Effects of Short-Term Improvement of Insulin Treatment and Glycemia on Hepatic Glycogen Metabolism in Type 1 Diabetes

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Insufficiently treated type 1 diabetic patients exhibit inappropriate postprandial hyperglycemia and reduction in liver glycogen stores. To examine the effect of acute improvement of metabolic control on hepatic glycogen metabolism, lean young type 1 diabetic (HbA_{1c} 8.8 ± 0.3%) and matched nondiabetic subjects (HbA_{1c} 5.4 ± 0.1%) were studied during the course of a day with three isocaloric mixed meals. Hepatic glycogen concentrations were determined noninvasively using in vivo ^{13}C nuclear magnetic resonance spectroscopy. Rates of net glycogen synthesis and breakdown were calculated from linear regression of the glycogen concentration time curves from 7:30–10:30 P.M. and from 10:30 P.M. to 8:00 A.M., respectively. The mean plasma glucose concentration was ~2.4-fold higher in diabetic than in nondiabetic subjects (13.6 ± 0.4 vs. 5.8 ± 0.1 mmol/l, $P < 0.001$). Rates of net glycogen synthesis and net glycogen breakdown were reduced by ~74% (0.11 ± 0.02 vs. 0.43 ± 0.04 mmol/l liver/min, $P < 0.001$) and by ~47% (0.10 ± 0.01 vs. 0.19 ± 0.01 mmol/l liver/min, $P < 0.001$) in diabetic patients, respectively. During short-term (24-h) intensified insulin treatment, the mean plasma glucose level was not different between diabetic and nondiabetic subjects (6.4 ± 0.1 mmol/l). Net glycogen synthesis and breakdown increased by ~92% (0.23 ± 0.04 mmol/l liver/min, $P = 0.017$) and by ~40% (0.14 ± 0.01 mmol/l liver/min, $P = 0.011$), respectively. In conclusion, poorly controlled type 1 diabetic patients present with marked reduction in both hepatic glycogen synthesis and breakdown. Both defects in glycogen metabolism are improved but not normalized by short-term restoration of insulinemia and glycemia. *Diabetes* 50:392–398, 2001

After a mixed meal, glucose is removed by the liver from the portal vein and the systemic circulation, and it is temporarily stored as glycogen (1). During the subsequent fasting period, the liver accounts for at least 80% of endogenous glucose production (2–4), 45% of which in healthy individuals is contributed by glucose derived from glycogen breakdown (5). Poorly controlled type 1 diabetic patients exhibit impaired suppression of endogenous glucose production after a mixed meal (6) and reduction in hepatic glycogen accumulation, which becomes most prominent after dinner (7). After an overnight fast, endogenous glucose production is also elevated and correlates with the degree of fasting hyperglycemia (8–10). However, no information is currently available on rates of glycogen breakdown during fasting in type 1 diabetes. It is of note that hepatic glycogenolysis also accounts for at least ~85% of the initial increase in endogenous glucose production during hypoglycemia (11) or glucagon exposure (12,13). Reduction in hepatic glycogen stores may therefore impair the rapid response of glucose production to hypoglycemia in type 1 diabetes. This is of particular clinical relevance, because intensive insulin therapy aiming at tight glycemic control in order to prevent diabetes-associated long-term complications increases the prevalence of hypoglycemic episodes (14,15).

Insufficient insulin replacement, decreased portal insulin-to-glucagon ratios and/or hyperglycemia-induced insulin resistance may account for the abnormalities of hepatic glucose metabolism in type 1 diabetes. However, hyperinsulinemic clamp tests and intensive insulin treatment completely normalized endogenous glucose production in some (6,8,16) but not in all studies (10). Even intraportal hyperinsulinemia does not reverse all of the alterations in hepatic carbon fluxes (17). In addition, chronic near-normoglycemia alone was not sufficient to reduce endogenous glucose production, which was suppressed only by plasma insulin concentrations as high as ~6 nmol/l (18). It has been suggested that only a near-physiological mode of insulin delivery by intermittent infusion will reactivate liver metabolism and reduce both HbA_{1c} levels and the frequency of hypoglycemic events in patients with brittle diabetes (19). Nevertheless, it is unknown at present whether or not improvement of both insulinemia and glycemia reverses the defects in hepatic glycogen metabolism in type 1 diabetes under physiological conditions of mixed meal ingestion.

Therefore, this study was designed with the following intentions: *1*) to determine rates of net hepatic glycogen synthesis after ingestion of a standardized mixed meal dinner, *2*) to determine rates of net hepatic glycogen breakdown during nighttime in type 1 diabetic and nondiabetic subjects, and *3*) to test the hypothesis that hepatic glycogen deposition can be completely restored to normal levels by short-term improvement of insulin treatment and glycemia for 24 h using

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FFA, free fatty acid; NMR, nuclear magnetic resonance.

variable intravenous insulin infusion. We applied ^{13}C nuclear magnetic resonance (NMR) spectroscopy to noninvasively follow the time course of hepatic glycogen concentrations in vivo (20). This method enables quantification of both net glycogen synthesis and breakdown during mixed meal ingestion (1,7,21) and avoids limitations of other experimental approaches such as liver biopsies (22), hepatic venous cannulation (23), or isotopic dilution methodology (6,24).

RESEARCH DESIGN AND METHODS

Type 1 diabetic patients (six men and one woman, age 30 ± 4 years, BMI 25.0 ± 0.9 kg/m^2, diabetes duration 9.6 ± 4.1 years, HbA_{1c} 8.8 ± 0.3%, mean insulin dosage 40 ± 4 U/day) were administered either a conventional insulin regimen consisting of two subcutaneous injections of a mixed insulin or multiple insulin injections. They had no history of hypoglycemic episodes for at least 1 week before the study. Individuals presenting diabetes-related complications were excluded from participation in this study. Nondiabetic subjects matched for age and body weight (six men and one woman, age 29 ± 2 years, BMI 24.6 ± 0.6 kg/m^2, HbA_{1c} 5.4 ± 0.1%) served as a control group. None had a family history of diabetes. Informed consent was obtained from all of the subjects after the nature and possible consequences of the study were explained to them. The protocol was reviewed and approved by the Human Ethics Committee of the University of Vienna Medical School.

Experimental protocol. All subjects were advised to ingest a carbohydrate-rich weight-maintaining diet and to refrain from strenuous physical exercise for at least 3 days before the study. Diabetic patients were instructed to omit NPH or Zn insulin and to correct plasma glucose concentrations with regular insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) for 24 h before admission. Nondiabetic subjects were studied once, whereas type 1 diabetic patients were studied twice: once during current metabolic control with correction of plasma glucose by subcutaneous insulin injection (protocol 1) and once during improved glycemic control by variable intravenous insulin infusion (protocol 2).

On the first study day, nondiabetic and diabetic subjects in protocol 1 were admitted to the clinical research facility at ~7:00 A.M. Teflon catheters were inserted in antecubital veins of the left arm for blood sampling. Three standard mixed meals (60% carbohydrate, 20% protein, and 20% fat) were served at 8:00 A.M. (720 kcal solid), 12:30 P.M. (710 kcal solid), and 5:00 P.M. (800 kcal liquid meal). During protocol 1, diabetic patients received subcutaneous insulin injections before the meals to avoid excessive increases of plasma glucose concentrations >22.2 mmol/l. Plasma glucose concentrations were measured every 15 min between 7:30 A.M. and midnight and, when appropriate, during nighttime.

During protocol 2, diabetic patients were admitted to the clinical research facility at ~12:00 A.M. on the day before the study. Teflon catheters were inserted in antecubital veins of the right and left arm for blood sampling and for insulin infusion, respectively. Insulin was administered as a variable intravenous infusion (0.2 to 1.5 mU · kg^{-1} · min^{-1}) to slowly lower the plasma glucose level and keep it between 5.56–6.67 mmol/l. On the next day, three standard mixed meals were served as described above. To achieve plasma glucose concentrations obtained in control subjects, insulin infusion rates were frequently adjusted based on the actual plasma glucose concentration and increased accordingly before ingestion of each meal. Plasma glucose concentrations were measured every 15 min during protocol 2.

In all studies, blood samples were taken every 2 h to allow determination of plasma concentrations of free insulin, C-peptide, glucagon, and free fatty acids (FFAs).

Analytical methods. Plasma glucose concentrations were measured using the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Fullerton, CA). HbA_{1c} was quantified by using high-performance liquid chromatography (Bio-Rad, Richmond, CA). Plasma concentrations of FFAs were determined by using a colorimetric assay (intra- and interassay CVs 4.3 and 5.7%) (Wako, Neuss, Germany). Free insulin (CVs ≤ 8%) (Pharmacia-Upjohn, Uppsala, Sweden), C-peptide (CVs ≤ 9%) (CIS, Gif-Sur-Yvette, France), and glucagon (CVs ≤ 8%) (Biochem, Freiburg, Germany) were determined by radioimmunoassay.

^{13}C NMR spectroscopy. After dinner, liver glycogen concentrations were determined in all subjects from 7:30 P.M. until the plateau was reached (~10:30 P.M.) and from 7:00 A.M. to 8:00 A.M. on the morning of the next day. These time periods were chosen because the difference in net hepatic glycogen accumulation between nondiabetic and diabetic subjects becomes maximal after dinner (7). In three subjects, the measurements were performed except for short breaks from 7:30 P.M. until 8:00 A.M. of the next morning to confirm linear decrease of liver glycogen concentrations between 10:30 P.M. and 8:00 A.M. (5).

In vivo ^{13}C NMR spectroscopy was performed on the 3 T Medspec 30/80-DBX system (Bruker Medical, Ettlingen, Germany) installed at the General Hospital of Vienna, Austria. A double-tuned ^1H (125.6 MHz) and ^{13}C (31.5 MHz) 10-cm circular coil was used for data collection. Subjects were lying in the supine position in the magnet with the coil positioned rigidly over the lateral aspect of the liver. The liver borders were determined by percussion, and the correct position of the coil was confirmed with a multislice gradient echoimage. Magnetic field homogeneity was optimized on the water signal to a line width of 60–80 Hz. Spectra were acquired using a modified 1D-ISIS sequence (20) without proton decoupling (pulse length 150 μs/135° in the coil plane, time of resonance 150 ms, acquisition time 25.6 ms, number of scans 5,000, total scan time 13 min). Spectra were zero filled to 4k, gaussian and exponentially filtered, and phase-corrected manually. Hepatic glycogen was quantified by integration of the C1 glycogen doublet at 100.5 ppm using the same frequency bandwidth for all spectra (± 300 Hz). Absolute quantification of the hepatic glycogen concentration was obtained by comparing the peak integral with that of a glycogen standard solution obtained under identical conditions. Corrections for loading and sensitive volume of the coil were performed.

Because hepatic glycogen synthase and phosphorylase are simultaneously active in humans, ^{13}C NMR spectroscopy does not measure total changes but does measure net changes in liver glycogen concentrations so that rates of net glycogen synthesis and net glycogenolysis can be assessed. Individual rates of net glycogen synthesis and net glycogen breakdown were calculated from linear regression of the net glycogen concentration-time curves between 7:30 and 10:30 P.M. and from 10:30 P.M. to 8:00 A.M., respectively.

Determination of liver volume. In all subjects, liver volumes were measured using magnetic resonance imaging in a 1.5 T Vision imager (Siemens, Munich, Germany) using a body array coil and inphase and postphase multislice FLASH imaging sequences. Slice number and position were chosen to cover the whole organ. Liver tissue was manually segmented, and the area of each region of interest was determined in each slice. Areas were added and multiplied by the sum of slice thickness (0.8 mm) and interslice distance (8 mm). Liver volumes were determined 2 h after dinner on the first study day.

Calculations and statistics. Data are means ± SE. One-way analysis of variance with Bartlett's test for equal variances and post hoc testing by the Newman-Keuls test was used for statistical comparisons between and within the different groups. In addition, data of type 1 diabetic patients before and during short-term intensified insulin treatment were compared using the paired Student's t test. Statistical significance was considered at $P < 0.05$. All calculations were performed using the Sigma Stat software package (Jandel, San Rafael, CA).

RESULTS

Plasma glucose. Fasting plasma glucose concentrations were approximately two times higher in poorly controlled type 1 diabetic than in nondiabetic subjects (11.55 ± 1.05 vs. 5.44 ± 0.12 mmol/l, poorly controlled diabetic subjects vs. control subjects, respectively; $P < 0.05$) (Fig. 1*A*). Overnight intravenous insulin infusion normalized fasting plasma glucose concentrations in the diabetic subjects (6.21 ± 0.18 mmol/l in insulin-infused diabetic subjects; NS vs. control subjects). During the course of the study, the mean plasma glucose concentration (7:30 A.M. to 8:00 A.M. of the next day) was markedly higher in poorly controlled diabetic subjects than in control subjects (13.61 ± 0.38 mmol/l in poorly controlled diabetic subjects vs. 5.76 ± 0.08 mmol/l in control subjects, $P < 0.001$), but was not different from control subjects during short-term intensified insulin treatment (6.39 ± 0.14 mmol/l in insulin-infused diabetic subjects; NS vs. control subjects; $P < 0.001$ vs. poorly controlled diabetic subjects). Similarly, the daytime mean plasma glucose concentration (8:00 A.M. to 7:00 P.M.) was higher in poorly controlled diabetic subjects than in control subjects (13.60 ± 0.40 vs. 5.79 ± 0.09 mmol/l, respectively; $P < 0.001$) and normalized during short-term intensified insulin treatment (6.34 ± 0.15 mmol/l in insulin-infused diabetic subjects; NS vs. control subjects). The mean plasma glucose concentration during nighttime (7:30 P.M. to 8:00 A.M.) was also higher in diabetic subjects than in control subjects (13.14 ± 1.29 vs. 5.40 ± 0.04 mmol/l, respectively; $P < 0.001$). During insulin infusion, the nighttime mean plasma glucose concentration markedly

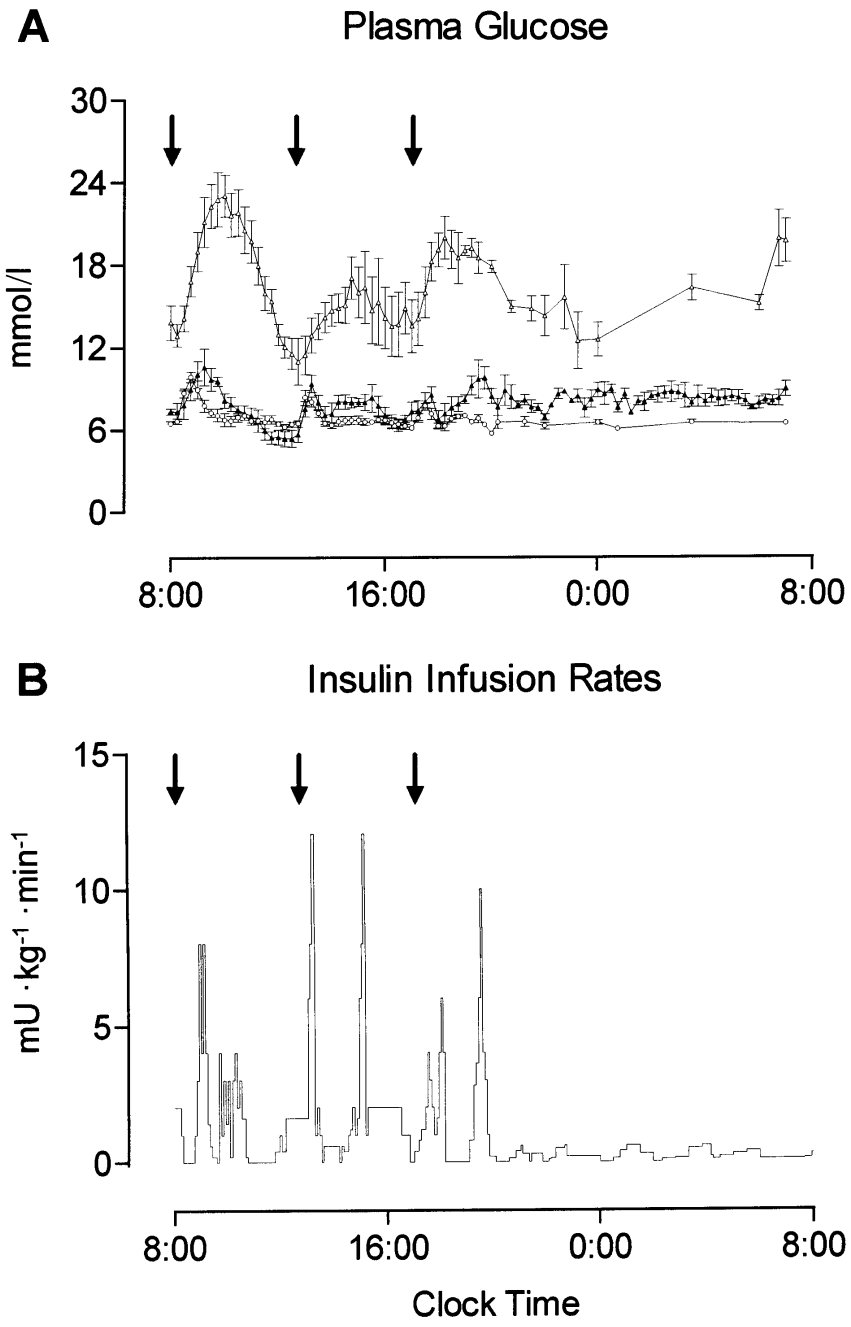

FIG. 1. *A*: **Time course of plasma glucose concentrations in nondiabetic control subjects (○) (n = 7) and poorly controlled type 1 diabetic subjects (n = 7) without intravenous insulin treatment and improved glycemic control (△) or with short-term (24-h) intensified intravenous insulin treatment and improved glycemic control (▲). During the study day, all subjects ingested three standard mixed meals (60% carbohydrate, 20% protein, and 20% fat) at 8:00 A.M. (720 kcal solid meal), 12:30 P.M. (710 kcal solid meal), and 5:00 P.M. (800 kcal liquid meal). Arrows indicate meals. Data are means ± SE.** *B*: **Rates of variable intravenous insulin infusion during short-term intensified insulin treatment of one type 1 diabetic patient (protocol 2, insulin-infused diabetic subject).**

improved (6.82 ± 0.31 mmol/l in insulin-infused diabetic subjects; $P < 0.001$ vs. poorly controlled diabetic subjects) and was not significantly different from glycemia in nondiabetic subjects. Peak individual rates of insulin infusion required to maintain such plasma glucose concentrations ranged from 5–12 $mU \cdot kg^{-1} \cdot min^{-1}$ and were adjusted in time periods as low as 5 min. Figure 1*B* depicts the time course of insulin infusion rates during short-term intensified insulin treatment in one representative diabetic subject.

Plasma C-peptide, insulin, glucagon, and FFAs. Fasting plasma C-peptide concentrations were markedly lower in diabetic subjects (0.32 ± 0.06 ng/ml in poorly controlled diabetic subjects vs. 0.26 ± 0.01 ng/ml in insulin-infused diabetic subjects, NS) than in nondiabetic subjects (1.61 ± 0.18 ng/ml; $P <$ 0.001 vs. poorly controlled diabetic subjects and insulin-infused diabetic subjects). Whereas plasma C-peptide concentrations were below the detection limit (0.25 ng/ml) in five diabetic patients, two patients exhibited detectable

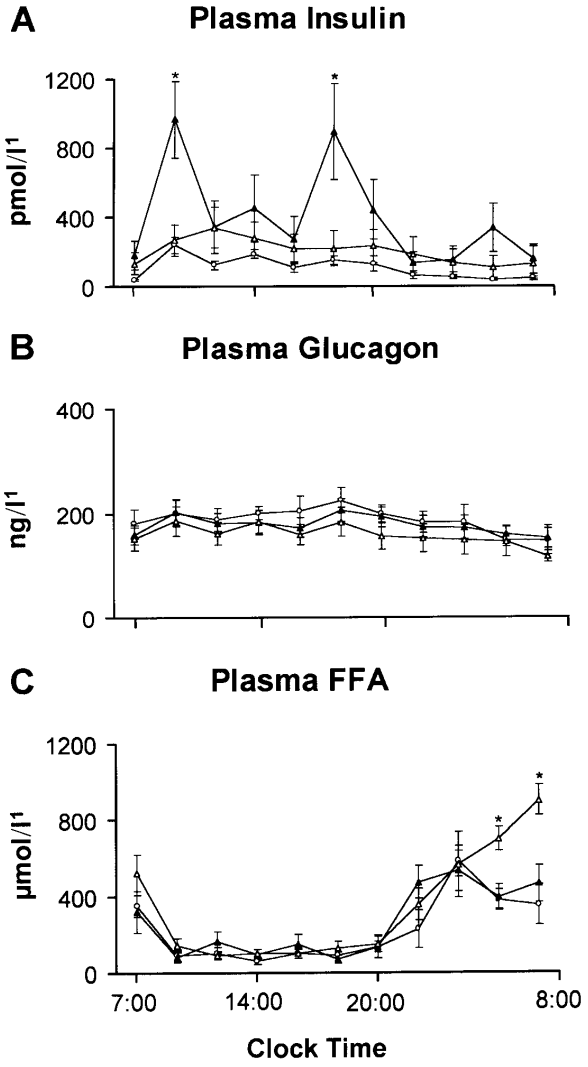

FIG. 2. Plasma concentrations of insulin (*A*), glucagon (*B*), and FFAs (*C*) in nondiabetic control subjects (○; *n* = 7) and poorly controlled type 1 diabetic subjects (*n* = 7) without intravenous insulin treatment and improved glycemic control (△) or with short-term (24-h) intensified intravenous insulin treatment and improved glycemic control (▲). Data are means ± SE.

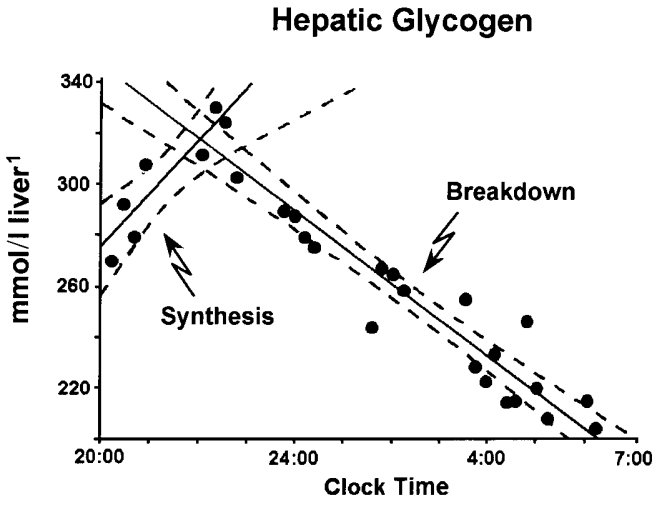

FIG. 3. Time course of hepatic glycogen concentrations in one nondiabetic control subject after ingestion of a 800-kcal liquid meal at 5:00 P.M. Rates of net glycogen synthesis and breakdown are represented by the slopes of lines determined from linear regression of the glycogen concentration time curves from 7:30–10:30 P.M. and from 10:30 P.M. to 8:00 A.M., respectively.

plasma C-peptide levels (0.57 and 0.54 ng/ml). Plasma C-peptide did not change from fasting levels in the diabetic subjects, but increased ($P < 0.0001$) in the control subjects. Following correction of glycemia by subcutaneous or intravenous insulin before 8:00 A.M., mean fasting plasma insulin was slightly, but not significantly, higher in diabetic subjects (134 ± 62 pmol/l in poorly controlled diabetic subjects and 180 ± 84 pmol/l in insulin-infused diabetic subjects) than in control subjects (40 ± 6 pmol/l) (Fig. 2*A*). During the study day, mean plasma insulin concentration was higher in diabetic patients during intensive insulin treatment (396 ± 87 pmol/l in insulin-infused diabetic subjects) than during poor metabolic control (205 ± 22 pmol/l in poorly controlled diabetic subjects, $P < 0.05$) and increased compared with nondiabetic control subjects (109 ± 20 pmol/l, $P < 0.01$). Plasma glucagon concentrations were not different in the fasting state (160 ± 24, 156 ± 21, and 181 ± 28 pg/ml in poorly controlled diabetic patients, insulin-infused diabetic patients, and control subjects, respectively) and throughout the studies (165 ± 7, 177 ± 5, and 187 ± 7 pg/ml in poorly controlled diabetic patients, insulin-infused diabetic patients, and control subjects, respectively) (Fig. 2*B*). Fasting plasma FFA concentrations were slightly higher in diabetic patients during poor metabolic control (524 ± 97 µmol/l in the poorly controlled diabetic subjects) than during intensified insulin treatment (319 ± 38 µmol/l in the insulin-infused diabetic subjects, NS) and control studies (353 ± 56 µmol/l, NS) (Fig. 2*C*). After 10:00 P.M., plasma FFA increased further only in diabetic patients during protocol 1 (698 ± 64 µmol/l in poorly controlled diabetic patients), ~1.8-fold higher than during protocol 2 (396 ± 66 µmol/l in insulin-infused diabetic patients; $P < 0.025$) and during control studies (380 ± 56 µmol/l; $P < 0.01$) performed at 3:30 A.M.

Hepatic glycogen synthesis and breakdown. Liver glycogen concentrations linearly increased from 8:00 A.M. to 11:00 P.M. Figure 3 illustrates the time course of liver glycogen concentrations and determination of rates of net glycogen synthesis by a line-fitting procedure in one nondiabetic subject. After dinner, hepatic glycogen concentrations were ~29% lower ($P < 0.001$) in poorly controlled diabetic subjects than in nondiabetic subjects (Table 1). Rates of net glycogen synthesis were ~74% lower ($P < 0.001$) in diabetic patients (Fig. 4*A*). Short-term improvement of metabolic control resulted in an increase of ~23% ($P < 0.01$) of maximal liver glycogen contents (Table 1), which was still reduced ($P < 0.05$) compared with control studies. Similarly, net glycogen synthesis doubled ($P = 0.017$ vs. poorly controlled diabetic subjects), but was ~52% lower ($P < 0.001$) than in nondiabetic subjects.

During overnight fasting, liver glycogen linearly decreased from 11:00 P.M. to 8:00 A.M. (Fig. 3). The maximum decrement was lower in poorly controlled diabetic subjects than in nondiabetic subjects (–58 ± 9 mmol/l liver vs. –113 ± 13 mmol/l liver; $P < 0.001$) and gradually increased during tight metabolic control (–82 ± 8 mmol/l liver in the insulin-infused diabetic subjects; $P < 0.05$ vs. poorly controlled diabetic subjects; $P < 0.01$ vs. control subjects). Rates of net glycogen breakdown were reduced by ~47% in poorly controlled diabetic

TABLE 1
Hepatic glycogen concentrations (in mmol/l liver) after ingestion of an 800-kcal liquid meal at 5:00 P.M. in seven nondiabetic and seven poorly controlled type 1 diabetic subjects without or with short-term intensified intravenous insulin treatment

	+3 h	*P*	+ 5.5 h	*P*	+14 h	*P*
Control subjects	273 ± 10		326 ± 10*		212 ± 12†	
Diabetic subjects						
Without insulin infusion	221 ± 10	<0.05 vs. CON	233 ± 9	<0.001 vs. CON	175 ± 9†	NS
With insulin infusion	257 ± 14	<0.05 vs. DM	287 ± 12‡	<0.01 vs. DM <0.05 vs. CON	205 ± 13†	NS

Data are means ± SE. *P < 0.001 vs. +3 h; †P < 0.001 vs. +3 h; ‡P < 0.01 vs. +3 h. DM, diabetic subjects without insulin infusion; CON, control subjects.

patients (P < 0.001 vs. control subjects), but improved during protocol 2 (P = 0.011 vs. insulin-infused and P < 0.05 vs. control subjects) (Fig. 4*B*). The following morning, hepatic glycogen had decreased (P < 0.001) in all protocols, with no differences among them (Table 1).

Mean liver volume was not different between diabetic and nondiabetic subjects (1,627 ± 102 cm^3 in the poorly controlled diabetic subjects vs. 1,502 ± 43 cm^3 in the control subjects, unpaired Student's t test: NS).

DISCUSSION

These results demonstrate a marked defect of net hepatic glycogen accumulation in poorly controlled type 1 diabetes. Although older studies using the biopsy technique reported increased glycogen deposition in such patients (25,26), the observed reduction by ~29% in peak liver glycogen content is in accordance with the only other study performed in type 1 diabetic patients under near-physiological conditions of mixed meal ingestion (7).

The glycemic profiles of poorly controlled type 1 diabetic patients exhibited a prolonged inappropriate increase of plasma glucose, particularly after breakfast. Most likely, this results from peripheral insulin resistance due to the diurnal secretion pattern of growth hormone (7,27) and is further supported by the observed nocturnal rise of plasma FFA, which strongly correlates with peripheral insulin resistance (28,29) and per se reduces skeletal muscle glucose uptake and glycogen synthesis (30,31). Although mean peripheral plasma insulin and glucagon concentrations were comparable with that of nondiabetic humans, portal hypoinsulinemia and a decreased insulin-to-glucagon ratio may account for the defect in postprandial glycogen accumulation. Net hepatic glycogen synthesis, as measured in this study, is the result of simultaneously active fluxes through glycogen synthase and phosphorylase (32), which are differentially regulated by glucose, insulin, and glucagon under in vivo conditions (33,34). Rates of net glycogen synthesis of nondiabetic subjects were in agreement with previous reports using either liver biopsies (35) or ^{13}C NMR spectroscopy (1,7,21). In the poorly controlled diabetic subjects, the prevailing hyperglycemia will primarily inhibit glycogen phosphorylase flux, while hypoinsulinemia and relative hyperglucagonemia in the portal vein may stimulate glycogen cycling (33,34). Furthermore, even short periods of hyperglycemia are sufficient to reduce nonoxidative glucose disposal (36). In addition to the effects of acute endocrine and metabolic changes, chronic alterations in the activities of hepatic glucoregulatory enzymes, such as glucokinase (37), may contribute to reduced glycogen synthesis. Such a defect was recently suggested in type 2 diabetic subjects, because both UDP-glucose flux and percent contribution of extracellular glucose to hepatic glycogen synthesis were reduced (38). The finding that glucokinase-deficient type 2 maturity-onset diabetes of young (MODY-2) patients exhibit only ~12% lower peak liver glycogen concentrations after dinner as compared with healthy subjects (21) also indicates that other enzymes, such as glycogen synthase (39,40), might add to defective glycogen accumulation in poorly controlled type 1 diabetes.

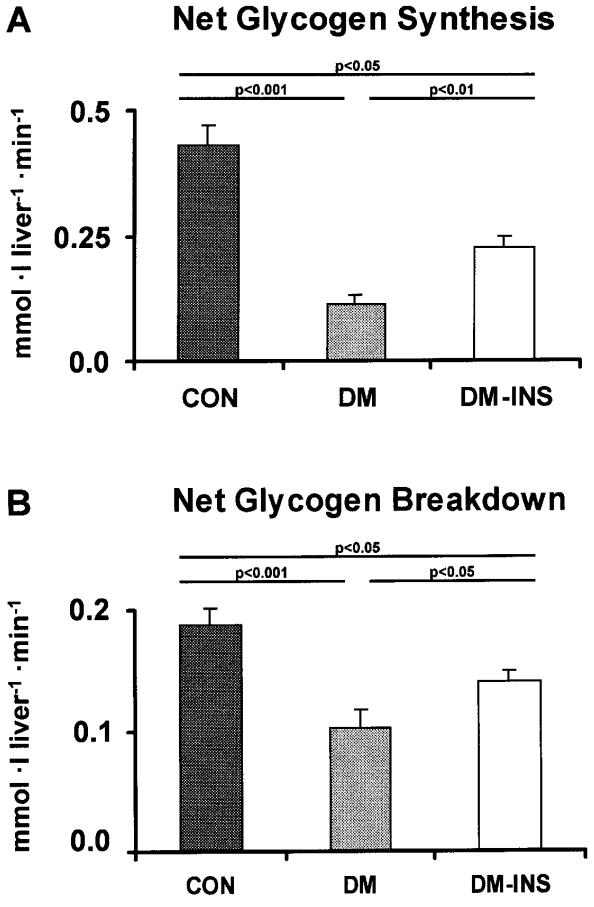

FIG. 4. Rates of net glycogen synthesis (*A*) and net glycogen breakdown (*B*) in livers of nondiabetic control subjects (n = 7) and poorly controlled type 1 diabetic subjects (n = 7) without or with short-term (24-h) intensified intravenous insulin treatment and improved glycemic control. Rates of hepatic glycogen synthesis and breakdown were calculated from linear regression of the glycogen concentration-time curves obtained after ingestion of a 800-kcal liquid meal. Data are means ± SE.

In addition, this study followed the time course of liver glycogen breakdown during overnight fasting. The decrease of intrahepatic glycogen concentrations was linear and allowed us to calculate rates of net glycogen breakdown (1,5,20). Corrections for changes in liver volume were not performed, because previous studies found no (5) or only minor loss (20) of liver volumes in subjects fasted for ≤64 h. Such decreases in liver size will result in changes of glycogen concentrations that are below the detection limit of the applied NMR technique. To the extent that simultaneous glycogen synthesis occurs, net glycogen breakdown will underestimate the flux through glycogen phosphorylase. Rates of glycogen breakdown in nondiabetic subjects (~0.19 mmol/l liver/min) were identical to previous reports (1,41), whereas no data were available for type 1 diabetes. The observed decrease of rates of net glycogen breakdown in poorly controlled type 1 diabetic patients may result from hyperglycemia that inhibits glycogen phosphorylase (34) and/or relative portal vein hyperglucagonemia (33). In addition, as individual net glycogenolytic rates are correlated with the initial hepatic glycogen concentration (5), the lower maximal postprandial liver glycogen concentrations may have contributed to reduced glycogen breakdown. Finally, the defects in liver glycogen metabolism could partly be because of the action of epinephrine, cortisol, and growth hormone, which stimulate splanchnic glucose output after glucose ingestion in humans (42,43) and worsen hyperglycemia in insulin-deficient dogs (44) and in human type 1 diabetes (45).

During hyperglycemic-hyperinsulinemic clamp tests, hepatic glycogen synthesis was normalized in 90%-pancreatectomized rats (46), as well as in five poorly controlled type 1 diabetic subjects (17). However, supraphysiological plasma concentrations of glucose (~17 and 9 mmol/l) and insulin (~3,300 and 400 pmol/l) were required to achieve this effect. Moreover, administration of somatostatin without concomitant glucagon infusion will have caused portal hypoglucagonemia in those type 1 diabetic patients (17), which favors net hepatic glycogen synthesis (33). In the present study, improvement of insulin replacement by variable intravenous insulin infusion resulted in glycemic profiles close to that of nondiabetic subjects. Under these conditions, more likely reflecting the aim of improved metabolic control in clinical practice, net glycogen accumulation of type 1 diabetic patients doubled, but was still half of that in control subjects. The failure to normalize glycogen synthesis could result, in part, from the decrease of ~57% of plasma glucose during short-term improvement of metabolic control. It is conceivable that hyperglycemia per se may compensate for defective glycogen synthesis in the poorly controlled diabetic patients. This could be because of glucose-induced glycogen synthesis following a portal glucose load (47) and/or because of reduction of simultaneous ongoing glycogenolysis (34). Alternatively, hyperglycemia may increase glycogen repletion by the indirect (gluconeogenic) pathway in diabetic rats (46) and humans who also exhibit reduced hepatic pyruvate oxidation (17).

One might speculate that infusion of insulin into a peripheral vein instead of physiological insulin delivery might be responsible for the difference between nondiabetic and type 1 diabetic subjects, despite their acutely improved metabolic control. During insulin infusion, mean plasma insulin concentration in the peripheral vein doubled and was ~190 pmol/l higher in the diabetic patients. Thus, portal vein insulin concentrations were likely to be similar in both groups (48). The observed increase in systemic insulin will decrease hepatic and renal gluconeogenesis, as well as lipolysis, which is mirrored by the observed decrease of plasma FFA concentrations. Consequently, endogenous glucose production should fall, net hepatic glucose output should approach zero (49), and glycogen synthesis should be maximal. It is of note that the efficacy of insulin to inhibit hepatic glucose production also depends on its pulsatile secretion pattern (50). However, in the present study, frequent adjustments of the insulin infusion at intervals as low as 5 min and peak rates of up to 12 mU · kg^{-1} · min^{-1} should have provided for near-physiological conditions (19,51). On the other hand, given comparable glycemic profiles in the absence of glucosuria, whole-body glucose disposal has to be similar in well- controlled diabetic and nondiabetic subjects. Therefore, in the well-controlled diabetic patients, the higher peripheral insulin concentration could have increased muscular glucose uptake, leaving less glucose available for hepatic glycogen formation.

Nevertheless, insufficient duration of normalized glycemia and insulinemia may account for the failure to completely restore glycogen synthesis and breakdown in our type 1 diabetic patients. Near-normalization of glycemia by intensive subcutaneous insulin therapy for ≥2 weeks was required to decrease tracer-determined glucose production to the normal range in the fasted (6,16,18) and in the postprandial state (6). This time period might be necessary to overcome hepatic insulin resistance, which is a common feature of insulin-dependent diabetes, despite physiological daily insulin requirements (8,52), as observed in our patients, whose nocturnal suppression of plasma FFA albeit indicates normal insulin sensitivity.

In conclusion, poorly controlled type 1 diabetic subjects present with *1*) markedly impaired hepatic glycogen synthesis after a mixed meal dinner, *2*) reduced glycogen breakdown during overnight fasting, and *3*) improved, but not normalized, glycogen synthesis and breakdown during refined insulinemia and glycemia. Postprandial enlargement of hepatic glycogen storage can provide more glucose for the immediate increase in endogenous glucose production during hypoglycemia counterregulation. Therefore, despite potentially increased frequency of hypoglycemia (14,15), intensive insulin therapy might rather serve to protect against severe hypoglycemic episodes.

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REFERENCES

1. Taylor R, Magnusson I, Rothman DL, Cline GW, Caumo A, Cobelli C, Shulman GI: Direct assessment of liver glycogen storage by 13C nuclear magnetic res-

onance spectroscopy and regulation of glucose homeostasis after a mixed meal in normal subjects. *J Clin Invest* 97:126–132, 1996
2. Cersosimo E, Judd RL, Miles JM: Insulin regulation of renal glucose metabolism in conscious dogs. *J Clin Invest* 93:2584–2589, 1994
3. Stumvoll M, Chintalapudi U, Perriello G, Welle S, Gutierrez O, Gerich J: Uptake and release of glucose by the human kidney: postabsorptive rates and responses to epinephrine. *J Clin Invest* 96:2528–2533, 1995
4. Ekberg K, Landau BR, Wajngot A, Chandramouli V, Efendic S, Brunengraber H, Wahren J: Contributions by kidney and liver to glucose production in the postabsorptive state and after 60 h of fasting. *Diabetes* 48:292–298, 1999
5. Petersen KF, Price T, Cline GW, Rothman DL, Shulman GI: Contribution of net hepatic glycogenolysis to glucose production during the early postprandial period. *Am J Physiol* 270:E186–E191, 1996
6. Pehling G, Tessari P, Gerich JE, Haymond MW, Service FJ, Rizza RA: Abnormal meal carbohydrate disposition in insulin-dependent diabetes: relative contributions of endogenous glucose production and initial splanchnic uptake and effect of intensive insulin therapy. *J Clin Invest* 74:985–991, 1984
7. Hwang JH, Perseghin G, Rothman DL, Cline GW, Magnusson I, Petersen KF, Shulman GI: Impaired net hepatic glycogen synthesis in insulin-dependent diabetic subjects during mixed meal ingestion: a ^{13}C nuclear magnetic resonance spectroscopy study. *J Clin Invest* 95:783–787, 1995
8. DeFronzo RA, Hendler R, Simonson D: Insulin resistance is a prominent feature of insulin-dependent diabetes. *Diabetes* 31:795–801, 1982
9. DeFronzo RA, Simonson D, Ferrannini E: Hepatic and peripheral insulin resistance: a common feature of type 2 (noninsulin-dependent) and type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 23:313–319, 1982
10. Yki Jarvinen H, Koivisto VA: Continuous subcutaneous insulin infusion therapy decreases insulin resistance in type 1 diabetes. *J Clin Endocrinol Metab* 58:659–666, 1984
11. Lecavalier L, Bolli G, Cryer P, Gerich J: Contributions of gluconeogenesis and glycogenolysis during glucose counterregulation in normal humans. *Am J Physiol* 256:E844–E851, 1989
12. Magnusson I, Rothman DL, Gerard DP, Katz LD, Shulman GI: Contribution of hepatic glycogenolysis to glucose production in humans in response to a physiological increase in plasma glucagon concentration. *Diabetes* 44:185–189, 1995
13. Cherrington AD, Williams PE, Shulman GI, Lacy WW: Differential time course of glucagon's effect on glycogenolysis and gluconeogenesis in the conscious dog. *Diabetes* 30:180–187, 1981
14. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
15. Bolli GB: How to ameliorate the problem of hypoglycemia in intensive as well as nonintensive treatment of type 1 diabetes. *Diabetes Care* 22 (Suppl. 2):B43–B52, 1999
16. Hother-Nielsen O, Schmitz O, Bak J, Beck Nielsen H: Enhanced hepatic insulin sensitivity, but peripheral insulin resistance in patients with type 1 (insulin-dependent) diabetes. *Diabetologia* 30:834–840, 1987
17. Cline GW, Rothman DL, Magnusson I, Katz LD, Shulman GI: 13C-nuclear magnetic resonance spectroscopy studies of hepatic glucose metabolism in normal subjects and subjects with insulin-dependent diabetes mellitus. *J Clin Invest* 94:2369–2376, 1994
18. Fasching P, Ratheiser K, Damjancic P, Schneider B, Nowotny P, Vierhapper H, Waldhäusl W: Both acute and chronic near-normoglycaemia are required to improve insulin resistance in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 36:346–351, 1993
19. Aoki TT, Benbarka MM, Okimura MC, Arcangeli MA, Walter RM Jr, Wilson LD, Truong MP, Barber AR, Kumagai LF: Long-term intermittent intravenous insulin therapy and type 1 diabetes mellitus. *Lancet* 342:515–518, 1993
20. Rothman DL, Magnusson I, Katz LD, Shulman RG, Shulman GI: Quantitation of hepatic glycogenolysis and gluconeogenesis in fasting humans with ^{13}C NMR. *Science* 254:573–576, 1991
21. Velho G, Petersen KF, Perseghin G, Hwang JH, Rothman DL, Pueyo ME, Cline GW, Froguel P, Shulman GI: Impaired hepatic glycogen synthesis in glucokinase-deficient (MODY-2) subjects. *J Clin Invest* 98:1755–1761, 1996
22. Nilsson LH, Hultman E: Liver glycogen in man: the effect of total starvation or a carbohydrate-poor diet followed by carbohydrate refeeding. *Scand J Clin Lab Invest* 32:325–330, 1973
23. Katz LD, Glickman MG, Rapoport S, Ferrannini E, DeFronzo RA: Splanchnic and peripheral disposal of oral glucose in man. *Diabetes* 32:675–679, 1983
24. Jackson RA, Roshania RD, Hawa MI, Sim BM, DiSilvio L: Impact of glucose ingestion on hepatic and peripheral glucose metabolism in man: an analysis based on simultaneous use of the forearm and double isotope techniques. *J Clin Endocrinol Metab* 63:541–549, 1986
25. Vaishnava H, Raju TR, Malik GB, Gulati PD: Hepatic glycogen studies in Indian diabetics. *Metabolism* 20:657–665, 1971
26. Manderson WG, McKiddie MT, Manners DJ, Stark JR: Liver glycogen accumulation in unstable diabetes. *Diabetes* 17:13–16, 1968
27. Cryer PE, Binder C, Bolli GB, Cherrington AD, Gale EA, Gerich JE, Sherwin RS: Hypoglycemia in IDDM. *Diabetes* 38:1193–1199, 1989
28. Perseghin G, Ghosh S, Gerow K, Shulman GI: Metabolic defects in lean nondiabetic offspring of NIDDM parents: a cross-sectional study. *Diabetes* 46:1001–1009, 1997
29. Jensen MD, Caruso M, Heiling V, Miles JM: Insulin regulation of lipolysis in nondiabetic and IDDM subjects. *Diabetes* 38:1595–1601, 1989
30. Roden M, Krssak M, Stingl H, Gruber S, Hofer A, Fürnsinn C, Moser E, Waldhäusl W: Rapid impairment of skeletal muscle glucose transport/phosphorylation by free fatty acids in humans. *Diabetes* 48:358–364, 1999
31. Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, Shulman GI: Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest* 97:2859–2865, 1996
32. Magnusson I, Rothman DL, Jucker B, Cline GW, Shulman RG, Shulman GI: Liver glycogen turnover in fed and fasted humans. *Am J Physiol* 266:E796–E803, 1994
33. Roden M, Perseghin G, Petersen KF, Hwang JH, Cline GW, Gerow K, Rothman DL, Shulman GI: The roles of insulin and glucagon in the regulation of hepatic glycogen synthesis and turnover in humans. *J Clin Invest* 97:642–648, 1996
34. Petersen KF, Laurent D, Rothman DL, Cline GW, Shulman GI: Mechanism by which glucose and insulin inhibit net hepatic glycogenolysis in humans. *J Clin Invest* 101:1203–1209, 1998
35. Nilsson LH, Hultman E: Liver and muscle glycogen in man after glucose and fructose infusion. *Scand J Clin Lab Invest* 33:5–10, 1974
36. Rossetti L, Giaccari A, DeFronzo RA: Glucose toxicity. *Diabetes Care* 13:610–630, 1990
37. Barzilai N, Rossetti L: Role of glucokinase and glucose-6-phosphatase in the acute and chronic regulation of hepatic glucose fluxes by insulin. *J Biol Chem* 268:25019–25025, 1993
38. Basu A, Basu R, Shah P, Vella A, Johnson CM, Nair KS, Jensen MD, Schwenk WF, Rizza RA: Effects of type 2 diabetes in the ability of insulin and glucose to regulate splanchnic and muscle glucose metabolism: evidence for a defect in hepatic glucokinase activity. *Diabetes* 49:272–283, 2000
39. Villar Palasi C, Guinovart JJ: The role of glucose 6-phosphate in the control of glycogen synthase. *FASEB J* 11:544–558, 1997
40. Pugazhenthi S, Khandelwal RL: Regulation of glycogen synthase activation in isolated hepatocytes. *Mol Cell Biochem* 149–150:95–101, 1995
41. Petersen KF, Krssak M, Navarro V, Chandramouli V, Hundal R, Schumann WC, Landau BR, Shulman GI: Contributions of net hepatic glycogenolysis and gluconeogenesis to glucose production in cirrhosis. *Am J Physiol* 276:E529–E535, 1999
42. Waldhäusl WK, Gasic S, Bratusch-Marrain P, Komjati M, Korn A: Effect of stress hormones on splanchnic substrate and insulin disposal after glucose ingestion in healthy humans. *Diabetes* 36:127–135, 1987
43. Bratusch Marrain PR, Gasic S, Waldhäusl WK, Nowotny P: The effect of growth hormone on splanchnic glucose and substrate metabolism following oral glucose loading in healthy man. *Diabetes* 33:19–25, 1984
44. Goldstein RE, Abumrad NN, Lacy DB, Wasserman DH, Cherrington AD: Effects of an acute increase in epinephrine and cortisol on carbohydrate metabolism during insulin deficiency. *Diabetes* 44:672–681, 1995
45. Perriello G, De Feo P, Torlone E, Fanelli C, Santeusanio F, Brunetti P, Bolli GB: Nocturnal spikes of growth hormone secretion cause the dawn phenomenon in type 1 (insulin-dependent) diabetes mellitus by decreasing hepatic (and extrahepatic) sensitivity to insulin in the absence of insulin waning. *Diabetologia* 33:52–59, 1990
46. Giaccari A, Rossetti L: Predominant role of gluconeogenesis in the hepatic glycogen repletion of diabetic rats. *J Clin Invest* 89:36–45, 1992
47. Pagliassotti MJ, Holste LC, Moore MC, Neal DW, Cherrington AD: Comparison of the time courses of insulin and the portal signal on hepatic glucose and glycogen metabolism in the conscious dog. *J Clin Invest* 97:81–91, 1996
48. Blackard WG, Nelson NC: Portal and peripheral vein immunoreactive insulin concentrations before and after glucose infusion. *Diabetes* 19:302–306, 1970
49. Cherrington AD, Edgerton D, Sindelar DK: The direct and indirect effects of insulin on hepatic glucose production in vivo. *Diabetologia* 41:987–996, 1998
50. Komjati M, Bratusch Marrain P, Waldhäusl W: Superior efficacy of pulsatile versus continuous hormone exposure on hepatic glucose production in vitro. *Endocrinology* 118:312–319, 1986
51. Lang DA, Matthews DR, Peto J, Turner RC: Cyclic oscillations of basal plasma glucose and insulin concentrations in human beings. *N Engl J Med* 301:1023–1027, 1979
52. Yki Jarvinen H, Koivisto VA: Natural course of insulin resistance in type I diabetes. *N Engl J Med* 315:224–230, 1986