Assessment of Hepatic Sensitivity to Glucagon in NIDDM
Use as a Tool to Estimate the Contribution of the Indirect Pathway to Nocturnal Glycogen Synthesis

Michael F. Nielsen, Steven Wise, Sean F. Dinneen, W. Frederick Schwenk, Ananda Basil, and Robert A. Rizza

NIDDM is associated with excessive rates of endogenous glucose production in both the postabsorptive and postprandial states. To determine whether this is due to an intrinsic increase in hepatic sensitivity to glucagon, 9 NIDDM and 10 nondiabetic subjects were studied on three occasions. On each occasion, glycogen was labeled the evening before the study with subjects ingesting meals containing [6-3H]galactose. Beginning at 6:00 A.M. on the following morning, somatostatin was infused to inhibit endogenous hormone secretion. Insulin concentrations were maintained constant at basal levels (defined as that necessary to keep glucose at ~5 mmol/l) in each individual. On one occasion, glucagon was infused at a rate of 0.65 ng · kg⁻¹ · min⁻¹ throughout the experiment, resulting in glucagon concentrations of ~130 pg/ml and a slow but comparable fall in endogenous glucose production with time in both groups. On the other two occasions, the glucagon infusion was increased at 10:00 A.M. to either 1.5 or 3.0 ng · kg⁻¹ · min⁻¹, resulting in an increase in glucagon concentrations to ~180 and 310 pg/ml, respectively. The increment in endogenous glucose production (i.e., area above basal) did not differ in diabetic and nondiabetic subjects during either the 1.5 ng · kg⁻¹ · min⁻¹ (0.75 ± 0.05 vs. 0.78 ± 0.048 mmol/kg) or 3.0 ng · kg⁻¹ · min⁻¹ (1.06 ± 0.066 vs. 1.10 ± 0.073 mmol/kg) glucagon infusions. In contrast, the amount of [6-3H]glucose released from glycogen was lower (P < 0.05) in the diabetic than nondiabetic subjects during both glucagon infusions.

The specific activity of glycogen, calculated as the integrated release of [6-3H]glucose divided by the integrated release of unlabeled glucose, was lower (P < 0.05) in diabetic subjects than in nondiabetic subjects during both glucagon infusions. The increment in endogenous glucose production (i.e., area above basal) did not differ in diabetic and nondiabetic subjects during either the 1.5 ng · kg⁻¹ · min⁻¹ (19.0 ± 3.9 vs. 41.4 ± 5.7 dpm/µmol) and 3.0 ng · kg⁻¹ · min⁻¹ (19.1 ± 3.1 vs. 36.5 ± 7.2 dpm/µmol) glucagon infusions, implying that a greater portion of the glucose released from glycogen was derived from the indirect pathway. We concluded that although NIDDM is not associated with an intrinsic alteration in hepatic sensitivity to glucagon, it does alter the relative contributions of the direct and indirect pathways to nocturnal glycogen synthesis. Diabetes 46:2007-2016, 1997

People with NIDDM have excessive rates of glucose production both before and after food ingestion (1–8). Because insulin inhibits and glucagon stimulates glucose production, the ratio of the two is believed to be a major determinant of the rate of hepatic glucose release (9–11). Increased rates of glucose production, therefore, could be due to insulin deficiency and/or glucagon excess. Previous studies have demonstrated that insulin concentrations are inappropriately low and glucagon concentrations inappropriately high in people with NIDDM when considered in light of the prevailing glucose concentrations (12–15). It is also well established (1–3,5) that NIDDM is commonly associated with hepatic insulin resistance (i.e., higher insulin concentrations are required to achieve the same biological effect). However, it is currently not known whether the hepatic response to glucagon is also abnormal.

A case can be made for either an increase or a decrease in hepatic sensitivity to glucagon in NIDDM. If indeed glucose production is predominantly determined by the portal-venous insulin-to-glucagon ratio, and if the ability of insulin to restrain endogenous glucose production in response to an increase in glucagon is impaired in NIDDM, then an increase in glucagon may be accompanied by a proportionately greater increase in glucose production in diabetic than in nondiabetic subjects. Although it could be argued that this is merely another manifestation of insulin resistance, the end result would be an enhanced response to glucagon. On the other hand, an argument can be made for a decreased response to glucagon. First, in vitro studies have suggested that glucagon binding and action on human liver is decreased in NIDDM (16). Second, mutations in the glucagon receptor have been reported to be present in at least a subset of individuals with NIDDM (17,18). These mutations have been shown to result in a reduced sensitivity to glucagon (19). Third, glucagon acutely increases endogenous glucose production, primarily by stimulating glycogenolysis (20,21). Hepatic glycogen content has been reported to be lower in diabetic than in nondiabetic subjects (22). If lesser amounts of...
glycogen are associated with lower rates of glycogenolysis, then the response to glucagon may also be lower.

In addition to differences in the rate of endogenous glucose production, the source of the released glucose also may differ in diabetic and nondiabetic subjects. Glycogen is formed by either direct or indirect pathways (23). In the former, glucose is taken up by the liver and converted to glucose-6-phosphate (G-6-P), then directly incorporated into glycogen; in the latter, G-6-P is first degraded to pyruvate, then reconverted via the gluconeogenic pathway back to G-6-P, which in turn is incorporated into glycogen. The contribution of the direct pathway to glycogen synthesis increases with increasing insulin and glucose concentrations, whereas the contribution of the indirect pathway increases following a fast when antecedent rates of gluconeogenesis are high (24–29). In an elegant series of experiments, Hwang et al. (30) used 13C nuclear magnetic resonance spectroscopy to demonstrate that flux through the gluconeogenic pathway relative to the direct pathway following a mixed meal was 1.7-fold greater in IDDM than in nondiabetic subjects (30). Similarly, Giaccari and Rossetti (31) showed that the contribution of the indirect pathway to glycogen synthesis during a hyperglycemic clamp was markedly increased in 90% of pancreatectomized diabetic rats (31). Currently it is not known whether NIDDM alters the relative contributions of the direct and indirect pathways to glucose production.

The present studies, therefore, were undertaken to determine whether the hepatic response to glucagon differs in NIDDM and nondiabetic subjects when differences in insulin action are controlled for by providing individually determined basal insulin concentrations. By prelabeling glycogen with [6-3H]glucose (derived from [6-3H]galactose ingested the evening before), these studies also afforded the opportunity to determine the source of the released glucose, since this tracer is detritiated during passage through the indirect pathway (32–34). We report that the ability of glucagon to stimulate endogenous glucose production did not differ in diabetic and nondiabetic subjects, indicating comparable hepatic sensitivity to glucagon. On the other hand, the amount of [6-3H]glucose released from glycogen was substantially lower in diabetic than in nondiabetic subjects, implying that the contribution of the indirect pathway to nocturnal glycogen synthesis was greater in diabetic than in nondiabetic subjects.

**RESEARCH DESIGN AND METHODS**

**Subjects.** After approval from the Mayo Institutional Review Board, 9 NIDDM and 10 nondiabetic volunteers gave written informed consent to participate in the study. The characteristics of the subjects are shown in Table 1. Age, sex, BMI, and percent body fat did not differ between groups. The mean GHB concentration (Glyc-Affin; Isolab, Akron, OH; normal range 4–7%) was higher in the diabetic than in the nondiabetic group. At the time of screening, all diabetic subjects were taking sulfonylureas, with one taking metformin. These agents were discontinued 3 weeks before the initial study. All subjects were in good health, had normal blood pressure, and were at stable weight. None regularly engaged in vigorous exercise or took any medication other than sulfonylureas. The nondiabetic subjects did not have any history of diabetes in first-degree relatives.

**Experimental design.** Volunteers were admitted to the Mayo General Clinical Research at 5:00 a.m. on the evening before the study. Subjects were asked to omit lunch on the day of admission in an effort to minimize hepatic glycogen. At 6:00 a.m., subjects ingested a 10 kcal/kg, 50% carbohydrate, 15% protein, and 35% fat meal containing 2 g/kg of dextrose mixed with 60 μCi of [6-3H]galactose in the form of gelatin. At 10:00 a.m. and 12:00 a.m., two additional evening snacks (2.75 kcal/kg as dextrose), each containing 1 g/kg of dextrose mixed with 30 μCi of [6-3H]galactose in the form of gelatin, were eaten to increase the labeling of glycogen and minimize the contribution of the indirect pathway to nocturnal glycogen synthesis; an 18-gauge catheter was inserted into a forearm vein. The diabetic subjects were infused with insulin to maintain euglycemia throughout the night (5,35). The nondiabetic subjects were infused with 0.9% saline.

At 6:00 a.m. the next morning, an 18-gauge catheter was inserted retrogradely into a contralateral dorsal hand vein. This hand was then placed in a heated Plexiglas box and maintained at a temperature of 35°C throughout the study to allow sampling of arterialized venous blood. Somatostatin (60 ng • kg⁻¹ • min⁻¹) and replacement infusions of glucagon (0.65 ng • kg⁻¹ • min⁻¹) and growth hormone (3 ng • kg⁻¹ • min⁻¹) were initiated at 6:00 a.m. Insulin also was infused in amounts necessary to maintain glucose concentrations constant at 5 mmol/L. The last adjustment of the insulin infusion rate was made at least 60 min before time zero. At 7:00 a.m., a primed-continuous infusion of [6,6-2H₂]glucose (6 mg/kg prime, 0.06 mg • kg⁻¹ • min⁻¹ continuous) was started for measurement of glucose turnover. The subjects were studied on three occasions. On one occasion, the glucagon infusion rate was maintained at 0.65 ng • kg⁻¹ • min⁻¹ throughout the study. On the other two occasions, the glucagon infusion rate was increased to 1.5 and 3.0 ng • kg⁻¹ • min⁻¹ at 10:00 a.m. and continued at the same rate for the next 4 h. The order of study was random, and studies were separated by at least 1 week. To maintain plasma [6,6-2H₂]glucose enrichment constant, the infusion rate of [6,6-2H₂]glucose was varied during the 1.5 and 3.0 ng • kg⁻¹ • min⁻¹ glucagon infusions (~180 to 0 min, 100–45 min, 180%, 45–60 min, 159%, 60–75 min, 133%, 75–95 min, 117%, and 90–240 min, 100%), in a manner that was anticipated to mimic the profile of release of glucose (36). Blood was sampled at regular intervals before and after infusion. For glucose infusion, an 18-gauge catheter was inserted into a forearm vein. The diabetic subjects were infused with insulin to maintain euglycemia throughout the night (5,35). The nondiabetic subjects were infused with 0.9% saline.

**Analysis.** Arterialized plasma samples were placed on ice, centrifuged at 4°C, separated, and stored at −20°C until assay. Glucose and lactate concentrations were measured using the glucose and lactate oxidase method (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin, C-peptide, and glucagon concentrations were measured by radioimmunoassay (Liscio Research, St. Louis, MO). Growth hormone concentrations were measured using reagents from ICN Biomedicals (Costa Mesa, CA). Free fatty acid concentrations were measured using a kit from Wako (Wako Pure Chemical Industries, Osaka, Japan). Plasma enrichment of [6,6-2H₂]glucose and specific activity of [6-3H]glucose were determined as previously described (35,37). Lean body mass and percent body fat were determined by dual-energy X-ray absorptiometry (DPX scanner; Lunar, Madison, WI).

To ensure that the ingested radiolabeled galactose was completely extracted by the liver, pilot studies were conducted as part of another study in seven NIDDM and six nondiabetic subjects. Each subject ingested a mixed meal (472 kcal; 45% carbohydrate, 40% fat, and 15% protein) that contained 100 μCi of [6-3H]galactose. Plasma galactose and glucagon specific activities were measured by high-performance liquid chromatography using two Amineex HPX-87H columns (Bio-Rad Laboratories, Richmond, CA) in series, with the mobile phase being 0.02 N sulfuric acid at a rate of 0.56 ml/min, as previously described (38). This resulted in separation of glucose from galactose (Fig. 1A). [6-3H]Galactose and [6-3H]Glucose radioactivity were measured in all postprandial plasma samples from one NIDDM and one nondiabetic subject. Thereafter, [6-3H]Galactose and [6-3H]Glucose radioactivity were measured only in samples obtained 60 min after food ingestion (i.e., the time of maximal appearance of [6-3H]Glucose). No [6-3H]Glucose was detected in any plasma sample (Fig. 1B).

**TABLE 1**

Characteristics of study subjects

<table>
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<tr>
<th>Subjects</th>
<th>Age (years)</th>
<th>Sex (M/F)</th>
<th>BMI (kg/m²)</th>
<th>Fat (%)</th>
<th>GHB (%)</th>
<th>Fasting glucose (mmol/L)</th>
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</thead>
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<tr>
<td>Diabetic</td>
<td>58 ± 2.8</td>
<td>6/3</td>
<td>29.4 ± 1.1</td>
<td>28.5 ± 3.2</td>
<td>9.6 ± 1.2</td>
<td>9.5 ± 1.7</td>
</tr>
<tr>
<td>Nondiabetic</td>
<td>53 ± 3.3</td>
<td>6/4</td>
<td>27.9 ± 0.9</td>
<td>28.4 ± 2.6</td>
<td>5.1 ± 0.1</td>
<td>5.1 ± 0.3</td>
</tr>
</tbody>
</table>

Data are means ± SD.

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*2008 DIABETES, VOL. 46, DECEMBER 1997*
Glucose Galactose
1 1
0 1
200 -
O
400 -'
600
Figure 1. A: high-performance liquid chromatographic separation of [3H]glucose from [14C]galactose when plasma is spiked with the two hexoses. B: representative chromatogram of plasma sampled 60 min after ingestion of the mixed meal containing [6-3H]galactose. No [6-3H]galactose was detected in plasma in this or any other postprandial sample. The x-axis shows elution time in minutes, and the y-axis shows disintegrations per minute of spiked standards (A) or plasma sample (B).

Calculations. The specific activity of [6-3H]glucose and molar percent excess (MPE) of [6,6-2H2]glucose were smoothed using the OOPSEG program of Bradley et al. (39). The rate of appearance of unlabeled glucose was calculated using the non-steady-state equations of Steele et al. (40)

$$E_G = \frac{R_{ch} - pV[C(t) - C(t_c)][MPE(t) - MPE(t_c)][t(t) - t_c(t)]}{[MPE(t_c) - MPE(t_c)]}$$

where $E_G$ is endogenous glucose production, $R_{ch}$ is the infusion rate of [6,6-3H]glucose, $C(t)$ and $C(t_c)$ represent the concentration of unlabeled glucose, and MPE$(t_c)$ and MPE$(t_c)$ are the plasma enrichment of [6,6-3H]glucose at times $t_c$ and $t_c$. The volume of distribution of glucose ($V$) was assumed to equal 200 ml/kg. The pool correction factor was assumed to be 0.65.

In addition, [6,6-3H]glucose was used to trace the rate of appearance of [6-3H]glucose as previously described (41). To do so, the above equation was again used, except the ratio of the plasma concentration of [6,6-3H]glucose (i.e., the concentration of the tracer in mmol/l) to the plasma concentration of [6-3H]glucose (i.e., the tracer concentration in dpm/l) was substituted for MPE, and the concentration of [6-3H]glucose (in dpm/l) was used for C.

RESULTS

Plasma glucagon, glucose, and insulin concentrations. Plasma glucagon concentrations did not differ in diabetic and nondiabetic subjects during the basal period or during the 0.65 (126 ± 1 vs. 134 ± 1 pg/ml), 1.5 (179 ± 3 vs. 176 ± 2 pg/ml), or 3.0 ng • kg⁻¹ • min⁻¹ (307 ± 5 vs. 311 ± 5 pg/ml) glucagon infusions (Fig. 2).

Plasma glucose concentrations were constant and equal in diabetic and nondiabetic subjects (5.24 ± 0.12 vs. 5.23 ± 0.12 mmol/l) during the basal period (Fig. 3). Glucose concentrations fell slightly but equally in both groups when the glucagon infusion was maintained constant at a rate of 0.65 ng • kg⁻¹ • min⁻¹ throughout the study. In contrast, glucose concentrations increased in both groups during the 1.5 and 3.0 ng • kg⁻¹ • min⁻¹ glucagon infusions. The area above basal did not differ in diabetic and nondiabetic subjects during either the 1.5 (192 ± 40 vs. 143 ± 52 mmol/l; $P = 0.48$) or 3.0 ng • kg⁻¹ • min⁻¹ glucagon infusions. The area above basal did not differ in diabetic and nondiabetic subjects during either the 1.5 (192 ± 40 vs. 143 ± 52 mmol/l; $P = 0.48$) or 3.0 ng • kg⁻¹ • min⁻¹ glucagon infusions. The area above basal did not differ in diabetic and nondiabetic subjects during either the 1.5 (192 ± 40 vs. 143 ± 52 mmol/l; $P = 0.48$) or 3.0 ng • kg⁻¹ • min⁻¹ glucagon infusions.

Both the basal insulin infusion rate (defined as that required to maintain glucose concentrations constant from 30 to 0 min) and the resultant insulin concentrations (0.44 ± 0.05 vs. 0.28 ± 0.03 mU • kg⁻¹ • min⁻¹ and 135 ± 22 vs. 79 ± 8 pmol/l, respectively) were greater ($P < 0.001$) in diabetic than in nondiabetic subjects (Fig. 4). Thus more insulin was required to achieve the same biological effect in diabetic than in nondiabetic subjects, thereby fulfilling the definition of insulin resistance. Insulin concentrations remained constant in both diabetic and nondiabetic subjects during all glucagon infusions.
ASSESSMENT OF HEPATIC SENSITIVITY TO GLUCAGON

Plasma C-peptide and growth hormone concentrations. Somatostatin suppressed endogenous insulin secretion in both groups, resulting in C-peptide concentrations that did not differ in diabetic or nondiabetic subjects during either the basal period (0.037 ± 0.006 vs. 0.049 ± 0.01 nmol/L, respectively) or during the glucagon infusions (Fig. 5). Growth hormone concentrations also were comparable (317 ± 217 vs. 28 ± 0.07 ng/ml, respectively) and constant in both diabetic and nondiabetic subjects on all three occasions (Fig. 5).

Endogenous glucose production. Basal endogenous glucose production tended to be lower in diabetic than in nondiabetic subjects (Fig. 7). When between-group differences in basal rates were tested for on each study day, they reached statistical significance on the 0.65 (P = 0.01) but not on the 1.5 (P = 0.15) or 3.0 ng·kg⁻¹·min⁻¹ (P = 0.09) glucagon infusion days.

Endogenous glucose production fell slowly but comparably over the 4 h of study in both groups during the 0.65 ng·kg⁻¹·min⁻¹ glucagon infusion. The response to glucagon (i.e., area above basal) did not differ in diabetic or nondiabetic subjects during either the 1.5 (0.75 ± 0.06 vs. 0.78 ± 0.05 nmol/kg; P = 0.75) or 3.0 ng·kg⁻¹·min⁻¹ (1.06 ± 0.07 vs. 1.10 ± 0.07 nmol/kg; P = 0.71) glucagon infusions.

Rate of release of [6-³H]glucose from glycogen. The basal rate of release of [6-³H]glucose from glycogen was lower (P < 0.001) in diabetic than in nondiabetic subjects (Fig. 8). In contrast to the increment in endogenous glucose production, which did not differ, the increment (area above basal) in release of [6-³H]glucose was lower (P < 0.05) in diabetic than in nondiabetic subjects during both the 1.5 (13.9 ± 2.8 vs. 32.9 ± 5.5 × 10⁵ dpm/kg) and 3.0 ng·kg⁻¹·min⁻¹ (19.2 ± 2.8 vs. 40.5 ± 8.5 × 10⁵ dpm/kg) glucagon infusions. The rate of release of [6-³H]glucose during the 0.65 ng·kg⁻¹·min⁻¹ glucagon infusion fell progressively in both groups over the 4 h of study.

Ratio of the rate of release of [6-³H]glucose to endogenous glucose production. The acute increase in endogenous glucose production during glucagon infusion is due almost entirely to an increase in glycogenolysis (20,21). This, plus the fact that metabolites of [6-³H]glucose are almost completely detritiated during gluconeogenesis (34), means that essentially all [6-³H]glucose released during the glucagon infusions was derived from glycogen. Therefore, the ratio of the area above basal of released [6-³H]glucose to the area above basal of released unlabeled glucose (i.e., endogenous glucose production) provides an index of the specific activity of [6-³H]glucose in glycogen prior to the glucagon infusions. This ratio was lower (P < 0.05) in diabetic than in nondiabetic subjects during both the 1.5 (19.0 ± 3.6 vs. 41.4 ± 5.7 dpm/μmol) and 3.0 ng·kg⁻¹·min⁻¹ (19.1 ± 3.1 vs. 36.5 ± 7.3 dpm/μmol) glucagon infusions (Fig. 9). Of note, the ratio observed during the 1.5 ng·kg⁻¹·min⁻¹ glucagon infusion did not differ from that observed during the 3.0 ng·kg⁻¹·min⁻¹ glucagon infusion in either group, implying consistent labeling of glycogen on the two study days.
Free fatty acid and lactate concentrations. Although basal values varied from day to day, the means of free fatty acid (0.28 ± 0.03 vs. 0.19 ± 0.02 mmol/L) and lactate (1.22 ± 0.12 vs. 1.09 ± 0.09 mmol/L) concentrations on the three occasions were higher (P < 0.05) in diabetic than in nondiabetic subjects (Table 2). Free fatty acid concentrations rose and lactate concentrations fell in both groups during the glucagon infusions.

DISCUSSION

NIDDM is associated with hepatic insulin resistance (1-5). The present studies indicate that the same is not true for glucagon. When differences in insulin action were controlled for by maintaining insulin concentrations at individually determined basal levels, glucagon-induced stimulation of endogenous glucose production did not differ in diabetic and nondiabetic subjects. On the other hand, the source of the released glucose in response to glucagon did differ between groups. Although the amount of unlabeled glucose that was released during the glucagon infusions was equivalent in both groups, the amount of [6-3H]glucose released from glycogen was substantially lower in diabetic than in nondiabetic subjects, implying that the contribution of the indirect pathway to nocturnal glycogen synthesis was greater in diabetic than in nondiabetic subjects.

Effects of glucagon on endogenous glucose production. Endogenous glucose production is inhibited by insulin and hyperglycemia and stimulated by glucagon (1-5,9-11). Therefore the response to glucagon is highly dependent on the prevailing glucose and insulin concentrations (9,11). Because hyperglycemia inhibits glucose production, we felt it essential to study the response to glucagon in the presence of matched glucose concentrations. A difference in response between hyperglycemic diabetic and euglycemic nondiabetic subjects would have been virtually impossible to interpret. Ideally, insulin concentrations also would have been the same in both groups. However identical glucose and insulin concentrations...
could be achieved only if insulin action also were equivalent between diabetic and nondiabetic subjects. This is not possible, given that NIDDM is associated with both hepatic and extrahepatic insulin resistance (1–5). Therefore, the same biological effect required higher insulin concentrations in diabetic than in nondiabetic subjects. To ensure that the response to glucagon was assessed in the presence of biologically equivalent amounts of insulin, the appropriate basal insulin concentration was individually determined on each occasion in each patient. We defined basal as the insulin concentration that exerted equivalent biological effects in the two groups. Because it is impossible to prove no difference, we cannot totally rule out the possibility that sensitivity to glucagon differs in NIDDM and nondiabetic subjects. However, the results of the present studies, taken in conjunction with those of Baron et al. (44), suggest that if such differences do exist, they are likely to be small.

Although it is becoming increasingly evident that both the kidney and the liver contribute to endogenous glucose production (45,46), Stumvoll et al. (47) recently showed that glucagon selectively stimulates hepatic but not renal glucose release. Glucagon causes only a transient increase in hepatic glucose release (48,49). The present studies show that this also occurs in people with NIDDM. The pattern of release of [6-3H]glucose closely mirrored that of endogenous glucose production in both NIDDM and nondiabetic subjects, indicating that the waning of glycogenolysis and hepatic glucose release occur in parallel. This conclusion is consistent with that previously derived using the hepatic vein catheterization technique in dogs and 13C nuclear magnetic resonance spectroscopy in humans (20,21).

Relative contribution of direct and indirect pathways to glycogen synthesis. Glycogen can be synthesized via either direct or indirect pathways (23). Studies in animals (24,25,27,28) and nondiabetic humans (26,29) indicate that the relative contribution of each is dependent on a variety of factors, including the duration of fast, the prevailing insulin...
In a series of preliminary experiments, we confirmed that when ingested in trace amounts as part of a mixed meal, [6-3H]galactose is completely extracted by the splanchnic bed in both NIDDM and nondiabetic subjects. Thus, once absorption of the meal is complete, the only source of subsequently released [6-3H]glucose is glycogen. In the current studies, because the last [6-3H]galactose was ingested 10 h prior to the test glucagon infusions, we feel confident that absorption was complete.

The amount of [6-3H]glucose released during glucagon infusion was 50% lower in diabetic than in nondiabetic subjects, whereas the amount of unlabeled glucose released was identical. Because glucagon acutely increases endogenous glucose production, primarily by stimulating glycogenolysis (20,21), this indicates that the ratio of [6-3H]glucose to unlabeled glucose (i.e., specific activity) within glycogen was lower in diabetic than in nondiabetic subjects. This could have occurred for several reasons. First, [6-3H]galactose was used to label glycogen the evening before. Lower hepatic uptake of [6-3H]galactose could have resulted in lower hepatic glycogen-specific activity. This did not appear to be the case, since, as discussed above, splanchnic extraction of [6-3H]galactose was the same (i.e., virtually complete) in both groups. Little, if any, galactose is utilized by the intestine (51). Thus equal splanchnic extraction implies that hepatic uptake of [6-3H]galactose also was equal in both groups. Second, the proportion of UDP-[6-3H]galactose entering the UDP-glucose pool that was hydrolyzed to [6-3H]glucose-1-phosphate could have been greater in diabetic than in nondiabetic subjects. Although we cannot totally exclude this possibility, flux of tracer in this direction would be anticipated to be trivial following meal ingestion in both groups, since grams of unlabeled glucose would be passing in the opposite direction through the same UDP-glucose pool into glycogen. Third, despite comparable incorporation of UDP-[6-3H]glucose into

and glucose concentrations, and the availability of gluconeogenic substrates. The approach used in the present studies took advantage of the unique metabolism of galactose to gain insight into the source of the glucose released from glycogen during the glucagon infusions (Fig. 10). After ingestion, galactose is extracted by the liver, phosphorylated, and converted to UDP-galactose, which in turn equilibrates with the hepatic UDP-glucose pool (50). This pool serves as the immediate precursor of glycogen (50). Thus ingested [6-3H]galactose is incorporated into glycogen as [6-3H]glucose.

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![Chart showing specific activity of glycogen](chart.png)

**TABLE 2**

<table>
<thead>
<tr>
<th>Free fatty acids (mmol/l) and lactate (mmol/l) concentrations following three different glucagon infusion rates</th>
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<td><strong>Free fatty acids</strong></td>
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<td>Diabetic</td>
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<td><strong>Lactate</strong></td>
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<td>0.6 ng · kg⁻¹ · min⁻¹</td>
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The cause of the greater contribution of the indirect pathway to glycogen synthesis in NIDDM subjects is not known, nor is it known whether the process occurs exclusively within the liver or involves increased substrate delivery from peripheral tissues to the liver. Previous experiments have shown that the relative contribution of the indirect pathway to glycogen synthesis is increased under conditions in which gluconeogenesis is also increased (25–31). This is logical because, under such conditions, the proportion of the G-6-P pool derived from gluconeogenesis increases relative to that derived from extracellular glucose. Gluconeogenesis is accelerated in untreated NIDDM subjects (22). Although the insulin infusion was started in the diabetic subjects concurrent with ingestion of the radiolabeled supper, the diabetic subjects were hyperglycemic on admission, and the infusion algorithm was not perfect. Therefore their glucose concentrations remained elevated during the first part of the night when glycogen synthesis presumably was at its peak. Although continued insulin infusion resulted in a fall in glucose concentrations toward nondiabetic levels, previous studies have shown that the ability of insulin to inhibit gluconeogenesis is impaired in NIDDM (5). In addition, free fatty acid and lactate concentrations, both of which can stimulate gluconeogenesis (28,55–57), continued to be higher in diabetic than in nondiabetic subjects on the morning of study. Alternatively, NIDDM has been reported to be associated with a decrease in hepatic glucokinase activity (58–60). If a decrease in glucokinase impaired hepatic uptake of extracellular glucose during the night, this could also have resulted in a lesser contribution of the direct pathway to glycogen synthesis in NIDDM subjects. Future studies will be required to distinguish between these, as well as other, possibilities.

In summary, glucagon-induced stimulation of endogenous glucose production did not differ in NIDDM and nondiabetic subjects when differences in insulin action were controlled by maintaining insulin concentrations at individually determined basal levels. These data argue strongly that the inappropriate elevations of endogenous glucose production commonly observed in NIDDM patients in both the postabsorptive and postprandial states are not due to an intrinsic increase in hepatic sensitivity to glucagon. On the other hand, although the amount of unlabeled glucose that was released during the glucagon infusions was equivalent in both groups, the amount of [6-3H]glucose released from glycogen was substantially lower in diabetic than in nondiabetic subjects, implying that the contribution of the indirect pathway to nocturnal glycogen synthesis was greater in diabetic than in nondiabetic subjects. Thus inhibitors of gluconeogenesis, in addition to lowering endogenous glucose production, may also decrease hepatic glycogen stores in diabetic subjects. Future studies will be required to determine whether this dual effect (if it occurs) improves glycemic control, and therefore would be considered as an advantage, or whether it makes people with NIDDM more vulnerable to hypoglycemia and therefore should be avoided.

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