Quantitative Contributions of Gluconeogenesis to Glucose Production During Fasting in Type 2 Diabetes Mellitus

Alexandre Wajngot, Visvanathan Chandramouli, William C. Schumann, Karin Ekberg, Paul K. Jones, Suad Efendic, and Bernard R. Landau

Contributions of gluconeogenesis to glucose production were determined between 14 to 22 hours into a fast in type 2 diabetics (n = 9) and age-weight-matched controls (n = 7); ages, 60.4 ± 2.3 versus 55.6 ± 1.2 years and body mass indices (BMI) 28.6 ± 2.3 versus 26.6 ± 0.8 kg/m^2. Production was measured using a primed-continuous [6,6-$^2H_2$]glucose infusion and gluconeogenesis from ^{2}H enrichment at carbons 2 and 5 of blood glucose on ^2H_2O ingestion. Plasma glucose concentration declined from 9.6 ± 0.6 at 14 hours to 7.3 ± 0.6 at 22 hours in the diabetics (P = .001) and from 5.4 ± 0.1 to 5.0 ± 0.1 in the controls (P < .05). Production from the 17th to 22nd hour declined 27.1% ± 0.6% in the diabetics versus 18.5% ± 0.8% in the controls (P = .001); from 10.4 ± 0.3 to 7.6 ± 0.2 versus 10.0 ± 0.4 to 8.2 ± 0.4 μmol/kg/min. Percent contributions of gluconeogenesis to production measured at 1 $\frac{1}{2}$- to 2-hour intervals beginning the 15th hour were 6.8% ± 1.0% more in the diabetics than controls. The quantity of glucose contributed by gluconeogenesis declined 19.8% ± 3.8% (P < .001) in the diabetics and 6.9% ± 2.3% in the controls (P = .05); 7.21 ± 0.32 to 5.74 ± 0.26 versus 6.20 ± 0.28 to 5.75 ± 0.24 μmol/kg/min. The contribution of glycogenolysis to production, estimated from the difference between production and gluconeogenesis, declined to the same extent in diabetic and control subjects, 40.7% ± 6.6% and 37.7% ± 4.1%; from 3.23 ± 0.35 to 1.86 ± 0.26 versus 3.81 ± 0.22 to 2.42 ± 0.28 μmol/kg/min. Thus, gluconeogenesis contributed more to glucose production in the diabetic than control subjects. Production and the contribution of gluconeogenesis declined more in the diabetic subjects during the fast. The factors regulating these changes remain uncertain.

Copyright © 2001 by W.B. Saunders Company

THE PATHOPHYSIOLOGY responsible for fasting hyperglycemia in type 2 diabetes is in debate.[1-3] The hyperglycemia has been attributed to increased glucose production in some studies and to impaired peripheral uptake in others. With the claim of excess glucose production have come data interpreted to indicate that the excess is due to increased gluconeogenesis.[4]

Evidence for increased production derives from several reports of positive correlations between glucose production and fasting plasma glucose concentration.[5,6] However, recent reports have brought into question the tracer methodology used to measure production. Thus, measurements made after tracer infusion of long duration or the giving of a prime of the tracer adjusted to the prevailing fasting glucose concentration, so as to achieve steady state more rapidly, suggest production may be normal or near normal in type 2 diabetes with plasma glucose concentration as high as 10 to 12 mmol/L.[7-9] Early estimates of the contribution of gluconeogenesis to glucose production were in conflict, a likely reflection of limitations in the methods then available for quantitation and in subject selection.[4,10]

A method for quantitating gluconeogenesis using ^2H_2O has been introduced.[11,12] It removes limitations of the other methods. We now have applied it to quantitate the contribution of gluconeogenesis to glucose production during fasting in type 2 diabetics with overnight fasting glucose concentrations about 10 mmol/L. Glucose production has been measured using [6,6-^{2}H]glucose with the giving of a prime adjusted to the glucose concentration. The ^2H_2O and [6,6-$^2H_2$]glucose were given together, so that glucose production and gluconeogenesis could be measured at the same time.

MATERIALS AND METHODS

Design and Subjects

In a first series of experiments, glucose production was quantitated in diabetic subjects. Production was measured in a 12-hour infusion and a 3-hour infusion, the latter using an adjusted prime. Jeng et al[8] reported the long duration of infusion a more satisfactory approach for quantitation. Quantitations were compared using [6-^{3}H]glucose and [6,6-$^2H_2$]glucose. Having obtained support for measurement of glucose production using [6,6-$^2H_2$]glucose in the 3-hour infusion, in a second series, along with production, gluconeogenesis was then measured using ^2H_2O.

Subjects were admitted to the Endocrine Research Unit of the Karolinska Hospital in the afternoon. Between 5 PM and 6 PM, each ate a dinner of 14 kcal/kg body weight composed of 48% carbohydrate, 19% protein, and 33% fat. They then fasted, except for the ingestion of water ad libitum and, for reasons to be noted, Coca-Cola Light (Coca-Cola, Atlanta, GA). In those subjects taking oral hypoglycemia agents for treatment of their diabetes, those medications were discontinued 1 week before study. For those taking insulin, daytime insulin was administered, but bedtime insulin was not given.

The study was approved by the Human Investigation Committees at the Karolinska Hospital and University Hospitals of Cleveland. Informed consent was obtained.

Quantitating Glucose Production

Eleven diabetics, 7 men and 4 women, fasting plasma glucose concentration 10.8 ± 0.7 mmol/L, were studied in the first series. They

From the Department of Endocrinology and Diabetology and the Division of Clinical Physiology, Karolinska Hospital, Stockholm, Sweden; and the Departments of Biochemistry, Epidemiology, and Biostatistics, Medicine, and Nutrition, Case Western Reserve University, Cleveland, OH.

Submitted December 14, 1999; accepted June 22, 2000.

Supported by Grant No. 72X-00034 from the Swedish Medical Research Council, Grant No. DK-14507 from the National Institutes of Health, and by the David S. Stein Foundation of the Jewish Federation of Cleveland.

Address reprint requests to Bernard R. Landau, MD, PhD, Department of Medicine, Case Western Reserve University School of Medicine, 10900 Euclid Ave, Cleveland, OH 44106-4951.

Copyright © 2001 by W.B. Saunders Company

0026-0495/01/5001-0003$10.00/0

doi:10.1053/meta.2001.19422

were 58.3 ± 2.7 years old with body mass indices (BMI) of 30.1 ± 0.6 kg/m^2. Known duration of their diabetes was 14.1 ± 1.4 years. Eight were being treated with insulin, and 3 of those also with an oral hypoglycemic agent. The other 3 were on treatment only with oral agents.

An infusion through a cannulated antecubital vein of [6-^{3}H]glucose (12 μc as a prime and 0.12 μc/min, purchased HPLC purified from NEN, Boston, MA) was begun at 11 PM and continued until 11 AM the following morning. Blood from a cannulated antecubital vein in the other arm was collected at 10-minute intervals from 10:30 to 11:00 AM for determination of the specific activities of glucose in the blood. Glucose and C-peptide concentrations were determined in plasma from the blood collected at 11 AM.

On another occasion, the subjects were treated identically except the infusion was over 3 hours. At 8 AM, an infusion of [6-^{3}H]glucose was begun at the rate of 0.12 μc/min, but with a prime also given at 8 AM of 12 μc multiplied by the plasma mmol/L glucose concentration at 8 AM divided by 5 mmol/L.[9] Blood was drawn for determination of plasma glucose concentration, glucose specific activity, at 10:30 to 11:00 AM and C-peptide concentration determinations again at 11 AM.

Three of the subjects infused with [6-^{3}H]glucose for 3 hours were also infused for 12 hours beginning at 11 PM with [6,6-$^2H_2$]glucose (99% ^{2}H enriched, purchased from Cambridge Isotopes Laboratories, Andover, MA) at the rate of 0.83 mg/min and with a prime of 83 mg. Thus, 3 subjects were infused for 12 hours with [6,6-$^2H_2$]glucose and for the last 3 hours with [6-^{3}H]glucose with a prime. In the blood samples collected at 10-minute intervals beginning at 10:30 AM, enrichments of [6,6-$^2H_2$]glucose, ie, the percent of the molecules of blood glucose with two ^{2}H bound to carbon 6, were then also determined.

In 6 of the subjects infused for 12 hours with [6-^{3}H]glucose, [6,6-$^2H_2$]glucose was infused for 3 hours beginning at 8 AM, again at the rate of 0.83 mg/min, but with a prime of 83 mg multiplied by the plasma mmol/L glucose concentration at 8 AM divided by 5 mmol/L.[9,12] Again, enrichments were also then determined in the bloods collected from 10:30 to 11 AM. Also, 5 of the subjects infused for 3 hours with [6-^{3}H]glucose were infused at the same time with the [6,6-$^2H_2$]glucose, again at 0.83 mg/min with the adjusted prime. Enrichments were then also determined, as well as glucose concentrations and specific activities.

Gluconeogenesis Contribution to Glucose Production

Nine diabetics, 1 woman and 8 men, 60.4 ± 2.3 years old, BMI 28.6 ± 2.3 kg/m^2, hemoglobin A_{1c} = 7.2 ± 0.4 (normal < 5.8) were studied. Known duration of their diabetes was 10.0 ± 1.7 years. Seven healthy normal male subjects (ages, 55.6 ± 1.2 years, and BMI 26.6 ± 0.8 kg/m^2, not significantly different from those of the diabetics) served as controls. Four of the diabetics were being treated with insulin, 4 with oral agents, and 1 with insulin and an oral agent. Three of the 9 subjects were also subjects in the first series.

At 11 PM, each subject drank 2.5 mL/kg body water of ^2H_2O (99.9% ^{2}H, purchased from Isotec, Miamisburg, OH) and again at 2 AM. Body water was estimated at 60% of body weight in the men and 50% in the women.[11] At 8 AM, the prime of [6,6-$^2H_2$]glucose was injected and the infusion begun (again a prime of 83 mg plasma multiplied by the plasma mmol/L glucose concentration at 8 AM/5 mmol/L and an infusion at a rate of 0.83 mg/min) into an antecubital vein and continued until 4 PM.

Blood, 20 mL, was drawn from the opposite arm at 9 AM, 11 AM, 12:30 AM, 2 PM, and 4 PM for determination of the ratio of ^{2}H bound to carbons 2 and 5 of blood glucose. Blood, 5 mL, was drawn at 10:40, 10:50, and 11 AM, and 3:40, 3:50, and 4 PM for determination of the enrichments of [6,6-^{2}H]glucose. Plasma glucose concentrations were also determined during the infusion period.

Six of the diabetic subjects served as controls for a study of the effect of phenylacetate on glucose production and gluconeogenesis.[13] The phenylacetate was given in Coca-Cola Light, 150 mL, at 11 AM, 11:30 AM, and noon to mask the taste of the phenylacetate. Therefore, while Coca-Cola Light is devoid of calories, all of the subjects studied ingested 150 mL at those times, so conditions were the same in controls and diabetics.

Plasma was collected at 8 AM for determination of enrichment in body water.

Analyses

Plasma glucose concentration was determined in duplicate using glucose oxidase (YSI 2300; Yellow Springs Instrument, Yellow Springs, OH). Enrichments of the hydrogens bound to carbons 2, 5, and 6 of blood glucose were determined as previously detailed.[11,12] Briefly, the supernatant, obtained after deproteinizing a blood sample by ZnSO4-Ba(OH)$_2$ addition, was deionized by passage through cation and anion exchange resins. Glucose in the effluent was isolated by high pressure liquid chromatography using a HPX 87P column (Bio-Rad Laboratories, Hercules, CA) with water at 80°C as solvent. To determine the percent of the glucose molecules having two ^{2}H at carbon 6, a sample of the glucose was oxidized with periodate and the formaldehyde formed, which contained carbon 6 with its 2 hydrogens, was converted to hexamethylenetetramine (HMT) by addition of ammonia. The HMT was assayed by gas chromatography-mass spectrometry for mass 142, ie, m + 2.

To determine the enrichment of the hydrogen bound to carbon 2, carbon 1 of a portion of the glucose was removed to form ribulose-5-P. The ribulose-5-P was reduced to a mixture of the polyol phosphates, ribitol-5-P, and arabitol-5-P. These were oxidized with periodate, yielding formaldehyde, which contained carbon 2 with its hydrogen. An HMT was made from the formaldehyde and assayed for mass 141, ie, m + 1. To determine the enrichment of the hydrogen bound to carbon 5, another portion of the glucose was oxidized to remove its carbon 6. The resulting xylose was also oxidized with periodate, yielding carbon 5 with its hydrogen in formaldehyde. Again, an HMT was formed and assayed for mass 141. Enrichments at carbons 2 and 5 of glucose from a subject were determined in quintuplicate.[12] Mean enrichment at carbon 2 was 0.46% with a coefficient of variation of 1.1%. Mean enrichment at carbon 5 was 0.19% with a coefficient of variation of 2.6%.

Background enrichments were measured in glucose from blood before ^2H_2O ingestion. HMTs, formed from formaldehyde from [6,6-$^2H_2$]glucose and [1-^{2}H]sorbitol of known enrichments, served as standards.[12] Regression lines had a r^2 coefficient of .997 ± .001 in the m + 2 assays (n = 4) and .999 ± .001 in the m + 1 assays (n = 6). The [1-^{2}H]sorbitol was prepared by reduction with $NaBH_4$ of [1-^{2}H]glucose, 98% enriched, also purchased from Cambridge Isotope Laboratories.[11]

Enrichment in plasma water, equated to that in body water, was measured by Metabolic Solutions, Nashua, NH, using an isotope ratio mass spectrometer.

The ^{3}H specific activity of blood glucose was determined also as previously described.[14] Plasma was deproteinized by addition of $ZnSO_4$ and Ba(OH)$_2$. The supernatant after centrifugation was deionized by passage through the ion exchange resins. A quantity of glucose in an evaporated portion of the effluent was then assayed for radioactivity in a liquid scintillation counter (Model 1600 TR; Packard Instrument, Meriden CT). Counting was for 20 minutes, in duplicate, resulting in a probable error of less than 2%.

Heparinized blood was kept on ice until centrifuged at 4°C. An aliquot of the plasma was used for determination of glucose. The remainder was frozen at −20°C for later analyses of C-peptide and insulin concentrations. C-peptide was measured by radioimmunoassay (RIA) using a commercially available kit (Novo-Nordisk Research, Bagsvaerd, Denmark). Immunoreactive insulin was assayed by RIA using our own antibodies, human insulin as a standard, and charcoal

Table 1. Comparison of Glucose Production Measured in Fasted Diabetic Subjects Infused for 3 Hours With or 12 Hours With [6-^{3}H]Glucose or [6,6-^{2}H]Glucose

Group	Glucose	Time (h)	Production	No.*
1	[6-^{3}H]	12	9.4 ± 0.3	11
	[6-^{3}H]	3	9.4 ± 0.4	
2	[6,6-^{2}H]	12	9.2 ± 0.2	3
	[6-^{3}H]	3	8.9 ± 0.1	
3	[6-^{3}H]	12	9.1 ± 0.5	6
	[6,6-^{2}H]	3	10.1 ± 0.6	
4	[6,6-^{2}H]	3	9.1 ± 0.6	5
	[6-^{3}H]	3	8.8 ± 0.6	

* Glucose production is expressed in μmol/kg body weight/min for the number of subjects.

addition to separate antibody-bound and free insulin.[15] Blood samples for assay of glucagon were collected in prechilled tubes containing Trasylol (Bayer, West Haven, CT) and EDTA. Assay was by the method of Faloona and Unger using the 30 K antibody.[16]

Calculations

Specific activities were calculated by dividing the dpm in the samples assayed by the amounts of glucose in the samples. To calculate the rate of glucose production using [6-^{3}H]glucose, the dpm of [6-^{3}H]glucose infused per minute was divided by the specific activity of the glucose in the blood in dpm/μmol. The specific activity used was the mean of the 4 determinations from the samples collected at 10:30 to 11 AM. The rate of glucose production using [6,6-$^2H_2$]glucose was calculated by multiplying the percent enrichment of [6,6-$^2H_2$]glucose infused, ie, 99%, by its rate of infusion, ie, 4.6 μmol/min, and dividing by the percent of [6,6-$^2H_2$]glucose enrichment in the blood glucose, determined by the assay of m + 2 at carbon 6, minus the rate of infusion. Enrichment at carbon 6 used in the calculation was the mean of the 4 determinations from the samples collected at 10:30 to 11 AM in the first series and of the 3 determinations from samples collected at 10:40 to 11 AM or at 3:40 to 4 PM in the second series. Results are expressed in μmoles/kg body weight/min. The percent contribution of gluconeogenesis to glucose production was set equal to 100 times the excess enrichment of the hydrogen bound to carbon 5 compared with that to carbon 2 of the glucose, ie, the C5/C2 ratio.[11,12] The rate glucose was formed by gluconeogenesis was calculated by multiplying the rate of glucose production by the percent contribution.

Statistics

Means ± SEM are recorded. Student's *t* test was used (independent groups for diabetics *v* controls and paired for comparisons over time) with a $P \leq .05$ considered significant. Coefficients of variation were calculated by dividing the standard deviations of the means by the means.

RESULTS

Table 1 records the glucose productions measured infusing [6-^{3}H]glucose and [6,6-^{2}H]glucose in the 11 diabetic subjects. Measurements were made at the end of 17 hours of fasting, ie, 11 AM, infusions having been at that time for 3 or 12 hours. Fasting plasma glucose concentration at 11 AM was 9.4 ± 0.4 mmol/L and C-peptide concentration 1.07 ± 0.11 nmol/L, in accord with the diagnosis in the subjects of type 2 diabetes. When the 11 subjects were infused with [6-^{3}H]glucose for 12 hours and with [6-^{3}H]glucose with a prime for 3 hours, there was no difference in glucose production. This was also the case when 3 of the subjects were infused for 12 hours with [6,6-^{2}H]glucose and from the 9th to 12th hour with [6-^{3}H]glucose; when 6 of the subjects infused with the [6-^{3}H]glucose for 12 hours were from the 9th to 12th hour infused with [6,6-^{2}H]glucose; and when 3 of the subjects infused with [6-^{3}H]glucose for 3 hours were infused with [6,6-^{2}H]glucose at the same time. Differences between the 2 estimates for the same subject in first group was 11.7% ± 3.4%, in the second group 4.5% ± 2.3%; in the third group 9.9% ± 2.3%, and in the fourth group 11.0% ± 3.7%.

The m + 2 excess at carbon 6 of blood glucose at 10:30 AM in the 3-hour infusions of [6,6-$^2H_2$]glucose was 96.3% ± 1.1% of that at 11 AM; in the 12-hour infusions, it was 96.4% ± 0.4%. The specific activity of blood glucose at 10:30 AM in the 3-hour infusion was 98.7% ± 0.7% of that at 11 AM. In the 12-hour infusion, it was 98.3% ± 1.2%

Table 2 records the plasma glucose concentration of the normal and diabetic subjects in whom the contribution of gluconeogenesis to glucose production was determined. Plasma glucose concentrations in the diabetics were after 14 hours of fasting 9.6 ± 0.6 mmol/L and declined over the next 8 hours of fasting to 7.6 ± 0.2 mmol/L ($P = .001$). In the normal subjects, concentrations declined from 5.4 to 5.0 mmol/L ($P < .05$).

The percent contribution of gluconeogenesis to glucose production gradually increased in both groups (Table 3); 52.6% ± 2.5% to 70.6% ± 2.6% in the normals and 62.5% ± 2.8% to 75.6% ± 3.1% in the diabetics. The means at all time points were higher in the diabetics than normals and significantly so at 12:30 PM ($P = .04$) and 2 PM ($P = .002$). The differences between normal and diabetic for the 5 times was 6.8% ± 1.0%.

Glucose production was not significantly different between the normals and diabetics at 11 AM, 10.0 ± 0.4 versus 10.4 ± 0.3 and at 4 PM, 8.2 ± 0.4 versus 7.6 ± 0.2 μmol/kg/min. However, glucose production over the 5-hour period declined more in the diabetics than controls (27.1% ± 0.6% *v* 18.5% ± 0.8%; $P = .001$). The quantity of glucose produced by gluconeogenesis, calculated by multiplying glucose production by the percent contribution of gluconeogenesis declined in the diabetics by 19.8% ± 3.7%, from 7.21 ± 0.32 μmol/kg/min at 11 AM to 5.74 ± 0.26 μmol/kg/min at 4 PM ($P = .001$). In the normals, it declined by 6.9% ± 2.3%, from 6.20 ± 0.28 at 11 AM to 5.75 ± 0.24 μmol/kg/min at 4 PM ($P = .05$). The decline in the diabetics was significantly more than in the normals ($P < .05$).

Thus, the production of gluconeogenesis in the diabetics

Table 2. Glucose Concentration (mmol/L) in Normal (n = 7) and Diabetic (n = 9) Subjects From 14 to 22 Hours of Fasting

Time	8 AM	9 AM	10 AM	11 AM	12:30 PM	2 PM	3 PM	4 PM
Normal	5.4 ± 0.1	5.4 ± 0.1	5.3 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.1 ± 0.1	5.0 ± 0.1
Diabetic	9.6 ± 0.6	9.6 ± 0.6	9.5 ± 0.7	9.5 ± 0.7	8.5 ± 0.7	8.0 ± 0.7	7.7 ± 0.7	7.3 ± 0.6

Table 3. Percent Gluconeogenesis (%GNG) Contributed to Glucose Production (GP) Measured in the Subjects of Table 2 From 15 to 22 Hours of Fasting and GP (μmol/kg/min) Measured at 17 Hours and 22 Hours

	%GNG					GP	
Time	9 AM	11 AM	12:30 AM	2 PM	4 PM	11 AM	4 PM
Normal	57.6 ± 2.5	61.9 ± 1.7	64.8 ± 1.5	63.5 ± 2.5	70.6 ± 2.6	10.0 ± 0.4	8.2 ± 0.4
Diabetic	62.5 ± 2.8	69.3 ± 2.9	71.1 ± 2.1	73.9 ± 1.7	75.6 ± 3.1	10.4 ± 0.3	7.6 ± 0.2

declined, so that after 22 hours of fasting, the absolute quantity of glucose produced by gluconeogenesis in the diabetics was not significantly more than in the normals (Fig 1). Glycogenolysis in the diabetics was at the rate of 3.23 ± 0.35 μmol/kg/min and declined by 40.7% ± 6.6% to 1.86 ± 0.26 μmol/kg/min at 4 PM. In the normals, the rate of glycogenolysis at 11 AM, 3.81 ± 0.22 μmol/kg/min, was not different from that in the diabetics. The percent decline, 37.7% ± 4.1%, and rate of glycogenolysis at 4 PM, 2.42 ± 0.28 μmol/kg/min, were also not significantly different from those in the diabetics (Fig 1).

The m + 2 excess at carbon 6 of blood glucose at 10:40 AM was 98.6% ± 1.3% and at 10:50 AM 98.0% ± 1.1% of that at 11 AM in the normals and 96.8% ± 0.7% and 99.2% ± 1.4% of that at 11 AM in the diabetics. At 3:40 and 3:50 PM for the normals, it was 101.2% ± 1.1% and 99.5% ± 1.5%, respectively, of that at 4 PM, and in the diabetic correspondingly 99.7% ± 1.2% and 100.9% ± 0.5%. Coefficient of variation for the 3 determinations for the subjects at 10:40 to 11 AM was 2.7% ± 0.3% and at 3:40 to 4 PM, 1.9% ± 0.3%.

In the diabetic subjects, enrichment at carbon 2 of blood glucose collected at 9 AM was 92.0% ± 1.7% and at 4 PM, 101.9% ± 3.4% of that in body water at 8 AM, which was 0.557% ± 0.010%. In the controls, the corresponding percentages were 95.0% ± 2.9%, 95.2% ± 2.4%, and 0.558% ± 0.013%. The mean increase of about 9% in the enrichment at carbon 2 of glucose in the diabetic subjects does not affect the estimates of gluconeogenesis, because those estimates depend on C5/C2 ratios. The C5/C2 ratio is unaffected by the C2/water ratio increasing with time.[12] The longer time needed for the enrichment at carbon 2 to approach that in body water in the diabetic than normal subjects is explained by the longer time needed for sufficient turnover of the larger glucose pool in the diabetic subjects.

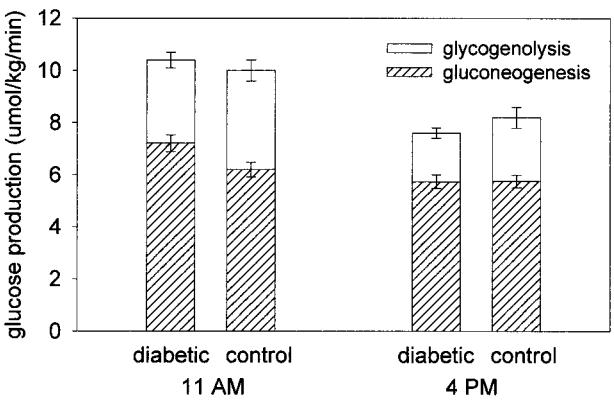

Fig 1. Contributions of glycogenolysis and gluconeogenesis to glucose production in diabetic and control subjects after fasting 17 hours (11 AM) and 22 hours (4 PM).

Insulin concentrations were not different in the diabetics and controls despite the differences in glucose concentrations (18 ± 4 *v* 18 ± 3 μU/mL at 8 AM and 16 ± 3 *v* 12 ± 2 μU/mL at 4 PM). Nor were C-peptide concentrations different (0.85 ± 0.15 *v* 1.10 ± 0.12 pmol/L at 8 AM and 0.79 ± 0.11 *v* 0.74 ± 0.08 pmol/L at 4 PM). However, insulin concentration was less in all 7 control subjects at 4 PM than 8 AM ($P = .05$), as was C-peptide concentration ($P = .01$). Glucagon concentration also did not differ significantly, but there was the suggestion of a higher concentration in the diabetics, particularly at 8 AM (33 ± 7 *v* 18 ± 3 at 8 AM and 23 ± 4 *v* 18 ± 2 at 4 PM).

DISCUSSION

Hother-Nielsen and Beck-Nielsen[9] calculated that in 6 published studies in which an adjusted prime-continuous infusion for 2 to 3 hours of [3-^{3}H]glucose was given to type 2 diabetic subjects with fasting plasma glucose concentrations of 8.3 to 11.0 mmol/L, glucose production rates were 5% to 18%, mean 12%, more than normal. In 6 studies in which a fixed prime was given and plasma glucose concentrations were from 10.1 to 12.2 mmol/L, production was 31% to 69%, mean 57%, more. Maggs et al[6] found, giving an adjusted prime-continuous infusion for 4 hours of [6,6-$^2H_2$]glucose, production rates about 15% more in type 2 diabetic subjects with fasting plasma glucose concentrations about 10 mmol/L than in normal subjects. Gastaldelli et al[17] found, compared with control subjects, production rates 16% more in obese (BMI, 30 to 32 kg/m^2) and 35% more in lean (BMI, 25 to 26 kg/m^2) type 2 diabetics with plasma glucose concentrations about 8.2 mmol/L when they were infused for 3 hours with [6,6-$^2H_2$]glucose using an adjusted prime. We found no significant increase in glucose production in diabetics subjects with fasting plasma glucose concentrations about 10 mmol/L. Chen et al[7] and Jeng[8] concluded that still more reliable estimates of production are obtained by infusing radioactive glucose for 12 hours. They found production normal in type 2 diabetic subjects with fasting glucose concentrations less than 10 mmol/L.[8] We detected no difference between measurements after 12 hours of infusion and after 3 hours with an adjusted prime.

We found about a 7% higher contribution of gluconeogenesis to glucose production in our diabetic than normal subjects. At 15 hours of fasting Gastaldelli et al[17] found in their lean diabetic subjects a 27% higher contribution than in controls (mean 64% *v* 47%) and in their obese diabetic subjects, a 10% higher contribution (68% *v* 62%). Consoli et al[18] concluded gluconeogenesis plays a predominant role in increasing glucose production in type 2 diabetics, but the [2-^{14}C]acetate method they used to arrive at that conclusion proved invalid.[19,20] How-

ever, they also reported gluconeogenesis measured by the incorporation into glucose of label from ^{13}C-alanine, ^{14}C-lactate, and ^{14}C-glycerol was markedly increased.[4,21,22] Rates of conversion to glucose were calculated by multiplying rates of glucose production by the ratio of the enrichment in plasma glucose to that in plasma alanine and by the ratios of specific activities in plasma glucose to those in plasma lactate and glycerol. The enrichment and specific activities of the gluconeogenic substrates in plasma are then assumed to reflect those at the site(s) of gluconeogensis and that they are relatively the same as in the plasma of the diabetic and normal subjects. Much of those increased rates of gluconeogenesis are attributable to the increased rates of glucose production they reported in their diabetic subjects. The subjects given ^{13}C-alanine and ^{14}C-lactate had a fasting plasma glucose concentration of 12.9 ± 0.6 mmol/L. Glucosuria in these subjects could have been significant, and production expected to be increased. Fasting concentration in the subjects given ^{14}C-glycerol was 10.9 ± 0.4 mmol/L. [2-^{3}H]Glucose was used to measure production.[22] Glucose cycling occurs in type 2 diabetes,[23] and this results in overestimations of production using [2-^{3}H]glucose.[24] Furthermore, while glucose production was stated to be 1.5 times more in the diabetics, the same amount of [2-^{3}H]glucose was infused into the normal and diabetic subjects and at steady state, the ^{3}H-specific activities of plasma glucose were the same. Thus, the data calculate to the same rates of glucose production in the diabetic and normal subjects.

Tayek and Katz,[25] from isotopomer analyses on infusing [U-$^{13}C_6$]glucose, estimated a contribution of gluconeogenesis of 46.6% in control and 48.8% in type 2 diabetic subjects with fasting plasma glucose concentrations about 11.8 mmol/L. Their estimates take on validity assuming in the normal and diabetic subjects extents of loss and dilution of label in the conversion to glucose of the labeled lactate formed from the [U-$^{13}C_6$]glucose were the same.[26-28] Hellerstein and associates[29,30] by mass isotopomers distribution analysis (MIDA), on infusing [2-^{13}C]glycerol estimated in type 2 diabetics a contribution of about 33.5% and in controls 36.5%. Limitations in the use of that method of analysis, resulting in underestimations, have been discussed.[28,31]

Magnusson et al[32] estimated an overall contribution of gluconeogenesis to glucose production of 88% in type 2 diabetic subjects during 23 hours of fasting. Gluconeogenesis was estimated by subtracting the net change in hepatic glycogen content during the fast, measured by nuclear magnetic resonance (NMR), from glucose production. The reasons for the estimates of the contribution in the diabetics by that NMR method being higher than by other methods relates to the definition of gluconeogenesis used. Gluconeogenesis is the production of glucose from noncarbohydrate precursors. The net rate of glycogenolysis is the difference between the rates of glycogenolysis and glycogenesis, ie, the rate of synthesis of glycogen. Subtracting that net rate from glucose production as done by Magnusson et al[32] then gives the sum of the rates of gluconeogenesis and glycogenesis, not just gluconeogenesis. Because the D_2O method gives estimates of rates of gluconeogenesis and glycogenolysis, by combining the 2 methods, the rate of glycogenesis can be estimated and hence the extent of glycogen cycling.

In the fasted normal subject, there is good evidence glycogen cycling, ie, simultaneous glyconeogenesis and glycogenolysis does not occur.[28,33] In accord with that, the NMR method gives estimates of the contribution of gluconeogenesis similar to that by the $^{2}H_2O$ method, ie, about 50%.[34,35] Glycogenesis has been concluded to occur in normal subjects in the fasted state.[36] Reference is to a study by Magnusson et al[37] titled and referred to as in the fasted state. However, glycogenesis was demonstrated while subjects, who had been fasted for 12 to 14 hours, were infused with glucose to maintain a plasma glucose concentration of ~9.5 mmol/L. In the diabetic in whom there is evidence of hyperglycemia, hyperinsulinemia and hyperglucagonemia glycogen cycling presumably occurs,[33] ie, there is glycogenesis.

The absolute quantity of glucose produced by gluconeogenesis and the percent by gluconeogenesis were significantly more in the diabetic than in the normal subjects at 17 hours of fasting. In the normal subjects, the absolute contribution of gluconeogenesis was only 6.9% ± 2.3% less at 22 than at 17 hours of fasting. That finding is in agreement with measurement of the contribution of gluconeogenesis to glucose production in normals previously reported.[12] In that study, gluconeogenesis was measured in 5 subjects after 14 to 16 hours of fasting and in 5 other subjects at 22 to 24 hours of fasting. Glucose productions at those times in that study were 10.2 ± 0.2 and 8.4 ± 0.4 μmol/kg/min compared with 10.0 ± 0.4 and 8.2 ± 0.4 at 17 hours and 22 hours in the present study. Absolute rates of gluconeogenesis in that study at both 14 to 16 hours and 22 to 24 hours were 5.5 ± 0.3 μmol/kg/min compared with 6.20 ± 0.28 and 5.75 ± 0.24 in the present study. In that study D_2O was ingested 3 hours before measurements were made and in the present study, 12 hours before. The similar results may then provide further support for negligible glycogen cycling in normal fasted subjects.

Glucose production and plasma glucose concentrations have previously been shown to progressively narrow between type 2 diabetic and normal subjects over a 20-hour period of fasting, albeit in diabetics with plasma glucose concentrations about 18 mmol/L.[7] Ours is the first study examining contributions of gluconeogenesis and glycogenolysis during such a fast. During the 5 hours from 17 hours to 22 hours, the contribution of gluconeogenesis, but not glycogenolysis, to glucose production declined more in the diabetic than normal subjects. As a result, at 22 hours of fasting, the absolute rates of gluconeogenesis in the normal and diabetic subjects were the same. Changes in hormonal milieu and substrates in the diabetic that could account for these differences from normal are still undefined. A higher glucagon concentration in the diabetics at 8 AM coupled to a decline in insulin secretion from 8 AM to 4 PM in the normals, but not diabetics, as evidenced by insulin and C-peptide concentrations, may be relevant. Lower hepatic glycogen content than normal has been found in type 2 diabetic subjects, but with fasting plasma glucose concentrations about 15 mmol/L.[32]

ACKNOWLEDGMENT

We thank nurses, Annica Clark and Kajsa Sundquist, for their care of the patients and for technical assistance.

REFERENCES

1. DeFronzo RA: Pathogenesis of type 2 (non-insulin dependent) diabetes mellitus: A balanced overview. Diabetologia 35:389-397, 1992

2. Beck-Nielsen H, Hother-Nielsen O, Vaag A, et al: Pathogenesis of type 2 (non-insulin dependent) diabetes mellitus: The role of skeletal muscle glucose uptake and hepatic glucose production in the development of hyperglycaemia. A critical comment. Diabetologia 37:217-231, 1994

3. Gerich JE: Is muscle the major site of insulin resistance in type 2 (non-insulin-dependent) diabetes mellitus? Diabetologia 34:607-610, 1991

4. Gerich JE: Gluconeogenesis in type 2 diabetes. Adv Exp Med Biol 334:253-288, 1993

5. DeFronzo RA, Ferrannini E, Simonson DC: Fasting hypoglycemia in non-insulin-dependent diabetes mellitus: Contributions of excessive hepatic glucose production and impaired tissue glucose uptake. Metabolism 38:387-395, 1989

6. Maggs DG, Buchanan TA, Burant CF, et al: Metabolic effect of troglitazone monotherapy in type 2 diabetes mellitus. Ann Intern Med 128:176-185, 1998

7. Chen Y-DI, Jeng C-Y, Hollenbeck CB, et al: Relationship between plasma glucose and insulin concentration, glucose production, and glucose disposal in normal subjects and patients with non-insulin-dependent diabetes. J Clin Invest 82:21-25, 1988

8. Jeng C-Y, Sheu WH-H, Fuh MM-T, et al: Relationship between hepatic glucose production and fasting plasma glucose concentration in patients with NIDDM. Diabetes 43:1440-1444, 1994

9. Hother-Nielsen O, Beck-Nielsen H: On the determination of basal glucose production rate in patients with type 2 (non-insulin-dependent) diabetes mellitus using primed-continuous 3-^{3}Hglucose infusion. Diabetologia 33:603-610, 1990

10. Landau BR: Estimating gluconeogenesis rates in NIDDM. Adv Exp Med Biol 334:209-220, 1993

11. Landau BR, Wahren J, Chandramouli V, et al: Contributions of gluconeogenesis to glucose production in the fasted state. J Clin Invest 98:378-385, 1996

12. Chandramouli V, Ekberg K, Schumann WC, et al: Quantifying gluconeogenesis during fasting. Am J Physiol 273:E1209-E1215, 1997

13. Wajngot A, Chandramouli V, Schumann WC, et al: A probing dose of phenylacetate does not affect glucose production and gluconeogenesis in humans. Metabolism 49:1211-1214, 2000

14. Ekberg K, Landau BR, Wajngot A, et al: Contributions by kidney and liver to glucose production in the postabsorptive state and after 60 h of fasting. Diabetes 48:292-298, 1999

15. Herbert V, Lau KS, Gottlieb CW, et al: Coated charcoal immunoassay of insulin. J Clin Endocrinol 25:1375-1384, 1965

16. Faloona GR, Unger RH: Glucagon radioimmunoassay, in Jaffe BM, Berman HR (eds): Methods of Hormone Radioimmunoassay. New York, NY, Academic, 1965, pp 317-330

17. Gastaldelli A, Baldi S, Pettiti M, et al: Influence of obesity and type 2 diabetes on gluconeogenesis and glucose output in humans: A quantitative study. Diabetes 49:1374-1380, 2000

18. Consoli A, Nurjhan N, Capani F, et al: Predominant role of gluconeogenesis in increased hepatic glucose production in NIDDM. Diabetes 38:550-557, 1989

19. Schumann WC, Magnusson I, Chandramouli V, et al: Metabolism of [2-^{14}C]acetate and its use in assessing hepatic Krebs cycle activity and gluconeogenesis. J Biol Chem 266:6985-6990, 1991

20. Consoli A, Gerich JE: Corrigendum for limitations in the use of [2-^{14}C]acetate for measuring gluconeogenesis in vivo. Diabetes 42: 1361-1362, 1993

21. Consoli A, Nurjahn N, Reilly JJ Jr, et al: Mechanism of increased gluconeogenesis in non-insulin-dependent diabetes mellitus. J Clin Invest 86:2038-2045, 1990

22. Nurjhan N, Consoli A, Gerich J: Increase lipolysis and its consequence on gluconeogenesis in non-insulin-dependent diabetes mellitus. J Clin Invest 89:169-175, 1992

23. Efendic S, Wajngot A, Vranic M: Increased activity of the glucose cycle in the liver: Early characteristic of type 2 diabetes. Proc Natl Acad Sci USA 82:2565-2569, 1985

24. Landau BR: Measuring glucose and fructose-6-phosphate cycling in liver in vivo. Metabolism 42:457-462, 1993

25. Tayek JA, Katz J: Glucose production, recycling, and gluconeogenesis in normals and diabetics: A mass isotopomer [U-^{13}C]glucose study. Am J Physiol 270:E709-E717, 1996

26. Landau BR: Commentary. Limitations in the use of [U-$^{13}C_6$]glucose to estimate gluconeogenesis. Am J Physiol 277:E408-E413, 1999

27. Radziuk J, Lee W-NP: Measurement of gluconeogenesis and mass isotopomer analysis based on [U-^{13}C]glucose. Am J Physiol 40:E199-E207, 1999

28. Landau BR: Quantifying the contribution of gluconeogenesis to glucose production in fasted human subjects using stable isotopers. Proc Nutr Soc 58:963-972, 1999

29. Hellerstein M, Neese R, Linfoot P, et al: Contribution from gluconeogenesis to fasting blood glucose measured by mass isotopomer distribution analysis in normal humans. Diabetes 44:153A, 1995 (suppl 1)

30. Christiansen M, Linfoot P, Neese R, et al: Gluconeogenesis in NIDDM: Effect of energy restriction. Diabetes 44:55A, 1995 (suppl 1)

31. Landau BR, Fernandez CA, Previs SF, et al: A limitation in the use of mass isotopomer distributions to measure gluconeogenesis in fasting humans. Am J Physiol 269:E18-E26, 1995

32. Magnusson I, Rothman DL, Katz L, et al: Increased rate of gluconeogenesis in type II diabetes mellitus. J Clin Invest 90:1323-1327, 1992

33. Petersen KF, Laurent D, Rothman DL, et al: Mechanism by which glucose and insulin inhibit net hepatic glycogenolysis in humans. J Clin Invest 101:1203-1209, 1998

34. Petersen KF, Price T, Cline GW, et al: Contribution of net hepatic glycogenolysis to glucose production during early postprandial period. Am J Physiol 270:E186-E191, 1996

35. Petersen KF, Krssak M, Navarro V, et al: Contributions of net hepatic glycogenolysis and gluconeogenesis to glucose production in cirrhosis. Am J Physiol 276:E529-E535, 1999

36. Fried R, Beckmann N, Keller U, et al: Early glycogenolysis and late glycogenesis in human liver after intravenous administration of galactose. Am J Physiol 270:G14-G19, 1996

37. Magnusson I, Rothman DL, Jucker B, et al: Liver glycogen turnover in fed and fasted humans. Am J Physiol 266:E796-E803, 1994