The Roles of Insulin and Glucagon in the Regulation of Hepatic Glycogen Synthesis and Turnover in Humans

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Abstract

To determine the respective roles of insulin and glucagon for hepatic glycogen synthesis and turnover, hyperglycemic clamps were performed with somatostatin [0.1 μg/(kg · min)] in healthy young men under conditions of: (I) basal (fasting) portal vein insulinemia-hypoglucagonemia, (II) basal portal vein insulinemia-basal glucagonemia, and (III) basal peripheral insulinemia-hypoglucagonemia. Synthetic rates, pathway (direct versus indirect) contributions, and percent turnover of hepatic glycogen were assessed by in vivo ^{13}C nuclear magnetic resonance spectroscopy during [1-^{13}C]glucose infusion followed by a natural abundance glucose chase in conjunction with acetaminophen to noninvasively sample the hepatic UDP-glucose pool. In the presence of hyperglycemia (10.4±0.1 mM) and basal portal vein insulinemia (192±6 pM), suppression of glucagon secretion (plasma glucagon, I: 31±4, II: 63±8 pg/ml) doubled the hepatic accumulation of glycogen (V_{syn}) compared with conditions of basal glucagonemia [I: 0.40±0.06, II: 0.19±0.03 mmol/(liter · min); $P < 0.0025$]. Glycogen turnover was markedly reduced (I: 19±7%, II: 69±12%; $P < 0.005$), so that net rate of glycogen synthesis increased approximately fivefold ($P < 0.001$) by inhibition of glucagon secretion. The relative contribution of gluconeogenesis (indirect pathway) to glycogen synthesis was lower during hypoglucagonemia (42±6%) than during basal glucagonemia (54±5%; $P < 0.005$). Under conditions of basal peripheral insulinemia (54±2 pM) and hypoglucagonemia (III) there was negligible hepatic glycogen synthesis and turnover. In conclusion, small changes in portal vein concentrations of insulin and glucagon independently affect hepatic glycogen synthesis and turnover. Inhibition of glucagon secretion under conditions of hyperglycemia and basal concentrations of insulin results in: (*a*) twofold increase in rate of hepatic glycogen synthesis, (*b*) reduction of glycogen turnover by ∼ 73%, and (*c*) augmented percent contribution of the direct pathway to glycogen synthesis compared with conditions of basal glucagonemia. (*J. Clin. Invest.* 1996. 97:642–648.) Key words: gluconeogenesis • somatostatin • acetaminophen • ^{13}C NMR spectroscopy

Introduction

After glucose ingestion, hepatic glycogen synthesis is a major fate for the glucose that is taken up by the liver (1) and defects in hepatic glycogen synthesis contribute to postprandial hyperglycemia of patients with poorly controlled insulin-dependent diabetes mellitus (IDDM)[1] (2). Both insulin deficiency and relative hyperglucagonemia may lead to these abnormalities of liver glycogen metabolism (3). Hepatic glycogen synthesis has been widely investigated in vitro (4–7), but because of the difficulties in examining this process in awake animals there are relatively little in vivo data (8). Before the advent of ^{13}C nuclear magnetic resonance (NMR) spectroscopy (9), measurements of hepatic glycogen content in humans by biopsy were limited to only a few time points (10), making detailed studies examining the hormonal regulation of hepatic glycogen synthesis in humans untenable.

The aims of this study were to evaluate the role of physiological glucagon concentrations for liver glycogen metabolism under conditions of hyperglycemia and low insulin concentrations to mimic the metabolic situation occurring in IDDM as well as to test whether or not suppression of glucagon secretion might augment hepatic glycogen synthesis and thereby offer a therapeutic option for patients with IDDM. To this end, ^{13}C NMR methods were applied to noninvasively measure hepatic glycogen contents in humans and to compare the effects of basal versus somatostatin (SRIF)-suppressed glucagon concentrations during hyperglycemia and basal peripheral or portal vein insulin concentrations on: (*a*) net hepatic glycogen synthesis, (*b*) glycogen turnover, i.e., simultaneous synthesis and breakdown of hepatic glycogen, and (*c*) the relative contributions of the direct (glucose → glucose-6-phosphate → glucose-1-phosphate → UDP-glucose → glycogen) and the indirect/gluconeogenic (C3 compounds → glucose-6-phosphate → glucose-1-phosphate → UDP-glucose → glycogen) pathway to hepatic glycogen synthesis. Infusion of [1-^{13}C]-enriched glucose was followed by a chase period with unenriched (natural abundance) glucose to estimate the rates of hepatic glycogen synthesis and turnover (11). To examine the pathway (direct versus indirect) contributions of hepatic glycogen synthesis, acetaminophen was used to noninvasively sample the intrahepatic UDP-glucose pool (12, 13).

Methods

Subjects. Eight healthy men (mean age 27±2 yr, mean body wt 77±2 kg, mean body mass index 24.1±0.5 kg/m², glycosylated hemoglobin 6.2±0.3%) with body weights between 87 and 105% of ideal body wt

(based upon 1983 Metropolitan Life Insurance tables) were studied during two experimental protocols (I and II), and three of them underwent protocol III. None of them had a family history of diabetes mellitus or any major diseases or was taking medication. Insulin dose–response experiments were performed in another three healthy men (mean age 24 ± 2 yr, mean body wt 78 ± 9 kg, mean body mass index 24.1 ± 2.3 kg/m^2, glycosylated hemoglobin $5.4\pm0.2\%$). The experimental protocols were reviewed and approved by the Human Investigation Committee of the Yale University School of Medicine, and informed consent was obtained from all subjects.

Experimental design. The subjects were given an isocaloric (33 kcal/kg body wt daily) diet consisting of 60% carbohydrate, 20% protein, and 20% fat prepared by the metabolic kitchen of the Yale/New Haven Hospital General Clinical Research Center for 3 d before the study. On the day before the study the subjects were admitted to the Clinical Research Center. After an overnight fast (12–14 h), one Teflon catheter was inserted in an antecubital vein for infusion of glucose and hormones at 6:30 a.m. Another catheter was placed in an antecubital vein of the opposite arm to allow blood withdrawal. The experiments were begun at 8:00 a.m. The different parts of this study were performed in random order and spaced by intervals of 3–12 wk.

Hyperglycemic clamp. Hyperglycemia was induced by the glucose clamp technique as described previously (14, 15). To inhibit endogenous insulin and glucagon secretion, an SRIF infusion [0.1 μg/(kg body wt · min)] was initiated 5 min before the start of the glucose-insulin infusion. Starting with time zero, insulin (Humulin-R; Eli Lilly & Co., Indianapolis, IN) was administered as a primed-continuous [0.6 mU/(kg body wt · min)] or continuous [0.15 mU/(kg body wt · min)] infusion to establish basal (fasting) portal vein or peripheral insulin concentrations, respectively. To define the dose–response relationship three subjects received primed-continuous insulin infusions at a rate of 0.3, 0.5, or 0.7 mU/(kg body wt · min), respectively. Simultaneously with the start of the insulin infusion, a prime-variable infusion of [1-^{13}C]glucose (20% enriched) was initiated to acutely raise and maintain the plasma glucose concentration by ~ 5 mM above baseline. At $t = 160$ min the infusate was switched to natural abundance glucose which was used as the infusate until the end of the experiment ($t = 245$ min). During protocol II [insulin dose: 0.6 mU/(kg body wt · min)], glucagon (Eli Lilly & Co.) was infused continuously [0.7 ng/(kg body wt · min)] starting with time zero to create conditions of basal plasma glucagon concentrations. This resulted in three experimental protocols: basal portal vein insulinemia-hypoglucagonemia (protocol I, $n = 8$), basal portal vein insulinemia-basal glucagonemia (protocol II, $n = 8$), and basal peripheral insulinemia-hypoglucagonemia (protocol III, $n = 3$). Based on the results of negligible glycogen accumulation and turnover, protocol III was discontinued after three experiments. All subjects ingested 1.5 grams of acetaminophen 5 min before the start of each glucose clamp test.

Plasma glucose concentrations were measured at 5-min intervals during the first hour and then every 7 or 8 min until the end of the clamp. The variable infusion of glucose (either ^{13}C-enriched or natural abundance) was adjusted to maintain the desired hyperglycemic plateau. Mean glucose infusion rates were calculated for 20-min intervals and corrected for urinary glucose loss.

Blood samples were taken every 15 min for determination of plasma insulin and glucagon concentrations and for gas chromatography-mass spectrometry (GC-MS) analysis of ^{13}C isotopic enrichment in plasma glucose and plasma acetaminophen-glucuronide. Urine was collected from 60 to 155 min and 155 to 245 min for isolation of acetaminophen-glucuronide.

In vivo ^{13}C NMR spectroscopic techniques. To assess rates of synthesis and breakdown of hepatic glycogen ^{13}C NMR spectra were acquired from 60 to 155 min and from 185 to 245 min. The measurements were limited to these time periods to minimize the subject discomfort resulting from lying motionless inside the 2.1-T 1-m bore spectrometer (Biospec I; Bruker Instruments, Inc., Billerica, MA). The ^{13}C NMR signals were obtained with a 9-cm circular ^{13}C observation coil and a 12 × 14-cm coplanar butterfly ^{1}H-decoupler coil

placed rigidly over the lateral aspect of the liver in the supine subject, as described previously (9). Briefly, the position of the coil over the liver was initially determined by percussion of the liver borders and then confirmed by imaging the liver from the surface coil with a multislice gradient echo image. The magnetic field homogeneity was optimized with the water signal obtained from the decoupling coil. Localized ^{13}C NMR liver spectra were obtained with a modified one-dimensional inversion-based sequence for surface suppression. Each spectrum consisted of 6,400 scans and required 15 min of signal averaging. Liver glycogen was quantified by integration of the C1 glycogen peak at 100.5 ppm using the same frequency bandwidth (250 Hz) for all spectra. The absolute concentration of hepatic glycogen was determined by comparing the peak integral (PI) of the C1 liver glycogen peak with that of a glycogen standard taken under identical conditions.

Analytical procedures. Plasma glucose concentrations were measured by the glucose oxidase method (Glucose analyzer II; Beckman Instruments, Inc., Fullerton, CA). Plasma immunoreactive insulin, glucagon, and C-peptide were measured using commercially available double-antibody radioimmunoassay kits (insulin: Diagnostic Systems Laboratories, Inc., Webster, TX; glucagon: Linco Research Inc., St. Charles, MO; C-peptide: Diagnostic Products Corp., Los Angeles, CA). Plasma lactate concentrations were measured by using the lactate dehydrogenase method (16). Plasma free fatty acid concentrations were determined using a microfluorimetric method (17). Plasma amino acid concentrations were measured by an automated amino acid analyzer (Dionex Corp., Sunnyvale, CA).

Plasma glucose and acetaminophen-glucuronide were derivatized to allow determination of ^{13}C atom percent enrichments (APE) by GC-MS. Plasma glucose was derivatized as the pentaacetate, after Ba(OH)$_2$/ZnSO$_4$ deproteinization and semipurification by anion/cation exchange chromatography (AG1-X8, AG50W-X8; Bio Rad Laboratories, Hercules, CA), as described previously (18). Plasma acetaminophen-glucuronide was derivatized by a modification of that used for amino acids (13). Plasma was deproteinized with Ba(OH)$_2$/ZnSO$_4$, the supernatant was freeze-dried, and the glucuronide moiety was derivatized as the *n*-butyl ester, triacetate.

For determination of ^{13}C isotopic enrichments by NMR, urine samples were freeze-dried and redissolved in water, and urine acetaminophen-glucuronide was semipurified by anion exchange chromatography (AG1-X8, 100–200 mesh, acetate form) as described previously (19). The purified acetaminophen-glucuronide was finally dissolved in a small volume of D$_2$O for NMR analysis.

GC-MS analysis. GC-MS analysis was performed with a Hewlett-Packard 5890 gas chromatograph (HP-1 capillary column, 12 m × 0.2 mm × 0.33 μm film thickness) interfaced to a Hewlett-Packard 5971A Mass Selective Detector operating in the positive chemical ionization mode with methane as reagent gas. For glucose pentaacetate and acetaminophen-glucuronide (*n*-butyl ester, triacetate), GC conditions were isothermal at 200°C. Ions with m/z 331, 332, and 333 were monitored for glucose C1 → C6 APE. For acetaminophen-glucuronide, ions with m/z 198/197 were monitored for determination of isotopic enrichment in C1 → C5, and m/z 299/300 and 359/360 for enrichment in C1 → C6.

Analytical NMR spectroscopy. ^{13}C isotopic enrichments in the individual carbons of the glucuronide moiety of acetaminophen-glucuronide collected from the urine samples were determined by ^{13}C NMR. To minimize any assumptions with regard to ^{13}C isotopic abundance of any carbon within the glucuronide moiety, isotopic abundance was determined from comparison of the ^{13}C NMR peak areas of the acetaminophen moieties of hepatically conjugated acetaminophen-glucuronide. ^{13}C isotopic enrichments in the acetaminophen moieties were assumed to be 1.1%. To minimize correction factors used to compensate for differences in nuclear Overhauser effect and relaxation times between Cs, comparison of NMR peak areas were limited to Cs of similar hybridization or NMR chemical shift. Peak areas of the acyl Cs of the acetaminophen were compared with the acyl Cs of the glucuronide. The isotopic enrichments of C1 of the glucu-

ronide moiety of acetaminophen-glucuronide were determined from the ratio of the PI of C1 of the glucuronide moiety with the mean PI of C2′ and C3′ (i.e., C2 and C3 of the aromatic ring) of the acetaminophen moiety. Additionally, the NMR acquisition parameters of peak width and delay between pulses were optimized to minimize correction factors and maximize the signal to noise ratio. A delay of 6.0 s between scans and a 30° pulse width were used to acquire all spectra obtained in this study. Correction factors ranged from 0.96 to 1.06. ^{13}C NMR chemical shifts of acetaminophen-glucuronide have been assigned from pure standards and mixtures, after taking into account the expected change in the ^{13}C chemical shift of unconjugated acetaminophen (13). Chemical shifts were referenced to trimethylsilylpropionate-(2,2,3,3-d$_4$) assigned to 0 ppm (MSD Isotopes, Montreal, Canada). The ^{13}C NMR chemical shift glucuronide C1, C2, and C6 have been assigned to 101.0, 73.2, and 176.1 ppm, respectively. In the acetaminophen moiety, C2′ (C6′) and C3′ (C5′) of the aromatic ring were assigned to 117.7 and 123.8 ppm (chemical shifts not specified).

Calculations and data analysis. The method for calculating rates of hepatic glycogen synthesis and simultaneous breakdown is based on measuring initial total (^{13}C + ^{12}C) glycogen concentration and changes in C1 glycogen peak integral (ΔPI; [11]). Briefly, the rate of hepatic accumulation of glycogen (V_{syn}) was assessed from the increase in [^{13}C]glycogen concentration during the initial [1-^{13}C]glucose infusion. The increase in total liver glycogen concentration (Δ[Gly]) for each 15-min interval is given as: $[\Delta PI \cdot a \cdot 100]/[(b \cdot APE\ glu) + 1.1]$ where a = [^{13}C]glycogen concentration per signal intensity unit (mmol/liter liver), $APE\ glu$ = atom percent excess ^{13}C over baseline in plasma glucose, and b = dilution factor. The dilution factor, b, represents the fraction of UDP-glucose formed by the direct pathway as determined from the ^{13}C APE in C1 and C6 of plasma glucose and of acetaminophen-glucuronide: $[(C1\ APE - C6\ APE)\ glucuronide]/[(C1\ APE - C6\ APE)\ glucose]$. The percentage of glycogen synthesized by the direct pathway (percent direct pathway) can be expressed as $b \cdot 100\%$. Since the increase in the total liver glycogen concentration was linear from t = 60–155 min during protocol I (r = 0.987), II (r = 0.953), and III (r = 0.963), the hepatic accumulation of glycogen [V_{syn}; mmol glycogen/(liter liver · min)] can be assessed from the slope of the line in a plot of the increase in total glycogen concentration versus time. To estimate a minimal rate of simultaneous glycogen breakdown, i.e., only the glycogen broken down that escapes the hexose-1-phosphate pool (V_{out}), the observed change in [1-^{13}C]glycogen concentration ([^{13}C-Gly]) during the unenriched glucose infusion (chase period; 185–245 min) is compared with the predicted change in [^{13}C-Gly], which is assuming constant glycogen synthesis without breakdown. The observed change in [1-^{13}C]glycogen concentration (Δ[^{13}C-Gly]$_o$) is given as: ΔPI · a, whereas the predicted increase in Δ[^{13}C-Gly]$_p$ during each 15-min (ΔT) of the chase period is given as: $\Delta T \cdot V_{syn} \cdot [(b \cdot APE\ glu) + 1.1]/100$. The difference between Δ[^{13}C-Gly]$_o$ and Δ[^{13}C-Gly]$_p$ represents the amount of [1-^{13}C]glycogen broken down (Δ[^{13}C-Gly]$_{out}$) during each time interval. The Δ[^{13}C-Gly]$_{out}$ is plotted versus time and the data are fitted to a line to give the average rate of [1-^{13}C]glycogen breakdown. To obtain a minimum estimate for the rate of total glycogen breakdown (V_{out}), the slope of that line is divided by the maximum fractional ^{13}C enrichment [$(b \cdot APE\ glu_{max}/100) + 0.011$] in C1 glycogen. The relative glycogen turnover (percentage) is then calculated as: $V_{out} \cdot 100/V_{syn}$, and the net rate of glycogen synthesis ($Vsyn_{net}$) is given as the difference between V_{syn} and V_{out}. The assumption made for these calculations will lead to underestimation of both liver glycogen accumulation which results from the rapid loss of glycogen preventing some newly formed glycogen to be detected in the [^{13}C]glycogen peak and of liver glycogen turnover to the extent that the unlabeled glycogen pool is also subject to turnover.

All data are presented as means±SEM. Linear regressions were calculated by the method of the least squares. Statistical comparisons between protocols I and II were made by the paired Student's t test. Data within a group or between the three protocols (I–III) were compared by ANOVA followed by the Student-Newman-Keuls post-hoc test.

Results

Plasma glucose, insulin, glucagon, C-peptide. Basal fasting concentrations of glucose (protocol I: 5.3±0.1, II: 5.3±0.2, III: 4.9±0.2 mM), insulin (I: 36±6, II: 36±6, III: 36±6 pM), and glucagon (I: 54±4, II: 53±5, III: 52±6 pg/ml) before the clamp studies were not different between the three experimental protocols. Within 15 min of starting the hyperglycemic clamp the desired level of hyperglycemia was achieved and then maintained until the end without differences between the protocols (I: 10.3±0.1, II: 10.4±0.1, III: 10.5±0.1 mM) (Fig. 1, *top*). Plasma insulin concentrations rapidly increased ($P < 0.0001$) and reached steady state values, which were not different between protocols I (192±12 pM) and II (192±12 pM) (Fig. 1, *middle*). During protocol III plasma insulin concentrations were slightly higher than the basal concentrations (54±2 vs. 36±6 pM, $P < 0.01$). SRIF resulted in a decrease by ∼ 39% of plasma glucagon concentrations during protocols I ($t = 15$–245 min: 31±4 pg/ml, $P < 0.0001$ vs. basal values) and III (32±7 pg/ml, $P = 0.0051$) (Fig. 1, *bottom*). When glucagon was infused during protocol II, plasma glucagon concentrations were kept at baseline values ($t = 15$–245 min: 63±8 pg/ml, NS vs. basal values), but were higher ($P < 0.005$) than during protocol I (Fig. 1, *bottom*). Basal C-peptide levels (I: 326±27, II: 361±24, III: 334±48 pM) were suppressed ($P < 0.0001$) during the clamp tests ($t = 125$–245 min), but were not different between protocols I (83±8 pM), II (94±7 pM), and III (84±16 pM).

Plasma lactate, free fatty acids, amino acids. Plasma lactate concentrations were similar before the experiments (protocol I: 0.88±0.07, II: 0.97±0.07, III: 0.87±0.08 mM) and increased during the insulin infusion (I: 1.37±0.06, II: 1.39±0.04, III: 0.98±0.06 mM). Baseline values of plasma free fatty acids were 430±36, 409±16, and 304±12 μM and were suppressed ($P < 0.0001$) to 164±12, 151±6, and 88±8 μM, respectively, under clamp conditions. Similarly, plasma concentrations of branch-chained amino acids (basal, I: 412±16, II: 438±21, III: 452±14 μM) decreased during protocols I (235±16 μM; $P < 0.0001$), II (234±14 μM, $P < 0.0001$), and III (372±5 μM, $P < 0.05$). Plasma gluconeogenic amino acids (I: 1,674±59, II: 1,731±40, III: 1,804±133 μM) were lower ($P < 0.001$) during steady state clamp conditions of protocols I (1,438±51 μM) and II (1,429±24 μM), but remained unchanged during protocol III (1,823±84 μM).

Glucose infusion rate. Under conditions of basal glucagon concentrations (protocol II) mean rates of glucose infusion were 14–18% lower compared with those during suppressed glucagon secretion (protocol I) (Table I). In the presence of basal peripheral vein concentrations of insulin (protocol III) the mean glucose infusion rate was reduced by 70% to 2.7±0.1 mg/(kg · min) compared with conditions simulating basal insulin concentrations in the portal vein (protocol II).

Basal glycogen, V_{syn}, V_{out}, $Vsyn_{net}$. Basal liver glycogen concentrations were similar before each study (protocol I: 359±22, II: 342±25, III: 294±37 mM). From $t = 60$ min to $t = 155$ min, total liver glycogen concentrations increased linearly during protocols I (r = 0.987), II (r = 0.953), and III (r = 0.963) (Fig. 2). The rates of hepatic glycogen synthesis, breakdown, and relative glycogen turnover are summarized in Table
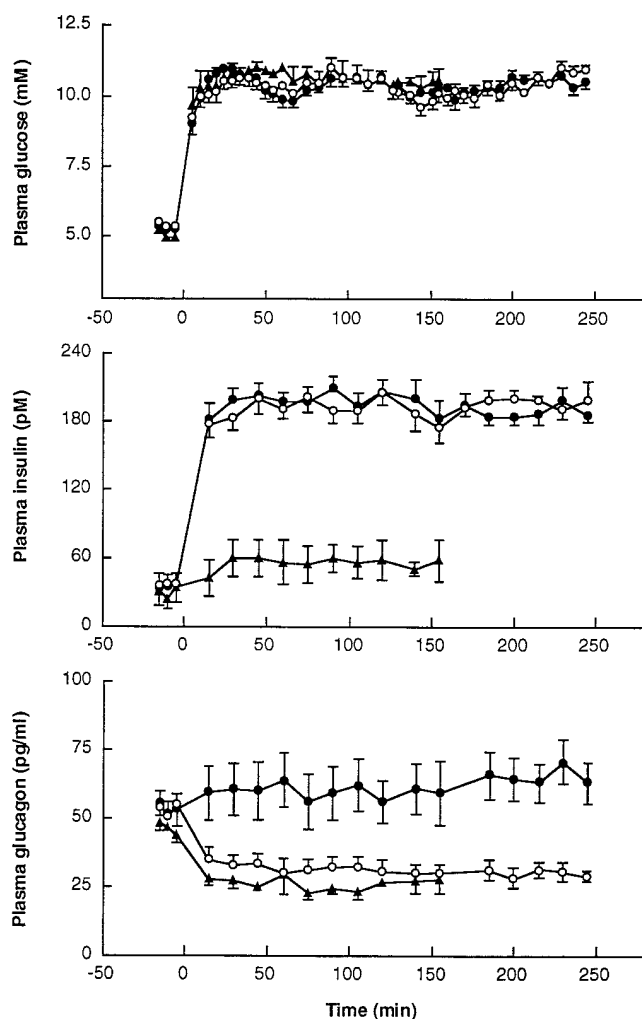
Figure 1. Time course of plasma glucose (*top*), insulin (*middle*), and glucagon (*bottom*) concentrations during hyperglycemic-SRIF [0.1 μg/(kg · min)]-insulin clamp studies in healthy humans. Protocol I (*n* = 8): insulin replacement [0.6 mU/(kg · min)] only (*open circles*); protocol II (*n* = 8): insulin [0.6 mU/(kg · min)] and glucagon replacement [0.7 ng/(kg · min)] (*filled circles*); protocol III (*n* = 3): low-dose insulin replacement [0.15 mU/(kg · min)] only (*filled triangles*).

II. Replacement of the basal glucagon concentration decreased the mean rate of hepatic glycogen synthesis (accumulation of glycogen) by 53% (*P* < 0.0025), while simultaneous glycogen turnover was increased more than threefold (*P* < 0.005). This resulted in reduction by 82% (*P* < 0.001) of the rate of net glycogen synthesis compared with the hypoglucagonemia group.

Under similar conditions of hyperglycemia and SRIF-suppressed glucagon secretion, accumulation of glycogen [V$_{syn}$: 0.07±0.03, 0.09, 0.30, 0.40±0.06, 0.44 mmol/(liter · min)] was stimulated by increasing the plasma insulin concentration (54±12, 129±4, 173±6, 192±6, 280±11 pM, respectively) (Fig. 3).

Pathways of hepatic glycogen synthesis. To obtain an estimate of the percent contribution of the pathways (direct versus indirect) to hepatic glycogen synthesis, the ratios of ^{13}C enrichments (APE) in plasma glucose and in plasma (urine) acetaminophen-glucuronide were compared. Plasma [13C]glucose

Protocol	60–120 min	120–180 min	180–240 min
Portal vein insulin (∼ 192 pM)			
Protocol I: − Glucagon	8.7±0.4	10.4±0.4	12.3±0.5
Protocol II: + Glucagon	7.4±0.5*	9.0±0.5‡	10.1±0.5§
Portal vein insulin (∼ 54 pM)			
Protocol III: − Glucagon	2.6±0.1§	2.7±0.2§	N.D.

Values are means±SE. *P* < 0.05, ‡*P* < 0.01, §*P* < 0.01 vs. protocols I and II. *N.D.*, not determined.

APE approached plateau values within 120 min and increased by < 9% until the end of the labeled glucose infusion. Mean maximum values of [13C]glucose enrichments were not different between protocols I (16.2±0.5%) and II (16.0±0.5%), but were lower during protocol III (9.8±0.3%). Both plasma and urine acetaminophen-glucuronide ^{13}C APE represent the UDP-glucose pool labeled by [13C]glucose and thereby indicate the percent contribution of the direct pathway to hepatic glycogen synthesis (11). The maximum ^{13}C enrichments in plasma acetaminophen-glucuronide corresponded well with the data obtained from urine (variation < 10%). During protocols I and II ^{13}C APE in plasma acetaminophen-glucuronide reached plateau values at *t* = 185 min and did not change significantly until the end of the experiment (*t* = 245 min: 9.5±0.9 vs. 8.2±1.1%). This indicates a time lag of 90 min between maximal plasma ^{13}C enrichments in glucose and in acetaminophen-glucuronide. The mean relative flux through the direct pathway (Fig. 4) for hepatic glycogen synthesis was higher during glucagon deficiency compared with basal concentrations of
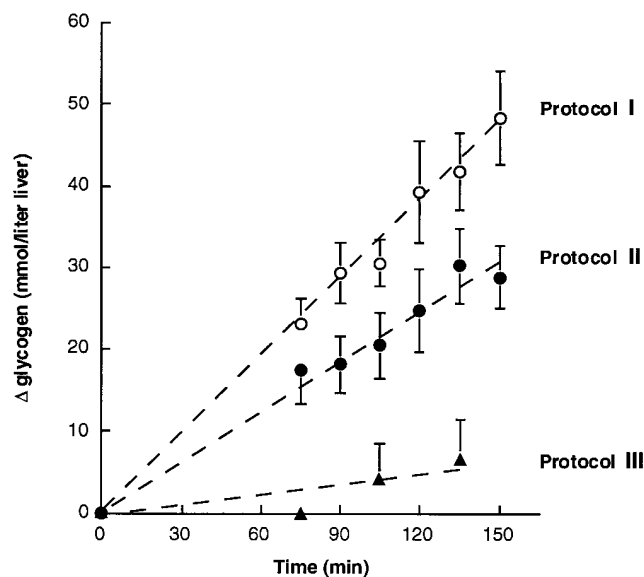

Figure 2. Increase in total liver glycogen over baseline values (Δ glycogen; mmol/liter liver) from 60 to 155 min during hyperglycemic-SRIF [0.1 μg/(kg · min)] clamp studies combined with insulin replacement [protocol I, *open circles*, and protocol II, *filled circles*: 0.6 mU/(kg · min); protocol III, *filled triangles*: 0.15 mU/(kg · min)] without (protocols I and III) or with glucagon replacement [protocol III: 0.7 ng/(kg · min)].

Table II. Rates of Hepatic Glycogen Synthesis (Vsyn), Glycogen Breakdown (Vout), and Net Glycogen Synthesis (Vsynnet) as well as Relative Turnover of Liver Glycogen

Protocol	V_{syn}	V_{out}	$Vsyn_{net}$	Glycogen turnover
		mmol/(liter · min)		%
Portal vein insulin ($\sim$ 192 pM)				
Protocol I: − Glucagon	0.40±0.06	0.07±0.03	0.33±0.05	19±7
Protocol II: + Glucagon	0.19±0.03*	0.12±0.03	0.06±0.03‡	69±12§

Values are means±SE. *$P < 0.0025$, ‡$P < 0.001$, §$P < 0.005$ vs. protocol I.

glucagon (I: 58±6 vs. 46±5%; $P = 0.0032$). It is of note that despite the lower relative flux through the indirect pathway, the absolute flux rates through this pathway were higher during protocol I than during protocol II [0.17±0.04 vs. 0.10±0.02 mmol/(liter · min)].

Discussion

In the presence of hyperglycemia, hypoglucagonemia, and conditions that approximate fasting peripheral insulin concentrations ($\sim$ 50 pM) there was only a small amount of [^{13}C]glucose incorporated into liver glycogen, indicating that there was a negligible amount of glycogen synthesis and glycogen turnover occurring. However, increasing the plasma insulin concentration to $\sim$ 190 pM elicited hepatic glycogen synthesis at a rate of $\sim$ 0.4 mmol/(liter · min). That insulin concentration is similar to values obtained directly from the portal vein in overnight fasted humans (20), but assuming a portal vein to peripheral vein insulin ratio of $\sim$ 3:1 (21), moderate hyperinsulinemia might have been present at the level of the portal vein. In a previous hyperglycemic-hyperinsulinemic clamp study using SRIF, only slightly higher glycogen synthetic rates [0.49 mmol/(liter · min)] were reported in the presence of peripheral and portal vein insulin concentrations of $\sim$ 410 pM (11). After a hyperglycemic clamp performed without SRIF resulting in a comparable level of hyperglycemia (9.4 mM), the mean glycogen synthetic rate was found to be $\sim$ 0.43 mmol/(liter · min) (11). Since this study was performed without SRIF infusion, portal vein insulin concentrations can be estimated to be

$\sim$ 780 pM from the peripheral mean plasma insulin concentration of $\sim$ 260 pM (21). Taken together along with the data of the present study, these results suggest that portal vein insulin concentrations in the range of $\sim$ 130–170 pM are required for half-maximal hepatic glycogen synthesis under conditions of intravenous glucose administration and suppressed glucagon secretion (Fig. 3). This insulin concentration range is close to the half-maximally effective concentration for suppression of hepatic glucose production (22–24). Since hepatic accumulation of glycogen might be expected to reflect glucose uptake by the liver (1), the present results are supported by reports on stimulation of net splanchnic glucose uptake during hypoglucagonemia at low plasma insulin concentrations in dogs (25) and healthy humans (26), but even at increased insulin levels in dogs (27).

This study also found that glucagon plays an important role in the regulation of hepatic glycogen synthesis under conditions of hyperglycemia and basal portal vein concentrations of insulin. Restoring the basal plasma glucagon concentration must have resulted in mild hypoglucagonemia in the portal vein (28), but still reduced net glycogen synthesis by $\sim$ 82%. This finding is in keeping with reports that hyperglycemia alone or in the presence of basal insulin concentrations causes little or no net hepatic/splanchnic glucose uptake (22, 25, 29, 30). The decrease in net hepatic glycogen synthesis could have occurred from decreased glycogen synthesis, increased glycogenolysis, or both. To examine these possibilities [^{13}C]glucose pulse–[^{12}C]glucose chase studies were performed. Restoring the basal plasma glucagon concentration in this study resulted
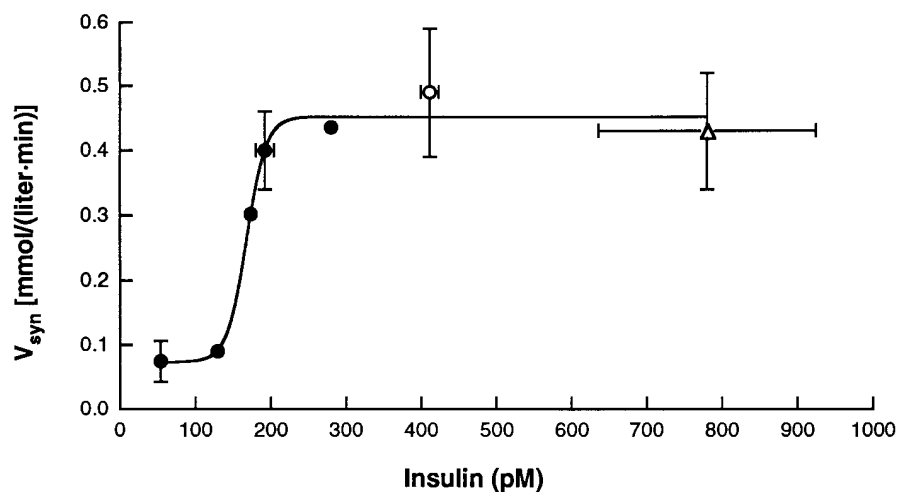

Figure 3. Concentration dependence of mean glycogen synthetic rate [mmol/(liter · min)] at varying (portal vein) plasma insulin concentrations (pM) under conditions of intravenous glucose infusion and suppressed glucagon secretion (*filled circles*). Additional data points are derived from Cline et al. (13) (*open circle*) and Magnusson et al. (11) (*open triangle*).
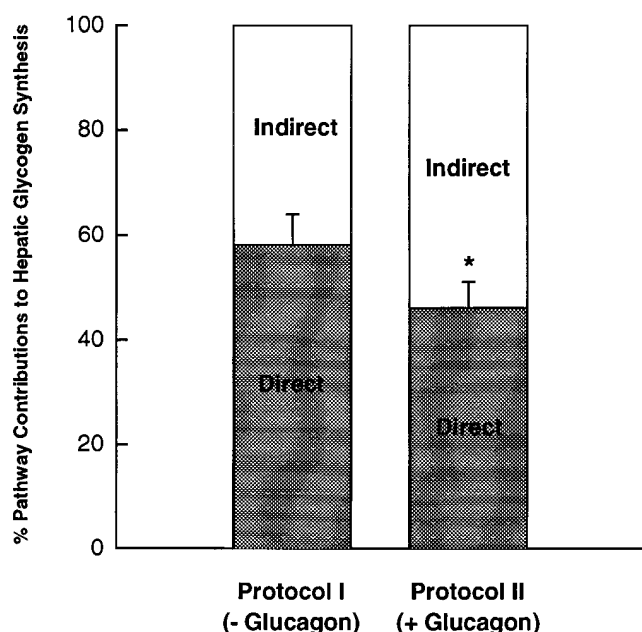
Figure 4. Relative contributions of the direct (*hatched bars*) and indirect pathway (*open bars*) to hepatic glycogen synthesis during hyperglycemic-SRIF [0.1 μg/(kg · min)]-insulin [0.6 mU/(kg · min)] clamp studies without (protocol I) or with (protocol II) glucagon replacement [0.7 ng/(kg · min)] in eight healthy men. *P = 0.0032 vs. protocol I.

in a 53% decrease in the accumulation of glycogen (V_{syn}) and a 71% increase of V_{out} under identical conditions of hyperglycemia and basal portal vein concentrations of insulin resulting in an almost fourfold increase in hepatic glycogen turnover. Overall decreases in V_{syn} accounted for ∼ 80% of the decrease in net hepatic glycogen synthesis.

The direct pathway accounted for ∼58% of the newly synthesized hepatic glycogen, when glucagon secretion was suppressed during hyperglycemia and plasma insulin concentrations of ∼ 190 pM. In a previous clamp study performed at comparable hyperglycemia and SRIF-suppressed glucagon secretion, but higher plasma insulin levels of ∼ 410 pM, the percent contribution of the direct pathway for hepatic glycogen synthesis was only slightly higher (∼ 65%; [13]). Similarly, during intravenous glucose infusion (plasma glucose of ∼ 9.3 mM) and plasma insulin concentrations of ∼ 260 pM, the contribution of the direct pathway to newly formed glycogen was ∼ 50% after an overnight fast (19). In this study, replacement of the basal plasma glucagon concentration resulted in an increase in the relative, but not in the absolute contribution of the indirect (gluconeogenic) pathway. In this context it is of note that higher concentrations of glucagon than used in this study are required to induce the well known stimulating effect on hepatic gluconeogenesis (31–34).

Defects in hepatic glycogen metabolism in subjects with IDDM have been demonstrated recently (2, 13). Although peripheral plasma glucagon concentrations in IDDM patients are typically only slightly higher than in healthy subjects (35, 36), the glucagon secretion pattern has been shown to be altered (37), and the insulin/glucagon ratio is substantially lower during mixed meal ingestion (2, 38). When SRIF or SRIF analogues were used to inhibit glucagon secretion in IDDM patients, differing results have been reported: most investigators found improvement of basal and postprandial hyperglycemia during glucagon deficiency (39–44), whereas others could not detect any effects of a SRIF analogue (SMS 201-995) on 24-h glucose and insulin profiles (43, 44). It is possible that these differences might be explained by incomplete inhibition of glucagon secretion (43, 44). While extrapancreatic effects of SRIF (analogues) on growth hormone secretion might have contributed to the improvement in the glycemic profile, suppression of glucagon secretion by the SRIF analogues WY-41,747 (45) or SMS 201-995 (46) combined with replacement of growth hormone was still able to decrease plasma glucose and insulin requirements after a single meal or during a day of mixed meal ingestion in IDDM patients. Metabolic effects of SRIF can be explained also by impairment of intestinal nutrient absorption (47). However, a recent report found that preventing the fall in plasma glucagon during SRIF infusion leads to higher postprandial glucose concentrations and greater integrated glycemic response in IDDM patients compared with conditions of glucagon suppression (48) which is consistent with the findings of the present study.

In summary, physiologic alterations in portal vein insulin and glucagon concentrations independently modify hepatic glycogen synthesis and turnover. Inhibition of glucagon secretion by SRIF under conditions of hyperglycemia and basal portal vein concentrations of insulin resulted in: (*a*) twofold increase in rate of hepatic glycogen synthesis, (*b*) reduction of glycogen turnover by ∼ 73%, and (*c*) augmented relative contribution of the direct pathway to glycogen synthesis when compared with conditions of basal glucagon concentrations. Thus, blocking secretion and/or action of glucagon at times of mixed meal ingestion may improve hepatic glycogen synthesis and thereby contribute to amelioration of postprandial hyperglycemia in IDDM patients who typically have relatively low concentrations of insulin and high concentrations of glucagon in the portal vein.

Acknowledgments

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