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: to examine the relationship between whole body insulin-mediated glucose disposal and the fasting plasma glucose concentration in non-diabetic individuals.

Research Design and Methods: 253 non-diabetic subjects with NGT, IFG, IGT and CGI received 75-gram OGTT 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-h plasma glucose concentrations in each group.

Results: TGD varied considerably between NGT and IFG individuals and displayed a strong inverse relationship with the 2h PG ($r=0.40$, $p<0.0001$) but not with the FPG. When IFG and NGT individuals were stratified based upon their 2-h PG concentration, the increase in 2h-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 that: (i) as in NGT, insulin-stimulated TGD varies considerably in IFG individuals; ii) the large variability in TGD in IFG and NGT individuals is related to the 2h-PG concentration; (iii) after adjustment for the 2h PG concentration, IFG subjects have comparable TGD to NGT individuals.

Isolated impaired fasting glucose (IFG) (FPG = 100–125; 2h PG < 140 mg/dl) and isolated impaired glucose tolerance (IGT) (2-hour 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), while 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 which have evaluated insulin resistance in IFG individuals using OGTT-derived indices of insulin resistance, eg, Matsuda index and HOMA-IR, have reported increased insulin resistance in IFG individuals (8–12). However, studies which directly measured insulin-mediated glucose uptake with the insulin clamp technique have reported conflicting

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Abbreviations:

results (4–7, 17, 18). Some studies have found similar rates of insulin-mediated glucose disposal in IFG to NGT (4–7), while 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 2h PG concentration is associated with a progressive decrease in the whole body insulin-mediated glucose disposal, and that the decrease in TGD is present with 2h PG concentrations that are well within the normal range (ie, $<$ 140 mg/dl) (19). Therefore, we hypothesize that TGD varies in IFG individuals based upon their 2h PG concentration. Thus, failing to match the 2h 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.

Research Design and Methods

Subjects. The participants included 253 nondiabetic Mexican American subjects, who received a 75 g OGTT and had their insulin-stimulated total body glucose disposal (TGD) measured with the euglycemic insulin clamp. Based on the OGTT, the 253 subjects were classified according to ADA criteria as having: (1) NGT (FPG $<$ 100 mg/ dl and 2-hour glucose $<$ 140 mg/dL, n = 121), (2) IFG (FPG = 100–125 mg/ dl and 2-hour glucose $<$ 140 mg/dL, n = 51), (3) IGT (FPG $<$ 100 mg/ dl and 2h PG = 140–199 mg/ dl, n = 30), and (4) Combined Glucose Intolerance (CGI, FPG = 100–125 mg/ dl and 2h 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 (ECG), 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 study in all subjects. The study protocol was approved by the Institutional Review Board of the University of Texas Health Science Center, San Antonio, 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 0800 hours following a 10–12 hour overnight fast.

OGTT

Before the start of the OGTT, a small polyethylene catheter was placed into an antecubital vein and blood samples were collected at –30, –15, 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 μCi)-continuous (0.25 μCi/min) infusion of 3-[^{3}H]glucose (DuPont NEN Life Science Products, Boston, MA) 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 $\cdot$ min^{-1} $\cdot$ 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 $<$ 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 (R_a) during the last hour of the euglycemic clamp was calculated with Steele's equation, as previously described (4). The rate of residual EGP during the insulin clamp was calculated by subtracting the rate of exogenous glucose infusion rate from the tracer-derived R_a. The insulin-stimulated rate of total glucose disposal (TGD) was calculated by adding the rate of residual EGP to the exogenous glucose infusion rate.

Values are expressed as mean ± SEM. 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 2h PG concentration, we created a linear regression model with the TGD as the dependent variable and the fasting or 2h PG concentration as the independent variable. Age, gender, 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 comparable TGD compared to NGT individuals while the TGD was markedly reduced in IGT and CGI groups. TGD varied considerably in both IFG and NGT individuals. When subjects in each group were stratified into 4 quartiles based upon the level of TGD, the most sensitive quartile in NGT group had 4-fold greater TGD compared to the most re-

Table 1. Characteristics of the study participants

	NGT	IFG	IGT	CGI	ANOVA
Number	121	51	30	51	
Age	40 ± 1	48 ± 2	42 ± 2	45 ± 2	<0.001
Sex (%F)	62	41	66	63	<0.01
BMI (kg/m^2)	30.0 ± 0.6	31.1 ± 0.9	32.3 ± 0.9	31.9 ± 0.9	<0.05
FPG (mg/dl)	91 ± 1	105 ± 1	92 ± 1	108 ± 1	<0.0001
2 h PG (mg/dl)	111 ± 2	118 ± 2	157 ± 3	164 ± 3	<0.0001
TGD (mg.kg^{-1}·min^{-1})	6.3 ± 0.3	6.5 ± 0.4	4.2 ± 0.5	4.7 ± 0.4	<0.001
SPI (μU/ml)	88 ± 3	98 ± 3	82 ± 4	86 ± 3	0.02
TGD/SSPI	7.1 ± 0.4	6.5 ± 0.4	4.4 ± 0.4	4.4 ± 0.3	<0.001
bHGP (mg/kg·min)	1.94 ± 0.06	1.89 ± 0.05	1.74 ± 0.06	1.76 ± 0.05	<0.05
rHGP (mg/kg·min)	0.09 ± 0.03	0.26 ± 0.07	0.17 ± 0.01	0.34 ± 0.07	<0.01

Value represents the mean ±SEM

sistant quartile (10.9 vs 2.7 mg/kg.min). Likewise, subjects in the most sensitive quartile in the IFG group had a ~3-fold greater TGD compared to 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 to 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 to 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

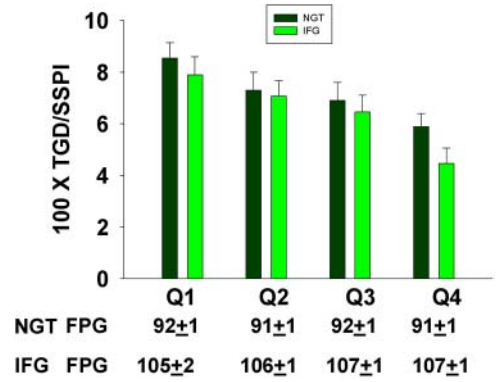

Figure 1. Stratification of NGT and IFG individuals by quartiles based upon their level of insulin-mediated glucose disposal measured with the insulin clamp. The fasting plasma glucose (FPG) concentration in each quartile is displayed at the bottom.

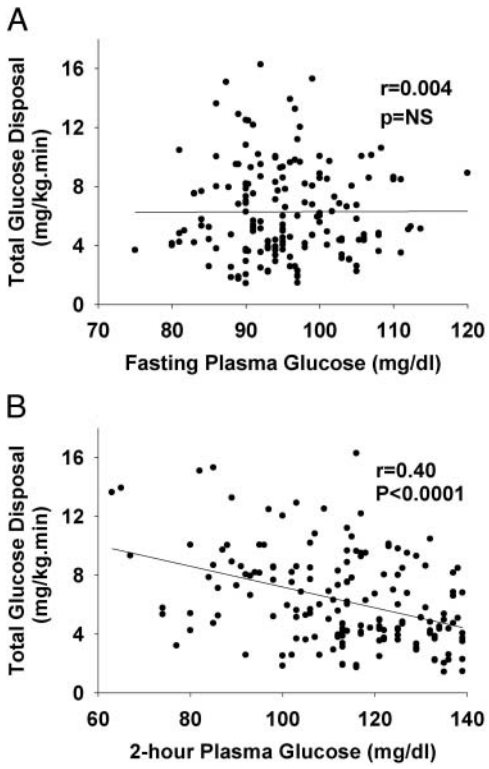

Figure 2. Relationship between insulin-mediated glucose disposal measured with the insulin clamp and the fasting (A) and 2-hour plasma glucose total body (B) concentration in subjects with NGT and IFG.

inverse correlation between TGD and the 2h PG concentration r = 0.40, $P < .0001$ (figure 2b).

To examine the contribution of variation in 2h PG concentration to the variability in insulin-stimulated TGD, we divided individuals in the NGT and IFG groups into 3 subgroups based upon their 2h-PG concentration (2h PG < 100, 100–119, 120–139 mg/dl) and compared the TGD in each group to that in subjects with 2h PG concentration of 140–199 mg/dl. Figure 3 demonstrates that in both IFG and NGT individuals, insulin-stimulated TGD progressively decreased as the 2h 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 insulin-stimulated TGD despite a FPG concentration that was comparable to NGT and IFG individuals, respectively.

To further examine the contribution of 2h 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 2h-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 2h 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 2h 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.

Table 2. Parameters of multiple linear regression model

Variable	Standardized β	P
Age (years)	−0.099	0.13
Gender (M *vs.* F)	0.015	0.81
BMI (kg/m^2)	−0.55	<0.0001
SSPI (μU/ml)	0.377	<0.0001
FPG (mg/dl)	0.046	0.53
2 h PG Concentration	−0.33	<0.0001

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 2h PG concentration and has no significant correlation with the FPG concentration (Figure 2). Each 10 mg/dl increase in 2h PG concentration is associated with 0.42 mg/kg.min decrease in TGD. Since 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 2h-PG concentration and this relationship is independent of the FPG concentration. Indeed, all studies which quantitated insulin-mediated TGD in IGT individuals consistently have documented a marked decrease compared to 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.

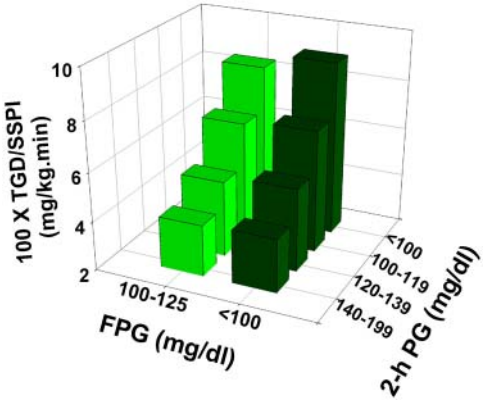

Figure 3. Insulin-mediated total body glucose disposal measured with the insulin clamp in NGT, IFG, IGT and CGI individuals stratified based upon the level of 2-hour plasma glucose concentration.

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 12–15 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 2h 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 2h PG concentration between 100–119 mg/dl (which is within the range considered to represent normal glucose tolerance) had a 28% decrease (from 8.53 to 6.2 mg/kg.min) in TGD compared to subjects with a 2h PG concentration < 100 mg/ dl. Similarly, IFG individuals with a 2h PG concentration be-

tween 100–119 had a 24% decrease (from 9.0 to 6.8 mg/kg.min) in TGD compared to IFG subjects with a 2h 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 2h PG concentration between IFG and NGT groups. Indeed, the two studies which have reported decreased insulin-mediated TGD in IFG vs NGT individuals included IFG individuals with a significantly greater 2h PG concentration compared to NGT subjects (15 and 34 mg/dl) (17, 18). The present results demonstrate that such a difference in 2h-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 resistance 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 to 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 to IFG individuals with a lower postprandial plasma glucose concentration (25). Collectively, these results demonstrate that the increase in 2h 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 non diabetic 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.

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