

Hepatic Glycogen Supercompensation Activates AMP-Activated Protein Kinase, Impairs Insulin Signaling, and Reduces Glycogen Deposition in the Liver

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OBJECTIVE—The objective of this study was to determine how increasing the hepatic glycogen content would affect the liver's ability to take up and metabolize glucose.

RESEARCH DESIGN AND METHODS—During the first 4 h of the study, liver glycogen deposition was stimulated by intraportal fructose infusion in the presence of hyperglycemic-normoinsulinemia. This was followed by a 2-h hyperglycemic-normoinsulinemic control period, during which the fructose infusion was stopped, and a 2-h experimental period in which net hepatic glucose uptake (NHGU) and disposition (glycogen, lactate, and CO₂) were measured in the absence of fructose but in the presence of a hyperglycemic-hyperinsulinemic challenge including portal vein glucose infusion.

RESULTS—Fructose infusion increased net hepatic glycogen synthesis (0.7 ± 0.5 vs. 6.4 ± 0.4 mg/kg/min; $P < 0.001$), causing a large difference in hepatic glycogen content (62 ± 9 vs. 100 ± 3 mg/g; $P < 0.001$). Hepatic glycogen supercompensation (fructose infusion group) did not alter NHGU, but it reduced the percent of NHGU directed to glycogen (79 ± 4 vs. 55 ± 6 ; $P < 0.01$) and increased the percent directed to lactate (12 ± 3 vs. 29 ± 5 ; $P = 0.01$) and oxidation (9 ± 3 vs. 16 ± 3 ; $P = \text{NS}$). This change was associated with increased AMP-activated protein kinase phosphorylation, diminished insulin signaling, and a shift in glycogenic enzyme activity toward a state discouraging glycogen accumulation.

CONCLUSIONS—These data indicate that increases in hepatic glycogen can generate a state of hepatic insulin resistance, which is characterized by impaired glycogen synthesis despite preserved NHGU. *Diabetes* 60:398–407, 2011

Although excessive hepatic glucose production contributes to fasting hyperglycemia (1,2), glucose intolerance is also a major defect in humans with diabetes mellitus. In response to a moderately sized oral glucose challenge, the liver normally takes up approximately one third of the ingested glucose, whereas the remaining two thirds escapes the splanchnic bed and is metabolized by other tissues of the body (3–5). Liver glucose disposal has consistently been shown to be

reduced in humans with diabetes mellitus (5–9), making it important to understand how this process is regulated and why it becomes dysfunctional.

Previous research has shown that net hepatic glucose uptake (NHGU) is regulated by a number of factors, including the glucose load to the liver, the hepatic sinusoidal insulin concentration, and the route of glucose delivery into the body. During euglycemic conditions, hyperinsulinemia alone does little to stimulate NHGU (10) or net glycogen synthesis (11), and only when pharmacologic levels of insulin are present in the face of euglycemia is NHGU significantly stimulated (10). However, when the glucose load to the liver is increased (i.e., hyperglycemia) by infusing glucose into a peripheral vein, hyperinsulinemia increases NHGU in a dose-dependent fashion (12). Despite this relationship between the hepatic glucose load and insulin, a rate of NHGU similar to that observed during the postprandial state (~ 5 – 6 mg/kg/min) can only be achieved during hyperglycemic/hyperinsulinemic conditions when a portion of the infused glucose is delivered via the hepatic portal vein (13,14), thereby creating a negative arterial-portal vein glucose gradient known as the “portal glucose signal.”

Some of the drugs now under development (e.g., glucokinase [GK] activators, glucagon receptor antagonists, and glycogen phosphorylase [GP] inhibitors) would reduce postprandial glucose excursions by stimulating hepatic glucose uptake and glycogen deposition. However, relatively little is known about the impact of hepatic glycogen content on the regulation of glucose metabolism in the liver in vivo. Our previous study (15) showed that acutely increasing the hepatic glycogen content by an increment similar to that seen after a meal did not impair the response of the liver (e.g., insulin signaling, NHGU, and net glycogen synthesis) to a subsequent hyperglycemic/hyperinsulinemic challenge. However, the increase in NHGU triggered by the increase in insulin was small (1.6 mg/kg/min), as was the increment in net glycogen synthesis (~ 1.0 mg/kg/min), raising the possibility that these stimuli (increased insulin and glucose) were not great enough to expose a defect caused by the increased glycogen content. Furthermore, the hepatic glycogen level, although high, was still within the normal diurnal range, leaving open the possibility that decrements in NHGU or net glycogen synthesis might not occur until the liver glycogen content is increased to a greater extent. Therefore, in the current study we increased the challenge to the liver by adding portal glucose delivery to the hyperglycemic/hyperinsulinemic challenge and further increased the hepatic glycogen content to determine whether excessive liver glycogen can alter hepatic glucose metabolism.

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RESEARCH DESIGN AND METHODS

Animals and surgical procedures. Studies were carried out on healthy, conscious 18-h fasted mongrel dogs of either sex with a mean weight of 22.5 ± 0.4 kg. All animals were maintained on a diet of meat and chow (34% protein, 14.5% fat, 46% carbohydrate, and 5.5% fiber based on dry weight; $\sim 1,700$ kcal/d). The animals were housed in a facility that met American Association for Accreditation of Laboratory Animal Care guidelines, and the protocol was approved by Vanderbilt University's Institutional Animal Care and Use Committee.

Two weeks before being studied, each dog underwent a laparotomy under general anesthesia (0.01 mg/kg buprenorphine presurgery and $\sim 2\%$ isoflurane inhalation anesthetic during surgery), and silicone catheters for sampling were inserted in the hepatic vein, hepatic portal vein, and a femoral artery as described previously (12). Catheters for intraportal infusion of hormones and substrates were placed in the splenic and jejunal veins (each of which empties into the portal vein), and ultrasonic flow probes (Transonic Systems, Ithaca, NY) were placed around the hepatic portal vein and the hepatic artery as described previously (12).

Two days before each study, blood was drawn to determine the leukocyte count and hematocrit for each animal. Animals were only studied if they had a leukocyte count $<16,000/\text{mm}^3$, a hematocrit $>35\%$, a good appetite (as evidenced by consumption of the entire daily ration), and normal stools.

On the morning of each study, catheters and flow probe leads were exteriorized from subcutaneous pockets using local anesthesia (2% lidocaine, Hospira, Lake Forest, IL). The contents of each catheter were aspirated and then flushed with saline. Angiocatheters (Deseret Medical, Becton Dickinson, Sandy, UT) were inserted into the cephalic and saphenous veins to allow infusion into the peripheral vasculature as desired. The animals stood comfortably in a Pavlov harness throughout the experiment.

Experimental design. Each experiment consisted of a 4-h liver glycogen loading period (-360 to -120 min), a 2-h control period (-120 to 0 min), and a 2-h experimental period (0–120 min). Each experiment was initiated at minute -360 by the infusion of somatostatin (SRIF; $0.8 \mu\text{g}/\text{kg}/\text{min}$; Bachem, Torrance, CA) into a peripheral vein to disable the endocrine pancreas (Fig. 1). This was accompanied by the intraportal replacement of both insulin ($0.3 \text{ mU}/\text{kg}/\text{min}$; Eli Lilly & Co., Indianapolis, IN) and glucagon ($0.55 \text{ ng}/\text{kg}/\text{min}$; Glucagon, Novo Nordisk, Bagsvaerd, Denmark) at basal rates. At the same time, the hepatic glucose load was doubled by infusing a 50% dextrose solution into a peripheral vein and either 0.7% saline (moderately elevated [MOD]; $n = 8$) or fructose at one of two rates ($0.4 \text{ mg}/\text{kg}/\text{min}$; HIGH; $n = 8$, or $1.0 \text{ mg}/\text{kg}/\text{min}$; SC; $n = 10$) into the portal vein. The latter was used so that we could stimulate NHGU and glycogen deposition without altering plasma insulin levels. Thus, by the start of the experimental period (i.e., at min 0), the hepatic glycogen content was moderately elevated (MOD group), markedly elevated (HIGH), or supercompensated (SC) relative to the level ($\sim 36 \text{ mg}/\text{g}$ liver) (16) seen after an 18-h fast. The 4-h glycogen loading period was followed by a 2-h hyperglycemic control period during which fructose was not infused, but the basal hormone levels and hyperglycemia were maintained. At -120 min, a *p*-aminohippuric acid infusion was started into a peripheral vein ($0.4 \text{ mg}/\text{kg}/\text{min}$) to allow the assessment of portal glucose infusate mixing with portal and hepatic vein blood during the experimental period. A [^{14}C]glucose infusion (for assessment of glucose oxidation) was begun at -90 min, so the tracer could equilibrate with blood glucose before the experimental period. During the 2-h experimental period (0–120 min), the intraportal infusion of insulin was increased to four times ($1.2 \text{ mU}/\text{kg}/\text{min}$) the basal rate, and 20% dextrose (mixed with *p*-aminohippuric acid) was infused intraportally at a rate of $4.0 \text{ mg}/\text{kg}/\text{min}$. The preexisting hepatic glucose load was

maintained during the experimental period by reducing the infusion of 50% dextrose into the peripheral vein as necessary. At the conclusion of the study, animals were killed with an overdose of pentobarbital, the abdomen was opened, and the positions of the catheter tips were verified. Liver and muscle biopsies were immediately freeze clamped and stored at -80°C .

Processing and analysis of samples. The processing of blood samples has been described (17). Plasma glucose was analyzed using the glucose oxidase method (Analox Instruments; Lunenburg, MA). Insulin, glucagon, lactate, glycerol, and nonesterified fatty acid concentrations were measured as previously described (14). Liver samples were pulverized under liquid nitrogen and assayed for liver glycogen (18) and triglyceride (TG) (19,20) as described previously. [^{14}C]glucose in plasma and $^{14}\text{CO}_2$ content in whole blood were measured as previously described (15). Western blot analyses were also carried out as previously described (15,21), as were hepatic glycogen synthase (GS) (under low or high glucose-6-phosphate [G6P], 0.16 and 6.67 mmol/L, respectively, with $0.13 \text{ mmol}/\text{L}$ of UDP-glucose) (22) and GP (23) activity measurements and G6P levels (24).

Real-time PCR methods. RNA extraction, cDNA synthesis, SDS-PAGE, and Western blotting were performed using standard methods (15,25). Real-time PCR was performed using canine-specific primers for GK (25) fatty acid synthase (FAS) (26), carnitine palmitoyltransferase (CPT) (27), and hypoxanthine phosphoribosyl transferase-1 (25). The PCR protocol consisted of a denaturing cycle at 95°C for 2 min followed by a 35-cycle amplification step (95°C for 30 s, $55\text{--}60^\circ\text{C}$ for 30 s, 72°C for 30 s). Expression of test genes were normalized relative to the housekeeping gene hypoxanthine phosphoribosyl transferase-1 using the Livak method as previously described (28). Analysis of data was performed using iCycler iQ Optical System Software Version 5.0 (Bio-Rad Laboratories, Inc., Hercules, CA).

Calculations and data analysis. Hepatic blood flow (HBF) was measured using ultrasonic flow probes. Net hepatic substrate balance, net hepatic fractional substrate extraction, sinusoidal insulin and glucagon levels, and hepatic glucose load were calculated as described previously (29). Plasma glucose levels were converted to whole blood values as described previously for the calculation of net hepatic glucose balance (NHGB) (30,31). Nonhepatic glucose uptake was calculated as the sum of NHGB and the exogenous glucose infusion rate (GIR) after correcting for the change in the glucose mass (32). Net hepatic glycogen synthesis was calculated as $(\text{NHGU} - \{\text{net hepatic lactate output [NHLO]} + \text{in glucose equivalents}\} + \text{hepatic glucose oxidation})$, where [NHGU] is the absolute value of NHGB. The hepatic glucose oxidation rate was calculated by dividing the net hepatic balance of $^{14}\text{CO}_2$ by the arterial [^{14}C]glucose specific activity.

Statistical analysis. All data are presented as mean \pm SEM, and statistical analyses were performed using SigmaStat (Aspire Software International; Ashburn, VA) software. Clamp data were analyzed using 2-way repeated-measures ANOVA (group \times time), and 1-way ANOVA was used to compare data from tissue analyses. Post hoc comparisons were made as appropriate, and statistical significance was $P < 0.05$.

RESULTS

Plasma hormone and glucose concentrations and hepatic glucose and lactate metabolism

Glycogen loading period. During the glycogen loading period, the arterial blood glucose level was $2\times$ basal in each group (Fig. 2A). On the other hand, the hepatic

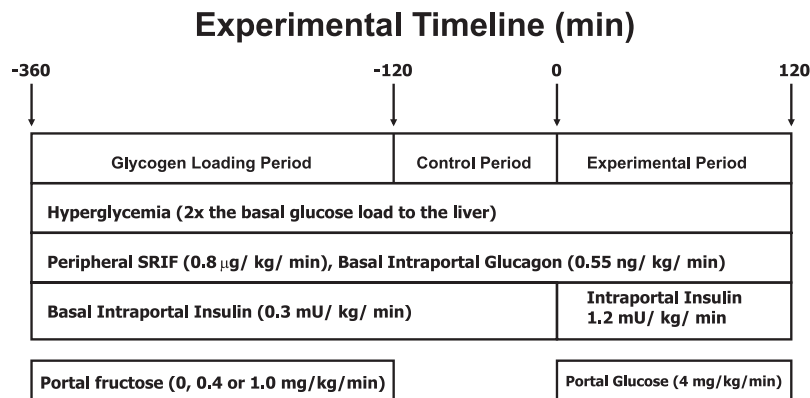


FIG. 1. Schematic representation of the study. Portal fructose infusion rates of 0, 0.4, and $1.0 \text{ mg}/\text{kg}/\text{min}$ represent the MOD, HIGH, and SC groups, respectively.

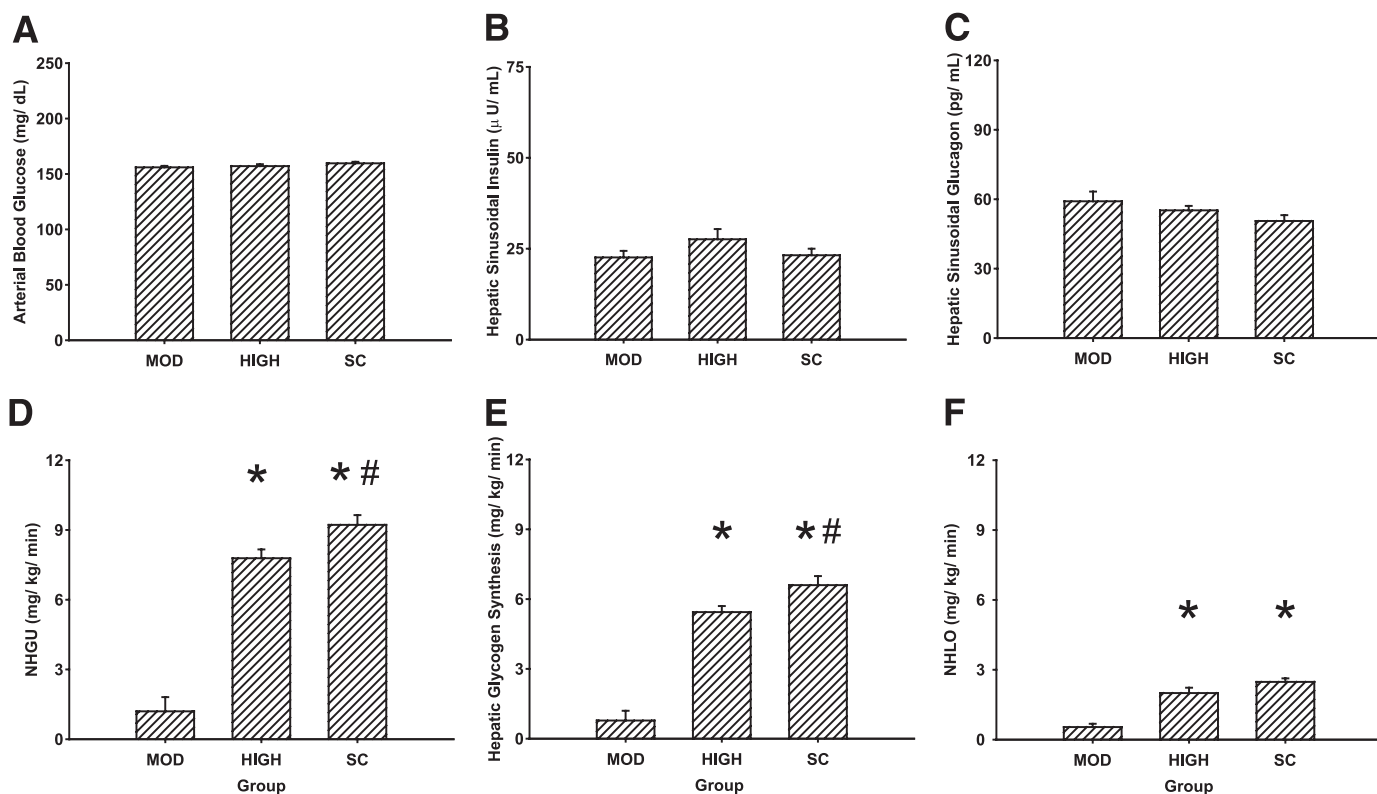


FIG. 2. Arterial blood glucose (A), hepatic sinusoidal insulin (B), hepatic sinusoidal glucagon (C), NHGU (D), net hepatic glycogen synthesis (E), and NHLO (in glucose equivalents) (F) during the final 20 min of the 4-h glycogen loading period. * $P < 0.05$ compared with MOD; # $P < 0.05$ compared with HIGH.

sinusoidal insulin and glucagon levels remained basal and similar in all groups (Fig. 2B and C), as did HBF (Table 1). NHGU, net glycogen synthesis, and NHLO were greater in both fructose infusion groups than in the saline group ($P < 0.001$; Fig. 2D–F). Likewise, both NHGU and net glycogen synthesis were greater when fructose was infused at the high rate as opposed to the low rate ($P < 0.05$).

Control and experimental periods. During the control and experimental periods, total HBF was similar in all groups (Table 1). Likewise, the arterial blood glucose level and hepatic glucose loads were also similar between groups (Fig. 3A and B). It was necessary to lower the arterial glucose level slightly when the portal glucose infusion was started to prevent the preexisting hepatic glucose load from changing. The hepatic sinusoidal insulin levels were increased fourfold at the outset of the experimental period, whereas the hepatic sinusoidal glucagon levels remained basal (Fig. 3C and D). As expected, NHGU was modest (~ 2.0 – 2.6 mg/kg/min) during the last 30 min of the control period regardless of treatment (Fig. 4A). During the experimental period, NHGU (Fig. 4A) and the hepatic fractional extraction of glucose (data not shown) increased rapidly and similarly in all groups. This increase in NHGU was accompanied by an increase in the rate of net glycogen synthesis and a reduction in NHLO (Fig. 4C), whereas the rate of hepatic glucose oxidation remained low in each group (Fig. 4D). The area under the curve (AUC) during the final hour (mg/kg \cdot 60 min) of the experimental period for NHLO (28 ± 7 , 45 ± 9 , and 58 ± 11 for MOD, HIGH, and SC, respectively) and glucose oxidation (15 ± 3 , 33 ± 3 , and 28 ± 4 , respectively) was significantly higher when liver glycogen was supercompensated

compared with MOD ($P < 0.05$), whereas the AUC for net glycogen synthesis (182 ± 39 , 219 ± 17 , and 113 ± 20 , respectively) was significantly lower ($P < 0.05$). By using AUC data over the final 60 min of the experimental period (Fig. 5), the percentage of NHGU accounted for by hepatic glucose oxidation, lactate production, and net glycogen synthesis averaged 9 ± 3 , 12 ± 3 , and $79 \pm 4\%$, respectively, in MOD, 11 ± 1 , 15 ± 3 , and $74 \pm 3\%$, respectively, in HIGH, and 16 ± 3 , 29 ± 5 , and $55 \pm 6\%$, respectively, in SC. The percent of NHGU accounted for by net glycogen synthesis and net lactate output was significantly different in SC compared with MOD ($P < 0.01$ for each).

Fat metabolism. Hyperglycemia modestly reduced arterial FFA and glycerol in all groups during the glycogen loading and control periods (Table 1). As expected, the fourfold increase in plasma insulin reduced both further. No significant differences were detected between groups at any time.

Glucose infusion rate and nonhepatic glucose uptake. During the glycogen loading period, the total GIR was higher in both fructose infusion protocols than in the saline infusion protocol. Likewise, it was higher in SC than HIGH ($P < 0.05$; Table 1). However, during the control period GIR returned to a similar rate in all groups. Likewise, the GIR increased similarly in response to insulin infusion and portal glucose delivery in all groups. Nonhepatic glucose uptake (Table 1) was not different between groups during the fructose infusion or control periods, and increased similarly during the experimental period in all groups.

Hepatic tissue data. There was a dose-dependent effect of fructose on the hepatic glycogen level measured at the end of the study (62 ± 9 , 86 ± 8 , and 100 ± 3 mg/g for

TABLE 1
Metabolic parameters and net substrate balances across the liver throughout the study

Group	Time (min)						Experimental period													
	Glycogen loading period			Control period			0		15		30		45		60		90		120	
Total hepatic blood flow (mL/kg/min)																				
MOD	25 ± 1	27 ± 2	26 ± 1	26 ± 1	26 ± 1	28 ± 2	28 ± 2	26 ± 1	26 ± 1	28 ± 2	27 ± 2	27 ± 2	27 ± 2	28 ± 2	28 ± 2	29 ± 2	29 ± 2	30 ± 2	30 ± 2	
HIGH	29 ± 2	30 ± 2	30 ± 2	30 ± 2	30 ± 2	30 ± 2	30 ± 2	30 ± 2	30 ± 2	30 ± 1	30 ± 2	30 ± 2	30 ± 2	32 ± 2	32 ± 2	32 ± 1	32 ± 1	32 ± 2	32 ± 2	
SC	30 ± 2	30 ± 3	29 ± 3	28 ± 2	30 ± 2	30 ± 2	30 ± 2	31 ± 3	31 ± 3	31 ± 2	31 ± 3	31 ± 3	31 ± 3	32 ± 3	32 ± 3	33 ± 3	33 ± 3	33 ± 2	33 ± 2	
Arterial blood lactate (μmol/L)																				
MOD	1,065 ± 159	1,028 ± 148	1,020 ± 116	1,019 ± 116	1,023 ± 134	1,009 ± 117	1,040 ± 118	1,040 ± 118	1,040 ± 118	1,058 ± 124	1,057 ± 139	1,057 ± 139	1,057 ± 139	966 ± 118	966 ± 118	971 ± 112	971 ± 112	974 ± 111	974 ± 111	
HIGH	1,453 ± 170	1,355 ± 136	997 ± 186	963 ± 100	841 ± 107	884 ± 101	985 ± 105	985 ± 105	985 ± 105	1,017 ± 73	933 ± 68	933 ± 68	933 ± 68	931 ± 91	931 ± 91	903 ± 89	903 ± 89	882 ± 63	882 ± 63	
SC	2,041 ± 220*#	2,132 ± 171*#	1,446 ± 164*#	1,188 ± 1198	1,102 ± 100#	1,051 ± 88	1,156 ± 88	1,156 ± 88	1,156 ± 88	1,132 ± 104	1,086 ± 76	1,086 ± 76	1,086 ± 76	1,006 ± 49	1,006 ± 49	942 ± 60	942 ± 60	1,005 ± 64	1,005 ± 64	
Arterial plasma FFA (μmol/L)																				
MOD	477 ± 77	429 ± 62	381 ± 63	381 ± 63	381 ± 63	392 ± 47	392 ± 47	392 ± 47	392 ± 47	188 ± 41	188 ± 41	188 ± 41	188 ± 41	127 ± 29	127 ± 29	91 ± 18	91 ± 18	74 ± 14	74 ± 14	
HIGH	342 ± 55	313 ± 40	392 ± 53	392 ± 53	392 ± 53	364 ± 52	364 ± 52	364 ± 52	364 ± 52	138 ± 22	138 ± 22	138 ± 22	138 ± 22	113 ± 31	113 ± 31	93 ± 21	93 ± 21	80 ± 10	80 ± 10	
SC	313 ± 38	290 ± 39	390 ± 49	390 ± 49	390 ± 49	385 ± 60	385 ± 60	385 ± 60	385 ± 60	189 ± 36	189 ± 36	189 ± 36	189 ± 36	132 ± 33	132 ± 33	128 ± 30	128 ± 30	117 ± 25	117 ± 25	
Net hepatic FFA uptake (μmol/kg/min)																				
MOD	1.6 ± 0.4	1.4 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	0.9 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	
HIGH	1.1 ± 0.2	0.9 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	0.0 ± 0.3	0.0 ± 0.3	0.0 ± 0.3	0.0 ± 0.3	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	
SC	1.1 ± 0.2	0.7 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.3	0.8 ± 0.3	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.4	0.2 ± 0.4	0.3 ± 0.1	0.3 ± 0.1	
Arterial blood glycerol (μmol/L)																				
MOD	49 ± 7	43 ± 5	48 ± 7	42 ± 7	54 ± 8	47 ± 6	39 ± 9	39 ± 9	39 ± 9	27 ± 5	29 ± 5	29 ± 5	29 ± 5	27 ± 4	27 ± 4	26 ± 5	26 ± 5	25 ± 7	25 ± 7	
HIGH	33 ± 6	29 ± 5	38 ± 6	42 ± 6	44 ± 4	45 ± 6	30 ± 4	30 ± 4	30 ± 4	23 ± 3	22 ± 4	22 ± 4	22 ± 4	22 ± 5	22 ± 5	25 ± 5	25 ± 5	21 ± 2	21 ± 2	
SC	34 ± 3	32 ± 3	40 ± 3	46 ± 7	44 ± 3	45 ± 4	37 ± 4	37 ± 4	37 ± 4	31 ± 4	28 ± 5	28 ± 5	28 ± 5	28 ± 5	28 ± 5	33 ± 9	33 ± 9	30 ± 6	30 ± 6	
Net hepatic glycerol uptake (μmol/kg/min)																				
MOD	1.1 ± 0.4	0.9 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	
HIGH	0.2 ± 0.4	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	1.1 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	
SC	1.1 ± 0.3	0.6 ± 0.1	0.7 ± 0.2	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.6 ± 0.2	0.8 ± 0.3	0.8 ± 0.3	0.7 ± 0.2	0.7 ± 0.2	
Exogenous glucose infusion rate (mg/kg/min)																				
MOD	4.4 ± 1.3	4.6 ± 1.2	4.6 ± 1.2	4.0 ± 0.7	4.3 ± 0.9	4.4 ± 0.7	6.4 ± 0.8	6.4 ± 0.8	6.4 ± 0.8	6.9 ± 1.1	8.8 ± 1.9	8.8 ± 1.9	8.8 ± 1.9	11.6 ± 2.2	11.6 ± 2.2	13.8 ± 2.2	13.8 ± 2.2	16.4 ± 2.1	16.4 ± 2.1	
HIGH	8.6 ± 0.7*	7.5 ± 0.6*	7.5 ± 0.6*	4.9 ± 0.8	4.7 ± 0.8	4.8 ± 0.8	7.3 ± 1.1	7.3 ± 1.1	7.3 ± 1.1	7.1 ± 1.1	9.0 ± 1.5	9.0 ± 1.5	9.0 ± 1.5	11.9 ± 2.5	11.9 ± 2.5	14.5 ± 2.3	14.5 ± 2.3	15.6 ± 2.5	15.6 ± 2.5	
SC	11.0 ± 0.6*#	10.3 ± 0.5*#	10.3 ± 0.5*#	5.8 ± 0.6*	4.9 ± 0.4	4.3 ± 0.2	5.2 ± 0.7	5.2 ± 0.7	5.2 ± 0.7	5.5 ± 0.6	8.8 ± 1.5	8.8 ± 1.5	8.8 ± 1.5	10.3 ± 1.5	10.3 ± 1.5	13.1 ± 2.0	13.1 ± 2.0	15.6 ± 1.9	15.6 ± 1.9	
Nonhepatic glucose uptake (mg/kg/min)																				
MOD	3.1 ± 1.1	3.1 ± 1.1	3.1 ± 1.1	2.8 ± 0.5	2.6 ± 0.7	2.5 ± 0.7	3.7 ± 0.9	3.7 ± 0.9	3.7 ± 0.9	4.2 ± 1.1	6.6 ± 1.8	6.6 ± 1.8	6.6 ± 1.8	8.7 ± 2.2	8.7 ± 2.2	10.2 ± 2.0	10.2 ± 2.0	12.1 ± 2.3	12.1 ± 2.3	
HIGH	0.9 ± 0.9	0.9 ± 0.7	0.9 ± 0.7	0.6 ± 0.6	1.4 ± 0.7	1.9 ± 0.6	4.1 ± 1.2	4.1 ± 1.2	4.1 ± 1.2	3.5 ± 1.1	4.1 ± 1.4	4.1 ± 1.4	4.1 ± 1.4	7.2 ± 2.2	7.2 ± 2.2	9.4 ± 2.1	9.4 ± 2.1	10.9 ± 2.3	10.9 ± 2.3	
SC	1.8 ± 0.9	2.6 ± 0.7	2.6 ± 0.7	2.1 ± 0.5	2.4 ± 0.4	2.1 ± 0.4	2.6 ± 0.7	2.6 ± 0.7	2.6 ± 0.7	3.6 ± 1.1	5.7 ± 1.2	5.7 ± 1.2	5.7 ± 1.2	7.1 ± 1.7	7.1 ± 1.7	9.5 ± 2.1	9.5 ± 2.1	11.8 ± 2.1	11.8 ± 2.1	

Data are mean ± SEM. * $P < 0.05$ compared with MOD; # $P < 0.05$ compared with HIGH.

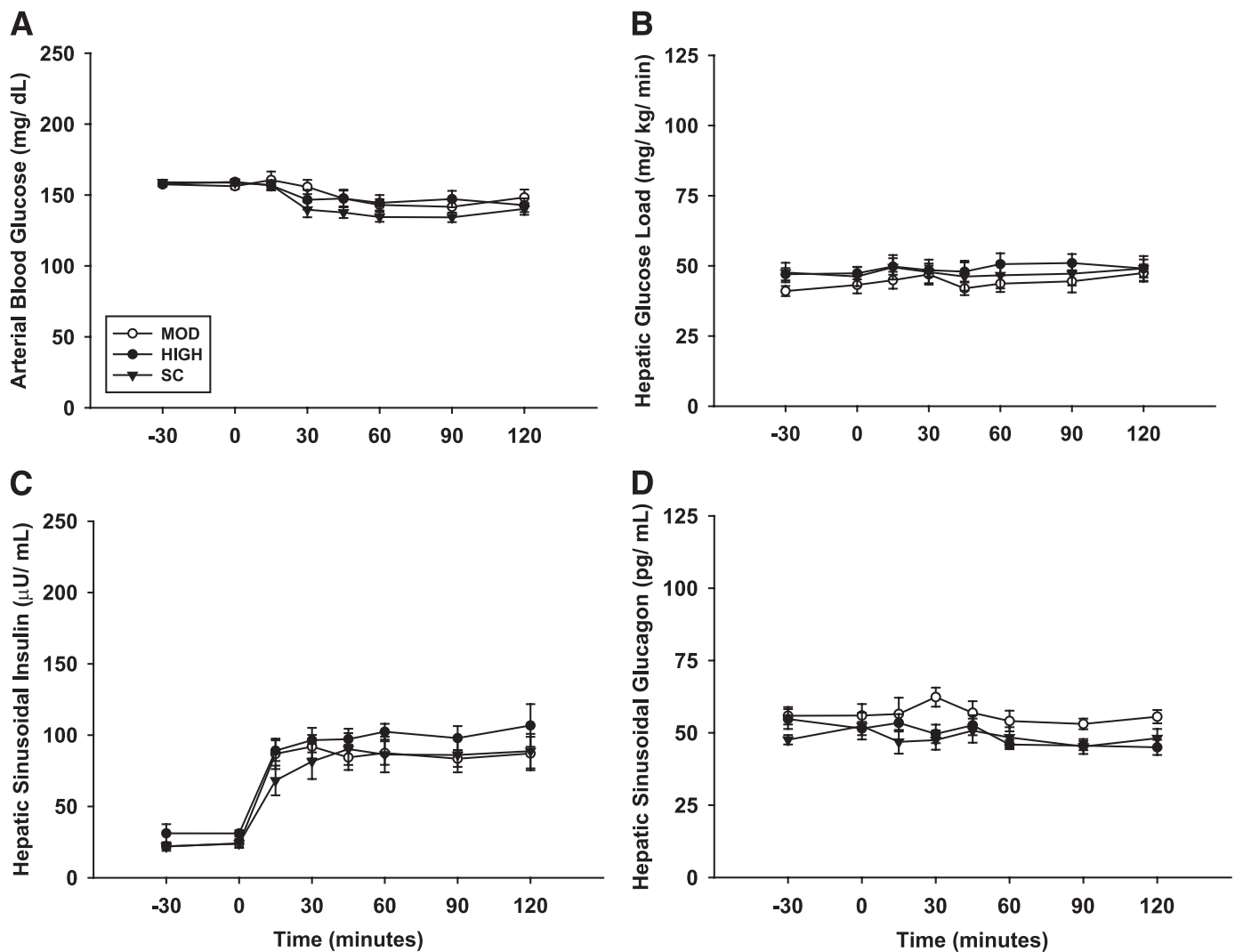


FIG. 3. Arterial blood glucose (A), glucose load to the liver (B), hepatic sinusoidal insulin (C), and hepatic sinusoidal glucagon (D) before and during the 120-min experimental period. No differences were detected among groups at any time point ($P > 0.05$).

MOD, HIGH, and SC, respectively; $P < 0.05$ between each group; Fig. 6A). On the other hand, the skeletal muscle glycogen level was similar in the three groups (8 ± 1 , 9 ± 1 , and 9 ± 1 mg/g, respectively; $P > 0.05$). Hepatic insulin signaling was reduced when the glycogen level was supercompensated as indicated by a 45% reduction in the phosphorylation state of Akt (Fig. 6B) and a 21% reduction in GSK3- β phosphorylation (Fig. 6C). This was associated with an increase in hepatic G6P content in SC compared with both MOD and HIGH (50 ± 7 , 66 ± 7 , and 176 ± 46 nmol/g in MOD, HIGH, and SC, respectively; $P < 0.01$ for each). In addition, there was a marked increase in AMP-activated protein kinase (AMPK)-Thr172 phosphorylation in SC compared with MOD (1.0 ± 0.3 , 1.1 ± 0.3 , and 2.3 ± 0.3 in MOD, HIGH, and SC, respectively; $P < 0.05$; Fig. 6D) and a reduction in liver GK mRNA (1.0 ± 0.1 , 1.1 ± 0.1 , and 0.6 ± 0.1 in MOD, HIGH, and SC, respectively; $P < 0.05$) in SC compared with MOD. The activity ratio of GS decreased 85% in SC compared with MOD ($P < 0.05$), whereas the activity ratio of GP was increased fourfold in SC compared with MOD ($P < 0.05$; Fig. 6E and F). Liver TG content was not significantly different among groups

(1.0 ± 0.2 , 1.1 ± 0.2 , and 1.5 ± 0.5 μ g TG/mg tissue for MOD, HIGH, and SC groups, respectively; $P > 0.40$). The slightly (but not significantly) higher TG value in the SC group was attributable to a single outlier (4.6 μ g/mg), the removal of which would make the SC group mean 1.0 ± 0.1 μ g/mg. Likewise, there were no differences in FAS protein or the phosphorylation state of ATP-citrate lyase, which are thought to be rate limiting for lipogenesis (data not shown). Furthermore, there was no indication of changes in the mRNA levels for selected enzymes involved in the regulation of lipid metabolism, such as CPT-1 (1.0 ± 0.2 , 0.7 ± 0.2 , and 0.9 ± 0.1) or FAS (1.0 ± 0.2 , 1.0 ± 0.1 and 1.2 ± 0.1) in MOD, HIGH, and SC, respectively.

DISCUSSION

The purpose of this study was to determine whether increasing the hepatic glycogen content can limit the uptake or storage of glucose by the liver. Our previous study (15) showed that an increase in hepatic glycogen by an amount similar to that seen after a single meal had no subsequent effect on NHGU or hepatic glucose disposition in response to a hyperglycemic/hyperinsulinemic clamp (i.e., without

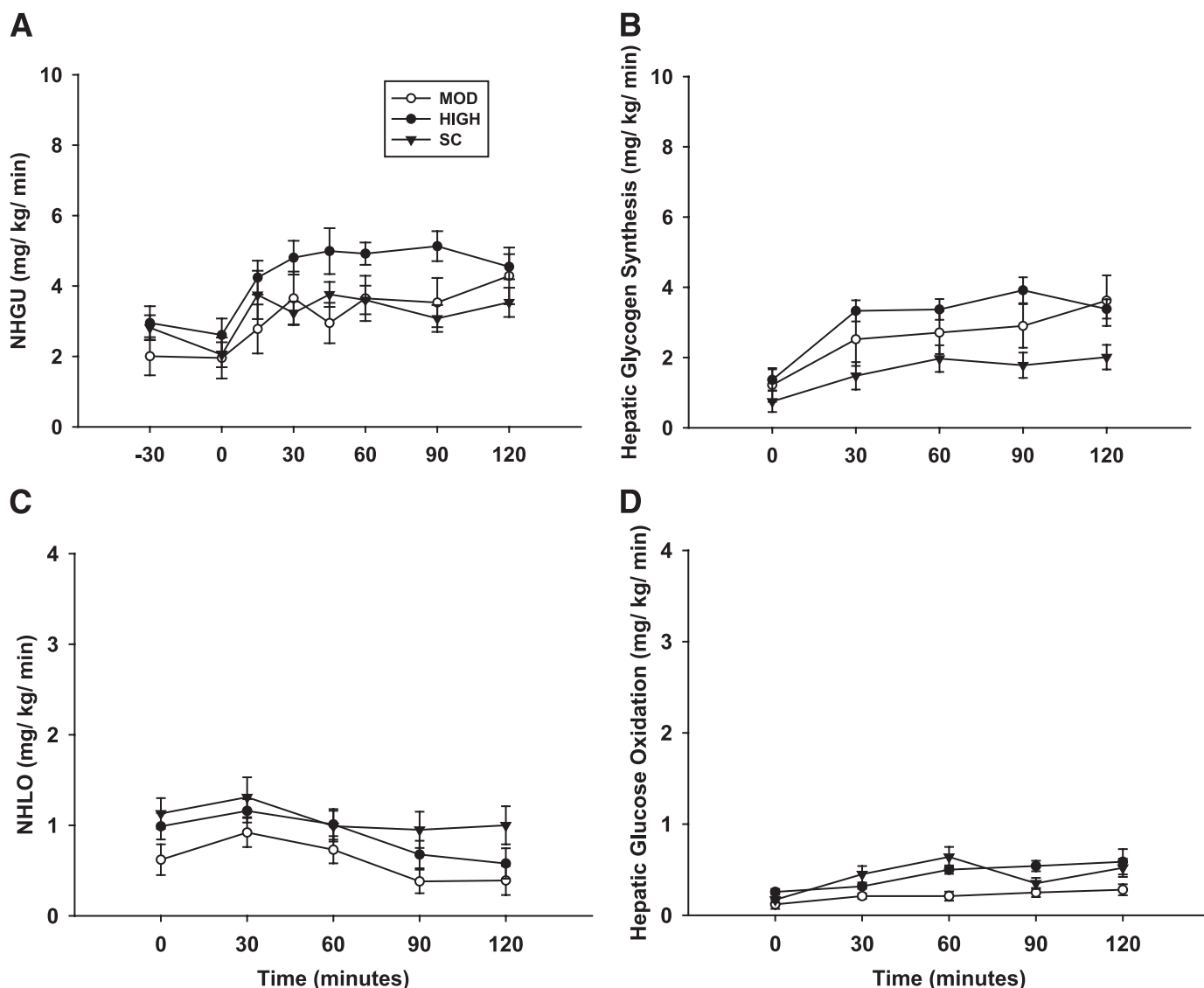


FIG. 4. NHGU (A), net hepatic glycogen synthesis (B), NHLO (in glucose equivalents) (C), and hepatic glucose oxidation (D) during the experimental period. AUC during the final hour of the experimental period for hepatic glucose oxidation ($P = 0.01$) and NHLO ($P = 0.02$) was lower in MOD compared with SC, whereas hepatic glycogen synthesis was higher ($P = 0.05$).

portal vein glucose infusion). In the current study, we increased the hepatic glycogen content even further, and we increased the challenge to the liver by adding portal glucose delivery to the hyperglycemic/hyperinsulinemic signals previously used. Our data show that although acutely supercompensating the hepatic glycogen content failed to affect NHGU, it reduced net hepatic glycogen synthesis, resulting in increased hepatic lactate production and glucose oxidation. Furthermore, these changes were associated with the generation of an insulin-resistant state in the liver, a significant increase in hepatic AMPK phosphorylation, and a shift in the activity of glycogen metabolizing enzymes to a state limiting further glycogen deposition.

Consistent with a previous study from our laboratory (15), a catalytic dose of fructose was infused on the background of hyperglycemia to load the liver with glycogen. When small quantities of fructose accompany hyperglycemia, hepatic glucose metabolism is augmented in both humans and dogs (33–35). In addition, it has been shown in the dog that fructose stimulates liver glucose

uptake in a curvilinear fashion up to a rate (e.g., ~ 12.5 mg/kg/min) (35) twice that generated during the postprandial state (~ 5 – 6 mg/kg/min) (13,14). The potency of fructose and the rapid diminution of its effect made it preferable to insulin for liver glycogen loading because the latter stimulates NHGU to a lower V_{max} (15) and produces intracellular signaling effects that can persist for a prolonged period. On the basis of our estimate of the rate of glycogen synthesis during the final experimental period and the hepatic glycogen level present at the end of the experiment, we can estimate that the glycogen level that existed before the insulin/glucose challenge was 52, 73, and 94 mg/g liver in MOD, HIGH, and SC, respectively. The fact that the net glycogen synthetic rates evident at the end of the glycogen loading period in the two fructose infusion groups (Fig. 2) were more similar than the liver glycogen levels before the experimental period is most likely explained by a slower rate of increase in glycogen synthesis over the 4-h loading period in the group with the lower fructose infusion rate and the more rapid return of

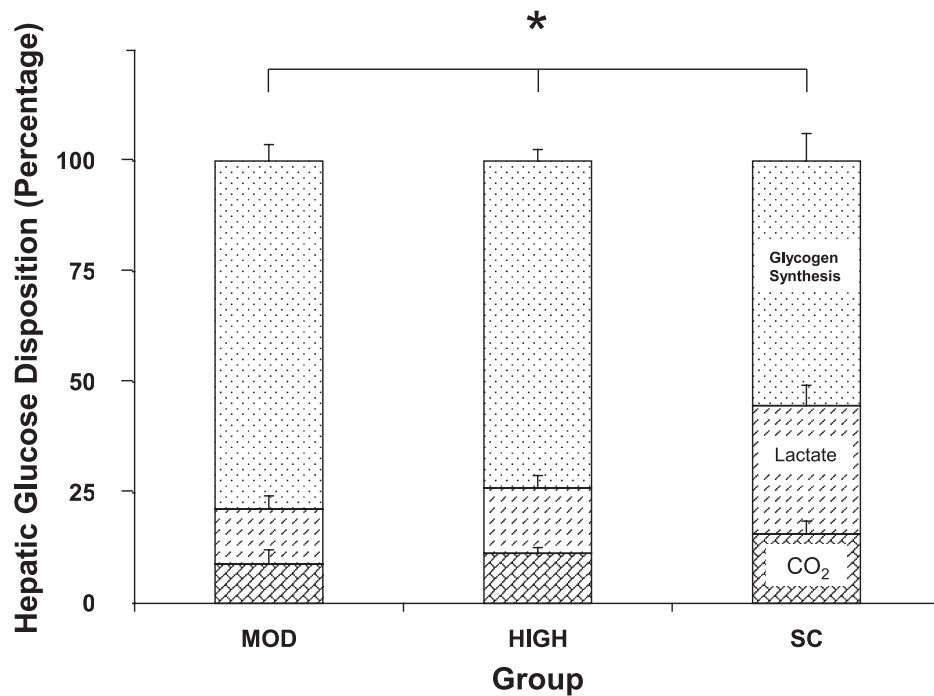


FIG. 5. The disposition of glucose taken up by the liver during the final hour of the experimental period into carbon dioxide (CO₂; bricked area), lactate (hatched area), and glycogen (dotted area). **P* < 0.05 for net hepatic glycogen synthesis and NHLO in SC compared with both HIGH and MOD.

glycogen synthesis to baseline after the withdrawal of the fructose infusion.

As noted earlier, we previously showed that moderate differences in liver glycogen content did not impair NHGU

or net liver glycogen synthesis seen in response to hyperglycemic/hyperinsulinemic conditions (15). This finding was in contrast with that of Galassetti et al. (16), who showed that although lowering the glycogen content by

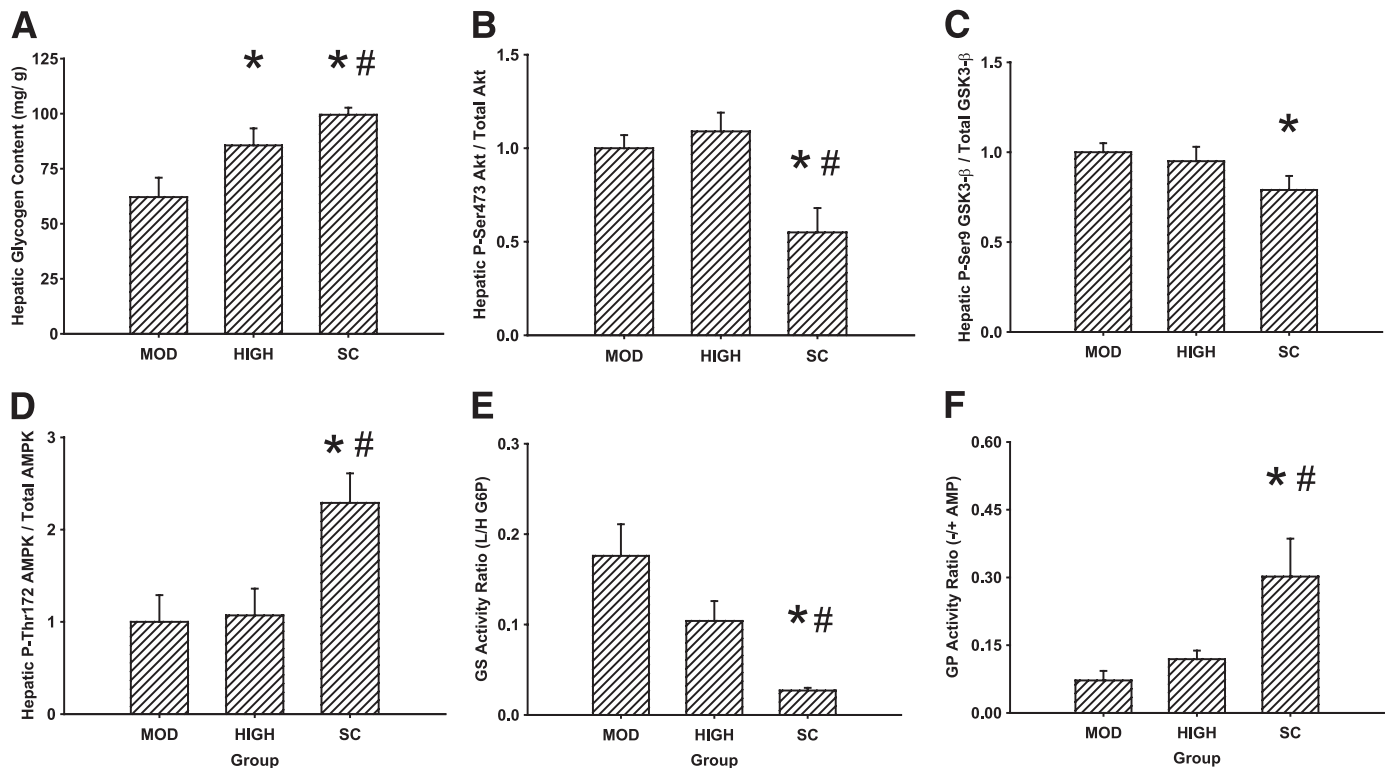


FIG. 6. Hepatic glycogen content (A), Akt (B), GSK3-β (C), and AMPK (D) phosphorylation and GS (E) and GP (F) activity ratios from liver biopsies taken at the conclusion of the experimental period. **P* < 0.05 compared with MOD; #*P* < 0.05 compared with HIGH.

prolonged fasting had no impact on NHGU, it did increase the proportion of NHGU that was converted to glycogen. Unfortunately, the prolonged fast undoubtedly produced changes in hepatic glucose metabolism that were independent of the glycogen content, making data interpretation difficult. Our current results demonstrate that although supercompensation of the liver's glycogen stores (i.e., 100 ± 3 vs. 62 ± 9 mg/g liver) had no effect on NHGU, it reduced net glycogen synthesis significantly, with 65% of this reduction being accounted for by increased lactate production, and the remainder by a near doubling of glucose oxidation. These data suggest that as glycogen stores become saturated, glucose taken up by the liver is directed away from glycogen in favor of the glycolytic pathway, such that lactate is subsequently released into the circulation to be metabolized by other tissues of the body. Our data, together with those of Galassetti et al. (16), suggest that hepatic glycogen synthesis displays autoregulatory behavior, being more efficient when the glycogen content is low (i.e., <30 mg/g liver) and less efficient when it is high (i.e., >100 mg/g liver) but of fixed efficiency over the normal diurnal range of glycogen concentrations.

In contrast with studies that have shown a reduction in muscle glucose uptake associated with increased glycogen content (36,37), liver glucose uptake likely remains unaffected by increased glycogen content because its glucose transport proteins are indigenous to the membrane, thereby making their activity independent of insulin signaling. However, we did see a reduction in GK mRNA in the SC group that was likely caused by impaired insulin signaling. Had the duration of the metabolic challenge period been >2 h, GK protein would likely have been reduced and liver glucose uptake would in turn be expected to decrease.

The decrease in glycogen deposition in the supercompensated liver is most likely explained both by the increase in AMPK phosphorylation and by the impairment in insulin signaling. Jørgensen et al. (38) showed that increasing the glycogen content of skeletal muscle led to large increases in GS phosphorylation at Ser7, thereby reducing its activity. In line with this, McBride and Hardie (39) hypothesized that glycogen loading (in both muscle and liver) increases the binding of AMPK to the non-reducing ends of the glycogen molecule's outer chains, and this close proximity to GS, which is also bound to glycogen, allows AMPK to reduce GS activity by phosphorylating it at Ser7. The AMPK activator AICAR has been reported to reduce insulin-mediated Akt and GSK3- β phosphorylation in muscle (40). Whether the increase in hepatic AMPK was responsible for the decrease in insulin signaling and GSK3- β phosphorylation in the current study remains unclear. Regardless, it seems likely that the decrease in GSK3- β phosphorylation that we observed could also have contributed to the decrease in GS activity. Future studies will be required to establish a cause and effect relationship among AMPK, hepatic insulin signaling, GS activity, and glycogen deposition.

In concert with the reduction in GS activity, there was a significant increase in GP activity, and undoubtedly these coordinated changes were responsible for the reduction in net hepatic glycogen synthesis. Previous studies have shown that AICAR has little effect on GP activity (i.e., the phosphorylation state of GP) (41–43). On the other hand, it is also worth noting that in a previous study, Hallgren et al. (44) showed that an increase in the intracellular particulate concentration, which is indicative of cell shrinkage, can cause an increase in GP activity by a magnitude similar to

that observed in response to glucagon. An approximate threefold increase in the hepatic glycogen content above basal may also be able to bring about such a response.

Despite the large reduction in GS activity, the liver was still able to incorporate more than one half of the glucose taken up in response to the hyperglycemic/hyperinsulinemic challenge into glycogen. This relative (albeit incomplete) preservation of the glycogen synthetic rate was probably a result of the 3.5-fold increase in hepatic G6P content that was generated by the "bottlenecking" of incoming glucose caused by the reduction in glycogen synthesis. The GS activity ratio (i.e., the ratio of glycogen synthesis at low compared with high G6P levels) represents the phosphorylation state of the enzyme and does not account for differences in allosteric effectors that may exist in vivo. In addition to being a potent stimulator of glycolysis and lactate production in the liver, G6P activates GS without regard to its state of phosphorylation (45). Thus, GS activity in vivo was probably greater in the SC group than is reflected by the in vitro assay because of the elevated G6P content. During the final 20 min of the glycogen loading period, the percentage of NHGU that was converted to glycogen in the two fructose infusion groups was similar (69 vs. 70% in HIGH and SC, respectively), despite a large calculated difference (59 ± 8 vs. 82 ± 3 mg/g liver) in hepatic glycogen content. The most likely explanation for this is that the hepatic glycogen content present at the end of the glycogen loading period in the supercompensated group still had not reached a level high enough to trigger the inhibition of glycogen deposition. The fact that there was only a small, nonsignificant effect of increased glycogen on glucose disposition in the HIGH group supports this possibility. On the other hand, it is also possible that the G6P effect on GS is not down-regulated by glycogen saturation, and it was probably this signal that triggered glycogen synthesis during the glycogen loading period.

Although fructose can be lipogenic in the liver, the normal rate of hepatic de novo lipogenesis (DNL) from carbohydrate precursors is low (46) and becomes dysregulated only after chronic fructose consumption (47,48). In line with this, Parks et al. (49) showed that a morning meal in which 50% of the caloric intake was from fructose did not affect DNL during a mixed meal consumed ~ 4.5 h later. In our study, fructose made up <5 and 10% of calories infused during the glycogen loading period in the HIGH and SC groups, respectively, and would therefore not have been expected to increase DNL significantly. The absence of differences in hepatic TG content and in FAS and CPT-1 mRNA, and the similarity of FAS and ATP-citrate lyase protein content and phosphorylation state in all groups support our conclusion that relevant quantities of carbon derived from NHGU do not end up in hepatic lipid stores.

In summary, our data show that although a modest increase in the hepatic glycogen content has little effect on liver glucose uptake or glycogen accretion, a marked increase in hepatic glycogen reduces net glycogen synthesis. Consequently, both lactate output and glucose oxidation by the liver increase. These changes are associated with impaired hepatic insulin signaling, increased AMPK phosphorylation, and a change in the activity of glycogen metabolizing enzymes toward a state that discourages further glycogen accumulation. Thus, as drugs that increase hepatic glycogen stores (e.g., GK activators, glucagon receptor antagonists, and GP inhibitors) are developed to treat postprandial hyperglycemia in patients with type 2

diabetes mellitus, care should be taken not to overfill the hepatic glycogen pool.

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J.J.W. collected all data and wrote the article. Z.A. assisted with all data collection. C.J.R. assisted with the collection of Western and mRNA data. M.S. assisted with all data collection. J.M.I. and P.J.R. performed *in vitro* assays and assisted with the data analysis. D.W.N. performed the surgeries. M.C.M. performed the initial studies and was involved with the study's conception. A.D.C. was involved in all intellectual and financial aspects of the study. All authors provided input during the writing of the article.

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