

Contribution of Hepatic and Extra-Hepatic Insulin Resistance to the Pathogenesis of Impaired Fasting Glucose: Role of Increased Rates of Gluconeogenesis

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Abstract

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; “pre-prandial”) or within thirty minutes 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 in IFG and NFG subjects implying hepatic and extra-hepatic insulin resistance. This was confirmed during “pre-prandial” insulin infusion when glucose disposal was lower ($p<0.05$) and EGP higher ($p<0.05$) in IFG than NFG subjects. Higher EGP was increased ($p<0.05$) rates of gluconeogenesis in IFG. EGP was comparably suppressed in IFG and NFG groups during “prandial” insulin infusion indicating hepatic insulin resistance was mild. Glucose disposal remained lower ($p<0.01$) in IFG than NFG subjects.

Conclusions: Hepatic and extra-hepatic insulin resistance contribute to fasting hyperglycemia in IFG with 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 thirty minutes of eating, extra-hepatic (but not hepatic) insulin resistance coupled with accompanying defects in insulin secretion is the primary cause of postprandial hyperglycemia.

Keywords: Prediabetes, fasting hyperglycemia, endogenous glucose production, gluconeogenesis, glycogenolysis

Abbreviations: IFG: impaired fasting glucose; NFG: normal fasting glucose; EGP: endogenous glucose production, OGTT: oral glucose tolerance test; FFA: free fatty acid concentrations

People with impaired fasting glucose, (IFG: fasting glucose 100 to 125 mg/dl) have a ~30 percent chance of developing diabetes over the next ten 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 OGTT 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 compared to individuals with normal fasting glucose 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 since the insulin concentrations achieved during the “low” dose insulin infusion used in that study to assess hepatic insulin action averaged ~900 pmol/l which 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 likely is relatively mild and therefore primarily impacts on 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.

In order 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 “pre-prandial”) or within thirty minutes 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 contribute 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 “pre-prandial” insulin infusion.

Research Design and Methods

Subjects

Characteristics of 31 subjects with impaired fasting glucose (IFG) and 28 subjects with normal fasting glucose (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 between 100 and 126 mg/dl, BMI between 20 and 40 kg/m², age between 40 and 70 years and were otherwise healthy. As previously reported (12,27) subjects were studied on three occasions in random order in which they ingested either a mixed meal, 75 grams of glucose or underwent a hyperinsulinemic euglycemic clamp during which they received either a 0.25 mU/kg/min (referred to as the “pre-prandial”) or 0.5 mU/kg/min (referred to as the “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.

Experimental design

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 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. After sampling blood for baseline enrichments, 1.67 mg per kg weight of body water ²H₂O was given in three divided doses at 1800, 2000 and 2200. Small sips of water (containing ²H₂O) were permitted overnight. At 0600, 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 mmol/l divided by 5.5 mmol/l times 12 μCi) continuous (0.12 μCi/min) infusion of [3-³H] glucose (New England Nuclear, Boston, MA) was started at 0700 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 (i.e., time 0) for 4 hours. An infusion of insulin also was started at a rate respectively of 0.25 mU.kg⁻¹.min⁻¹ or 0.50 mU.kg⁻¹.min⁻¹ in the subjects receiving either a “pre-prandial” (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 hours of the study as previously described (28). In addition, the basal [$3\text{-}^3\text{H}$] 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 [$3\text{-}^3\text{H}$] glucose specific activity. (IFG: 0-60 min: 90%, 60-240 min: 40%; NFG: 0-60 min 80%, 60-240 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). 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 percent body fat and visceral fat as previously described (31). Plasma [$3\text{-}^3\text{H}$] 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 et al (32-34).

Calculations

The mean concentrations and rates from -30 to 0 minutes and 210-240 minutes 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 \pm 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 (Figure 1)

Fasting glucose concentrations were higher ($p < 0.001$) in the IFG than 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 vs. 5.1 ± 0.1 mmol/l and 5.5 ± 0.1 vs. 5.0 ± 0.1 mmol/l respectively during the final thirty minutes of the "pre-prandial" and "prandial" insulin infusions.

Fasting insulin concentrations were higher ($p < 0.001$) in the IFG than the NFG subjects (48 ± 5 vs. 27 ± 2 pmol/l). Despite identical infusion rates, insulin concentrations during the final thirty minutes of the "pre-prandial" insulin infusion were higher ($p < 0.01$) in the IFG than NFG subjects (79 ± 4 vs. 60 ± 3 pmol/l) indicating lower rates of insulin

clearance. On the other hand, insulin concentrations did not differ in the IFG and NFG subjects during the “prandial” insulin infusion (147 ± 9 vs. 134 ± 11 pmol/l).

Fasting C-peptide concentrations were higher ($p < 0.001$) in the IFG than the NFG subjects (0.57 ± 0.03 vs. 0.40 ± 0.02 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 “pre-prandial” (0.06 ± 0.00 vs. 0.04 ± 0.00 nmol/l) and “prandial” (0.04 ± 0.00 vs. 0.03 ± 0.00 nmol/l) insulin infusions.

Fasting glucagon concentrations did not differ in IFG and NFG groups (70 ± 5 vs. 64 ± 4 pg/ml). Glucagon infusion, started at time zero, resulted in constant and comparable plasma glucagon concentrations during both the “pre-prandial” (70 ± 4 vs. 63 ± 3 pg/ml) and “prandial” (65 ± 3 vs. 63 ± 4 pg/ml) insulin infusions.

Glucose infusion rate and glucose specific activity (Figure 2)

The glucose infusion rates required to maintain euglycemia were lower ($p < 0.01$) in the IFG than NFG subjects during the final thirty minutes of both the “pre-prandial” (8 ± 3 vs. 21 ± 3 $\mu\text{mol/kg/min}$) and “prandial” (17 ± 3 vs. 32 ± 4 $\mu\text{mol/kg/min}$) insulin infusions documenting the presence of insulin resistance. Of note, in contrast to all NFG subjects, 7 of 15 IFG subjects did not require any exogenous glucose during the “pre-prandial” 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 (Figure 3)

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 $\mu\text{mol/kg/min}$) implying hepatic insulin resistance. This was confirmed during the “pre-prandial” insulin infusion when endogenous insulin secretion was suppressed and similar portal insulin and glucagon concentration 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 $\mu\text{mol/kg/min}$). 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 $\mu\text{mol/kg/min}$).

As with EGP, glucose disappearance following an overnight fast did not differ in IFG and NFG subjects (15.3 ± 0.4 vs. 15.6 ± 0.4 $\mu\text{mol/kg/min}$). Since insulin concentrations were higher, this also implied insulin resistance. This was confirmed during the “pre-prandial” insulin infusion when glucose disappearance was lower ($p < 0.05$) in the IFG group than the NFG group (18.6 ± 2.6 vs. 28.8 ± 2.7 $\mu\text{mol/kg/min}$). However, in contrast to EGP, glucose disappearance also was lower ($p < 0.01$) in the IFG than NFG group during the “prandial” insulin infusion (23.0 ± 2.2 vs. 35.2 ± 3.8 $\mu\text{mol/kg/min}$).

Contribution of gluconeogenesis and glycogenolysis to EGP after an overnight fast and during the “pre-prandial” insulin infusion (table 2 and Figure 4)

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). Of

note, since the rate of glucose disappearance was lower in the IFG than NFG subjects during the “pre-prandial” insulin infusion, clearance of plasma C5 glucose and C2 glucose also was lower in IFG than NFG subjects. This resulted in plasma C5 glucose and C2 glucose enrichments that were higher ($p < 0.001$) in IFG than NFG subjects (table 2).

The rate of gluconeogenesis, calculated by multiplying the plasma ratio of C5 glucose to C2 glucose times EGP (shown in figure 4 as a reference), did not differ in the IFG and NFG subjects following an overnight fast (11.2 ± 0.5 vs. $11.9 \pm 0.5 \mu\text{mol/kg/min}$). On the other hand, the rate of gluconeogenesis was higher ($p < 0.05$) in the IFG than NFG during the “pre-prandial” (7.3 ± 0.6 vs. $5.0 \pm 0.9 \mu\text{mol/kg/min}$) insulin infusion. Glycogenolysis, calculated by subtracting gluconeogenesis from EGP did not differ in IFG and NFG subjects either following an overnight fast (3.8 ± 0.3 vs. $3.8 \pm 0.5 \mu\text{mol/kg/min}$) or during the “pre-prandial” insulin infusion (2.9 ± 0.5 vs. $2.2 \pm 0.5 \mu\text{mol/kg/min}$).

Plasma FFA concentrations (Figure 5)

As with EGP, fasting plasma FFA did not differ in IFG and NFG subjects (0.57 ± 0.03 vs. $0.56 \pm 0.03 \text{ mmol/l}$) despite higher insulin concentrations implying insulin resistance. This was confirmed during the “pre-prandial” insulin infusion when plasma FFA were higher ($p < 0.05$) in IFG than NFG subjects (0.30 ± 0.06 vs. $0.12 \pm 0.02 \text{ mmol/l}$). On the other hand, FFA concentrations did not differ in IFG and NFG subjects (0.17 ± 0.03 vs. $0.13 \pm 0.02 \text{ mmol/l}$) 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 FFA correlated with both fasting EGP ($r = 0.36$; $P < 0.005$) and 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 “pre-prandial” ($r = 0.67$; $P < 0.001$) 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 “pre-prandial” ($r = 0.76$; $P < 0.001$ 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 “pre-prandial” insulin infusion.

Discussion

The data from the present study establish that people with IFG have hepatic insulin resistance that is due to impaired insulin induced suppression of EGP due, at least in part, to 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 “pre-prandial” and “prandial” insulin infusion providing strong experimental support for involvement of extra-hepatic 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 again are 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 beta 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 were slightly but significantly higher in the IFG than 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) then 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 “pre-prandial” infusion. However, since fasting insulin concentrations differed in the IFG and NFG group, this represented an ~ 2.2 fold increase in peripheral insulin concentrations in the IFG group and an 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 NFG subjects during the “pre-prandial” 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 “pre-prandial” insulin infusion. Taken together, the data from the present study conducted in Caucasians and those from Weyer et al conducted in Pima Indians (14) indicate that people with IFG have hepatic insulin resistance.

Insulin also indirectly inhibits EGP by lowering plasma FFA (42,43). Somatostatin resulted in near complete suppression of C-peptide in both groups documenting effective inhibition of endogenous hormone secretion. Since the “pre-prandial” insulin infusion was designed to approximate portal insulin concentrations, it resulted in an increase in peripheral insulin concentrations which in turn caused plasma FFA to fall in both groups. However, suppression of FFA was blunted in the IFG subjects indicating insulin resistance. Since elevated FFA

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 “pre-prandial” insulin infusion. Additional studies will be required to specifically address this hypothesis.

We did not use a 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 “pre-prandial” 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 are both impaired in people with IFG and therefore suggest that future studies determining whether agents that lower FFA 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 “pre-prandial” insulin infusion (32). The contribution of gluconeogenesis and glycogenolysis to EGP following an overnight fast did not differ in IFG and NFG subjects whether expressed as percent or 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 “pre-prandial” portal concentrations, rates of gluconeogenesis were greater in IFG than NFG subjects accounting for the associated increase in EGP. Due to the

feed back 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 relatively large amounts of exogenous glucose that were required to maintain euglycemia. Therefore we do not know if 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 also is 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 if regulation of glycogenolysis also is abnormal in people with IFG.

Insulin induced stimulation of glucose disposal also was impaired in IFG subjects. However, in contrast to EGP, glucose disposal was lower in the IFG than NFG subjects during both the “pre-prandial” 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 if 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 minutes of eating a mixed meal or drinking 75 grams of 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 NFG subjects during the six hours after meal ingestion indicating 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 "pre-prandial" insulin infusion, the IFG subjects have hepatic as well as extrahepatic insulin resistance. As discussed above, since the hepatic insulin resistance is relatively mild, its contribution to postprandial hyperglycemia appears to be minimal since the post-prandial increase in glucose and insulin concentrations that occurs after eating results in rapid and near complete suppression of glucose production (12). This differs from

situation present 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 beta 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 both were greater in the IFG than 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 both were greater in the IFG than NFG lends further support to the concept that adiposity and its associated metabolic abnormalities contributes 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 NFG subjects during the pre-prandial 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 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 to 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 subjects than NFG subjects in the fasting state but were essentially completely suppressed by somatostatin during the “pre-prandial” 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 extra-hepatic insulin resistance to fasting and postprandial hyperglycemia in people with IFG likely will 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, at least in part, to 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 extra-hepatic 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 occurs in people with IFG, is due at least in part to abnormal regulation of gluconeogenesis, and likely contributes to the eventual development of overt diabetes mellitus in susceptible individuals.

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Table 1
Subject Characteristics

	NFG	IFG
Gender (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*
TBW (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*
2hr pp (mmol/l)	7.8±0.3	9.7±0.4*

Values are means ± SE. M: male; F: female; BMI: body mass index; TBW: total body weight, FPG: fasting plasma glucose at time of screening; 2hr pp: 2 hour oral glucose tolerance test value.

* P<0.05 vs. NFG

Table 2
 Plasma C5 glucose and C2 glucose enrichment after an overnight fast and during the “pre-prandial” 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
“Pre-prandial”			
NFG	0.18±0.02	0.28±0.04	0.70±0.05
IFG	0.33±0.02*	0.47±0.03*	0.72±0.03

Values are means ± SE. *P<0.001 vs. NFG

Legends

Figure 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 as pre-prandial and $0.5 \text{ mU}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ referred as prandial in the NFG and IFG group.

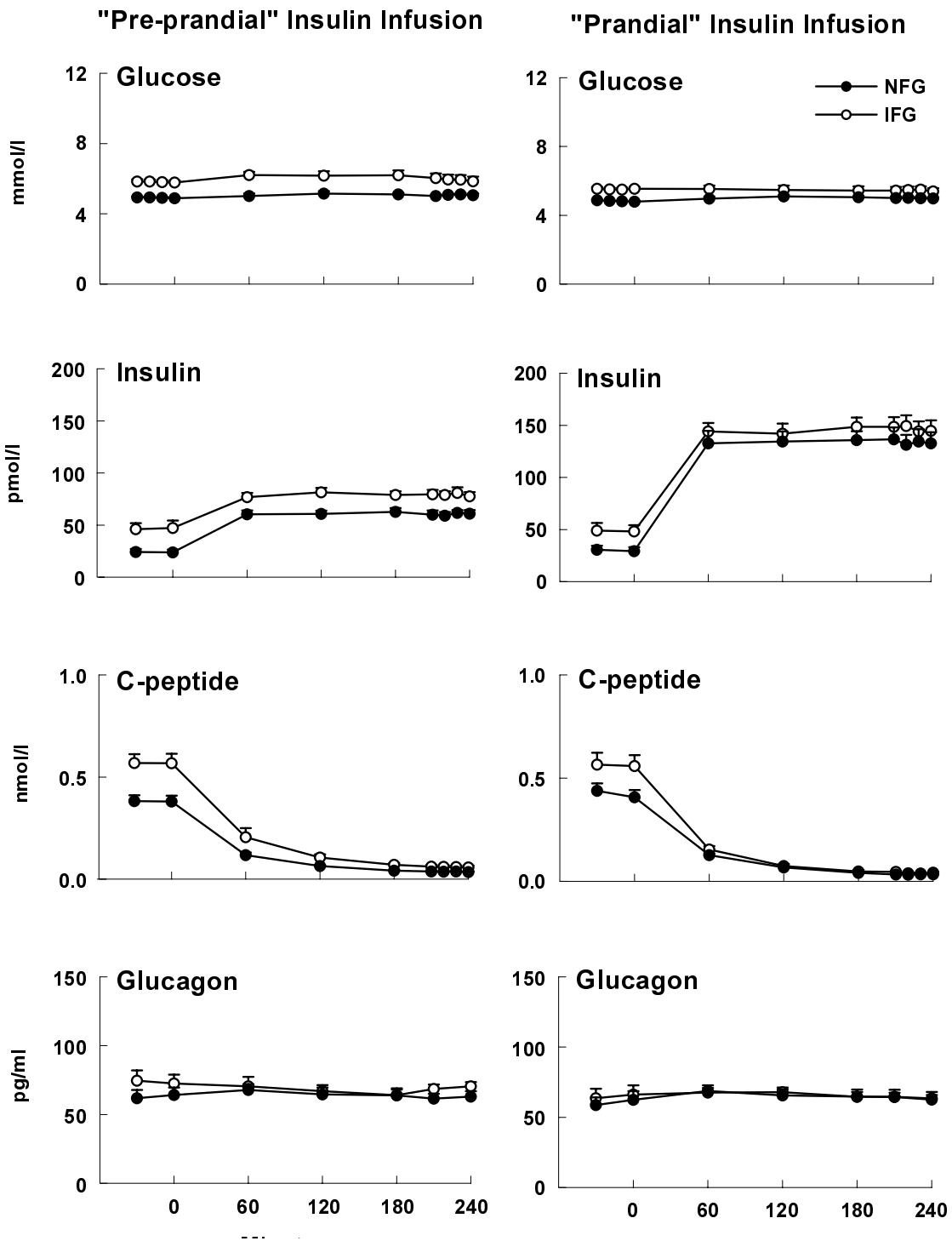


Figure 2.

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 as pre-prandial (right side) and 0.5 mU.kg⁻¹.min⁻¹ referred as prandial (left side) in the NFG and IFG group.

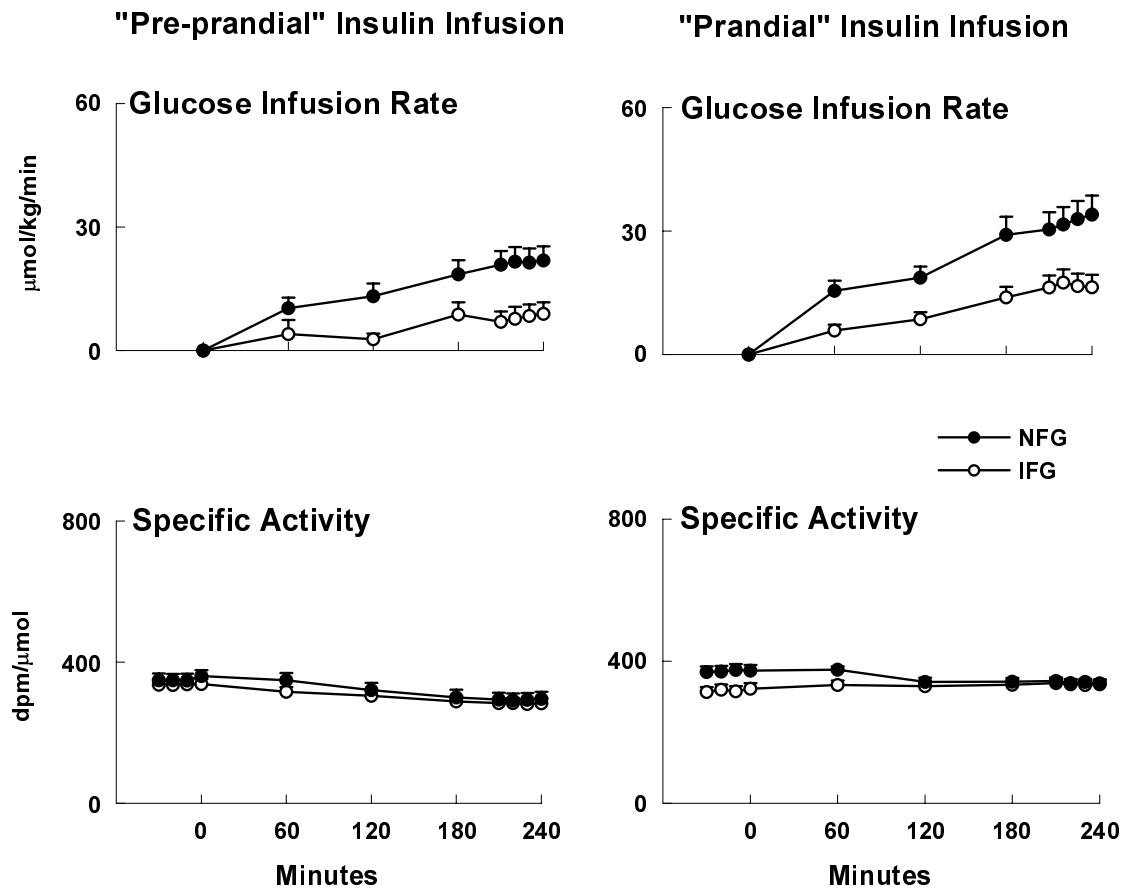


Figure 2

Figure 3.

Rates of EGP and rates for glucose disappearance observed before insulin infusion in all subjects with IFG or NFG and during pre-prandial insulin replacement in one group and prandial insulin replacement in the other group.

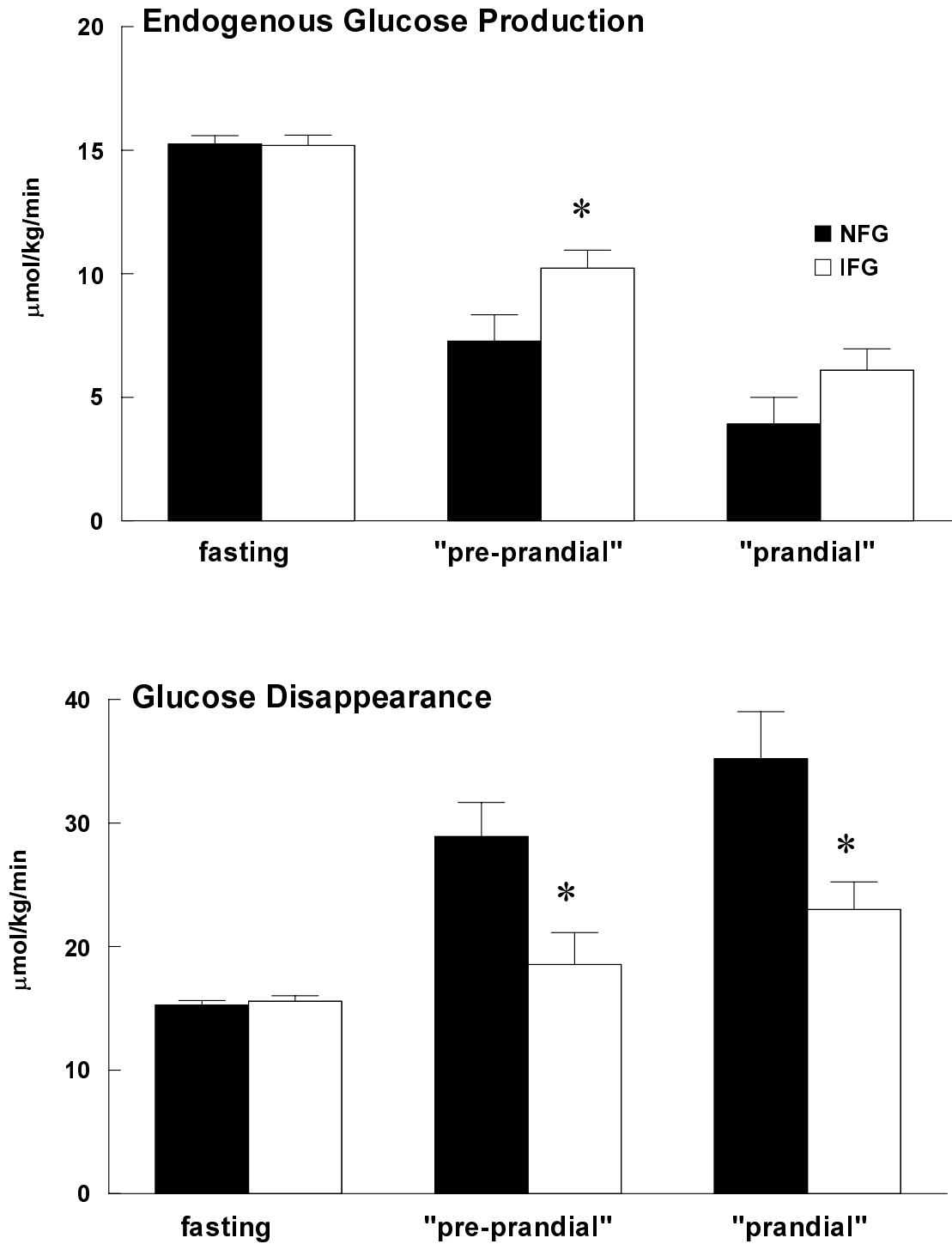


Figure 3

Figure 4.
Rates of EGP, gluconeogenesis and glycogenolysis observed before insulin infusion and during pre-prandial insulin replacement.

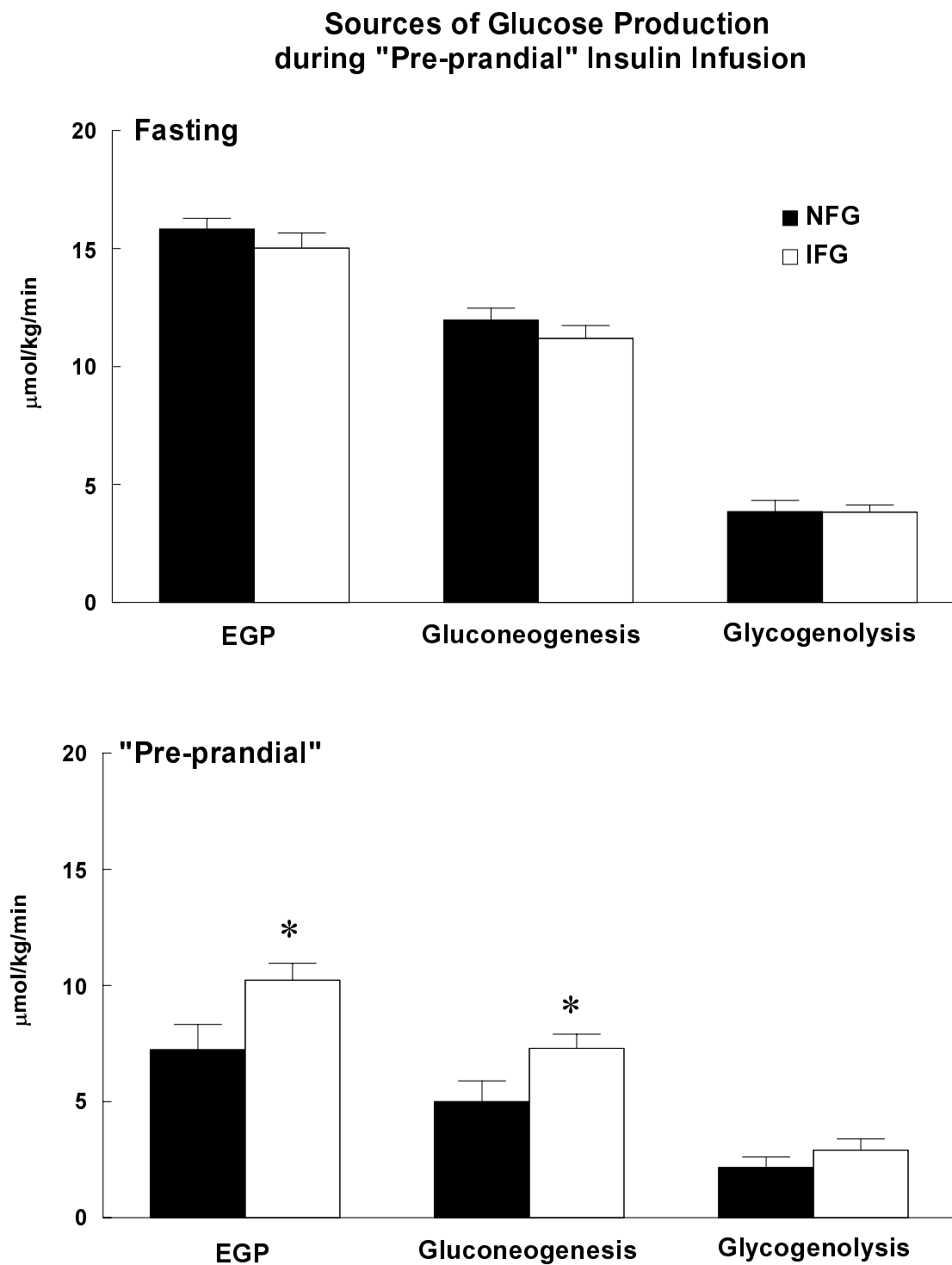


Figure 4

Figure 5.

Plasma FFA concentrations observed before insulin infusion in all subjects with IFG or NFG and during pre-prandial and prandial insulin replacement.

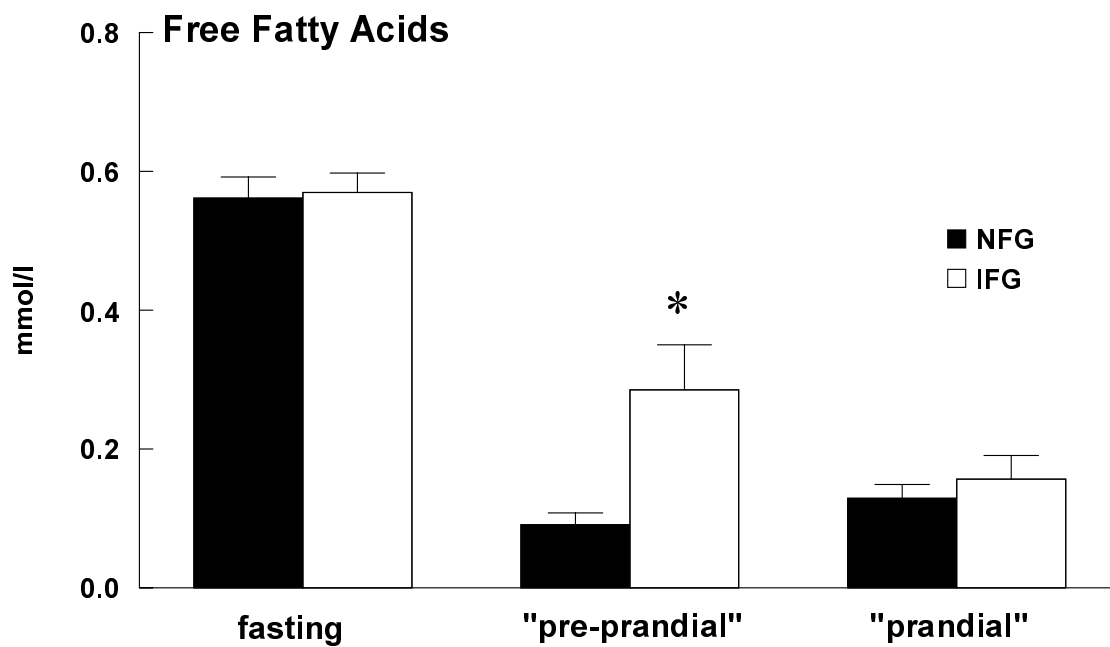


Figure 5