Priming Effect of a Morning Meal on Hepatic Glucose Disposition Later in the Day

Mary Courtney Moore,[1,2] Marta S. Smith,[1] Ben Farmer,[1,2] Guillaume Kraft,[1] Masakazu Shiota,[1,2] Phillip E. Williams,[2,3] and Alan D. Cherrington[1,2]

METABOLISM

We used hepatic balance and tracer ([^{3}H]glucose) techniques to examine the impact of "breakfast" on hepatic glucose metabolism later in the same day. From 0–240 min, 2 groups of conscious dogs (n = 9 dogs/group) received a duodenal infusion of glucose (GLC) or saline (SAL), then were fasted from 240–360 min. Three dogs from each group were euthanized and tissue collected at 360 min. From 360–600 min, the remaining dogs underwent a hyperinsulinemic (4× basal) hyperglycemic clamp (arterial blood glucose 146 ± 2 mg/dL) with portal GLC infusion. The total GLC infusion rate was 14% greater in dogs infused with GLC than in those receiving SAL (AUC$_{360–600min}$ 2,979 ± 296 vs. 2,597 ± 277 mg/kg, respectively). The rates of hepatic glucose uptake (5.8 ± 0.8 vs. 3.2 ± 0.3 mg · kg^{-1} · min^{-1}) and glycogen storage (4.7 ± 0.6 vs. 2.9 ± 0.3 mg · kg^{-1} · min^{-1}) during the clamp were markedly greater in dogs receiving GLC compared with those receiving SAL. Hepatic glycogen content was ~50% greater, glycogen synthase activity was ~50% greater, glycogen phosphorylase activity was ~50% lower, and the amount of phosphorylated glycogen synthase was 34% lower, indicating activation of the enzyme, in dogs receiving GLC compared with those receiving SAL. Thus, morning GLC primed the liver to extract and store more glucose in the presence of hyperinsulinemic hyperglycemia later in the same day, indicating that breakfast enhances the liver's role in glucose disposal in subsequent same-day meals.

A number of studies, but not all, have suggested a metabolic advantage to consuming versus skipping breakfast (1–7). In particular, insulin insensitivity and the risk of overweight/obesity have been reported to be lower among those who regularly eat breakfast versus those who skip breakfast (8–11). In a study in which the same meal was given to normal individuals three times throughout the day, the excursions of glucose and insulin were twice as great in the morning as at midday or in the evening (12). Moreover, consumption of the largest meal of the day in the morning, rather than the evening, was associated with lower glycemia over 24 h in a small group of lean, healthy volunteers (13). These findings suggest a possible "priming" effect of breakfast on glucose metabolism throughout the remainder of the day.

The liver is an especially important organ in glucose metabolism, by virtue of its ability to both take up and release glucose as appropriate to maintain glucose homeostasis. The liver is responsible for the uptake of as much as one-third of a moderate-sized oral or duodenal glucose load, and its role in glucose homeostasis can be seen to be even greater when its full response (i.e., including suppression of net hepatic glucose output) is considered (14,15). The extent to which glucose metabolism by the liver later in the same day is affected by breakfast consumption remains unknown. Assessment of hepatic glucose balance in humans is not practical, given the difficulty in accessing the hepatic portal vein. Hepatic vascular access is also a problem in rodent models because of the small size of the vessels. A model using conscious, chronically catheterized dogs, on the other hand, allows for a robust assessment of both net and absolute hepatic glucose balance and whole-body glucose disposition (16).

We hypothesized that breakfast intake has a priming effect on the liver as a result of the excursions in glycemia and/or insulin it brings about, initiating molecular changes that facilitate net hepatic glucose uptake (NHGU) and storage

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[1]Department of Molecular Physiology & Biophysics, Vanderbilt University School of Medicine, Nashville, TN

[2]Diabetes Research and Training Center, Vanderbilt University School of Medicine, Nashville, TN

[3]Department of Surgery, Vanderbilt University School of Medicine, Nashville, TN

Corresponding author: Mary Courtney Moore, genie.moore@vanderbilt.edu.

during subsequent meals in the same day. As a first step in examining this hypothesis, we delivered saline or glucose via duodenal infusion in the morning and then assessed hepatic glucose metabolism later in the day. We used a canine model to precisely quantify NHGU and glycogen synthesis, and to examine molecular markers associated with glucose metabolism in the liver.

RESEARCH DESIGN AND METHODS

Animal Care and Surgical Procedures

Adult mongrel dogs (10 male and 8 female; 20.6 ± 0.6 kg) acquired from a U.S. Department of Agriculture–licensed vendor were studied. The protocol was approved by the Vanderbilt University Institutional Animal Care and Use Committee, and the animals were housed and cared for according to AAALAC International guidelines. The dogs were fed once daily a chow-and-meat diet in an amount calculated to maintain weight (17), and they were maintained on a 12-h light/12-h dark cycle.

Approximately 16 days before the study, each dog underwent surgery under general anesthesia to insert sampling catheters in the femoral artery, hepatic portal vein, and left common hepatic vein; blood flow probes around the portal vein and hepatic artery; a splenic vein and a jejunal vein catheter to allow infusion into the portal circulation; and an infusion catheter in the inferior vena cava. In addition, a duodenal infusion catheter was inserted. All procedures were previously described (15,17).

On the day before the study, all dogs were fed their regular diet at noon, and any remaining food was removed within an hour. All dogs ate at least 75% of the daily ration the day before study.

Experimental Design

Figure 1 provides an outline of the study design. On the morning of the study, at the onset of the light cycle, the duodenal and arterial catheters were removed from their subcutaneous pockets under local anesthesia, and a continuous 4-h (0–240 min) infusion of 20% glucose at 10 mg · kg^{-1} · min^{-1} was initiated via the duodenal catheter in nine dogs to mimic a morning meal (GLC group). Another nine animals received an intraduodenal infusion of normal saline at a rate identical (in mL · kg^{-1} · min^{-1}) to the infusion received by the group receiving glucose (SAL group). Arterial glucose and insulin concentrations were monitored in the groups during the 4-h duodenal infusion. The duodenal infusions ended at 240 min. At that point, six dogs in each group were prepared for a subsequent clamp period. The flow probes and the remainder of the infusion and sampling catheters were exteriorized and peripheral venous access was established (18). A primed (38 μCi), continuous (0.38 μCi/min) infusion of [^{3}H]glucose was initiated via a peripheral vein; after a 90-min tracer equilibration period (240–330 min), postinfusion fasting blood samples were collected from the sampling catheters from 330–360 min ("preclamp" period). Three dogs that had undergone duodenal glucose infusion and three that had received duodenal saline infusion did not receive [^{3}H]glucose but were euthanized at 360 min to collect hepatic tissues; these are referred to as the GLC$_{no-clamp}$ and SAL$_{no-clamp}$ groups.

From 360–600 min, all dogs in the GLC and SAL groups (n = 6 dogs/group) underwent a hyperinsulinemic-hyperglycemic clamp with portal vein glucose infusion to mimic the conditions during absorption of a later meal. During the clamp, somatostatin (0.8 μU · kg^{-1} · min^{-1}) was infused via the peripheral vein to suppress endocrine pancreatic secretion, and regular insulin (four times basal values; 1.2 mU · kg^{-1} · min^{-1}) and glucagon (basal; 0.57 ng · kg^{-1} · min^{-1}) were replaced via intraportal infusion. Glucose (20%) was infused continuously at 4 mg · kg^{-1} · min^{-1} into the portal circulation. This provided a constant, readily quantifiable load of glucose to the liver. In conjunction with this, a primed, continuous infusion of 50% glucose was administered via a peripheral vein to create and maintain hyperglycemia; the infusion rate was adjusted as required, on the basis of 0.2-mL samples taken every 5 min, to clamp arterial blood glucose at 150 mg/dL. We previously demonstrated that this dual infusion method is effective in clamping glucose delivery to both the liver and the peripheral tissues at very constant values (see, e.g, ref. 19). Larger samples were taken every 15–30 min from the artery, portal vein, and hepatic vein
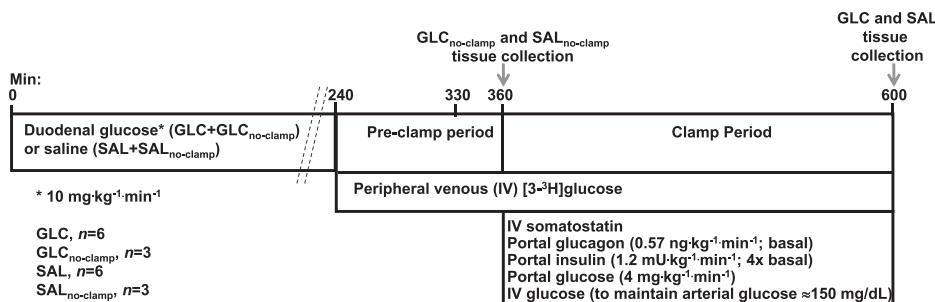

Figure 1—Study design. The dogs were randomly assigned to duodenal infusion of either glucose or saline. After 4 h of duodenal infusion, there was a 2-h period before the clamp, followed by a 4-h hyperinsulinemic-hyperglycemic clamp with portal glucose infusion. The dogs receiving no clamp were euthanized and tissue collected at 360 min. IV, intravenous.

catheters to allow measurement of hormones and substrates. At the end of the study, each animal was deeply anesthetized with sodium pentobarbital while all infusions continued, and tissues from three liver lobes were rapidly (within 3 min) freeze-clamped in situ and stored at −80°C for later analysis. The dogs were then euthanized.

Analyses

Real-time PCR and Western Blotting: Enzyme Activities
RNA isolation, cDNA synthesis, quantitative PCR primers and analysis, and Western blotting procedures were performed as described previously (20). Antibodies were purchased from Cell Signaling Technology, with the exception of the protein targeting to glycogen (PTG) antibody (PPP1R3C), which was obtained from Santa Cruz Biotechnology. ImageJ software (http://rsb.info.nih.gov/ij/) was used for quantification. Quantities of specific mRNAs and proteins of interest were normalized to that of RPL32 and β-actin, respectively (Sigma-Aldrich). Glucokinase (GK), glycogen synthase (GS), and glycogen phosphorylase (GP) activities were determined as previously described (21).

Biochemical Analysis
Plasma glucose, [³H]glucose, glucagon, insulin, and non-esterified fatty acid (NEFA) levels, and blood lactate and glycerol concentrations, were measured using standard methods, as described previously (19). The method described by Keppler and Decker (22) was used to determine hepatic glycogen concentrations.

Lipids were extracted from hepatic tissue as described by Folch et al. (23). Lipids were recovered from the filtered extracts in the chloroform phase. Individual lipid classes were separated by thin-layer chromatography and then scraped from the plates and methylated (24). The methylated fatty acids were extracted and analyzed using gas chromatography. Inclusion of lipid standards with odd-chain fatty acids permitted quantitation of the amount of lipid in the sample. Dipentadecanoyl phosphatidylcholine (C15:0), diheptadecanoin (C17:0), trieicosenoin (C20:1), and cholesteryl eicosenoate (C20:1) were used as standards.

Calculations
Unidirectional hepatic glucose uptake (HGU) and non-HGU were calculated with [³H]glucose, as described previously (19). Net hepatic substrate balances, net hepatic carbon retention, and non-HGU were also calculated with cold glucose concentrations using the arteriovenous difference method (19). An indirect method was used to calculate NHGU in order to reduce any error introduced by streaming infusate in the portal vein (19). Hepatic sinusoidal plasma insulin and glucagon concentrations were calculated as previously described (19). Glycogen synthesis via the direct pathway was calculated by dividing hepatic [³H]-labeled glycogen by the average inflowing plasma [³H]glucose-specific radioactivity (19). Net hepatic carbon retention, previously shown to be a reliable index of glycogen synthesis, was calculated as described previously (19).

Statistical Analyses
Data are expressed as means ± SEMs. Two-way ANOVA with or without a repeated measures design was used (SigmaStat; Systat, Richmond, CA), and post hoc analysis was performed using the Student-Newman-Keuls multiple comparisons test. A P value <0.05 was considered significant.

RESULTS

Morning Infusion Period
Arterial insulin and glucose concentrations before the morning infusion period did not differ between the SAL and $SAL_{no\text{-}clamp}$ versus the GLC and $GLC_{no\text{-}clamp}$ groups (Table 1). In the SAL and $SAL_{no\text{-}clamp}$ groups, blood glucose concentrations drifted down slightly but significantly over the course of the infusion, whereas insulin concentrations did not change significantly. In the GLC and $GLC_{no\text{-}clamp}$ groups, concentrations of both glucose and insulin were highest early in the duodenal glucose infusion period, with glucose levels at 60 min nearly 1.5-fold basal values and insulin concentrations nearly seven times basal values. By 180 min, the glucose and insulin concentrations had fallen to only 130% and 157% of basal values, respectively. The blood glucose concentrations remained stable from 180 to 240 min, whereas at 240 min the insulin concentrations

Table 1—Venous blood glucose and plasma insulin concentrations before (0 min) and during (60–240 min) the morning infusion period

Parameter and groups	0 min	60 min	120 min	180 min	240 min
Blood glucose (mg/dL)					
SAL + $SAL_{no\text{-}clamp}$	87 ± 1	87 ± 2	86 ± 2	83 ± 1†	84 ± 2
GLC + $GLC_{no\text{-}clamp}$	87 ± 3	122 ± 5*†	111 ± 6*†	108 ± 6*†	107 ± 11*†
Plasma insulin (μU/mL)					
SAL + $SAL_{no\text{-}clamp}$	9 ± 1	7 ± 1	9 ± 2	7 ± 1	8 ± 1
GLC + $GLC_{no\text{-}clamp}$	7 ± 2	47 ± 24*†	26 ± 1*†	11 ± 1*†	21 ± 3*†

Data are mean ± SEM. SAL + $SAL_{no\text{-}clamp}$ includes six dogs that underwent the clamp (SAL) and three dogs from which tissue was harvested before the clamp ($SAL_{no\text{-}clamp}$). GLC + $GLC_{no\text{-}clamp}$ includes six dogs that underwent the clamp (GLC) and three dogs from which tissue was harvested before the clamp ($GLC_{no\text{-}clamp}$). *$P < 0.05$ vs. SAL + $SAL_{no\text{-}clamp}$; †$P < 0.05$- vs. 0-min sample in the same group.

were approximately threefold the basal values. Blood glucose and plasma insulin concentrations were significantly greater during duodenal infusion of glucose compared with saline, as anticipated (Table 1). Glucagon concentrations remained unchanged from basal values throughout the 240-min period in all dogs (data not shown). In addition, cortisol concentrations did not change over time during the morning period within the groups and did not differ between treatments (mean for the period 2.7 ± 0.2 vs. 2.3 ± 0.2 µg/dL in SAL + SAL$_{no-clamp}$ vs. GLC + GLC$_{no-clamp}$ groups, respectively; P = 0.11).

Data Before and During the Clamp Period

Hormone and Hepatic Blood Flow Data

The plasma insulin concentrations during the preclamp and afternoon clamp periods did not differ significantly between the SAL and GLC groups (Table 2). The clamp period concentrations of insulin were approximately fourfold higher than the preclamp values in both groups, mimicking postprandial conditions. The arterial plasma glucagon and cortisol concentrations were not different during the preclamp and clamp periods, and they did not differ between the two groups at any time (Table 2). In addition, hepatic blood flow did not differ between the SAL and GLC groups at any time (Table 2).

Glucose Data

Blood glucose concentrations were no different between the SAL and GLC groups during the preclamp or the clamp periods. In keeping with this, the hepatic glucose loads were virtually identical in the two groups (Fig. 2A and B). During the preclamp period, all animals were in a state of net hepatic glucose output (1.4 ± 0.1 and 1.6 ± 0.2 mg · kg^{-1} · min^{-1} in the SAL and GLC groups, respectively; P = 0.72) (Fig. 2C); with the onset of the hyperinsulinemic-hyperglycemic clamp in the presence of portal

glucose infusion, they shifted to NHGU, with the mean rate in the GLC group ~2.4 mg · kg^{-1} · min^{-1} greater than that in the SAL group (P < 0.05). Unidirectional (tracer-determined) HGU was near zero in both groups during the basal period (Fig. 2D). HGU increased markedly in both groups with the onset of the clamp, with mean rates of 5.0 ± 0.5 vs. 2.9 ± 0.2 mg · kg^{-1} · min^{-1} in the GLC and SAL groups, respectively (Fig. 2D).

The total glucose infusion rate (the fixed-rate portal vein infusion plus the variable peripheral glucose infusion required to maintain equivalent hyperglycemia) was slightly but not significantly greater in the GLC group versus the SAL group (mean clamp period rates: 14.4 ± 1.4 vs. 13.3 ± 1.5 mg · kg^{-1} · min^{-1}, respectively; P = 0.28) (Fig. 2E). Non-HGU increased throughout the clamp period in both groups, with no significant difference in the rates between groups (mean 8.6 ± 1.8 and 9.9 ± 1.5 mg · kg^{-1} · min^{-1} in the GLC and SAL groups, respectively) (Fig. 2F). In addition, net hepatic carbon retention, an index of glycogen synthesis, was increased >50% in the GLC group versus the SAL group (Fig. 2G).

Lactate, Glycerol, and NEFA

Arterial blood lactate concentrations increased in both groups with the onset of the clamp period and did not differ between groups (Fig. 3A). Both groups exhibited net hepatic lactate uptake during the late-morning fasting period and rapidly switched to net hepatic lactate output (NHLO) after the start of the clamp (Fig. 3B). The rates of NHLO were higher in the GLC group than in the SAL group throughout the clamp period (P < 0.05), such that the area under the curve (AUC) of the change in NHLO from the fasting period after breakfast was 46% greater in the GLC than the SAL group (P < 0.05).

The arterial concentrations of glycerol and NEFA declined quickly with the onset of the clamp in both groups and remained low throughout the clamp, indicating no difference in lipolytic suppression. In addition, no between-group differences in glycerol or NEFA uptake by the liver occurred at any time (Fig. 3C–F).

Liver Tissue Analyses

Glycogen, Triglyceride, and mRNA Data

Hepatic glycogen concentrations at the end of study did not differ among the SAL, SAL$_{no-clamp}$, and GLC$_{no-clamp}$ groups (Fig. 4A). By contrast, the concentrations in the GLC group were ~40% greater than in the SAL and SAL$_{no-clamp}$ groups and 28% greater than in the GLC$_{no-clamp}$ group (P < 0.05 for the GLC group vs. all other groups). Glycogen synthesis via the direct pathway was ~50% greater in the GLC versus the SAL group (P = 0.08) (Fig. 4B).

The total hepatic triglyceride content at the end of the clamp did not differ between the SAL and GLC groups, averaging 713 ± 27 µg/g tissue in both groups. In addition, the fatty acid composition of hepatic triglycerides did not differ significantly between groups, with one

Table 2—Arterial plasma insulin, glucagon, and cortisol concentrations and hepatic blood flow during the preclamp and clamp periods

Parameter and group	Before the clamp (330–360 min)	Clamp period (360–600 min)
Arterial insulin (µU/mL)		
SAL	5 ± 1	19 ± 2
GLC	6 ± 1	23 ± 2
Arterial glucagon (ng/L)		
SAL	30 ± 4	31 ± 5
GLC	40 ± 4	39 ± 4
Arterial cortisol (µg/dL)		
SAL	2.7 ± 0.6	3.4 ± 0.8
GLC	1.7 ± 0.5	2.8 ± 0.7
Total hepatic blood flow (mL · kg^{-1} · min^{-1})		
SAL	34 ± 1	31 ± 2
GLC	30 ± 3	30 ± 4

Data are mean ± SEM (n = 6 dogs/group) for all time points during the indicated period. There were no significant differences between groups.
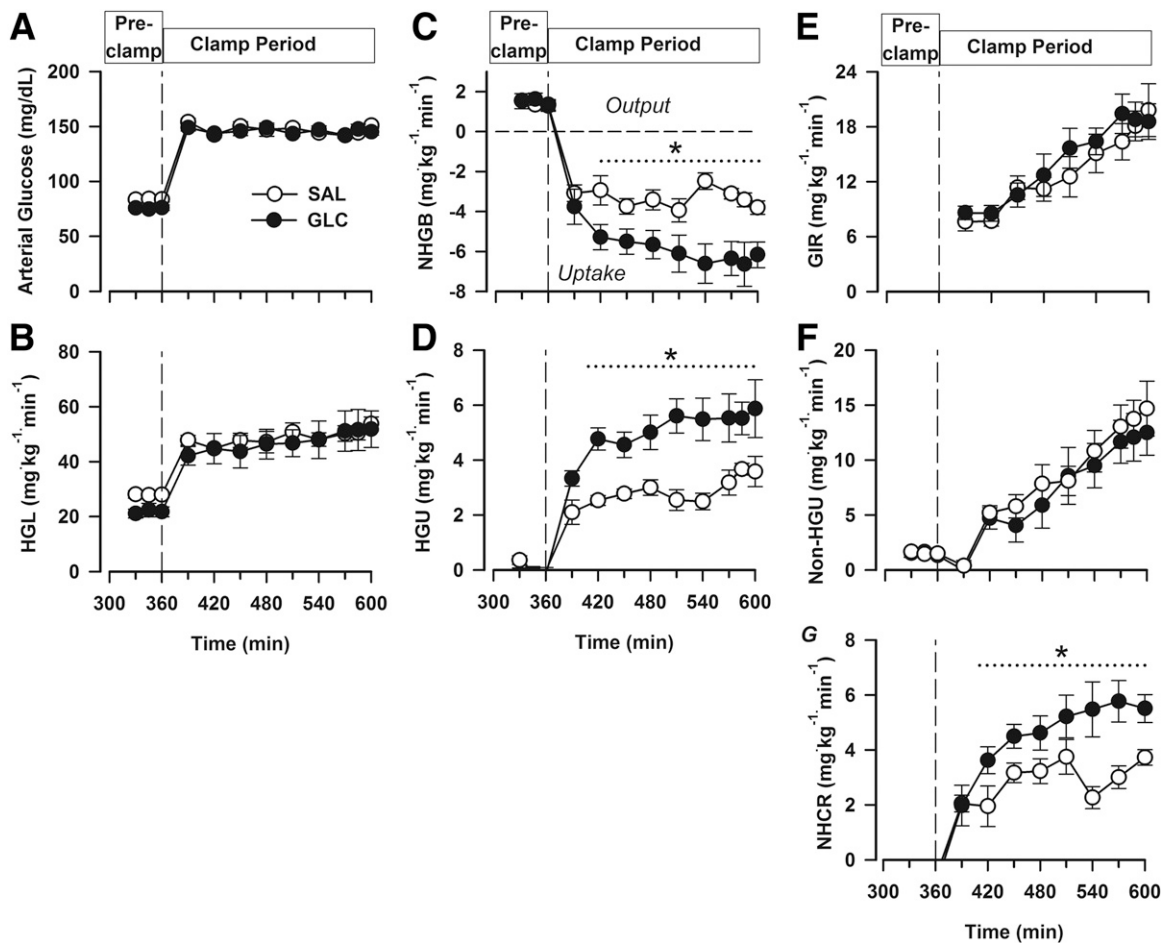
Figure 2—Data from the fasting period following the morning infusion (before the clamp [Pre-clamp]; 330–360 min) and during a subsequent hyperinsulinemic-hyperglycemic clamp with intraportal glucose infusion (clamp period). Arterial blood glucose (A), hepatic glucose load (HGL; B), net hepatic glucose balance (NHGB; C), unidirectional (tracer-determined) HGU (D), glucose infusion rate (GIR; E), non-HGU (F), and net hepatic carbon retention (NHCR, in milligrams glucose equivalents per kilogram per minute) (G). From 0–240 min (data not shown), the groups (n = 6 dogs/group) received a duodenal infusion of saline (white circles) or glucose (black circles). *$P < 0.05$ between groups.

exception. None of the livers from the SAL group exhibited detectable levels of 18:3ω3 (α-linolenic acid [ALA]), whereas ALA was quantifiable in all livers in the GLC group, albeit at the lower limit of detection (Table 3).

The SAL, GLC, and GLC$_{no-clamp}$ groups exhibited significant enhancement in GK mRNA compared with the SAL$_{no-clamp}$ group; the increase in the GLC group was 77% greater than that in the SAL group and double that in the GLC$_{no-clamp}$ group (Fig. 4C). This suggests that the impact of the morning glucose infusion was to double the GK message evident during the afternoon clamp.

Western Blotting and Enzyme Activities

Despite the enhancement in GK mRNA, relative GK protein did not differ significantly among the four treatment groups (Fig. 4D). Nevertheless, the SAL$_{no-clamp}$ group exhibited reduced GK activity ($P < 0.05$) in comparison with all three of the other groups, which did not differ significantly from one another (Fig. 4E). The amount of phosphorylated GS (pGS) protein was significantly reduced in the three test groups compared with the SAL$_{no-clamp}$ group, and the GLC

group had significantly lower ($P < 0.05$) relative pGS protein than the SAL group (Fig. 4F).

GS activity was increased by morning glucose infusion (GLC$_{no-clamp}$ group) and by the clamp (SAL group), and the combination of the two (GLC group) brought about a further significant increase in GS activity (Fig. 4G). While GP activity was not altered in the GLC$_{no-clamp}$ and SAL groups, the combination of morning glucose delivery and the afternoon clamp reduced it by ~50% (Fig. 4H). Thus, the GS activity–to–GP activity ratio (GS-I [activity of glucose-6-phosphate–independent form]/GPa [active form]) increment was >3.5-fold greater in the GLC group, with the results of GLC$_{no-clamp}$ deducted from it, compared with the data from the SAL group, with the results from SAL$_{no-clamp}$ deducted from it ($P < 0.05$) (Fig. 4I).

Relative phosphorylated Akt (pAkt) protein was enhanced in all other groups versus the SAL$_{no-clamp}$ group ($P < 0.05$) (Fig. 4J). The enhancement in the GLC$_{no-clamp}$ group was significantly reduced in comparison with that in both the SAL and GLC groups, which did not differ
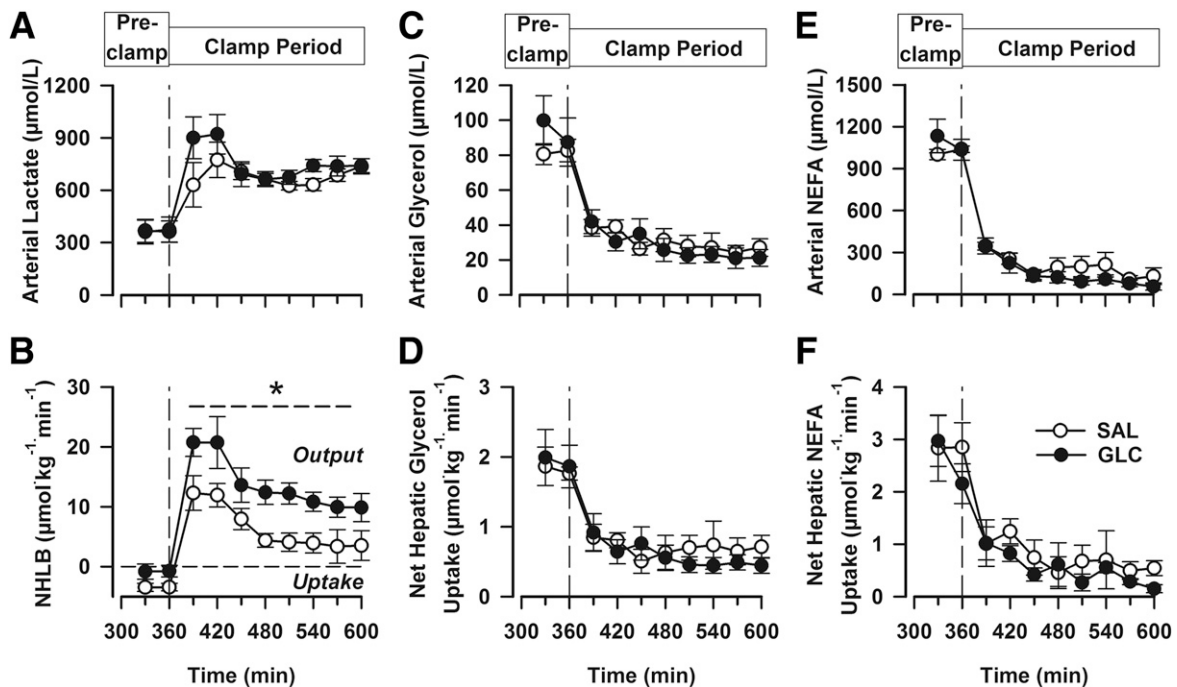
Figure 3—Data from the fasting period following the morning infusion (before the clamp [Pre-clamp]; 330–360 min) and during a subsequent hyperinsulinemic-hyperglycemic clamp with intraportal glucose infusion (clamp period). Arterial blood lactate (*A*), net hepatic lactate balance (NHLB; *B*), arterial blood glycerol (*C*), net hepatic glycerol uptake (*D*), arterial plasma NEFA uptake (*E*), and net hepatic NEFA uptake (*F*). From 0–240 min (data not shown), the groups (*n* = 6 dogs/group) received a duodenal infusion of saline (white circles) or glucose (black circles). *P < 0.05 between groups.

from one another. Moreover, PTG expression did not differ significantly among any of the groups (Fig. 4*K*).

DISCUSSION

In this study we used duodenal glucose versus saline infusions in the morning as surrogates for breakfast and no breakfast intake, respectively, and used hyperinsulinemic-hyperglycemic clamp conditions in the presence of the portal glucose signal as a surrogate for a later meal. This ensured that the GLC and SAL groups would have indistinguishable pancreatic hormone concentrations and hepatic glucose loads during the final study period, an important element of the study design since these factors are key regulators of HGU (16). HGU, NHGU, net hepatic carbon retention, and terminal hepatic glycogen concentrations were all at least 50% greater in the GLC group versus the SAL group, consistent with a priming effect of a morning carbohydrate load on the liver. The GLC group did not display a global increase in glucose disposal in comparison with the SAL group, even though the total glucose infusion rate was ~1 mg · kg^{-1} · min^{-1} (~9%) greater in the GLC group (P = 0.28). The enhancement of liver glucose uptake (~2.4 mg · kg^{-1} · min^{-1}) in the GLC group was offset by a tendency toward a reduction in the non-HGU rate in the same group (1.3 mg · kg^{-1} · min^{-1}, or ~15%; P = 0.28), which is probably the result of an increase in first-pass glucose extraction by the liver. This is consistent with our previous observation of a reduction

in muscle glucose uptake in response to a glucose portal signal (25). These data suggest that the benefits of morning hyperglycemia and/or hyperinsulinemia were largely centered on hepatic metabolism and enhanced glycogen deposition. Indeed, the difference in hepatic glycogen deposition accounted for ~86% of the difference in HGU between groups, with hepatic glycolysis—that is, net hepatic lactate production (Fig. 3)—accounting for an additional ~13–14%. We demonstrated that hepatic glucose oxidation is remarkably constant (~0.3 mg glucose · kg^{-1} · min^{-1}) under a variety of experimental clamp conditions (26–28) and thus would account for a very small amount of HGU in both the SAL and GLC groups.

Suppression of circulating glycerol and NEFA levels was equivalent in the SAL and GLC groups. Because lipolysis is highly sensitive to hyperinsulinemia in a healthy state (29), this was not unexpected. Nevertheless, these findings are consistent with the impact of morning hyperinsulinemia and hyperglycemia being focused on the liver. Although ALA was detectable in the livers of dogs in the GLC group, but not those in the SAL group, it is not clear whether this could have acutely affected HGU. Chronic consumption of a high-ALA diet in mice fed a high-fat diet was associated with reduced hepatic glucose production, in association with lower levels of steatosis (30). Also, oral and intravenous glucose tolerance were modestly improved in Zucker diabetic fatty rats fed a high-ALA diet for 8 weeks, although this was not associated with altered expression of
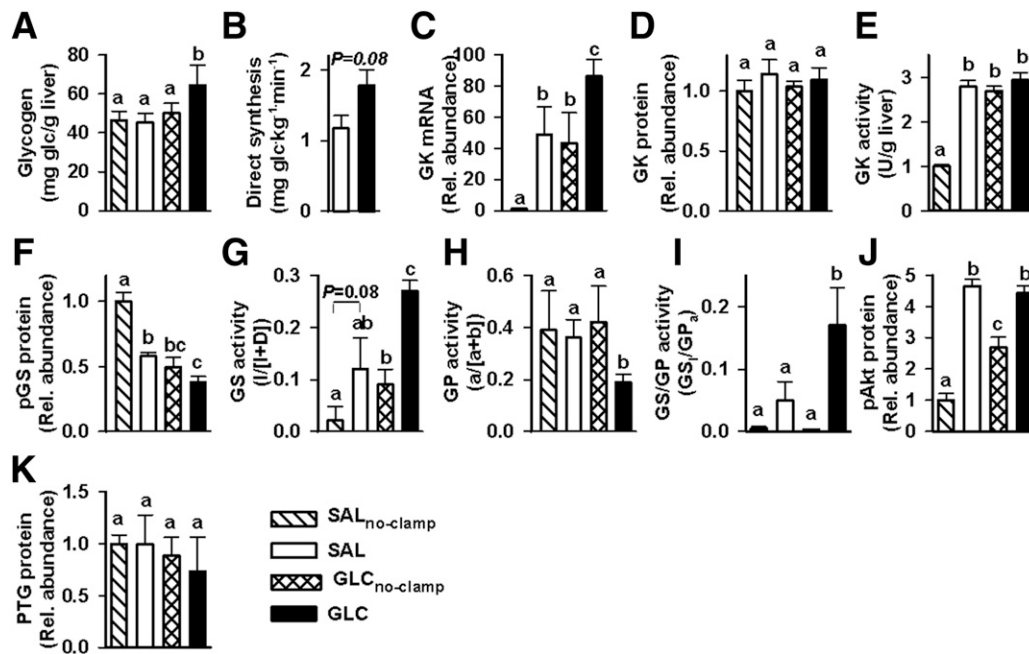
Figure 4—Hepatic tissue analyses: glycogen concentrations (*A*), glycogen synthesis via the direct pathway (*B*), GK mRNA (*C*), relative (Rel.) GK protein (*D*), GK activity (*E*), pGS protein (*F*), GS activity (active/total) (*G*), GP activity (active/total) (*H*), GS activity relative to that of GP (*I*), pAKT protein (*J*), and relative PTG protein (*K*) in the SAL$_{no\text{-}clamp}$ (hatched bars), SAL (white bars), GLC$_{no\text{-}clamp}$ (cross-hatched bars), and GLC (black bars) groups. Samples were taken at the end of study (360 min for the no-clamp groups and 600 min for the SAL and GLC groups). RNA and protein levels in the SAL, GLC$_{no\text{-}clamp}$, and GLC groups are expressed relative to those in the SAL$_{no\text{-}clamp}$ group. Direct glycogen synthesis is not shown for the no-clamp groups because they received no [^{3}H]glucose. Data marked with different lowercase letters differed significantly from one another ($P < 0.05$), whereas those marked with the same letter were not significantly different from one another.

any of the hepatic inflammatory markers examined (31). The acute effect of changes in hepatic ALA have not, to our knowledge, been examined.

Hepatic glycogen concentrations, net hepatic carbon retention, and glycogen synthesis via the direct pathway were all higher in the GLC group than in the SAL group. Given the metabolic importance of GK to the liver (32,33), the impact of insulin on GK transcription (34), and the long half-life of GK once it is elevated (35), we originally speculated that the insulin excursion associated with morning hyperglycemia might bring about an increase in GK transcription and a resulting increase in GK protein. Consistent with this, the increase in GK mRNA in the GLC group was approximately equal to the sum of the increases in the SAL and GLC$_{no\text{-}clamp}$ groups; thus, the mRNA response to morning glucose infusion seemed to persist to

360 min, with the afternoon clamp having an additive effect. In spite of the difference in mRNA, neither GK protein nor the activity of the enzyme differed between the SAL and GLC groups, indicating that an impact of the morning glucose infusion was not additive on either GK protein or GK activity. This suggests that GK protein and its activity were maximally stimulated by either the morning duodenal glucose infusion or the afternoon clamp, and if there was any preferential impact on GK in the GLC group, it lies in stimulating the translocation of the enzyme.

In contrast to our findings with GK, the activity of GS was significantly enhanced and that of GP was significantly reduced in the GLC group versus the others, resulting in significantly greater GS activity relative to GP activity in the GLC group compared with all other groups. Our previous work (36) and that of others (37)

Table 3—Hepatic triglyceride composition

	Fatty acid species (µg/g tissue)									
Group	14:0	16:0	16:1	18:0	18:1ω9	18:1ω7	18:2	18:3ω6	18:3ω3	20:4
SAL	12 ± 2	195 ± 37	24 ± 3	62 ± 10	214 ± 36	46 ± 20	111 ± 22	15 ± 9	–	34 ± 7
GLC	14 ± 1	186 ± 29	26 ± 2	69 ± 7	232 ± 18	24 ± 2	120 ± 16	4 ± 2	5 ± 1	33 ± 6

Samples (*n* = 6/group) were collected at the end of the clamp period. There were no significant differences between groups for any of the species detected in both groups. The species 18:3ω3 was not detected in the SAL group; in the GLC group, it appeared at the lower limit of detection. 14:0, tetradecanoic acid; 16:0, hexadecanoic acid; 16:1, 9-tetradecenoic acid; 18:0, octadecanoic acid; 18:1ω9, (Z)-octadec-9-enoic (oleic) acid; 18:1ω7, 11-octadecenoic acid; 18:2, 9,12-octadecadienoic (linoleic) acid; 18:3ω6, all-*cis*-6,9,12-octadecatrienoic acid; 18:3ω3, all-*cis*-9,12,15-octadecatrienoic (α-linolenic) acid; 20:4, all-*cis*-5,8,11,14-icosatetraenoic acid.

demonstrated the importance of suppressing GP activity relative to GS activity in the stimulation of HGU and hepatic glucose storage. We examined the hepatic expression of pAkt and PTG in an attempt to identify other regulatory proteins that might have affected glucose uptake and storage in the SAL and GLC groups, but we found no evidence for a differential effect.

The glucose and insulin excursions in response to standardized midday and evening meals are significantly smaller than those following breakfast in normal men (12). Moreover, when obese or overweight women were randomly assigned to diets causing isocaloric weight loss, with 500 kcal at lunch and either 700 kcal at breakfast and 200 kcal at dinner, or 200 kcal at breakfast and 700 at dinner, the group consuming the larger breakfast exhibited significantly reduced fasting glucose, insulin, and HOMA–insulin resistance in comparison with the group consuming the larger evening meal after 12 weeks of treatment (11). In addition, the insulin and glucose AUCs were significantly reduced at all three meals in the group eating a larger breakfast versus that eating the larger dinner (11). Given the importance of the liver in determining glucose and meal tolerance (38), the marked improvement in meal tolerance on the day when a larger breakfast was consumed is consistent with enhanced HGU on that day.

Most (39–44), but not all (12,45), data from healthy humans indicate that meal or glucose tolerance has a diurnal pattern, such that it decreases over the course of the day in subjects receiving three daily meals or glucose loads. This has been suggested to be related to a loss of insulin sensitivity and a decrease in the β-cell response to glucose from morning to evening (44). A decline in meal/glucose tolerance from morning to evening has been observed in subjects with impaired fasting glucose (46) but not in obese subjects with normal glucose tolerance (44), non–insulin-treated individuals with type 2 diabetes (47), or adults with type 1 diabetes (48). The current study differs from these previous studies in that it was not aimed at examining diurnal changes in either glucose tolerance or β-cell responsiveness, but instead was specifically designed to quantify the impact of morning hyperinsulinemia and hyperglycemia, brought about by a duodenal glucose infusion, on hepatic glucose metabolism in the same day. It remains to be determined whether hyperinsulinemia, hyperglycemia, or both are key elements in the enhancement of the HGU and hepatic glucose storage observed in the GLC group.

Individuals with diabetes have had lower postprandial levels of hepatic glycogen than normal controls in some (12,49,50), but not all (51–54), investigations. The degree of diabetes control seems to be a key determinant of postprandial hepatic glycogen content (54). Decreased hepatic glycogen might increase the vulnerability of people with type 1 diabetes to hypoglycemia, particularly during the nocturnal period. We recently showed that a liver-brain counterregulatory axis that is responsive to the mass of hepatic glycogen affects the counterregulatory response

to hypoglycemia (28). Our data suggest that an increase in insulinemia and/or glycemia early in the day might improve hepatic glycogen stores and possibly reduce the risk of hypoglycemia during the night.

In conclusion, our data indicate that a morning glucose load exerts a priming effect on the liver, such that glucose uptake and storage are enhanced during a "meal" (i.e., a period of hyperinsulinemia and hyperglycemia) later in the day. Our findings also suggest that the quality and quantity of breakfast intake may be especially important in optimizing hepatic glycogen reserves, thus potentially reducing the risk of hypoglycemia in those with diabetes (28).

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