Circulating Fibroblast Growth Factor-21 (FGF-21) is Elevated in Impaired Glucose Tolerance and Type 2 Diabetes and Correlates with Muscle and Hepatic Insulin Resistance

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**Objective:** FGF-21 is highly expressed in the liver and regulates hepatic glucose production and lipid metabolism in rodents. However, its role in the pathogenesis of T2DM in humans remains to be defined. The aim of this study was to quantitate circulating plasma FGF-21 levels and examine their relationship with insulin sensitivity in subjects with varying degrees of obesity and glucose tolerance.

**Research Design and Methods:** Forty one (41) subjects; 8 lean normal-glucose-tolerant (NGT), 9 obese NGT, 12 impaired fasting glucose/impaired glucose tolerance (IFG/IGT), and 12 T2DM subjects received an oral glucose tolerance test (OGTT) and euglycemic hyperinsulinemic clamp (80 mU/m²•min) combined with 3-[³H] glucose infusion.

**Results:** T2DM, IGT and obese NGT subjects were insulin resistant compared to lean NGT subjects. Plasma FGF-21 levels progressively increased from 3.9±0.3 ng/ml in lean NGT subjects to 4.9±0.2 in obese NGT subjects to 5.2±0.2 in IGT and to 5.3 ± 0.2 in T2DM subjects. FGF-21 levels correlated inversely with whole body (primarily reflects muscle) insulin sensitivity (r=-0.341, p=0.029) and directly with the hepatic insulin resistance index (r=0.432, p=0.028). FGF-21 levels also correlated with measures of glycemia: FPG (r=0.312, p=0.05), 2-h plasma glucose (r=0.414, p=0.01), and HbA₁c (r=0.325, p=0.04).

**Conclusion:** Plasma FGF-21 levels are increased in insulin resistant states and correlate with hepatic and whole body (muscle) insulin resistance. FGF-21 may play a role in pathogenesis of hepatic and whole body insulin resistance in T2DM.
Fibroblast growth factors (FGFs) represent a group of peptides that regulate diverse biological functions including cell differentiation, cell growth and angiogenesis (1; 2). Recently, a subfamily of FGFs that interact with nuclear receptors has been identified, that play an important role in liver, bone and adipose tissue metabolism (3; 4). This subfamily contains FGF-19 which regulates energy expenditure (5; 6), FGF-23 that regulates phosphate metabolism and excretion (7), and the recently described FGF-21 which regulate glucose homeostasis (8; 9).

FGF-21 is a novel protein that has been implicated in the regulation of lipid and glucose metabolism under fasting and ketotic conditions (9; 10). In murine models FGF21 was reported to be expressed predominantly in liver (11), but its expression has also been reported in adipose tissue and pancreatic beta cells (12). In a primate model of diabetes, Kharitonenkov et al (9) reported a reduction in plasma glucose, insulin, triglyceride, LDL cholesterol and HDL cholesterol levels following 6 weeks of recombinant FGF-21 administration. In diet-induced obese mice, FGF-21 reversed hepatic steatosis and improved insulin sensitivity (13). In adipose tissue, FGF21 was shown to increased glucose uptake (9). Based on these observations FGF-21 has been proposed as a potential therapeutic agent for T2DM in humans (14). However, few studies in humans have examined the relationship between FGF-21 and glucose/lipid metabolism. Chen et al reported that patients with newly diagnosed T2DM had significantly higher plasma FGF-21 concentrations than non-diabetic controls, and FGF-21 negatively correlated with fasting plasma glucose and BMI (15). More recently, Zhang et al found that FGF-21 concentrations are elevated in obese nondiabetic individuals compared to lean healthy controls and that the circulating correlated positively with adiposity and fasting insulin and negatively with HDL cholesterol (16). Conversely, in patients with anorexia nervosa plasma FGF-21 concentrations are decreased and increased following weight gains (17). In the present study, we examined the relationship between plasma FGF-21 concentrations and direct measurements of peripheral and hepatic insulin sensitivity in subjects with varying degrees of obesity and glucose tolerance.

RESEARCH DESIGN AND METHODS

Forty-one subjects participated in the study: 8 lean normal glucose tolerant (NGT), 7 obese NGT, 13 impaired glucose tolerant (IGT) and 13 T2DM. All subjects were in good general health based on medical history, physical examination, screening blood chemistry and hematologic tests, urinalysis, and electrocardiogram. Weight was stable in all subjects (±2 lb) for at least 3 months before the study. None of the subjects participated in any heavy exercise, and they were instructed not to engage in any vigorous exercise for at least 3 days before the study. None of the non-diabetic subjects were taking medications known to affect lipid glucose metabolism. Subjects who ever had received insulin or thiazolidinediones were excluded. Each study volunteer received: i) oral glucose tolerance test (OGTT) and ii) hyperinsulinemic euglycemic insulin clamp with 3-[3H]-glucose to examine both hepatic and peripheral (primarily reflects muscle) insulin sensitivity. The purpose, nature, and potential risks of the study were explained to all subjects, and written voluntary consent was obtained before their participation. All research procedures were approved by the Institutional Review Board of University of Texas Health Science Center at San Antonio.

OGTT: Baseline blood samples for determination of plasma glucose, free fatty acid (FFA), insulin, and C-peptide
concentrations were drawn at -30, -15, and 0 min. At time zero subjects ingested 75 grams of glucose in 300 ml orange-flavored water, and plasma glucose, FFA and insulin were measured at 15-min intervals for 2 h.

Hyperinsulinemic Euglycemic Clamp: All studies were conducted in the General Clinical Research Center of UTHSCSA and began at 0700 h after a 12-h overnight fast. A prime (25 µCi)-continuous (0.25 µCi/min) infusion of 3-[³H]-glucose was started, and 2 h (3 h for diabetic subjects) were allowed for isotopic equilibration. In T2DM, the priming dose of tritiated glucose was increased in proportion to the increase in fasting plasma glucose concentration. At the end of the tracer equilibration period, a primed-continuous insulin infusion (80 mU/m²•min) was started and plasma glucose was measured every 5 min. Based on the negative feedback principle, a variable infusion of 20% glucose was adjusted to maintain plasma glucose concentration constant at each subjects fasting glucose level in the control group (18). In diabetic subjects, plasma glucose concentration was allowed to decrease to 100 mg/dl, at which level it was maintained.

Analytical Determinations: Plasma glucose was measured at bedside with the glucose oxidase method (Beckman Instruments, Fullerton, CA). Plasma insulin concentration was measured by radioimmunoassay (Diagnostic Products, Los Angeles, CA). Tritiated glucose specific activity was determined on deproteinized plasma samples as previously described (19). Plasma FFA concentration was determined by an enzymatic calorimetric quantification method (Wako Chemicals, Nuess, Germany). Plasma FGF-21 concentrations were measured by radioimmunoassay (Phoenix Pharmaceuticals Inc, Burlingam, CA) at baseline in duplicate from plasma collected prior to start of the euglycemic insulin clamp. This assay has been reported to cross-react specifically with human FGF-21 (100%). The intra and inter-assay CV were 3.6 % and 3.9 %, respectively. Serum creatinine was measured with an automated enzymatic assay and GFR was estimated with the Cockcroft-Gault formula (20).

Calculations: Under steady-state postabsorptive conditions, the rate of endogenous glucose appearance (Ra) was calculated as the 3-[³H] glucose infusion rate (dpm/min) divided by the steady-state plasma 3-[³H] glucose specific activity (dpm/mg). During the euglycemic insulin clamp, the rate of whole-body glucose appearance (Ra) was calculated with Steele’s equation (21) using a distribution volume of 250 ml/kg. EGP was calculated by subtracting the exogenous glucose infusion rate from Ra. The rate of insulin-mediated whole-body glucose disposal (Rd) was determined by adding the rate of residual EGP to the exogenous glucose infusion rate. In the post absorptive state, fasting plasma insulin is the primary determinant of endogenous glucose production (22). The hepatic insulin resistance index was calculated as the product of EGP and fasting plasma insulin concentration (23). Similarly, since fasting insulin concentration is the most important regulator of fasting plasma FFA concentration, adipocyte insulin resistance was calculated as the product of fasting plasma FFA and fasting plasma insulin concentration (24).

Statistical Analysis: Data was expressed as mean ± SEM, unless otherwise specified. SPSS ver. 15 statistical package (Chicago, IL) was used for all calculations. Pearson’s or Spearman’s correlations were used to examine the relationship between plasma FGF-21 levels and markers of insulin sensitivity, as well as with anthropometric parameters. ANOVA with post hoc analysis with Bonferroni correction was used to
compare significant differences between groups.

RESULTS

Study population and clinical characteristics: T2DM subjects were slightly, but not significantly older than NGT subjects. BMI was similar in obese NGT, IGT and T2DM subjects. T2DM individuals had significantly higher fasting plasma glucose, plasma insulin and triglycerides and HbA1c compared to lean NGT subjects (Table 1). T2DM had significantly lower HDL cholesterol concentration compared to NGT. Subjects with IGT and T2DM had significantly lower whole body (primarily muscle) glucose uptake compared to lean NGT subjects (Table-1). Hepatic insulin resistance (EGP × fasting Insulin) in obese NGT subjects was slightly but not significantly elevated compared to NGT subjects. However IFG/IGT and T2DM individuals displayed significantly greater hepatic insulin resistance (p<0.05). Similarly, the adipocyte insulin resistance (FFA × fasting Insulin) was significantly higher in subjects with IFG/IGT and T2DM.

Plasma FGF-21 changes in relation to glucose tolerance: Plasma of FGF-21 was higher in obese NGT versus lean NGT subjects (4.92±0.17 vs 3.88±0.30 ng/ml, p=0.04). Subjects with IGT (5.22±0.23 ng/ml p<0.05 vs. lean NGT) and T2DM (5.27 ±0.23, p<0.05 vs. lean NGT) also had increased plasma (Figure-1). Plasma FGF-21 concentration correlated with HbA1c (r=0.325, p=0.04), FPG (r=0.312, p=0.05), 2-h glucose (r=0.414, p=0.01). There was a direct association between and BMI (r=0.456, p<0.001) in the entire group. A recent report demonstrated elevated plasma FGF-21 levels in patients with chronic kidney disease (25). We did not observe any correlation between plasma FGF-21 and either GFR (r=0.089, p=ns) or serum creatinine (r=0.277, p=0.08).

Relationship between FGF-21 and whole body and hepatic insulin resistance: The insulin-stimulated rate of glucose disposal (Rd) correlated inversely with plasma FGF-21 concentration (-0.421, p<0.01). A positive correlation also was observed between FGF-21 level and hepatic insulin resistance index (0.344, p=0.02) and adipocyte insulin resistance index (0.318, p=0.045).

DISCUSSION

FGF-21 was discovered during a high throughput assay for secreted proteins which increased glucose uptake in 3T3L-1 adipocytes (9). Subsequent studies showed that administration of recombinant FGF-21 in rodent models of diabetes and in diabetic rhesus monkeys improved the blood glucose and the lipid profile (4; 9; 13). However, in none of these studies were the plasma levels of FGF-21 measured.

In the present study, we demonstrate that plasma FGF-21 levels are elevated in insulin resistant states (obesity, IGT/IFG, T2DM) and are inversely correlated with both peripheral and hepatic insulin sensitivity. This is consistent with two other reports in humans which demonstrated elevated plasma FGF-21 concentration in obesity, IGT and T2DM (15; 16). The novelty of our study is that we demonstrate for the first time in human, that the increase in plasma FGF-21 levels are strongly correlated with the severity of whole body (primarily reflects muscle) and hepatic insulin resistance.

Our study is in agreement with two previous studies in Asians in which increased plasma FGF-21 levels were observed in newly diagnosed, drug naïve diabetic subjects and in treated T2DM subjects (26). In a Chinese population, plasma FGF-21 levels correlated with markers of the insulin resistance (metabolic) syndrome (16). However, this later study did not measure either hepatic or peripheral insulin sensitivity.
In rodent models FGF-21 stimulates glucose uptake in 3TL3 adipocytes and increases GLUT-4 expression in adipocytes. Arner et al has demonstrated that human FGF-21 inhibits lipolysis in human adipocytes and suggested that this may contribute to the protein’s insulin sensitizing effect in humans (27). A synergistic interaction has been described between FGF-21 and rosiglitazone to stimulate glucose uptake (28). Contrary to these observations, in the present study plasma FGF-21 concentrations were positively correlated with adipocyte insulin resistance. With regard to the liver, in animal models FGF-21 has been shown to be expressed primarily in liver and its glucose lowering effects of FGF-21 has been suggested to be mediated by its actions on liver (9; 11). In contrast, in the present study we demonstrate a positive correlation between elevated FGF-21 levels and hepatic insulin resistance.

The apparently divergent results of the current study in humans and previous studies in animals could reflect a true species difference in the metabolic effects of FGF-21 in man versus animals or may be less contradictory than they appear. Thus, the elevated plasma FGF-21 levels in insulin resistant states may simply reflect a compensatory response to offset the peripheral and/or hepatic insulin resistance and not be a cause of the insulin resistance. Since our observations are cross-sectional in nature, it is not possible to establish a cause and effect relationship, i.e. what is primary and what is secondary. It also is not possible to distinguish whether the increased plasma FGF-21 levels in obese, IGT/IFG, and T2DM subjects is related to the insulin resistance or obesity, since all three groups had similarly elevated FGF-21 levels. Further studies will be required to further elucidate the role of FGF-21 in glucose homeostasis and whether FGF-21 will sensitize target issues (liver, adipocytes, muscle) to insulin, as has been reported in animal models of diabetes.

Recent studies have suggested that plasma FGF-21 concentrations are affected by the glomerular filtration rate and therefore may be related to the level of renal function (25). Patients undergoing dialysis have significantly increased plasma FGF-21 levels compared to control subjects and this is independent of the glucose/lipid metabolic status (25). Although none of the participants enrolled in our study had impaired renal function, GFR spanned a wide range. Nonetheless, we did not find a significant relationship between plasma FGF-21 concentration and estimated GFR. Thus, in our sample FGF-21 concentrations are unlikely to be affected by this parameter.

In summary, elevated plasma FGF-21 concentrations in humans appear to be related to the presence of hepatic and peripheral insulin resistance. Whether the increase in plasma FGF-21 represents a compensatory effect to offset the insulin resistance or is a causative factor in the development of insulin resistance is to be determined.

**Figure Legends**

*Figure 1.* Plasma FGF-21 concentration in lean NGT, obese NGT, IGT, and T2DM subjects. Data are mean ± SEM. * p<0.05

*Figure 2.* Correlation between plasma FGF-21 concentration and insulin-stimulated glucose disposal during the hyperinsulinemic euglycemic clamp.
Figure 3. Correlation between plasma FGF-21 concentration and hepatic insulin resistance index

REFERENCES:


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<th>Lean NGT (n=8)</th>
<th>Obese NGT (n=9)</th>
<th>IFG/IGT (n=12)</th>
<th>T2DM (n=12)</th>
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<td><strong>Age (y)</strong></td>
<td>40±5</td>
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<td><strong>Systolic BP (mmHg)</strong></td>
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<td><strong>HbA1c (%)</strong></td>
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<td><strong>FPG (mg/dl)</strong></td>
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<td>96±3</td>
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<td><strong>2-h Glucose (mg/dl)</strong></td>
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<td>102 ± 7</td>
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<td><strong>FPI (µU/ml)</strong></td>
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<td><strong>F-FFA (µEq/l)</strong></td>
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<td>585±36*</td>
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<td><strong>Rd (mg/kg•min)</strong></td>
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<td><strong>Hepatic Insulin Resistance Index</strong></td>
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<td><strong>Adipocyte Insulin Resistance Index</strong></td>
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<td>5.4±1.2*†</td>
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Data are expressed as mean ± SEM. BP= Blood Pressure; FPG= fasting plasma glucose; FPI= fasting plasma insulin; FFA= fasting plasm free fatty acid; Rd= insulin-stimulated rate of glucose disposal; HOMA-IR = HOMA insulin resistance; * = p<0.05 vs. Lean NGT; † = p<0.05 vs. Obese NGT.
Figure 1

**Bar Graph**

- NGT Lean
- NGT Obese
- IGT
- T2DM

FGF-21 (ng/ml)

**Legend:**
- * indicates statistical significance.

**Figure 1**

**Scatter Plot**

- FGF-21 (ng/ml) vs. Rd (mg/kg.min⁻¹)

- Correlation coefficient: \( r = -0.421 \)
- p-value: \( p = 0.007 \)
Figure 2

![Graph showing the relationship between Hepatic Insulin Resistance Index and FGF-21 (ng/ml). The correlation coefficient (r) is 0.344 and the p-value is 0.034.](image-url)

- Hepatic Insulin Resistance Index
- FGF-21 (ng/ml)