Low-Dose Oral Glyburide Reduces Glucose Production Rates in Patients With Impaired Fasting Glucose

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Impaired fasting glucose (IFG) is commonly seen in the US population. Approximately 20% of patients with IFG can progress to develop type 2 diabetes mellitus (DM-2) within 1 year. In the recent diabetes prevention study, lifestyle changes reduced the progression to only 8% per year, and metformin reduced the progression from IFG to DM-2 from 20% to 11% per year. Sulfonylurea therapy in DM-2 increases β-cell function and fails to accelerate the 4% loss in function observed per year. Low-dose sulfonylurea therapy for IFG may be an effective treatment to delay the onset of type 2 diabetes if the treatment does not cause hypoglycemia. A very low dose of glyburide (20 μg/kg body weight) was given orally to 15 nondiabetic volunteers in an attempt to describe its effects on glucose production rates (GPR), blood glucose concentrations, and conterregulatory hormone profile. Six of the volunteers had IFG (mean ± SEM, 115 ± 1.8 mg/dL), and 9 had a normal fasting glucose (NFG) (94 ± 2.3 mg/dL). Fasting blood glucose (FBG) decreased more in IFG after glyburide when compared with the NFG group (29% ± 2.4% *v* 17% ± 3.5%, *P* < .05). Patients with IFG had a larger insulin response to glyburide than those with NFG (17.7 ±3 *v* 10.7 ± 2.9 μU/mL; *P* < .05). The IFG patients also had a greater decrease in GPR (19% ± 4%) than seen with the normals (12% ± 3%, *P* < .05). The steeper decrease in GPR may have been due to a greater insulin response to oral glyburide in those with IFG. Low-dose glyburide increases insulin's effect on the liver.

IN 1997, THE American Diabetes Association (ADA) lowered the diagnostic criteria for type 2 diabetes mellitus (DM-2) to a fasting blood glucose (FBG) concentration equal to or greater than 126 mg/dL. At the same time, they adopted new criteria for the diagnosis of impaired fasting glucose (IFG), which is a FBG concentration above 109 and below 126 mg/dL on 2 occasions.[1] Patients with IFG have an increased risk for DM-2. In a recent study, the progression rate for patients with IFG was 22.3 cases/100-patient-year, which is equal to 20%/year.[2]

Recent work with lifestyle changes has resulted in a 60% reduction in the progression rate from IFG to DM-2 (20% to 8% per year). Metformin treatment reduced the progression rate to only 11% per year.[2] Lifestyle and the use of additional diabetic medications may result in a greater reduction in the progression of IFG to DM-2. Earlier work with tolbutamide delayed the onset of DM-2 in those patients who did not withdraw from the study due to severe hypoglycemia.[3] An ongoing trial is testing whether glimepiride will reduce the progression from IFG to DM-2.[4]

The purpose of this study was to test the effect of low-dose oral glyburide (20 μg/kg body weight) on blood glucose concentration, glucose production and counterregulatory hormone levels in IFG. Our overall goal was to document the short-term action of low-dose glyburide administration in patients with IFG.

MATERIALS AND METHODS

All volunteers consented to the Institutional Review Board (IRB) approved protocol and were admitted to the General Clinical Research Center (GCRC) for 3 days. Fifteen volunteers with a normal performance status and lung cancer (8 adeno, 4 small cell, and 3 squamous cell) began a supervised diet on day 1 that delivered 1.5 times the basal energy expenditure, 1.0 g of protein per kilogram body weight, and a 300-g carbohydrate intake per day. No one had a history of diabetes or liver disease. Six volunteers with IFG had an age of 57 ± 2 (mean ± SEM) years. Nine volunteers with normal fasting glucose (NFG) concentrations had an average age of 54 ± 5 years. There was no difference in weight or body mass index (BMI) between the 2 groups.

On day 2, the volunteers were given a 5-hour oral 75-g glucose tolerance test (OGTT). At baseline (6 AM) and every hour, blood measurements were obtained for glucose, insulin, and C-peptide. After the OGTT, dietary intake was a minimum of 300 g carbohydrate intake per day. This is to adequately replete glycogen stores for the next morning's test to measure fasting glucose production rates (GPR).

On day 3 after an overnight fast, a combined 100% pure 6-^{3}H-glucose (25 μCi, 14.4 μCi per hour) was started at 8 AM for an 8-hour infusion period. The catheter was placed in the right anticubital vein. Blood was drawn from an arterilized left hand vein every 30 minutes between hours 3 to 4 and 7 to 8 of the study for the measurement of plasma 6-^{3}H glucose specific activity as described previously.[5] There was no change in the steady state conditions for plasma glucose specific activities between hours 3 to 4 and 7 to 8. Specific activity changed less than 5% over the 1-hour period when GPR was determined. Therefore, steady state equations were used to determine GPR and glucose clearance.[5]

In this study, we administered a low dose of glyburide to assess its effect on blood glucose, glucose production, carbohydrate oxidation, and fat oxidation. We provided 10 μg/kg body weight (approximately 0.7 mg) as an oral administration of glyburide at hour 4 followed by 5-μg/kg body weight at hours 5 and 6. Each dose of glyburide was given with a large glass of water to promote absorption. Patients were not given food to obtain a maximal effect on FBG. The maximum effect was determined as the difference in the baseline glucose concentration and lowest obtained after taking glyburide. This was done also to evaluate the safety of this dose of glyburide in volunteers who may not always have food intake.

Blood measurements also included glucose by a glucose oxidase method and insulin, C-peptide, glucagon, GH, and cortisol concentrations by standardized radioimmunoassay methods.[5] Catecholamines

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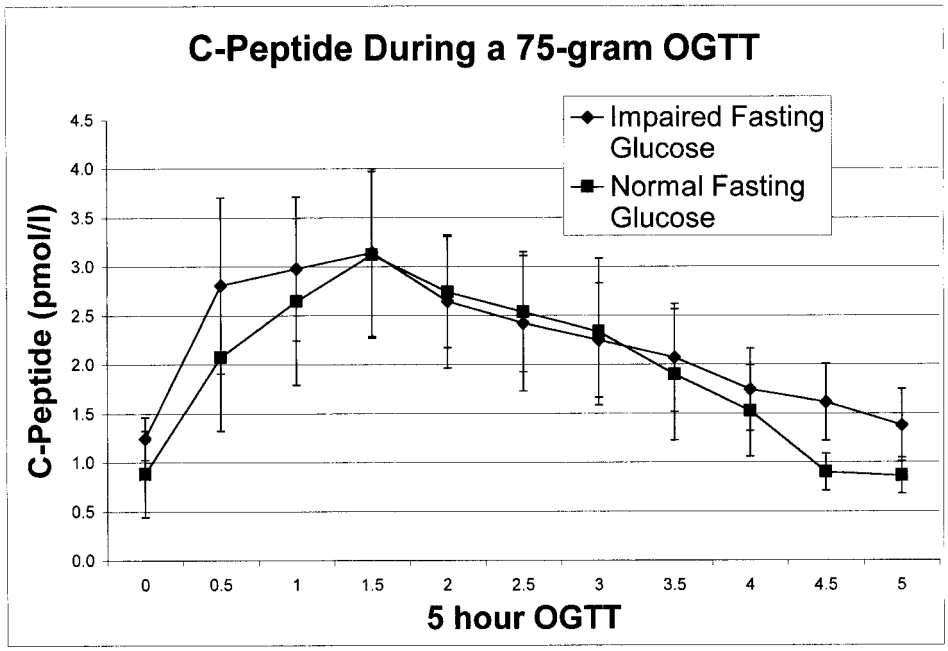

Fig 1. C-peptide response to a 75-g OGTT. Overall, the C-peptide response was similar in the IFG and NFG groups.

were performed by thin layer chromatography.[6] Resting oxygen consumption and carbon dioxide production were measured every 30 minutes from hour 3 to hour 8 using an indirect respiratory calorimetry method.[7] Oxygen and carbon dioxide concentrations were analyzed by a gas mass spectrometer (Perkin-Elmer MGA-1100, Norwalk, CT). The carbohydrate and fat oxidation were determined by indirect calorimetry.[7]

Data Analysis

Mean baseline values (hours 3 to 4) were compared with final values (hours 7 to 8) by analysis of variance (ANOVA) with significance defined at a P value less than .05. All volunteers completed the 3-day study.

RESULTS

Six of the volunteers had IFG (FBG >109 mg/dL), and 9 had a NFG. The HOMA, a marker of insulin resistance, was increased in those patients with IFG (6.1 ± 3.0 *v* 2.5 ± 0.6, $P <$ .05). During the OGTT on day 2, insulin and C-peptide secretion were similar between the 2 groups. Figure 1 demonstrates the C-peptide response between the 2 groups during a 75-gOGTT. The insulin response was similar (data not shown). The blood glucose concentrations during the OGTT are shown in Table 1. Glucose concentration was significantly greater in the IFG group at time 0 and time 30 minutes, but not at any of the other times during the OGTT.

On day 3, all patients consumed approximately 0.7 mg glyburide at hour 4 and 0.35 mg at hours 5 and 6 during an extended 8-hour study. After glyburide ingestion, IFG patients had a maximum decrease in blood glucose (29% ± 2% *v* 17% ± 4% ($P <$ .05; Fig 2). Glyburide administration increased insulin only in the IFG group within 30 minutes and remained elevated up to 2 hours after starting glyburide (Fig 3). Glyburide maintained C-peptide concentrations and prevented the normal decrease in C-peptide that occurs with extended fasting alone (Fig 4).

Glyburide administration resulted in a decrease in GPR in the IFG group (2.05 ± 0.19 to 1.65 ± 0.21; $P <$ .05) and in the NFG group (2.30 ± 0.11 to 2.03 ± 0.14 mg/kg/min). The decrease in GPR was greater for the IFG group (19.2% ± 4.1% *v* 12.2% ± 3.2%; $P <$ .05). There was a significant correlation between the percent decrease in plasma glucose and the percent decrease in GPR (r = .69; $P <$ .05).

Glucose clearance was unchanged with glyburide treatment in the IFG group (2.03 ± 0.03 to 1.98 ± 0.29 mL/kg/min) and NFG group (2.55 ± 0.15 to 2.44 ± 0.18 mL/kg/min, hours 3 to 4 and 7 to 8, respectively).

Glyburide had no effect on carbohydrate oxidation in either group (Table 2). Fat oxidation increased over time from 0.20 ± 0.08 to 0.42 ± 0.10 mg/kg/min; $P <$ .01). The increase was similar in both groups.

Two volunteers in each group reduced blood glucose to between 65 and 68 mg/dL. Plasma epinephrine concentrations doubled in both groups after glyburide administration (Fig 5),

Table 1. Blood Glucose (mg/dL) Response to a 75-g OGTT

	NFG	IFG
Time 0	101 ± 3	112 ± 7*
Time 30	157 ± 13	177 ± 5*
Time 60	174 ± 14	189 ± 11
Time 90	178 ± 13	184 ± 13
Time 120	139 ± 8	165 ± 21
Time 150	121 ± 14	136 ± 18
Time 180	108 ± 14	126 ± 16
Time 210	97 ± 7	122 ± 19
Time 240	97 ± 6	99 ± 8
Time 270	84 ± 4	91 ± 4
Time 300	81 ± 6	90 ± 4

NOTE. Data are mean ± SEM.
*$P <$ .05 *v* NFG.

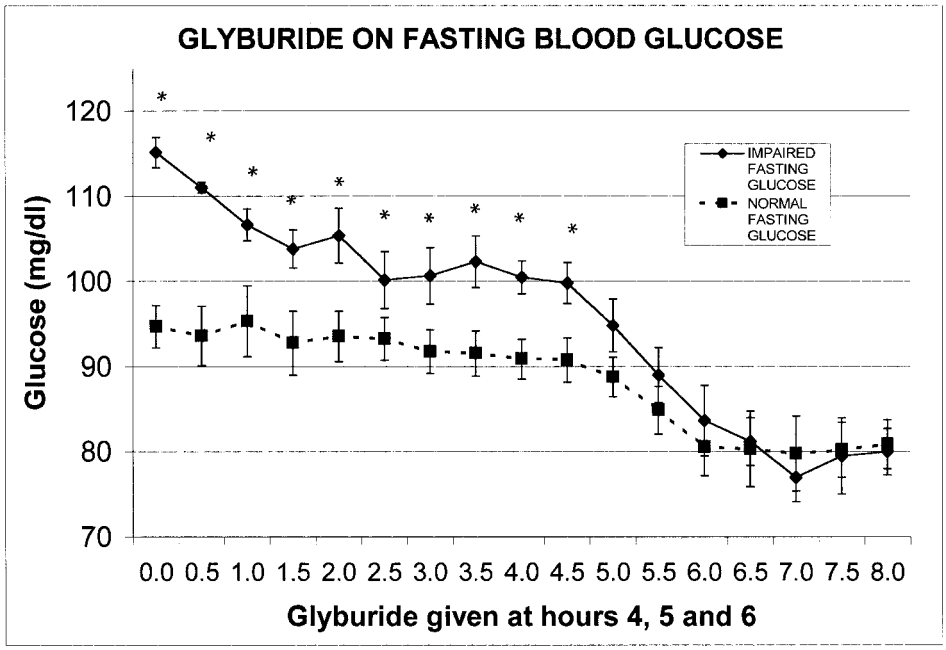

Fig 2. All volunteers fasted from 10 PM the night before until completion of the 8-hour study. This figure plots the blood glucose from hour 0 (8 AM) to hour 8 (4 PM). At hour 4 (12:00 noon), all volunteers ingested 10 μg/kg body weight glyburide. At hours 5 (1 PM) and 6 (2 PM), they ingested 5 μg/kg body weight glyburide. The dashed line indicates those patients with NFG, and the normal line is for the group with IFG. Data are listed as mean ± SEM.

but the volunteers were without hypoglycemic symptoms. Glyburide had no effect on norepinephrine, cortisol, glucagon, or growth hormone (GH) concentrations in either group (data not shown).

DISCUSSION

IFG and Coronary Artery Disease

Recently patients with IFG and coronary artery disease (CAD) have been found to have a similar increased risk for mortality as seen in type 2 diabetes mellitus with CAD (20% *v* 24%).[8] Patients with IFG may need to be treated as if they have the same risk for CAD as patients with type 2 diabetes mellitus. Individuals with type 2 diabetes mellitus have a 5.8-fold increased risk for myocardial infarction as compared with nondiabetic individuals.[9]

Developing new treatment strategies for patients with IFG is also important because 20% of patients with IFG progress to type 2 diabetes mellitus per year.[2] Metformin alone can reduce the incidence of diabetes from 20% to 11%.[2] Sulfonylurea treatment may prevent the progression to type 2 diabetes in

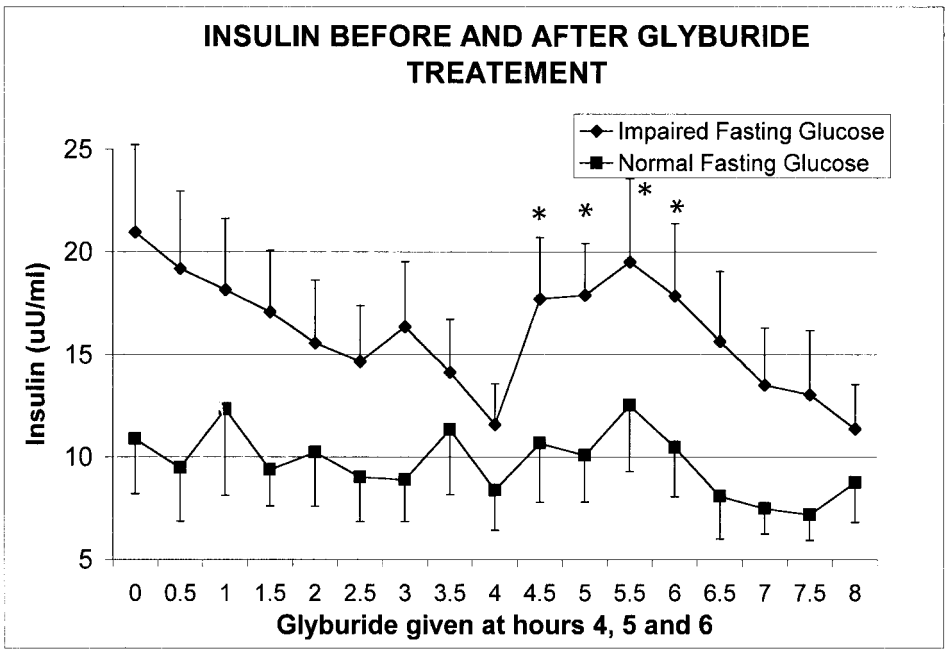

Fig 3. Insulin concentration before and after glyburide administration. Before glyburide administration, the serum insulin concentration decreased in between hour 0 and hour 4 in the IFG group, but not in the NFG group. Serum insulin increased in the IFG group after ingestion of glyburide at hour 4 (noon). The asterisk indicates a significant increase in serum insulin concentrations after starting glyburide in the IFG group.

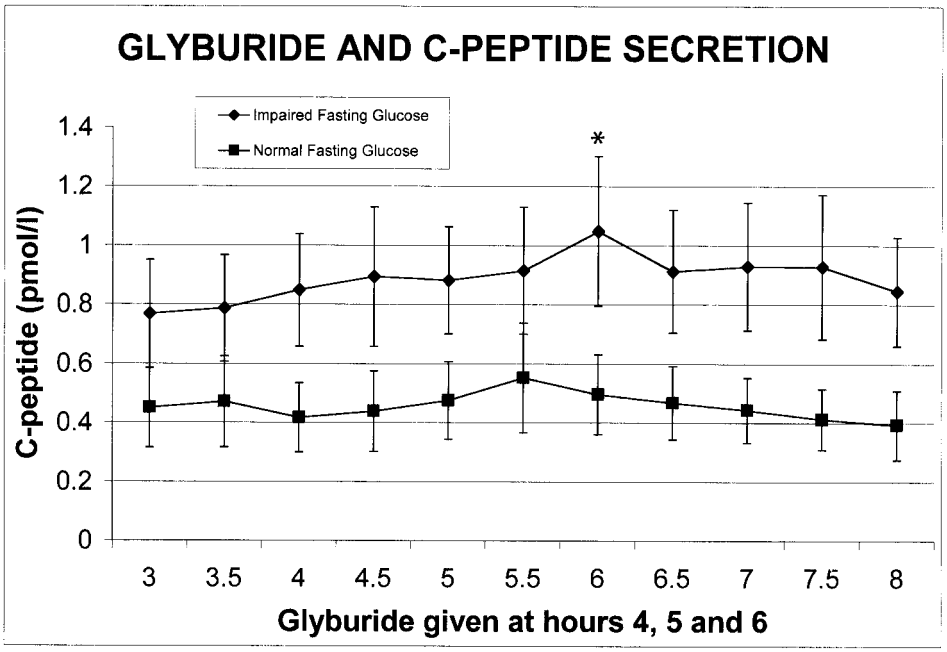

Fig 4. C-peptide secretion before and after ingestion of glyburide at hour 4 (noon). Overall, the C-peptide concentration was increased in the IFG group before glyburide administration. C-peptide concentration increased at hour 6 (2 hours after starting glyburide). Neither of the groups had the expected decrease in C-peptide seen with fasting alone (0.7 to 0.5 pmol/L).

volunteers who can safely take tolbutamide.[3] Low doses of glyburide were given in this study to test for safety and physiologic effects in patients with IFG.

Glyburide on FBG Concentrations

In individuals with NFG, blood glucose concentration reduces by approximately 1% per hour between 12 to 20 hours of fasting.[10,11] In patients with diabetes, the decrease in blood glucose is between 3% and 4.5% per hour.[12-14] A total of 10 mg glyburide increases this rate to 7.6% per hour.[14]

In the NFG group, FBG decreased by only 0.3% per hour before administration of the glyburide. Approximately 1.4 mg glyburide increases this decrease to 3% per hour in those volunteers with a normal FBG concentration (Fig 2). In the IFG group, before glyburide, the decrease in blood glucose averaged of 3.1% per hour. Glyburide treatment in the IFG group increased the decrease to 5.1% per hour ($P < .05$, Fig 2). The increased response to low-dose glyburide treatment seen in those with IFG may be useful information for planning clinical trials with the use of sulfonylureas to delay the onset of type 2 diabetes mellitus.

β-cell function decreases approximately 4% per year as part of the natural disease progression of type 2 diabetes mellitus.[15] It is unclear when the loss begins, but it may start 10 years before the onset of diabetes mellitus.[1,15] Patients with IFG may also lose 4% of their β-cell function per year. Sulfonylurea treatment increases insulin secretion in type 2 diabetes without increasing the rate of β-cell loss.[15] It is unknown if sulfonylurea treatment in patients with IFG would alter the onset of β-cell failure (accelerate or reduce)? A large clinical trial using 1 mg glimepride in volunteers with both IFG and impaired glucose tolerance (IGT) is underway in Sweden. One of the study goals is to prevent the progression to type 2 diabetes mellitus.[4]

Table 2. Carbohydrate Oxidation After Glyburide Administration

	Baseline (hour 3 to 4)	After Glyburide (hour 7 to 8)
NFG (umol/kg/min)	11.7 ± 1.0	11.7 ± 1.8
IFG (umol/kg/min)	11.3 ± 4.3	8.5 ± 1.9

NOTE. mean ± SEM; no significant difference.

Glyburide and GPR

Glyburide appears to have a direct effect on the liver to reduce gluconeogenesis and to increase glycogen synthesis.[16,17] Glyburide administration to patients with type 1 diabetes reduces the amount of insulin infusion required to maintain euglycemia.[18] In type 2 diabetes, the addition of oral sulfonylureas is associated with a greater reduction in endogenous GPR even though serum insulin concentrations were not increased.[19] This suggests that glyburide potentiates insulin's action to suppress GPR.

In an earlier study using the same dose of glyburide, GPR decreased over a 4-hour period greater than that seen with fasting alone ($-16\% \pm 2\%$ *v* $-8\% \pm 4\%$ *v* $P < .001$[10]). In the current study, glyburide treatment reduced GPR by 15% in the entire group, which was greater than the 8% seen with fasting alone.[10] The IFG group had a greater insulin secretion (17.7 ± 3.0 *v* 10.7 ± 2.9 μU/mL, $P < .05$). This may explain the greater decrease in GPR observed in the IGF group compared with the NFG group ($-19\% \pm 4\%$ *v* $-12\% \pm 3\%$, respectively, $P < .05$).

Glyburide can reduce GPR by increasing hepatic glycogen synthase and by decreasing glycogenolysis.[16,20] This effect is distal to the tyrosine kinase activation site of the β subunit. Increasing glycogen synthesis and reducing glycogenolysis would result in a reduced GPR.

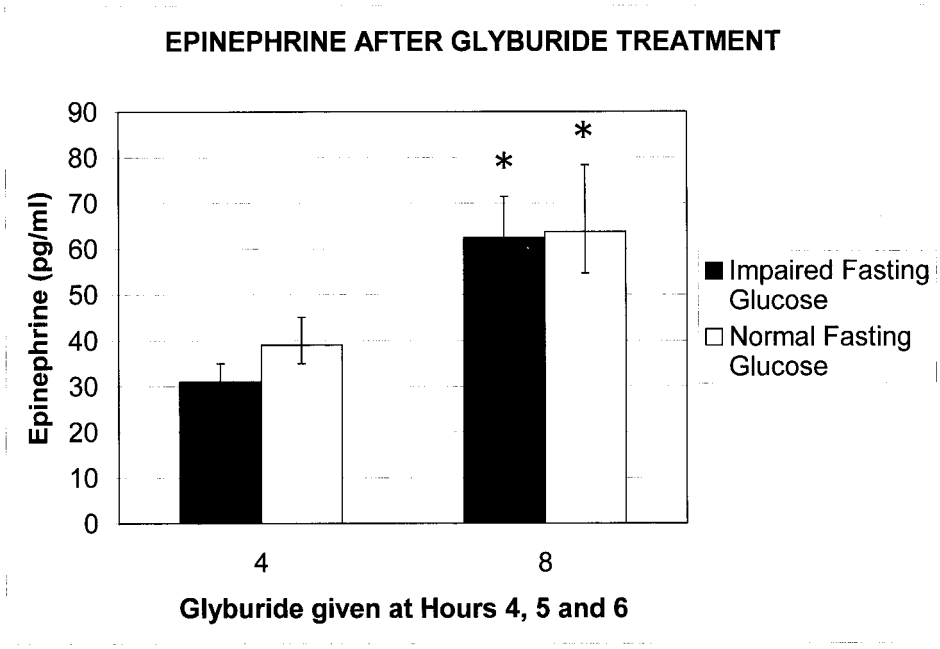

Fig 5. The increase in epinephrine concentrations seen after administration of glyburide is shown. The concentrations listed were for hours 4 (before) and hours 8 (4 hours after starting glyburide). Additional values at hours 5, 6, and 7 were similar to that observed at hour 8 (data not shown).

Besides glyburide's effect on glycogen, it also inhibits gluconeogenesis by uncoupling the livers' oxidative phosphorylation process.[21] This results in a decrease in pyruvate carboxylase activity, which could also contribute to the observed decrease in GPR. In an isolated liver perfusion system, glyburide reduces gluconeogenesis without insulin in the system.[17] Therefore, glyburide's effect on GPR may have been medicated, in part, by a direct effect on gluconeogenesis, glycogenolysis, or both.

The increase in epinephrine concentration suggests that the depth of the decrease in blood glucose may have resulted in a mild secretion of epinephrine. A larger dose of glyburide may result in symptomatic hypoglycemia. However, recent data would suggest that low-dose epinephrine infusion that result in similar values observed in this study (Fig 4) blunt the decrease in blood glucose concentrations by reducing glucose clearance.[22] However, no such change in glucose clearance was observed in the current study.

Glyburide Effects on Carbohydrate and Lipid Oxidation

Insulin administration reduces fat oxidation[18,23] and increases glucose oxidation.[23,24] Fat oxidation increased in both groups, which suggests that the small increase in insulin concentration was insufficient to suppress fat oxidation. Although there was a significant increase in insulin concentrations seen in the IFG group after glyburide, fat oxidation increased, and there was no effect on glucose (carbohydrate) oxidation. In fact, because glucose availability and glucose production decreased, one might expect a decrease in glucose oxidation. There was a slight, but not significant, decrease in glucose oxidation in the IGF group given low-dose glyburide. The change seen was likely due to a prolonged fasting state and not due to insulin action.

In summary, low-dose glyburide administration is associated with a greater decrease in GPR in individuals with an impaired FBG concentration (IFG) as compared with patients who have a normal glucose concentration. Glyburide treatment results in a 3% per hour decrease in GPR in volunteers with NFG and 5% per hour decrease in GPR in volunteers with IFG. The larger decrease in GPR may be due to the greater insulin response seen in IFG. Similar to the greater fall in GPR, the maximum decrease in FBG was also greater in the IFG group (7% *v* 4% per hour). While low-dose glyburide was safely tolerated in our fasting volunteers, longer-term studies need to be completed before it should be considered for clinical use in volunteers with IFG.

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