Glucagon Dose-Response Curve for Hepatic Glucose Production and Glucose Disposal in Type 2 Diabetic Patients and Normal Individuals

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This study sought to examine whether enhanced hepatic sensitivity to glucagon contributes to impaired glucose homeostasis in subjects with type 2 diabetes mellitus (T2DM). Eight T2DM and 9 age-, weight-, and gender-matched nondiabetic subjects received a 4-hour glucagon infusion at the rates of 0.2, 0.5, 2, 6, and 8 ng · kg^{-1} · min^{-1} while maintaining the plasma insulin concentration constant at the basal level with exogenous infusions of somatostatin and insulin. On the evening prior to study, diabetic subjects received a low-dose insulin infusion at a rate designed to maintain euglycemia and this infusion rate was continued until the end of the glucagon infusion study on the following day. Each glucagon infusion study was performed on a separate day and in random order. 3-^{3}H-glucose was infused in all studies to measure endogenous glucose production (EGP) and the rate of whole body glucose disposal. During the first 2 hours (0 to 120 minutes) of glucagon infusion, EGP increased sharply in both groups, and the initial rate of rise in EGP was higher in control versus diabetic subjects. During the last 2 hours (120 to 240 minutes) of glucagon infusion, EGP in the diabetics tended to be higher than controls during the 3 lower glucagon infusion rates and this difference reached statistical significance ($P < .05$ to .01) during the 6 and 8 ng · kg^{-1} · min^{-1} infusions. During the 2 hours following cessation of glucagon (240- to 360-minute time period), the stimulation of glucose disappearance from plasma was impaired ($P < .05$) during all 5 glucagon infusion rates in the diabetics compared to controls. We conclude that in T2DM patients, the initial (0 to 120 minutes) stimulation of hepatic glucose output (which primarily reflects glycogenolysis) by glucagon is not enhanced in T2DM patients. The late (120 to 240 minutes) stimulation of hepatic glucose output (which primarily reflects gluconeogenesis) by glucagon tends to be increased, especially at supraphysiologic plasma glucagon concentrations.

IN MANY,[1-5] but not all,[6,7] individuals with type 2 diabetes mellitus (T2DM), the plasma glucagon concentration is increased in absolute terms and, when viewed relative to the prevailing hyperglycemia and hyperinsulinemia,[3,8,9] the majority of T2DM patients have fasting hyperglucagonemia. Following nutrient ingestion, an elevated plasma glucagon concentration in T2DM patients is more apparent. After an oral glucose load, the plasma glucagon concentration falls in normal subjects, whereas in T2DM subjects no significant decline occurs,[4,8] and some diabetic patients even manifest a paradoxical increase.[10-13] Following amino acid infusion[14,15] or protein ingestion,[16] T2DM individuals experience a much larger increment in plasma glucagon concentration than nondiabetic control subjects.

Fasting and postprandial glucagon concentrations play an important role in the regulation of hepatic glucose production. In normal humans and dogs, the basal plasma glucagon concentration is responsible for the maintenance of approximately half of basal hepatic glucose production.[9,17,18] Similarly, in T2DM patients, inhibition of basal glucagon secretion with somatostatin reduces the elevated basal rate of hepatic glucose production by approximately 50% to 60%.[5] In patients with impaired glucose tolerance[19] and T2DM,[20,21] the suppression of hepatic glucose production following glucose ingestion is impaired and the magnitude of the defect in suppression of hepatic glucose production is correlated with the increase in plasma glucagon concentration and the glucagon:insulin molar ratio. In normal human volunteers, we have reported that 48 hours of physiologic hyperglucagonemia is associated with an increased rate of basal hepatic glucose production, impaired insulin-mediated suppression of hepatic glucose production, and defective glucose disposal by peripheral tissues.[22] Conversely, chronic glucagon deficiency is associated with a reduced rate of hepatic glucose production, enhanced suppression of hepatic glucose production by insulin, and increased peripheral tissue sensitivity to insulin.[23,24] Similarly, in T2DM patients, inhibition of glucagon secretion with somatostatin is associated with a marked improvement in fasting glucose levels, while glucagon replacement causes a deterioration in glycemic control.[25,26] In nondiabetic subjects who are rendered hypoinsulinemic with somatostatin, minimal elevations in the plasma glucagon concentration augment hepatic glucose production, and plasma glucose levels rise by 30% to 40%.[27]

Taken collectively, these results indicate that in T2DM individuals, fasting plasma glucagon levels are inappropriately elevated, fail to suppress normally in response to hyperglycemia/hyperinsulinemia, and play an important role in the maintenance of increased basal hepatic glucose production and fasting hyperglycemia. These observations have taken on renewed interest with the development of novel agents that inhibit glucagon secretion and/or antagonize the actions of glucagon.[28-30] It is surprising, therefore, that only 1 previous study[7] has examined hepatic sensitivity to glucagon in T2DM patients, and these investigators concluded that the liver sensi-

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Submitted June 18, 2001; accepted March 29, 2002.

Supported by Juvenile Diabetes Foundation International fellowship Grant No. 391253, the GRECC, funds from the VA Medical Research Fund, NIH Grant No. DK24092, and General Clinical Research Center Grant No. RR01346.

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0026-0495/02/5109-0006$35.00/0

doi:10.1053/meta.2002.34700

tivity to glucagon was not altered. However, this study focused only on the lower part of the glucagon-hepatic glucose production dose-response curve and their T2DM patients had normal fasting plasma glucagon levels, in contrast to the elevated concentrations reported by most investigators.[1-5] The present study examined endogenous (primarily hepatic) glucose production, the great majority (≥75% to 80%) of which is derived from the liver,[21] and tissue glucose disposal in response to varying doses of glucagon, spanning both the physiologic and pharmacologic range in T2DM and nondiabetic control subjects.

MATERIALS AND METHODS

Subjects

Eight T2DM (2 females, 6 males) and 9 healthy nondiabetic control (3 females, 6 males) subjects, who were matched for age, gender, sex, and body mass index to the diabetics (Table 1), participated in the study. All T2DM patients had their diabetes diagnosed after the age of 30 years. Four diabetic patients were taking a sulfonylurea agent, which was discontinued 3 days before each study. No subject ever had received insulin or any other oral antidiabetic agent. No subject had any evidence of cardiovascular, pulmonary, renal, hepatic, or other major organ system disease as determined by history, physical examination, and routine laboratory tests. No subject was taking any medication known to adversely affect glucose metabolism. Body weight was stable for at least 3 months prior to study in each subject. A weight-maintaining diet containing at least 200 g of carbohydrate per day was consumed for 3 days before each study. The purpose and potential risks involved in the study were carefully explained to all subjects before obtaining their written voluntary consent. The study protocol was reviewed and approved by the Institutiional Review Board of the University of Texas Health Science Center at San Antonio.

Study Design

T2DM subjects were admitted to the Diabetes Research Unit of the General Clinical Research Center (GCRC) of the Audie L. Murphy Veterans Administration Hospital at 5 PM on the day before each study. Nondiabetic control subjects reported to the GCRC at 7:30 AM on the morning of the day of the study. After a supper containing 1,200 calories (50% carbohydrate, 35% fat, 15% protein), diabetic patients received an overnight low-dose insulin (Novo Nordisk, Copenhagen, Denmark) infusion at the rate of 0.1 to 0.4 mU · kg^{-1} · min^{-1} to normalize the basal rate of endogenous glucose production (EGP) and to maintain euglycemia (90 to 95 mg/dL). This allowed us to compare the increments in EGP and plasma glucose concentration in T2DM and control subjects who started with comparable rates of glucose production and fasting glucose levels. At 7:30 AM on the following morning, a 20-gauge catheter was inserted retrogradely into a vein on the dorsum of the hand and the hand was placed in a hot box heated to 60°C. In diabetic subjects the antecubital vein catheter that was used for the overnight insulin infusion also was used for the infusion of all test substances. On the evening prior to study, control subjects also consumed a dinner containing 1,200 calories with 50% carbohydrate, 35% fat, and 15% protein. In control subjects on the morning of the study, catheters were placed retrogradely into a vein on the dorsum of the hand and into an ipsilateral antecubital vein. In all subjects, a somatostatin (Bachem, Torrance, CA) infusion was begun at 8 AM at the rate of 0.5 mg/h and continued throughout the study to inhibit endogenous insulin and glucagon secretion. In control subjects, an infusion of insulin (0.07 mU · kg^{-1} · min^{-1}) designed to replace basal insulin levels, was started at the same time as the somatostatin. In T2DM patients, the rate of insulin infusion needed to maintain euglycemia between 5 AM and 8 AM was continued throughout the study. Basal levels of glucagon were not replaced because, in pilot studies, this was associated with a progressive rise in EGP and plasma glucose concentration. Without basal glucagon replacement during the 210-minute equilibration period (8 AM to 11:30 AM), the plasma glucose concentration easily was maintained constant at approximately 90 mg/dL by means of a variable glucose infusion. In all subjects a constant rate of glucose infusion was achieved during the last 60 minutes before the start of glucagon infusion. This constant glucose infusion rate was continued until the end of the study. One hour after starting the somatostatin infusion, a prime (15 μCi) continuous (0.15 μCi/min) infusion of 3-^{3}H-glucose (New England Nuclear, DuPont, Boston, MA) was started and maintained until completion of the study. After 3.5 hours of somatostatin infusion, glucagon (Eli Lilly, Indianapolis, IN) was infused for 240 minutes and this was followed by a 120-minute post-glucagon recovery period. Glucagon was infused at varying rates increase the plasma glucagon concentration through the physiologic (0.2 to 2.0 ng/kg · min glucagon infusion rates) and pharmacologic (6 and 8 ng/kg · min infusion rates) range (see below). Each glucagon infusion was carried out on a separate day in random order, with a 7- to 10-day interval between studies. Glucagon was infused at 0.2 ng · kg^{-1} · min^{-1} (3 T2DM and 5 control subjects), 0.5 ng · kg^{-1} · min^{-1} (7 T2DM and 6 control subjects), 2 ng · kg^{-1} · min^{-1} (8 T2DM and 9 control subjects), 6 ng · kg^{-1} · min^{-1} (7 T2DM and 6 control subjects), and 8 ng · kg^{-1} · min^{-1} (7 T2DM and 7 control subjects). Because supraphysiologic plasma glucagon levels can be observed during stress and during glucagon administration to combat hypoglycemia, we have examined both physiologic and supraphysiologic glucagon infusion rates.

Table 1. Clinical Characteristics of Control and T2DM Subjects

	T2DM	Controls
No.	8	9
Gender (M/F)	3/5	2/7
Age (yr)	45 ± 2	44 ± 3
Duration of diabetes	5 ± 1	—
Ideal body weight (%)*	127 ± 5	118 ± 5
Body mass index (kg/m^2)	29 ± 2	27 ± 1
Fasting plasma glucose (mg/dL)	173 ± 45†	91 ± 4

*According to the 1983 Metropolitan Life Insurance Tables.
†$P < .01$.

Analytic Determinations

The plasma glucose concentration was determined by the glucose oxidation method using a Beckman Glucose Analyzer 2 (Beckman, Fullerton, CA). Plasma free fattty acid (FFA) and lactate concentrations were determined by standard fluorometric methods.[21] Tritiated glucose radioactivity was determined as previously described after precipitation of plasma proteins using the Somogyi procedure.[22] Plasma insulin was measured by a solid-phase radioimmunoassay (Coat-A-Count, Diagnostic Products, Los Angeles, CA). Plasma C-peptide was measured by a specific radioimmunoassay (C-peptide 125 I RIA Kit, Incstar, Stillwater, MN). Plasma glucagon concentration was measured by radioimmunoassay using double antibody (Glucagon Double Antibody, Diagnostic Products, Los Angeles, CA).

Calculations and Statistics

The rates of endogenous glucose appearance (Ra), glucose disappearance (Rd), and the metabolic clearance rate of glucose were determined by using Steele's non–steady-state equation[31] with a distribution volume of 0.65. The rate of EGP was calculated by subtracting the infusion rate of exogenous glucose from the tracer-derived Ra. Since more than 80% to 85% of endogenous glucose appearance is from the

Table 2. Peripheral Plasma Insulin Concentration (μU/mL) During Each Glucagon Infusion Rate

Glucagon Infusion Rate	Basal	Time (min) 60	120	180	240	300	360
0.2 ng/kg · min							
T2DM	15 ± 3	13 ± 3	15 ± 2	15 ± 2	14 ± 2	15 ± 2	17 ± 3
Controls	6 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 1	7 ± 2	8 ± 2
0.5 ng/kg · min							
T2DM	15 ± 5	17 ± 7	15 ± 5	17 ± 6	16 ± 5	16 ± 6	16 ± 5
Controls	6 ± 1	6 ± 1	6 ± 1	8 ± 1	7 ± 1	7 ± 1	8 ± 1
2 ng/kg · min							
T2DM	16 ± 5	10 ± 3	16 ± 4	15 ± 4	14 ± 4	15 ± 4	16 ± 4
Controls	5 ± 1	5 ± 1	6 ± 1	7 ± 1	8 ± 1	7 ± 1	7 ± 1
6 ng/kg · min							
T2DM	12 ± 2	11 ± 2	13 ± 2	14 ± 2	13 ± 2	13 ± 3	16 ± 4
Controls	8 ± 1	8 ± 1	10 ± 2	10 ± 3	10 ± 2	9 ± 2	10 ± 2
8 ng/kg · min							
T2DM	20 ± 8	18 ± 8	18 ± 6	17 ± 6	18 ± 6	17 ± 6	17 ± 6
Controls	6 ± 1	7 ± 1	10 ± 1	11 ± 1	11 ± 2	8 ± 1	7 ± 1

NOTE. All values are the mean ± SEM. The basal plasma insulin concentration for T2DM patients represents the value obtained in the morning after the overnight somatostatin/insulin infusion.

liver[32] and since glucagon does not appear to stimulate renal glucose production,[33] we have employed the terms endogenous glucose production and hepatic glucose production interchangeably in the following discussion. The response of hepatic glucose production to various doses of glucagon was analyzed by fitting the Michaelis-Menten equation type reaction, using a nonlinear least-square method. The glucagon sensitivity index was defined as the initial slope of the dose-response curve in the Michaelis-Menten equation, as suggested by Bradley and Bergman.[34] The portal vein insulin concentration (I_{pv}) was estimated as follows: $(I_{pv})_{ss} = (Ip)_{ss} + (I_{pv})_o\,[(CP)_o - (CP)_{ss}]/(CP)$, where I_{pv} and I_p are the portal vein and peripheral plasma insulin concentrations, respectively, CP is the arterialized plasma C-peptide concentration, and the subscripts ss and o indicate the steady state and baseline, respectively. The ratio of the basal portal insulin concentration to peripheral insulin concentration is assumed to be 3:1.[35]

All data are expressed as the mean ± SEM. Statistical analyses were performed using the StatView II program (Abacus Concepts, Berkeley, CA). Multifactorial analysis of variance (ANOVA) for repeated measures over 1 factor (time) was employed a priori to test for changes with time and between groups. If this analysis revealed significant differences, the Student's *t* test for unpaired data was used to assess differences between groups.

RESULTS

Plasma Hormone Concentrations

In the basal state both the peripheral and estimated portal plasma insulin concentrations required to maintain normoglycemia were 2- to 3-fold greater in T2DM compared to control subjects (Tables 2 and 3). During the 4-hour glucagon infusion period both the peripheral (Table 2) and calculated portal (Table 3) plasma insulin concentrations remained constant in the T2DM group during all 5 glucagon infusion rates (0 to 240 minutes) and there were no statistically significant differences in the integrated areas under the curves for the change from baseline for the plasma insulin concentration (Table 2) or for the calculated portal insulin concentration from 0 to 240 min-

Table 3. Calculated Portal Insulin Concentration (μU/mL) During Each Glucagon Infusion Rate

Glucagon Infusion Rate	Basal	Time (min) 60	120	180	240	300	360
0.2 ng/kg · min							
T2DM	15 ± 2	13 ± 5	15 ± 1	15 ± 4	14 ± 1	15 ± 3	17 ± 4
Controls	10 ± 1	9 ± 2	10 ± 3	10 ± 3	11 ± 3	15 ± 5	13 ± 3
0.5 ng/kg · min							
T2DM	17 ± 6	18 ± 7	17 ± 5	18 ± 6	18 ± 6	18 ± 5	22 ± 9
Controls	8 ± 1	10 ± 2	11 ± 2	10 ± 2	10 ± 1	10 ± 1	11 ± 2
2 ng/kg · min							
T2DM	18 ± 5	11 ± 3	17 ± 4	17 ± 4	16 ± 4	16 ± 4	18 ± 4
Controls	7 ± 1	6 ± 1	8 ± 1	11 ± 1	12 ± 2	13 ± 2	10 ± 2
6 ng/kg · min							
T2DM	14 ± 2	12 ± 2	14 ± 3	16 ± 3	16 ± 3	14 ± 3	21 ± 8
Controls	10 ± 1	11 ± 1	19 ± 2	20 ± 3	18 ± 4	19 ± 4	16 ± 3
8 ng/kg · min							
T2DM	21 ± 8	19 ± 8	14 ± 6	20 ± 6	20 ± 6	19 ± 6	19 ± 6
Controls	8 ± 1	9 ± 1	19 ± 2	23 ± 3	25 ± 3	19 ± 4	12 ± 1

NOTE. All values are the mean ± SEM.

Table 4. Basal Plasma Glucagon Concentration and the Increment (or Decrement) in Plasma Glucagon Concentration From Baseline (pg/mL) During Each Glucagon Infusion Rate During the 60- to 360-Minute Time Period

Glucagon Infusion Rate	Basal	Time (min)					
		60	120	180	240	300	360
0.2 ng/kg · min							
T2DM	68 ± 8	5 ± 7	2 ± 6	3 ± 11	4 ± 6	3 ± 2	4 ± 2
Controls	69 ± 16	13 ± 5	18 ± 5	8 ± 3	5 ± 8	2 ± 7	3 ± 4
0.5 ng/kg · min							
T2DM	76 ± 9	26 ± 12	27 ± 11	25 ± 14	34 ± 12	−4 ± 5	−11 ± 12
Controls	65 ± 15	23 ± 4	27 ± 4	22 ± 6	18 ± 7	−1 ± 4	1 ± 1
2 ng/kg · min							
T2DM	70 ± 7	64 ± 12	58 ± 16	66 ± 15	48 ± 11	16 ± 11	22 ± 10
Controls	58 ± 6	81 ± 13	97 ± 22	86 ± 12	69 ± 12	2 ± 8	8 ± 9
6 ng/kg · min							
T2DM	57 ± 4	232 ± 39	270 ± 36	243 ± 20	236 ± 18	7 ± 4	14 ± 6
Controls	59 ± 11	185 ± 57	253 ± 21	210 ± 60	177 ± 58	8 ± 9	3 ± 10
8 ng/kg · min							
T2DM	62 ± 6	300 ± 49	318 ± 30	376 ± 48	304 ± 49	13 ± 6	3 ± 8
Controls	61 ± 10	288 ± 33	279 ± 25	306 ± 33	356 ± 55	21 ± 8	12 ± 10

NOTE. Glucagon was infused from 0 to 240 minutes. All values are the mean ± SEM.

utes between any of the 5 glucagon infusion protocols. In control subjects, both the peripheral (Table 2) and calculated portal (Table 3) plasma insulin concentrations remained constant during the 3 lowest glucagon infusion rates and there were no statistically significant differences in the integrated areas under the curve for the peripheral plasma insulin concentration or for the calculated portal insulin concentration from 0 to 240 minutes. However, during the 6- and 8-ng · kg^{-1} · min^{-1} glucagon infusion protocols in controls subjects, the plasma C-peptide levels increased modestly (data not shown) and the calculated portal plasma insulin concentration increased significantly to levels similar to those in the diabetic group (Table 3). There were no statistically significant differences in the integrated areas under the curve for the change in peripheral plasma insulin concentration during the 6- and 8-ng/kg · min glucagon infusion rates in the controls, but there was a small but significant increment in the integrated area under the curve for the calculated portal insulin concentration ($P < .05$ to .01). During the 0.2-, 0.5-, and 2.0-ng · kg^{-1} · min^{-1} glucagon infusion rates, the calculated portal plasma insulin concentrations were significantly greater in diabetics compared to controls (Table 3). During all glucagon infusion rates, the peripheral plasma insulin concentration was, on mean, approximately 1.5-fold greater in diabetic versus control subjects (Table 2).

Plasma glucagon levels, measured before somatostatin infusion, were slightly higher in T2DM subjects than in controls (112 ±8 *v* 97± 5 pg/mL; $P < .10$). The plasma glucagon concentration increased in proportion to the rate of glucagon infusion, attaining comparable plasma levels in control and T2DM subjects (Table 4). The calculated plasma glucagon clearance rate was similar in the 2 groups (T2DM, 24 ± 2 mL · kg^{-1} · min^{-1}; controls, 25 ± 2 mL · kg^{-1} · min^{-1}). Sixty minutes after stopping the glucagon infusion, the plasma glucagon concentration returned to baseline values, where they remained until the end of the study (Table 4).

Plasma Glucose, Free Fatty Acid, and Lactate Concentrations

Overnight insulin infusion resulted in similar fasting plasma glucose concentrations in T2DM (92 ± 1 mg/dL) and control (93 ± 1 mg/dL) subjects and these levels were maintained constant throughout the equilibration period in both groups (coefficient of variation, < 5%) (Fig 1). Figure 2 illustrates the change in plasma glucose concentration in response to the various glucagon infusion rates. The plasma glucose concentration rose in a dose-related manner in control and T2DM subjects. In both control and diabetic subjects, the plasma glucose concentration increased steeply during the initial 0 to 60 minutes of glucagon infusion, tended to plateau during the subsequent hour (60 to 120 minutes), and remained constant

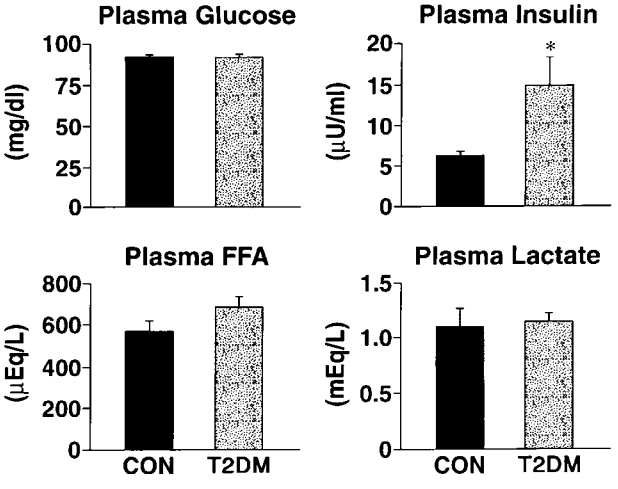

Fig 1. Plasma concentrations of glucose, insulin, FFA, and lactate during the basal equilibration period in control (CON) and T2DM individuals. *$P < .001$.

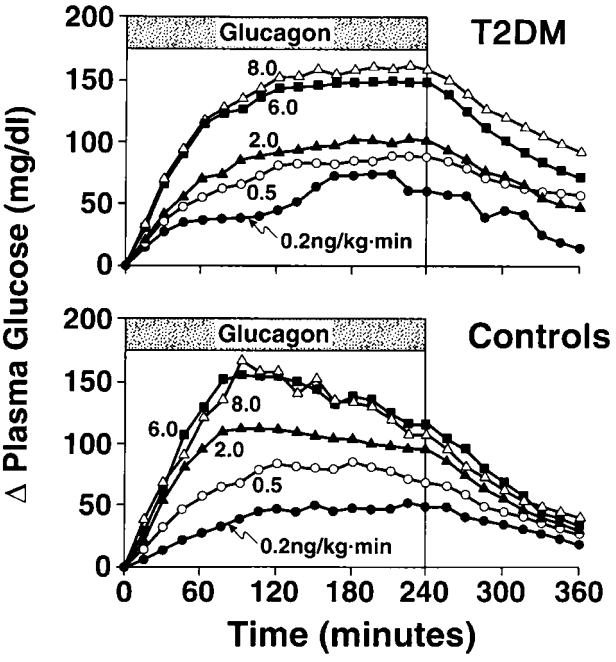

Fig 2. Time course of increment in plasma glucose concentration above baseline in T2DM patients (top) and control subjects (bottom) in response to a 4-hour glucagon infusion administered at rates of 0.2 (□), 0.5 (●), 2 (○), 6 (■), and 8 ng · kg^{-1} · min^{-1} (△) and during the 2 hours after cessation of the glucagon infusion.

during the last 2 hours (120 to 140 minutes) of glucagon infusion. In both control and diabetic subjects, the maximal increment in plasma glucose concentration was attained with the 6-ng · kg^{-1} · min^{-1} glucagon infusion and no further increase was observed with the-8 ng · kg^{-1} · min^{-1} infusion rate (Fig 2). Cessation of glucagon infusion was followed by a rapid and progressive decline in the plasma glucose concentration in both groups; during the 4 highest glucagon infusion studies, the rate of decline in plasma glucose concentration was slower ($P < .05$ to .01) in T2DM versus control subjects (Fig 2).

Since 3 different 2-hour periods (plasma glucose rise, plasma glucose plateau, and plasma glucose decline) were readily apparent, their respective incremental areas under the curve in response to the 5 glucagon infusion rates were determined (Fig 3). This analysis showed that the mean increment in plasma glucose concentration during hours 0 and 2 of glucagon infusion was similar in controls and diabetic subjects. During the 2-hour post-glucagon recovery period, the increment in plasma glucose concentration was greater in T2DM versus control subjects during each glucagon infusion rate and this difference reached statistical significance ($P < .01$) during the 2 highest glucagon infusion rates.

Following the overnight insulin infusion, plasma lactate concentration was similar in T2DM patients and controls (Fig 1). Glucagon had no effect on the plasma lactate concentration, which was similar in T2DM and control subjects during each glucagon infusion rate (data not shown). After the overnight insulin infusion, the plasma FFA concentration was slightly, although not significantly higher in T2DM than in control subjects (Fig 1). These slightly higher plasma FFA levels persisted in the diabetic subjects during all 4 glucagon infusion rates (data not shown).

Endogenous (hepatic) Glucose Production

The plasma glucose response to glucagon infusion is the result of the balance between EGP and glucose utilization by all tissues in the body. These processes were separately quantitated by the tracer technique. During the basal equilibration period, EGP was slightly greater in T2DM (1.37 ± 0.06 mg · kg^{-1} · min^{-1}) compared to control (1.15 ± 0.08 mg · kg^{-1} · min^{-1}) individuals ($P < .05$), even though the plasma insulin concentration was more than 2-fold elevated ($P < .01$) in the diabetic group.

In response to each glucagon infusion rate, a biphasic response in EGP occurred with a prompt rise in EGP during the initial 0 to 60 minutes of hyperglucagonemia, a subsequent decline during the 60- to 120-minute period, and the achievement of a plateau during the 120- to 140-minute period of glucagon infusion. After cessation of the glucagon infusion, EGP promptly returned to baseline values in both groups. A representative pattern of EGP response to glucagon (6 ng · kg^{-1} · min) is shown in Fig 4.

The incremental response in EGP during hours 0 and 2 of glucagon infusion and during the 2-hour post-glucagon recovery period is shown in Fig 5. In controls, the rise in EGP during the initial 0 to 120 minutes was greater than in T2DM subjects during the 0.2-, 0.5-, and 2.0-ng · kg^{-1} · min^{-1} glucagon infu-

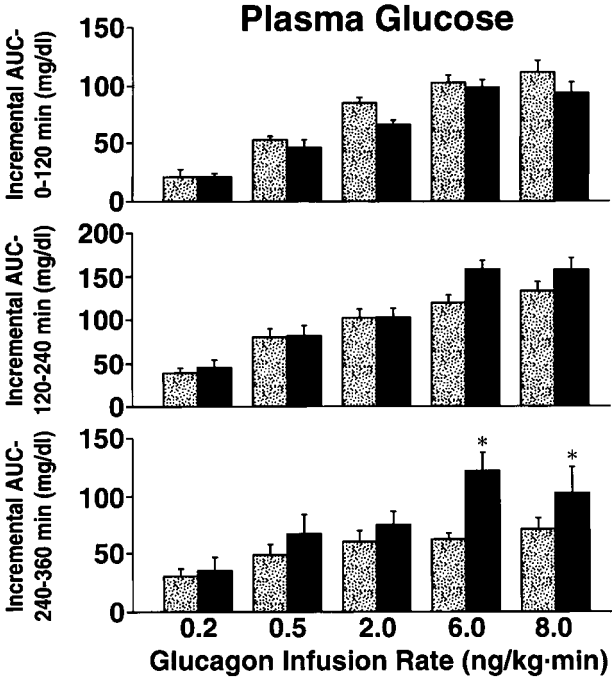

Fig 3. Incremental area under the curve of plasma glucose concentration during the time periods 0 to 120 minutes (top), 120 to 240 minutes (middle), and 240 to 360 minutes (bottom). Glucagon was infused from 0 to 240 minutes. Control subjects are shown by the stippled bars and T2DM patients by the solid bars.

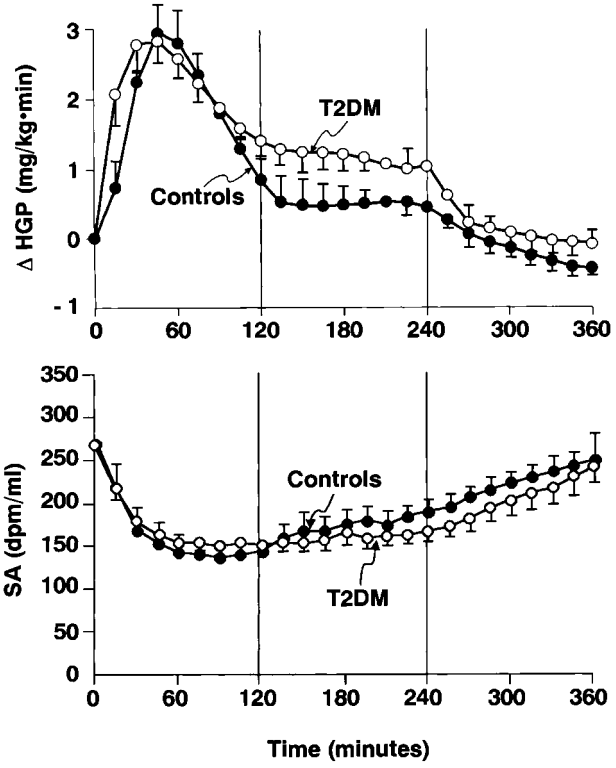

Fig 4. Increment in hepatic glucose production (top) in response to the 6 ng · kg^{-1} · min^{-1} glucagon infusion in control subjects (●) and T2DM patients (○). The time courses of plasma tritiated glucose specific activity (SA) in control and T2DM are shown in the bottom panel.

sions ($P < .05$). In the diabetic group, EGP during the 120- to 140-minute period tended to be greater than in controls during the 4 highest glucagon infusion rates and this difference achieved statistical significance ($P < .05$ to .01) during the 6-ng · kg^{-1} · min^{-1} and 8-ng · kg^{-1} · min^{-1} glucagon infusion rates (Fig 5). After cessation of the glucagon infusion, EGP decreased rapidly and similarly in both groups. During the initial 2 hours (0 to 120 minutes) of glucagon infusion, the initial rate of rise (slope) of EGP in controls (0.12 ± 0.05 *v* 0.04 ± 0.01 mg · kg^{-1} · min^{-1} per pg glucagon/mL, $P < .05$) was significantly greater than in the diabetic group. There was no difference in the Vmax for EGP between diabetics and controls during the 0- to 120-minute time period.

Total Body Glucose Disposal (Rd)

During the basal equilibration period, total body glucose disposal (1.65 ± 0.8 *v* 1.69 ± 0.05 mg · kg^{-1} · min^{-1}) and plasma glucose clearance rate (1.78 ± 0.08 *v* 1.83 ± 0.06 mL · kg^{-1} · min^{-1}) were similar in T2DM and control subjects. During the 2 highest glucagon infusion rates, there was a significant and similar increase in Rd in the T2DM and control groups ($P < .05$ *v* baseline) (Fig 6), even though the plasma insulin concentration failed to increase whatsoever in diabetic subjects and increased only minimally in controls (Table 2). This increase in Rd most likely was secondary to the resultant hyperglycemia and the mass action effect of glucose to promote its own uptake. During the 120- to 140-minute period of glucagon infusion, the increment in Rd tended to be less in diabetic versus control subjects during all glucagon infusion rates and this difference achieved statistical significance during the highest glucagon infusion rate (Fig 6). During the 2 hour post-glucagon recovery period the increment in Rd was significantly ($P < .05$ to .01) reduced in T2DM versus control subjects for each glucagon infusion rate. The impairment in tissue glucose disposal is supported by the significant reduction ($P < .01$ to .05) in metabolic clearance rate of glucose in T2DM patients (data not shown).

DISCUSSION

The present study was undertaken to compare hepatic sensitivity to glucagon in T2DM individuals and an appropriately matched group of healthy control subjects. Both hyperglycemia[36,37] and hyperinsulinemia[35] exert a suppressive effect on hepatic glucose output and enhance total body glucose utilization. In a previous study[38] designed to examine the transient response of the liver to an increment in plasma glucagon concentration, we demonstrated that, within the physiologic range of hyperglycemia and hyperinsulinemia, an increase in the plasma glucose concentration was considerably more potent than insulin in blunting the glucagon-stimulated rise in hepatic glucose output. Therefore, in the present study diabetic subjects received an overnight low-dose insulin infusion to ensure normal fasting plasma glucose levels prior to the start of the glucagon infusion. Under these conditions, the initial slope of

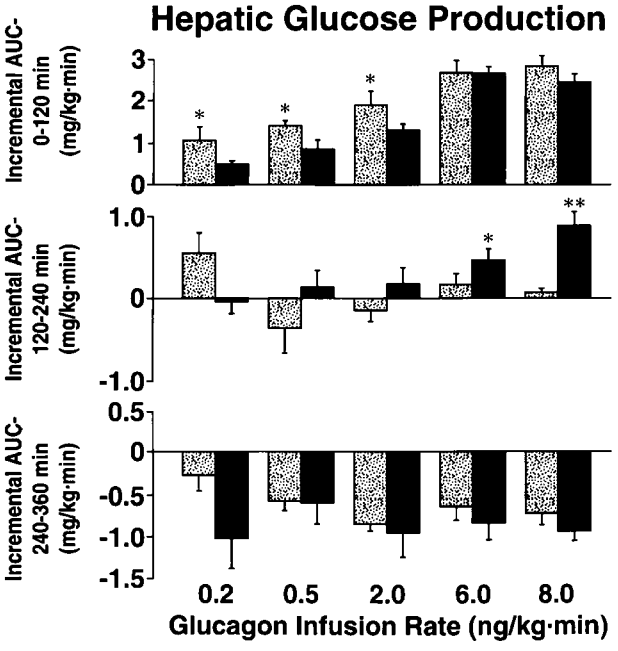

Fig 5. Incremental area under the curve of hepatic glucose production during the time periods 0 to 120 minutes (top), 120 to 240 minutes (middle), and 240 to 360 minutes (bottom). Glucagon was infused from 0 to 240 minutes. Control subjects are shown by the stippled bars and T2DM patients by the solid bars. *$P < .05$, **$P < .01$.

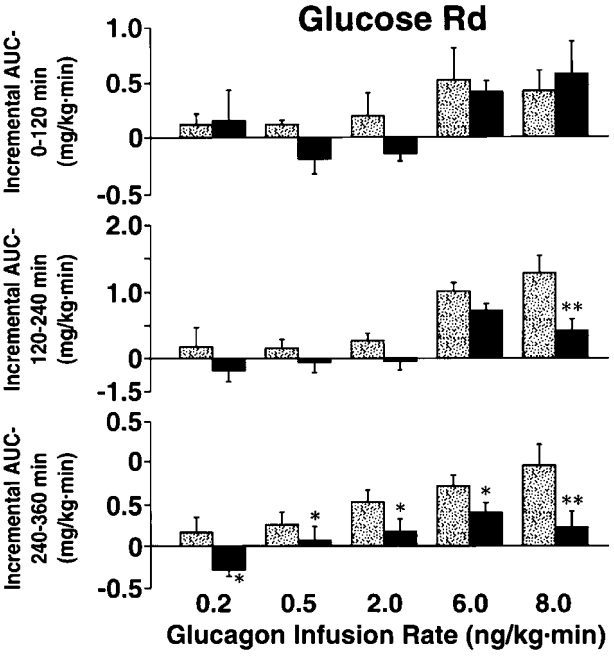

Fig 6. Incremental area under the curve for the rate of plasma glucose disappearance (Rd) during the time periods 0 to 120 minutes (top), 120 to 240 minutes (middle), and 240 to 360 minutes (bottom). Glucagon was infused from 0 to 240 minutes. Control subjects are shown by the stippled bars and T2DM patients by the solid bars. *$P < .05$, **$P < .01$.

the curve relating the plasma glucagon concentration to total EGP provides an index of hepatic sensitivity to glucagon.[34,39] Constant levels of insulin were maintained during all studies by infusing somatostatin to inhibit endogenous insulin secretion and by continuing the overnight insulin infusion required to achieve normoglycemia in the diabetic subjects. Control subjects also received somatostatin with replacement of basal insulin levels on the morning of the study. Consistent with the well-described hepatic insulin resistance in T2DM patients,[35,40] normoglycemia was obtained at the expense of a higher (by 88%) plasma insulin concentration. Under ideal conditions, one would like the plasma insulin and glucose concentrations to be similar in the diabetic and control groups. However, this only could be achieved if insulin action was equivalent in the 2 groups. This is not possible, since T2DM subjects exhibit both hepatic and peripheral resistance to insulin.[35] Because we wished to examine the initial slope of the curve relating plasma glucagon to EGP in subjects with similar basal rates of EGP and fasting glucose concentrations in order to derive an index of hepatic sensitivity to glucagon,[34,39] and because the hyperglycemic response to glucagon is more potent than hyperinsulinemia in inhibiting glucagon stimulatory effect on hepatic glucose output,[38] we felt that it was essential to restore normoglycemia in the diabetic group with a low-dose overnight insulin infusion prior to the start of glucagon infusion. We chose not to replace basal plasma growth hormone levels, since no metabolic effects of acute growth hormone deficiency are known to occur over the short time period of the present study.[41]

Glucagon exerts a biphasic effect on hepatic glucose output with a rapid, though evanescent, stimulation of glycogenolysis[18,38] and a delayed, but persistent stimulation of gluconeogenesis.[18,39] Consistent with these previous observations, the present results also demonstrated a biphasic response of EGP, in which most of the stimulatory effect of glucagon on EGP was exerted during the initial 2 hours and only a modest stimulatory action occurred during hours 2 through 4. There was no significant difference in the cumulative rise in EGP during the 4-hour period of glucagon infusion in T2DM patients and control subjects. During the initial 2 hours of the 0.2-, 0.5-, and 2-ng $\cdot kg^{-1} \cdot min^{-1}$ glucagon infusion rates, the rise in EGP was significantly lower in the diabetic versus control group and this was reflected by a lower initial rate of rise (slope) of the line relating EGP to the plasma glucagon concentration in the diabetic group (0.04 ± 0.01 *v* 0.12 ± 0.02 mg $\cdot kg^{-1} \cdot min^{-1}$ per pg of glucagon $\cdot mL^{-1}$, $P < .01$; combined analysis of 0.2-, 0.5-, and 2.0-ng $\cdot kg^{-1} \cdot min^{-1}$ physiologic glucagon infusion studies). Thus, within the physiologic range of plasma glucagon concentrations and within the limits of the present experimental design, we were unable to demonstrate an increased sensitivity of hepatic glucose production to glucagon in T2DM individuals. To the contrary, the initial stimulatory effect of glucagon on hepatic glucose production was reduced in T2DM. Several explanations could account for this observation. First, and most importantly, studies using magnetic resonance imaging spectroscopy[42] have shown that the liver glycogen concentration in subjects with T2DM is significantly reduced compared to appropriately matched control subjects. Since the initial response (0 to 120 minutes) to glucagon primarily reflects the stimulation of glycogenolysis in the liver, a reduced hepatic glycogen concentration in the T2DM group could account for the blunted response to hyperglucagonemia. Alternatively, the blunted early glycemic response to glucagon could reflect a defect in the mechanisms responsible for the activation of glycogenolysis by glucagon. A third explanation that could, in part, explain the blunted rise in EGP in the diabetic group during the 0.2-, 0.5-, and 2.0-ng $\cdot kg^{-1} \cdot min^{-1}$ glucagon infusion, is the presence of mild portal hyperinsulinemia that resulted from the overnight insulin infusion. However, this would require that a plasma insulin concentration that exerted similar biological effects in the diabetic and control groups prior to the dose-response glucagon infusions, no longer did so during the glucagon infusions. Although this possibility cannot be excluded, it seems unlikely. Thus, our results are consistent with those of Nielsen et al,[7] who also demonstrated a blunted rise in EGP in response to glucagon infusion. One could interpret our results, as well as those of Nielsen et al,[7] to indicate that hepatic sensitivity to glucagon is not increased in T2DM patients. However, if hepatic glycogen levels are reduced in T2DM patients, this may have obscured an enhanced glycogenolytic response during the first 2 hours of glucagon infusion

Although the cumulative 4-hour incremental rise in EGP in response to glucagon was similar in T2DM and control subjects, noticeable differences were detected when the dynamic response to glucagon was considered. As stated above, the initial rate of rise in EGP in response to glucagon was more rapid in control versus T2DM subjects, and the calculated

glucagon sensitivity (slope of the line relating EGP to the plasma glucagon concentration) was significantly higher (0.12 ± 0.02 *v* 0.04 ± 0.01 mg · kg^{-1} · min^{-1} per pg · mL^{-1} of glucagon, $P < .01$ during the 3 lower physiologic glucagon infusion rates). In contrast, during the last 2 hours of glucagon infusion the increment in EGP in response to glucagon tended to be higher in the diabetic versus control group during the 0.5- and 2.0-ng · kg^{-1} · min^{-1} glucagon infusion rates, and this difference reached statistical significance during the 2 highest (6 and 8 ng · kg^{-1} · min^{-1}) infusion rates (Figs 4 and 5). These findings suggest a differential responsiveness of glycogenolysis and gluconeogenesis to glucagon, ie, control subjects have a more rapid rate of glycogen breakdown, while diabetics manifest a more persistent stimulation of de novo glucose synthesis, which is most apparent in response to supraphysiologic elevations in the plasma glucagon concentration. The significantly higher rate of EGP in T2DM patients during the last 2 hours of glucagon infusion (6 and 8 ng · kg^{-1} · min^{-1}) is consistent with the increase in hepatic gluconeogenic activity that has been demonstrated in diabetic subjects by other investigators.[42-44] Alternatively, it cannot be excluded that the increased late stimulation of EGP in response to the 2 highest glucagon infusion rates reflects an altered, ie, delayed, timing of the hormone effect on glycogenolysis.

In the only previous study that examined hepatic sensitivity to glucagon in T2DM patients, Nielsen et al[7] did not observe an augmented late (2 to 4 hours) response to the hormone. However, the highest glucagon infusion rate used by these investigators was 2.0 ng · kg^{-1} · min^{-1}. Thus, they would not have been able to detect the enhanced sensitivity to the higher glucagon infusion rates employed in the present study. Consoli et al have suggested that increased muscle efflux of lactate, that occurs secondarily to insulin resistance and impaired glucose oxidation in peripheral tissues, is responsible for the accelerated rate of hepatic gluconeogenesis in T2DM subjects.[44] The plasma lactate concentration was measured in our study and we found no difference between controls and T2DM patients. Although definite conclusions cannot be drawn without measurement of lactate turnover, the failure to observe any increase in plasma lactate concentration suggests that substrate, at least lactate, availability does not play an important role in the greater elevation of EGP in response to glucagon infusion during the 120- to 240-minute time period in T2DM subjects. In contrast, plasma FFA levels were modestly increased in T2DM individuals, both in the basal state and during glucagon infusion. Increased FFA availability may have contributed to the augmented late response of EGP to glucagon via several mechanisms. Accelerated FFA oxidation has been shown to cause an allosteric activation of the enzymes involved in gluconeogenesis[45] and to provide an energy source for gluconeogenesis.[46] Lastly, hepatic insulin resistance or some other metabolic disturbance that is fundamental to the diabetic state, ie, enhanced expression of the genes involved in gluconeogenesis,[47] could be responsible for the accelerated rate of hepatic glucose output in response to pharmacological plasma glucagon concentrations.

During the 2-hour post-glucagon recovery period, the increment in whole body glucose disposal was significantly reduced in diabetic subjects (Fig 6), and this was paralleled by a marked reduction in the plasma glucose clearance rate (data not shown). The blunted increment in whole body glucose disposal also was evident during the last 2 hours of the glucagon infusion period. These observations indicate that the greater hyperglycemic response observed during the late phases of high-dose glucagon infusion (6 and 8 ng · kg^{-1} · min^{-1}) and post-glucagon infusion periods (Fig 3) is the result of a modest increase in EGP, combined with a severe defect in tissue glucose utilization. Since plasma insulin concentrations in the range of 8 to 16 μU/mL do not stimulate tissue glucose uptake,[48] the impaired tissue glucose utilization in T2DM patients most likely reflects the inability of hyperglycemia to promote glucose utilization through a mass action effect.[36] It also is possible that 4 hours of hyperglucagonemia per se impairs tissue glucose uptake. We previously have shown that 24 to 48 hours of hyperglucagonemia causes a modest reduction in peripheral tissue sensitivity to insulin.[22]

In conclusion, the present results are consistent with those of Nielsen et al[7] and do not suggest that hepatic sensitivity to a physiologic increment in plasma glucagon concentration is altered in T2DM patients. However, in response to a pharmacologic increase in the plasma glucagon concentration, T2DM subjects demonstrate an excessive increase in hepatic glucose production during the 120-to 240-minute time period, suggesting an enhanced sensitivity of gluconeogenesis to glucagon. Lastly, the reduced rate of glucose disposal following cessation of the glucagon infusion is consistent with an impaired ability of hyperglycemia to promote glucose uptake in T2DM individuals.

REFERENCES

1. Reaven G, Chen Y-D, Golay A, et al: Documentation of hyperglucagonemia throughout the day in nonobese patients with non-insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 64:106-110, 1987

2. Boden G, Soriano M, Hoeldtje R, et al: Counter regulatory hormone release and glucose recovery after hypoglycemia in non-insulin-dependent diabetic patients. Diabetes 32:1055-1059, 1983

3. Unger R, Aguilar-Parada E, Muller W, et al: Studies of pancreatic alpha cell function in normal and diabetic subjects. J Clin Invest 49:837-839, 1970

4. Ohneda A, Watanabe K, Horigome K, et al: Abnormal response of pancreatic glucagon to glycemic changes in diabetes mellitus. J Clin Endocrinol Metab 46:504-510, 1978

5. Baron A, Schaeffer L, Shragg P, et al: Role of hyperglucagonemia in maintenance of increased rates of hepatic glucose output in type II diabetics. Diabetes 36:274-283, 1987

6. Shah P, Basu A, Basu R, et al: Impact of lack of suppression of glucagon on glucose tolerance in humans. Am J Physiol 277:E283-E290, 1999

7. Nielsen MF, Wise S, Dinneen SF, et al: Assessment of hepatic sensitivity to glucagon in NIDDM. Use as a tool to estimate the contribution of the indirect pathway to nocturnal glycogen synthesis. Diabetes 46:2007-2016, 1997

8. Muller W, Faloona G, Aguilar-Parada E, et al: Abnormal alpha-cell function in diabetes; responses to carbohydrate and protein ingestion. N Engl J Med 282:109-115, 1970

9. Cherrington AD: Control of glucose uptake and release by the liver in vivo. Diabetes 48:1198-1214, 1999

10. Buchanan K, McCarrol A: Abnormalities of glucagon metabolism in untreated diabetes mellitus. Lancet 2:1394-1395, 1972

11. Day J, Anderson J: Abnormalities of glucagon metabolism in diabetes mellitus. J Clin Endocrinol Metab 2:211-217, 1973

12. Butler RC, Rizza RA: Contribution to postprandial hyperglycemia and effect on initial splanchnic glucose clearance on hepatic glucose cycling in glucose-intolerant or NIDDM patients. Diabetes 40:73-81, 1991

13. Mitrakou A, Kelly D, Veneman T, et al: Contribution of abnormal muscle and liver glucose metabolism to postprandial hyperglycemia in NIDDM. Diabetes 39:1381-1390, 1990

14. Aronoff S, Bennett P, Rushforth N, et al: Arginine-stimulated hyperglucagonemia in diabetic Pima Indians. Diabetes 25:404-407, 1976

15. Dimitriadis G, Pehling G, Gerich J: Abnormal glucose modulation of islet A- and B-cell responses to arginine in non-insulin-dependent diabetes mellitus. Diabetes 34:541-547, 1985

16. Gerich J, Lorenzi M, Karam J, et al: Abnormal pancreatic glucagon secretion and postprandial hyperglycemia in diabetes mellitus. JAMA 234:159-165, 1975

17. Liljenquist J, Mueller G, Cherrington A, et al: Evidence for an important role of glucagon in the regulation of hepatic glucose production in normal man. J Clin Invest 59:369-374, 1977

18. Cherrington AD, Chiasson JL, Liljenquist JE, et al: The role of insulin and glucagon in the regulation of basal glucose production in the postabsorptive dog. J Clin Invest 58:1407-1418, 1976

19. Mitrakou A, Kelley D, Mokan M, et al: Role of reduced suppression of glucose production and diminished early insulin release in impaired glucose tolerance. N Engl J Med 326:22-29, 1992

20. Mitrakou A, Kelley D, Veneman T, et al: Contribution of abnormal muscle and liver glucose metabolism to postprandial hyperglycemia in NIDDM. Diabetes 39:1381-1390, 1990

21. Ferrannini E, Simonson DC, Katz LD, et al: The disposal of an oral glucose load in patients with non-insulin dependent diabetes mellitus. Metabolism 37:79-85, 1988

22. DelPrato S, Castellino P, Simonson D, et al: Hyperglucagonemia and insulin-mediated glucose metabolism. J Clin Invest 79:547-556, 1987

23. DelPrato S, Tiengo A, Baccaglini U, et al: Effect of insulin replacement on intermediary metabolism in diabetes secondary to pancreatectomy. Diabetologia 25:252-259, 1983

24. Vigili de Kreutzenberg S, Maifreni L, et al: Glucose turnover and recycling in diabetes secondary to total pancreatectomy. Effect of glucagon infusion. J Clin Endocrinol Metab 70:1023-1029, 1990

25. Raskin P, Unger RH: Hyperglucagonemia and its suppression. Importance in metabolic control of diabetes. N Engl J Med 299:433-435, 1978

26. Sherwin RS, Fisher M, Hendler R, et al: Hyperglucagonemia and blood glucose regulation in normal, obese, and diabetic subjects. N Engl J Med 299:455-461, 1976

27. Lins PE, Wajngot A, Adamson U, et al: Minimal increase in glucagon levels enhance glucose production in man with partial hypoinsulinemia. Diabetes 32:633-666, 1987

28. Drucker DJ: Glucagon-like peptides. Diabetes 47:159-169, 1998

29. Holst JJ, Deacon CF: Inhibition of the activity of dipeptidyl peptidase IV as a treatment for type 2 diabetes. Diabetes 47:1663-1670, 1995

30. Smith RA, Sisk R, Lockhart P, et al: Isolation of glucagon antagonists by random molecular mutagenesis and screening. Mol Pharmacol 43:741-748, 1993

31. Steele R, Wall J, deBodo R, et al: Measurement of size and turnover rate of body glucose pool by the isotope dilution method. Am J Physiol 187:15-24, 1956

32. Stumvoll M, Meyer C, Mitrakou A, et al: Renal glucose production and utilization: new aspects in humans. Diabetologia 40:749-757, 1997

33. Stunvoll M, Meyer C, Kreier M, et al: Effects of glucagon on renal and hepatic glutamine gluconeogenesis in normal postabsorptive humans. Metabolism 47:1227-1232, 1998

34. Bradley D, Bergman R: Hepatic glucagon sensitivity and fasting glucose concentration in normal dogs. Am J Physiol 262:E539-E545, 1992

35. Groop L, Bonadonna R, DelPrato S, et al: Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus. Evidence for multiple sites of insulin resistance. J Clin Invest 84:205-213, 1989

36. Del Prato S, Simonson DC, Sheehan P, et al: Studies on the mass effect of glucose in diabetes. Evidence for glucose resistance. Diabetologia 40:687-697, 1997

37. Liljenquist J, Mueller G, Gherrington A, et al: Hyperglycemia per se (insulin and glucagon withdrawn) can inhibit hepatic glucose production in man. J Clin Endocrinol Metab 48:117-126, 1982

38. Ferrannini E, DeFronzo R, Shwerwin R: Transient hepatic response to glucagon in man: Role of insulin and hyperglycemia. Am J Physiol 242:E73-E81, 1982

39. Stevenson R, Steiner K, Davis M, et al: Similar dose responsiveness of hepatic glycogenolysis and gluconeogenesis to glucagon in vivo. Diabetes 36:382-389. 1987

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

41. Gerich J, Lorenzi M, Bier D, et al: Effects of physiologic levels of glucagon and growth hormone on human carbohydrate and lipid metabolism. J Clin Invest 57:875-884, 1976

42. Magnusson I, Rothman D, Katz L, et al: Increased rate of gluconeogenesis in type II diabetes mellitus. A 13 C nuclear magnetic resonance study. J Clin Invest 90:1323-1327, 1992

43. Gastadelli A, Baldi S, Pettit M, et al: Influence of obesity and type 2 diabetes on gluconeogenesis and glucose output in humans: A quantitative study. Diabetes 49:1367-1373, 2000

44. Consoli A, Nurjhan N, Reilly J, et al: Contribution of liver and skeletal muscle to alanine and lactate metabolism in humans. Am J Physiol 259:E677-E684, 1990

45. Williamson J, Kreisberg R, Felts P: Mechanism for the stimulation of gluconeogenesis by fatty acids in perfused rat liver. Proc Natl Acad Sci USA 56:247-254, 1966

46. Ferrannini E, Groop L: Hepatic glucose production in insulin-resistant states. Diab Metab Rev 5:711-725, 1989

47. Granner D, O'Brien R, Imai E, et al: Complex hormone response unit regulating transcription of the phosphoenolpyruvate carboxykinase gene: From metabolic pathways to molecular biology. Rec Prog Horm Res 47:319-348, 1991

48. Zierler K, Rabinowitz D: Effect of very small concentrations of insulin on forearm metabolism. J Clin Invest 43:950-962, 1964