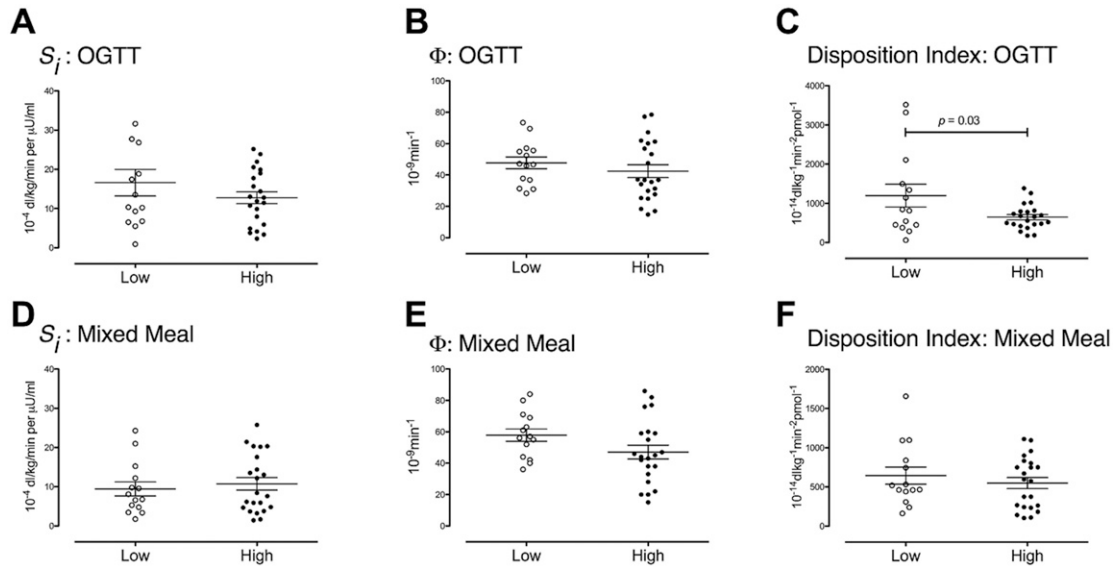
#### F. Relationship of Postprandial Glucose Concentrations With Meal $R_a$ and DI

Peak glucose concentrations observed during the OGTT and the mixed meal did not correlate ( $R^2 < 0.01$ ) with peak (Fig. 5A) and integrated meal appearance (Fig. 5B). Integrated postchallenge glucose concentrations during either challenge also did not correlate with meal appearance (data not shown).

Alternatively, peak glucose during the OGTT was inversely correlated with DI ( $R^2 = 0.10$ ,  $P = 0.05$ ; Fig. 5C). A similar relationship ( $R^2 = 0.14$ ,  $P = 0.02$ ; Fig. 5D) of peak glucose with DI was observed during the mixed meal.

### 3. Discussion

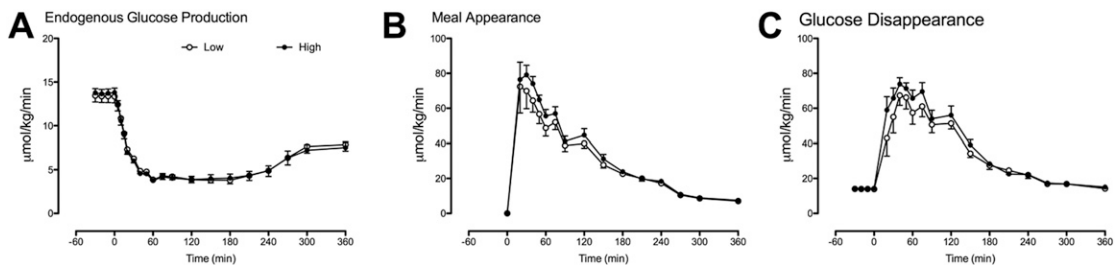
We report that the appropriateness of insulin secretion, as assessed by the DI, is decreased in individuals with elevated 1-hour glucose concentrations. In contrast, the rate of systemic meal glucose appearance did not differ between groups. Additionally, net insulin action, suppression of EGP, glucose disposal, and postprandial glucagon concentrations also did not differ. Taken together, these data indicate that in humans with NFG/NGT, elevated 1-hour postchallenge glucose concentrations are due to impaired  $\beta$ -cell function.



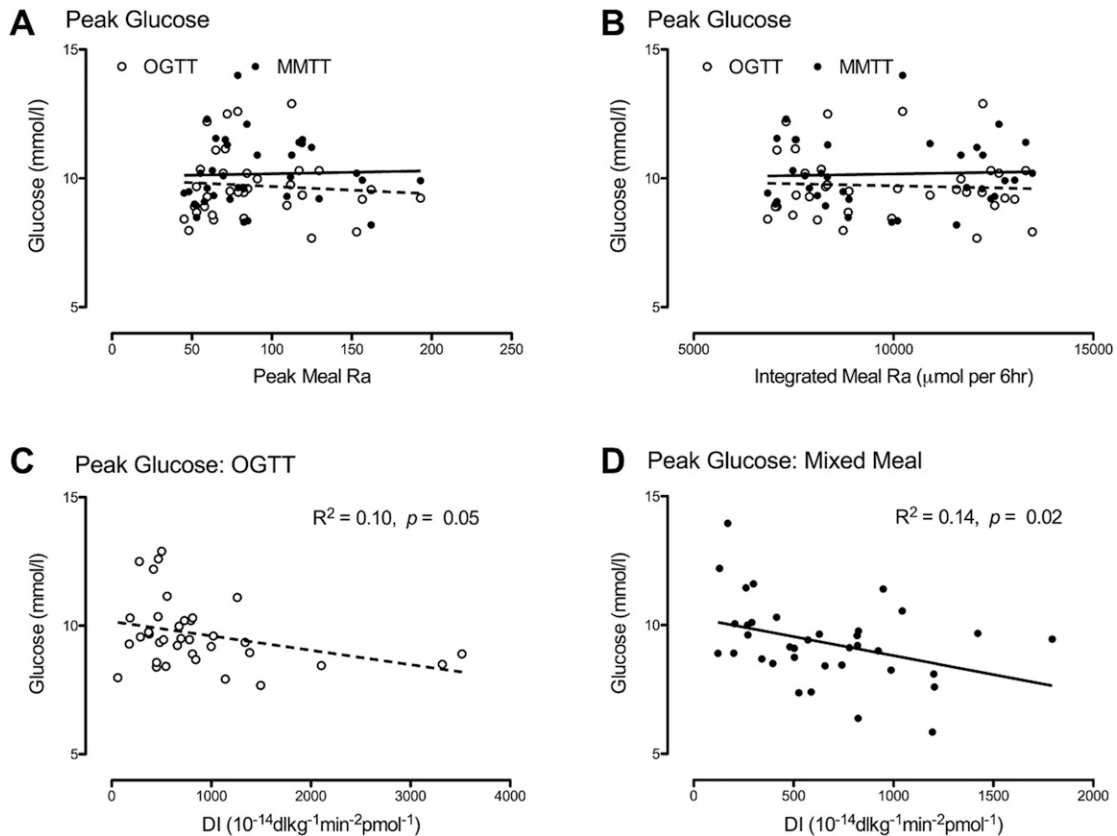
**Figure 3.** Insulin action ( $S_i$ ; A and D),  $\Phi$  (B and E), and DI (C and F) after a 75-g oral glucose challenge and after a mixed meal test in subjects with low ( $\circ$ ) and high ( $\bullet$ ) 60-min glucose during a 75-g oral glucose challenge.

Longitudinal, population-based studies have demonstrated that 60-minute glucose after a 75-g oral glucose challenge is a better predictor of progression to diabetes than is fasting or 120-minute glucose values. Indeed, Abdul-Ghani *et al.* [8] reported that nearly 20% of normal glucose-tolerant subjects with a glucose value  $\geq 155$  mg/dL develop type 2 diabetes during a 7- to 8-year period. Postchallenge hyperglycemia has been assumed to reflect defects in insulin secretion and action. Indeed, some investigators have reported that subjects with NGT, but with a 60-minute glucose value  $\geq 155$  mg/dL, have impaired insulin sensitivity and  $\beta$ -cell dysfunction [12]. However, more recently, Fiorentino *et al.* [10] suggested that high 60-minute glucose concentrations may reflect increased gut absorption of glucose as implied by increased expression of SGLT-1 in these subjects with otherwise normal fasting and 2-hour glucose concentrations.

In the present experiment we used state-of-the-art tracer-based methodology [24] to measure the systemic appearance of ingested glucose, which is a composite of the rate of appearance of glucose in the proximal intestine, intestinal glucose absorption, and splanchnic glucose extraction. We report no difference in peak or integrated meal appearance when subjects were categorized by their 60-minute glucose concentrations after a 75-g OGTT. Alternatively, peak glucose concentrations were inversely correlated with the DI, which expresses  $\Phi$  as a function of prevailing insulin action [26]. Although it is theoretically possible that accelerated gastric emptying and/or rapid intestinal glucose absorption could be exactly offset by a temporally equal increase in hepatic glucose uptake resulting in no change in the



**Figure 4.** Endogenous glucose production (A), the rate of the systemic appearance of ingested glucose (Meal  $R_a$ ; B) and  $R_d$  (C) in subjects with low ( $\circ$ ) and high ( $\bullet$ ) 60-min glucose during the labeled mixed meal test.



**Figure 5.** Relationship of peak glucose during an oral glucose challenge (OGTT;  $\circ$ /dotted line) and during the mixed meal tolerance test (MMTT;  $\bullet$ /solid line) with peak (A) and integrated (B) rates of meal appearance. Peak glucose was inversely correlated with DI during the OGTT (C;  $\circ$ /dotted line) and during the MMTT (D;  $\bullet$ /solid line).

systemic rate of meal appearance, we think this to be very unlikely because hepatic glucose uptake is known to be decreased (rather than increased) in people with overt diabetes [33], suggesting that it would be implausible for hepatic glucose uptake to be enhanced earlier in the evolution of diabetes. Taken together with the prior reports that meal appearance does not differ in people with NGT, IGT, or type 2 diabetes [5], these data suggest that elevated 1-hour postchallenge glucose concentrations can be explained by defects in  $\beta$ -cell function.

The rate of gastric emptying can contribute to the variability of postprandial glucose concentrations [34]. The carbohydrate component of the labeled mixed-meal challenge used in this series of experiments is likely to empty similar to a liquid at body temperature, as suggested by the similar time to peak glucose as observed with an OGTT. Although the emptying rates for liquids and solids differ significantly [35], liquid emptying may be dependent on fasting gastric volume [36]. However, there is little evidence to suggest that fasting gastric volume is affected by glucose tolerance status. Additionally, the time to peak glucose concentrations did not differ between groups, suggesting that different rates of appearance of glucose in the proximal small intestine cannot explain differences in postprandial glucose concentrations.

The splanchnic tissues exposed to ingested glucose, including the liver, extract some amount prior to its appearance in the systemic circulation. This is stimulated by hyperglycemia and hyperinsulinemia, conditions extant after a meal or oral challenge. Previously we demonstrated that splanchnic glucose extraction is unaffected by type 1 diabetes [14] but decreased in type 2 diabetes [33]. In keeping with prior studies, peak and integrated meal rates of appearance did not differ between the high and low groups, suggesting that if



impaired  $\beta$ -cell function may have decreased splanchnic meal extraction, its effects were not detected in this sample size.

Multiple factors other than the rate of meal absorption can alter glucose tolerance [37]. In addition to permitting measurement of meal appearance, the labeled mixed meal also enables simultaneous measurement of postprandial suppression of EGP and stimulation of glucose uptake. None of these parameters differed between the groups. Furthermore, neither fasting nor postprandial glucagon concentrations differed between groups. In contrast,  $\beta$ -cell function, measured as the DI, was decreased in the individuals with an elevated 1-hour post-OGTT glucose concentration. Additionally, in the group as a whole, the higher either the post-OGTT or post-mixed meal peak glucose, the lower the DI, again indicating that appropriateness of  $\beta$ -cell function for the prevailing insulin action is a strong determinant of the degree to which glucose concentrations rise following carbohydrate ingestion.

It is notable that in our study the absolute value of the DI was lower in the individuals with an elevated 1-hour post-OGTT glucose concentration, but it was not lower in the same individuals following ingestion of a mixed meal. This contrasts with prior data reporting reciprocal changes in insulin secretion and action after a mixed meal as compared with an OGTT so that the net DI is unchanged [38]. Additionally, Marini *et al.* [12] used an IV glucose tolerance test to conclude that  $\beta$ -cell function was impaired in individuals with characteristics similar to the subjects in our high group. However, unlike in our experiment, insulin action was impaired in this group, possibly owing to increased age, weight, and fasting glucose compared with people in the low group from their cohort. Alternatively, whereas glucose is the sole nutrient in an OGTT, a mixed meal contains other nutrients, including protein and fat, which also can modulate insulin secretion and gastrointestinal motility. Future studies are required to determine the extent to which, if any, of these factors offset subtle defects in glucose-stimulated insulin secretion.

In conclusion, we demonstrate that in individuals with NFG/NGT who have 60-minute glucose after an OGTT  $>155$  mg/dL, the rate of systemic appearance of meal-derived glucose does not differ from that observed in subjects with a 60-minute glucose after an OGTT  $<155$  mg/dL. This implies that increased rates of gut glucose absorption cannot explain postchallenge glucose concentrations. Additionally, net insulin action, postprandial suppression of EGP, postprandial stimulation of glucose uptake, as well as fasting and postprandial glucagon concentrations also did not differ. The DI was decreased in subjects with elevated 1-hour glucose after an OGTT, and this inversely correlated with peak glucose concentrations after both an OGTT and a mixed meal. These data indicate that the elevated 1-hour (and peak postprandial) glucose concentrations observed during an OGTT, in subjects with NFG/NGT, are likely due to a defect in  $\beta$ -cell function, potentially presaging the subsequent development of prediabetes and, eventually, overt type 2 diabetes. Nevertheless, it remains plausible that other factors such as gastric absorption and splanchnic glucose uptake contribute to the pathogenesis of prediabetes and require further study.

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