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Research Article

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Impaired Net Hepatic Glycogen Synthesis in Insulin-dependent Diabetic Subjects during Mixed Meal Ingestion

A ^{13}C Nuclear Magnetic Resonance Spectroscopy Study

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Abstract

Hepatic glycogen concentration was measured in six subjects with insulin-dependent diabetes mellitus (IDDM) and nine weight-matched control subjects using ^{13}C nuclear magnetic resonance spectroscopy during a day in which three isocaloric mixed meals were ingested. The relative fluxes of the direct and indirect (3 carbon units → → glycogen) pathways of hepatic glycogen synthesis were also assessed using [1-^{13}C]glucose in combination with acetaminophen to noninvasively sample the hepatic UDP-glucose pool. Mean fasting hepatic glycogen content was similar in the two groups. After each meal, hepatic glycogen content increased, peaking 4–5 h after the meal in both groups. By 11:00 p.m. the IDDM subjects had synthesized only 30% of the glycogen that was synthesized by the control group [IDDM subjects, net increment = 44±20 (mean±SE) mM; control subjects, net increment = 144±14 mM; $P < 0.05$]. After breakfast the flux through the gluconeogenic pathway relative to the direct pathway of hepatic glycogen synthesis was 1.7-fold greater in the IDDM subjects (59±4%) than in the control subjects (35±4%, $P < 0.0003$). In conclusion, under mixed meal conditions, subjects with poorly controlled IDDM have a major defect in net hepatic glycogen synthesis and augmented hepatic gluconeogenesis. The former abnormality may result in an impaired glycemic response to counterregulatory hormones, whereas both abnormalities may contribute to postprandial hyperglycemia. (*J. Clin. Invest.* 1995. 95:783–787.) Key words: glycogenolysis • gluconeogenesis • NMR spectroscopy • diabetes mellitus

Introduction

Until recently the only way to measure hepatic glycogen content in humans was by needle biopsy or by direct sampling of liver during abdominal surgery. Because of the invasiveness of these procedures, little is known about the role of hepatic glycogen metabolism in maintaining glucose homeostasis in either normal individuals or patients with type I or insulin-dependent diabetes mellitus (IDDM).[1] Because glucose is taken up by the liver after a meal and stored as glycogen, it is possible that alterations in hepatic glycogen synthesis may contribute to postprandial hyperglycemia in IDDM patients. Furthermore, because mobilization of hepatic glycogen is the primary mechanism that counters hypoglycemia, decreased hepatic glycogen stores might be an important predisposing factor for the development of severe hypoglycemia in IDDM patients.

Recent developments in ^{13}C nuclear magnetic resonance (NMR) spectroscopy have made it possible to measure hepatic glycogen content in humans noninvasively (1, 2). This technique was used in the present study to measure changes in net hepatic glycogen concentration in both normal and IDDM subjects throughout a day in which three isocaloric mixed meals were ingested. In addition, the relative contributions of the direct (glucose → glucose-6-phosphate → glucose-1-phosphate → UDP-glucose → glycogen) and indirect [3-carbon units (derived either intra- or extrahepatically) → → phosphoenolpyruvate → → glucose-6-phosphate → glucose-1-phosphate → UDP-glucose → glycogen] pathways of hepatic glycogen synthesis were assessed using [1-^{13}C]glucose in the meal in combination with acetaminophen to noninvasively sample the hepatic UDP-glucose pool (3). Recent studies have found that in normal subjects fasted overnight the direct and indirect pathways contribute equally to hepatic glycogen synthesis during a glucose infusion (3) and that fluxes through these pathways are regulated by substrate and hormonal concentrations (4–6) as well as by diet (3, 7). The effect of ingesting a mixed meal on the pathways of hepatic glycogen synthesis has not yet been evaluated. Furthermore, it might be anticipated that the relative flux through these pathways of liver glycogen synthesis would be altered in IDDM subjects in response to chronically altered concentrations of glucose, insulin, and glucagon.

Methods

Subjects. Nine healthy control subjects (9 male, mean age±SE was 22±1 yr, mean weight 78±3 kg, mean BMI 23.4±1.0 kg/m^2) and six C-peptide negative type I diabetic subjects (4 male, 2 female, mean age 34±2 yr, mean weight 73±3 kg, mean BMI 23.5±2.4 kg/m^2, mean duration of diabetes 16±4 yr) were studied. All of the IDDM subjects were on an insulin regimen consisting of a subcutaneous injection of regular insulin (2–14 units, Humulin R; Lilly, Indianapolis, IN) administered 20–30 min before each meal and a subcutaneous injection of intermediate (8–12 units, Humulin NPH; Lilly) or long acting (8–36 units, Humulin Ultralente; Lilly) insulin given at bedtime, with the exception of two IDDM subjects who also had a subcutaneous injection of intermediate (8–12 units, Humulin NPH; Lilly) in the morning in

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1. *Abbreviations used in this paper:* IDDM, insulin-dependent diabetes mellitus; NMR, nuclear magnetic resonance.

addition to their bedtime dose of NPH insulin. None of the IDDM subjects had any renal complications from diabetes and all were in fair and stable control (mean glycosylated hemoglobin was 10.2±0.3%, normal range 4–8%) for ≥ 6 mo before the study. Written consent was obtained from all subjects after explanation of the purpose, nature, and potential risks of the study. The protocol was reviewed and approved by the Human Investigation Committee of Yale University School of Medicine.

Experimental protocol. For 3 d before the study (outpatient days 1–3) and during the 2 study days (inpatient days 4 and 5) the subjects ingested a weight maintenance diet (35 kcal/kg per d), which was prepared by the Yale/New Haven Hospital Clinical Research Center metabolic kitchen. The proportion of calories was divided according to the American Diabetes Association guidelines: 60% carbohydrate, 20% protein, and 20% fat, and the calories were distributed equally among three meals with no snacks given between the meals. This diet was similar to the diet that the diabetic subjects followed before being enrolled in the study. In consultation with a physician, the diabetic subjects adjusted their insulin doses in their usual fashion. Fasting, 4:00 p.m., and bedtime blood glucose concentrations during this period were similar to what the diabetic subjects normally observed with home glucose monitoring while eating their usual diet, although postprandial glucose concentrations were somewhat higher.

On the evening of day 3 the subjects were admitted to the Yale/New Haven Hospital Clinical Research Center and underwent ^{13}C NMR measurements of hepatic glycogen content the following day. The basal hepatic glycogen concentration was measured at 7:00 a.m. before breakfast. Subjects had their breakfast at 8:00 a.m., lunch at 1:00 p.m., and dinner at 6:00 p.m. following the same schedule as the previous 3 d. Meals were consumed completely within 20 min. Diabetic subjects took the same dose of regular insulin before each meal that they took on day 3. On day 4, NMR measurements of hepatic glycogen were performed continuously in 30-min blocks starting ~ 2 h after each meal and continuing just before the next meal to capture the greatest increment in hepatic glycogen concentration.

To minimize subject motion during the NMR measurements, which would have reduced the quality of the glycogen spectra, blood sampling was performed on day 5. At 6:00 a.m. on day 5, a catheter was inserted into an antecubital vein for blood sampling and the subjects ingested the identical diet at the same times as they did on day 4. The diabetic subjects took the same amount of insulin at the same time as they did on day 4. Blood samples were obtained before each meal and every 15 min thereafter for measurement of plasma glucose and ^{13}C glucose enrichment and every 30 min for insulin, C-peptide, and glucagon. To evaluate the contributions of the direct and indirect pathways to hepatic glycogen synthesis, 10 g of [1-^{13}C]glucose (99% enriched) was substituted for 10 g of glucose in the breakfast and administered along with 300 mg of acetaminophen to each subject (3). Urine was collected at hourly intervals following breakfast for assessment of the ^{13}C enrichment in the acetaminophen-glucuronide.

In vivo ^{13}C NMR spectroscopy. All measurements were performed in a 2.1-T 1-m-bore spectrometer (Biospec, Bruker, Billerica, MA) as previously described (1). Briefly, a 9-cm circular ^{13}C coil was used as a transmitter/receiver coil and a 12 × 14-cm butterfly proton coil was used for shimming, scout images, and proton decoupling during ^{13}C NMR acquisition. The liver position in the supine subject was confirmed from a mutislice axial FLASH image (8). Signals from the liver were acquired by image selected *in vivo* spectroscopy localization technique to remove signals from the surface above the liver (9). A 4-ms hyperbolic secant pulse was used to invert the 2.5 cm of the skin surface, and as a result signals from the surface were eliminated by adding scans with an alternate inversion pulse (10). A 180° pulse at the coil center was used as an excitation pulse and it was calibrated each time using a 2-cm sphere containing ^{13}C-enriched formic acid that was placed at coil center. Each proton decoupled ^{13}C NMR spectrum consisted of 12,800 scans that were acquired for 30 min. All spectra were processed and analyzed in an identical fashion. Hepatic glycogen was quantified by integration of the C1 glycogen peak at 100.5 ppm with a computer (Aspect 3000, Bruker) using the same frequency bandwidth for all spectra (250 Hz). Absolute quantitation of hepatic glycogen was determined by comparing the peak integral of the C1 liver glycogen peak with the C1 glycogen peak integral of a glycogen standard taken under identical conditions. Finally, minor corrections (< 10%) were made for incomplete space filling of the surface coil by the liver by comparison of the axial images of the glycogen phantom with that of the subject's liver.

Analytical procedures. Plasma glucose concentration was determined with a glucose analyzer (Glucose Analyzer II; Beckman, Fullerton, CA). Plasma immunoreactive insulin (free insulin for the IDDM subjects), C-peptide, and glucagon were measured with double antibody radioimmunoassay techniques using commercially available kits (insulin, Diagnostic Systems Laboratories, Webster, TX; C-peptide, Diagnostic Products Corporation, Los Angeles, CA; and glucagon, ICN Biomedicals Inc., Costa Mesa, CA). Glycosylated hemoglobin was measured using an ion exchange chromatography method (Isolab, Akron, OH).

The relative contributions of the direct and indirect pathway to hepatic glycogen synthesis were determined using acetaminophen to noninvasively sample the hepatic UDP-glucose pool as previously described (3). Briefly, plasma samples were deproteinized with $Ba(OH)_2$ and $ZnSO_4$ and deionized by passage through a mixed bed ion exchange resin column. After evaporation, the residual glucose was derivatized with pyridine/acetic anhydride (1:2 in volume) to yield glucose pentaacetate. Total ^{13}C enrichment of plasma glucose was determined by chemical ionization mass spectral analysis of the pentaacetate glucose derivative performed on a gas chromatography-mass spectrometry (HP 5971 GC-MS; Hewlett Packard Co., Palo Alto, CA) (11). Urine acetaminophen-glucuronide was semipurified by anion exchange chromatography. After freeze-drying, the sample was redissolved in a minimum volume of water, deproteinized with sodium sulfate and methanol, and filtered. The solvent was removed on a rotovapor (Buchi). Urinary acetaminophen-glucuronide ^{13}C enrichment was determined by both gas chromatography-mass spectrometer and ^{13}C NMR spectroscopy. The isolated and purified acetaminophen-glucuronide was placed in a 5-mm NMR tube, and ^{13}C NMR spectroscopy was done at 125.76 MHz (AM500 Bruker). NMR chemical shifts of the glucuronide C1, C2, and C6 were assigned to 101.0, 73.2, and 176.1 ppm, respectively. In the acetaminophen moiety, C2′(C6′) and C3′(C5′) of the aromatic ring were assigned to 117.7 and 123.8 ppm. The percent of glycogen synthesized by the direct pathway was calculated by the following equation: % Direct Pathway = C1 − C6 acetaminophen-glucuronide/C1 − C6 plasma glucose, where C1, C6 acetaminophen-glucuronide and C1, C6 plasma glucose represent the ^{13}C enrichments in the C1 and C6 positions of the glucuronide and plasma glucose, respectively.

All values are expressed as means±SE. Comparisons between groups were done using the unpaired Student's *t* test and comparisons within the group were made using the paired Student's *t* test or analysis of variance with Student-Newman-Keuls post hoc testing.

Results

The time courses for mean plasma glucose, insulin, and glucagon concentration during day 5 in both control and IDDM subjects are shown in Fig. 1. Despite following identical diets, both fasting and postprandial plasma glucose concentrations (*top*) were markedly higher in the IDDM subjects compared with the control subjects. The peak plasma glucose concentrations after breakfast (control subjects, 143±5 mg/dl; IDDM subjects, 398±27 mg/dl) were greater than the peak plasma glucose concentrations after the other meals in both normal subjects (lunch, 123±4 mg/dl; dinner, 117±3 mg/dl; both $P < 0.05$ compared with breakfast) and IDDM subjects (lunch, 293±37 mg/dl; dinner, 261±8 mg/dl; both $P < 0.05$ compared with breakfast) even though the meals were isocaloric and contained an identical composition of carbohydrate, protein, and fat. This

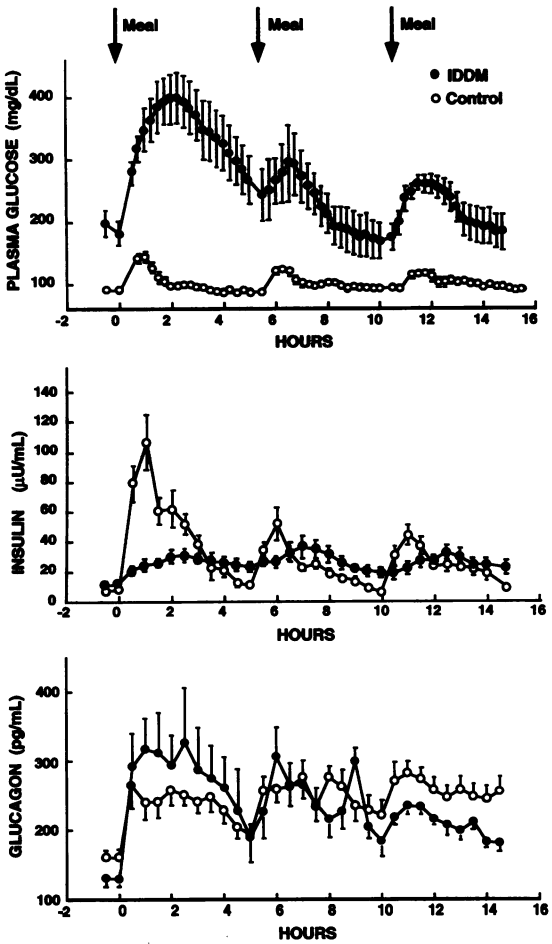

Figure 1. Time course for plasma glucose concentration (*top*), insulin concentration (*middle*), and glucagon concentration (*bottom*) in normal and IDDM subjects during a day in which three isocaloric mixed meals were ingested 5 h apart. (To convert plasma glucose to mmol/liter, multiply by 0.05551 and to convert plasma insulin to pmol/liter, multiply by 6.0.)

most likely reflects a component of peripheral insulin resistance secondary to an early morning rise in growth hormone concentration (12, 13) and is consistent with the higher peak plasma insulin concentrations (*middle*) observed in the normal subjects after breakfast (107±18 μU/ml) compared with after lunch (52±11 μU/ml, $P < 0.05$) and dinner (44±7 μU/ml, $P < 0.05$). Although there was no difference in the mean plasma glucagon concentration between the two groups at any time (*bottom*), the mean plasma glucagon concentration was inappropriately elevated in the IDDM subjects considering their level of hyperglycemia. The concentration of plasma C-peptide measured before and after each meal was undetectable in all of the IDDM subjects.

Fig. 2 shows the time course for mean hepatic glycogen concentration in both control and IDDM subjects. In the control subjects, the mean fasting (7:00 a.m.) hepatic glycogen concentration was 274±11 mM and increased continuously throughout the day without any detectable decrease after breakfast or lunch. Hepatic glycogen concentration peaked at ~ 4 h after dinner, resulting in a net increment of 144±14 mM from breakfast (Table I). In the IDDM subjects mean fasting hepatic glycogen

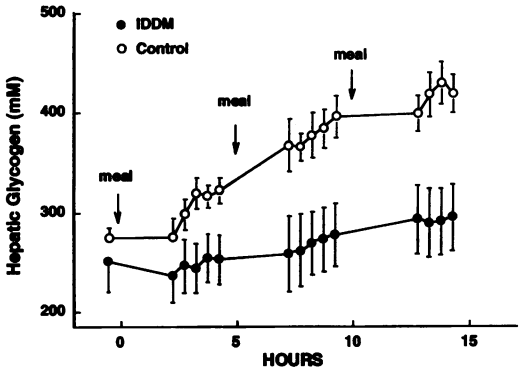

Figure 2. Time course for hepatic glycogen concentration (mM) in both normal and IDDM subjects during a day in which three isocaloric mixed meals were ingested 5 h apart.

concentration was slightly, but not significantly, lower (250±30 mM) than in the control subjects. Although the overall pattern of net hepatic glycogen synthesis was similar in the IDDM and the control subjects, the net amount of hepatic glycogen synthesized by the IDDM subjects was significantly less than in the control subjects such that by 4 h after dinner when hepatic glycogen stores were at their maximum, the IDDM subjects had stored only ~ 30% of the hepatic glycogen that was made by the control subjects, assuming similar liver volumes in both groups (Table I).

Fig. 3 shows the mean relative flux of the direct pathway for hepatic glycogen synthesis after breakfast. During the first 4 h after breakfast the relative flux through the direct pathway of hepatic glycogen synthesis was between 1.6- and 1.8-fold greater in the control subjects compared with the IDDM subjects. The relative flux through the direct pathway increased in both groups such that by the last interval (4–5 h), 71±10% and 51±10% of hepatic glycogen was synthesized by the direct pathway in the control and IDDM groups, respectively. Assuming linear rates of hepatic glycogen synthesis over the 5 h after breakfast, it can be estimated that the control subjects synthesized ~ 65±4% of their hepatic glycogen during this time by the direct pathway compared with ~ 41±4% ($P < 0.0003$) for the IDDM subjects.

Discussion

Prior studies of hepatic glycogen metabolism in humans have been limited to mostly invasive biopsy techniques and therefore

Table I. Hepatic Glycogen Concentration (mM) in Control and IDDM Subjects

	Fasting	4 h after breakfast	4 h after lunch	4 h after dinner	Maximum increment between dinner and fasting
Control	274±11	321±12‡	395±21§	420±19	144±14
IDDM	250±30	252±25*	277±32*	294±33*	44±20*

Values are means±SE. * $P < 0.05$ compared with control subjects at the same time. ‡ $P < 0.05$ compared with control subjects fasting. § $P < 0.003$ compared with control subjects 4 h after breakfast.

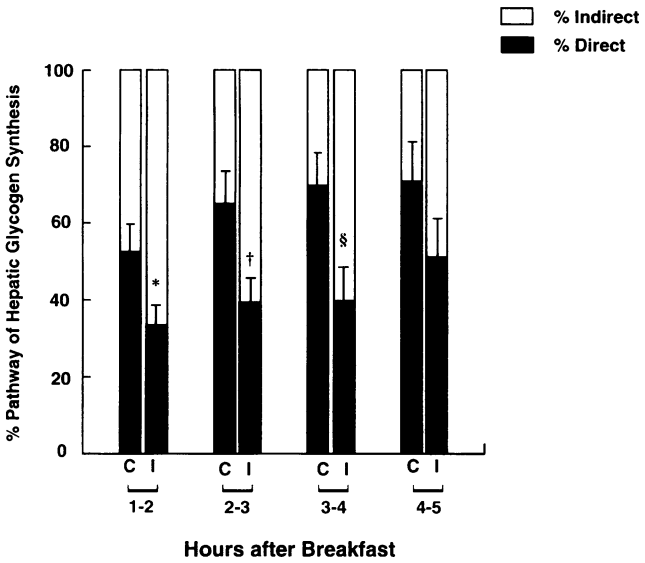

Figure 3. Relative fluxes of the direct and indirect pathways of hepatic glycogen synthesis determined at four 1-h intervals after breakfast in control (*C*) and IDDM (*I*) subjects. (Data are shown for only 7 of the normal subjects because there was not enough acetaminophen-glucuronide to process in two of the subjects.) $*P = 0.06$, $\dagger P = 0.05$, $\S P = 0.03$ compared with control subjects at the same time point.

confined to only a few measurements during the experiment. The present study is the first to examine net hepatic glycogen synthesis in humans throughout the course of a day in which three isocaloric mixed meals were ingested. The results demonstrate that hepatic glycogen stores peak between 4 and 5 h after each meal in both normal and IDDM subjects and that it increments progressively throughout the day. If meals are ingested 5 h apart, hepatic glycogen stores reach a peak just when the next meal is begun, resulting in negligible net hepatic glycogenolysis during the waking hours (8:00 a.m. to 11:00 p.m.). This suggests that under these conditions glucose absorption from meals and possibly gluconeogenesis account for the majority of glucose appearance in the body during the waking hours of the day and that net hepatic glycogenolysis contributes significantly to whole body glucose production mostly during the night (11:00 p.m. until breakfast).

These studies also found that during a day in which three mixed meals are ingested, poorly controlled IDDM patients have a profound defect in net hepatic glycogen synthesis such that by the end of the day they synthesized less than one third the amount of glycogen than did the control subjects. Because net hepatic glycogen synthesis is a balance between glycogen synthase and phosphorylase activity, these results could be explained by abnormalities in either or both of these enzymes. We recently found that hepatic glycogen synthase and glycogen phosphorylase can be simultaneously active in humans under conditions of net glycogen synthesis resulting in glycogen cycling (14). It is possible that poorly controlled IDDM subjects have higher rates of hepatic glycogen cycling following meal ingestion resulting in less net glycogen accumulation. It is also possible that acquired defects in hepatic glucokinase activity play a role in limiting net hepatic glycogen synthesis (15).

The gluconeogenic pathway was relatively much more important for synthesizing hepatic glycogen in the IDDM subjects, because by the end of breakfast ~ 60% of the glycogen was derived from gluconeogenesis in the IDDM subjects compared to ~ 35% in the control subjects. Although it is likely that the lower insulin:glucagon ratio in the IDDM subjects played an important role in causing these results, it is also possible that chronic alterations in hepatic glucose metabolism acquired as a result of the diabetes were responsible. The percentage of hepatic glycogen synthesized by the direct pathway tended to increase in both normal and IDDM subjects after breakfast, suggesting that these pathways are inducible over the course of a few hours. These results are in good agreement with a previous study that found the percent of hepatic glycogen synthesized by the direct pathway during a glucose infusion was ~ 50% in normal subjects after an overnight fast and that this flux increased to ~ 70% when the glucose infusion was repeated 4 h after breakfast (3). Whether the defects in hepatic glycogen synthesis observed in the IDDM subjects after an overnight fast can be reversed by more aggressive treatment with insulin remains to be determined. Although it may be possible to normalize plasma glucose concentrations by giving more insulin, it is very difficult to normalize portal vein insulin concentrations by giving insulin peripherally without causing peripheral hyperinsulinemia, which has the potential for causing hypoglycemia (16, 17).

Defective hepatic glycogen synthesis and increased rates of gluconeogenesis have important clinical implications for patients with IDDM. First, because much of the glucose that is taken up by the liver after a meal is converted to hepatic glycogen (18), any decrease in net hepatic glycogen synthesis would be expected to exacerbate postprandial hyperglycemia. Second, normal glucose tolerance after a meal depends on normal suppression of hepatic glucose production (19). Because hepatic glycogenolysis has been shown in anesthetized dogs to be more sensitive to inhibition by insulin than gluconeogenesis, the increased activity of hepatic gluconeogenesis observed in the IDDM subjects would also be expected to contribute to postprandial hyperglycemia (20). Finally, these results show that IDDM subjects have relatively lower hepatic glycogen stores throughout most of the day and this deficiency becomes most prominent in the late evening. Because mobilization of hepatic glycogen accounts for most of the increase in hepatic glucose production that results from an increase in plasma epinephrine (21) or glucagon concentration (22, 23), reduced hepatic glycogen stores may be an important factor limiting the ability of the IDDM patient to respond to hypoglycemia. This may become especially evident during the late evening and early morning hours when hepatic glycogenolysis has a major role in maintaining normal glucose production and differences between hepatic glycogen content between the normal and IDDM subjects are at their greatest.

In summary, we examined net hepatic glycogen synthesis in both normal and IDDM subjects throughout a day in which three isocaloric mixed meals were ingested 5 h apart and found (*a*) in both normal and IDDM subjects net hepatic glycogenolysis contributed minimally to whole body glucose production during the waking day (8:00 a.m. to 11:00 p.m.) and became appreciable only during the evening hours, (*b*) in normal subjects approximately half of the glycogen that was synthesized after breakfast occurred by the direct pathway, and (*c*) poorly controlled IDDM subjects had augmented hepatic gluconeogenesis and impaired net hepatic glycogen synthesis such that by the end of the day they had synthesized less than one third the

amount of hepatic glycogen as the control subjects. Decreased hepatic glycogen reserves in the later part of the day may be an important factor contributing to impaired glycemic response to counterregulatory hormones in IDDM patients, whereas augmented gluconeogenesis may be an important predisposing factor to postprandial hyperglycemia. The degree to which these abnormalities can be reversed with more aggressive insulin therapy remains to be determined.

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