To examine the mechanism by which metformin lowers endogenous glucose production in type 2 diabetic patients, we studied seven type 2 diabetic subjects, with fasting hyperglycemia (15.5 ± 1.3 mmol/l), before and after 3 months of metformin treatment. Seven healthy subjects, matched for sex, age, and BMI, served as control subjects. Rates of net hepatic glycogenolysis, estimated by $^{13}$C nuclear magnetic resonance spectroscopy, were combined with estimates of contributions to glucose production of gluconeogenesis and glycogenolysis, measured by labeling of blood glucose by $^2$H from ingested $^2$H$_2$O. Glucose production was measured using $[6,6-^2$H$_2]$glucose. The rate of glucose production was twice as high in the diabetic subjects as in control subjects (0.70 ± 0.05 vs. 0.36 ± 0.03 mmol · m$^{-2}$ · min$^{-1}$), and metformin reduced that rate by 36% (to 0.38 ± 0.03 mmol · m$^{-2}$ · min$^{-1}$, $P < 0.0001$). Metformin reduced that rate by 24% (to 0.53 ± 0.03 mmol · m$^{-2}$ · min$^{-1}$, $P = 0.0009$) and fasting plasma glucose concentration by 30% (to 10.8 ± 0.9 mmol/l, $P = 0.0002$). The rate of gluconeogenesis was three times higher in the diabetic subjects than in the control subjects (0.59 ± 0.03 vs. 0.18 ± 0.03 mmol · m$^{-2}$ · min$^{-1}$) and metformin reduced that rate by 36% (to 0.38 ± 0.03 mmol · m$^{-2}$ · min$^{-1}$, $P = 0.01$). By the $^2$H$_2$O method, there was a twofold increase in rates of gluconeogenesis in diabetic subjects (0.42 ± 0.04 mmol · m$^{-2}$ · min$^{-1}$), which decreased by 53% after metformin treatment (0.28 ± 0.03 mmol · m$^{-2}$ · min$^{-1}$, $P = 0.0002$). There was no glycogen cycling in the control subjects, but in the diabetic subjects, glycogen cycling contributed to 25% of glucose production and explains the differences between the two methods used. In conclusion, patients with poorly controlled type 2 diabetes have increased rates of endogenous glucose production, which can be attributed to increased rates of gluconeogenesis. Metformin lowered the rate of glucose production in these patients through a reduction in gluconeogenesis. 

**Diabetes** 49:2063–2069, 2000

---

**RESEARCH DESIGN AND METHODS**

**Subjects.** Nine subjects with history of type 2 diabetes (seven men and two women, aged 55 ± 4 years, weight 90 ± 5 kg, body surface area 2.1 ± 0.1 m$^2$) were studied before and after 3 months of treatment with metformin. At baseline, these subjects were in poor glycemic control, as reflected by fasting plasma glucose concentration of 15.3 ± 1.1 mmol/l and glycosylated hemoglobin of 12.9 ± 1.2%. All of the patients were initially screened to rule out any other systemic disease or any biochemical evidence of abnormal renal or hepatic functions. Patients with a history of alcohol abuse, symptomatic coronary heart disease, current use of insulin for glycemic control, significant hepatic enzyme elevation (>2 times the upper limit of normal), serum creatinine >1.5 mg/dl, or a metallic device/fragment in their body were excluded from the study. Because the purpose of the study was to assess the mechanism by which metformin lowers glucose production, two diabetic subjects whose glucose production failed to decrease after metformin treatment were excluded.

Although it is generally agreed that metformin reduces fasting plasma glucose concentrations by reducing rates of hepatic glucose production (1,2), its effect on the relative contributions of hepatic glycogenolysis and gluconeogenesis remains controversial. Some studies conclude that metformin works mostly by reducing rates of gluconeogenesis (3); others, that it works by reducing rates of hepatic glycogenolysis (4,5).

Because of limitations of the methods used in the previous studies to assess gluconeogenesis and glycogenolysis, we used two independent and complementary methods to assess these processes in patients with poorly controlled type 2 diabetes before and after 3 months of metformin therapy. $^{13}$C nuclear magnetic resonance (NMR) spectroscopy was used to directly measure rates of net hepatic glycolysis, in combination with $[6,6-^2$H$_2]$glucose administration, to calculate the rates of endogenous glucose production (6). Rates of gluconeogenesis were estimated by subtracting the rates of net hepatic glycolysis from the rates of endogenous glucose production. In addition, fractional contributions of gluconeogenesis and glycogenolysis were estimated from the ratios of the $^2$H enrichments of the hydrogens bound to C5 and C2 of blood glucose after oral intake of $^2$H$_2$O (7,8). Rates of gluconeogenesis were calculated by multiplying those fractional contributions by the rates of glucose production. In addition, because the NMR method measures rates of net hepatic glycolysis whereas the $^2$H$_2$O method measures rates of total hepatic glycolysis (net hepatic glycolysis + glycogen cycling), estimates of glycogen cycling were made by subtracting rates of net hepatic glycolysis (NMR measured) from the rates of total hepatic glycolysis ($^2$H$_2$O measured).
The subjects were brought by wheelchair to the Yale Magnetic Resonance Center at 10:45 p.m. They were placed in a 2.1 T NMR spectrometer (Bruker Biospec Spectrometer; Billerica, MA), where liver glycogen concentrations were measured periodically from 11:00 p.m. to 7:00 a.m. the next morning with 13C NMR spectroscopy. The subjects also drank 5 ml/kg body water of 1H2O (99.9% 2H; Isotec, Miamisburg, OH), divided into four equal portions and given 45 min apart. The first dose was given at midnight and the last dose at 2:15 a.m. (9). Blood samples were drawn at 10-min intervals for the determination of enrichment of plasma [6,6-2H2]glucose, plasma glucose, and other hormone concentrations (as determined from the formate sphere as a calibration standard. Resonances from the metabolic unit of liver glycogen have been shown to be ~100% visible in vivo by this method). Reproducibility of the glycogen concentration measurement has been assessed in an earlier study, with a coefficient of variation between the pairs of measurements of ~7%. The standard deviation in the 13C NMR glycogen concentration measurement due to spectral noise was ±5% (6).

**Analyzes.** Plasma glucose concentrations were measured by a glucose oxidase spectrophotometric enzyme instrument (Gluco-Trol 850, Ames, Elkhart, IN). Plasma lactate concentrations were measured using a lactate dehydrogenase method (11). Plasma insulin, glucagon, C-peptide, and cortisol concentrations were measured using commercial double-antibody radioimmunoassay kits (insulin: Diagnostic Systems Laboratories, Webster, TX; glucagon: Linco Research, St. Charles, MO; cortisol: Diagnostic Product, Los Angeles, CA). Plasma free fatty acid concentrations were determined using a microfluorometric method (12).

**2H2O method.** Body water was calculated as 50% of body weight in women and 60% in men (13). Enrichments of the hydrogen in C2 and C5 of blood glucose were determined by procedures previously described (7,8). ZnSO4 (0.3N) and Ba(OH)2 (0.3N) were added to the blood to precipitate protein and the mixture was centrifuged. The supernatant was deionized by passage through a column of AG 1–8 X in the formate form and AG 50W –8 X in the hydrogen form (Bio-Rad Laboratories, Hercules, CA). Glucose in the effluent was isolated by high-performance liquid chromatography using a Bio-Rad HPX –87P column with water as solvent at 80°C and a flow rate of 0.5 ml/min. An aliquot of the glucose was converted to xylose. The xylose was oxidized with periodate to yield C5 of glucose with its hydrogen in formaldehyde. Hexamethylenetramine (HMT) was prepared from the formaldehyde and assayed for mass spectrometry using a chromatograph–mass spectrometer (GCMS) (7,8,14). HMTs from [1-H]oribitol of known enrichments 0.125 to 1.0% provided standards for the assay. Another aliquot of the glucose was converted to ribulose–5-phosphate, which was reduced to a mixture of ribitol–5-phosphate and arabinitol–5-phosphate. These were also oxidized with periodate, yielding formaldehyde containing C2 with its hydrogen. Again the formaldehyde was condensed to form an HMT, which was assayed by GCMS for mass m+1. Enrichments of plasma water were determined by Metabolic Solutions, (Nashua, NH) using an isotope ratio mass spectrometer. plasma water weight was assumed to be 94% of plasma volume (7,15).

For determining glucose production, plasma glucose collected at 10-min intervals in the last hour of infusion of [6,6-2H2]glucose infusion was derivatized as the penta-acetate, following Ba(OH)2/ZnSO4 deproteinization and semipurification by anion/cation exchange chromatography (AG 1–8 X, AG 50W–8 X, Bio-Rad Laboratories, Richmond, CA). Plasma free fatty acid concentrations were determined using a microfluorometric method (12).

**Calculations**

**Endogenous glucose production rates.** Rate of endogenous glucose production (mmol · m–2 · min–1) equals the infusion rate × (enrichment/100–enrichment–1). The infusion rate is the rate that [6,6-2H2]glucose is infused
TABLE 1
Enrichments in plasma glucose after 200–230 min of [6,6-\(^2\)H\(_2\)]glucose infusion as a percentage of enrichment at 240 min (11:30 A.M.)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>200</th>
<th>210</th>
<th>220</th>
<th>230</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>98.9 ± 4.3</td>
<td>104 ± 5.5</td>
<td>99.3 ± 3.3</td>
<td>97.4 ± 3.8</td>
</tr>
<tr>
<td>Diabetic subjects before metformin</td>
<td>104.3 ± 4.5</td>
<td>99 ± 6.0</td>
<td>102.8 ± 3.4</td>
<td>106.8 ± 5.6</td>
</tr>
<tr>
<td>Diabetic subjects after metformin</td>
<td>94 ± 1.0</td>
<td>92.8 ± 3.0</td>
<td>95 ± 3.2</td>
<td>98.8 ± 2.7</td>
</tr>
</tbody>
</table>

Data are means ± SE. *Mean percent excess enrichments at 240 min in control subjects was 1.14% in diabetic subjects pretreatment, 0.82%; and in diabetic subjects postmetformin 1.02%. Natural abundance was less than one-seventh of these enrichments.

\[\text{(mmol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}), \text{enrichment}^{\text{mm}} \text{is the enrichment of the hydrogen bound to carbon 6 of the [6,6-\(^2\)H\(_2\)] glucose (i.e., 99%) and enrichment}^{\text{plasma}} \text{is the percent enrichment in the hydrogen bound to carbon 6 of plasma glucose.}

**RESULTS**

**Subject characteristics and chemistries.** Baseline characteristics of control and diabetic subjects are shown in Table 2. Weight, age, and body surface area were not significantly different between the two groups. There was also no significant difference in insulin concentrations, but the diabetic subjects were relatively insulinopenic in the presence of a threefold higher fasting plasma glucose concentration (Table 3). Plasma triglyceride levels were significantly higher in the diabetic subjects. Plasma concentrations of glucagon, lactate, cortisol, free fatty acids, total cholesterol, HDL, and LDL were similar.

**Endogenous glucose production and clearance rate.** The rate of endogenous glucose production in the diabetic subjects was twice that of the control subjects. (Table 4). The glucose clearance rate was significantly decreased in diabetic subjects compared with that in the control subjects.
TABLE 3  
Plasma metabolite concentrations

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>Diabetic subjects premetformin</th>
<th>Diabetic subjects postmetformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (pmol/l)</td>
<td>63 ± 8</td>
<td>77 ± 13</td>
<td>88 ± 13</td>
</tr>
<tr>
<td>C-peptide (nmol/l)</td>
<td>0.67 ± 0.11</td>
<td>0.70 ± 0.10</td>
<td>0.75 ± 0.10</td>
</tr>
<tr>
<td>Glucagon (ng/l)</td>
<td>48 ± 6</td>
<td>64 ± 7</td>
<td>60 ± 5</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>0.65 ± 0.09</td>
<td>0.93 ± 0.14</td>
<td>1.13 ± 0.25</td>
</tr>
<tr>
<td>Free fatty acids (µmol/l)</td>
<td>639 ± 65</td>
<td>894 ± 132</td>
<td>627 ± 89*</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>475 ± 57</td>
<td>570 ± 103</td>
<td>472 ± 79</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.5 ± 0.3</td>
<td>5.8 ± 0.5</td>
<td>4.4 ± 0.2†</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.1 ± 0.3</td>
<td>2.6 ± 0.6§</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.4 ± 0.3</td>
<td>1.1 ± 0.04</td>
<td>1.0 ± 0.06</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>3.5 ± 0.4</td>
<td>3.2 ± 0.3</td>
<td>2.4 ± 0.2</td>
</tr>
</tbody>
</table>

Data are means ± SE. *P = 0.01 vs. premetformin; †P = 0.04 vs. premetformin; ‡P = 0.04 vs. control subjects.

bound to C2 of blood glucose at 8:30 A.M. was 93 ± 2.5% of the enrichment in plasma water in the control subjects, 95.3 ± 1.7% in the diabetic subjects before treatment, and 94.6 ± 2.3% after treatment. This is in accord with essentially complete equilibration of the hydrogen bound to carbon 2 of glucose with that in body water (7,8). Measurements of the C5-to-C2 ratios were made during the period that glycogen contents were measured (i.e., from 7 to 15 h of fasting). However, technical limitations prevented measurement of glucose production during that time. Instead, production was measured during the 18th h of fasting. Those differences in time could affect the quantitation to some extent, but not the conclusions from those quantitations. It is known that glucose production decreases with duration of fasting. In normal subjects, it decreased ~20% from 14 to 22 h of fasting (7). In type 2 diabetic subjects with fasting plasma glucose concentrations ~10 mmol/l, production declined 25% from 17 to 22 h of fasting (A. Wajngot, V.C., W.C.S., K. Ekberg, P.K. Jones, S. Efendic, B.R.L., unpublished data). Therefore, at 8:30 A.M., production might have been 10–15% more than that at 11:30 A.M. However, m+2 enrichments used to estimate production were not different over a 40-min interval ending at 11:30 A.M. (Table 1). The C5-to-C2 ratio also increased with time (Table 5), by perhaps 1–2% per hour, and the ratio in glucose collected at 8:30 A.M. was then a composite of the ratios in glucose entering the blood at that time with decreasing amounts from the earlier times. Thus, the ratio at 8:30 A.M., used for calculation, is also an underestimate by a small amount. Justification for assuming a constant rate of net hepatic glycogenolysis, from midnight to 7 A.M., is evidenced in the figure.

**Hepatic glycogen cycling.** There was no detectable hepatic glycogen cycling in the control subjects (Table 4). In contrast, there was a significant amount of hepatic glycogen cycling in the poorly controlled type 2 diabetic subjects, accounting for ~25% of endogenous glucose production (Table 4).

**Indirect calorimetry.** There were no significant differences between the two groups in the basal rates of carbohydrate, lipid, or protein oxidation (data not shown).

**Metformin treatment.** After 3 months of metformin treatment, there was a 30% decrease in fasting plasma glucose concentration and glycosylated hemoglobin levels compared with the pretreatment values (Table 2). There was no significant change in body weight or plasma insulin, C-peptide, glucagon, lactate, and cortisol levels. Metformin treatment resulted in a 24% reduction in cholesterol and a 30% reduction in free fatty acid concentrations in plasma (Table 3).

Three months of metformin treatment also lowered post-treatment glucose production by 25% in comparison with pretreatment (Table 4). There was no significant increase in the glucose clearance rate (46.9 ± 4.5 ml · m⁻² · min⁻¹ before metformin vs. 50.9 ± 3.6 ml · m⁻² · min⁻¹ after metformin). Both hepatic glycogen content and the rate of net hepatic glycogenolysis tended to increase with metformin treatment, as determined by ¹³C NMR. There was no significant change in the rate of total hepatic glycogenolysis as determined indirectly by the ²H₂O method. The rate of gluconeogenesis as determined by ¹³C NMR was 37% less after treatment. Similarly, there was a 33% reduction in the rate of gluconeogenesis measured with the ²H₂O method. There was also a trend for metformin to cause a 40% reduction in rates of hepatic glycogen cycling (Table 4). Metformin treatment did not significantly change the rates of carbohydrate, lipid, or protein oxidation (data not shown).

**DISCUSSION**

Increased rates of glucose production are strongly correlated with increased fasting plasma glucose concentrations in patients with type 2 diabetes (18–21). We found that the rate of glucose production was increased twofold in the diabetic subjects as compared with that in the control subjects, which
glycogenolysis, whereas the $\text{H}_2\text{O}$ method measures rates of neogenesis as determined by both methods. This decrease in glucose production could be entirely accounted for by a reduction in the rates of gluconeogenesis, which is consistent with the results of previous studies (2,22,24,25). This decrease in glucose production could be entirely attributed to an approximately threefold increase in the rate of gluconeogenesis as assessed by the $\text{C}^13\text{C}$ NMR method and mostly to a roughly twofold increase in the rate of gluconeogenesis as assessed by the $\text{H}_2\text{O}$ method. These results are consistent with our previous study examining the rates of gluconeogenesis in normal and poorly controlled type 2 diabetic subjects, using $\text{C}^13\text{C}$ NMR (23). Metformin treatment resulted in a 25–30% reduction in fasting plasma glucose concentrations and glucose production, which is consistent with the results of previous studies (2,22,24,25). This decrease in glucose production could be entirely accounted for by a reduction in the rates of gluconeogenesis as determined by both methods.

Because the NMR method measures rates of net hepatic glycogenolysis, whereas the $\text{H}_2\text{O}$ method measures rates of total hepatic glycogenolysis (net hepatic glycogenolysis + hepatic glycogen cycling), estimates of hepatic glycogen cycling were made by subtracting rates of net hepatic glycogenolysis (NMR measured) from the rates of total hepatic glycogenolysis ($\text{H}_2\text{O}$ measured). There was no detectable hepatic glycogen cycling in the control subjects, which is consistent with an earlier study using these two methods (26). In contrast, there was considerable glycogen cycling in the poorly controlled diabetic subjects, which accounted for ~25% of endogenous glucose production (Table 4). Hyperglucagonemia and hyperinsulinemia, which are typically present in patients with type 2 diabetes, have both been shown to promote glycogen cycling (27–29). Although the plasma concentrations of these hormones tended to be higher in the diabetic subjects than in the control subjects, the differences were not significant; however, it is likely that differences in portal vein concentrations of these hormones were much greater.

Three different studies have previously examined the effect of metformin on the rates of net hepatic glycogenolysis and gluconeogenesis in patients with type 2 diabetes with conflicting results. Stumvoll et al. (3) studied 10 obese diabetic individuals before and after 16 weeks of treatment with metformin (2,550 mg/day), using $\text{C}^14\text{C}$ lactate to estimate the rates of gluconeogenesis. Using this approach, these investigators found that metformin decreased endogenous glucose production through a 37% reduction in rates of gluconeogenesis. However, because of unknown dilution of the $\text{C}^14\text{C}$ label in the tricarboxylic acid cycle, this approach does not accurately quantify rates of gluconeogenesis. In addition, the diabetic subjects lost on average ~3 kg of body wt during this study, which may have obscured the independent role of metformin in lowering the rates of glucose production.

Cusi et al. (4) used the same $\text{C}^13\text{C}$ lactate approach to assess rates of gluconeogenesis in 20 type 2 diabetic subjects before and after 15 weeks of metformin treatment (2,500 mg/day) in a randomized double-blind placebo-controlled trial. Fifteen of the subjects were taking sulfonylureas at the time of enrollment. This medication was continued during the trial, and metformin was added. These investigators reported that metformin treatment significantly decreased glucose production, but they found no change in the contribution of gluconeogenesis from lactate. They therefore concluded that metformin decreased glucose production by inhibiting hepatic glycogenolysis. It is unclear why these investigators arrived at a result opposite to that of Stumvoll et al. (3) despite using the identical approach.

More recently, Christiansen et al. (5) examined the mechanism of metformin’s action in diabetic subjects using mass isotopomer distribution analysis to estimate the rates of gluconeogenesis using $\text{C}^2\text{H}^3\text{C}$ glycerol. Five obese subjects (BMI 38 ± 3) with type 2 diabetes were studied before and after 4 weeks of treatment with metformin. In this study, gluconeogenesis contributed only ~24% of overall glucose production in the diabetic subjects. These investigators therefore concluded that an increased rate of glycogenolysis was entirely responsible for the increased rate of glucose production, which is contrary to the current and previous $\text{C}^13\text{C}$ NMR studies (23). These investigators also found that metformin treatment led to a 17% reduction in the rate of glucose production, with no change in the absolute rate of gluconeogenesis and concluded that this occurred through a 21% reduction in hepatic glycogenolysis. It is unclear why Christiansen et al. obtained the opposite results from those obtained in the present study. However, recent studies have demonstrated limitations in their approach, resulting in underestimation of gluconeogenic rates (30–33).

The data from the present study are consistent with in vitro studies demonstrating an inhibitory effect of metformin. 

![Graph](image-url)
on gluconeogenesis. Radziuk et al. (34) reported that metformin inhibited gluconeogenesis in perfused liver, primarily through inhibition of hepatic lactate uptake. Argaud et al. (35) found that metformin decreased ATP concentration in isolated rat hepatocytes. Because ATP is an allosteric inhibitor of pyruvate kinase, these investigators hypothesized that the metformin-induced reduction in glucose production was the result of increased pyruvate kinase flux. However, Large and Beylot (36) observed no decrease in ATP concentration or in the uptake of gluconeogenic precursors by perfused livers obtained from fasted streptozocin-injected diabetic rats after metformin treatment. They hypothesized that metformin decreased gluconeogenic flux through inhibition of pyruvate carboxylase–phosphoenolpyruvate carboxykinase activity and possibly through increased conversion of pyruvate to alanine. However, it should be noted that all of these in vitro studies used very high doses of metformin (250–350 mg/kg), which are 8- to 12-fold higher than the doses used in the treatment of diabetic patients.

Metformin treatment also lowered the plasma free fatty acid concentrations by 30% in the diabetic subjects, which is consistent with the results of some (37–39) but not all previous studies (3.4, 40). Plasma free fatty acids have been shown to play an important role in the regulation of hepatic glucose production (41–44). It is possible that reduced plasma free fatty acid concentration after metformin treatment also contributed to the reduced rates of gluconeogenesis.

In summary, we examined the effect of metformin treatment on rates of total and net hepatic glycogenolysis, gluconeogenesis, and hepatic glucose in a poorly controlled type 2 diabetic patients who were matched to healthy control subjects using 13C NMR and 2H2O techniques. We found the following: 1) Poorly controlled diabetic subjects had a twofold increase in rates of glucose production and extensive hepatic glycogen cycling accounting for ~25% of their glucose production; 2) the increased rate of glucose production in the diabetic subjects could be attributed to an increased rate of gluconeogenesis; and 3) metformin treatment in the diabetic subjects decreased rates of glucose production through a reduction in the rate of gluconeogenesis.

ACKNOWLEDGMENTS

This work was supported by grants from the U.S. Public Health Service (R01 DK-49230, P30 DK-45735, R01 DK-14507, and M01 RR-00125), the Endocrine Fellows Foundation, and an unrestricted grant from Bristol-Myers Squibb. R.S.H. was the recipient of an Endocrine Fellows Foundation grant. K.F.P. was the recipient of a K-23 award from the National Institutes of Health.

We thank Veronika Walton and Laura Burden for their expert technical assistance and the staff of the Yale-New Haven Hospital GCRC.

REFERENCES