Alterations in Postprandial Hepatic Glycogen Metabolism in Type 2 Diabetes

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Decreased skeletal muscle glucose disposal and increased endogenous glucose production (EGP) contribute to postprandial hyperglycemia in type 2 diabetes, but the contribution of hepatic glycogen metabolism remains uncertain. Hepatic glycogen metabolism and EGP were monitored in type 2 diabetic patients and nondiabetic volunteer control subjects (CON) after mixed meal ingestion and during hyperglycemic-hyperinsulinemic-somatostatin clamps applying ^{13}C nuclear magnetic resonance spectroscopy (NMRS) and variable infusion dual-tracer technique. Hepatocellular lipid (HCL) content was quantified by ^{1}H NMRS. Before dinner, hepatic glycogen was lower in type 2 diabetic patients (227 ± 6 vs. CON: 275 ± 10 mmol/l liver, $P < 0.001$). After meal ingestion, net synthetic rates were 0.76 ± 0.16 (type 2 diabetic patients) and 1.36 ± 0.15 mg · kg^{-1} · min^{-1} (CON, $P < 0.02$), resulting in peak concentrations of 283 ± 15 and 360 ± 11 mmol/l liver. Postprandial rates of EGP were ~0.3 mg · kg^{-1} · min^{-1} (30–170 min; $P < 0.05$ vs. CON) higher in type 2 diabetic patients. Under clamp conditions, type 2 diabetic patients featured ~54% lower ($P < 0.03$) net hepatic glycogen synthesis and ~0.5 mg · kg^{-1} · min^{-1} higher ($P < 0.02$) EGP. Hepatic glucose storage negatively correlated with HCL content ($R = -0.602$, $P < 0.05$). Type 2 diabetic patients exhibit *1*) reduction of postprandial hepatic glycogen synthesis, *2*) temporarily impaired suppression of EGP, and *3*) no normalization of these defects by controlled hyperglycemic hyperinsulinemia. Thus, impaired insulin sensitivity and/or chronic glucolipotoxicity in addition to the effects of an altered insulin-to-glucagon ratio or increased free fatty acids accounts for defective hepatic glycogen metabolism in type 2 diabetic patients. *Diabetes* 53:3048–3056, 2004

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EGP, endogenous glucose production; FFA, free fatty acid; HCL, hepatocellular lipid; NMRS, nuclear magnetic resonance spectroscopy.

Under normal life conditions, meals are ingested every few hours, therefore rendering humans in the postprandial state for approximately half of the day. Knowledge of glucose metabolism after meal ingestion is central to the understanding of the pathophysiology and characterization of potential therapeutic targets in the diabetic state. Many previous studies in type 2 diabetic patients demonstrated defects in skeletal muscle glucose metabolism during experimental hyperinsulinemic hyperglycemia (1,2) and recently also after meal ingestion (3).

Nevertheless, the liver is almost exclusively responsible for endogenous glucose production (EGP), which correlates with the degree of hyperglycemia after an overnight fast (4–8) and is less suppressed in type 2 diabetic patients after meal ingestion (6,9–13). Invasive hepatic vein catheter studies suggested that splanchnic glucose uptake is decreased in type 2 diabetic patients (14,15). Impaired insulin-stimulated hepatic glucose uptake was confirmed in type 2 diabetic patients by positron emission tomography (16). Using a needle biopsy technique, Beringer and Thaler (17) demonstrated decreased hepatic glycogen concentrations in elderly diabetic patients. Noninvasive ^{13}C nuclear magnetic resonance spectroscopy (NMRS) studies found lower hepatic glycogen breakdown and increased gluconeogenesis in type 2 diabetic patients (18,19), but the contribution of liver glycogen metabolism to postprandial hyperglycemia and its sensitivity to insulin in type 2 diabetic patients remains uncertain.

Under steady-state conditions (clamp technique), the portal vein insulin, glucagon, and free fatty acid (FFA) concentrations regulate hepatic glucose metabolism in nondiabetic humans (20–25). Furthermore, increased liver fat accumulation (hepatocellular lipid [HCL] content) was found to correlate negatively with peripheral insulin sensitivity and insulin-dependent suppression of EGP (7,26). However, the impact of these factors on hepatic glycogen metabolism in type 2 diabetic patients is uncertain.

Thus, this study examined hepatic glycogen metabolism in type 2 diabetic patients and matched nondiabetic volunteers *1*) after mixed meal ingestion and *2*) during hyperglycemic-hyperinsulinemic-somatostatin clamps. We used noninvasive ^{13}C NMRS (18,27,28) to directly quantify net postprandial hepatic glycogen synthesis (protocol A) and variable infusion dual-tracer technique (6,27,29) to assess the postprandial time course of EGP (protocol B).

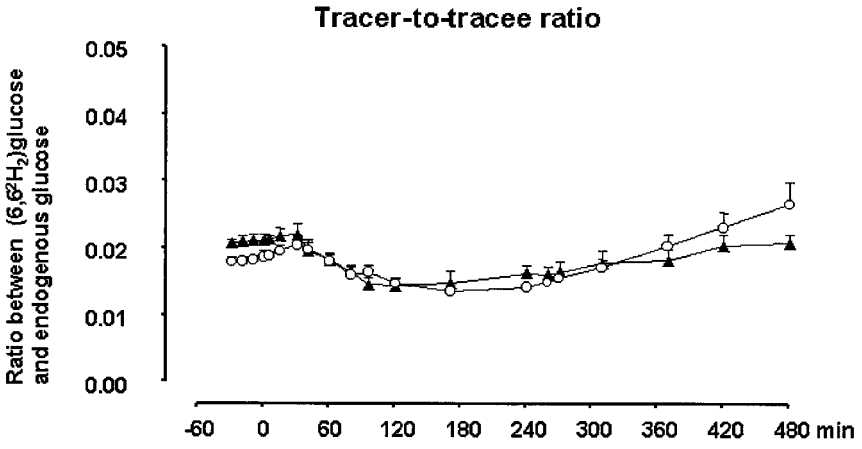

FIG. 1. Time course of ratio between plasma [6,6-$^2H_2$]glucose concentration and endogenous glucose concentration during the mixed-meal test in type 2 diabetic patients (○) and CON (▲).

For evaluation of the role of hepatic insulin sensitivity in type 2 diabetic patients, we also measured flux through hepatic glycogen synthase, simultaneous flux through hepatic glycogen phosphorylase, and EGP under conditions of matched hyperglycemia, hyperinsulinemia, hypoglucagonemia, and decreased plasma FFA concentrations (clamp test, protocol C).

RESEARCH DESIGN AND METHODS

We studied seven type 2 diabetic patients (five men/two women, age 56 ± 3 years, BMI 26.9 ± 0.6 kg/m^2, known diabetes duration 7 ± 2 years, HbA_{1c} 7.1 ± 0.3%, triglycerides 254 ± 49 mg/dl) and seven glucose-tolerant humans (five men/two women, age 49 ± 2 years, BMI 25.8 ± 0.9 kg/m^2, HbA_{1c} 5.2 ± 0.1%, triglycerides 139 ± 32 mg/dl) during mixed-meal protocols (protocols A and B). Subsequently, we studied six type 2 diabetic patients (five men/one woman, age 53 ± 4 years, BMI 26.1 ± 0.7 kg/m^2, known diabetes duration 6 ± 2 years, HbA_{1c} 7.4 ± 0.1%, triglycerides 221 ± 15 mg/dl) and six healthy humans (four men/two women, age 55 ± 4 years, BMI 27.5 ± 0.7 kg/m^2, HbA_{1c} 5.4 ± 0.1%, triglycerides 102 ± 17 mg/dl) during hyperglycemic-hyperinsulinemic clamp tests (protocol C). Four type 2 diabetic patients and four nondiabetic volunteer control subjects (CON) participated in all three protocols. Type 2 diabetic patients had no history of insulin or thiazolidinediones therapy and stopped their hypoglycemic agents for at least 3 days before each protocol. Female participants were either postmenopausal or were studied in the first half of their menstrual cycle. All volunteers were on an isocaloric diet and refrained from physical exercise for at least 3 days before the protocols, which were spaced by at least 3-week intervals. Informed consent to protocols, approved by the local ethics board, was obtained from all participants.

Mixed-meal tests (protocols A and B). All volunteers were examined under identical postprandial conditions, once for liver glycogen measurement (protocol A) and again for assessing EGP (protocol B). On both occasions, they were served breakfast (231 kcal, 7:00 A.M.), lunch (208 kcal, 11:00 A.M.), and dinner (400 ml liquid meal, 652 kcal; carbohydrate/protein/fat: 83.6 g/23.2 g/24.0 g, 5:00 P.M.).

Protocol A. Hepatic glycogen concentrations were measured using ^{13}C NMRS (28,30) before (4:00–4:30 P.M.) and after dinner (6:00–7:00 P.M., 8:00–9:00 P.M., 10:00–11:00 P.M., 12:00–1:00 A.M., and 7:00–8:00 A.M.). Liver volume measurements were performed independently on a clinical 1.5-T MR scanner (24).

Protocol B. At 3:00 P.M., a primed (20 μmol/kg per 5 mmol/l) continuous (0.020 μmol · kg^{-1} · min^{-1}) infusion of [6,6-$^2H_2$]glucose (99% enriched) was started (28). At 5:00 P.M. (0 min), the test meal was ingested in which 10 g glucose were replaced by [1-^{13}C]glucose (99% enriched). After dinner, the [6,6-$^2H_2$]glucose infusion rate was stepwise adjusted according to a protocol developed in pilot experiments (n = 5), which was then applied in both groups (0–3 min: 100%; 3–8 min: 60%; 8–18 min: 20%; 18–110 min: 10%; 110–180 min: 20%; 180–280 min: 30%; 280–320 min: 50%; 320–380 min: 60%, 380–480 min: 70%). This infusion protocol aimed at minimizing variations of the tracer-to-tracee ratios of endogenous glucose (Fig. 1).

The percent contribution of the direct (glucose → glucose-6-phosphate → UDPglucose → glycogen) and indirect (pyruvate → glucose-6-phosphate → glycogen) pathways to glycogen synthesis was determined using the glucuronide probe technique with 500 mg acetaminophen given orally at 4:30 P.M. (28). From 4:45 P.M. to 1:00 A.M., 22 plasma samples were drawn for measurement of ^{13}C and ^2H glucose enrichments.

Clamp test (protocol C). Type 2 diabetic patients were admitted the evening before the study, and plasma glucose concentrations were normalized overnight using intravenous insulin infusion (Actrapid; Novo Nordisk, Vienna, Austria) (28). Nondiabetic volunteers were admitted at 6:00 A.M. the day of the study.

At 6:30 A.M. (−150 min), a primed-continuous infusion of [6,6-^{2}H]glucose was started for measurement of EGP (28). At 9:00 A.M. (0 min), a hyperglycemic-hyperinsulinemic-pancreatic clamp test was initiated by somatostatin (−5 to 300 min: 0.1 μg · kg^{-1} · min^{-1}; UCB Pharma, Vienna, Austria) and insulin (0–8 min: 80 mU · min^{-1} · m^{-2} body surface area; 8–300 min: 40 mU · min^{-1} · m^{-2} body surface area; Actrapid) infusion. Plasma glucose was raised and maintained at ~180 mg/dl by primed (0.2 g/kg) variable dextrose infusion (20% wt/vol) enriched with [1-^{13}C]glucose (20% wt/wt) and [6,6-$^2H_2$]glucose (2% wt/wt). For assessment of the flux through hepatic glycogen phosphorylase, the [1-^{13}C]glucose-labeled infusion was switched to natural abundance [1-^{12}C]glucose infusion at 150 min (21–23). Hepatic glycogen concentrations were measured before the clamps (−30 to 0 min), from 60 to 150 min and from 210 to 300 min during the clamps with ^{13}C NMRS, as previously published (28,30). Contributions of the direct and gluconeogenic pathways to glycogen synthesis were assessed with 1,000 mg acetaminophen given orally at −30 min (27,28,31). HCL content was measured before the clamp test. During the clamps, plasma samples for measurement of ^{13}C enrichments in glucose and glucuronide and ^2H enrichments in glucose were drawn at 15-min intervals.

Liver ^{13}C and ^1H NMRS. Localized ^{13}C NMR spectra were obtained in the 3T/80 cm Medspec (Bruker Biospin, Ettlingen, Germany) with a 10-cm circular $^{13}C/^1H$ transmitter/receiver coil placed over the lateral aspect of the liver, applying a modified one-dimensional inversion-based sequence (28,30). Absolute glycogen concentrations were quantified by comparing the C1 glycogen peak (100.5 ppm) integral of liver spectra with that of a glycogen standard taken under identical conditions. To eliminate the contribution of [1-^{13}C]glucose to the [1-^{13}C]glycogen spectral line intensity during labeled glucose infusion in protocol C, only the left half of the [1-^{13}C]glycogen doublet in the spectra was integrated. Corrections for loading of the coil and sensitive volume of the coil were performed (28).

Localized ^1H NMR spectra of the liver were acquired using the same magnetic resonance system, coil, and patient placement inside the magnet applying the breath-hold–triggered stimulated echo acquisition mode sequence (7). HCL content was quantified by integration of the CH_2 and CH_3 group resonances and is expressed in arbitrary units (AU) as a percentage of total ^1H NMRS signals (water + lipid).

Analytical procedures. Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Fullerton, CA). Plasma FFAs were assayed microfluorimetrically (Wako Chemicals, Richmond, VA). Plasma immunoreactive insulin, C-peptide, and glucagon were measured by commercially available radioimmunoassays (24,32,33). Atom percent excess of ^2H and ^{13}C in plasma glucose and ^{13}C atom percent excess in plasma acetaminophen glucuronide were quantified with gas and liquid chromatography–mass spectroscopy, respectively (28).

Calculations

Hepatic glycogen metabolism. Rates of net hepatic glycogen synthesis and subsequent net glycogenolysis during protocol A were calculated from linear regression of the individual glycogen concentration time curves over respective time periods of increasing (6:00 to ~9:00 P.M. in CON and 6:00–10:30 P.M. in type 2 diabetic patients) and decreasing (10:00 P.M. to 8:00 A.M.) glycogen concentrations (28,30). The time point of the peak glycogen concentration

was calculated from the intersection of regression lines of increasing and decreasing glycogen concentrations.

In protocol C, flux through hepatic glycogen synthase (V_{Syn}) was assessed using previously published equations (21–23,34) from linear regression of the increments in hepatic glycogen concentration. These increments were calculated in 7-min intervals between 60 and 150 min of [1-^{13}C]glucose infusion from the increase of the [1-^{13}C]glycogen peak intensity, corrected by actual ^{13}C plasma glucose enrichment above the natural abundance and by the contribution of the direct glycogen synthesis pathway. The percent contribution of the direct pathway to glycogen synthesis was calculated as the ratio between differences in ^{13}C enrichments at the C1 and C6 positions of glucuronide and glucose (28).

Simultaneous flux through hepatic glycogen phosphorylase (V_{Out}) was calculated by subtracting the observed from the predicted change in [1-^{13}C]-glycogen concentrations during the chase period (210–300 min), assuming constant synthase flux and highest possible ^{13}C enrichment in the C1 glycogen (21–23,34). Relative glycogen turnover (%) was calculated as ($V_{Out} \times 100$)/V_{Syn}. The rate of net hepatic glycogen synthesis (V_{SynNet}) was determined as the difference between the synthase (V_{Syn}) and simultaneous phosphorylase (V_{Out}) flux (21–23,34). All rates describing hepatic glycogen metabolism were converted from mmol/l liver per minute into mg · kg^{-1} · min^{-1} by using individual data on liver volume and body weight, which did not affect the results of the statistical analyses.

Rates of gluconeogenesis (protocols A and B, 11:00 P.M. to 1:00 A.M.) were calculated as the difference between the mean individual rates of EGP and net hepatic glycogenolysis and are given as percent contribution to EGP (30).

EGP protocol B. The plasma glucose concentration after a meal is the sum of exogenous (meal-derived) and endogenous (due to EGP) glucose concentrations. The exogenous glucose can be calculated on the basis of its proportionality to meal-derived [1-^{13}C]glucose. Subtracting exogenous glucose from total plasma glucose concentration yields the endogenous glucose concentration. Rates of appearance of endogenous glucose (EGP) were quantified with a variable infusion double-tracer technique (6,27) and calculated from plasma [6,6-$^{2}H_2$]glucose concentrations and endogenous glucose concentrations using Steele's non–steady state equation (35). EGP is derived by first expressing the disappearance rate parameter, k_{01}, from the mass balance equation of [6,6-$^{2}H_2$]glucose tracer and then using it in the mass balance of endogenous glucose (29). The time course of [6,6-$^{2}H_2$]glucose concentration and the ratio of [6,6-$^{2}H_2$]glucose to endogenous glucose concentration were smoothed using an algorithm based on stochastic nonparametric deconvolution (36).

Protocol C. Basal rates of EGP were calculated by dividing the tracer ([6,6-$^{2}H_2$]glucose) infusion rate times tracer enrichment by the tracer enrichment in plasma glucose and subtracting the tracer infusion rate. Rates of EGP during the clamp test were calculated for 15-min intervals using Steele's non–steady-state equation to calculate the rate of appearance of overall glucose and subtracting the mean glucose infusion rate of that 15-min interval from the overall rate of appearance of glucose (37).

Whole-body glucose metabolism. During protocol C, insulin-stimulated whole-body glucose disposal was calculated from glucose infusion rates and [6,6-$^{2}H_2$]glucose dilution, both corrected for EGP (1).

Statistical analysis. Data are given as means ± SE. Student's two-tailed unpaired t test was used for group comparison. Two-way repeated-measures ANOVA and Bonferroni/Dunnet post hoc test were used for statistical analysis of the time course EGP. Because metabolites and hormones did not differ within the groups between the protocols, individual data of every participant at each time point from both protocols were pooled for presentation. Statistical significance was considered at $P < 0.05$.

RESULTS

Mixed-meal test. Plasma glucose concentrations were ~69% higher in type 2 diabetic patients before dinner (150 ± 12 vs. CON: 89 ± 2 mg/dl, $P < 0.001$) (Fig. 2*A*) and remained elevated throughout the study. Plasma FFA concentrations were less suppressed in type 2 diabetic patients after dinner (mean 60–240 min: 255 ± 21 vs. CON: 115 ± 14 μmol/l, $P < 0.001$) (Fig. 2*B*). In type 2 diabetic patients, plasma insulin was higher before (11 ± 1 vs. CON: 6 ± 1 μU/ml, $P < 0.01$), but was lower after the dinner (120 min; 36 ± 5 vs. CON: 93 ± 18 μU/ml, $P < 0.01$), whereas plasma glucagon was higher in type 2 diabetic patients (30 min: 108 ± 4 vs. CON: 82 ± 6 pg/ml, $P < 0.01$) (Fig. 2*C* and *D*).

Hepatic glycogen concentrations were lower in type 2 diabetic patients before dinner (type 2 diabetic patients: 227 ± 6 vs. CON: 275 ± 10 mmol/l, $P < 0.001$, Fig. 3*A*) and remained unchanged for 1 h after dinner (type 2 diabetic patients: 227 ± 10 vs. CON: 265 ± 11 mmol/l, $P < 0.001$). Liver glycogen increased afterward to mean individual peak values of 283 ± 15 mmol/l in type 2 diabetic patients and 360 ± 11 mmol/l in CON ($P < 0.01$), with a time lag of ~55 min between the groups (calculated peak time; type 2 diabetic patients: 302 ± 15 min vs. CON: 247 ± 17 min; $P < 0.05$). The difference between lowest individual preprandial and highest individual postprandial glycogen concentration was 86 ± 7 mmol/l in CON and 56 ± 12 mmol/l in type 2 diabetic patients ($P < 0.05$). Rates of net glycogen synthesis (Fig. 4*A*) were ~44% lower in type 2 diabetic patients (0.76 ± 0.16 vs. CON: 1.36 ± 0.15 mg · kg^{-1} · min^{-1}, $P < 0.05$). The direct pathway contributed similarly to glycogen synthesis in both study groups (type 2 diabetic patients: 60 ± 4% vs. CON: 56 ± 2%, $P = 0.514$). Overnight, hepatic glycogen concentrations decreased linearly, reaching values of ~215 mmol/l in both groups in the morning (Fig. 3*A*). Rates of net glycogenolysis were ~50% lower in type 2 diabetic patients (0.37 ± 0.08 vs. CON: 0.75 ± 0.09 mg · kg^{-1} · min^{-1}, $P < 0.01$) (Fig. 4*A*).

Before dinner, EGP was ~13% higher in type 2 diabetic patients (1.95 ± 0.07 vs. CON: 1.73 ± 0.07 mg · kg^{-1} · min^{-1}, $P < 0.05$) (Fig. 4*B*). After dinner, EGP decreased in both groups but remained ~0.3 mg · kg^{-1} · min^{-1} higher in type 2 diabetic patients from 30–170 min (0.98 ± 0.11 vs. CON: 0.68 ± 0.05 mg · kg^{-1} · min^{-1}, $P < 0.05$). Time course of EGP reached the nadir in CON at 60–90 min, but only at 240 min in type 2 diabetic patients, i.e., the maximum suppression of EGP occurred delayed in type 2 diabetic patients. Thereafter, EGP was similar in both groups and returned to ~60–70% of predinner values at 480 min. Rates of postabsorptive gluconeogenesis in the time period of 11:00 P.M. to 1:00 A.M. represented 67 ± 8% of EGP in type 2 diabetic patients but only 43 ± 4% ($P < 0.05$) of EGP in CON.

Clamp tests. During the hyperglycemic-hyperinsulinemic clamp tests, plasma glucose, FFAs, insulin, and glucagon were not different between both groups (Fig. 2*E–H*). Whole-body glucose uptake was ~37% lower in type 2 diabetic patients (180–300 min: type 2 diabetic patients: 8.03 ± 0.35 mg · kg^{-1} · min^{-1} vs. CON: 12.73 ± 0.48 mg · kg^{-1} · min^{-1}; $P < 0.05$).

Before the clamp test, both study groups exhibited similar hepatic glycogen concentrations (type 2 diabetic patients: 218 ± 10 vs. CON: 234 ± 9 mmol/l, $P = 0.28$, Fig. 3*B*). During the clamp, hepatic glycogen concentrations increased linearly in both groups (Fig. 3*B*), but V_{Syn} was ~46% lower in type 2 diabetic patients (0.63 ± 0.12 vs. CON: 1.17 ± 0.15 mg · kg^{-1} · min^{-1}, $P < 0.05$), with similar contribution of the direct pathway in both groups (type 2 diabetic patients: 60 ± 10% vs. CON: 65 ± 2%, $P = 0.416$). Simultaneous V_{Out} (Fig. 5*A*) was not different (type 2 diabetic patients: 0.21 ± 0.07 vs. CON: 0.23 ± 0.11 mg · kg^{-1} · min^{-1}, $P = 0.895$), resulting in ~54% lower rates of net hepatic glycogen synthesis (Fig. 5*A*) in type 2 diabetic patients (0.42 ± 0.10 vs. CON: 0.91 ± 0.16 mg · kg^{-1} · min^{-1}, $P < 0.05$). Relative hepatic glycogen turnover was

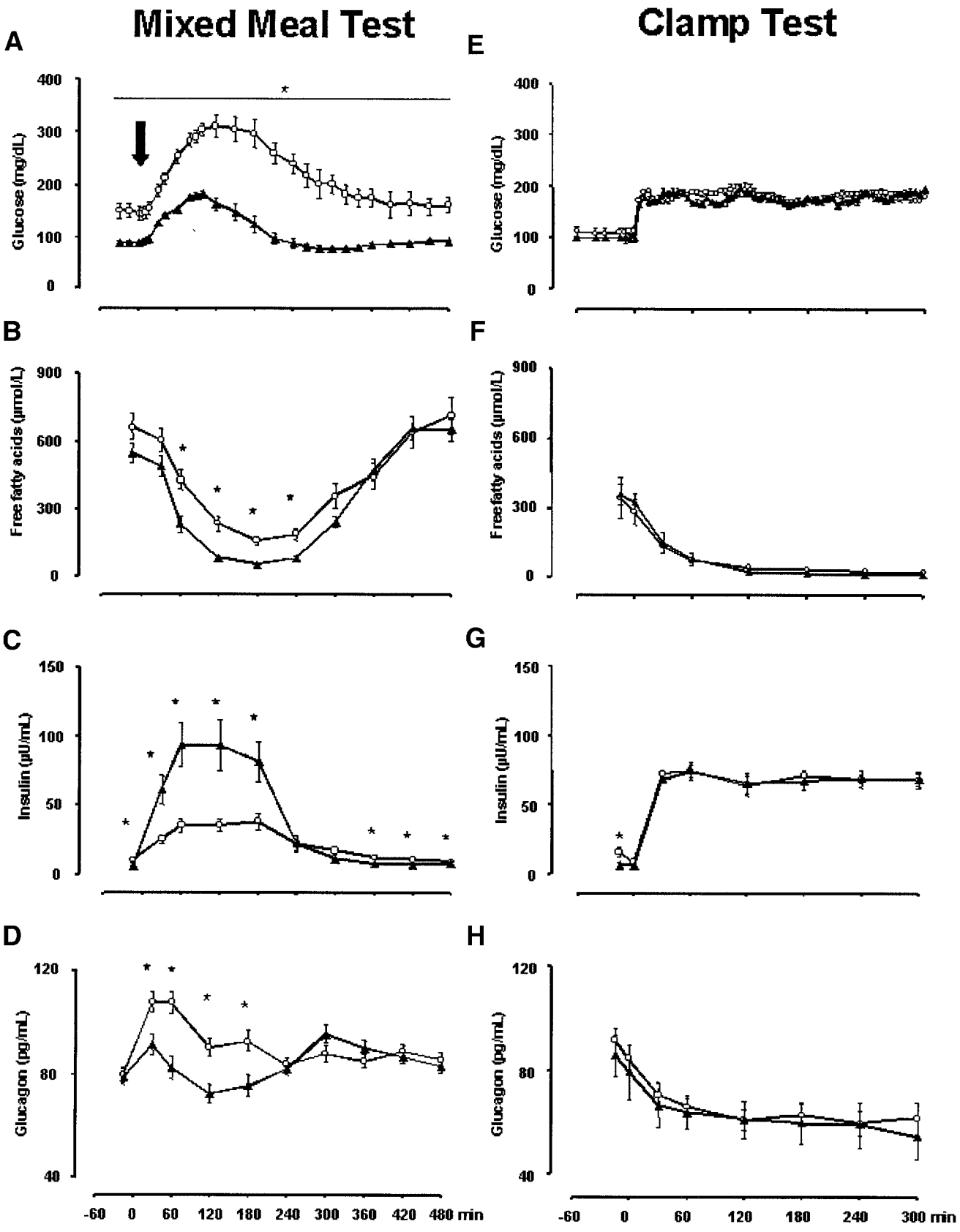

FIG. 2. Plasma concentrations of glucose, FFAs, and major glucoregulatory hormones in type 2 diabetic patients (○) and CON (▲) after ingestion of a mixed-meal dinner (arrow) (*A–D*) and during hyperglycemic-hyperinsulinemic-somatostatin clamp tests (*E–H*). *$P < 0.05$ for type 2 diabetic patients vs. CON.

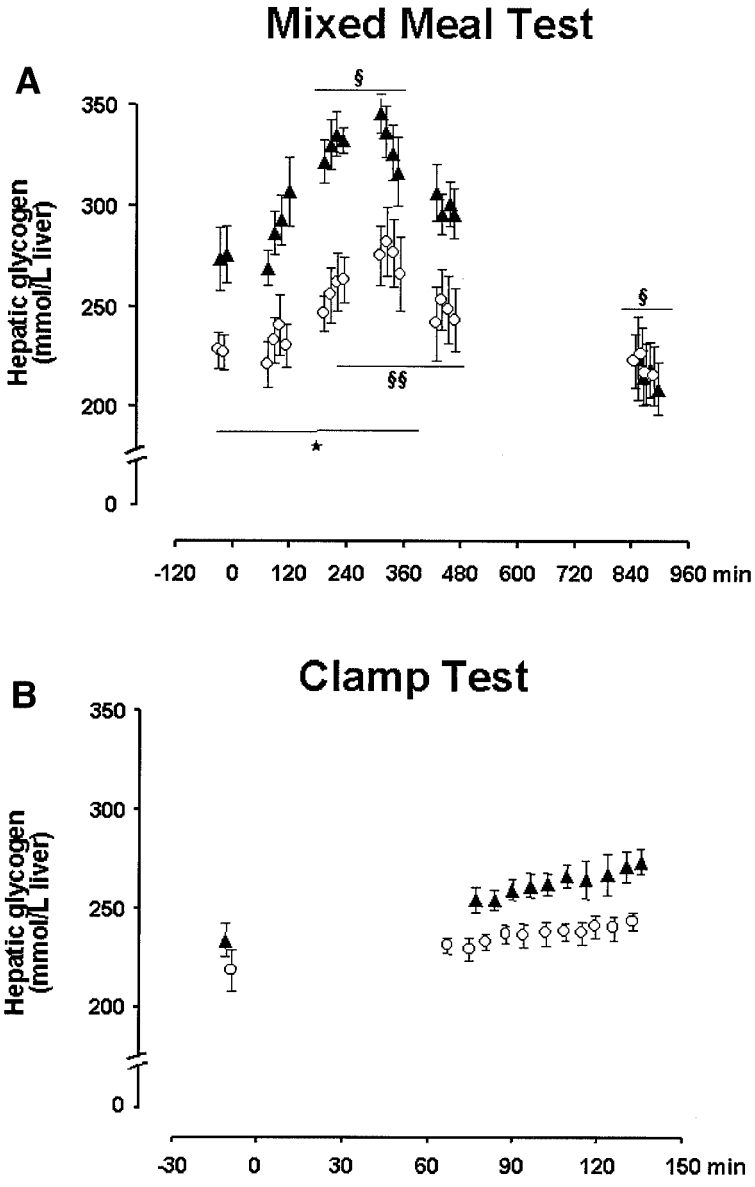

FIG. 3. Time course hepatic glycogen concentration during a mixed-meal test (*A*) (protocol A, −120 to 960 min) and during the pulse period of the clamp test (*B*) (protocol C, −30 to 150 min) in type 2 diabetic patients (○) and CON (▲). *A*: **P* < 0.05, type 2 diabetic patients vs. CON; §*P* < 0.05 vs. basal in CON; §§*P* < 0.05 vs. basal in type 2 diabetic patients. *B*: Mean Pearson coefficient for linear regression of measured hepatic glycogen concentrations versus time was *R* = 0.96 ± 0.01 in CON and *R* = 0.77 ± 0.12 in type 2 diabetic patients.

slightly but not significantly increased in type 2 diabetic patients (31 ± 11% vs. CON: 19 ± 11%, $P = 0.460$).

EGP was ~30% higher in type 2 diabetic patients before the clamp (type 2 diabetic patients: 2.38 ± 0.10 vs. CON: 1.83 ± 0.05 mg · kg^{-1} · min^{-1}, $P < 0.01$) and remained so during the clamp (120–300 min: type 2 diabetic patients: 0.53 ± 0.05 vs. CON: 0.04 ± 0.04 mg · kg^{-1} · min^{-1}, $P < 0.02$) (Fig. 5*B*).

Hepatocellular lipid content was three times higher in type 2 diabetic patients (9.9 ± 2.5 AU vs. CON: 2.8 ± 0.7 AU, $P < 0.05$) and negatively correlated across the whole study population with rates of net hepatic glycogen synthesis ($R = -0.602$, $P < 0.05$) as well as with rates of whole-body glucose uptake ($R = -0.576$, $P < 0.05$) during the clamp test.

DISCUSSION

This study provides the time course of cumulative glycogen storage and rates of net hepatic glycogen synthesis in type 2 diabetic patients under physiological conditions of mixed-meal ingestion as well as under that of experimental stimulation of glycogen accumulation. Postprandial glycogen synthesis is reduced in mildly overweight type 2 diabetic patients and is accompanied by a temporarily impaired EGP suppression. Because these defects of postprandial hepatic glucose metabolism of type 2 diabetic patients were observed during decreased insulin-to-glucagon ratios and impaired FFA suppression, we also assessed hepatic glucose metabolism under conditions of hyperinsulinemic hyperglycemia combined with low glucagon and FFA concentrations. Under these conditions, glycogen synthesis and suppression of EGP remained clearly impaired in type 2 diabetic patients.

Postprandial conditions. After dinner, glycogen accumulation rose by ~86 mmol/l above preprandial concentration in CON in ~4.5 h, accounting for storage of ~26 g carbohydrates in the liver, which is similar to previous findings in nondiabetic humans (27). In type 2 diabetic patients, glycogen concentrations rose only by 56 mmol/l liver within ~5.5 h after meal ingestion, resulting in storage of ~17 g carbohydrates in the liver. Thus, type 2 diabetic patients exhibit reduction of net hepatic glycogen

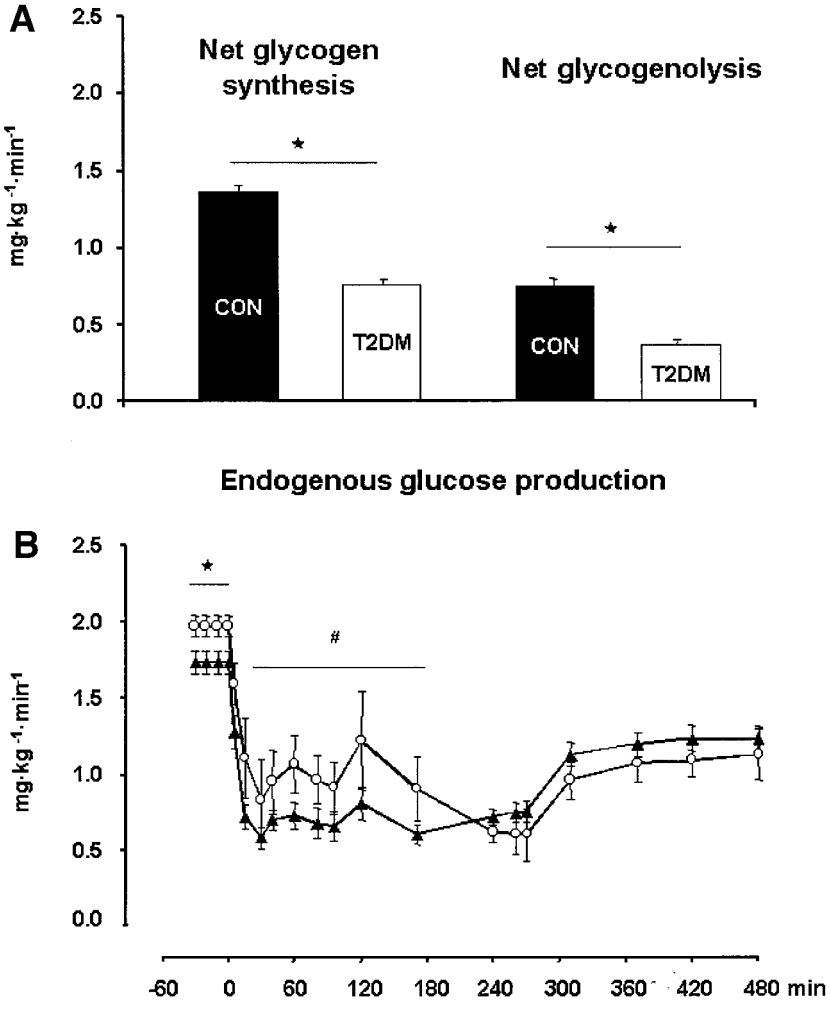

FIG. 4. Rates of net hepatic glycogen synthesis and subsequent net hepatic glycogenolysis (*A*) and time course of EGP (*B*) in type 2 diabetic patients (T2DM) (○) and CON (▲) after ingestion of a mixed-meal dinner at zero time. **P* < 0.01, #*P* < 0.05, type 2 diabetic patients vs. CON.

accumulation by ~35%, suggesting that decreased hepatic glycogen storage contributes to their postprandial hyperglycemia.

A similar defect was observed in glucokinase-deficient maturity-onset diabetes of the young (MODY)-2 patients (38), in whom the impaired hepatic glucokinase activity is held responsible for the reduction in the contribution of glucose (the direct pathway) to hepatic glycogen synthesis (38). Of note, in the present study, the relative contribution of the direct pathway was unchanged in type 2 diabetic patients after the meal as well as during the clamp test. Previously, lower contributions of the direct pathway were reported (15,39), which could be due to the metabolic characteristics of these type 2 diabetic patients (higher HbA_{1c} and BMI) and lower insulin-to-glucagon ratios compared with the present study. In agreement with previous studies (18,19), type 2 diabetic patients also exhibited decreased net glycogenolysis and increased contribution of gluconeogenesis to glucose production during the postabsorptive period.

It is well known that EGP correlates with the degree of hyperglycemia after overnight fasting (4–8). Here we report that EGP of type 2 diabetic patients is also increased in the afternoon after a standardized breakfast and lunch, which is in contrast to normalized rates of EGP of type 2 diabetic patients after prolonged fasting (8). After dinner, EGP was rapidly suppressed in both groups but remained ~0.3 $mg \cdot kg^{-1} \cdot min^{-1}$ higher during the first 3 h in type 2 diabetic patients, i.e., the maximum suppression of EGP occurs later in type 2 diabetic patients, providing for an additional ~5 g glucose released into the circulation, thus also contributing to postprandial hyperglycemia. This time course of EGP suppression is similar to previous reports (6,10,29). Earlier studies, first describing higher rates of postprandial EGP in type 2 diabetic patients (10–12), observed more pronounced defects of EGP suppression. These differences can probably be attributed to methodological uncertainties in the assessment of metabolite fluxes under rapidly changing conditions in the classic double-tracer approach, which can be reduced by the variable infusion double-tracer technique (27,29,40).

After meal ingestion, type 2 diabetic patients had lower plasma insulin but higher glucagon and FFA concentrations than CON, as previously reported (10–12,41). Increased plasma FFAs impair EGP suppression (42) and affect autoregulation of hepatic glucose metabolism (24). The molar portal vein insulin-to-glucagon ratio, as estimated from peripheral concentrations (43,44), was ~50% lower after the meal in type 2 diabetic patients. Additionally to a possible impairment of hepatic insulin sensitivity, the lower insulin-to-glucagon ratio could have contributed to the decreased net glycogen synthesis (21,23) and EGP suppression (20,45). On the other hand, type 2 diabetic patients with markedly higher postprandial insulin secre-

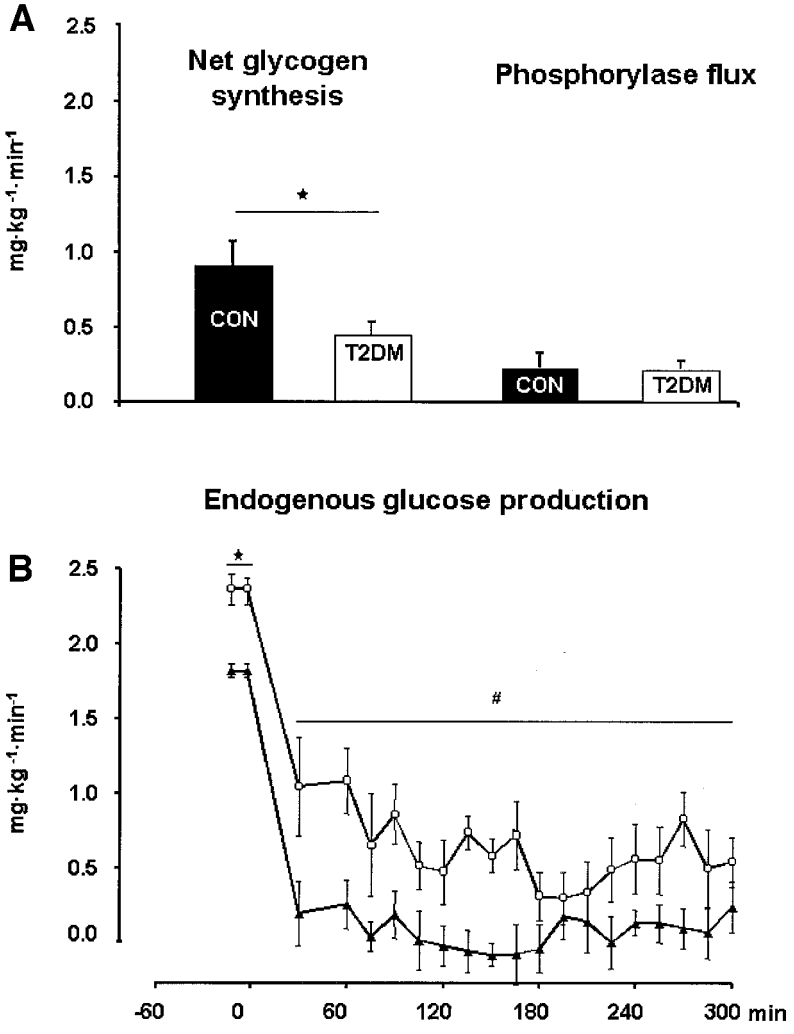

FIG. 5. V_{SynNet}, V_{Out} (*A*), and time course of EGP (*B*) in type 2 diabetic (T2DM) (○) and CON (▲) during hyperglycemic-hyperinsulinemic-somatostatin clamp tests. **P* < 0.01, #*P* < 0.05, type 2 diabetic patients vs. CON.

tion exhibit a comparable defect of EGP suppression (6). Thus, excessive postprandial hyperglycemia per se limited observed defects in hepatic glucose metabolism (46,47). Taken together, impaired net hepatic glycogen synthesis and insufficient EGP suppression resulted in decreased hepatic glucose uptake and increased amounts of glucose released into the circulation in type 2 diabetic patients.

Hyperglycemic-hyperinsulinemic clamp conditions. The decreased net hepatic glycogen synthesis of type 2 diabetic patients after meal ingestion can result from two distinct mechanisms: *1*) decreased V_{Syn} and/or *2*) simultaneously increased V_{Out} (21,23,48). Previous reports demonstrated that hyperglycemia per se does not markedly stimulate net hepatic glycogen synthesis, even in young healthy men (21,23). In addition, hyperinsulinemia per se is capable of stimulating hepatic glucose uptake in humans to some extent (23,25), and stepwise increases in the portal vein insulin concentration during hyperglycemia lead to an insulin concentration–dependent increase in net hepatic glycogen synthesis (21). Thus, we assumed that combined hyperglycemia and hyperinsulinemia are required to substantially stimulate net hepatic glycogen synthesis in the presence of hypoglucagonemia.

To evaluate hepatic glucose metabolism independently of type 2 diabetes–associated alterations in postprandial metabolite and hormonal responses, glycogen metabolism, as well as rates of EGP and whole-body glucose disposal, were assessed under matched conditions of hyperglycemia, portal vein insulin-to-glucagon ratios, and similar plasma FFA concentrations.

V_{SynNet} values observed in CON are in line with those obtained in nondiabetic humans under similar conditions (21–23). In type 2 diabetic patients, we observed ~54% reduced rates of net hepatic glycogen synthesis. Because the V_{Out} was comparable between type 2 diabetic patients and CON, the lower V_{SynNet} values are entirely explained by ~46% reduced V_{Syn} in type 2 diabetic patients, which under clamp conditions, cannot be explained by short-term differences in the metabolic or hormonal environment between type 2 diabetic patients and CON (Fig. 1). The comparable phosphorylase flux supports previous findings of similar hepatic glucagon sensitivity in type 2 diabetic patients and nondiabetic humans (49,50). During the hyperglycemic-hyperinsulinemic pancreatic clamp test, rates of EGP also remained higher in type 2 diabetic patients, which is in line with persistent decreased insulin sensitivity at the level of the liver (4,10).

In line with previous reports (7,26,51), HCL content was increased in type 2 diabetic patients and inversely correlated to insulin-stimulated whole-body glucose uptake during the clamp test. Furthermore, HCL content inversely correlated with V_{SynNet}, suggesting that HCL content can

serve as a marker not only of peripheral but also of hepatic insensitivity to insulin action.

During the clamp test, the ~40% reduction in rates of whole-body glucose uptake indicated peripheral insulin resistance in type 2 diabetic patients. Of note, V_{SynNet} contributed by ~6% and ~8% to whole-body glucose disposal in type 2 diabetic patients and CON, respectively, supporting the contention that skeletal muscle accounts for ~90% of whole-body glucose disposal under clamp conditions (1). Because both glucose disposal and net hepatic glycogen synthesis were similarly decreased by 40–50% in type 2 diabetic patients of our study and skeletal muscle glycogen synthesis is reduced by 55% in type 2 diabetic patients under clamp conditions (1), glycogen synthesis seems to be equally insensitive to insulin in liver and skeletal muscle.

In contrast to current knowledge of the importance of skeletal muscle insulin resistance in the development of overt type 2 diabetic patients, there is still debate on the causality between the defects in hepatic glucose metabolism and the onset of type 2 diabetic patients. There are some arguments in favor of the hypothesis that the defects in hepatic glycogen metabolism are secondary to type 2 diabetic patients and could result from effects of acute and/or chronic hyperglycemia, increased availability of lipids, and alterations of secretion of major glucoregulatory hormones. Both the blunted rise of insulin-to-glucagon ratios and higher plasma FFA concentrations likely contributed to the impaired net hepatic glycogen synthesis in type 2 diabetic patients during the mixed-meal study. When insulin, glucagon, glucose, and FFA concentrations were successfully matched during the clamp protocol, hepatic glycogen synthesis was also impaired, indicating an additional defect aside from the effects of metabolic and hormonal differences. Moreover, the impact of chronic changes in glycemia and lipidemia, frequently termed "glucolipotoxicity," on liver metabolism cannot be completely excluded from the results of the clamp test.

Chronically increased availability of both circulating (FFAs and triglycerides) and intracellular (HCL) lipids are frequently observed in different nondiabetic insulin-resistant populations as well as in type 2 diabetic patients (52,53) and are associated with defects in hepatic and/or whole-body glucose metabolism (26,52–55). Thus, increased HCL content in type 2 diabetic patients and its correlation with net hepatic glycogen synthesis as observed in our study indicates that fat accumulation in liver could play a role in the development of defects of hepatic glycogen metabolism in type 2 diabetic patients.

In conclusion, we show that type 2 diabetic patients exhibit a marked reduction of net hepatic glycogen synthesis after mixed-meal ingestion. Furthermore, even stimulation of glycogen synthesis by combined hyperinsulinemic hyperglycemia is unable to overcome this defect of hepatic glycogen metabolism. Thus, impaired insulin sensitivity and/or chronic glucolipotoxicity in addition to any effects of altered insulin-to-glucagon ratio or increased FFA accounts for defective hepatic glucose metabolism in type 2 diabetic patients.

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