

Contribution of Hepatic and Extrahepatic Insulin Resistance to the Pathogenesis of Impaired Fasting Glucose

Role of Increased Rates of Gluconeogenesis

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OBJECTIVE—To determine the contribution of hepatic insulin resistance to the pathogenesis of impaired fasting glucose (IFG).

RESEARCH DESIGN AND METHODS—Endogenous glucose production (EGP) and glucose disposal were measured in 31 subjects with IFG and 28 subjects with normal fasting glucose (NFG) after an overnight fast and during a clamp when endogenous secretion was inhibited with somatostatin and insulin infused at rates that approximated portal insulin concentrations present in IFG subjects after an overnight fast (~80 pmol/l, “preprandial”) or within 30 min of eating (~300 pmol/l, “prandial”).

RESULTS—Despite higher ($P < 0.001$) insulin and C-peptide concentrations and visceral fat ($P < 0.05$), fasting EGP and glucose disposal did not differ between IFG and NFG subjects, implying hepatic and extrahepatic insulin resistance. This was confirmed during preprandial insulin infusion when glucose disposal was lower ($P < 0.05$) and EGP higher ($P < 0.05$) in IFG than in NFG subjects. Higher EGP was due to increased ($P < 0.05$) rates of gluconeogenesis in IFG. EGP was comparably suppressed in IFG and NFG groups during prandial insulin infusion, indicating that hepatic insulin resistance was mild. Glucose disposal remained lower ($P < 0.01$) in IFG than in NFG subjects.

CONCLUSIONS—Hepatic and extrahepatic insulin resistance contribute to fasting hyperglycemia in IFG with the former being due at least in part to impaired insulin-induced suppression of gluconeogenesis. However, since hepatic insulin resistance is mild and near-maximal suppression of EGP occurs at portal insulin concentrations typically present in IFG subjects within 30 min of eating, extrahepatic (but not hepatic) insulin resistance coupled with accompanying defects in insulin secretion is the primary cause of postprandial hyperglycemia. *Diabetes* 56:1703–1711, 2007

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EGP, endogenous glucose production; FFA, free fatty acid; IFG, impaired fasting glucose; NFG, normal fasting glucose.

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People with impaired fasting glucose (IFG; fasting glucose 100–125 mg/dl) have an ~30% chance of developing diabetes over the next 10 years (1–3).

Numerous studies have shown that IFG is associated with defects in both insulin secretion and action that are particularly evident when IFG is accompanied by impaired glucose tolerance or oral glucose tolerance test–diagnosed diabetes (4–11). However, few studies have addressed the pathogenesis of fasting hyperglycemia that is characteristic of IFG. We and others have reported that despite higher insulin and glucose concentrations, EGP either does not differ (4,12,13) or is elevated (14) in individuals with IFG in comparison with individuals with normal fasting glucose (NFG) concentrations. This implies the presence of hepatic insulin resistance and is similar to the situation observed in people with overt type 2 diabetes where EGP is excessive when considered in the light of prevailing glucose and insulin concentrations (15–17). However, to our knowledge, the only study that has formally assessed hepatic insulin action in people with IFG is that of Weyer et al. (14). In that study, suppression of EGP during hyperinsulinemic-euglycemic clamp was impaired in young Native American subjects with IFG (14). This observation was remarkable because the insulin concentrations achieved during the “low” dose insulin infusion used in that study to assess hepatic insulin action averaged ~900 pmol/l, and these concentrations are several-fold higher than those that result in maximal suppression of EGP in less insulin-resistant populations (18–20).

EGP has been shown to correlate with fasting glucose concentration when people with normal fasting glucose and diabetes are included in the same analysis (21,22). Therefore, the concept that IFG is caused by an inappropriately elevated rate of EGP is appealing. However, in a recent series of experiments, we noted that whereas EGP did not differ in subjects with IFG and NFG in the fasting state despite higher insulin and glucose concentrations, implying hepatic insulin resistance, suppression of EGP was normal following ingestion of a mixed meal with the associated postprandial hyperglycemia being entirely explained by lower rates of glucose disposal due to impaired insulin-induced stimulation of glucose uptake (12). This led us to speculate that if subjects with IFG have hepatic insulin resistance, it is likely relatively mild and therefore

TABLE 1
Subject characteristics

	NFG	IFG
Sex (M/F)	11/17	16/15
Age (years)	51.5 ± 1.6	54.5 ± 1.4
BMI (kg/m ²)	27.8 ± 0.6	31.5 ± 0.9*
Total body fat (kg)	80.1 ± 1.9	94.0 ± 3.5*
Height (m)	1.70 ± 0.02	1.72 ± 0.02
Body fat (%)	35.7 ± 1.9	38.3 ± 1.6
Body fat (kg)	28.1 ± 1.7	36.2 ± 2.0*
Fat-free mass (kg)	47.5 ± 2.0	53.7 ± 2.3*
Visceral fat (cm ²)	112.4 ± 10.3	197.7 ± 17.7*
FPG (mmol/l)	5.1 ± 0.1	6.1 ± 0.0*
2-h preprandial OGTT (mmol/l)	7.8 ± 0.3	9.7 ± 0.4*

Data are means ± SEM. FPG, fasting plasma glucose at time of screening; OGTT, 2-h oral glucose tolerance test value. **P* < 0.05 vs. NFG.

primarily impacts glucose homeostasis when insulin concentrations are low (e.g., after an overnight fast) but has little influence on glucose tolerance when insulin (and glucose concentrations) are high (e.g., after eating). We also speculated that if peripheral tissues are more insulin resistant than the liver, then impaired insulin-induced stimulation of glucose uptake would contribute to both fasting and postprandial hyperglycemia in subjects with IFG.

To address these questions, EGP and glucose disposal were measured in subjects with IFG and NFG following an overnight fast and during a hyperinsulinemic-euglycemic clamp when insulin was infused at rates designed to approximate insulin concentrations typically present in the portal vein of subjects with IFG following an overnight fast (~80 pmol/l, referred to as “preprandial”) or within 30 min of eating a carbohydrate-containing meal (~300 pmol/l, referred to as “prandial”). Endogenous insulin and glucagon secretion were inhibited with somatostatin, and replacement amounts of insulin and glucagon were infused in order to achieve comparable portal concentrations in both groups. Since impaired insulin-induced suppression of gluconeogenesis and perhaps glycogenolysis contributes to the pathogenesis of hepatic insulin resistance in people with diabetes (23–26), gluconeogenesis and glycogenolysis were measured using the deuterated water method in the fasting state and during the preprandial insulin infusion.

RESEARCH DESIGN AND METHODS

Characteristics of 31 subjects with IFG and 28 subjects with NFG studied are given in Table 1. Three additional subjects (IFG, *n* = 1; NFG, *n* = 2) were excluded from this analysis due to somatostatin-induced nausea that precluded completion of the study. Subjects were Caucasian, in good health, at a stable weight, and did not engage in vigorous physical exercise. Subjects were part of a larger study examining mechanisms of postprandial hyperglycemia, results of which have been reported in part elsewhere (12,27). The subjects were selected by using the Mayo Clinic electronic medical record system to search for all individuals who had fasting glucose levels between 100 and 126 mg/dl, BMI between 20 and 40 kg/m², and age between 40 and 70 years and were otherwise healthy. As previously reported (12,27), subjects were studied on three occasions in random order at which time they ingested either a mixed meal or 75 g glucose or underwent a hyperinsulinemic-euglycemic clamp during which they received either a 0.25 mU · kg⁻¹ · min⁻¹ (preprandial) or 0.5 mU · kg⁻¹ · min⁻¹ (prandial) insulin infusions. The study was approved by the Mayo Institutional Review Board, and all subjects gave informed written consent to participate in the study.

All subjects were instructed to follow a weight-maintenance diet containing 55% carbohydrate, 30% fat, and 15% protein for at least 3 days before the study date. Subjects were admitted to the general clinical research center at

1700 h on the evening before the study and ate a standard 10 kcal/kg meal (55% carbohydrate, 30% fat, and 15% protein) between 1830 and 1900 h. After sampling blood for baseline enrichments, 1.67 mg/kg wt of body water ²H₂O was given in three divided doses at 1800, 2000, and 2200 h. Small sips of water (containing ²H₂O) were permitted overnight. At 0600 h, an 18-gauge cannula was inserted in a forearm vein for tracer and hormone infusions. Another cannula was inserted in a retrograde fashion in a dorsal hand vein of the opposite arm, and the hand was placed in a heated box (~55°C) to enable sampling of arterialized venous blood.

A primed (fasting glucose in millimoles per liter divided by 5.5 mmol/l × 12 μCi) continuous (0.12 μCi/min) infusion of [³-³H]glucose (New England Nuclear, Boston, MA) was started at 0700 h and continued until the end of the study. A constant infusion containing somatostatin (60 ng · kg⁻¹ · min⁻¹), glucagon (0.65 ng · kg⁻¹ · min⁻¹), and growth hormone (3 ng · kg⁻¹ · min⁻¹) was started at 1000 h (i.e., time 0) for 4 h. An infusion of insulin also was started at a rate, respectively, of 0.25 or 0.50 mU · kg⁻¹ · min⁻¹ in the subjects receiving either a preprandial (15 IFG and 13 NFG) or prandial (16 IFG and 15 NFG) insulin infusion. Dextrose (D₅₀W) containing [³-³H]glucose was infused as necessary to maintain plasma glucose concentrations at ~5.0 mmol/l over the 4 h of the study, as previously described (28). In addition, the basal [³-³H]glucose infusion was adjusted downward beginning at time 0 in a manner mimicking the anticipated pattern of fall of EGP in an effort to further minimize the change in plasma [³-³H]glucose specific activity. (IFG: 0–60 min, 90%; 60–240 min, 40%; NFG: 0–60 min, 80%; 60–240 min, 60%) as previously described [29,30].) Arterialized venous blood samples were collected at regular intervals for measurement of glucose, tracer, and hormone concentrations.

Analytical techniques. Plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at –20°C until assay. Glucose concentration was measured using a glucose oxidase method (Yellow Springs Instrument, Yellow Springs, OH). Insulin concentrations were measured using a chemiluminescence assay with reagents obtained from Beckman (Access Assay; Beckman, Chaska, MN). Glucagon and C-peptide concentrations were measured by radioimmunoassay using reagents supplied by Linco Research (St. Louis, MO). Free fatty acid (FFA) concentrations were measured using a calorimetric assay (COBAS; Roche Diagnostics, Indianapolis, IN). Body composition was measured using dual-energy X-ray absorptiometry (DPX scanner; Lunar, Madison, WI) and computerized absorption tomography with cuts at L2/3 and T11/12 to determine percentage body fat and visceral fat, as previously described (31). Plasma [³-³H]glucose specific activity was measured using liquid scintillation counting as previously described (18). Enrichments of deuterium on the 2nd and 5th carbon of plasma glucose were measured according to the method developed by Landau and coworkers (32–34).

Calculations. The mean concentrations and rates from –30 to 0 min and 210–240 min were used for analysis. All rates are expressed per kilogram of fat-free mass. Glucose appearance and disappearance were calculated using the steady-state equations of Steele et al. (35). EGP during the clamp was calculated by subtracting the glucose infusion rate from the tracer-determined rate of glucose appearance. The rate of gluconeogenesis was calculated by multiplying the plasma ratio of C5 and C2 glucose enrichments times the rate of EGP (32,33). Glycogenolysis was calculated by subtracting the rate of gluconeogenesis from EGP (26,36,37).

Statistical analysis. Data in the text and figures are expressed as means ± SE. Student’s unpaired *t* test was used to compare results between IFG and NFG groups. *P* < 0.05 was considered statistically significant.

RESULTS

Plasma glucose, insulin, C-peptide, and glucagon concentrations. Fasting glucose concentrations were higher (*P* < 0.001) in the IFG than in the NFG subjects (5.7 ± 0.1 vs. 4.9 ± 0.0 mmol/l) and remained higher (*P* < 0.05), averaging 5.9 ± 0.3 versus 5.1 ± 0.1 mmol/l and 5.5 ± 0.1 versus 5.0 ± 0.1 mmol/l, respectively, during the final 30 min of the preprandial and prandial insulin infusions (Fig. 1).

Fasting insulin concentrations were higher (*P* < 0.001) in the IFG than in the NFG subjects (48 ± 5 vs. 27 ± 2 pmol/l). Despite identical infusion rates, insulin concentrations during the final 30 min of the preprandial insulin infusion were higher (*P* < 0.01) in IFG than in NFG subjects (79 ± 4 vs. 60 ± 3 pmol/l), indicating lower rates of insulin clearance. On the other hand, insulin concentra-

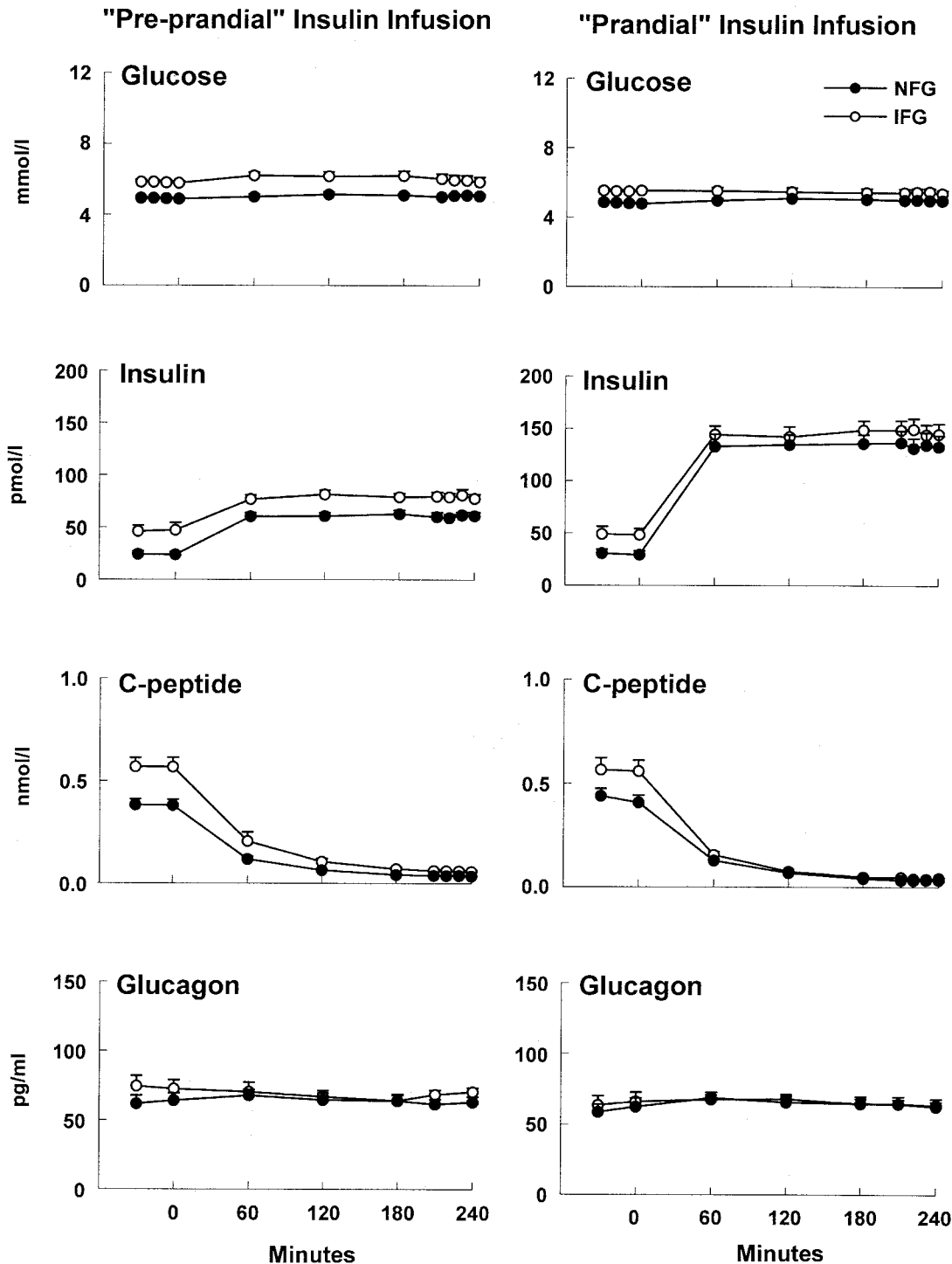


FIG. 1. Plasma glucose, insulin, C-peptide, and glucagon concentrations observed when insulin was infused at rates of $0.25 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (referred to as "preprandial") and $0.5 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (referred to as "prandial") in the NFG and IFG groups.

tions did not differ in the IFG and NFG subjects during the prandial insulin infusion (147 ± 9 vs. $134 \pm 11 \text{ pmol/l}$).

Fasting C-peptide concentrations were higher ($P < 0.001$) in the IFG than in the NFG subjects (0.57 ± 0.03 vs. $0.40 \pm 0.02 \text{ nmol/l}$). The somatostatin infusion, started at time 0, resulted in comparable and near-complete suppression of C-peptide concentrations in the IFG and NFG subjects during both the preprandial (0.06 ± 0.00 vs. $0.04 \pm 0.00 \text{ nmol/l}$) and prandial (0.04 ± 0.00 vs. $0.03 \pm 0.00 \text{ nmol/l}$) insulin infusions.

Fasting glucagon concentrations did not differ in IFG and NFG groups (70 ± 5 vs. $64 \pm 4 \text{ pg/ml}$). Glucagon infusion, started at time 0, resulted in constant and comparable plasma glucagon concentrations during both the preprandial (70 ± 4 vs. $63 \pm 3 \text{ pg/ml}$) and prandial (65 ± 3 vs. $63 \pm 4 \text{ pg/ml}$) insulin infusions.

Glucose infusion rate and glucose specific activity. The glucose infusion rates required to maintain euglycemia were lower ($P < 0.01$) in the IFG than in the NFG subjects during the final 30 min of both the preprandial

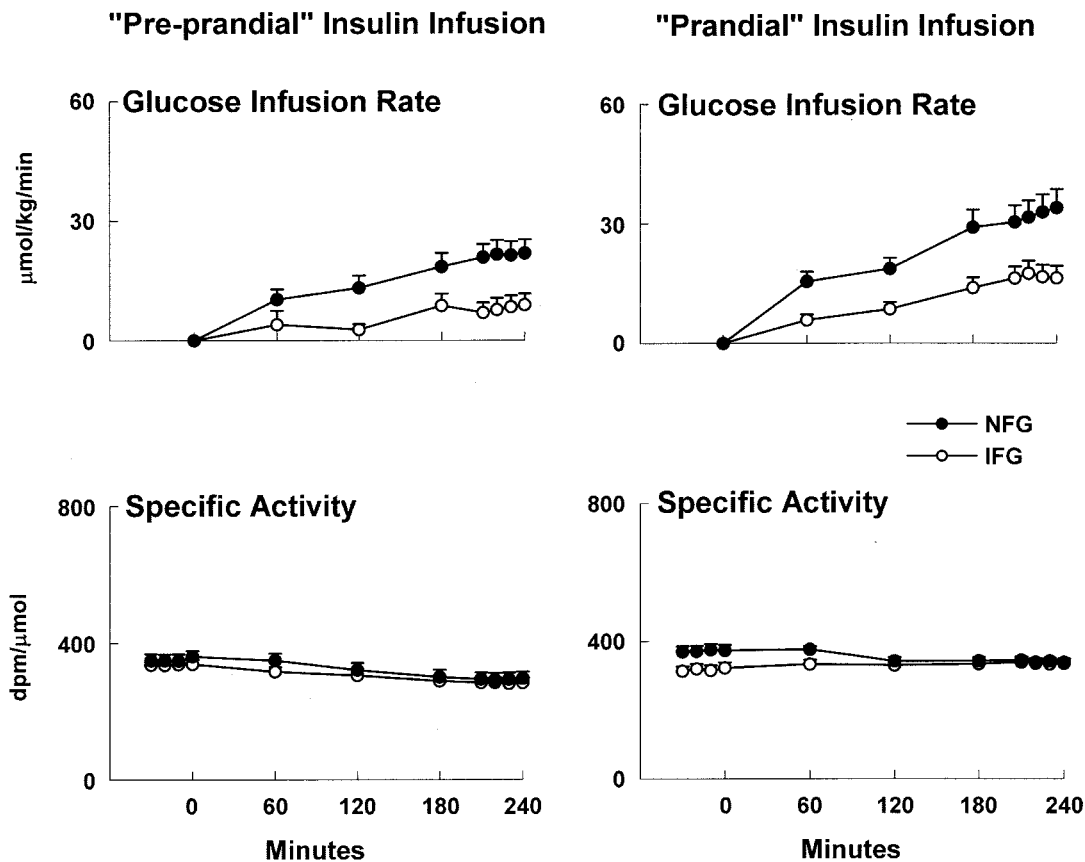


FIG. 2. The glucose infusion rates required to maintain euglycemia and the plasma [3-³H]glucose specific activity observed when insulin was infused at rates of 0.25 mU · kg⁻¹ · min⁻¹, referred to as "preprandial," and 0.5 mU · kg⁻¹ · min⁻¹, referred to as "prandial," in the NFG and IFG groups.

(8 ± 3 vs. 21 ± 3 μmol · kg⁻¹ · min⁻¹) and prandial (17 ± 3 vs. 32 ± 4 μmol · kg⁻¹ · min⁻¹) insulin infusions, documenting the presence of insulin resistance (Fig. 2). Of note, in contrast to all NFG subjects, 7 of 15 IFG subjects did not require any exogenous glucose during the preprandial insulin infusion. In addition, 3 of 16 IFG subjects did not require any exogenous glucose during the prandial insulin infusion. Plasma glucose specific activity remained constant throughout the study, enabling accurate measurement of glucose turnover in both IFG and NFG subjects.

Endogenous glucose production and glucose disappearance. Despite higher insulin concentrations, EGP following an overnight fast did not differ in IFG and NFG subjects (15.2 ± 0.4 vs. 15.3 ± 0.3 μmol · kg⁻¹ · min⁻¹), implying hepatic insulin resistance (Fig. 3). This was confirmed during the preprandial insulin infusion when endogenous insulin secretion was suppressed and similar portal insulin and glucagon concentrations were present in both groups. Under these conditions, EGP was greater (*P* < 0.05) in IFG subjects than in NFG subjects (10.2 ± 0.7 vs. 7.3 ± 1.1 μmol · kg⁻¹ · min⁻¹). On the other hand, the higher insulin concentrations achieved during the prandial insulin infusion resulted in comparable suppression of EGP in IFG and NFG subjects (6.1 ± 0.9 vs. 3.9 ± 1.1 μmol · kg⁻¹ · min⁻¹).

As with EGP, glucose disappearance following an overnight fast did not differ between IFG and NFG subjects (15.3 ± 0.4 vs. 15.6 ± 0.4 μmol · kg⁻¹ · min⁻¹). Since insulin concentrations were higher, this also implied insulin resistance. This was confirmed during the preprandial

insulin infusion when glucose disappearance was lower (*P* < 0.05) in the IFG group than in the NFG group (18.6 ± 2.6 vs. 28.8 ± 2.7 μmol · kg⁻¹ · min⁻¹). However, in contrast to EGP, glucose disappearance also was lower (*P* < 0.01) in the IFG than in the NFG group during the prandial insulin infusion (23.0 ± 2.2 vs. 35.2 ± 3.8 μmol · kg⁻¹ · min⁻¹).

Contribution of gluconeogenesis and glycogenolysis to EGP after an overnight fast and during the preprandial insulin infusion. Plasma C5 glucose and C2 glucose enrichment and the ratio of the plasma C5 glucose to C2 glucose enrichment did not differ in the IFG and NFG subjects following an overnight fast (Table 2 and Fig. 4). Of note, since the rate of glucose disappearance was lower in the IFG than in the NFG subjects during the preprandial insulin infusion, clearance of plasma C5 glucose and C2 glucose also was lower in IFG than in NFG subjects. This resulted in plasma C5 glucose and C2 glucose enrichments that were higher (*P* < 0.001) in IFG than in NFG subjects (Table 2).

The rate of gluconeogenesis, calculated by multiplying the plasma ratio of C5 glucose to C2 glucose times EGP (shown in Fig. 4 as a reference), did not differ in the IFG and NFG subjects following an overnight fast (11.2 ± 0.5 vs. 11.9 ± 0.5 μmol · kg⁻¹ · min⁻¹). On the other hand, the rate of gluconeogenesis was higher (*P* < 0.05) in the IFG than in the NFG subjects during the preprandial (7.3 ± 0.6 vs. 5.0 ± 0.9 μmol · kg⁻¹ · min⁻¹) insulin infusion. Glycogenolysis, calculated by subtracting gluconeogenesis from EGP did not differ between IFG and NFG subjects either following an overnight fast (3.8 ± 0.3 vs. 3.8 ± 0.5

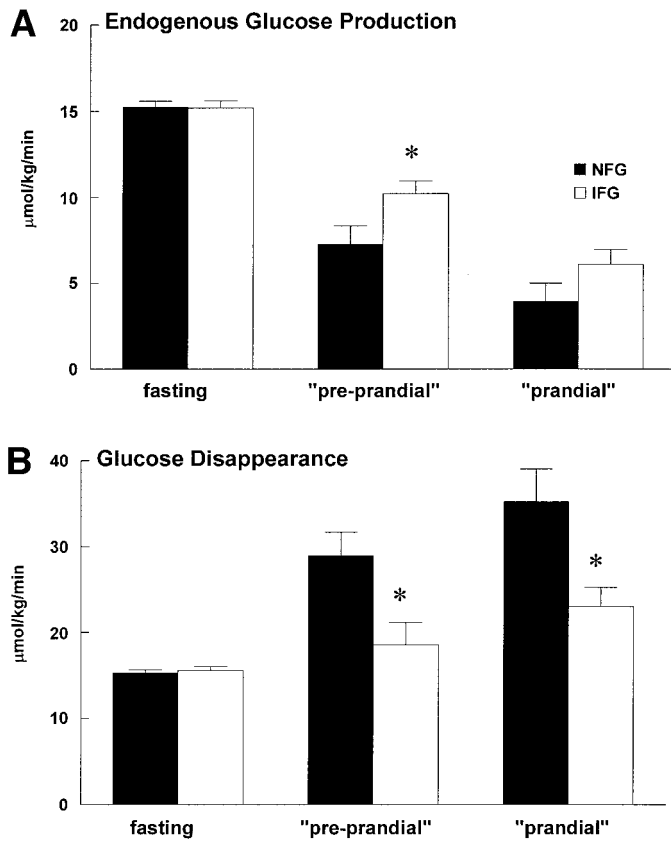


FIG. 3. Rates of EGP (A) and glucose disappearance (B) observed before insulin infusion in all subjects with IFG or NFG and during preprandial insulin replacement in one group and prandial insulin replacement in the other group.

$\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) or during the preprandial insulin infusion (2.9 ± 0.5 vs. $2.2 \pm 0.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$).

Plasma FFA concentrations. As with EGP, fasting plasma FFA did not differ between IFG and NFG subjects (0.57 ± 0.03 vs. 0.56 ± 0.03 mmol/l) despite higher insulin concentrations, implying insulin resistance (Fig. 5). This was confirmed during the preprandial insulin infusion when plasma FFAs were higher ($P < 0.05$) in IFG than in NFG subjects (0.30 ± 0.06 vs. 0.12 ± 0.02 mmol/l). On the other hand, FFA concentrations did not differ in IFG and NFG subjects (0.17 ± 0.03 vs. 0.13 ± 0.02 mmol/l) during the prandial insulin infusion.

Correlations. Fasting EGP and fasting gluconeogenesis did not correlate with fasting glucose, insulin, or C-peptide. On the other hand, when the IFG and NFG subjects were considered as a single group, fasting FFAs correlated with both fasting EGP ($r = 0.36$; $P < 0.005$) and

TABLE 2
Plasma C5 glucose and C2 glucose enrichment after an overnight fast and during the preprandial insulin infusion

	C5 glucose	C2 glucose	C5/C2 glucose
Fasting			
NFG	0.36 ± 0.04	0.47 ± 0.03	0.76 ± 0.03
IFG	0.38 ± 0.02	0.51 ± 0.03	0.74 ± 0.02
Preprandial			
NFG	0.18 ± 0.02	0.28 ± 0.04	0.70 ± 0.05
IFG	$0.33 \pm 0.02^*$	$0.47 \pm 0.03^*$	0.72 ± 0.03

Data are means \pm SE. * $P < 0.001$ vs. NFG.

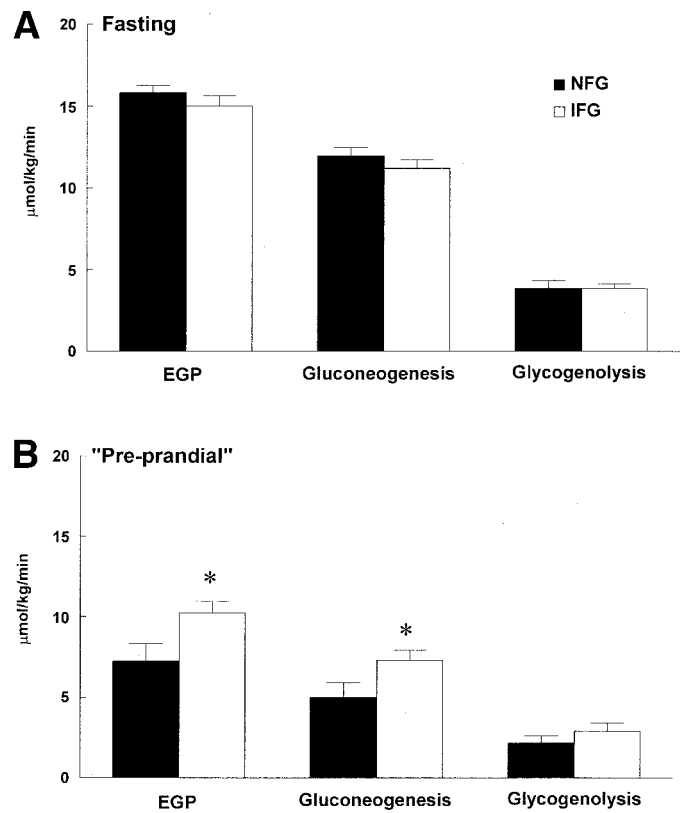


FIG. 4. Rates of EGP, gluconeogenesis, and glycogenolysis observed before insulin infusion (A) and during preprandial insulin replacement (B).

fasting gluconeogenesis ($r = 0.55$; $P < 0.005$). Plasma FFA concentrations were inversely related to glucose disappearance in the fasting state ($r = 0.36$; $P < 0.005$), during the preprandial ($r = 0.67$; $P < 0.001$) and prandial ($r = 0.45$; $P < 0.05$) insulin infusions. Visceral fat and total body fat also were inversely related to glucose disappearance during both the preprandial ($r = 0.76$; $P < 0.001$; and $r = 0.38$; $P < 0.05$) and prandial ($r = 0.54$; $P < 0.01$; and $r = 0.49$; $P < 0.01$) insulin infusions. In contrast, none of the above variables correlated with either EGP or gluconeogenesis during the preprandial insulin infusion.

DISCUSSION

The data from the present study establish that people with IFG have hepatic insulin resistance due to impaired insu-

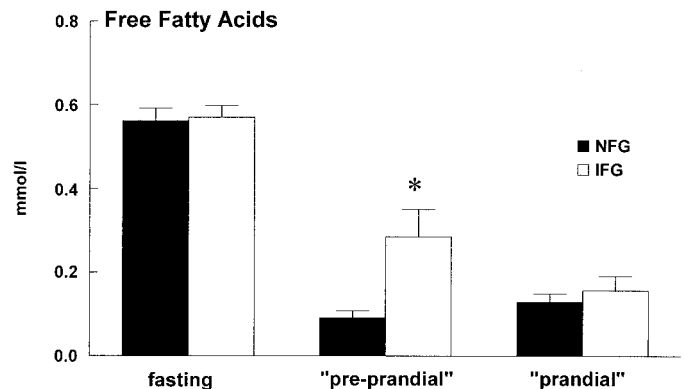


FIG. 5. Plasma FFA concentrations observed before insulin infusion in all subjects with IFG or NFG and during preprandial and prandial insulin replacement.

lin-induced suppression of EGP due to, at least in part, an increase in the rate of gluconeogenesis. On the other hand, since the severity of hepatic insulin resistance is relatively mild, EGP is comparably suppressed in IFG and NFG subjects at insulin concentrations commonly observed immediately after eating (10,12), indicating that while hepatic insulin resistance likely contributes to fasting hyperglycemia in subjects with IFG, it is unlikely to be the cause of postprandial hyperglycemia. In contrast, insulin-induced stimulation of glucose disposal was impaired during both preprandial and prandial insulin infusion, providing strong experimental support for involvement of extrahepatic insulin resistance in the pathogenesis of fasting as well as postprandial hyperglycemia in people with IFG.

Glucose concentrations rise when the rate of glucose entering exceeds that leaving the circulation. The increase in glucose concentration stimulates insulin secretion, which combined with the effects of the rising glucose concentration inhibits EGP and increases glucose disposal. Glucose concentrations continue to rise until EGP and glucose disposal are again equal. Therefore, the degree of fasting hyperglycemia in people with IFG will be determined by interaction between the severity of insulin resistance and the ability of the β -cell to respond to an increase in glucose concentration. In the present study, hepatic insulin resistance was relatively mild and fasting insulin and C-peptide concentrations slightly but significantly higher in the IFG than in the NFG subjects. This indicates that IFG subjects were able to secrete enough additional insulin to at least partially compensate, albeit at a slightly elevated fasting glucose concentration, for the presence of insulin resistance.

Insulin inhibits glucose production both directly by altering the activity of hepatic enzymes and indirectly by decreasing the rate of delivery of substrates such as FFA from peripheral tissues to the liver (38,39). The experimental design used in the present study sought to assess insulin action in IFG and NFG subjects under conditions in which portal insulin and glucagon concentrations were comparable and equal in the two groups and approximated those present in IFG subjects after an overnight fast. Assuming a portal to hepatic insulin gradient of ~ 1.6 (40,41), the peripheral insulin concentration of 48 pmol/l present in IFG in the fasting state before the clamp was associated with a portal insulin concentration of ~ 77 pmol/l. If so, this value is very close to the insulin concentration of ~ 80 pmol/l achieved in IFG subjects during the preprandial infusion. However, since fasting insulin concentrations differed in the IFG and NFG groups, this represented an ~ 2.2 -fold increase in peripheral insulin concentrations in the IFG group and a 1.7-fold increase in the NFG group. On the other hand, the pattern of change in portal glucagon concentrations is less clear. Since the peripheral glucagon concentrations remained essentially unchanged following initiation of the somatostatin and glucagon infusions, we presume that portal glucagon concentrations decreased. If so, this would increase the portal insulin-to-glucagon ratio, thereby enhancing suppression of EGP. From the perspective of the present experiments, since the peripheral and therefore portal venous insulin concentrations were higher in IFG than in NFG subjects during the preprandial insulin infusion and since EGP was still higher in the former, this represents a conservative error regarding the degree of hepatic insulin resistance that was present during the preprandial insulin infusion.

Taken together, the data from the present study conducted in Caucasians and those from Weyer et al. (14) conducted in Pima Indians indicate that people with IFG have hepatic insulin resistance.

Insulin also indirectly inhibits EGP by lowering plasma FFAs (42,43). Somatostatin resulted in near-complete suppression of C-peptide in both groups, documenting effective inhibition of endogenous hormone secretion. Since the preprandial insulin infusion was designed to approximate portal insulin concentrations, it resulted in an increase in peripheral insulin concentrations, which in turn caused plasma FFAs to fall in both groups. However, suppression of FFA was blunted in the IFG subjects, indicating insulin resistance. Since elevated FFAs impair the ability of insulin to inhibit glycogenolysis (44), this may account for the slightly higher rates of glycogenolysis observed in the IFG subjects during the preprandial insulin infusion. Additional studies will be required to specifically address this hypothesis.

We did not use an FFA tracer. Therefore, we do not know whether this solely was due to impaired inhibition of lipolysis and/or to an accompanying defect in FFA clearance. Consistent with previous reports (23,45), fasting FFA correlated with fasting EGP, implying but not proving causality. On the other hand, FFA did not correlate with EGP during the preprandial insulin infusion, likely because of the very low FFA concentrations and near-complete suppression of EGP in the NFG subjects. In any case, these data clearly indicate that insulin-induced suppression of FFA and EGP is impaired in people with IFG and therefore suggest that future studies determining whether agents that lower FFA and also normalize fasting glucose in people with IFG would be of considerable interest.

EGP equals the sum of gluconeogenesis and glycogenolysis. The deuterated water method was used to measure gluconeogenesis in the fasting state and during the preprandial insulin infusion (32). The contribution of gluconeogenesis and glycogenolysis to EGP following an overnight fast did not differ between IFG and NFG subjects, whether expressed as a percentage or as absolute rates of production. However, as with EGP, when endogenous insulin and glucagon secretion were inhibited by somatostatin and insulin was infused so as to produce preprandial portal concentrations, rates of gluconeogenesis were greater in IFG than in NFG subjects, accounting for the associated increase in EGP. Due to the feedback loops discussed above, the lack of difference or only a minimal difference in gluconeogenesis in the fasting state when glucose and insulin concentrations differed that is readily evident when concentrations are more closely matched is similar to the situation observed in people with diabetes (23). We did not attempt to assess gluconeogenesis during the prandial insulin infusion due to concern regarding our ability to accurately measure C5 enrichment in the NFG subjects during infusion of the relatively large amounts of exogenous glucose required to maintain euglycemia. Therefore, we do not know whether despite comparable rates of EGP, the contribution of gluconeogenesis to EGP also was higher in IFG subjects. If so, the difference must have been small, since suppression of EGP was near complete in both groups during the prandial infusion. While the present data indicate that insulin-induced suppression of gluconeogenesis is impaired in people with IFG, we cannot rule out the possibility that suppression of glycogenolysis is also impaired, since the

insulin doses used resulted in near-maximal suppression in both groups. Furthermore, the number of subjects in each group was relatively small. Future studies will be required to determine whether regulation of glycogenolysis is also abnormal in people with IFG.

Insulin-induced stimulation of glucose disposal was also impaired in IFG subjects. However, in contrast to EGP, glucose disposal was lower in the IFG than in the NFG subjects during both the preprandial and prandial insulin infusions. Since glucose was clamped in the euglycemic range, the lower rates of disposal presumably reflected decreased muscle glucose uptake (46,47). Of note, we did not generate full insulin dose response curves. Therefore, we do not know whether glucose disposal in IFG subjects eventually would have equaled that observed in NFG subjects at higher insulin concentrations. Even if it did, several arguments still favor decreased glucose uptake as the primary cause of postprandial hyperglycemia in people with IFG. First, insulin-induced stimulation of glucose disposal was markedly reduced at insulin concentrations of ~ 150 pmol/l, levels similar to those observed in the same subjects within 30–60 min of eating a mixed meal or drinking 75 g glucose (12,27). Second, insulin action measured in the same individuals on a separate occasion using the labeled “minimal” meal model was lower in IFG than in NFG subjects during the 6 h after meal ingestion, indicating that there was not a late compensatory increase in glucose disposal (12). Third, there is a substantial lag between the time when plasma insulin concentrations increase and interstitial insulin concentrations increase (48). This combined with lower overall uptake would further blunt the rate of rise of glucose uptake in IFG subjects, thereby hindering disposition of the meal-derived glucose that rapidly enters the systemic circulation after eating a carbohydrate-containing meal.

However, as is evident during the preprandial insulin infusion, the IFG subjects have hepatic as well as extrahepatic insulin resistance. As discussed above, because the hepatic insulin resistance is relatively mild, its contribution to postprandial hyperglycemia appears to be minimal, since the postprandial increase in glucose and insulin concentrations that occurs after eating results in rapid and near-complete suppression of glucose production (12). This differs from the present situation following an overnight fast when glucose and insulin concentrations are only slightly elevated. Under these circumstances, hepatic insulin resistance becomes germane, leading to an excessive rate of hepatic glucose release, which in turn causes glucose concentrations to rise until the rate of glucose production again equals glucose disposal, albeit now at an “abnormal” glucose concentration (IFG). Taken together, these data indicate that the relative contribution of hepatic and extrahepatic insulin resistance to glucose intolerance will differ depending on the prevailing insulin concentration, which in turn will be determined by the extent to which β -cell function is preserved in people with IFG.

As with all experiments, the present studies suffer from certain limitations. Visceral fat and total body fat were both greater in the IFG than in the NFG subjects. Therefore, glucose turnover was expressed per kilogram of fat-free mass to avoid the confounding that would occur if the denominator differed between groups due to differing amounts of fat. The fact that visceral fat and total body fat were both greater in the IFG than in the NFG subjects lends further support to the concept that adiposity and its associated metabolic abnormalities contribute to the

pathogenesis of IFG. However, additional studies will be required to specifically address this hypothesis. Consistent with previous reports that IFG is more common in men than women (49), there were slightly (but not significantly) more men than women in the IFG than NFG groups (51 vs. 39%). However, the overall conclusion that IFG subjects are more insulin resistant than NFG subjects remained unchanged when the men and women were analyzed separately. Glucose production was higher and glucose disposal lower in the IFG than in the NFG subjects during the preprandial insulin infusion. On the other hand, despite that fact that most IFG subjects required no exogenous glucose, glucose concentrations remained higher in the IFG than in the NFG subjects. Since hyperglycemia per se suppresses glucose production and enhances glucose uptake (29,30,46,47), if glucose concentrations in the NFG subjects had been raised to levels matching those present in the IFG subjects, presumably rates of glucose production would have been even lower and disposal higher in the NFG subjects. This would have further accentuated the differences in production and disposal between the IFG and NFG subjects.

The experimental design (i.e., pancreatic clamp) assumes that hepatic extraction (and therefore portal concentrations) of glucagon is the same in the IFG and NFG groups. Comparable peripheral glucagon concentrations during the exogenous replacement glucagon infusion supports but does not prove this assumption. It also assumes that hepatic sensitivity to glucose is the same in the IFG and NFG subjects. We have previously demonstrated that this assumption is valid in people with overt type 2 diabetes (50). However, to our knowledge it has not formally been tested in people with IFG. C-peptide concentrations were higher in the IFG than in the NFG subjects in the fasting state but were essentially completely suppressed by somatostatin during the preprandial and prandial insulin infusions, resulting in portal insulin concentrations that closely approximated those in the peripheral circulation. This clearly will not be the case under conditions of daily living, when portal insulin concentrations will be higher than peripheral concentrations and will rapidly change, particularly after food ingestion. Therefore, the actual contribution of hepatic and extrahepatic insulin resistance to fasting and postprandial hyperglycemia in people with IFG will likely vary considerably depending on the amount of residual insulin secretion, the severity of insulin resistance, and the type of food eaten.

In summary, the present study establishes that people with IFG have hepatic insulin resistance that is due to, at least in part, impaired insulin-induced suppression of gluconeogenesis. The defect appears to be mild, since EGP is rapidly suppressed by the rising insulin and glucose concentrations that occur within a few minutes after eating. On the other hand, insulin-induced stimulation of glucose disposal was reduced at insulin concentrations up to ~ 150 pmol/l, indicating that extrahepatic insulin resistance, by lowering glucose disposal at insulin concentrations throughout the physiologic range, likely contributes to the pathogenesis of postprandial as well as fasting hyperglycemia. The defects in insulin action in IFG subjects were associated with impaired insulin-induced suppression of plasma FFA, implying but not proving a causal role. These data indicate that hepatic insulin resistance occurring in people with IFG is due to, at least in part, abnormal regulation of gluconeogenesis and likely contrib-

utes to the eventual development of overt diabetes in susceptible individuals.

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