# Portal Vein Glucose Entry Triggers a Coordinated Cellular Response That Potentiates Hepatic Glucose Uptake and Storage in Normal but Not High-Fat/High-Fructose–Fed Dogs

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The cellular events mediating the pleiotropic actions of portal vein glucose (PoG) delivery on hepatic glucose disposition have not been clearly defined. Likewise, the molecular defects associated with postprandial hyperglycemia and impaired hepatic glucose uptake (HGU) following consumption of a high-fat, high-fructose diet (HFFD) are unknown. Our goal was to identify hepatocellular changes elicited by hyperinsulinemia, hyperglycemia, and PoG signaling in normal chow-fed (CTR) and HFFD-fed dogs. In CTR dogs, we demonstrated that PoG infusion in the presence of hyperinsulinemia and hyperglycemia triggered an increase in the activity of hepatic glucokinase (GK) and glycogen synthase (GS), which occurred in association with further augmentation in HGU and glycogen synthesis (GSYN) in vivo. In contrast, 4 weeks of HFFD feeding markedly reduced GK protein content and impaired the activation of GS in association with diminished HGU and GSYN in vivo. Furthermore, the enzymatic changes associated with PoG sensing in chow-fed animals were abolished in HFFD-fed animals, consistent with loss of the stimulatory effects of PoG delivery. These data reveal new insight into the molecular physiology of the portal glucose signaling mechanism under normal conditions and to the pathophysiology of aberrant postprandial hepatic glucose disposition evident under a diet-induced glucose-intolerant condition. *Diabetes* 62:392–400, 2013

Neither hyperinsulinemia (physiologic) nor hyperglycemia alone is sufficient to stimulate hepatic glucose uptake (HGU) (1,2). Likewise, an elevation in plasma insulin coincident with hyperglycemia resulting from the infusion of glucose into a peripheral vein is insufficient to elicit the peak rates of HGU and glycogen synthesis (GSYN) that occur in response to oral or enteral glucose delivery (1,3–7). Conversely, delivery of glucose into the hepatic portal vein significantly amplifies HGU and GSYN in the rat (8,9), dog (4,7,10,11), and human (5,12). The augmentation of HGU elicited by the intraportal route of glucose delivery has been attributed to a signal generated in the presence of a negative arterial-portal venous glucose gradient, termed the portal glucose signal (13). Working in concert with the level of insulin (10) and the load of glucose reaching the liver (14), the portal glucose signal orchestrates a coordinated metabolic response favoring enhanced HGU and GSYN following ingestion of a glucose-containing meal (15).

Postprandial hyperglycemia is one of the sequelae of diabetes that contributes to the elevation of hemoglobin $A_{1c}$ associated with the disease (16). It is due in part to inappropriate suppression of hepatic glucose production (HGP) coupled with inadequate stimulation of HGU, underscoring the key role of the liver in regulating postprandial glucose metabolism (17–19). Previously, Basu et al. (20,21) demonstrated that splanchnic (which comprises the gut and liver tissues) glucose uptake (SGU) and hepatic GSYN were markedly diminished in type 2 diabetic subjects compared with nondiabetic control subjects despite equivalent elevations in plasma insulin and glucose. Furthermore, portal vein glucose (PoG) delivery (by way of enteral glucose infusion) in the presence of hyperinsulinemia was ineffective in normalizing the reduced rates of SGU and GSYN in diabetic subjects (20). The authors (20,21) postulated that a defect in glucokinase (GK) was linked to the aberrant hepatic response in type 2 diabetic individuals, but cellular evidence supporting this claim was not provided.

Recently, our laboratory developed a large animal model of glucose intolerance by feeding dogs a high-fat/high-fructose diet (HFFD; 52/17% of total energy, respectively) (22). We used an HFFD because it mirrors a Western diet, which contains foods that are replete with saturated fat and simple sugars, and has been associated with an increased risk for the development of type 2 diabetes (23,24). Chronic consumption of an HFFD resulted in postprandial hyperglycemia and diminished net HGU in response to an oral mixed-meal challenge (25) or a hyperinsulinemic-hyperglycemic (HIHG) clamp (22). However, the cellular changes associated with PoG delivery and diet-induced diminished hepatic responsiveness to postprandial cues were not identified. Thus, the first goal of this study was to assess the changes in hepatic GK, glycogen synthase (GS), and glycogen phosphorylase (GP) activity elicited by PoG delivery in the presence of a physiologic rise in glucose and insulin. The second goal was to identify a cellular explanation for the defect in HGU associated with consumption of an HFFD.

## RESEARCH DESIGN AND METHODS

**Animal care and surgical procedures.** The protocol was approved by the Vanderbilt University Institutional Animal Care and Use Committee, and the

animals were housed and cared for according to Association for Assessment and Accreditation of Laboratory Animal Care International guidelines. Adult male mongrel dogs were fed a standard meat (Kal Kan, Franklin, TN) and laboratory chow diet (control [CTR], $n = 15$) or a high-fat, high-fructose diet (HFFD, $n = 16$; PMI Nutrition TestDiet, St. Louis, MO) for 4 weeks, as described elsewhere (22). After 2 weeks of feeding, a subset of dogs (CTR, $n = 10$; HFFD, $n = 11$) underwent a laparotomy to insert sampling catheters into the hepatic portal vein, the left common hepatic vein, and the femoral artery and to insert infusion catheters into a jejunal and splenic vein (4). Ultrasonic blood flow cuffs (Transonic Systems, Ithaca, NY) were secured around the hepatic artery and hepatic portal vein for the measurement of hepatic blood flow, as described previously (4). After 4 weeks of feeding, another subset of dogs (CTR, $n = 5$; HFFD, $n = 5$) was killed following an 18-h fast for the acquisition of liver biopsies under basal conditions. All dogs studied were healthy, as indicated by: 1) hematocrit >35%; 2) leukocyte count <18,000/mm$^3$; 3) good appetite; and 4) normal stools.

**Experimental design.** After 4 weeks of feeding, HIHG clamp experiments with (+) or without (−) PoG infusion were conducted on four groups (CTR−PoG [$n = 5$], HFFD−PoG [$n = 5$], CTR+PoG [$n = 5$], and HFFD+PoG HFFD [$n = 6$]) of 18-h–fasted dogs that had been fed one can of meat just prior to fasting (Fig. 1). Experiments consisted of a 100-min equilibration period (−120 to −20 min), a 20-min control period (−20 to 0 min), and a 180-min HIHG period divided into two subperiods (P1, 0–90 min; P2, 90–180 min). At −120 min, a priming dose of [3-$^3$H] glucose (38 μCi) was given, followed by a constant infusion of [3-$^3$H] glucose (0.38 μCi/min). At time 0, a constant infusion of somatostatin (0.8 μg/kg/min; Bachem, Torrance, CA) was started in a leg vein, and insulin and glucagon were then replaced intraportally at threefold basal (1.2 mU/kg/min; Lilly) and basal (0.55 ng/kg/min; Novo Nordisk) rates, respectively. A variable infusion of 50% dextrose was started in a leg vein in order to double the hepatic glucose load (HGL). In P2, normal saline (−PoG) or 20% dextrose (+PoG; 4.0 mg/kg/min) was infused intraportally. In the +PoG groups, the peripheral glucose infusion rate was adjusted as needed to clamp the HGL to that in P1. At the end of the study, animals were anesthetized with sodium pentobarbital, and liver sections were freeze-clamped and stored at −80°C.

**Real-time PCR and Western blot analysis.** RNA isolation, cDNA synthesis, quantitative PCR primers and analysis, and Western blotting procedures were performed as described previously (26,27). The GK antibody was a gift from Dr. Masakazu Shiota (Vanderbilt University School of Medicine, Molecular Physiology and Biophysics Department). All other antibodies were purchased from Santa Cruz Biotechnology (actin and GK regulatory protein [GKRP]) or Cell Signaling Technology (GS). Test protein bands were quantified using ImageJ software (National Institutes of Health; http://rsb.info.nih.gov/ij/).

**Enzyme activity determination.** GK activity was assessed as described previously (28). GS activity ratio was calculated as activity measured at low glucose-6-phosphate (160 μmol) versus high glucose-6-phosphate (6.7 mmol/L) concentrations, with low uridine diphosphate glucose (134 μmol), as described previously (29). GP activity was assessed by measuring the incorporation of [14C]-glucose from [14C]-glucose-1-phosphate into glycogen in the absence or presence of 2 mmol/L AMP (30).

**Analytical procedures.** Plasma glucose, [$^3$H]-glucose, glucagon, insulin, and nonesterified fatty acid (NEFA) levels and blood lactate and glycerol concentrations were measured using standard methods as described previously (31,32). Liver glycogen levels were determined using the amyloglucosidase method described by Keppler and Decker (33).

**Calculations.** Unidirectional HGU was calculated by multiplying the hepatic fractional extraction of [$^3$H]-glucose (HFrExG*) by the HGL (mg/kg/min). HFrExG* (unitless) was determined by dividing the hepatic [$^3$H]-glucose balance by the hepatic [$^3$H]-glucose load according to the following equation:

$$G^*_H \times BF_H - [[(G^*_A \times BF_A) + (G^*_P \times BF_P)])/[(G^*_A \times BF_A) + (G^*_P \times BF_P)],$$
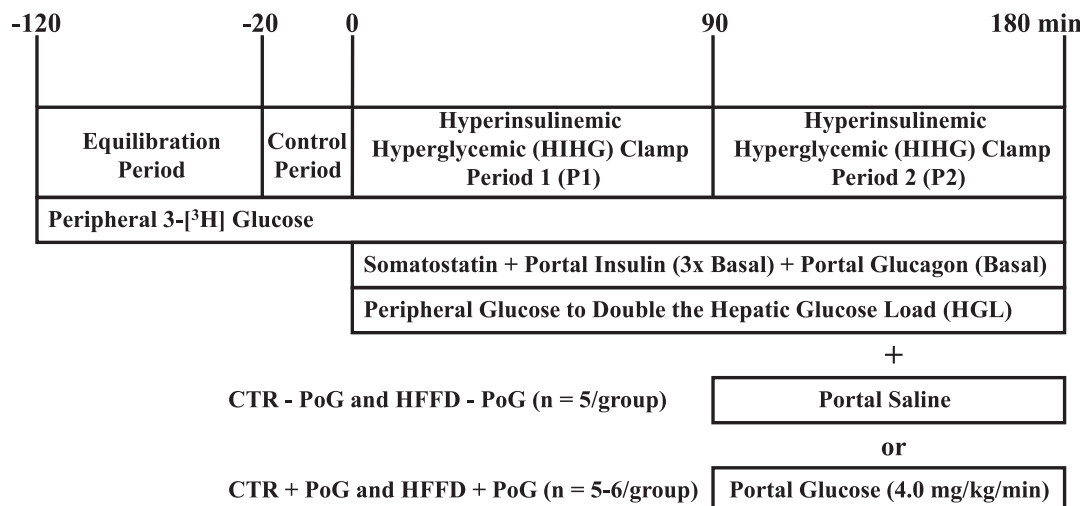
where $G^*_A$, $G^*_P$, and $G^*_H$ represent [$^3$H]-glucose values (dpm/mL) in the artery, portal, and hepatic veins, respectively, and $BF_A$, $BF_P$, and $BF_H$ represent blood flow (ml/kg/min) in the hepatic artery, portal vein, and liver, respectively. We converted plasma [$^3$H]-glucose values to blood [$^3$H]-glucose using previously established conversion factors (7). HGL was calculated according to the following equation:

$$HGL = G_A \times BF_H + PoG \, inf - GUG,$$

where $G_A$ represents the unlabeled blood glucose concentration in the artery (mg/mL), $BF_H$ represents total hepatic blood flow, PoGinf represents the portal glucose infusion rate (mg/kg/min), and GUG represents the uptake of glucose by the gut (mg/kg/min). GUG was calculated as follows:

$$([G^*_A - G^*_P]/G^*_A) \times (G_A \times BF_P),$$

where $([G^*_A - G^*_P] / G^*_A)$ represents the fractional extraction of [$^3$H]-glucose across the gut, $G_A$ represents the unlabeled blood glucose concentration in the



|  | -120 | -20 | 0 | 90 | 180 min |
|---|---|---|---|---|---|

**FIG. 1. Schematic representation of the HIHG clamp protocol.** The protocol consisted of basal control (−20–0 min) and experimental periods (P1: 0–90 min; P2: 90–180 min). Somatostatin and 3-[$^3$H] glucose were infused peripherally, insulin (threefold basal) and glucagon (basal) were infused intraportally, and glucose was infused peripherally at a variable rate to increase the HGL twofold basal during P1 and P2. During P2, glucose was also infused intraportally to activate PoG signaling.

Equilibration Period | Control Period | Hyperinsulinemic Hyperglycemic (HIHG) Clamp Period 1 (P1) | Hyperinsulinemic Hyperglycemic (HIHG) Clamp Period 2 (P2)

Peripheral 3-[$^3$H] Glucose

Somatostatin + Portal Insulin (3x Basal) + Portal Glucagon (Basal)

Peripheral Glucose to Double the Hepatic Glucose Load (HGL)

+

CTR - PoG and HFFD - PoG (n = 5/group) — Portal Saline

or

CTR + PoG and HFFD + PoG (n = 5-6/group) — Portal Glucose (4.0 mg/kg/min)

Performed in 18-h-fasted conscious dogs.
CTR = Normal Chow Control Group; HFFD = High-Fat, High-Fructose group; - PoG = no Portal Vein Glucose Infusion; + PoG = with Portal Vein Glucose Infusion

artery, and $BF_P$ represents blood flow in the portal vein, respectively. It should be stressed that this approach yields HGU rather than net HGU.

Net hepatic substrate balances were calculated with the arteriovenous difference method as described previously (22). Positive net hepatic substrate balance values indicate net production, and negative values indicate net uptake. In the +PoG groups, a correction factor was applied to net hepatic glucose balance (NHGB) to account for the percent recovery of the PoG infusate in the hepatic vein. HGP was calculated as the difference between NHGB and HGU. Plasma insulin and glucagon levels entering the hepatic sinusoids were calculated as described elsewhere (27). The incorporation of glucose into glycogen through the direct pathway was calculated by dividing hepatic [$^3$H]-labeled glycogen by the average inflowing plasma [$^3$H]-glucose specific activity, as described previously (34).

**Statistical analyses.** All data are presented as means ± SEM. Two-way ANOVA with or without repeated measures design was used (SigmaStat; Systat, Richmond, CA), and post hoc analysis was performed using the Student-Newman-Keuls multiple comparisons test. Significance was established when $P$ was <0.05.

## RESULTS

**Plasma hormone concentrations.** During transition from the control to experimental period, arterial and hepatic sinusoidal insulin concentrations (μU/mL; CTR−PoG: 22 ± 1 and 68 ± 6, HFFD−PoG: 23 ± 1 and 67 ± 3, CTR+PoG: 23 ± 4 and 78 ± 10, HFFD+PoG: 27 ± 1 and 81 ± 11; $P$ < 0.05 vs. basal period) were increased to similar levels in all four groups, whereas arterial and hepatic sinusoidal glucagon concentrations were kept basal throughout the study (Fig. 2A–D).

**Blood glucose and HGL.** During P1, arterial blood glucose concentrations were similar in all four groups (mg/dL; CTR−PoG: 159 ± 3, HFFD−PoG: 161 ± 3, CTR+PoG: 161 ± 4, HFFD+PoG: 166 ± 2; $P$ < 0.05 vs. basal period) in order to double the HGL (mg/kg/min; CTR−PoG: 37 ± 3, HFFD-PoG: 36 ± 3, CTR+PoG: 37 ± 2, HFFD+PoG: 37 ± 4; $P$ < 0.05 vs. basal period) (Fig. 3A and B). During P2, arterial glucose concentrations were clamped at a slightly

reduced level in +PoG groups (mg/dL; CTR: 147 ± 3, HFFD: 152 ± 2) to maintain a doubling of the HGL in the presence of PoG infusion (Fig. 3A and B).

**HGU, production, and net balance.** HGU and HGP were similar among all four groups during the control period (Fig. 3C and D and Table 1). In response to hyperinsulinemia and hyperglycemia, HGU increased to a similar rate in both CTR groups during P1, reaching a peak of 1.5 ± 0.3 and 1.8 ± 0.3 mg/kg/min in the −PoG and +PoG groups, respectively ($P$ < 0.05 vs. basal period). When coupled with near complete suppression of HGP, a robust switch from net hepatic glucose output (NHGO) to uptake (NHGU) occurred in both CTR groups (Table 1). In the CTR−PoG group, mean rates of HGU and NHGU (1.6 ± 0.1 and 1.5 ± 0.2 mg/kg/min, respectively) during P2 were similar to those in P1 (Fig. 3C and Table 1). In contrast, PoG delivery rapidly augmented HGU in the CTR+PoG group, with a significant increase occurring 15 min after the start of PoG infusion (HGU, mg/kg/min; CTR−PoG: 1.4 ± 0.2 vs. CTR+PoG: 2.2 ± 0.3, $P$ < 0.05), and an eventual peak of 3.0 ± 0.3 mg/kg/min (Fig. 3D). Given that HGP was already suppressed, the significant increase in NHGU seen in response to PoG delivery was accounted for by the increase in HGU (Table 1).

In contrast to chow-fed animals, 4 weeks of HFFD feeding rendered the liver resistant to the stimulatory effects of hyperinsulinemia, hyperglycemia, and PoG delivery on HGU. As a result, mean rates of HGU in HFFD-fed animals during P1 and P2 (mg/kg/min; 0.4 ± 0.1 and 0.6 ± 0.2 in HFFD−PoG and 0.5 ± 0.1 and 0.6 ± 0.2 in HFFD+PoG, respectively) changed minimally from those observed during the control period and were significantly diminished relative to rates observed in the corresponding CTR groups (Fig. 3C and D and Table 1). Furthermore, HGP was incompletely suppressed in both HFFD groups during P1,
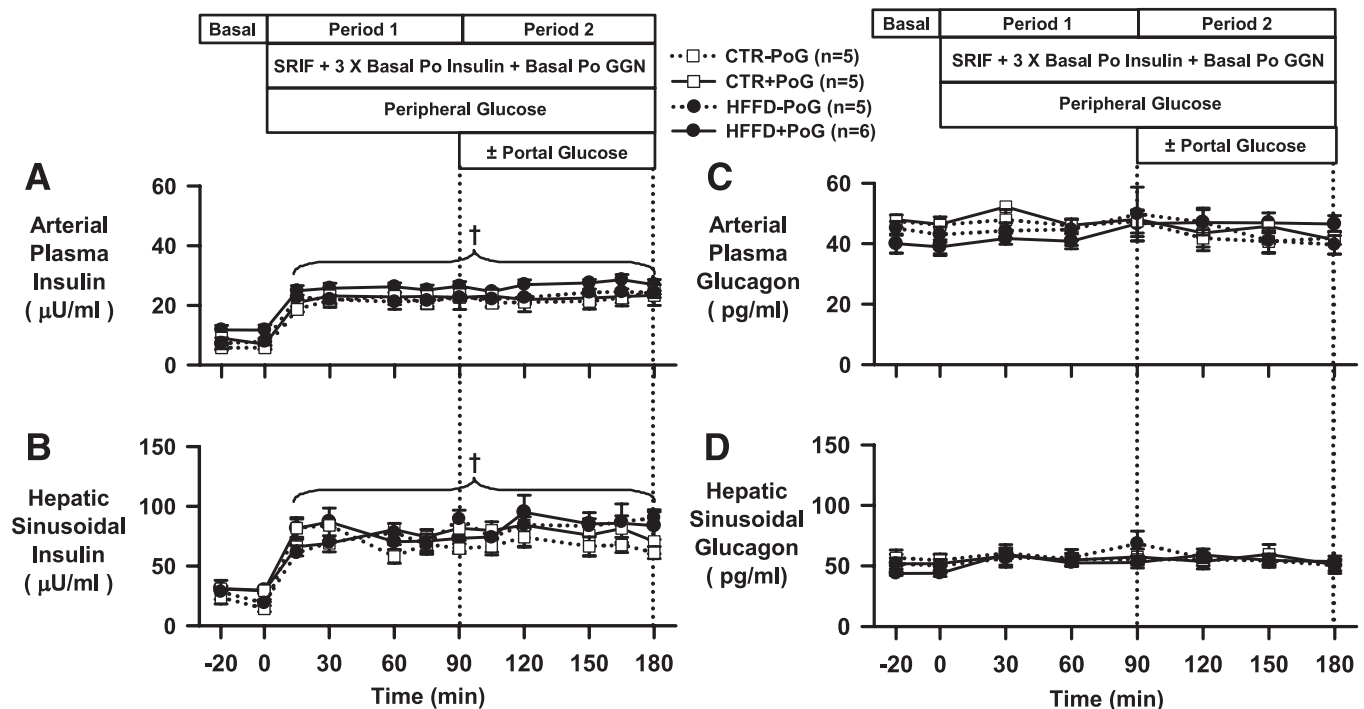


FIG. 2. Plasma hormone concentrations during HIHG clamps in CTR and HFFD groups. Arterial plasma insulin (*A*) and glucagon (*C*) and hepatic sinusoidal insulin (*B*) and glucagon (*D*) during the control (−20–0 min) and experimental periods (0–180 min) of HIHG clamps conducted in 18-h fasted dogs after 4 weeks of feeding a CTR (CTR−PoG, *n* = 5; CTR+PoG, *n* = 5; open squares) or HFFD (HFFD−PoG, *n* = 5; HFFD+PoG, *n* = 6; filled circles). Data are means ± SEM. †*P* < 0.05 vs. basal period. GGN, glucagon; Po, intraportal; SRIF, somatostatin.
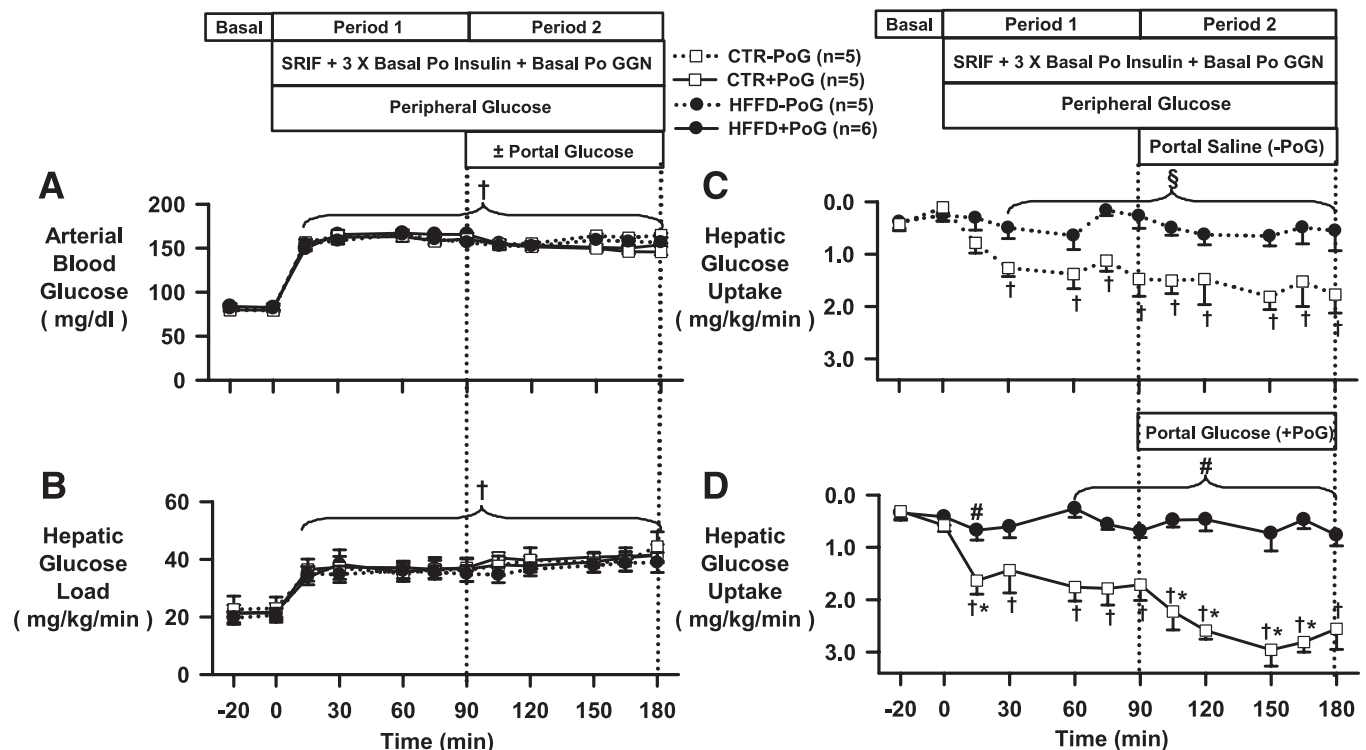
**FIG. 3. Arterial blood glucose, HGL, and HGU during HIHG hyperglycemic clamps in CTR and HFFD groups. Arterial blood glucose (*A*), HGL (*B*), and HGU in the portal saline (*C*) and portal glucose (*D*) groups during the control (−20–0 min) and experimental periods (0–180 min) of HIHG clamps conducted in 18-h–fasted dogs after 4 weeks of feeding a CTR (CTR−PoG, *n* = 5; CTR+PoG, *n* = 5; open squares) or HFFD (HFFD−PoG, *n* = 5; HFFD+PoG, *n* = 6; filled circles). Data are means ± SEM. \*$P < 0.05$ vs. corresponding CTR group; †$P < 0.05$ vs. basal period; §$P < 0.05$, HFFD−PoG vs. CTR; #$P < 0.05$, HFFD+PoG vs. CTR. GGN, glucagon; Po, intraportal; SRIF, somatostatin.**

resulting in sustained NHGO despite the presence of hyperinsulinemia and hyperglycemia (Table 1). In the absence of PoG delivery during P2, there was no further suppression of HGP in the HFFD−PoG group. Thus, mean rates of HGU and HGP remained similar to those in P1, and the liver failed to take up glucose in net sense (Fig. 3*C* and *D* and Table 1). In contrast, PoG delivery suppressed HGP further in the HFFD+PoG group ($P < 0.05$, P2 vs. P1), although it did not augment HGU (Fig. 3*D* and Table 1). As a result, NHGB fell to a value not significantly different from zero (Table 1).

**Lactate metabolism.** All groups exhibited net hepatic lactate uptake during the control period (Table 2). Coincident with the increase in HGU during P1, there was a significant increase in arterial blood lactate concentrations in both CTR groups that reflected a switch from net hepatic lactate uptake to output. Net hepatic lactate output waned during P2 in the absence of PoG infusion, whereas in its presence, it was sustained at an elevated rate (Table 2). In contrast, both HFFD groups exhibited net hepatic lactate uptake for the duration of the study, although it was somewhat reduced relative to the control period during both P1 and P2 (Table 2).

**Glycerol and NEFA metabolism.** During the control period, arterial blood glycerol and plasma NEFA concentrations and their net hepatic balances were similar among the four groups. During P1 and P2, their levels and hepatic uptakes declined in response to hyperinsulinemia, with the steady-state values being slightly lower in the CTR groups (Table 2).

**Hepatic GK and GKRP.** GK mRNA levels were similar between CTR and HFFD groups under basal fasted

conditions (Fig. 4*A*). The combination of hyperinsulinemia and hyperglycemia stimulated six- and sevenfold increases in GK expression in CTR and HFFD groups, respectively ($P < 0.05$ vs. basal CTR), but this did not manifest as an increase in GK protein or activity (Fig. 4*B* and *C*). Strikingly, PoG delivery stimulated a significantly greater increase in GK expression in both diet groups (26- and 24-fold increase in CTR and HFFD groups, respectively; $P < 0.05$ vs. basal CTR). In addition, PoG infusion was associated with a significant increase in GK protein and enzymatic activity in chow-fed animals (Fig. 4*B*). In HFFD-fed dogs, in contrast, GK protein levels and activity were markedly reduced ($P < 0.05$) regardless of the experimental condition. Intriguingly, GKRP levels were also significantly lower in HFFD-fed animals (Fig. 4*D*).

**Hepatic glycogen metabolism.** Under basal fasted conditions, the phosphorylation of GS (Ser641) and the activity ratios of GS and GP were not different between groups. In the CTR−PoG group, hyperinsulinemia and hyperglycemia produced a significant decrease (35%) in GS phosphorylation (Ser641) (Fig. 5*A*). Accordingly, the activity ratio of GS increased approximately fivefold ($P < 0.05$ vs. basal), whereas that of GP was reduced by 40% (Fig. 5*B* and *C*). Thus, when GS and GP were themselves expressed as a ratio (GS/GP), there was an eightfold increase from basal ($P < 0.05$) concomitant with an increase in liver glycogen levels (Fig. 5*D* and *E*). Although portal glucose delivery did not produce a further decrement in GS phosphorylation (Ser641), it did stimulate a further increase in the GS activity ratio (~40%; $P < 0.05$ vs. CTR−PoG) and an additional small decrease in the GP activity ratio (Fig. 5*B* and *C*). As a result, the GS/GP activity ratio was elevated 12-fold

TABLE 1
Mean values for HGU, HGP, and NHGB during the control and experimental periods of an HIHG clamp in CTR−PoG, HFFD−PoG, CTR+PoG, and HFFD+PoG groups

| Group | Control period | Experimental P1 | Experimental P2 |
|---|---|---|---|
| **HGU (mg/kg/min)** | | | |
| CTR−PoG | 0.27 ± 0.12 | 1.31 ± 0.17* | 1.64 ± 0.13*† |
| HFFD−PoG | 0.32 ± 0.11 | 0.35 ± 0.07§ | 0.58 ± 0.21§ |
| CTR+PoG | 0.44 ± 0.11 | 1.67 ± 0.29* | 2.73 ± 0.22*‡¶ |
| HFFD+PoG | 0.38 ± 0.10 | 0.53 ± 0.05‖ | 0.61 ± 0.17‖ |
| **HGP (mg/kg/min)** | | | |
| CTR−PoG | 1.35 ± 0.14 | 0.04 ± 0.36* | 0.10 ± 0.22* |
| HFFD−PoG | 1.78 ± 0.29 | 0.90 ± 0.21* | 0.79 ± 0.39* |
| CTR+PoG | 2.11 ± 0.36 | 0.21 ± 0.26* | 0.31 ± 0.29* |
| HFFD+PoG | 1.94 ± 0.30 | 1.33 ± 0.18*‖ | 0.47 ± 0.42*‡ |
| **NHGB (mg/kg/min)** | | | |
| CTR−PoG | 1.09 ± 0.06 | −1.27 ± 0.21* | −1.54 ± 0.17* |
| HFFD−PoG | 1.46 ± 0.23 | 0.55 ± 0.24*§ | 0.21 ± 0.24*§ |
| CTR+PoG | 1.66 ± 0.26 | −1.47 ± 0.14* | −2.42 ± 0.36*‡¶ |
| HFFD+PoG | 1.57 ± 0.25 | 0.80 ± 0.16*‖ | −0.14 ± 0.38*‡‖ |

Values are means ± SEM; $n = 5$ in CTR−PoG, HFFD−PoG, and CTR+PoG groups and $n = 6$ in the HFFD+PoG group. Dogs were 18-h fasted prior to study. Control period: −20–0 min; experimental P1: 0–90 min; P2: 90–180 min. Positive values for NHGB indicate net glucose production; negative values indicate net glucose uptake. *$P < 0.05$, experimental period vs. control period. †$P < 0.05$, ‡$P < 0.01$, experimental P1 vs. experimental P2. §$P < 0.05$, HFFD−PoG vs. CTR−PoG. ‖$P < 0.05$, HFFD+PoG vs. CTR+PoG. ¶$P < 0.05$, +PoG vs. corresponding −PoG group.

in the presence of PoG infusion ($P < 0.05$ vs. CTR−PoG), and liver glycogen levels were increased to a significantly greater extent than in its absence (Fig. 5D and E).

In the HFFD−PoG group, in contrast, hyperinsulinemia and hyperglycemia did not produce a decrease in GS phosphorylation (Ser641). Although the activity ratio of GS increased approximately threefold ($P < 0.05$ vs. basal), the rise was significantly reduced relative to the increase seen in the corresponding CTR group. The activity ratio of GP changed minimally from basal (Fig. 5B and C). Consequently, the GS/GP activity ratio and the change in liver glycogen were significantly lower in the HFFD−PoG group than in the CTR−PoG group (Fig. 5D and E). Furthermore, PoG delivery produced no further change in GS phosphorylation (Ser641) or in the GS, GP, or GS/GP activity ratios. Although terminal liver glycogen levels were slightly increased in the HFFD+PoG group, the change in glycogen induced by PoG delivery was markedly less in HFFD-fed dogs than in CTR-fed dogs (Fig. 5E). Notably, HFFD feeding was associated with significantly less (~70%; $P < 0.05$) incorporation of glucose into glycogen through the direct pathway, in agreement with diminished HGU and GK activity and impaired activation of GS (Fig. 5F).

**Liver triglyceride content.** There was no effect of diet or experimental condition on total liver triglyceride levels (μg/mg liver; basal CTR: 2.5 ± 0.4, basal HFFD: 1.6 ± 0.1, HIHG−PoG CTR: 1.3 ± 0.2, HIHG−PoG HFFD: 1.8 ± 0.4, HIHG+PoG CTR: 1.4 ± 0.2, HIHG+PoG HFFD: 1.1 ± 0.2).

## DISCUSSION

This study revealed that activation of liver GK and GS occurs in response to hepatic PoG delivery in the presence of a physiologic rise in plasma glucose and insulin. Conversely, HFFD feeding was associated with a marked reduction in liver GK content and activity and an impairment in the activation of GS in response to hyperinsulinemia, hyperglycemia, and PoG infusion. Our findings, although not definitively proving causality, thus provide compelling biochemical evidence implicating these changes in the increased postprandial hyperglycemia and diminished hepatic glucose disposal associated with chronic consumption of a diet high in fat and fructose (22,25).

**Metabolic and cellular response of the liver to hyperglycemia and hyperinsulinemia in CTR animals.** Consistent with previous observations (1,4,7,11), a physiologic rise in glucose and insulin in the absence of PoG delivery resulted in a dynamic switch from NHGO to NHGU. This was due to near complete suppression of HGP concomitant with significant stimulation of HGU. Although a sixfold increase in hepatic GK expression accompanied hyperinsulinemia in the CTR−PoG group, this did not result in an increase in the functional amount of GK protein within 3 h. Previously, Iynedjian et al. (35) demonstrated in rats, and Ramnanan et al. (26) more recently in dogs, that a lag time exists between the transcriptional induction of GK by insulin and subsequent accumulation of GK protein, thus probably explaining this asynchrony. Nevertheless, hyperglycemia and hyperinsulinemia also stimulated the dephosphorylation and activation of GS while triggering the dephosphorylation and inactivation of GP, which culminated in an increase in the deposition of carbon as glycogen within the liver. These data are concordant with previous results from Pagliassotti et al. (7) in dogs and Petersen et al. (36) in humans.

**Metabolic and cellular response of the liver to PoG delivery in CTR animals.** PoG infusion triggered a significantly greater increase in net HGU, as reported previously by our laboratory (4,7,10,11). This was solely attributable to further augmentation of HGU, given that HGP was completely suppressed prior to PoG delivery. Intriguingly, PoG infusion increased GK mRNA 24-fold above basal, which did result in a significant increase in GK protein by 3 h. This occurred despite the fact that the level and duration of hyperinsulinemia, the canonical hormonal inducer of liver GK (35), was equivalent among the groups. Thus, activation of glucose signaling in the portal vein under HIHG conditions triggered a significantly greater increase in GK mRNA such that after 90 min of this signaling, GK protein was increased. This finding is at odds with a previous report by Iynedjian et al. (37) in which hyperglycemia had no effect on the induction of GK expression by insulin in cultured liver cells. Notably, total hepatic denervation ablates the response of the liver to PoG delivery, suggesting that the PoG signal is neurally mediated (38). Thus, it is likely that the induction of liver GK by PoG delivery is also under neural control, such that its effect would only be detected in the intact organism. Recently, Ramnanan et al. (39) demonstrated that a selective rise in brain insulin levels induced a threefold increase in hepatic GK mRNA that was ablated with intracerebroventricular infusion of a phosphatidylinositol-3 kinase inhibitor. These data raise the possibility that efferent signals sent to the liver might contribute to the transcriptional regulation of hepatic GK in response to a meal, although the precise nature of the neural or humoral factor mediating this response is currently unknown. In addition, our findings do not shed light on whether PoG delivery stimulated the translocation of GK in vivo, as we have suggested previously (8,40), but they clearly demonstrate that GK expression and activity are both augmented by PoG delivery. Likewise, PoG infusion triggered

TABLE 2
Mean values for lactate, glycerol, and NEFA concentrations, and their net hepatic balance during the control and experimental periods of an HIHG clamp in the CTR−PoG, HFFD−PoG, CTR+PoG, and HFFD+PoG groups

| Group | Control period (min) −20–0 | Experimental P1 (min) 60 | 75 | 90 | Experimental P2 (min) 150 | 165 | 180 |
|---|---|---|---|---|---|---|---|
| **Arterial blood lactate (μmol/L)** | | | | | | | |
| CTR−PoG | 325 ± 43 | 100 ± 71* | 972 ± 22* | 970 ± 39* | 850 ± 45* | 873 ± 79* | 853 ± 66* |
| HFFD−PoG | 472 ± 154 | 504 ± 54‡ | 579 ± 65‡ | 658 ± 87 | 811 ± 132‡ | 762 ± 83‡ | 783 ± 85‡ |
| CTR+PoG | 397 ± 111 | 663 ± 87* | 628 ± 74*† | 642 ± 88* | 643 ± 78* | 689 ± 87* | 693 ± 121* |
| HFFD+PoG | 318 ± 42 | 422 ± 44 | 386 ± 45 | 394 ± 50 | 444 ± 57† | 452 ± 59†§ | 466 ± 45† |
| **Net hepatic lactate balance (μmol/kg/min)** | | | | | | | |
| CTR−PoG | −6.6 ± 0.6 | 7.2 ± 1.6* | 6.9 ± 1.9* | 3.8 ± 2.3* | 2.7 ± 0.9* | 1.9 ± 1.0* | 1.2 ± 1.0* |
| HFFD−PoG | −7.3 ± 2.0 | −4.1 ± 1.4*‡ | −3.4 ± 1.3*‡ | −3.8 ± 1.4*‡ | −4.0 ± 1.0*‡ | −4.6 ± 0.3‡ | −4.7 ± 0.3‡ |
| CTR+PoG | −5.3 ± 0.3 | 8.3 ± 1.9* | 7.0 ± 1.7* | 6.3 ± 1.3* | 5.1 ± 0.6*† | 4.4 ± 0.8*† | 5.0 ± 0.8*† |
| HFFD+PoG | −6.3 ± 1.1 | −3.6 ± 0.9§ | −2.8 ± 0.9*§ | −2.5 ± 0.9*§ | −3.3 ± 0.7*§ | −3.6 ± 1.0§ | −3.6 ± 0.7§ |
| **Arterial blood glycerol (μmol/L)** | | | | | | | |
| CTR−PoG | 81 ± 12 | 27 ± 6* | 31 ± 1* | 27 ± 6* | 23 ± 10* | 31 ± 12* | 29 ± 10* |
| HFFD−PoG | 92 ± 15 | 62 ± 18* | 62 ± 15* | 57 ± 15* | 68 ± 19*‡ | 51 ± 17* | 53 ± 18* |
| CTR+PoG | 72 ± 6 | 32 ± 4* | 27 ± 3* | 23 ± 1* | 29 ± 3* | 29 ± 5* | 29 ± 3* |
| HFFD+PoG | 98 ± 12 | 56 ± 6* | 51 ± 7* | 45 ± 4* | 55 ± 6* | 49 ± 6* | 50 ± 7* |
| **Net hepatic glycerol balance (μmol/kg/min)** | | | | | | | |
| CTR−PoG | −1.7 ± 0.4 | −0.5 ± 0.2* | −0.4 ± 0.1* | −0.4 ± 0.1* | −0.3 ± 0.1* | −0.6 ± 0.2* | −0.6 ± 0.2* |
| HFFD−PoG | −1.7 ± 0.4* | −1.0 ± 0.4* | −1.1 ± 0.3* | −1.0 ± 0.4* | −1.2 ± 0.4* | −1.0 ± 0.3* | −0.9 ± 0.5* |
| CTR+PoG | −1.7 ± 0.1 | −0.7 ± 0.2* | −0.5 ± 0.1* | −0.5 ± 0.1* | −0.7 ± 0.1* | −0.6 ± 0.1* | −0.8 ± 0.1* |
| HFFD+PoG | −2.0 ± 0.4 | −1.0 ± 0.2* | −1.0 ± 0.3* | −0.8 ± 0.2* | −1.0 ± 0.3* | −0.8 ± 0.2* | −0.9 ± 0.2* |
| **Arterial plasma NEFA (μmol/L)** | | | | | | | |
| CTR−PoG | 924 ± 127 | 99 ± 25* | 116 ± 26* | 114 ± 42* | 63 ± 10* | 103 ± 33* | 89 ± 28* |
| HFFD−PoG | 791 ± 119 | 212 ± 61* | 203 ± 64* | 152 ± 44* | 146 ± 56* | 117 ± 38* | 107 ± 33* |
| CTR+PoG | 831 ± 73 | 154 ± 30* | 136 ± 27* | 118 ± 33* | 84 ± 17* | 109 ± 30* | 102 ± 20* |
| HFFD+PoG | 852 ± 115 | 261 ± 51* | 207 ± 46* | 168 ± 31* | 169 ± 43* | 139 ± 25* | 174 ± 39* |
| **Net hepatic NEFA balance (μmol/kg/min)** | | | | | | | |
| CTR−PoG | −2.7 ± 0.5 | −0.1 ± 0.1* | −0.2 ± 0.1* | −0.2 ± 0.1* | −0.1 ± 0.1* | −0.3 ± 0.2* | −0.2 ± 0.1* |
| HFFD−PoG | −2.3 ± 0.3 | 0.0 ± 0.3* | −0.5 ± 0.3* | −0.3 ± 0.1* | −0.5 ± 0.4* | −0.3 ± 0.1* | −0.3 ± 0.1* |
| CTR+PoG | −2.5 ± 0.2 | −0.2 ± 0.2* | −0.1 ± 0.1* | −0.2 ± 0.1* | −0.2 ± 0.1* | −0.3 ± 0.1* | −0.3 ± 0.1* |
| HFFD+PoG | −2.9 ± 0.5 | −0.9 ± 0.2* | −0.7 ± 0.1* | −0.5 ± 0.2* | −0.7 ± 0.2* | −0.3 ± 0.1* | −0.5 ± 0.2* |

Values are means ± SEM; $n = 5$ in CTR−PoG, HFFD−PoG, and CTR+PoG groups and $n = 6$ in the HFFD+PoG group. Dogs were 18-h fasted prior to study. Positive values for balance data indicate net hepatic production; negative values indicate net hepatic uptake. *$P < 0.05$, experimental period vs. control period. †$P < 0.05$, +PoG vs. corresponding −PoG group. ‡$P < 0.05$, CTR−PoG vs. HFFD−PoG. §$P < 0.05$, CTR+PoG vs. HFFD+PoG.

a significantly greater increase in GS activity, although this was not associated with further diminution of GS phosphorylation on Ser641. Given that GS contains several other phosphorylation sites that regulate its activity (41,42), the effect of the PoG signal on the phosphorylation state of GS could have been mediated by dephosphorylation of sites distinct from that of Ser641. Nevertheless, these data suggest that PoG signaling produces a significantly greater increase in HGU and GSYN by augmenting the activity of GK and GS under HIHG conditions.

**Metabolic and cellular response of the liver to hyperglycemia and hyperinsulinemia in HFFD animals.** In stark contrast to the response observed in chow-fed dogs, HFFD-fed dogs exhibited impaired suppression of HGP and virtually no stimulation of HGU in response to hyperinsulinemia and hyperglycemia. Despite a sevenfold increase in GK mRNA levels in the HFFD−PoG group, GK

protein content and activity were significantly reduced relative to those in chow-fed dogs. In agreement with these findings, livers of dogs in the HFFD−PoG group continued net hepatic lactate uptake for the duration of the study. Because phosphorylation of glucose by GK is thought to be rate-limiting for HGU, this is likely to explain the impaired HGU caused by the HFFD (43,44). Although the possibility cannot be excluded that a substrate-mediated increase in GK flux and hepatic glucose cycling occurred in the HFFD−PoG group, it did not translate into an increase in committed glucose uptake and carbon retention by the liver. In addition, the phosphorylation of GS (Ser641) was not reduced from basal, and the activity ratio of GS in the presence of hyperinsulinemia and hyperglycemia was significantly lower in HFFD-fed dogs than in the corresponding CTR group. Consequently, the change in liver glycogen levels was minimal. Although our data do not prove causality,
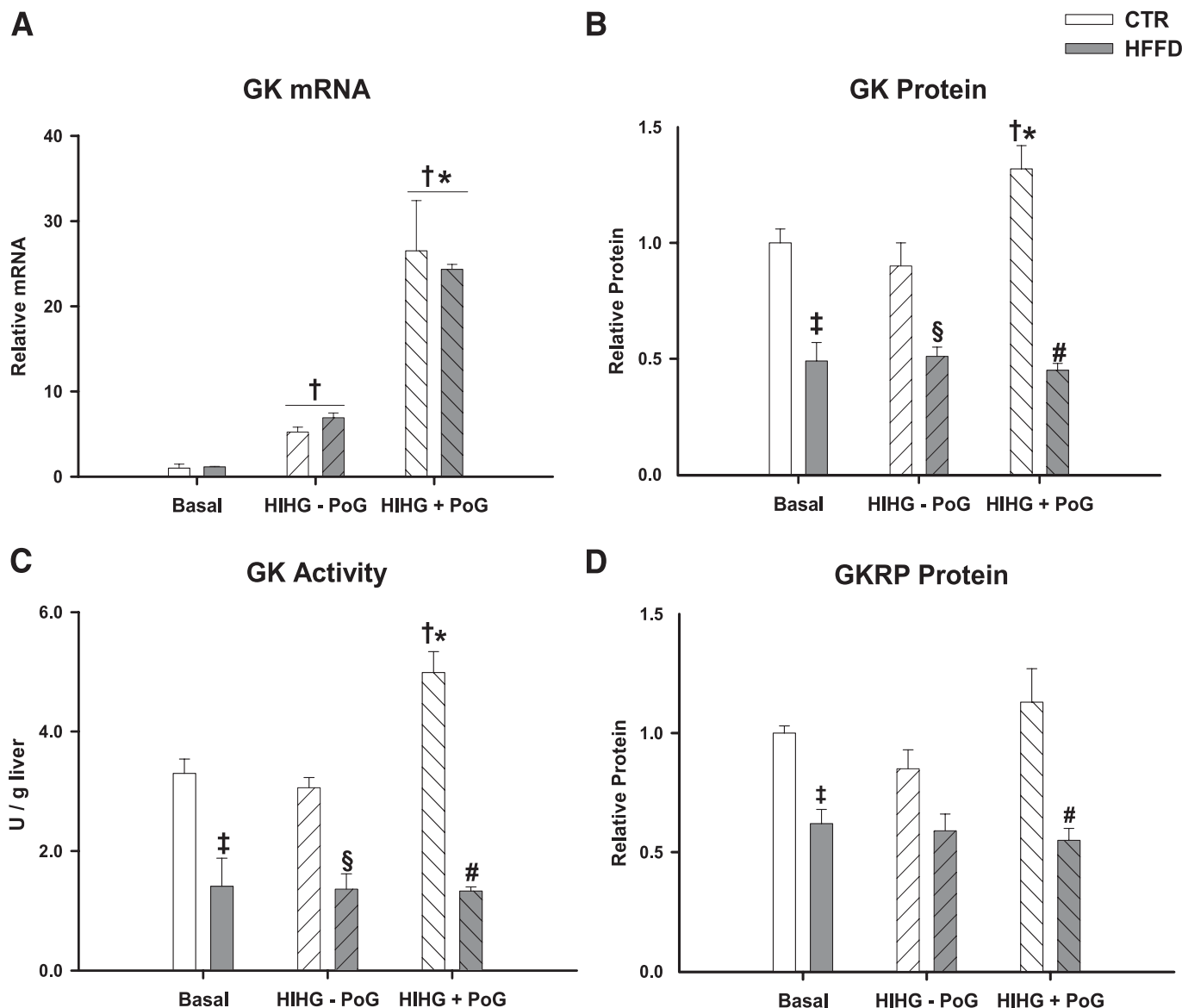
**FIG. 4. Hepatic GK and GKRP in CTR and HFFD groups.** Levels of GK mRNA (*A*) and protein (*B*), GK activity (*C*), and levels of GKRP (*D*). *A*, *B*, and *D* are expressed relative to levels observed in basal CTR animals. Data are means ± SEM; *n* = 5 to 6 per group. *$P < 0.05$ vs. corresponding CTR group; †$P < 0.05$ vs. basal CTR; ‡$P < 0.05$, basal HFFD vs. CTR; §$P < 0.05$, HFFD−PoG vs. CTR; #$P < 0.05$, HFFD+PoG vs. CTR.

they clearly suggest that impaired regulation of hepatic GK, GS, and GP explains the deficit in HGU and storage following consumption of a HFFD.

**Metabolic and cellular response of the liver to portal glucose delivery in HFFD animals.** PoG delivery in the presence of hyperinsulinemia and hyperglycemia triggered suppression of residual HGP in the HFFD+PoG group, but did not stimulate HGU. As a result, NHGB decreased to a rate not significantly different from zero. Likewise, PoG delivery had no impact on net hepatic lactate balance, on the GS or GP activity ratios, or on the incorporation of glucose into glycogen through the direct pathway, consistent with lack of stimulation of HGU and GSYN in the HFFD+PoG group. Although PoG infusion induced the expression of hepatic GK in the HFFD+PoG group, the increase in GK protein content and activity seen in response to PoG delivery in chow-fed animals was abolished in HFFD-fed animals, as their GK protein levels were decreased by >50%. Likewise, the ability of PoG delivery to activate GS was

abolished in the HFFD group. Thus, 4 weeks of HFFD feeding abrogated the stimulatory effects of hyperglycemia, hyperinsulinemia, and PoG delivery on hepatic GK and GS activity. Collectively, these data are in agreement with those of Basu et al. (20), who demonstrated that impaired SGU in type 2 diabetic individuals is not dependent on the route of glucose delivery. Furthermore, Basu et al. (20,21) provided convincing metabolic data to suggest that a defect in hepatic GK was linked to impaired SGU in subjects with type 2 diabetes, given that the contribution of extracellular glucose to the uridine diphosphate glucose pool was markedly reduced in type 2 diabetic subjects despite the presence of hyperinsulinemia and hyperglycemia. Although our diet-induced model of glucose intolerance is not directly comparable to individuals with type 2 diabetes, our GK findings provide new cellular evidence in support of the claim that defective regulation of liver GK is associated with postprandial hyperglycemia and diminished hepatic glucose disposition.
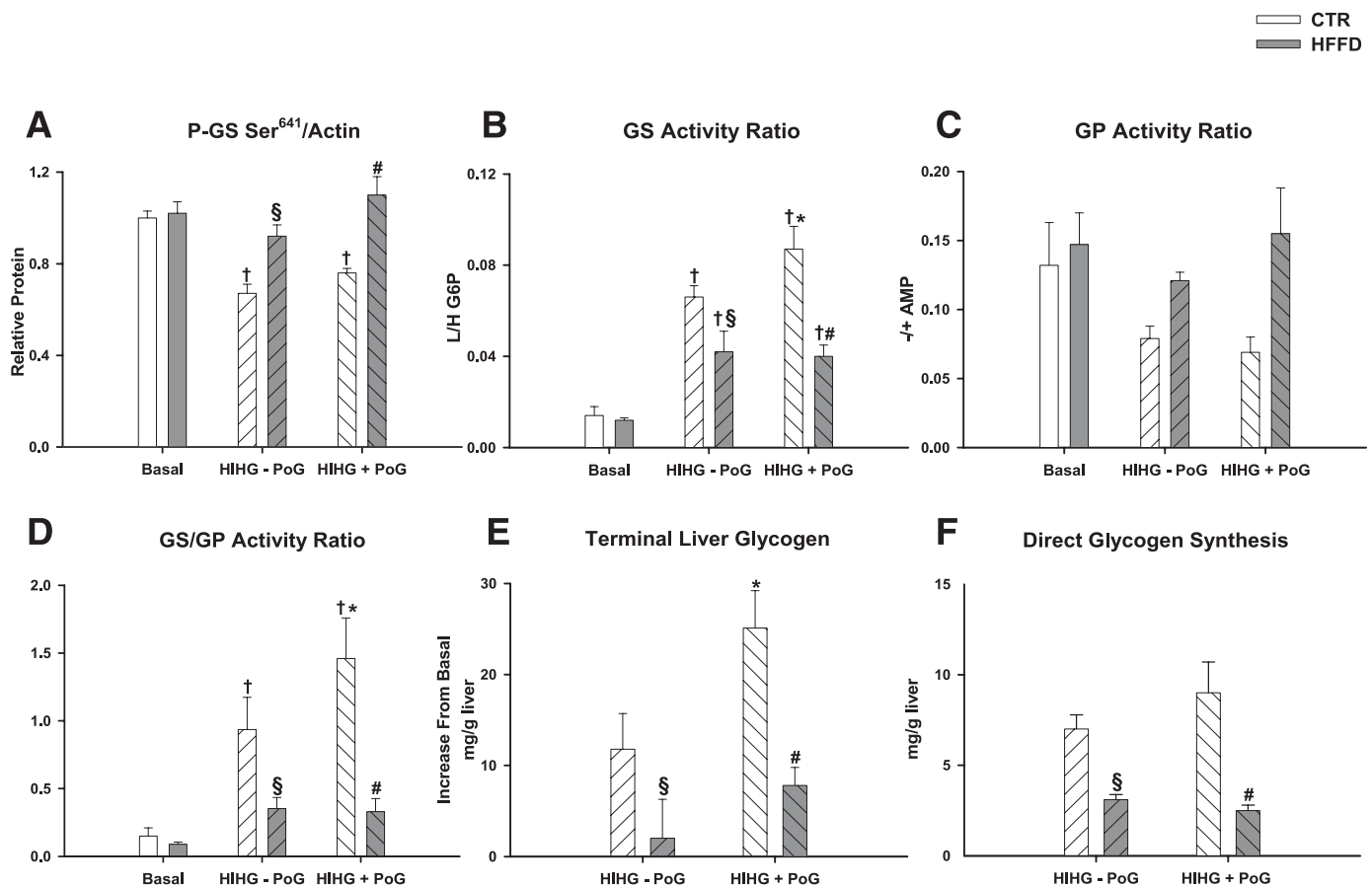
FIG. 5. Markers of hepatic glycogen metabolism in CTR and HFFD groups. Phosphorylation of GS on Ser641 (*A*) relative to levels observed in basal CTR animals. Activity ratios of GS (*B*), GP (*C*), and GS/GP (*D*). Calculated increment in liver glycogen from basal (*E*). Glycogen synthesized through the direct pathway (*F*). Data are means ± SEM; *n* = 5 to 6 per group. *$P < 0.05$ vs. corresponding CTR group; †$P < 0.05$ vs. basal CTR; §$P < 0.05$, HFFD−PoG vs. CTR; #$P < 0.05$, HFFD+PoG vs. CTR.

Normal GK mRNA expression concomitant with diminished GK protein content suggests that the decrease in GK protein occurred posttranscriptionally. Interestingly, liver GKRP protein levels were significantly reduced in our HFFD-fed dog model as well. Studies conducted in GKRP-deficient mice (45,46) demonstrated that they have decreased GK protein despite normal basal and insulin-stimulated GK mRNA, suggesting that GKRP exerts a permissive effect on the level of GK protein through a posttranscriptional mechanism. Although we cannot directly compare our findings in the HFFD-fed dog to those of a genetically modified mouse model, it is tempting to speculate that reduced levels of GKRP in HFFD-fed animals might have contributed to the posttranscriptional decline in GK protein.

Altogether, our findings reveal new insight into the molecular physiology of the portal glucose signaling mechanism under normal conditions and the pathophysiology of aberrant postprandial hepatic glucose disposition evident in response to diet-induced glucose intolerance. These data support the possibility that nutritional modulation of hepatic GK might be a common pathogenic factor underlying the manifestation of disturbances in postprandial hepatic glucose flux and GSYN.

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