

# Liver Glycogen Loading Dampens Glycogen Synthesis Seen in Response to Either Hyperinsulinemia or Intraportal Glucose Infusion

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The purpose of this study was to determine the effect of liver glycogen loading on net hepatic glycogen synthesis during hyperinsulinemia or hepatic portal vein glucose infusion in vivo. Liver glycogen levels were supercompensated (SCGly) in two groups (using intraportal fructose infusion) but not in two others (Gly) during hyperglycemic-normoinsulinemia. Following a 2-h control period during which fructose infusion was stopped, there was a 2-h experimental period in which the response to hyperglycemia plus either 4× basal insulin (INS) or portal vein glucose infusion (PoG) was measured. Increased hepatic glycogen reduced the percent of glucose taken up by the liver that was deposited in glycogen ( $74 \pm 3$  vs.  $53 \pm 5\%$  in Gly+INS and SCGly+INS, respectively, and  $72 \pm 3$  vs.  $50 \pm 6\%$  in Gly+PoG and SCGly+PoG, respectively). The reduction in liver glycogen synthesis in SCGly+INS was accompanied by a decrease in both insulin signaling and an increase in AMPK activation, whereas only the latter was observed in SCGly+PoG. These data indicate that liver glycogen loading impairs glycogen synthesis regardless of the signal used to stimulate it. *Diabetes* 62:96–101, 2013

**I**n humans, one-third of the glucose ingested during an oral challenge is taken up by the liver, whereas the remaining two-thirds escape the splanchnic bed to be metabolized elsewhere (1–3). This process is reduced in humans with type 2 diabetes (2,4,5), thereby highlighting the importance of understanding how this complex process is regulated in the normal state and why it becomes dysfunctional in the diseased state.

When hyperglycemia is accompanied by hyperinsulinemia (6) and the presence of a negative arterial-portal vein glucose gradient [also called the portal glucose signal (7)], both net hepatic glucose uptake (NHGU) and glycogen synthesis are stimulated to a maximal physiological level. Furthermore, both insulin's and the portal glucose signal's ability to stimulate NHGU and glycogen synthesis are additive (6). Although the mechanisms by which both insulin and the portal glucose signal stimulate the uptake of glucose and glycogen synthesis in the liver are not fully understood, both are thought to involve the translocation of glucokinase from the nucleus to the cytosol, where glucose phosphorylation occurs (8), as well as the reciprocal coordination of the

activities of glycogen synthase (GS) and glycogen phosphorylase (GP).

Drugs are being developed to reduce postprandial glucose excursions by stimulating hepatic glucose uptake and glycogen deposition. However, questions remain about the possible deleterious effect that loading the liver with glycogen could have on hepatic glucose fluxes during the postprandial state. In a previous study (9) when hepatic glycogen was increased from 64 to 100 mg/g, hepatic glycogen synthesis was reduced in response to hyperglycemic-hyperinsulinemia plus the portal glucose signal. This reduction in glycogen synthesis was accompanied by reduced insulin signaling, an increase in AMPK phosphorylation, and subsequent dysregulation of the activity of both GS and GP toward states discouraging further glycogen accretion. Were the impairment in glycogen synthesis a function of reduced insulin signaling, the glycogen synthetic rate should only be reduced in response to hyperinsulinemia and remain unchanged in response to the portal glucose signal. In contrast, if the increase in AMPK activation causes the reduction in glycogen synthesis, then the glycogen synthetic rate seen in response to either hyperinsulinemia or portal vein glucose infusion should be reduced. Therefore, the purpose of the current study was to determine the effect of hepatic glycogen supercompensation on insulin- or portal glucose signal-stimulated increases in hepatic glycogen synthesis.

## RESEARCH DESIGN AND METHODS

**Animals and surgical procedures.** Studies were carried out on 18-h fasted dogs with a mean weight of  $22.6 \pm 0.4$  kg. The animals were housed in a facility that met Association for Assessment and Accreditation of Laboratory Animal Care International 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 to permit placement of catheters for intraportal infusions and blood sampling across the liver (6). Ultrasonic flow probes (Transonic Systems, Ithaca, NY) were placed around the hepatic portal vein and the hepatic artery to measure blood flow.

**Experimental design.** Experiments consisted of a 4-h liver glycogen loading period (–360 to –120 min), a 2-h control period (–120–0 min), and a 2-h experimental period (0–120 min) and were initiated by the infusion of somatostatin ( $0.8 \mu\text{g}/\text{kg}/\text{min}$ ; Bachem, Torrance, CA) into a peripheral vein to disable the endocrine pancreas. 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 ( $0.55 \text{ ng}/\text{kg}/\text{min}$ ; Novo Nordisk, Bagsvaerd, Denmark) at basal rates. At the same time, blood glucose was doubled by infusing 50% dextrose into a peripheral vein and either saline (Gly;  $n = 17$ ) or fructose ( $1.0 \text{ mg}/\text{kg}/\text{min}$ ; SCGly;  $n = 17$ ) into the hepatic portal vein, the latter to stimulate hepatic glycogen deposition. The glycogen-loading period was followed by a 2-h hyperglycemic control period during which fructose infusion was stopped but basal hormone levels were maintained. A  $^{14}\text{C}$ -glucose infusion was begun at –90 min to assess hepatic glucose oxidation. During the 2-h experimental period (0–120 min), seven of the animals in the SAL group (Gly+INS) and eight animals in the SCGly group (SCGly+INS) received an intraportal infusion of

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insulin that was four times (1.2 mU/kg/min) basal. Another eight animals in the SAL group (Gly+PoG) and seven animals in the SCGly group (SCGly+PoG) received an intraportal infusion of 20% dextrose at a rate of 4.0 mg/kg/min. Two animals from each of the Gly and SCGly groups were used for control studies to assess basal molecular biology. In these animals, hyperglycemia was maintained in the presence of basal insulin and glucagon until 120 min. The only measured parameter that differed between these animals was liver glycogen content (Fig. 4A), so data were combined to provide values for the time-control group (CON;  $n = 4$ ). At the conclusion of the study, all animals were killed with pentobarbital. Liver biopsies were stored at  $-80^{\circ}\text{C}$ . The processing and analysis of blood samples and tissue analyses were performed as described previously (9).

**Calculations and data analysis.** Net hepatic substrate balance and sinusoidal hormone levels were calculated as described earlier (10–12). Net hepatic glycogen synthesis and hepatic glucose oxidation were also measured as previously described (9), as were glucose-6-phosphate (G6P) content (13) and GS and GP activities (14,15).

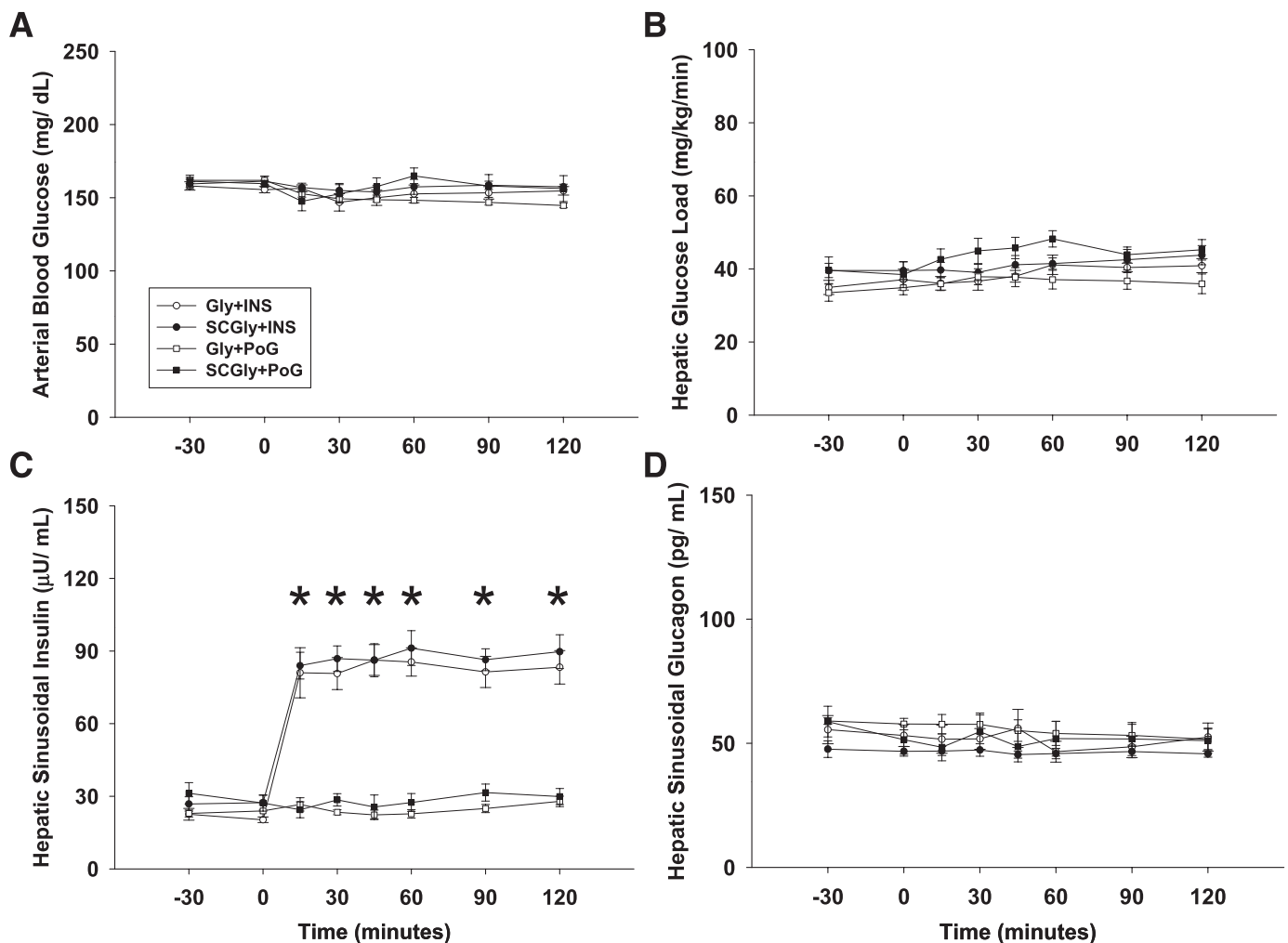
**Statistical analysis.** All data are presented as mean  $\pm$  SEM. Data were analyzed using repeated-measures ANOVA. Post hoc comparisons were made as appropriate, and statistical significance was  $P < 0.05$ .

## RESULTS

**In vivo metabolic data.** During both the control and experimental periods, the arterial blood glucose level and the glucose load to the liver were not different among groups (Fig. 1). At the outset of the experimental period (i.e., min

0), the hepatic sinusoidal insulin concentration was raised fourfold in the Gly+INS and SCGly+INS groups and maintained at a basal value in Gly+PoG and SCGly+PoG (Fig. 1C). In all four groups, the hepatic sinusoidal glucagon levels remained basal throughout the study (Fig. 1D). NHGU was modest during the final 30 min of the control period in all groups (Fig. 2A) and increased along with net glycogen synthesis in all groups over the 2-h test period (Fig. 2B). Nevertheless, the area under the curve for glycogen synthesis over the final hour of the experimental period was lower in SCGly+INS and SCGly+PoG than in their respective saline-infused groups ( $P < 0.05$ ). The reduction in net glycogen synthesis was primarily accounted for by increased hepatic lactate output (Fig. 2C;  $P < 0.05$ ). When expressed as a percentage of NHGU, glycogen synthesis was reduced in both SCGly groups compared with their respective saline-infused controls, whereas lactate output was increased (Fig. 3;  $P < 0.05$ ).

**Hepatic tissue data.** Large differences in hepatic glycogen existed between the Gly and SCGly groups at the end of the experimental period (Fig. 4A). Compared with CON, a rise in insulin increased the GS activity ratio nearly threefold (Fig. 4B), an effect that was completely suppressed in the presence of glycogen loading. In response to



**FIG. 1.** Arterial blood glucose (A), glucose load to the liver (B), hepatic sinusoidal insulin (C), and hepatic sinusoidal glucagon (D) prior to and during the 120-min experimental period. No differences were detected among groups at any time point ( $P > 0.05$ ). \* $P < 0.05$  in both INS groups compared with both PoG groups.

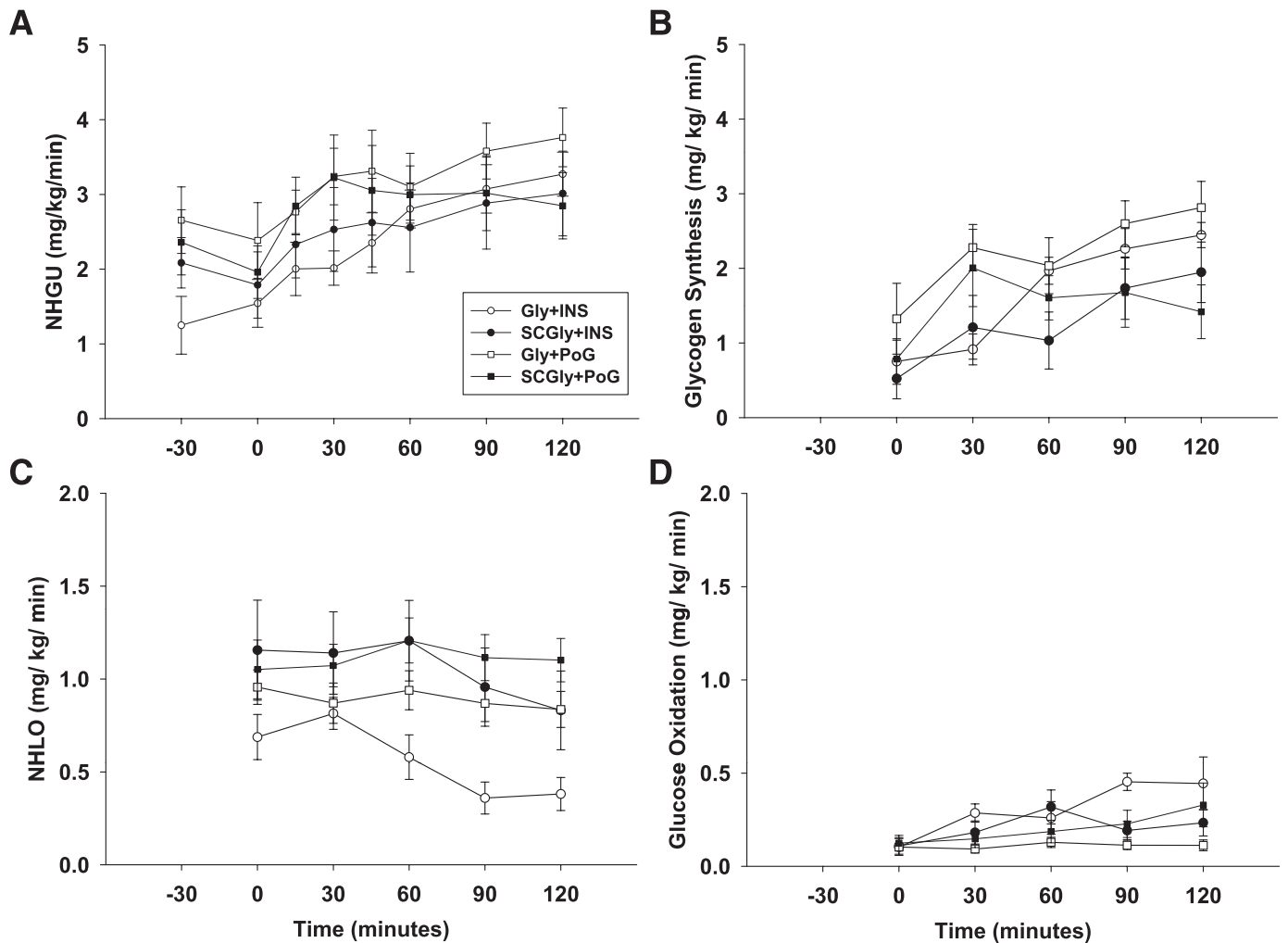


FIG. 2. NHGU (A), net glycogen synthesis (B), net hepatic lactate output (NHLO) (C), and hepatic glucose oxidation (D) during the experimental period.

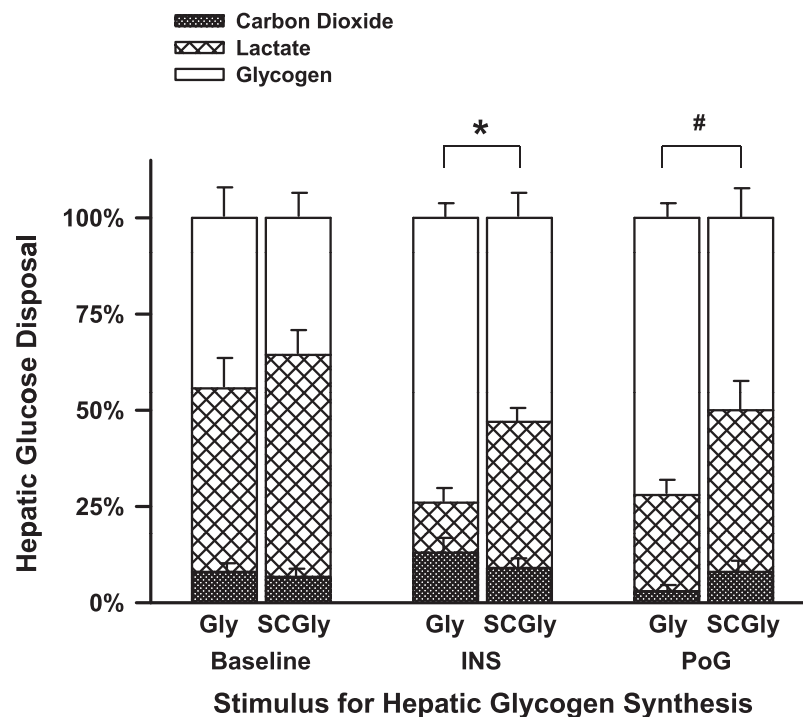
intraportal glucose infusion, the GS activity ratio in Gly+PoG remained at a value not different from that in CON. However, the GS activity ratio was further suppressed in the glycogen-loaded liver ( $P < 0.05$ ). GP activity was suppressed by 56% compared with CON in response to hyperinsulinemia, an effect that was completely blocked by liver glycogen loading (Fig. 4C;  $P < 0.05$ ). Intraportal glucose infusion had no effect on GP activity, and glycogen loading also failed to alter it. Hyperinsulinemia doubled Ser473 Akt phosphorylation in Gly+INS compared with CON (Fig. 4D;  $P < 0.05$ ). However, similar to its effect on GS activity, glycogen loading reduced phospho-Akt to a level similar to that seen in CON. In response to intraportal glucose infusion, hepatic Akt phosphorylation did not increase significantly and thus was not diminished by glycogen loading. Hepatic AMPK Thr172 phosphorylation was not increased above CON in Gly+INS or Gly+PoG. However, it was increased in both SCGly+INS and SCGly+PoG compared with their respective saline groups (Fig. 4E;  $P < 0.05$ ). The increase in AMPK phosphorylation occurred in spite of similar AMP, ADP, and ATP levels in all groups (data not shown). Portal glucose infusion was associated with increased hepatic G6P levels, whereas hyperinsulinemia was not. However, glycogen loading

increased G6P levels in both SCGly groups compared with their respective control groups (Fig. 4F;  $P < 0.05$ ).

## DISCUSSION

In humans with type 2 diabetes, liver glucose uptake and glycogen synthesis during the postprandial state are reduced compared with normal individuals, thereby contributing to glucose intolerance (2,4,5). Hepatic glucose uptake in response to a meal is triggered by three primary stimuli: 1) an increase in the glucose load to the liver, 2) hyperinsulinemia, and 3) the portal glucose signal (6,16,17). In a previous study (9), we showed that during hyperglycemic-hyperinsulinemia plus the portal glucose signal, increasing the hepatic glycogen level from 62 to 100 mg/g reduced the proportion of glucose taken up by the liver that was incorporated into glycogen from 79 to 55%. However, it remains unclear which signal (i.e., insulin or portal glucose delivery) was impaired.

At the end of the hyperglycemic control period (0 min), the percent of glucose taken up by the liver that was deposited in glycogen was 44 and 36% in the Gly and SCGly groups, respectively. In Gly, this was increased to 74% by insulin and 72% by portal glucose infusion, with an offsetting decrease in lactate release. On the contrary, when



**FIG. 3.** The disposition of glucose taken up by the liver in Gly and SCGly groups during the final 30 min of the hyperglycemic control period (baseline), the final hour of the experimental period during hyperinsulinemia (INS), and portal vein glucose infusion (PoG). NHGU during the baseline period averaged  $2.0 \pm 0.3$  mg/kg/min in both the Gly and SCGly groups ( $P > 0.05$ ). During the final hour of the experimental period, NHGU averaged  $3.0 \pm 0.3$  and  $2.8 \pm 0.6$  mg/kg/min in Gly+INS and SCGly+INS, respectively ( $P > 0.05$ ), and  $3.5 \pm 0.4$  and  $3.0 \pm 0.4$  mg/kg/min in Gly+PoG and SCGly+PoG, respectively ( $P > 0.05$ ). \* $P < 0.05$  for net hepatic glycogen synthesis and net hepatic lactate output in SCGly+INS compared with Gly+INS; # $P < 0.05$  for net hepatic glycogen synthesis, net hepatic lactate output, and hepatic glucose oxidation in SCGly+PoG compared with Gly+PoG.

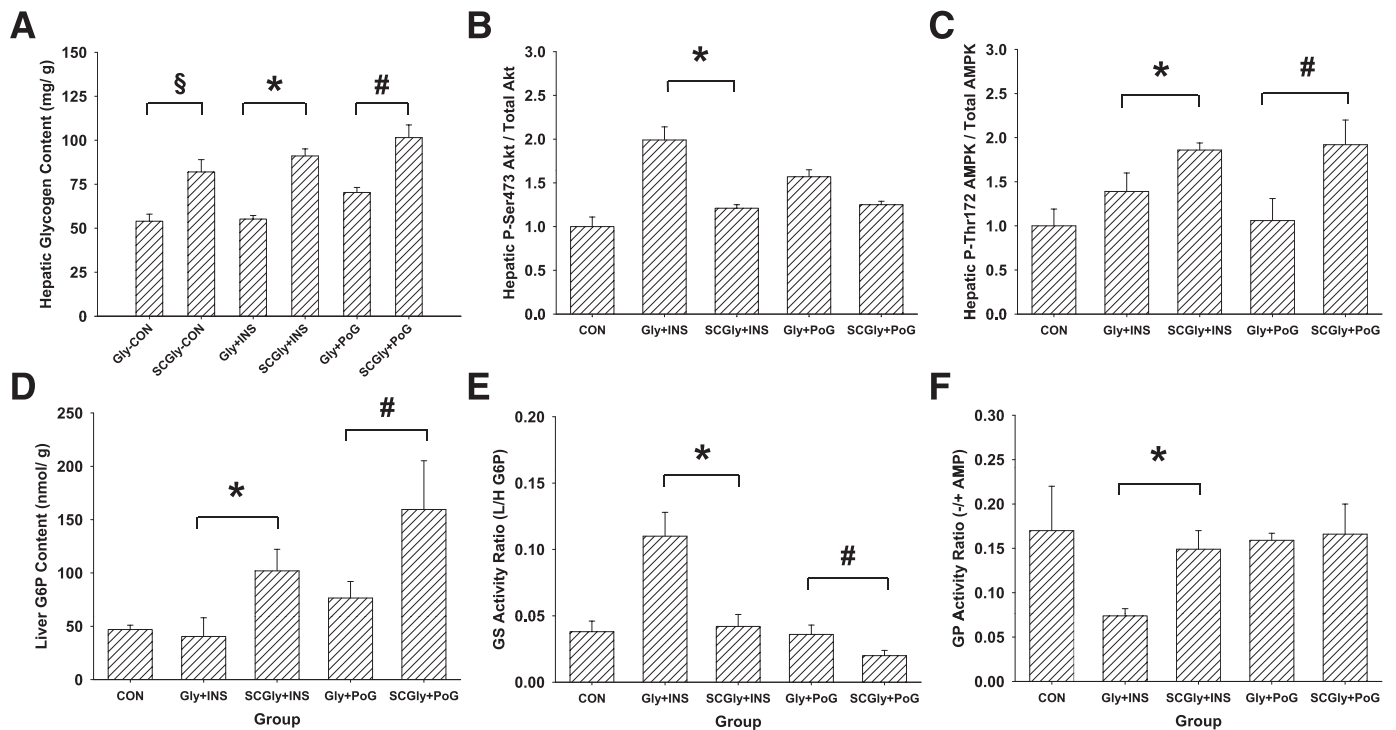
liver glycogen was supercompensated (SCGly), the percent of extracted glucose that was deposited in glycogen was only 53 and 50% during hyperinsulinemia or portal glucose infusion, respectively. Thus, regardless of the signal, the liver's ability to store incoming glucose as glycogen was reduced by glycogen loading.

In the absence of liver glycogen loading, portal glucose infusion (Gly+PoG) caused a rise in NHGU and glycogen synthesis despite no change in the activity ratios of GS or GP and no change in Akt or AMPK phosphorylation. However, the ability of intraportally derived glucose to promote its own incorporation into glycogen by a push mechanism led to a twofold increase in G6P content, a metabolite that allosterically activates and inhibits the activities of GS and GP, respectively (18–21). Liver glycogen loading (SCGly+PoG) caused an increase in AMPK phosphorylation without lowering Akt phosphorylation. In turn, the GS activity ratio was significantly reduced in the absence of a change in GP activity. These data suggest that the rise in AMPK activity most likely caused the reduction in GS activity due to its ability to phosphorylate and inactivate the enzyme (22). In contrast, the rise in AMPK had no apparent effect on the activity of GP, a finding that is consistent with previous data that the AMPK activator AICAR does not reduce GP activity in vivo (23,24). The G6P content doubled as a result of the reduction in net glycogen synthesis in SCGly+PoG. However, despite this increase, glycogen synthesis remained impaired, suggesting a compromised ability of G6P to allosterically regulate GS and/or GP activity (18).

In response to hyperinsulinemia (Gly+INS), both NHGU and glycogen synthesis rose to levels seen in response to

the portal glucose signal. As expected, the rise in insulin led to a rise and fall in the activity ratios of GS and GP, respectively. In turn, liver G6P content remained low due to the pull mechanism generated in response to the changes in glycogenic enzyme activity. However, in response to glycogen loading, AMPK activation rose and insulin signaling was completely ablated, thereby leading to GS and GP activity ratios similar to those seen in the basal, unstimulated state. Because the percent reduction in GS activity in response to glycogen loading was similar in both SCGly groups, it further supports the notion that the increase in AMPK activity was responsible (22). It is alternatively possible that the increase in AMPK activity reduced GS activity by impairing insulin signaling, as AICAR has been shown to have this effect in muscle (25). However, the presence of a reduction in GS activity in SCGly+PoG despite the absence of a reduction in insulin signaling argues against this possibility.

In addition to the reduction in GS activity ratio seen in response to hyperinsulinemia as a result of glycogen loading, GP activity was also increased. Whereas hyperinsulinemia clearly reduced GP activity in Gly+INS, this effect was completely absent in the glycogen-loaded liver as GP activity in SCGly+INS was not different from CON or either PoG group in which insulin levels remained basal. Because there was no change in GP activity in either PoG group, even with the increase in AMPK activation in SCGly+PoG, we conclude that the reduction in insulin signaling in SCGly+INS was responsible for the increase in GP activity seen with liver glycogen loading. This conclusion is supported by previous research indicating that GP activity is not regulated by AMPK (23,24). As was the case during



**FIG. 4.** Hepatic glycogen content (panel *A*), hepatic Akt (*B*), and AMPK (*C*) phosphorylation, hepatic G6P content (*D*), and glycogen synthase (GS) (*E*; the activity ratio in the presence of low and high G6P levels) and GP (panel *F*) activity ratios from liver biopsies taken at the conclusion of the experimental period. Gly-CON ( $n = 2$ ) and SCGly-CON ( $n = 2$ ) in panel *A* are hyperglycemic control animals that did not receive hyperinsulinemia or portal vein glucose infusion during the experimental period. In all other panels, these data were merged ( $n = 4$ ) due to the absence of any difference among the groups. Total GS activity (GS+; 6.7 mmol G6P, 4.4 mmol uridine 5'-diphosphate-glucose) was not different among groups and averaged  $21.6 \pm 0.7$  nmol uridine 5'-diphosphate-glucose/mg/min. Likewise, total GP activity (GP+; 2 mmol AMP) was similar among groups and averaged  $0.097 \pm 0.003$   $\mu$ mol G1P/mg/min. \* $P < 0.05$  in SCGly+INS compared with Gly+INS; # $P < 0.05$  in SCGly+PoG compared with Gly+PoG; § $P < 0.05$  in SCGly-CON compared with Gly-CON.

portal glucose infusion, glycogen loading led to a doubling of hepatic G6P content, to a level similar to that seen in response to portal glucose infusion in the nonloaded liver. Despite this increase in G6P, liver glycogen synthesis remained compromised, which further supports our conclusion that glycogen loading impairs the ability of G6P to regulate GS and/or GP activity.

In summary, our data show that acute hepatic glycogen loading equipotently reduced net glycogen synthesis by the liver during hyperinsulinemia or portal glucose infusion. Despite this similarity, the manner in which excessive glycogen accretion impairs glycogen synthesis in response to either signal appears to be different. On the one hand, glycogen loading likely reduces insulin-stimulated glycogen synthesis by AMPK-mediated reductions in GS activity and by increased GP activity from impaired insulin signaling. On the other hand, portal glucose signal-induced glycogen synthesis appears to be reduced by AMPK-mediated reductions in GS activity and a compromised ability of G6P to allosterically regulate the activities of GS and/or GP. Thus, as treatments are developed to treat glucose intolerance and type 2 diabetes by increasing hepatic glucose uptake, care should be taken not to overfill the hepatic glycogen pool due to its deleterious effects on hepatic glucose metabolism.

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J.J.W. wrote the manuscript and collected all data. Z.A. and G.K. assisted with data collection. C.J.R. assisted with the collection of Western data. J.M.I. and P.J.R. performed in vitro assays and assisted with the data analysis. M.S. and M.L. assisted with data collection. A.D.C. was involved in all intellectual and financial aspects of the study. All authors provided input during the writing of the manuscript. A.D.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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