Glucose-Mediated Glucose Disposal at Baseline Insulin Is Impaired in IFG

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Objective: To quantify glucose-mediated glucose disposal with and without basal insulin replacement and insulin-mediated glucose disposal in subjects with impaired fasting glucose (IFG).

Research Design and Methods: We used the hyperglycemic/pancreatic clamp and stepped euglycemic clamp techniques to quantify glucose disposal and suppression of endogenous glucose production (EGP) in those with normal glucose tolerance (NGT; n = 14) and those with IFG (n = 14).

Results: Total body glucose-mediated glucose uptake, measured with the hyperglycemic/pancreatic clamp, was not significantly affected by the basal plasma insulin levels in subjects with IFG and those with NGT. Compared with subjects with NGT, those with IFG had significantly lower glucose-mediated glucose uptake (by 15%) during the hyperglycemic clamp performed with and without basal insulin replacement. In contrast, insulin-mediated glucose disposal was comparable in both groups. The suppression of EGP by hyperglycemia was similar in both groups. However, the suppression of EGP by insulin was attenuated in those with IFG compared with those with NGT.

Conclusions: The results of the present study have demonstrated that (i) glucose-mediated glucose disposal is impaired in those with IFG; (ii) insulin-mediated glucose uptake in IFG is normal; and (iii) insulin action to suppress EGP is impaired. **(*J Clin Endocrinol Metab* 104: 163–171, 2019)**

Impaired fasting glucose (IFG) [fasting plasma glucose (FPG), 100 to 125 mg/dL] is a prediabetic state. Compared with subjects with normal glucose tolerance (NGT), individuals with IFG manifest a fivefold greater risk of progression to type 2 diabetes mellitus (T2DM) (1). Previous clinical studies (2–8) have documented multiple metabolic abnormalities in those with IFG, including insulin resistance in the liver and impaired first-phase insulin secretion. However, conflicting results have been reported regarding insulin sensitivity in the skeletal muscle of those with IFG. Some studies have reported normal or near-normal insulin sensitivity in the skeletal muscle (3, 4, 9). However, other studies have reported decreased muscle insulin sensitivity in those with IFG compared with subjects with NGT (5, 10). Although some studies have reported decreased insulin sensitivity in subjects with IFG compared with those with NGT, the 2-hour plasma glucose (PG) concentration in those with IFG was significantly greater than that in subjects with NGT in these studies (5, 10). We (3, 11), and others (4, 7–10), have demonstrated that the increase in the 2-hour PG concentration in the nondiabetic range is associated with a progressive decline in insulin-mediated glucose disposal. Furthermore, when those with IFG and those with NGT were matched for the 2-hour PG value, both groups had comparable insulin sensitivity measured with the euglycemic insulin clamp (11).

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Abbreviations: bEGP, basal endogenous glucose production; BMI, body mass index; dpm, disintegrations per minute; EGP, endogenous glucose production; FPG, fasting plasma glucose; IFG, impaired fasting glucose; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PG, plasma glucose; Ra, rate of glucose appearance; T2DM, type 2 diabetes mellitus; TGD, total glucose disposal.

Although previous studies have identified multiple metabolic defects in subjects with IFG (2–17), none of these defects (*e.g.*, decreased first-phase insulin secretion) can explain the etiology of the elevated FPG concentration. The rate of basal endogenous (primarily reflecting hepatic) glucose production (bEGP) is the principal determinant of the FPG concentration (18). We (2) and others (5–7) have previously demonstrated that subjects with IFG will manifest moderate to severe hepatic insulin resistance. However, the elevated fasting plasma insulin concentration offsets the hepatic insulin resistance and maintains the rate of bEGP in the normal range. The vast majority (3–8, 12, 13), although not all (14, 17), previous studies have failed to document a substantial increase in the bEGP rate in those with IFG compared with subjects with NGT. Investigators who reported a small elevation in the bEGP in some studies (14, 17) also have reported a normal bEGP rate in other studies (5, 8, 13). A normal rate of bEGP in subjects with IFG in the presence of an elevated FPG concentration suggests a decreased glucose clearance rate. Consistent with this, we (19) have previously demonstrated that those with IFG will manifest a decreased basal rate of glucose clearance during the fasting state.

Because glucose disposal during the fasting state is insulin independent (*i.e.*, glucose-mediated) (20), the aim of the present study was to use the hyperglycemic/pancreatic clamp technique to quantify total body glucose-mediated glucose uptake in those with IFG and compare the results with those from subjects with NGT. We also used a three-step euglycemic insulin clamp to quantify insulin-mediated total body glucose uptake at a physiologic plasma insulin concentration to rule out the possibility that differences in the plasma insulin concentration contributed to the conflicting results regarding insulin sensitivity in subjects with IFG.

Research Design and Methods

Subjects

A total of 14 subjects with NGT and 14 with isolated IFG according to the American Diabetes Association criteria (21) participated in the present study. All the subjects were in good general health as determined by the medical history, physical examination, screening blood test, urinalysis, and electrocardiographic results. No subjects were participating in an excessively heavy exercise program or took any medication known to affect glucose tolerance. Their weight had been stable (±3 lb) during the 3-month period before the study. The institutional review board of our institution approved the study protocol, and all 14 subjects provided written informed consent before participation.

Study design

All studies were performed in the morning after a 10- to 12-hour overnight fast at the Clinical Research Center at the Texas Diabetes Institute. Each test was performed on a separate day with an interval of 1 to 3 weeks. Each subject received (i) a 75-g oral glucose tolerance test (OGTT) to document glucose tolerance status; (ii) dual energy x-ray absorptiometry scan to quantify total body fat and lean body mass; (iii) a three-step euglycemic insulin clamp; (iv) a hyperglycemic clamp (+100 mg/dL) with somatostatin infusion and replacement of basal glucagon and insulin levels; and (v) a hyperglycemic clamp (+100 mg/dL) with somatostatin infusion and replacement of basal glucagon without basal insulin replacement.

OGTT

Before the OGTT, a catheter was placed into an antecubital vein, and blood samples were collected at −30, −15, 0, 30, 60, 90, and 120 minutes for measurement of the PG and insulin concentrations.

Three-step euglycemic insulin clamp

Before the start of the insulin clamp, a catheter was placed into an antecubital vein for infusion of all test substances. A second catheter was inserted retrogradely into a vein on the dorsum of the hand, and the hand was placed into a thermoregulated box heated to 70°C. All subjects received a prime (40 μCi)-continuous (0.4 μCi/min) infusion of 3-[^{3}H] glucose (DuPont NEN Life Science Products, Boston, MA). After a 2-hour basal tracer equilibration period, the subjects received a prime-continuous insulin infusion at the rate of 10 mU/min^{-1} · m^{-2} for 100 minutes. At 100 minutes, the insulin infusion rate was increased to 20 mU/min^{-1} · m^{-2} for an additional 100 minutes. Finally, at 200 minutes, the insulin infusion rate was increased to 40 mU/min^{-1} · m^{-2}. During the last 30 minutes of the basal equilibration period, plasma samples were taken at 5- to 10-minute intervals for determination of the PG and insulin concentrations and tritiated glucose radioactivity. During the insulin infusion, PG concentration was measured every 5 minutes, and a variable infusion of 20% glucose was adjusted, using the negative feedback principle, to maintain the PG concentration at each subject's FPG level, with a coefficient of variation <5%. Plasma samples were collected every 5 to 10 minutes from 60 to 100 minutes, 160 to 200 minutes, and 260 to 300 minutes for the determination of the PG and insulin concentrations and tritiated glucose-specific activity.

Hyperglycemic clamp with basal insulin replacement

Before the start of the hyperglycemic clamp, a catheter was placed into an antecubital vein for infusion of all test substances. A second catheter was inserted retrogradely into a vein on the dorsum of the hand, and the hand was placed into a thermoregulated box heated to 70°C. At −120 minutes, a prime (40 μCi)-continuous (0.4 μCi/min) infusion of 3-[^{3}H] glucose (DuPont NEN Life Science Products) was started to quantify the rate of total body glucose appearance and disappearance. At −60 minutes, a somatostatin infusion (rate, 750 μg/h) was started to inhibit pancreatic hormone secretion. The basal plasma insulin and glucagon concentrations were replaced with insulin (0.1 mU/kg/min) and glucagon (0.3 ng/kg/min) infusions. The infusions of 3-[^{3}H] glucose, somatostatin, insulin, and glucagon were continued until the end of the study. At time 0, the PG concentration was increased and maintained for 90 minutes at 100 mg/dL greater than the fasting level (*i.e.*, from ~100 to 200 mg/dL) with a variable infusion of 20% glucose. Plasma samples were collected every 5 to 10 minutes for 30 minutes before the start of the glucose infusion and from

60 to 90 minutes for the determination of the PG, glucagon, and insulin concentrations and tritiated glucose-specific activity. Urine was collected from 0 to 90 minutes, and urinary glucose excretion was subtracted from the total glucose disposal (TGD) to obtain the tissue glucose uptake.

Hyperglycemic clamp without basal insulin replacement

On a separate day, the hyperglycemic clamp was repeated as described in the previous section with one difference. After the start of the somatostatin infusion, only basal glucagon (0.3 ng/kg/min) was replaced. Insulin was not infused in this study.

Analytical determinations

The PG concentration was determined using the glucose oxidase method (Analox Glucose Analyzer; Analox Instruments, Lunenburg, MA). Plasma insulin and glucagon concentrations were determined by radioimmunoassay (Diagnostic Products, Los Angeles, CA). Plasma [^{3}H] glucose radioactivity was determined on barium hydroxide/zinc sulfate–precipitated plasma extracts, as previously described (3, 22).

Calculations and statistical analysis

During the insulin clamp, the bEGP rate was calculated as the [3-^{3}H] glucose infusion rate [disintegrations per minute (dpm)/min] divided by the steady-state plasma [3-^{3}H] glucose-specific activity (dpm/mg). After insulin infusion, non–steady-state conditions for [3-^{3}H] glucose will prevail, and total rate of glucose appearance (Ra) in the systemic circulation was computed using Steele equation (23) during the last 30 minutes of each step (*i.e.,* from 70 to 100, 170 to 200, and 270 to 300 minutes), as previously reported (22). The residual rate of endogenous glucose production (EGP) during each clamp step was calculated by subtracting the glucose infusion rate from Ra during the same period. During the hyperglycemic clamp, the bEGP rate was calculated as the [3-^{3}H] glucose infusion (dpm/min) divided by the steady-state plasma [3-^{3}H] glucose-specific activity (dpm/mg). After glucose infusion, non–steady-state conditions for [3-^{3}H] glucose will prevail, and the total Ra in the systemic circulation was computed using Steele equation during the last 30 minutes of the clamp (*i.e.,* from 60 to 90 minutes (Supplemental Fig. 1). TGD was calculated by subtracting the urinary glucose excretion from the rate of glucose disappearance during the same period. The glucose clearance rate during the last 30 minutes of the hyperglycemic clamp was calculated by dividing the TGD by the mean PG concentration during the same period. The residual rate of EGP during the last 30 minutes of the clamp was calculated by subtracting the glucose infusion rate from the Ra during that period.

Because most glucose uptake occurs in lean tissues and to account for differences in the lean body mass among the individuals, the rates of glucose appearance and disappearance are expressed as mg/min/kg of lean body mass (fat-free mass).

Data are presented as the mean ± SEM. Differences between the mean values were tested using the Student *t* test. Statistical significance was determined at $P < 0.05$

Results

Subject characteristics

As per the study design, the subjects with IFG had a significantly greater FPG concentration compared with those with NGT (107 ± 2 mg/dL vs 89 ± 1 mg/dL; $P < 0.0001$). No substantial differences were found between the NGT and IFG groups (Table 1) in age, sex, body mass index (BMI), or percentage of body fat. The 2-hour PG during the OGTT was similar in the IFG and NGT groups (112 ± 4 mg/dL vs 108 ± 5 mg/dL; $P = 0.57$; Fig. 1).

Basal glucose uptake with basal insulin replacement

Before the start of the somatostatin infusion, the fasting plasma insulin and glucagon concentrations during the hyperglycemic/pancreatic clamp were 12 ± 1 vs 8 ± 1 μU/mL ($P = 0.02$) and 38 ± 4 vs 43 ± 5 pg/mL ($P = 0.71$) in the IFG and NGT groups, respectively. During the somatostatin infusion, the plasma insulin concentrations were similar in the IFG and NGT groups (10 ± 1 vs 8 ± 1 μU/mL). Also, the plasma glucagon concentrations were similar in the IFG and NGT groups during the somatostatin infusion (20 ± 3 vs 25 ± 5 ng/L), respectively. During the last 30 minutes of the hyperglycemic clamp (*i.e.,* 60 to 90 minutes), the mean PG (197 ± 2 vs 206 ± 2 mg/dL), insulin (11 ± 1 and 9 ± 1 μU/mL), and glucagon (18 ± 3 vs 23 ± 5 pg/mL) concentrations were comparable in the IFG and NGT groups (Fig. 2A and 2B).

Compared with NGT, the total body glucose disposal during the hyperglycemic clamp was 15.5% lower in the subjects with IFG (4.93 ± 0.16 vs 5.83 ± 0.35 mg/kgFFM/min; $P < 0.05$; Fig. 3A). Thus, the glucose clearance rate during the hyperglycemic clamp in the subjects with IFG subjects was reduced by 25.5% compared with that in those with NGT (2.21 ± 0.18 vs 2.96 ± 0.18 mg/kgFFM/min; $P = 0.004$; Fig. 3B). Furthermore, TGD during the hyperglycemic clamp inversely and significantly correlated with the FPG concentration ($r = -0.60$; $P = 0.001$; Fig. 3C).

The bEGP rate during the hyperglycemic/pancreatic clamp was 3.32 ± 0.11 and 3.35 ± 0.12 mg/kgFFM/min ($P = 0.86$) in the subjects with NGT and IFG, respectively, and was suppressed similarly during the last 30 minutes of the hyperglycemic clamp (Table 2).

Table 1. Patient Characteristics

Characteristic	NGT	IFG	*P* Value
Age, y	33 ± 4	39 ± 5	0.14
Sex			0.31
Male	5	8	
Female	7	4	
BMI, kg/m^2	28.7 ± 2.1	30.8 ± 1.3	0.18
Body fat, %	35 ± 2	33 ± 3	0.73
FPG, mg/dL	89 ± 1	107 ± 2	<0.0001
2-h PG, mg/dL	108 ± 5	112 ± 4	0.57
Fasting insulin, μU/mL	8 ± 1	12 ± 1	0.02
Fasting FFA, mM	0.47 ± 0.05	0.49 ± 0.05	0.76

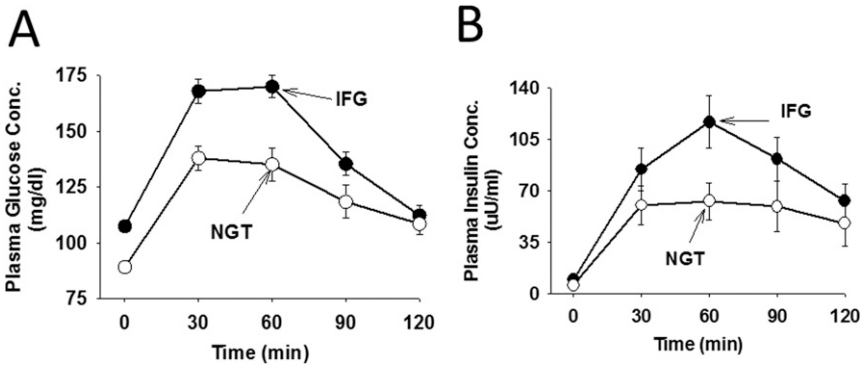

Figure 1. Plasma (A) glucose and (B) insulin concentrations during the OGTT in those with IFG and NGT. Conc., concentration.

Basal glucose uptake without basal insulin replacement

Before the start of the somatostatin infusion, the fasting plasma insulin and glucagon concentrations during the pancreatic clamp were 10 ± 1 vs 6 ± 1 μU/mL ($P = 0.03$) and 48 ± 4 vs 41 ± 5 pg/mL ($P = 0.39$) in the IFG and NGT groups, respectively. During the somatostatin infusion, the plasma insulin concentration was significantly reduced to a similar level in the subjects with IFG and NGT (5 ± 1 vs 4 ± 1 μU/mL, respectively; $P = 0.0004$ and $P = 0.02$ vs fasting, respectively). The plasma glucagon concentrations were similar in the IFG and NGT groups during the somatostatin infusion (31 ± 5 vs 23 ± 3 pg/mL, respectively; $P = 0.11$). During the last 30 minutes of the hyperglycemic clamp (*i.e.*, 60 to 90 minutes), the mean PG (201 ± 3 vs 210 ± 3 mg/dL), insulin (5 ± 1 and 4 ± 1 μU/mL), and glucagon (32 ± 3 vs 22 ± 5 pg/mL) concentrations did not differ significantly between the IFG and NGT groups (P = NS for all; Fig. 2C and 2D).

The TGD during the hyperglycemic clamp without basal insulin replacement was 7% and 6% less than that in the hyperglycemic clamp with the insulin infusion in the NGT and IFG groups, respectively. However, the difference did not reach statistical significance ($P = 0.31$ and $P = 0.49$). Nonetheless, compared with NGT, the TGD during the hyperglycemic clamp without basal insulin replacement was lower in the subjects with IFG by 15% (4.62 ± 0.24 vs 5.42 ± 0.27 mg/kgFFM/min; $P < 0.05$). Thus, the glucose clearance rate during the hyperglycemic clamp in the subjects with IFG was reduced by 25.5% compared with that in the subjects with NGT (2.17 ± 0.11 vs 2.76 ± 0.11 mL/kgFFM/min, respectively; $P = 0.003$; Fig. 3B). Furthermore, the TGD rate during the hyperglycemic clamp inversely and significantly correlated with the FPG concentration ($r = -0.62$; $P < 0.001$; Fig. 3D). The bEGP rate during the hyperglycemic clamp/pancreatic clamp was 3.53 ± 0.14 and 3.23 ± 0.07 mg/kgFFM/min ($P = 0.85$) in the NGT and IFG groups, respectively, and was suppressed similarly during the hyperglycemic clamp (Table 2).

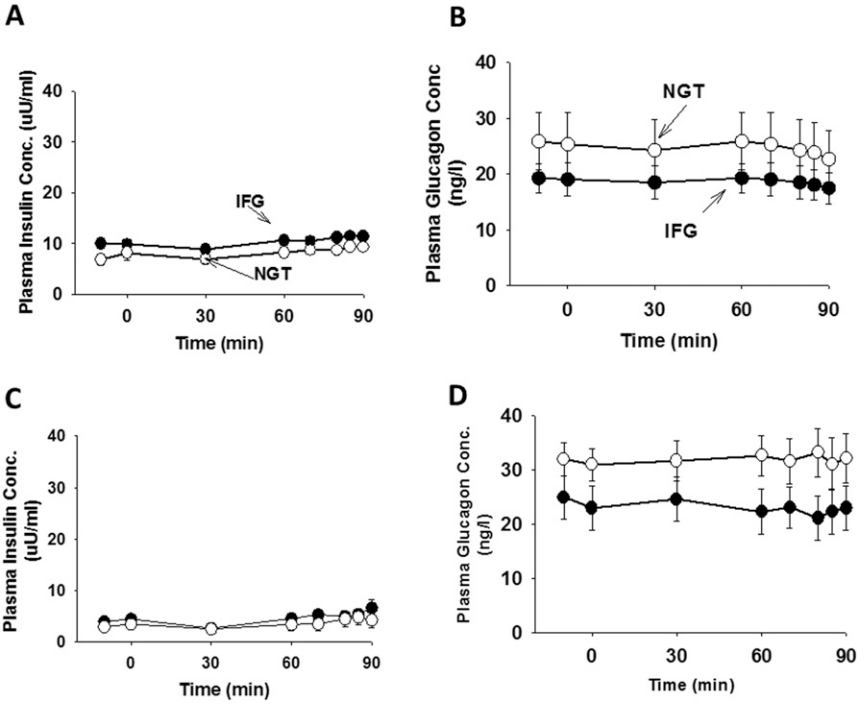

Figure 2. Plasma (A) insulin and (B) glucagon concentrations during the hyperglycemic clamp (C) with basal insulin replacement and (D) without insulin replacement. Conc., concentration.

Insulin-mediated glucose disposal

The fasting plasma insulin concentration during the euglycemic insulin clamp was 8 ± 1 and 12 ± 1 μU/mL ($P < 0.05$) in the NGT and IFG groups, respectively. During the euglycemic clamp, the steady-state plasma insulin concentrations were comparable in the NGT and IFG groups (21 ± 2 vs 24 ± 2 μU/mL, 36 ± 4 vs 40 ± 4 μU/mL, and 63 ± 4 vs 67 ± 4 μU/mL measured 70 to 100, 170 to 200, and 270 to 300 minutes, respectively).

Total body insulin-stimulated glucose disposal during the time periods 70 to 100 minutes, 170 to 200 minutes, and 270 to 300 minutes was similar in the IFG and IGT groups (Fig. 4A). The bEGP rate was comparable in the IFG

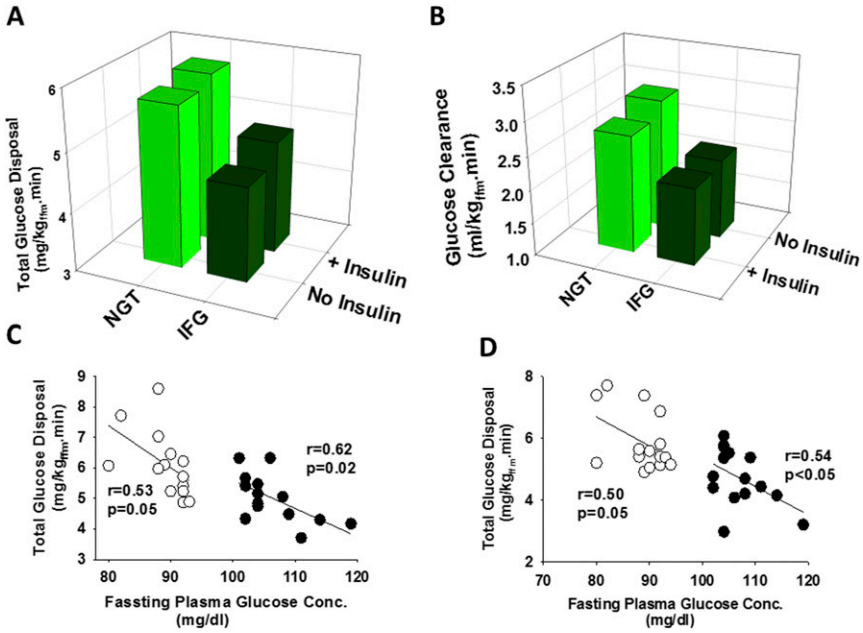

Figure 3. Total body (A) glucose-mediated glucose disposal and (B) glucose clearance rate during the last 30 min of the hyperglycemic clamp with and without basal insulin replacement. Relationship between TGD and the FGG concentration during the last 30 min of the hyperglycemic clamp in subjects with NGT (open symbols) and IFG (closed symbols) (C) with basal insulin replacement and (D) without basal insulin replacement. Conc., concentration.

and NGT groups (2.93 ± 0.11 vs 3.21 ± 0.41 mg/kgFFM/min; P = NS). However, the suppression of EGP at 70 to 100 minutes and at 170 to 200 minutes was impaired in the subjects with IFG compared with those with NGT (Fig. 4B).

Discussion

The results of the present study have two major findings. The total body glucose-mediated glucose disposal, measured with the hyperglycemic/pancreatic clamp technique, was significantly reduced in the subjects with IFG compared with that in the subjects with NGT. Glucose disposal during the hyperglycemic clamp was reduced by ~15% in the subjects with IFG compared with those with NGT, just as was the glucose clearance (Fig. 3; Table 2). Furthermore, lowering the fasting plasma insulin concentration by ~60% did not cause any substantial change in glucose disposal, indicating that glucose disposal during the fasting state is not sensitive to changes in the fasting plasma insulin concentration. These results provide strong evidence in support of decreased glucose-mediated glucose uptake in subjects with IFG (*i.e.*, glucose resistance). We (24), and others (25), have previously demonstrated decreased total body glucose-mediated glucose uptake in those with T2DM (*i.e.*, glucose resistance). The present study has extended these observations and, to the best of our knowledge, is the first to demonstrate a similar defect in subjects with IFG.

Table 2. Metabolic Parameters During Hyperglycemic Clamp

	NGT		IFG	
Parameter	**Hyperglycemic Clamp With Insulin**	**Hyperglycemic Clamp Without Insulin**	**Hyperglycemic Clamp With Insulin**	**Hyperglycemic Clamp Without Insulin**
bHGP, mg/kgFFM/min	3.32 ± 11	3.53 ± 0.14	3.35 ± 0.12	3.23 ± 0.07
FPG, mg/dL	89 ± 2	88 ± 2	109 ± 2[a]	107 ± 1[a]
SSPG, mg/dL	197 ± 2	201 ± 3	206 ± 2	210 ± 3
TGD at 200 mg/dL, mg/kgFFM/min	5.83 ± 0.35	5.42 ± 0.27	4.93 ± 0.16[b]	4.62 ± 0.24[b]
ΔGU/ΔG	2.33 ± 0.28	1.89 ± 0.16	1.60 ± 0.20[b]	1.34 ± 0.19[b]
Fasting glucose clearance	3.79 ± 0.15	4.01 ± 0.15	3.12 ± 0.12[c]	3.02 ± 0.07[a]
Glucose clearance, mL/kgFFM/min	2.96 ± 0.18	2.76 ± 0.11	2.21 ± 0.11[c]	2.17 ± 0.11[c]
rHGP, mg/kgFFM/min	2.08 ± 0.21	2.18 ± 0.25	1.71 ± 0.18	2.10 ± 0.21
SSPI (60–90 min, μL/mL	11 ± 1	5 ± 1[d]	10 ± 1	4 ± 1[d]
SSPG (60–90 min), pg/mL	17 ± 3	32 ± 4[d]	21 ± 5	22 ± 4
Fasting C-peptide, ng/mL	2.0 ± 0.2	1.9 ± 0.2	2.3 ± 0.1[b]	2.4 ± 0.2[b]
SSPC-Pep (60–90 min), pg/mL	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1

Abbreviations: bHGP, basal hepatic glucose production; rHGP, residual hepatic glucose production; SSPC-Pep, steady state plasma C-peptide concentration; SSPG, steady-state plasma glucagon; SSPI, steady-state plasma insulin; ΔGU/ΔG, increase in glucose uptake divided by the increase in plasma glucose concentration during the clamp, factored by 100.

[a] $P < 0.001$ vs NGT.

[b] $P < 0.05$ vs NGT.

[c] $P < 0.01$ vs NGT.

[d] $P < 0.05$ vs with insulin replacement.

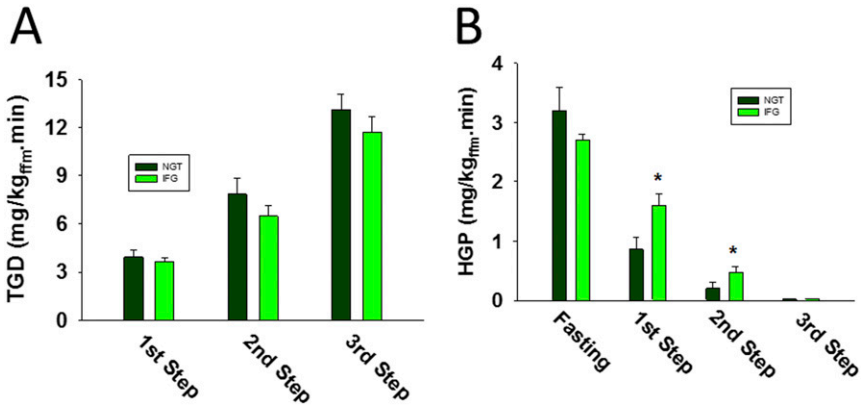

Figure 4. (A) TGD during the last 30 min of each step of the euglycemic hyperinsulinemic clamp in those with IFG and NGT. (B) Rate of EGP during the fasting state and during the last 30 min of each step of the euglycemic clamp in those with IFG and NGT. HGP, hepatic glucose production.

The FPG concentration is determined by the balance between the basal rate of EGP and the basal rate of tissue glucose uptake. Because basal glucose uptake is independent of the basal insulin level and is primarily driven by the mass action of glucose, it is unlikely to be affected by impaired first-phase insulin secretion, which has consistently been reported in subjects with IFG (2–4). The present study has demonstrated that glucose-mediated glucose uptake during the fasting state is significantly reduced in those with IFG. However, consistent with previous studies (2–8, 12, 13, 25), the basal rate of EGP, measured during both the euglycemic insulin clamp and the hyperglycemic clamp, was comparable in those with IFG and NGT. Thus, decreased basal glucose uptake in the presence of a normal rate of basal glucose production leads to an increase in the FPG (*i.e.*, IFG). Stated otherwise, the FPG in those with IFG increases to a level at which the basal rate of glucose-mediated glucose uptake matches the basal rate of glucose production.

It could be argued that hepatic insulin resistance results in an increase in EGP, which increases the FPG. However, such a scenario requires an elevated bEGP rate in subjects with IFG compared with those with NGT. Because bEGP in IFG has been reported to be comparable to that in subjects with NGT in the vast majority of studies (3–8, 12, 13), including the present study, this scenario seems unlikely to explain the pathophysiology of IFG. Furthermore, DeFronzo *et al.* (18, 26) have shown that bEGP does not increase until the FPG concentration exceeds 140 mg/dL. However, other investigators have demonstrated that the bEGP rate does not increase until the FPG concentration exceeds 160 mg/dL (27).

Because most glucose disposal during an intravenous glucose infusion (*e.g.*, hyperglycemic clamp) occurs in skeletal muscle, the results of the present study suggest that glucose-mediated glucose uptake in skeletal muscle in those with IFG is impaired. Ciaraldi *et al.* (28), measuring the arteriovenous glucose balance across the leg, demonstrated that the decrease in total body basal glucose uptake in T2DM is associated with a decrease in basal leg glucose uptake. Thus, they demonstrated that decreased basal glucose uptake in patients with T2DM (25, 28) is, at least in part, due to decrease basal muscle glucose uptake. The results of the present study have extended these observations and demonstrated that the defect in glucose-mediated glucose uptake is present at the IFG stage.

Studies in experimental animals have shown that GLUT1 is the primary glucose transporter responsible for glucose-mediated glucose uptake during the fasting state (29). Decreased gene expression and protein levels of GLUT1 have been reported in association with decreased glucose-mediated glucose uptake in patients with T2DM (28) and in animal models of diabetes (30–32).

Nielsen *et al.* (33) reported that glucose-mediated glucose disposal in response to an increase in the PG concentration from 100 to 135 mg/dL was comparable in those with T2DM and control subjects and that the decrease in glucose-mediated glucose disposal becomes evident only when the PG concentration exceeds 135 mg/dL. Because the magnitude of increase in the PG concentration in the hyperglycemic clamp was larger (+100 mg/dL; *i.e.*, from 100 to 200 mg/dL), it was impossible to determine in the present study at which PG concentration the decrease in glucose-mediated glucose uptake became evident in those with IFG. However, in a previous study of individuals with T2DM (24), we demonstrated that, after a 50-mg/dL increment in the PG concentration, glucose-mediated glucose uptake was substantially reduced. When these previous results (24) are considered together with the substantial reduction in both fasting glucose clearance and glucose clearance under hyperglycemic conditions (+100 mg/dL) in the present study (Table 2), we can conclude that the reduction in glucose-mediated glucose disposal is present throughout the range of PG concentrations in those with IFG and T2DM.

We recently have shown decreased splanchnic glucose uptake in subjects with IFG compared with those with NGT (34). The present study has extended these observations to indicate that total body glucose-mediated glucose uptake is impaired in subjects with IFG.

The second finding of the present study was that, unlike glucose-mediated glucose uptake, insulin-mediated

glucose uptake will be normal to near normal in subjects with IFG. Previous studies (2, 3, 9), which quantitated insulin-mediated glucose disposal with the euglycemic clamp in subjects with IFG, used a high insulin infusion rate (80 mU/m^2/min) and reported normal to near normal insulin-mediated glucose uptake. The present study used lower insulin infusion rates and demonstrated that, over a range of physiologic plasma insulin concentrations (20 to 60 μU/mL), insulin-mediated glucose uptake in subjects with NGT and IFG will be similar, excluding the possibility that the high insulin infusion rate used in previous studies (2, 3, 9) had overcome a defect in insulin sensitivity in subjects with IFG. Although some studies have reported decreased insulin sensitivity in subjects with IFG compared with those with NGT, the 2-hour PG concentration in subjects with IFG was significantly greater than that in those with NGT in these studies (5, 10). We (3, 11), and others (4, 7–10), have demonstrated that the increase in the 2-hour PG concentration in the nondiabetic range is associated with progressive decline in insulin-mediated glucose disposal. Furthermore, when subjects with IFG and NGT were matched for the 2-hour PG value, as in the present study, both groups had comparable insulin sensitivity measured with the euglycemic insulin clamp (11).

Both glucose uptake and glucose metabolism are impaired in patients with T2DM [reviewed in (35)]. We previously have shown that most insulin-mediated glucose uptake is directed toward nonoxidative pathways and glucose-mediated glucose uptake is primarily oxidized (20). Because glucose-mediated glucose uptake is reduced in subjects with IFG and insulin-mediated glucose uptake is normal or near normal, we hypothesized that the decrease in glucose-mediated glucose uptake in those with IFG will result in decreased glucose oxidation. Consistent with this, previous studies (36) have demonstrated that a small increase in the FPG concentration will shift fuel consumption toward fat oxidation.

Unlike insulin action in skeletal muscle, hepatic insulin sensitivity was impaired in subjects with IFG. The suppression of hepatic glucose production by insulin was markedly reduced in the subjects with IFG by ~50% (Fig. 4) during the first and second steps of the euglycemic insulin clamp. This observation demonstrates the presence of moderate to severe hepatic insulin resistance in subjects with IFG and is consistent with previous studies from our group (2, 11) and others (4–7, 12), which demonstrated an increase in the hepatic insulin resistance index (product of bEGP and fasting plasma insulin) during the basal state in those with IFG. However, the suppression of hepatic glucose production by glucose during the hyperglycemic clamp was similar in both groups.

Because of the intensive experimental procedures used in the present study, a relatively small number of subjects was included. Because the number of subjects was small (14 with NGT vs 14 with IFG), the statistical power to reach absolute conclusions is uncertain. Another limitation was that, by chance, the FPG concentration of the participants with IFG was in the low range of IFG (mean, 108 mg/dL; range, 102 to 119 mg/dL). Nonetheless, the difference in the mean FPG concentration between those with IFG and NGT was relatively large (18 mg/dL). Although the BMI of the subjects with IFG was slightly greater than the BMI of those with NGT, the difference was not statistically significant. Because glucose disposal and clearance were expressed per fat-free mass, it is unlikely that the small insignificant difference in the BMI could have affected the results of the present study. Furthermore, neither glucose-mediated glucose disposal nor glucose clearance during the hyperglycemic clamp correlated with the BMI.

In conclusion, the results of the present study have demonstrated that subjects with IFG manifest decreased total body glucose-mediated glucose uptake (glucose resistance) during the fasting state, which, in the presence of normal endogenous (primarily hepatic) glucose production, will lead to an increase in the FPG concentration. Insulin-mediated glucose uptake in subjects with IFG is normal over a wide range of PG concentrations but insulin-mediated suppression of endogenous (hepatic) glucose production is impaired.

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References

1. Unwin N, Shaw J, Zimmet P, Alberti KG. Impaired glucose tolerance and impaired fasting glycaemia: the current status on definition and intervention. *Diabet Med*. 2002;**19**(9):708–723.
2. Abdul-Ghani MA, Tripathy D, DeFronzo RA. Contributions of beta-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. *Diabetes Care*. 2006;**29**(5):1130–1139.
3. Abdul-Ghani MA, Jenkinson CP, Richardson DK, Tripathy D, DeFronzo RA. Insulin secretion and action in subjects with impaired fasting glucose and impaired glucose tolerance: results from the Veterans Administration Genetic Epidemiology Study. *Diabetes*. 2006;**55**(5):1430–1435.
4. Weyer C, Bogardus C, Pratley RE. Metabolic characteristics of individuals with impaired fasting glucose and/or impaired glucose tolerance. *Diabetes*. 1999;**48**(11):2197–2203.
5. Bock G, Chittilapilly E, Basu R, Toffolo G, Cobelli C, Chandramouli V, Landau BR, Rizza RA. Contribution of hepatic and extrahepatic insulin resistance to the pathogenesis of impaired fasting glucose: role of increased rates of gluconeogenesis. *Diabetes*. 2007;**56**(6):1703–1711.
6. Cali' AM, Bonadonna RC, Trombetta M, Weiss R, Caprio S. Metabolic abnormalities underlying the different prediabetic phenotypes in obese adolescents. *J Clin Endocrinol Metab*. 2008;**93**(5):1767–1773.
7. Bacha F, Lee S, Gungor N, Arslanian SA. From pre-diabetes to type 2 diabetes in obese youth: pathophysiological characteristics along the spectrum of glucose dysregulation. *Diabetes Care*. 2010;**33**(10):2225–2231.
8. Bock G, Dalla Man C, Campioni M, Chittilapilly E, Basu R, Toffolo G, Cobelli C, Rizza R. Pathogenesis of pre-diabetes: mechanisms of fasting and postprandial hyperglycemia in people with impaired fasting glucose and/or impaired glucose tolerance. *Diabetes*. 2006;**55**(12):3536–3549.
9. Wasada T, Kuroki H, Katsumori K, Arii H, Sato A, Aoki K. Who are more insulin resistant, people with IFG or people with IGT? *Diabetologia*. 2004;**47**(4):758–759.
10. Kim SH, Reaven GM. Isolated impaired fasting glucose and peripheral insulin sensitivity: not a simple relationship. *Diabetes Care*. 2008;**31**(2):347–352.
11. Winner D, Norton L, Kanat M, Arya R, Fourcaudot M, Hansis-Diarte A, Tripathy D, DeFronzo RA, Jenkinson CP, Abdul-Ghani M. Strong association between insulin-mediated glucose uptake and the 2-hour, not the fasting plasma glucose concentration, in the normal glucose tolerance range. *J Clin Endocrinol Metab*. 2014;**99**(9):3444–3449.
12. Ter Horst KW, Gilijamse PW, Ackermans MT, Soeters MR, Nieuwdorp M, Romijn JA, Serlie MJ. Impaired insulin action in the liver, but not in adipose tissue or muscle, is a distinct metabolic feature of impaired fasting glucose in obese humans. *Metabolism*. 2016;**65**(5):757–763.
13. Perreault L, Man CD, Hunerdosse DM, Cobelli C, Bergman BC. Incretin action maintains insulin secretion, but not hepatic insulin action, in people with impaired fasting glucose. *Diabetes Res Clin Pract*. 2010;**90**(1):87–94.
14. Perreault L, Bergman BC, Playdon MC, Dalla Man C, Cobelli C, Eckel RH. Impaired fasting glucose with or without impaired glucose tolerance: progressive or parallel states of prediabetes? *Am J Physiol Endocrinol Metab*. 2008;**295**(2):E428–E435.
15. Tripathy D, Carlsson M, Almgren P, Isomaa B, Taskinen MR, Tuomi T, Groop LC. Insulin secretion and insulin sensitivity in relation to glucose tolerance: lessons from the Botnia Study. *Diabetes*. 2000;**49**(6):975–980.
16. Hanefeld M, Koehler C, Fuecker K, Henkel E, Schaper F, Temelkova-Kurktschiev T; Impaired Glucose Tolerance for Atherosclerosis and Diabetes study. Insulin secretion and insulin sensitivity pattern is different in isolated impaired glucose tolerance and impaired fasting glucose: the risk factor in Impaired Glucose Tolerance for Atherosclerosis and Diabetes study. *Diabetes Care*. 2003;**26**(3):868–874.
17. Basu R, Barosa C, Jones J, Dube S, Carter R, Basu A, Rizza RA. Pathogenesis of prediabetes: role of the liver in isolated fasting hyperglycemia and combined fasting and postprandial hyperglycemia. *J Clin Endocrinol Metab*. 2013;**98**(3):E409–E417.
18. DeFronzo RA, Ferrannini E, Simonson DC. Fasting hyperglycemia in non-insulin-dependent diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake. *Metabolism*. 1989;**38**(4):387–395.
19. Jani R, Molina M, Matsuda M, Balas B, Chavez A, DeFronzo RA, Abdul-Ghani M. Decreased non-insulin-dependent glucose clearance contributes to the rise in fasting plasma glucose in the non-diabetic range. *Diabetes Care*. 2008;**31**(2):311–315.
20. Del Prato S, Riccio A, Vigili de Kreutzenberg S, Dorella M, Tiengo A, DeFronzo RA. Basal plasma insulin levels exert a qualitative but not quantitative effect on glucose-mediated glucose uptake. *Am J Physiol*. 1995;**268**(6):E1089–E1095.
21. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*. 1997;**20**(7):1183–1197.
22. Abdul-Ghani MA, Jani R, Chavez A, Molina-Carrion M, Tripathy D, Defronzo RA. Mitochondrial reactive oxygen species generation in obese non-diabetic and type 2 diabetic participants. *Diabetologia*. 2009;**52**(4):574–582.
23. Steele R, Wall JS, De Bodo RC, Altszuler N. Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *Am J Physiol*. 1956;**187**(1):15–24.
24. Del Prato S, Matsuda M, Simonson DC, Groop LC, Sheehan P, Leonetti F, Bonadonna RC, DeFronzo RA. Studies on the mass action effect of glucose in NIDDM and IDDM: evidence for glucose resistance. *Diabetologia*. 1997;**40**(6):687–697.
25. Basu A, Basu R, Shah P, Vella A, Johnson CM, Nair KS, Jensen MD, Schwenk WF, Rizza RA. Effects of type 2 diabetes on the ability of insulin and glucose to regulate splanchnic and muscle glucose metabolism: evidence for a defect in hepatic glucokinase activity. *Diabetes*. 2000;**49**(2):272–283.
26. DeFronzo RA. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes*. 1988;**37**(6):667–687.
27. Jeng CY, Sheu WH, Fuh MM, Chen YD, Reaven GM. Relationship between hepatic glucose production and fasting plasma glucose concentration in patients with NIDDM. *Diabetes*. 1994;**43**(12):1440–1444.
28. Ciaraldi TP, Mudaliar S, Barzin A, Macievic JA, Edelman SV, Park KS, Henry RR. Skeletal muscle GLUT1 transporter protein expression and basal leg glucose uptake are reduced in type 2 diabetes. *J Clin Endocrinol Metab*. 2005;**90**(1):352–358.
29. Olson AL, Pessin JE. Structure, function, and regulation of the mammalian facilitative glucose transporter gene family. *Annu Rev Nutr*. 1996;**16**(1):235–256.
30. Miele C, Formisano P, Condorelli G, Caruso M, Oriente F, Andreozzi F, Tocchetti CG, Riccardi G, Beguinot F. Abnormal glucose transport and GLUT1 cell-surface content in fibroblasts and skeletal muscle from NIDDM and obese subjects. *Diabetologia*. 1997;**40**(4):421–429.
31. Marette A, Atgié C, Liu Z, Bukowiecki LJ, Klip A. Differential regulation of GLUT1 and GLUT4 glucose transporters in skeletal muscle of a new model of type II diabetes. The obese SHR/N-cp rat. *Diabetes*. 1993;**42**(8):1195–1201.
32. Kraegen EW, Sowden JA, Halstead MB, Clark PW, Rodnick KJ, Chisholm DJ, James DE. Glucose transporters and in vivo glucose

uptake in skeletal and cardiac muscle: fasting, insulin stimulation and immunoisolation studies of GLUT1 and GLUT4. *Biochem J*. 1993;**295**(Pt 1):287–293.
33. Nielsen MF, Basu R, Wise S, Caumo A, Cobelli C, Rizza RA. Normal glucose-induced suppression of glucose production but impaired stimulation of glucose disposal in type 2 diabetes: evidence for a concentration-dependent defect in uptake. *Diabetes*. 1998;**47**(11):1735–1747.
34. Alatrach M, Agyin C, Adams J, DeFronzo RA, Abdul-Ghani MA. Decreased basal hepatic glucose uptake in impaired fasting glucose. *Diabetologia*. 2017;**60**(7):1325–1332.
35. Abdul-Ghani MA, DeFronzo RA. Pathogenesis of insulin resistance in skeletal muscle. *J Biomed Biotechnol*. 2010;**2010**:476279.
36. Malin SK, Viskochil R, Oliver C, Braun B. Mild fasting hyperglycemia shifts fuel reliance toward fat during exercise in adults with impaired glucose tolerance. *J Appl Physiol (1985)*. 2013;**115**(1):78–83.