Postprandial and Fasting Hepatic Glucose Fluxes in Long-Standing Type 1 Diabetes

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OBJECTIVE—Intravenous insulin infusion partly improves liver glucose fluxes in type 1 diabetes (T1D). This study tests the hypothesis that continuous subcutaneous insulin infusion (CSII) normalizes hepatic glycogen metabolism.

RESEARCH DESIGN AND METHODS—T1D with poor glycemic control (T1Dp; HbA_{1c}: 8.5 ± 0.4%), T1D with improved glycemic control on CSII (T1Di; 7.0 ± 0.3%), and healthy humans (control subjects [CON]; 5.2 ± 0.4%) were studied. Net hepatic glycogen synthesis and glycogenolysis were measured with in vivo ^{13}C magnetic resonance spectroscopy. Endogenous glucose production (EGP) and gluconeogenesis (GNG) were assessed with [6,6-$^2H_2$]glucose, glycogen phosphorylase (GP) flux, and gluconeogenic fluxes with ^2H_2O/paracetamol.

RESULTS—When compared with CON, net glycogen synthesis was 70% lower in T1Dp (P = 0.038) but not different in T1Di. During fasting, T1Dp had 25 and 42% higher EGP than T1Di (P = 0.004) and CON ($P < 0.001$; T1Di vs. CON: P = NS). GNG was 74 and 67% higher in T1Dp than in T1Di (P = 0.002) and CON (P = 0.001). In T1Dp, GP flux (7.0 ± 1.6 μmol · kg^{-1} · min^{-1}) was twofold higher than net glycogenolysis, but comparable in T1Di and CON (3.7 ± 0.8 and 4.9 ± 1.0 μmol · kg^{-1} · min^{-1}). Thus T1Dp exhibited glycogen cycling (3.5 ± 2.0 μmol · kg^{-1} · min^{-1}), which accounted for 47% of GP flux.

CONCLUSIONS—Poorly controlled T1D not only exhibits augmented fasting gluconeogenesis but also increased glycogen cycling. Intensified subcutaneous insulin treatment restores these abnormalities, indicating that hepatic glucose metabolism is not irreversibly altered even in long-standing T1D. ***Diabetes*** **60:1752–1758, 2011**

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Received 7 July 2010 and accepted 28 March 2011.

DOI: 10.2337/db10-1001. Clinical trial reg. no. NCT00481598, clinicaltrials.gov.

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db10-1001/-/DC1.

The liver is responsible for raising endogenous glucose production (EGP) to maintain constant plasma glucose concentrations mainly via gluconeogenesis during fasting (1) or via glycogenolysis as first-line response to hypoglycemia (2). Patients with type 1 diabetes (T1D) not only have blunted glycogen synthesis (3,4) but also impaired glycogenolysis during hypoglycemia (5). This, in concert with impaired counterregulatory hormonal responses, leads to a diminished defense against hypoglycemia, one of the major concerns of insulin treatment.

Short-term normoglycemia, induced by investigator-controlled variable intravenous insulin infusion, improves nocturnal hepatic glycogen synthesis in well-controlled T1D (6). In everyday life, patients with T1D attempt to achieve near-normoglycemia by intensified insulin therapy using frequent insulin injections or continuous subcutaneous insulin infusion (CSII) pumps. However, the effects on pathways of hepatic glucose metabolism during the critical nocturnal fed-to-fasting transition have hitherto not been described under real life conditions.

The postprandial fluxes of hepatic glucose metabolism during the first 6 h after meal ingestion can be described by a metabolic model (Fig. 1) and were measured in this study in all groups. In addition to net substrate fluxes such as conversion of glycogen to glucose, the model includes the futile exchange between hepatic glycogen and glucose-6-phosphate (G6P) that is driven by simultaneous glycogen synthase (GS) and glycogen phosphorylase (GP) fluxes, a process known as glycogen cycling (7). Although net glycogenolytic fluxes have been previously characterized in T1D (4,6), no measurements of glycogen cycling have been reported in these patients. Aside from its possible relevance to metabolic alterations in T1D, glycogen cycling dilutes the enrichment of hepatic G6P from gluconeogenic tracers independently of net glycogenolytic flux. Thus, with such methodologies, the contribution of glycogenolysis to EGP may be overestimated, whereas that of gluconeogenesis is correspondingly underestimated. Resolution of glycogen cycling from net glycogenolytic and gluconeogenic contributions requires that the gluconeogenic tracer assay be supplemented by an independent measurement of either net glycogenolysis (in vivo ^{13}C magnetic resonance spectroscopy [MRS]) or absolute GS flux (isotope dilution of labeled galactose at the level of uridine diphosphate UDP-glucose [7]).

Glycogen cycling and net substrate fluxes were resolved for humans with type 2 diabetes (T2D) or liver cirrhosis by integrating direct in vivo ^{13}C MRS measurements of net glycogen depletion with isotopic tracer measurements of

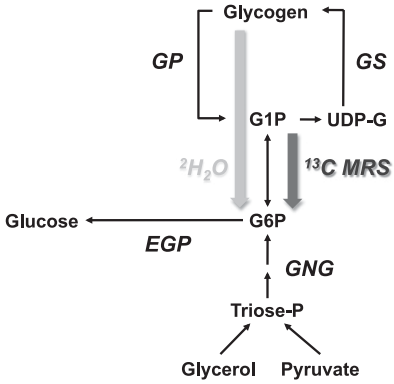

FIG. 1. Metabolic model representing fluxes between G6P, glycogen, glucose, and the parameters of glycogenolytic flux derived by ^2H_2O and ^{13}C MR methods. Component fluxes include GS flux, GP, GNG, and EGP. The in vivo ^{13}C MRS assay measures the net loss of hexose from the pool of glycogen metabolites (i.e., net glycogenolysis), and GNG is calculated as EGP − net glycogenolysis. Net glycogenolysis represents the difference between GP and GS, hence the fraction of EGP derived from net glycogenolytic flux is equal to (GP − GS)/EGP. The ^2H_2O method measures the fractional contribution of GP to EGP flux. When GS is zero, net glycogenolysis and GP are equal. During glycogen cycling, where GS is active, GP is higher than net glycogenolysis.

EGP, gluconeogenesis, and GP fluxes (8,9). Here, we tested the hypothesis that patients with CSII-treated T1D should have normal hepatic glycogen metabolism by applying an integrated approach comprising direct ^{13}C MRS measurements of liver glycogen and simultaneous assessment of fluxes through GP, glycogen cycling, EGP, and GNG using [6,6-$^2H_2$]glucose and ^2H_2O/glucuronide.

RESEARCH DESIGN AND METHODS

Volunteers. Twenty-two volunteers were included, comprising patients with long-standing C-peptide–negative T1D and T1D poor (T1Dp) or T1D improved (T1Di) glycemic control and nondiabetic humans without family history of diabetes (control subjects [CON]) (Table 1). All participants underwent clinical examination including medical history and laboratory tests to exclude general internal, endocrine, and metabolic diseases. They were sedentary and had comparable habitual physical activity as assessed with a self-administered questionnaire (10). After detailed information, all participants consented in writing to the study, which had been reviewed and approved by the Ethical Board of the City of Vienna and registered at ClinicalTrials.gov (NCT00481598).

Study protocol. Patients with T1Dp were treated by multiple daily insulin injections and instructed to omit NPH- or Zn-insulin and only use regular insulin to control blood glucose for 24 h before the experiment. Although CON were studied once, patients with T1Dp who responded to CSII with HbA_{1c} levels ≤7.2% for at least 5 months were studied before and after switching to CSII. Six of the 10 patients did not meet this criterion and were therefore excluded from the T1Di. Another four patients on CSII with target glycemic control for at least 5 months were recruited and studied once.

After admission to the clinical research unit, all volunteers received intravenous catheters (Vasofix, Braun; http://www.bbraun.com/index.cfm) in antecubital veins of both arms for blood sampling and infusions at ~7:00 A.M. Three standard mixed meals (60% carbohydrate, 20% protein, 20% fat) were served at 8:00 A.M. (737 kcal solid), 1:00 P.M. (735 kcal solid), and 6:40 P.M. (823 kcal liquid meal) (4,6). Patients received subcutaneous insulin injections before meals and when necessary to avoid excessive plasma glucose excursions. ^{13}C MRS was performed in 60-min sessions to monitor liver glycogen at 5:30 P.M., 11.30 P.M., 2:00 A.M., and 7:00 A.M., respectively.

At 10:30 P.M., a primed-continuous (0.05 mg · kg^{-1} · min^{-1}) infusion of [6,6-$^2H_2$]glucose was started and continued until 6:00 A.M. the next day. To confirm isotopic steady state, blood samples were drawn to measure tracer enrichments at 12:30, 12:40, and 12:50 A.M.; at 3:10, 3:20, and 3:30 A.M.; and at 6:30, 6:40, and 6:50 A.M.

At 11:00 P.M., all volunteers drank a total of 3 g/kg body water of ^2H_2O (99.9% enriched; Cambridge Isotope Laboratories, Andover, MA). Body water was assumed to be 60% in men and 50% in women (11,12). Thereafter, they had free access to drinking water containing 0.3% ^2H_2O to maintain isotopic equilibrium in body water. At 03:00 A.M., they ingested 1,000 mg paracetamol. Between 5:30 and 6:00 A.M., MRS was performed to measure hepatic lipid content (HLC) and phosphorus compounds. Between 06:00 A.M. and 08:00 A.M., urine was collected and evaporated for measuring recovery of paracetamol glucuronide (13). Blood was drawn to measure glucose every 30 min between 7:00 A.M. and 12:00 P.M. and then at timed intervals to determine [6,6-$^2H_2$]glucose enrichments, metabolites, and hormones.

Measurement of glucuronide ^2H enrichment in urine water. Paracetamol glucuronide was converted to monoacetone glucose (MAG) as described (13,14). MAG was dissolved in acetonitrile/water (90/10% vol/vol) for NMR analysis. Urinary paracetamol glucuronides were obtained in sufficient yields for NMR analysis from 8 of 8 healthy subjects, 7 of 10 T1Dp, and 5 of 8 T1Di. Proton-decoupled ^2H NMR spectra were acquired with an 11.75-T Varian Unity 500 system equipped with a 5-mm broadband probe. For samples with low amounts of MAG, spectra were obtained with a 14.1T VNMR600 spectrometer equipped with a 3-mm microprobe (Varian, Palo Alto, CA). Spectra were acquired at 50°C without field-frequency lock with 90° pulse angle, 10-ppm sweep width, and 1.6-s acquisition time (Fig. 2). Between 11,100 and 50,000 free induction decays (FID) were acquired per sample for collection times of 6–27 h. Fully relaxed ^1H NMR spectra were obtained under the same conditions with 45° pulse width, 3-s acquisition time, and a 20-s delay. Each ^1H spectrum was acquired with a single FID. ^2H enrichment of urine water was analyzed by ^2H NMR (15). All NMR spectra were analyzed using the curve-fitting routine supplied with the NUTS PC-based NMR spectral analysis program (Acorn NMR, Fremont, CA).

MRS and magnetic resonance imaging. Volunteers were examined in supine position for MRS on a 3-T Bruker Biospin (Ettlingen, Germany) and for magnetic resonance imaging (MRI) on a Siemens TIM Trio (Erlangen, Germany).

^1H MRS was used for the determination of HCL using single voxel spectroscopy (STEAM) (16), jMRUI (17), and AMARES (18). ^{13}C MRS was used for quantification of liver glycogen with a circular, 10-cm diameter surface coil placed over the lateral aspect of the liver, which was confirmed by MRI (4). One session consisted of four to five 10-min blocks (19) during which 4,096 ^{13}C FIDs were acquired every 150 ms (pulse-acquire experiment, 500 μs adiabatic excitation pulse). For absolute quantification the experiment was also performed on a glucose solution of known concentration and volume. Supplemental Figure 1 shows example spectra of one CON, T1Dp, and T1Di, respectively.

Liver volume was quantified by acquiring axial T_1-weighted MRI of the abdomen during one breath-hold and analyzed using home-built semi-automatic software (20). Mean liver volume was not different between groups (T1Dp: 1,602 ± 380; T1Di: 1,402 ± 164; CON: 1,451 ± 366 cm^3; P = NS).

TABLE 1
Anthropometric and fasting blood parameters (means ± SD [95% CI]) in patients with T1Dp or T1Di glycemic control and in CON

	T1Dp	T1Di	CON
N total (female/male)	10 [4/6]	8 [3/5]	8 [4/4]
Age (years)	35 ± 10 [28–42]	35 ± 10 [27–43]	31 ± 11 [21–40]
BMI (kg/m^2)	25 ± 3 [23–27]	26 ± 3 [24–28]	24 ± 4 [21–28]
Physical activity	2.8 ± 0.6 [2.2–3.3]	2.6 ± 0.8 [1.9–3.3]	2.9 ± 0.6 [2.3–3.4]
Diabetes duration (years)	20 ± 13 [11–30]	17 ± 14 [4–29]	—
Hemoglobin A_{1c} (%)	8.5 ± 0.4 [8.1–8.8]†	7.0 ± 0.3 [6.7–7.2]‡	5.2 ± 0.4 [4.9–5.5]*
Glucose (mmol/L)	10.6 ± 3.2 [8.3–12.9]	7.6 ± 3.3 [4.9–10.3]	5.0 ± 0.4 [4.7–5.4]§

Mean physical activity is calculated from rating on a scale ranging from 1 [low] to 5 [high degree of activity] and given in arbitrary units. *CON vs. T1Dp $P < 0.001$; †T1Dp vs. T1Di $P < 0.001$; ‡CON vs. T1Di $P < 0.001$; §CON vs. T1Dp $P = 0.001$.

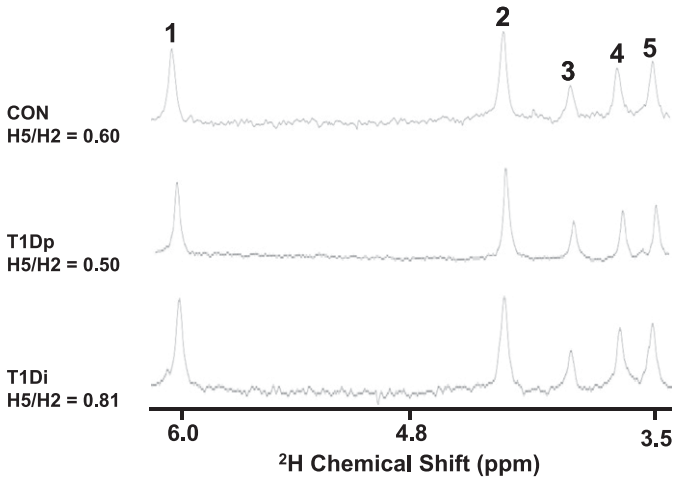

FIG. 2. ^{2}H NMR spectra of urinary glucuronides following derivatization to MAG from a healthy control subject, a diabetic patient with poor glycemic control (T1Dp), and a diabetic patient with good glycemic control (T1Di). The number above each signal represents its positional origin in the MAG molecule. The ratio of signal 5 and signal 2 areas (H5-to-H2) is also shown.

Metabolites and hormones. Plasma glucose was measured using the glucose oxidase method (Glucose Analyzer II; Beckmann Instruments, Fullerton, CA). Plasma insulin, C-peptide, and glucagon were determined by double-antibody radioimmunoassays (Linco, St. Charles, MO; http://www.millipore.com/); HbA_{1c} using HPLC (Bio-Rad, Hercules, FL); and triglycerides, total, HDL-, and LDL-cholesterol using routine laboratory methods (Abbot Aeroset LN09D05–01, A1522460). Free fatty acids (FFA) were assayed microfluorimetrically (Wako Chem; http://www.wakousa.com/) in orlistat-treated samples to prevent in vitro lipolysis (21). Plasma growth hormone was measured by immunoradiated matrix assay (DiaSorin, Saluggia Vercelli, Italy; http://www.diasorin.com).

Calculations. EGP was measured using isotopic dilution, whereas net hepatic glycogenolysis (GLYnet) was simultaneously measured by in vivo ^{13}C MRS, yielding net GNG as the difference between EGP and GLYnet (22). Rates of net hepatic glycogen synthesis during the absorptive period were calculated from linear regression analysis of glycogen concentrations measured by ^{13}C MRS from immediately before the meal at 5:30 P.M. until peak levels were reached (4).

Rates of GLYnet during the postabsorptive period (6–12 h after dinner) were calculated from linear regression of glycogen concentrations measured from peak levels until 8:00 A.M. the following morning. Rates of EGP (in $\mu mol \cdot kg^{-1} \cdot min^{-1}$) were calculated by dividing the [6,6-$^2H_2$]glucose infusion rate times tracer enrichment by the percent tracer enrichment in plasma and subtracting the tracer infusion rate (23). Rates of GNG (in $\mu mol \cdot kg^{-1} \cdot min^{-1}$) were estimated as follows (24): GNG = EGP − GLYnet.

Fractional GP flux contribution to EGP is given as 1 − (H5/H2) where H5-to-H2 is the ratio of glucuronide position 5 to position 2 enrichment from ^2H_2O (8). Absolute GP flux (in $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was calculated as follows: GP = EGP × [1 − (H5/H2)].

Given certain assumptions on the order of glucosyl uptake and release from glycogen (7), GP flux reflects the sum of GLYnet and GS fluxes (8,9). When GS activity is negligible (i.e., no glycogen cycling), GP flux by ^2H_2O equals GLYnet flux by in vivo ^{13}C MRS (7–9). However, in the presence of glycogen cycling when both GP and GS fluxes are active, GP is higher than that of GLYnet, with the difference being accounted for by GS (Fig. 1).

Glycogen cycling activity during net glycogen breakdown, defined as the GS flux ($\mu mol \cdot kg^{-1} \cdot min^{-1}$), was estimated as follows: GS = GP − GLYnet.

These calculations assume that *1*) all EGP was derived from hepatic G6P with no relevant extrahepatic glucose production, i.e., kidney, and *2*) during glycogen cycling, G6P molecules labeled in position 5 from the gluconeogenic pathway that are recruited for glycogen synthesis are trapped in glycogen and are not recycled back to G6P during the glycogenolysis step (7).

Areas under the curve of plasma glucose concentrations (AUCglu) were estimated with the trapezoidal rule for defined time periods (25).

Statistics. All analyses were performed using SPSS 14.0 software (SPSS; http://www.spss.com). Data are presented as means ± SDs with 95% CIs. Between-group comparisons were performed using ANOVA with Tukey post hoc testing or the two-tailed Student t test when appropriate. Linear correlations are Pearson product-moments correlations (R, P). Spearman correlations were used for parameters with skewed distribution. P values of < 0.05 were considered to indicate significant differences.

RESULTS

Baseline data. Anthropometric variables were comparable between groups (Table 1). Plasma glucose was higher in T1Dp than in the other groups. HbA_{1c} was lower in T1Di than in T1Dp but higher than in CON. HCL were similar in all groups (T1Dp, 2.5 ± 1.6%; T1Di, 2.5 ± 1.5%; CON, 3.9 ± 2.4%; P = NS).

Fasting period. Plasma glucose was more than twofold greater in T1Dp than in CON and tended to be higher (P = 0.162) in T1Di than in CON (Table 1). Fasting insulin tended to be lower in CON (T1Dp 123 ± 74, T1Di 188 ± 253, CON 81 ± 51 pmol/L; T1Dp vs. CON P = 0.829, T1Di vs. CON P = 0.339, T1Dp vs. T1Di P = 0.627) (Fig. 3*C*). Plasma glucagon (T1Dp 55 ± 15, T1Di 72 ± 19, CON 59 ± 19 ng/L; Fig. 3*D*) and FFA were similar (T1Dp 564 ± 374, T1Di 778 ± 463, CON 354 ± 145 μmol/L; P = NS; Fig. 3*B*). Plasma cortisol (T1Dp 8.4 ± 5.4, T1Di 6.7 ± 4.9, CON 9.4 ± 6.6 μg/dL; P = NS) and growth hormone (T1Dp 3.1 ± 1.8, T1Di 4.4 ± 2.0, CON 2.5 ± 2.9 ng/mL; P = NS) were also comparable across the groups.

Postprandial period (0–6 h after dinner). Mean plasma glucose was lower in T1Di than in T1Dp but about twofold higher than in CON (T1Dp vs. CON $P < 0.001$, T1Di vs. CON $P < 0.001$, T1Dp vs. T1Di P = NS; Fig. 3*A*). AUCglu was comparable between T1Dp and T1Di (T1Dp 1,088 ± 345, T1Di 1,290 ± 488, CON 175 ± 119 $mmol \cdot L^{-1} \cdot min^{-1}$; T1Dp vs. T1Di P = NS, T1Dp vs. CON $P < 0.001$, T1Di vs. CON $P < 0.001$). Plasma FFA decreased in all groups (T1Dp 57 ± 31%, T1Di 23 ± 10%, CON 33 ± 16%; T1Dp P = 0.023, T1Di P = 0.013, CON P = 0.002 vs. predinner values; Fig. 3*B*). Plasma insulin and glucagon, peripheral insulin-to-glucagon ratios (Fig. 3*C* and *D*), cortisol (T1Dp 9.3 ± 4.7, T1Di 6.3 ± 3.6, CON 7.1 ± 3.6 μg/dL; P = NS), and growth hormone (T1Dp 3.1 ± 2.6, T1Di 3.0 ± 3.7, CON 2.2 ± 2.4 ng/mL; P = NS) were not different between groups.

T1Di accumulated slightly more hepatic glycogen upon meal ingestion than T1Dp, but hepatic glycogen did not differ between groups (T1Dp vs. CON P = 0.270, T1Dp vs. T1Di P = 0.159, T1Di vs. CON P = 0.952; Table 2). Net hepatic glycogen synthesis was lower in T1Dp than in CON (1.0 ± 1.8 vs. 3.3 ± 2.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; P = 0.038) but not different between CON and T1Di (2.7 ± 1.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; P = 0.782; Fig. 4).

Postabsorptive period (6–12 h after dinner). Mean plasma glucose was highest in T1Dp (T1Dp 14.7 ± 2.3, T1Di 9.7 ± 1.8, CON 4.9 ± 0.3 mmol/L; T1Dp vs. CON $P < 0.001$, T1Dp vs. T1Di $P < 0.001$, T1Di vs. CON $P < 0.001$; Fig. 3*A*). AUCglu was comparable between T1Dp and T1Di (T1Dp 757 ± 400, T1Di 1,041 ± 452, CON 85 ± 45 $mmol \cdot L^{-1} \cdot min^{-1}$; T1Dp vs. T1Di P = NS, T1Dp vs. CON $P < 0.001$, T1Di vs. CON $P < 0.001$). Plasma insulin was higher in T1Di compared with CON (T1Dp 19 ± 12, T1Di 33 ± 30, CON 10 ± 4 pmol/L; T1Di vs. CON P = 0.05; Fig. 3*C*). Plasma FFA (T1Dp 574 ± 244, T1Di 552 ± 446, CON 399 ± 198 μmol/L; P = NS; Fig. 3*B*), cortisol (T1Dp 12.5 ± 3.7, T1Di 16.6 ± 5.9, CON 13.0 ± 5.3 μg/dL; P = NS), and growth hormone (T1Dp 3.8 ± 4.5, T1Di 3.6 ± 3.7, CON 4.7 ± 7.3 ng/mL; P = NS) did not differ between groups.

GLYnet varied considerably but was not different between groups (T1Dp 3.3 ± 1.6, T1Di 5.3 ± 3.2, CON

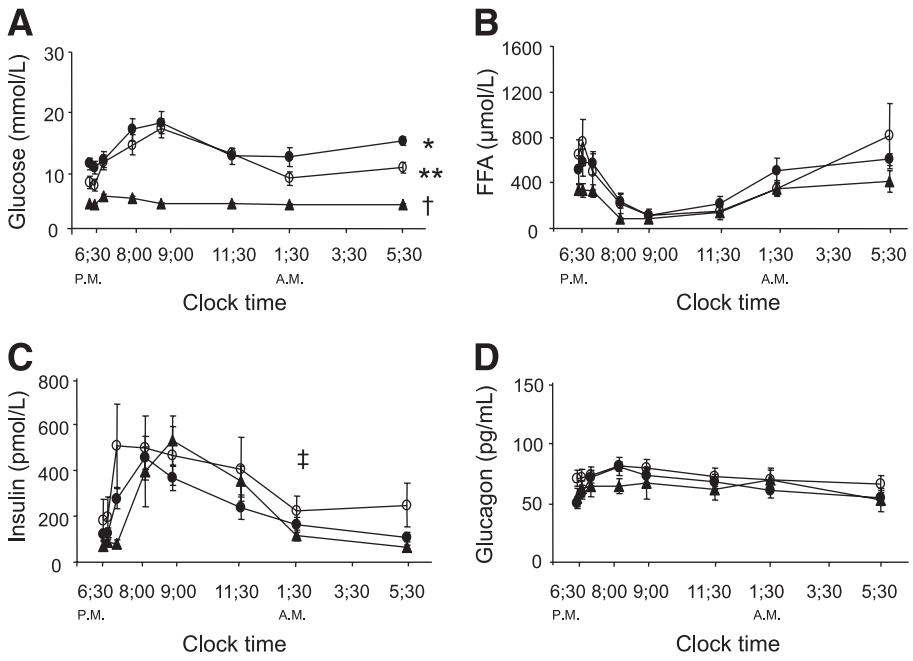

FIG. 3. Plasma concentrations of glucose (*A*), FFA (*B*), insulin (*C*), and glucagon (*D*) in CON (▲), T1Dp (●), and T1Di (○). Mean plasma glucose from 6:30 P.M. until 5:30 A.M. is shown. *T1Dp vs. CON $P < 0.001$; **T1Dp vs. T1Di $P = 0.017$; †CON vs. T1Di $P < 0.001$; plasma insulin concentration at 1:30 A.M. ‡CON vs. T1Di $P = 0.036$. Data are presented as means ± SEM.

4.0 ± 1.5 μmol · kg^{-1} · min^{-1}; T1Dp vs. CON $P = 0.787$, T1Dp vs. T1Di $P = 0.161$, T1Di vs. CON $P = 0.479$; Fig. 4). EGP was higher in T1Dp (16.5 ± 2.3 μmol · kg^{-1} · min^{-1}) than in T1Di and CON (13.2 ± 1.3 vs. 11.6 ± 2.0 μmol · kg^{-1} · min^{-1}; T1Dp vs. T1Di $P = 0.004$, T1Dp vs. CON $P < 0.001$). Accordingly, GNG was greater in T1Dp compared with both T1Di and CON (T1Dp 13.2 ± 3.0, T1Di 7.9 ± 3.1, CON 7.6 ± 2.3 μmol · kg^{-1} · min^{-1}; T1Dp vs. T1Di $P = 0.002$, T1Dp vs. CON $P = 0.001$).

GP flux from ^2H_2O/glucuronide was lower in T1Di than in T1Dp (T1Dp 7.0 ± 1.6, T1Di 3.7 ± 0.8, CON 4.9 ± 1.0 μmol · kg^{-1} · min^{-1}; T1Dp vs. T1Di $P = 0.005$, T1Dp vs. CON P = NS; Fig. 4). Glycogen cycling was highest in T1Dp (T1Dp 3.5 ± 2.0, T1Di −2.3 ± 1.9, CON 1.0 ± 1.3 μmol · kg^{-1} · min^{-1}; T1Dp vs. T1Di $P = 0.008$, T1Dp vs. CON P = NS). In T1Dp, glycogen cycling accounted for 47% of total GP flux. This degree of glycogen cycling activity resulted in substantial overestimation of the contribution of glycogenolytic flux to EGP by ^2H_2O compared with the fractional glycogenolytic contribution by in vivo ^{13}C MRS (43 ± 8 vs. 20 ± 9%; $P < 0.001$). In contrast, both methods yielded equivalent glycogenolytic contributions to EGP in both T1Di and CON groups reflecting less glycogen cycling. For T1Di, GLYnet and GP flux accounted for 41 ± 19 and 28 ± 6% of EGP. For CON, the corresponding values were 35 ± 9 and 41 ± 6% of EGP. Supplemental Table 1 shows the individual fluxes of GLYnet and glycogen phosphorylase (GLYphos) fluxes.

Correlation analyses. EGP correlated positively with glycogen cycling ($R^2 = 0.347$; $P = 0.008$; Fig. 5*A*), HbA_{1c} ($R^2 = 0.525$; $P < 0.001$; Fig. 5*B*) and fasting plasma glucose ($R^2 = 0.382$; $P = 0.001$). Insulin requirements (T1Dp: 43 ± 12 units/24 h, T1Di: 54 ± 14 units/24 h; P = NS) correlated positively with BMI ($R^2 = 0.257$; $P = 0.032$) and negatively with GP flux ($R^2 = 0.445$; $P = 0.025$) across all T1D.

DISCUSSION

This study shows that long-term improvement of glycemic control using CSII-pump improves abnormal hepatic glucose metabolism in T1D even despite short-term hyperglycemia. To mimic everyday life, the patients with T1D applied insulin subcutaneously throughout the study days. This is in contrast with previous studies, where short-term normoglycemia was achieved by variable intravenous insulin infusion and only at supra-physiological plasma insulin concentrations (4,6). Thus, although our study recorded marked improvements in average plasma glucose and HbA_{1c} levels, it could not obtain strict normoglycemia even in the well-controlled T1Di. Nevertheless, CSII pump therapy improved hepatic glucose metabolism, as seen by raised rates of glycogen synthesis during meal ingestion and more effective suppression of EGP and GNG during fasting in T1Di.

During fasting, healthy humans appear to have only minimal glycogen cycling activity as reported (8,15) and confirmed by the current study. Acute hyperglycemia per se

TABLE 2
Hepatic glycogen concentrations before and after ingestion of an 823-kcal mixed meal at 6:30 P.M. in T1Dp, T1Di, and CON

	Before dinner	+5 h	+7.5 h	+12.5 h
T1Dp	190 ± 66 [143–237]	209 ± 68 [161–257]	210 ± 55 [170–250]	157 ± 40 [129–185]
T1Di	209 ± 47 [170–248]	270 ± 62 [219–322]	263 ± 74 [201–325]	173 ± 43 [137–209]
CON	197 ± 67 [141–253]	260 ± 74 [198–323]	225 ± 76 [161–288]	174 ± 57 [126–222]

Values are means ± SD [95% CI] in micromoles per liter liver.

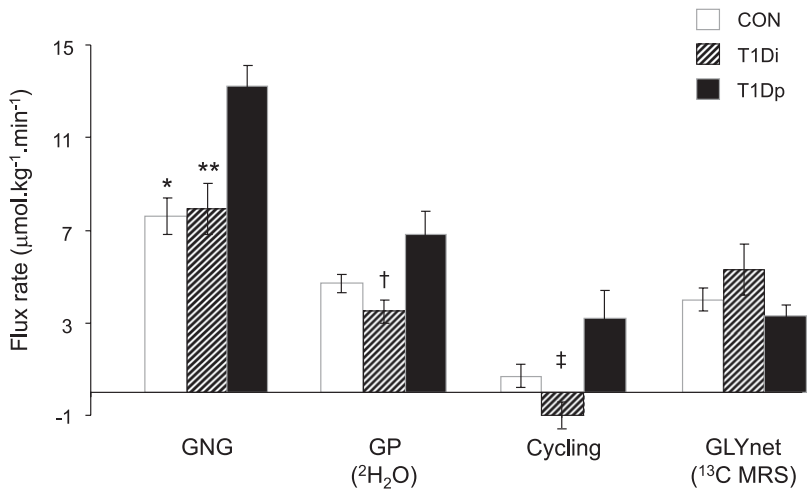

FIG. 4. GNG, GP, glycogen cycling, and GLYnet in healthy CON (white bars), T1Di (hatched bars), and T1Dp (black bars). *CON vs. T1Dp $P = 0.001$; **T1Di vs. T1Dp $P = 0.002$; †T1Di vs. T1Dp $P = 0.021$; ‡T1Di vs. T1Dp $P = 0.02$. Data are presented as means ± SEM.

inhibits GP flux in healthy subjects (25). Stingl et al. (26) showed modest glycogen cycling during hyperglycemic-hyperinsulinemic clamps mimicking postprandial conditions in healthy subjects. Other studies of healthy subjects demonstrated that hyperinsulinemia and relative hypoglucagonemia may stimulate glycogen cycling (25,27). Although its clinical relevance is not entirely clear, glycogen cycling may serve to limit the accumulation of liver glycogen at high glycogen concentrations (28). Under hyperglycemic-hyperinsulinemic conditions, on the other hand, glycogen cycling may be the principal mechanism to inhibit GLYnet and EGP (27). In contrast, patients with T2D exhibit substantial glycogen cycling of ~25% of EGP after overnight fasting (8). In the fed state, this could explain lower glycogen stores and thus contribute to postprandial hyperglycemia (7).

Insulin and glucagon are also potent regulators of glycogenolysis, as indicated by reduced glycogen breakdown during hyperinsulinemia and hypoglucagonemia (25,27,29). It was hypothesized that complete normalization of glycogen metabolism could only be achieved by raising the insulin-to-glucagon ratio (6). In the current study, peripheral insulin and glucagon levels were comparable between CON and T1D, and within the range of other studies (30). Because all T1D were C-peptide negative, portal insulin concentrations and portal insulin-to-glucagon ratios were about twofold higher in CON than in T1D (31). Thus one might speculate that glycogen synthesis should have been higher in CON compared with either group of T1D, which was not the case in the current study.

The marked decrease in plasma FFA levels after dinner in all groups suggests normal insulin sensitivity at least with respect to lipolysis. One could argue that significant improvement of hepatic glycogenolysis after CSII pump treatment was not achieved, because glycemic control was insufficient compared with previous studies (6). Then again, glycogen synthesis was significantly improved as well as EGP was reduced, along with gluconeogenesis. This leaves acute hyperglycemia as the most probable cause for the incomplete restoration of glycogenolysis in line with previous clinical data (32). Petersen et al. (27) reported accordingly that in healthy humans, hyperglycemia per se reduces net hepatic glycogenolysis primarily through inhibition of GP flux. Hyperinsulinemia, on the other hand, also inhibits glycogenolysis, but presumably via the activation of glycogen synthase.

It is intriguing that EGP and GNG were near normal in T1Di despite short-term hyperglycemia. Bock et al. (33)

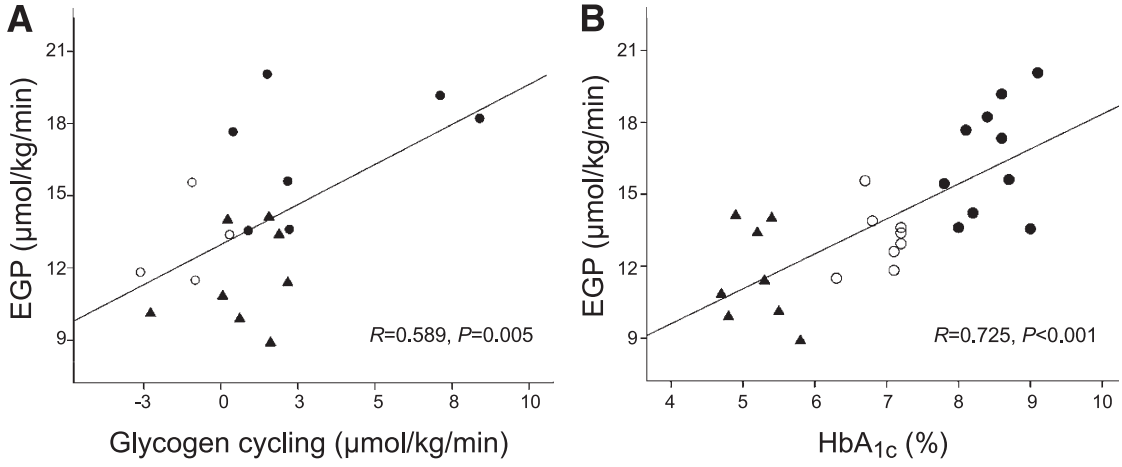

FIG. 5. Relationship between EGP and glycogen cycling (*A*) and HbA_{1c} (*B*) across all groups: patients with poorly controlled type 1 diabetes (●), improved glycemic control (○), and nondiabetic CON (▲).

showed in subjects with impaired fasting glucose that higher EGP as a result of GNG can be suppressed by portal insulin levels of ~300 pmol/L, whereas glucose disposal was still lower than in subjects with normal fasting glucose. This indicates extrahepatic, but not hepatic insulin resistance. Bischof et al. (6) demonstrated that even during near normoglycemic long- and short-term control as induced by manually adjusted variable insulin infusion, patients with T1D displayed peripheral, but not hepatic, insulin resistance, as seen by normal EGP despite augmented contribution of GNG to glycogen synthesis. This could be partly because of not completely normalized control of immediate prandial glucose concentrations. Taken together, our data indicate that intensified glycemic control improved hepatic insulin resistance, whereas short-term hyperglycemia might have contributed to diminished peripheral insulin resistance.

Although EGP and GNG in T1Di could be restored to normal levels, glycogen metabolism failed to improve significantly, suggesting that some alterations might be irreversible in long-standing T1D. In T2D, there is evidence for a defect in the rapid suppression of hepatic glucose production by blood glucose (glucose effectiveness) (34,35). Hyperglycemia regulates hepatocellular G6P (36) in such a way that the maintenance of intracellular G6P homeostasis is achieved at the expense of aggravated hyperglycemia (glucose toxicity) (37). To this end, high G6P levels suppress the expression of glucokinase (36) but increase the expression of G6P (38). Since G6P, in concert with glucose (39), inactivates GP and thereby glycogenolysis, perturbation in the concentration of this metabolite might lead to declined glucose effectiveness in diabetes (40). Glycogen synthesis also might be negatively affected by the deranged sensitivity of GP to glucose because both elevation of G6P and inactivation of GP are needed to activate glycogen synthase in concert (40). Notably, inhibition of GP and activation of glycogen synthase are not necessarily coupled and coordinated in reciprocal fashion, as also demonstrated by increased glycogen cycling under hyperinsulinemia in healthy humans (27) and in insufficiently treated T2D (15) as well as in our T1Dp.

Other than the liver, the kidney also is able to release glucose into the circulation. In healthy humans, the kidney makes at most only a minor contribution to postabsorptive glucose homeostasis and accounts for 20–25% of whole-body glucose turnover only after 60-h fasting (41). Because both renal and hepatic glucose release are increased at least in T2D (42), our measurements may partly overestimate hepatic glucose production.

In healthy subjects, the ^2H_2O/glucuronide method yielded high signal-to-noise spectra and reliable ^2H NMR enrichment data because of the abundance of urinary glucuronide. Based on previous studies (13,43–45), the low glucuronide yields in T1Di were unexpected and precluded a rigorous evaluation of fluxes for this group. Our previous experience is that T1Dp tend to generate the lowest yields of urinary glucuronides, in particular those with urinary glucose excretion. Supplemental figure 2 gives the individual fluxes of ^2H NMR spectra of urinary glucuronide following derivatization to monoacetone glucose. In CON, net hepatic glycogenolysis accounted for about one-third of EGP and glycogen cycling fluxes were minimal. EGP and gluconeogenic flux ranges were in good agreement with estimates from previous studies of overnight-fasted subjects (46). T1Dp presented a different profile of fasting hepatic glucose fluxes with greater EGP and hepatic gluconeogenic rates but normal net hepatic glycogen hydrolysis fluxes as reported (47,48). Moreover, in T1Dp, glycogen cycling fluxes tended to be increased relative to CON. As a result of glycogen cycling, the contribution of hepatic glycogenolysis to EGP measured by ^{13}C MRS (20 ± 9%) was significantly lower than the GP flux estimated by ^2H_2O/glucuronide (43 ± 8% of EGP). Thus, for T1Dp, the decoupling of GP flux from GLYnet means that the ^2H_2O measurement per se may overestimate the fractional contribution of hepatic glycogenolysis to EGP. The ^2H enrichment profile of glucose or glucuronide from ^2H_2O does not provide any independent information on glycogen cycling. Therefore, resolution of GP and glycogenolytic fluxes requires that the ^2H_2O measurement be accompanied by an independent measurement of net glycogenolysis via ^{13}C MRS. Alternatively, these parameters can be resolved if the ^2H_2O measurement is accompanied by an assay of UDP-glucose synthesis flux via a primed infusion of labeled galactose (net glycogenolysis being estimated as the difference between the calculated GP and UDP-glucose synthesis rates).

For the T1Di, where both glucuronide and ^{13}C MRS data were obtained, EGP and GNG fluxes were reduced and matched those of healthy CON. Moreover, glycogen cycling fluxes were relatively low and reduced in comparison with those from the T1Dp. In T1Di, GP fluxes (by ^2H_2O/glucuronide) reflected GLYnet (by ^{13}C MRS) so that both methods gave consistent estimates of hepatic glycogenolytic contributions to EGP (27 ± 8 by ^2H_2O/glucuronide vs. 35 ± 17% by ^{13}C MRS).

One limitation of this study is that CSII treatment did not normalize postprandial glycemia in T1Di. This is likely because of the high-carbohydrate liquid dinner, which was chosen to allow for comparison with previous studies in patients with T2D and nondiabetic humans. We also refrained from correcting blood glucose in very short time intervals by intravenous insulin to mimic the everyday life situation. Particularly, near-normoglycemic control with subcutaneous insulin infusions remains challenging during ingestion of an 823-kcal high-carbohydrate drink to maximize glycogen synthesis. In the face of different plasma glucose levels, hyperglycemia per se might have diminished glucose effectiveness, which can be restored even in poorly controlled T2D by short-term normoglycemia (35). Furthermore, portal vein insulin levels and insulin-to-glucagon ratios were likely lower in T1D than in CON, which will favor glycogenolysis. However, further raising of insulin doses would have markedly increased the risk of hypoglycemia in the T1Di. Nevertheless, improvement of glucose and insulin concentrations was sufficient to suppress both GNG and EGP in T1Di.

Taken together, poorly controlled T1D not only exhibits augmented fasting gluconeogenesis but also increased glycogen cycling. Intensified subcutaneous insulin treatment restores these abnormalities, indicating that hepatic glucose metabolism is not irreversibly altered even in long-standing T1D.

ACKNOWLEDGMENTS

This study was supported in part by the Juvenile Diabetes Research Foundation International (JDRFI 1-2006-74), the European Foundation for the Study of Diabetes, the Schmutzler-Stiftung, the Skröder-Stiftung, the German Research Foundation (SFB 512), and the German Federal Ministry of Education and Research to the German Center for Diabetes Research.

No potential conflicts of interest relevant to this article were reported.

M.K. researched data and wrote the manuscript. J.J. researched data, contributed to discussion, and reviewed and edited the manuscript. A.I.S., C.B., A.L., G.K.-B., J.S., M.C., P.N., V.C., and M.W. researched data. M.R. wrote, reviewed, and edited the manuscript and contributed to discussion.

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