

# Increased Rate of Gluconeogenesis in Type II Diabetes Mellitus

## A $^{13}\text{C}$ Nuclear Magnetic Resonance Study

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### Abstract

To quantitate hepatic glycogenolysis, liver glycogen concentration was measured with  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy in seven type II diabetic and five control subjects during 23 h of fasting. Net hepatic glycogenolysis was calculated by multiplying the rate of glycogen breakdown by the liver volume, determined from magnetic resonance images. Gluconeogenesis was calculated by subtracting the rate of hepatic glycogenolysis from the whole body glucose production rate, measured using  $[6\text{-}^3\text{H}]\text{glucose}$ . Liver glycogen concentration 4 h after a meal was lower in the diabetics than in the controls;  $131\pm 20$  versus  $282\pm 60$  mmol/liter liver ( $P < 0.05$ ). Net hepatic glycogenolysis was decreased in the diabetics,  $1.3\pm 0.2$  as compared to  $2.8\pm 0.7$   $\mu\text{mol}/(\text{kg body wt} \times \text{min})$  in the controls ( $P < 0.05$ ). Whole body glucose production was increased in the diabetics as compared to the controls,  $11.1\pm 0.6$  versus  $8.9\pm 0.5$   $\mu\text{mol}/(\text{kg body wt} \times \text{min})$  ( $P < 0.05$ ). Gluconeogenesis was consequently increased in the diabetics,  $9.8\pm 0.7$  as compared to  $6.1\pm 0.5$   $\mu\text{mol}/(\text{kg body wt} \times \text{min})$  in the controls ( $P < 0.01$ ), and accounted for  $88\pm 2\%$  of total glucose production as compared with  $70\pm 6\%$  in the controls ( $P < 0.05$ ). In conclusion: increased gluconeogenesis is responsible for the increased whole body glucose production in type II diabetes mellitus after an overnight fast. (*J. Clin. Invest.* 1992. 90:1323–1327.) Key words: glycogenolysis • glucose turnover • liver glycogen • fasting • liver volume

### Introduction

Type II diabetes mellitus is characterized by fasting hyperglycemia and an excessive, prolonged rise in the plasma glucose concentration after glucose or meal ingestion (1–3). The fasting hyperglycemia has been attributed to result mostly from an increased rate of glucose production (1, 2), which could result from an increased rate of hepatic glycogenolysis and/or gluconeogenesis. Increased gluconeogenesis has been suggested to be the predominant factor (4), but the methods for its quantification depend on several assumptions of uncertain validity (5). Rates of net hepatic glycogenolysis have not been measured in type II diabetic patients.

We have recently developed a new method to measure directly net rates of hepatic glycogenolysis noninvasively in humans using natural abundance  $^{13}\text{C}$  nuclear magnetic resonance (NMR)<sup>1</sup> spectroscopy to measure glycogen concentrations and magnetic resonance imaging to measure liver volume (6). By subtracting the glycogenolytic rate from the total glucose production rate, determined using tritiated glucose turnover, the rate of gluconeogenesis can be calculated.

The aim of this study was to apply this new method to quantitate rates of net hepatic glycogenolysis and gluconeogenesis in type II diabetic patients and healthy control subjects after an overnight fast.

### Methods

**Subjects.** Seven subjects, five males and two females, with type II diabetes mellitus, and five healthy control subjects, four males and one female, were studied. All were within 20% of their ideal body weight according to the Metropolitan Life Insurance tables. The control subjects were matched to the diabetics with respect to age and body mass index; the mean ages of the diabetic and control subjects were  $57\pm 4$  and  $61\pm 5$  yr, respectively, and their mean body mass indexes were  $28\pm 1$  and  $25\pm 2$   $\text{kg}/\text{m}^2$ , respectively. The mean duration of diabetes, calculated from the first record of plasma glucose concentration  $> 7.8$  mmol/liter was  $13\pm 4$  yr. All the diabetic subjects fulfilled the criteria for the diagnosis of diabetes mellitus as established by the National Diabetes Data Group. Five were treated with oral sulfonylurea agents and two were treated with insulin. The oral medication was discontinued three days before the study and the insulin treatment was stopped the evening before the study. Their mean hemoglobin  $\text{A}_{1\text{C}}$  level was  $12\pm 1\%$  (normal range 4–8%), and their mean fasting plasma glucose concentration after the discontinuation of medication was  $14.6\pm 1.2$  mmol/liter. One of the diabetic subjects took diltiazem for mild angina; however, the medication was not taken the day of the study. Two of the other diabetic subjects were treated with a  $\beta$ -blocking agent for hypertension, and that medication was withdrawn one to three days before the study. None of the control subjects was taking any medication or had a family history of diabetes. Informed consent was obtained from all subjects after the purpose, nature, and potential risks of the study were explained to them. The protocol was reviewed and approved by the Human Investigation Committee of the Yale University School of Medicine.

**Experimental protocol.** For three days before the study all subjects received a standardized, high carbohydrate diet consisting of 60% carbohydrate, 20% protein, 20% fat, and the daily energy content was 30–33 kcal/kg body wt. At 5 p.m. the day before the study all subjects were admitted to the clinical research center at Yale-New Haven Hospital, where they were given a liquid meal that consisted of 60.5% carbohydrate, 16% protein, and 23.5% fat, supplying a total amount of 650 kcal in 640 ml. Subjects then fasted for 23 h, during which time they were given only water to drink ad lib. 4 h after the liquid meal was ingested, at 9 p.m., a  $^{13}\text{C}$  NMR measurement of liver glycogen concen-

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1. Abbreviation used in this paper: NMR, nuclear magnetic resonance.

tration was performed and the same measurement was repeated the next day at 6:30 a.m., 10 a.m., 12:30 p.m., and 3:30 p.m. (i.e., at 13.5, 17, 19.5, and 22.5 h into the fast). At 7:30 a.m. magnetic resonance imaging to measure liver volume was performed. Teflon catheters were placed in an antecubital vein and in a retrodorsal hand vein. Baseline blood samples for determination of plasma glucose, insulin, glucagon, and cortisol concentrations were drawn from the hand vein at 9 a.m. Directly thereafter a prime continuous infusion of [6-<sup>3</sup>H]glucose (25 μCi, 0.25 μCi/min, 0.2 ml/min; New England Nuclear, Boston, MA) was started through the catheter placed in the antecubital vein. The [6-<sup>3</sup>H]glucose was purified using HPLC, and its purity was shown to be > 98%. Blood samples for determination of plasma glucose tritium specific activity were drawn every 10 min between 11:30 a.m. and noon and between 2:30 and 3:00 p.m. from the catheter placed in a retrodorsal hand vein. The hand was inserted into a heated box (70°C) to ensure arterialization of the venous blood. The [6-<sup>3</sup>H]glucose infusion was discontinued at 3:00 p.m. Blood samples for determination of plasma glucose, insulin, glucagon, and cortisol concentrations were drawn from the hand vein again at the end of the study.

Continuous indirect calorimetry was performed between 11:30 a.m. and noon and between 2:30 and 3 p.m. as previously described (7). The nonprotein respiratory quotient was obtained from the tables of Lusk, according to which the respiratory quotient for 100% oxidation of fat is 0.707 and that for the oxidation of carbohydrates is 1.00 (8).

**NMR spectroscopic and imaging techniques.** The method for measuring liver glycogen concentration by <sup>13</sup>C NMR spectroscopy has been described in detail (6). In brief, a 9-cm <sup>13</sup>C observation coil and a 12 × 14-cm coplaner butterfly <sup>1</sup>H decoupler coil was placed over the lateral aspect of the liver in the supine subject. A 6-mm thick Lucite plate was placed between the coil and the chest. The subject was then placed inside an NMR spectrometer (1 m bore, 2.1 T; BioSpec, Bruker Instruments, Inc., Billerica, MA), and the liver was positioned within the homogenous volume of the magnet. The magnetic field homogeneity was optimized with the water signal, and the position of the coil was confirmed by imaging the liver from the surface coil, using a multislice gradient echo image. Localized <sup>13</sup>C NMR liver spectra were obtained using a modified one-dimensional inversion-based sequence for surface suppression. Each measurement required 15-30 min of signal averaging. Local heating from decoupling radio frequency, calculated using a magnetic vector potential model, was < 4 W/kg tissue. The subjects were removed from the magnet between each measurement.

Imaging of all subjects to determine the liver volume was performed in a 1.5 T magnet (Signa; General Electric Co., Milwaukee, WI) as previously described (6).

**Analytical procedures.** Plasma glucose concentration was measured by the glucose oxidase method using a glucose oxidase analyzer (Beckman Instruments Inc., Fullerton, CA). Plasma tritiated glucose specific activity was determined after isolation of glucose by ion exchange chromatography as previously described (9). Plasma immunoreactive insulin and glucagon concentrations were measured with double antibody radioimmunoassay techniques (Ventrex Laboratories Inc., Portland, ME, and ICN Biomedicals Inc., Costa Mesa, CA). Plasma cortisol concentration was also measured with a radioimmunoassay technique (Clinical Assay, Baxter Healthcare, Cambridge, MA).

**Calculations.** The rate of total glucose production was calculated by dividing the [6-<sup>3</sup>H]glucose infusion rate (disintegrations per minute [dpm]/min) by the plasma glucose tritium specific activity (dpm/mmol) after 6 h of [6-<sup>3</sup>H]glucose infusion. Rate of net hepatic glycogenolysis was calculated for each individual by finding the best fit of the glycogen concentration measured between 4 and 22 h of fasting to a line by method of least squares. The slope was multiplied by liver volume to obtain the absolute rate of net glycogenolysis. The gluconeogenic rate was then calculated by subtracting the rate of hepatic glycogenolysis from the rate of total glucose production.

All values are expressed as means ± SE. Comparisons were made using paired and unpaired two-tailed *t* test and analysis of variance

when appropriate. Linear regression analysis was applied to assess linearity of the decrease in liver glycogen content during the fast.

## Results

Plasma glucose, insulin, glucagon, and cortisol concentrations are recorded in Table I. Plasma glucose concentration was significantly elevated throughout the study in the diabetic as compared to the control subjects. Plasma insulin, glucagon, and cortisol concentrations were not significantly different between the groups; however, plasma cortisol concentration decreased significantly (*P* < 0.05) between 16 and 23 h of fasting in both groups.

The respiratory quotient was 0.82 ± 0.01 in the diabetic and 0.83 ± 0.03 in the control subjects when measured between 11:30 a.m. and noon, and did not change when measured between 2:30 and 3 p.m. The estimated metabolic rate was 1,620 ± 69 kcal/24 h in the diabetic and 1,720 ± 126 kcal/24 h in the control subjects when measured between 11:30 a.m. and noon and did not change when estimated at the later time point.

Fig. 1 shows typical decoupled <sup>13</sup>C NMR spectra of liver glycogen from a diabetic and a control subject four hours after ingesting the liquid meal. The C1 peak of glycogen was clearly delineated in the spectrum at 100.4 parts per million. The standard deviation in the determination of liver glycogen concentration due to the spectral noise was ± 13 mmol/liter liver. The mean time course for the liver glycogen concentration in the two study groups during the fast are shown in Fig. 2. The initial concentration, measured four hours after the liquid meal, was lower in the diabetic as compared to the control subjects (131 ± 20 mmol/liter liver versus 282 ± 60 mmol/liter liver, *P* < 0.05). There was a significant decline in liver glycogen concentration in both groups during the fast (*P* < 0.01). The mean rate of glycogen breakdown was higher in the control subjects than in the diabetic subjects (10.5 ± 2.0 versus 4.6 ± 0.9 mmol/[liter liver × h], *P* < 0.05). After 22.5 hours of fasting, liver glycogen concentration was 69 ± 15 mmol/liter liver and 98 ± 23 mmol/liter liver in the diabetic and control subjects,

Table I. Plasma Glucose, Insulin, Glucagon, and Cortisol Concentrations in Type II Diabetic Patients and Healthy Controls after 16 and 23 h of Fasting

	16 h	23 h
Plasma glucose concentration, mmol/liter		
Controls	5.4 ± 0.2	5.1 ± 0.2
DM	14.6 ± 1.2*	10.6 ± 0.8*
Plasma insulin concentration, pmol/liter		
Controls	62 ± 11	43 ± 6
DM	93 ± 20	145 ± 73
Plasma glucagon concentration, ng/liter		
Controls	91 ± 12	86 ± 17
DM	98 ± 9	76 ± 10
Plasma cortisol concentration, nmol/liter		
Controls	552 ± 107	276 ± 69 <sup>‡</sup>
DM	368 ± 30	209 ± 13 <sup>‡</sup>

\* *P* < 0.001 compared to controls; <sup>‡</sup> *P* < 0.05 compared to 16 h of fasting.

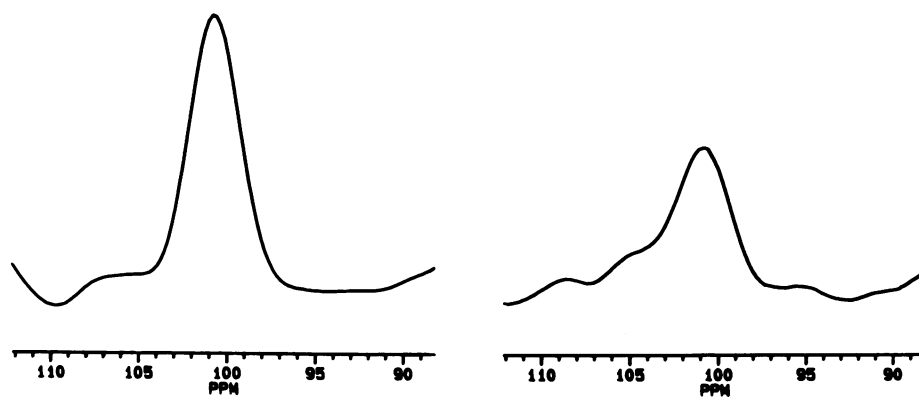


Figure 1. Typical  $^{13}\text{C}$  NMR spectrum of the C1 position of liver glycogen from one control subject (left panel) and one type II diabetic patient (right panel) four hours after the liquid meal.

respectively (NS). The mean liver volume, measured after 14.5 hours of fasting, was similar in both groups,  $1.42 \pm 0.10$  liter and  $1.19 \pm 0.13$  liter in the diabetic and control subjects, respectively.

The contribution of net hepatic glycogenolysis and gluconeogenesis to the rate of total glucose production are summarized in Table II. The mean rate of net hepatic glycogenolysis was less in the diabetic as compared to the control subjects ( $1.3 \pm 0.2 \mu\text{mol}/[\text{kg body wt} \times \text{min}]$  versus  $2.8 \pm 0.7 \mu\text{mol}/[\text{kg body wt} \times \text{min}]$ ,  $P < 0.05$ ). Total glucose production was measured after 22 hours of fasting, following six hours of  $[6\text{-}^3\text{H}]\text{glucose}$  infusion. The mean rate of total glucose production was increased by 25% in the diabetic as compared to the control subjects, ( $11.1 \pm 0.6 \mu\text{mol}/[\text{kg body wt} \times \text{min}]$  versus  $8.9 \pm 0.5 \mu\text{mol}/[\text{kg body wt} \times \text{min}]$ ,  $P < 0.05$ ). As a consequence of the decreased rate of hepatic glycogenolysis and increased rate of total glucose production in the diabetic subjects, the rate of gluconeogenesis was found to be increased in the diabetic as compared to the control subjects ( $9.8 \pm 0.7$  versus  $6.1 \pm 0.5 \mu\text{mol}/[\text{kg body wt} \times \text{min}]$ ,  $P < 0.01$ ), and accounted for  $88 \pm 2\%$  of the rate of total glucose production in the diabetic and for  $70 \pm 6\%$  in the control subjects ( $P < 0.05$ ).

## Discussion

$^{13}\text{C}$  NMR spectroscopy provides a direct noninvasive method to measure liver glycogen concentrations in humans. In combi-

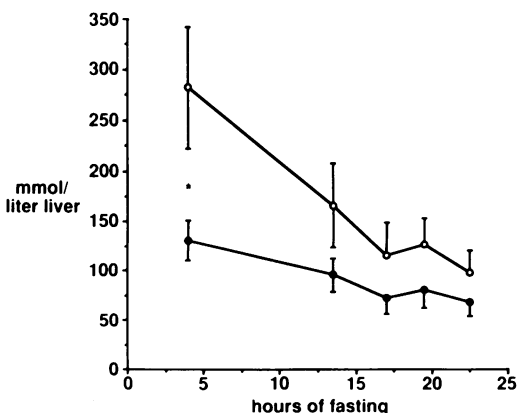


Figure 2. Time course for the mean liver glycogen concentration in the diabetics (●) and controls (○).

nation with magnetic resonance imaging to measure liver volume this method was applied in the present study to quantitate rates of net hepatic glycogenolysis in type II diabetic patients and healthy control subjects during 23 hours of fasting.

Liver glycogen concentration was initially lower in the diabetic as compared to the control subjects. This finding does not appear to be related to the dietary conditions, since all subjects ate a standardized diet for three days before the study. It is possible that the diabetic subjects have a defect in liver glycogen synthesis as well as in muscle glycogen synthesis which has previously been shown (10). Liver glycogen synthesis has not been assessed directly in type II diabetic patients; however, splanchnic glucose uptake is decreased after glucose administration (3). Net hepatic glycogenolysis was calculated by multiplying the mean rate of glycogenolysis, measured by  $^{13}\text{C}$  NMR in  $\text{mmol}/(\text{liter liver} \times \text{min})$ , by the liver volume. In a previous study the liver volume was shown to decrease by on average 23% during 67 hours of fasting in healthy young subjects (6). It is therefore likely that the liver volume also decreased in the present study. The measurement of liver volume was performed in the middle of the fast to compensate for that change. To the extent the liver volume decreased during the first 14.5 hours of fasting the rate of glycogenolysis will be underestimated in both the diabetic and the control subjects.

The rate of total glucose production was increased by an average of 25% in the diabetic as compared to the control subjects when measured after six h infusion of the  $[6\text{-}^3\text{H}]\text{glucose}$ . When glucose production was calculated from the plasma tritiated glucose specific activity measured after 2.5 h of  $[6\text{-}^3\text{H}]\text{glucose}$

Table II. Rates of Total Glucose Production, Hepatic Glycogenolysis, and Gluconeogenesis in Type II Diabetic Patients and Healthy Controls during 23 h of Fasting

	Controls	DM
Glucose production $\mu\text{mol}/(\text{kg body wt} \times \text{min})$	$8.9 \pm 0.5$	$11.1 \pm 0.6^*$
Hepatic glycogenolysis $\mu\text{mol}/(\text{kg body wt} \times \text{min})$	$2.8 \pm 0.7$	$1.3 \pm 0.2^*$
Gluconeogenesis $\mu\text{mol}/(\text{kg body wt} \times \text{min})$	$6.1 \pm 0.5$	$9.8 \pm 0.7^\ddagger$
Gluconeogenesis (percentage of glucose production)	$70 \pm 6\%$	$88 \pm 2\%^*$

\*  $P < 0.05$ ;  $^\ddagger P < 0.01$ .

glucose infusion, the rate was significantly higher in both the diabetic subjects and in the control subjects (diabetics,  $15.8 \pm 1.2$  as compared to  $11.1 \pm 0.6$   $\mu\text{mol}/[\text{kg body wt} \times \text{min}]$  at 6 h [ $P < 0.001$ ]; controls,  $10.2 \pm 0.5$  as compared to  $8.9 \pm 0.5$   $\mu\text{mol}/[\text{kg body wt} \times \text{min}]$  at 6 h [ $P < 0.001$ ]). The most likely reason for these higher rates of glucose production is that steady state in [ $^3\text{H}$ ]glucose specific activity was not achieved after a 2.5-hour equilibration time (11). In support of this we found that the plasma glucose specific activity increased significantly by  $25 \pm 13\%$  in the diabetic subjects ( $P < 0.01$ ) and by  $9 \pm 3\%$  in the control subjects ( $P < 0.05$ ) when measured from 11:30 a.m. to noon. The plasma glucose specific activity increased by only  $5 \pm 1\%$  and  $1 \pm 4\%$  in the diabetic and control subjects, respectively, when measured at the later time period.

As a consequence of the increased rate of total glucose production and lower rate of net hepatic glycogenolysis in the diabetic subjects, the rate of gluconeogenesis was significantly increased, and accounted for  $88 \pm 2\%$  of total glucose production during the first 23 h of fasting, as compared to  $70 \pm 6\%$  in the control group. Consoli et al. found increased rates of gluconeogenesis in non-insulin dependent diabetic patients as compared to controls, but the percent contribution of gluconeogenesis was reported to be lower than in the present study in both groups (4). They found that gluconeogenesis from phosphoenolpyruvate accounted for 54% of total glucose production in the diabetic as compared with only 29% in the control subjects. However, they used a complicated isotopic approach to measure gluconeogenesis, the validity of which has been questioned (5). Furthermore, their method did not take into account gluconeogenesis from: (a) glycerol; (b) glyconeogenic amino acids that enter the TCA cycle other than through pyruvate; or (c) the kidney. In regard to this last possibility, net production of glucose by the kidneys was not detected in the initial phase (12–14 h) of fasting in normal subjects or type I diabetic patients (12), but this has not been evaluated in type II diabetic patients.

In the present study the rate of glycogenolysis was measured over a 20-h period and the rate of total glucose production was measured in the end of that period. To the extent that total glucose production declined during the fast, the rate of gluconeogenesis may be underestimated in both groups.

Regression analysis of the decrease in glycogen concentration with time was performed for each individual subject to assess if the rates were linear. All control subjects and five of the diabetic subjects had linear rates of glycogenolysis ( $P < 0.05$ ). In two of the diabetic subjects liver glycogen decreased during the initial 13.5 h and then plateaued. By using the mean rate of glycogenolysis in the calculation of gluconeogenesis in these two subjects we may overestimate the rate of glycogenolysis at the time when glucose production was measured, i.e., after 6 h of tracer infusion. Therefore, in these two subjects gluconeogenesis might have been underestimated and consequently the differences between them and the control subjects might have been underestimated.

The diabetic subjects had a slightly, although not statistically significant, higher body mass index than the control subjects. To address the possible effects of obesity on the rate of gluconeogenesis, body mass index was correlated to the rate of gluconeogenesis and the percent contribution of gluconeogenesis. No correlation between these parameters could be found in either the diabetic or the control group.

With an increased rate of gluconeogenesis in type II diabetic subjects, it might be expected that there is an increased uptake of gluconeogenic precursors by the liver. When splanchnic uptake of alanine, lactate, pyruvate, and glycerol was measured in a group of patients with mild type II diabetes, only glycerol uptake was increased in these patients, whereas the fractional extraction of these substrates was comparable in the diabetic and control groups (3). However, these were patients with mild diabetes who had no increase in splanchnic glucose production as compared to the control subjects. Splanchnic uptake of gluconeogenic precursors have not been measured in patients with poorly controlled type II diabetes, but Cori cycling has been found to be increased in type II diabetic subjects (13) as well as the conversion of lactate and alanine into glucose (14).

The mean initial liver glycogen concentration in the control subjects in this study was  $\sim 30\%$  lower than in a previous study of young healthy subjects (6). The 30% contribution of glycogenolysis to total glucose production observed in the control group in the present study was also somewhat lower than the 36% we observed in that study (6). The differences might be explained by the dietary conditions preceding the fast. Both groups were fed a standardized diet of the same dietary composition, but the caloric intake was on average 35% higher in the young healthy subjects. In addition, since the rate of hepatic glycogenolysis is expressed for kilograms body weight and the control subjects in this study were heavier than the young subjects, the difference will be enhanced. Alternatively, these parameters could depend on other factors such as age and physical activity. When basal liver glycogen concentration was measured in biopsies from healthy subjects, a wide range in concentration was reported, which was attributed to different dietary intake (15).

In summary, we found liver glycogen concentrations to be lower in patients with type II diabetes mellitus than in control subjects four hours after a standard meal. During 23 hours of fasting, whole body glucose production was increased and the rate of net hepatic glycogenolysis decreased in the diabetics. We conclude that the increased rate of glucose production in patients with type II diabetes is due to enhanced gluconeogenesis.

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