

Strong Association Between Insulin-Mediated Glucose Uptake and the 2-Hour, Not the Fasting Plasma Glucose Concentration, in the Normal Glucose Tolerance Range

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Aim: The aim of this study was to examine the relationship between whole-body insulin-mediated glucose disposal and the fasting plasma glucose concentration in nondiabetic individuals.

Research Design and Methods: Two hundred fifty-three nondiabetic subjects with normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance, and combined glucose intolerance received a 75-g oral glucose tolerance test and euglycemic hyperinsulinemic clamp. Total glucose disposal (TGD) during the insulin clamp was compared in IFG and NGT individuals and was related to fasting and 2-hour plasma glucose concentrations in each group.

Results: TGD varied considerably between NGT and IFG individuals and displayed a strong inverse relationship with the 2-hour plasma glucose (PG; $r = 0.40$, $P < .0001$) but not with the fasting PG. When IFG and NGT individuals were stratified based on their 2-hour PG concentration, the increase in 2-hour PG was associated with a progressive decrease in TGD in both groups, and the TGD was comparable among NGT and IFG individuals.

Conclusion: The present results indicate the following: 1) as in NGT, insulin-stimulated TGD varies considerably in IFG individuals; 2) the large variability in TGD in IFG and NGT individuals is related to the 2-hour PG concentration; and 3) after adjustment for the 2-hour proglucagon concentration, IFG subjects have comparable TGD with NGT individuals. (*J Clin Endocrinol Metab* 99: 3444–3449, 2014)

Isolated impaired fasting glucose (IFG) [fasting plasma glucose (FPG) = 100–125; 2 h plasma glucose (PG) < 140 mg/dL] and isolated impaired glucose tolerance (IGT) (2 h plasma glucose concentration = 140–199; FPG < 100 mg/dL) are intermediate states in the transition of glucose tolerance from normal to overt diabetes (1). Thus, both IFG and/or IGT are recognized as prediabetic states. Although IFG and IGT are associated with a similar increased future risk of type 2 diabetes mellitus (T2DM) (2, 3), we and others have shown that they represent two different clinical entities with distinct pathophysiologic

abnormalities (4–14). Subjects with IGT manifest skeletal muscle insulin resistance and a decrease in both first- and second-phase insulin secretion (4–7, 9, 13), whereas subjects with IFG manifest normal muscle insulin sensitivity but impaired hepatic insulin sensitivity (4–7) and impaired first-phase insulin secretion with intact second-phase insulin secretion (5, 9, 13, 15, 16). Although the impairment in first-phase insulin secretion is a consistent finding in IFG individuals, controversy exists regarding muscle insulin resistance in IFG. Studies that have evaluated insulin resistance in IFG individuals using oral glu-

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Abbreviations: BMI, body mass index; CGI, combined glucose intolerance; FPG, fasting plasma glucose; IFG, isolated impaired fasting glucose; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PG, plasma glucose; SSPI, steady-state plasma insulin concentration; T2DM, type 2 diabetes mellitus; TGD, total glucose disposal.

glucose tolerance test (OGTT)-derived indices of insulin resistance, eg, Matsuda index and homeostasis model assessment index of insulin resistance, have reported increased insulin resistance in IFG individuals (8–12). However, studies that directly measured insulin-mediated glucose uptake with the insulin clamp technique have reported conflicting results (4–7, 17, 18). Some studies have found similar rates of insulin-mediated glucose disposal in IFG to normal glucose tolerance (NGT) (4–7), whereas others have reported a decreased insulin-mediated glucose disposal in IFG (17, 18). The reason for this inconsistency in whole-body insulin-mediated glucose disposal in IFG individuals is unclear. We previously reported that in nondiabetic individuals, the increase in 2-hour PG concentration is associated with a progressive decrease in the whole-body insulin-mediated glucose disposal and that the decrease in total glucose disposal (TGD) is present with 2-hour PG concentrations that are well within the normal range (ie, < 140 mg/dL) (19). Therefore, we hypothesize that TGD varies in IFG individuals based on their 2-hour PG concentration. Thus, failing to match the 2-hour PG concentration between IFG vs NGT subjects could, at least in part, account for the inconsistencies in insulin-stimulated glucose disposal reported in the literature. The aim of the present study is to test this hypothesis.

Subjects and Methods

Subjects

The participants included 253 nondiabetic Mexican American subjects, who received a 75-g OGTT and had their insulin-stimulated TGD measured with the euglycemic insulin clamp. Based on the OGTT, the 253 subjects were classified according to American Diabetes Association criteria as having the following: 1) NGT (FPG < 100 mg/dL and 2 h glucose < 140 mg/dL, n = 121); 2) IFG (FPG = 100–125 mg/dL and 2 h glucose < 140 mg/dL, n = 51); 3) IGT (FPG < 100 mg/dL and 2 h PG = 140–199 mg/dL, n = 30); and 4) combined glucose intolerance (CGI; FPG = 100–125 mg/dL and 2 h PG = 140–199 mg/dL, n = 51).

All subjects had normal liver, cardiopulmonary, and kidney function as determined by medical history, physical examination, screening blood tests, electrocardiogram, and urinalysis. No subject was taking any medication known to affect glucose tolerance. Body weight was stable (± 2 kg) for at least 3 months before the study in all subjects. The study protocol was approved by the Institutional Review Board of the University of Texas Health Science Center San Antonio (San Antonio, Texas), and informed written consent was obtained from all subjects before their participation. All studies were performed at the General Clinical Research Center of the University of Texas Health Science Center at 8:00 AM following a 10- to 12-hour overnight fast.

Oral glucose tolerance test

Before the start of the OGTT, a small polyethylene catheter was placed into an antecubital vein and blood samples were collected at –30, –15, and 0. Subjects then ingested 75 g of glucose, and blood samples were obtained at 30, 60, 90, and 120 minutes. Plasma glucose and insulin concentrations were measured on all blood samples.

Euglycemic insulin clamp

Before the start of the insulin clamp, a primed (25 μ Ci)-continuous (0.25 μ Ci/min) infusion of 3- 3 H]glucose (DuPont NEN Life Science Products) was started and continued to the end of the study. After a 2-hour basal tracer equilibration period, subjects received a prime-continuous (240 pmol/min $^{-1}$ ·m $^{-2}$) insulin infusion for 240 minutes. During the insulin infusion, plasma glucose concentration was measured every 5 minutes, and a variable infusion of 20% glucose was adjusted, based on the negative-feedback principle, to maintain the plasma glucose concentration at each subject's fasting glucose level with a coefficient of variation less than 5%. Plasma samples were collected every 30 minutes from 0 to 180 minutes and every 5–10 minutes from 180 to 240 minutes for the determination of plasma glucose and insulin concentrations and tritiated glucose-specific activity.

Calculations and statistical analysis

The rate of glucose appearance during the last hour of the euglycemic clamp was calculated with Steele's equation, as previously described (4). The rate of residual endogenous glucose production during the insulin clamp was calculated by subtracting the rate of exogenous glucose infusion rate from the tracer-derived glucose appearance. The insulin-stimulated rate of TGD was calculated by adding the rate of residual endogenous glucose production to the exogenous glucose infusion rate.

Values are expressed as mean \pm SEM. An ANOVA was used to compare the mean of more than two groups. Simple Pearson's correlation was used to assess the correlation between two variables. To evaluate the change in TGD associated with the change in the fasting vs 2-hour PG concentration, we created a linear regression model with the TGD as the dependent variable and the fasting or 2-hour PG concentration as the independent variable. Age, gender, body mass index (BMI), and the steady-state plasma insulin concentration during the last hour of the clamp were included as independent variables.

Results

Table 1 presents the clinical and metabolic characteristics of the study participants. Subjects with IFG had compa-

Table 1. Characteristics of the Study Participants

	NGT	IFG	IGT	CGI	ANOVA
n	121	51	30	51	
Age, y	40 ± 1	48 ± 2	42 ± 2	45 ± 2	<.001
Sex (%F)	62	41	66	63	<.01
BMI, kg/m ²	30.0 ± 0.6	31.1 ± 0.9	32.3 ± 0.9	31.9 ± 0.9	<.05
FPG, mg/dL	91 ± 1	105 ± 1	92 ± 1	108 ± 1	<.0001
2-Hour PG, mg/dL	111 ± 2	118 ± 2	157 ± 3	164 ± 3	<.0001
TGD, mg/kg ⁻¹ ·min ⁻¹	6.3 ± 0.3	6.5 ± 0.4	4.2 ± 0.5	4.7 ± 0.4	<.001
SPI, μU/mL	88 ± 3	98 ± 3	82 ± 4	86 ± 3	.02
TGD/SSPI	7.1 ± 0.4	6.5 ± 0.4	4.4 ± 0.4	4.4 ± 0.3	<.001
bHGP, mg/kg·min	1.94 ± 0.06	1.89 ± 0.05	1.74 ± 0.06	1.76 ± 0.05	<.05
rHGP, mg/kg·min	0.09 ± 0.03	0.26 ± 0.07	0.17 ± 0.01	0.34 ± 0.07	<.01

Abbreviations: HGP, hepatic glucose production; rHGP, residual HGP; bHGP, basal HGP; SSPI, steady state plasma insulin. Value represents the mean ± SEM.

able TGD compared with NGT individuals, whereas the TGD was markedly reduced in the IGT and CGI groups. TGD varied considerably in both IFG and NGT individuals. When subjects in each group were stratified into four quartiles based on the level of TGD, the most sensitive quartile in the NGT group had 4-fold greater TGD compared with the most resistant quartile (10.9 vs 2.7 mg/kg·min). Likewise, subjects in the most sensitive quartile in the IFG group had an approximately 3-fold greater TGD compared with subjects in the most resistant quartile (9.8 vs 3.5 mg/kg·min). Moreover, the level of TGD in each quartile in IFG individuals was comparable with their counterpart in the NGT group (Figure 1), despite the fact that IFG subjects in each quartile had a 12–16 mg/dL greater FPG concentration compared with their NGT counterparts. When IFG and NGT subjects were pooled into one group, the TGD did not correlate with the FPG concentration (Figure 2A). However, there was a strong inverse correlation between TGD and the 2-hour PG concentration ($r = 0.40$, $P < .0001$) (Figure 2B).

To examine the contribution of variation in the 2-hour PG concentration to the variability in insulin-stimulated TGD, we divided individuals in the NGT and IFG groups

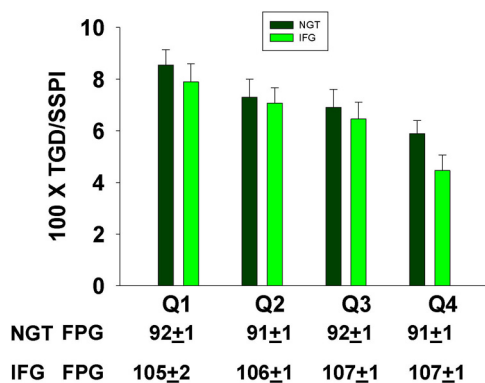


Figure 1. Stratification of NGT and IFG individuals by quartiles (Q) based on their level of insulin-mediated glucose disposal measured with the insulin clamp. The FPG concentration in each quartile is displayed at the bottom.

into three subgroups based on their 2-hour-PG concentration (2 h PG < 100, 100–119, 120–139 mg/dL) and compared the TGD in each group with that in subjects with the 2-hour PG concentration of 140–199 mg/dL. Figure 3 demonstrates that in both IFG and NGT individuals, insulin-stimulated TGD progressively decreased as the 2-hour PG concentration increased. Moreover, in each subgroup, TGD was comparable in IFG and NGT individuals. Furthermore, subjects with IGT and CGI had a further decrease in the insulin-stimulated TGD despite a FPG concentration that was comparable with the NGT and IFG individuals, respectively.

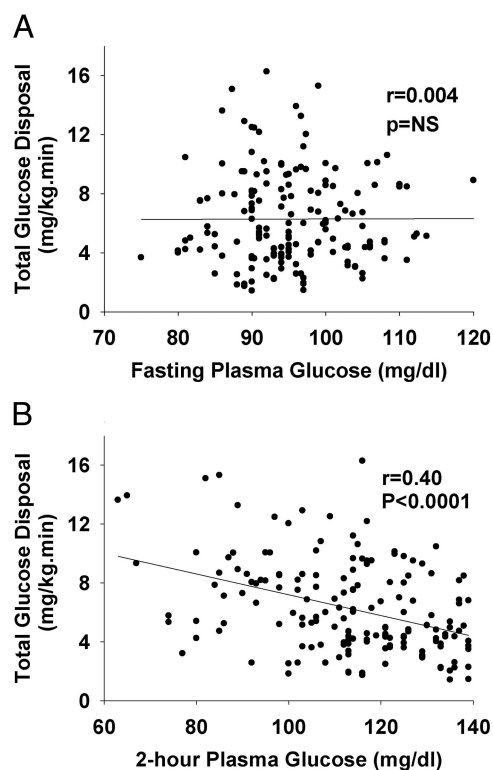


Figure 2. Relationship between insulin-mediated glucose disposal measured with the insulin clamp and the fasting (A) and 2-hour plasma glucose total body (B) concentration in subjects with NGT and IFG.

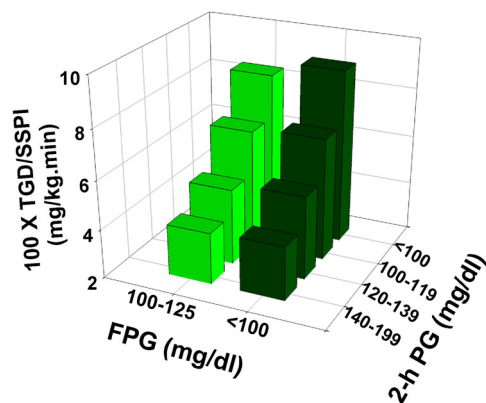


Figure 3. Insulin-mediated total body glucose disposal measured with the insulin clamp in NGT, IFG, IGT, and CGI individuals stratified based on the level of the 2-hour plasma glucose concentration.

To further examine the contribution of 2-hour PG vs FPG concentration to the variability in TGD, we created a multiple linear regression model with TGD as the dependent variable and FPG and 2-hour PG concentrations as independent variables. After adjustment for age, gender, BMI, and steady-state plasma insulin concentration (SSPI) during the last hour of the clamp, only SSPI, BMI, and the 2-hour PG concentration were significant independent predictors of insulin-stimulated TGD (Table 2), and this model explained 50% of the variance in TGD. Each 10 mg/dL increase in 2-hour PG concentration was associated with 0.423 mg/kg·min decrease in TGD. Conversely, a 5 mg/dL increase in the FPG concentration was associated with a small (0.08 mg/kg·min), nonsignificant increase in TGD.

Discussion

The present results demonstrate that, in both NGT and IFG individuals, insulin-stimulated TGD, measured with the euglycemic insulin clamp, is strongly and inversely related to the 2-hour PG concentration and has no significant correlation with the FPG concentration (Figure 2). Each 10 mg/dL increase in 2-hour PG concentration is associated with 0.42 mg/kg·min decrease in TGD. Because most glucose disposal during the euglycemic insulin clamp

takes place in skeletal muscle, this observation indicates that impaired insulin-mediated glucose disposal in skeletal muscle, ie, muscle insulin resistance, is strongly related to the increase in 2-hour PG concentration, and this relationship is independent of the FPG concentration. Indeed, all studies that quantitated insulin-mediated TGD in IGT individuals consistently have documented a marked decrease compared with NGT subjects (4–7, 9, 13). The lack of correlation between the FPG and TGD can be explained by the fact that the fasting plasma glucose level is determined by the balance between the rate of basal hepatic glucose production and the basal rate of tissue glucose uptake (20), which primarily takes place in insulin insensitive tissues, eg, brain and splanchnic tissue. Therefore, it is not surprising that the level of insulin-mediated glucose uptake is unrelated to the FPG concentration.

Consistent with previous studies (21, 22), the present study results demonstrate that NGT subjects have considerable variability in skeletal muscle insulin sensitivity. Our results also demonstrate that a similar variability in insulin-stimulated TGD also is present in IFG individuals (Figure 1). However, when subjects with IFG and NGT are matched for their 2-hour PG concentration (Figure 3), TGD was very similar despite a 12- to 15-mg/dL difference in FPG level, indicating that the increase in FPG per se is not associated with reduced insulin sensitivity in skeletal muscle. Conversely, an increase in 2-hour PG concentration that is considered to be well within the normal range (<140 mg/dL) was associated with a marked and similar decrease in insulin-stimulated TGD in both NGT and IFG individuals. Thus, an NGT subject with a 2-hour PG concentration between 100 and 119 mg/dL (which is within the range considered to represent NGT) had a 28% decrease (from 8.53 to 6.2 mg/kg·min) in TGD compared with subjects with a 2-hour PG concentration less than 100 mg/dL. Similarly, IFG individuals with a 2-hour PG concentration between 100 and 119 mg/dL had a 24% decrease (from 9.0 to 6.8 mg/kg·min) in TGD compared with IFG subjects with a 2-hour PG concentration < 100 mg/dL. Thus, much of the inconsistency regarding skeletal muscle insulin sensitivity in IFG subjects can be explained by differences in the 2-hour PG concentration between the IFG and NGT groups. Indeed, the two studies that have reported decreased insulin-mediated TGD in IFG vs NGT individuals included IFG individuals with a significantly greater 2-hour PG concentration compared with NGT subjects (15 and 34 mg/dL) (17, 18).

The present results demonstrate that such a difference in the 2-hour-PG concentration between the two groups could account for an 11% and 25% decrease in TGD in IFG individuals independent of the FPG level. Insulin re-

Table 2. Parameters of Multiple Linear Regression Model

Variable	Standardized β	P Value
Age, y	-.099	.13
Gender, male vs female	.015	.81
BMI, kg/m ²	-.55	<.0001
SSPI, μ U/mL	.377	<.0001
FPG, mg/dL	.046	.53
2-Hour PG concentration	-.33	<.0001

sistance is a core defect in T2DM and the presence of insulin resistance is an independent predictor of future T2DM risk (23). The results of the present study demonstrate that insulin resistance in NGT and prediabetic individuals (IGT and IFG) is related to the 2-hour PG concentration and not to the FPG concentration. Increased future T2DM risk in IFG compared with NGT individuals is therefore most likely explained by the strong correlation between the FPG and 2-hour PG concentrations. In the present study, the correlation between the FPG and 2-hour PG concentration was 0.39 ($P < .0001$). Consistent with this hypothesis, we previously have shown that when IFG and NGT subjects are matched for their postprandial plasma glucose concentration level, they have similar future T2DM risk (24). Moreover, NGT individuals with higher postprandial plasma glucose levels had a significantly greater T2DM risk compared with IFG individuals with a lower postprandial plasma glucose concentration (25). Collectively these results demonstrate that the increase in 2-hour PG concentration indicates insulin resistance in skeletal muscle and thus serves as a risk factor of future T2DM risk independent of the FPG concentration.

In conclusion, the results of the present study demonstrate that insulin-mediated glucose uptake measured with the insulin clamp, which represent insulin sensitivity in skeletal muscle, correlates with the 2-hour plasma glucose concentration during the OGTT, not with the FPG concentration, in the nondiabetic range, and this relationship is a continuum. Therefore, the 2-hour plasma glucose, not the FPG, represents a marker of insulin resistance in skeletal muscle in nondiabetic individuals.

Acknowledgments

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