Hepatic Glucose Metabolism in Late Pregnancy
Normal Versus High-Fat and -Fructose Diet

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Net hepatic glucose uptake (NHGU) is an important contributor to postprandial glycemic control. We hypothesized that NHGU is reduced during normal pregnancy and in a pregnant diet-induced model of impaired glucose intolerance/gestational diabetes mellitus (IGT/GDM). Dogs (n = 7 per group) that were nonpregnant (N), normal pregnant (P), or pregnant with IGT/GDM (pregnant dogs fed a high-fat and -fructose diet [P-HFF]) underwent a hyperinsulinemic-hyperglycemic clamp with intraportal glucose infusion. Clamp period insulin, glucagon, and glucose concentrations and hepatic glucose loads did not differ among groups. The N dogs reached near-maximal NHGU rates within 30 min; mean ± SEM NHGU was 105 ± 9 μmol·100 g liver^{-1}·min^{-1}. The P and P-HFF dogs reached maximal NHGU in 90–120 min; their NHGU was blunted (68 ± 9 and 16 ± 17 μmol·100 g liver^{-1}·min^{-1}, respectively). Hepatic glycogen synthesis was reduced 20% in P versus N and 40% in P-HFF versus P dogs. This was associated with a reduction (>70%) in glycogen synthase activity in P-HFF versus P and increased glycogen phosphorylase (GP) activity in both P (1.7-fold greater than N) and P-HFF (1.8-fold greater than P) dogs. Thus, NHGU under conditions mimicking the postprandial state is delayed and suppressed in normal pregnancy, with concomitant reduction in glycogen storage. NHGU is further blunted in IGT/GDM. This likely contributes to postprandial hyperglycemia during pregnancy, with potential adverse outcomes for the fetus and mother. ***Diabetes*** **62:753–761, 2013**

Net hepatic glucose balance is the sum of two processes, hepatic glucose output (HGO) and hepatic glucose uptake (HGU), which often occur simultaneously. Net hepatic glucose uptake (NHGU) is observed after ingestion of a glucose load or mixed meal in healthy individuals as a result of suppression of HGO and stimulation of HGU. In normal-weight pregnant women without diabetes but not obese women without diabetes and women with gestational diabetes mellitus (GDM), HGO under hyperinsulinemic-euglycemic clamp conditions remains sensitive to suppression by hyperinsulinemia (1–3). However, the effect of pregnancy (either in normal-weight or obese women or in women with diabetes) on HGU under postprandial conditions remains virtually unexplored. After a mixed meal, NHGU is indistinguishable between pregnant and nonpregnant rabbits (4) and higher in pregnant than in nonpregnant dogs (5). However, glucose and insulin concentrations differ between pregnant and nonpregnant animals after mixed-meal feedings, making it impossible to draw a firm conclusion about the impact of pregnancy on postprandial hepatic glucose disposal. The dog is a useful model for examining the liver's role in postprandial metabolism (6), and many aspects of glucose metabolism in the normal dog in late pregnancy are similar to those in human (7–9). Therefore, the current study compares NHGU and glycogen storage in conscious normal pregnant and nonpregnant dogs as well as a diet-induced canine model of impaired glucose tolerance (IGT) or GDM in the presence of fixed glucose and pancreatic hormone concentrations similar to those observed in the postprandial period. We hypothesized that NHGU and glycogen synthesis are reduced in normal pregnant versus nonpregnant dogs under these conditions. We further hypothesized that hepatic glucose disposal would be impaired more in IGT/GDM than in normal pregnancy.

RESEARCH DESIGN AND METHODS

Animals 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 guidelines. Three groups of nulliparous female dogs (11–16 months old) were studied: nonpregnant (N) (n = 7), normal pregnant (P) (n = 9), and pregnant with IGT/GDM (pregnant dogs fed a high-fat and -fructose diet [P-HFF]) (n = 7). The N and P groups consumed a diet of laboratory chow and meat (~7,740 kJ/day; protein 31%, carbohydrate 43%, fat 26%) (9) throughout the study, and the P-HFF group consumed the same diet until the second trimester. In weeks 4–5 of gestation (total gestation ~9 weeks), the dogs in the P-HFF group were placed on a high-fat and -fructose diet (~9,042 kJ/day; protein 19%, fat 60%, carbohydrate 21% [with 12% of total energy derived from fructose]), which they consumed for a total of 3 weeks. The timing was chosen because most gestation-related impairments of glucose metabolism become evident during the second or third trimester, and we previously showed that consumption of this diet in mid- to late pregnancy results in IGT or GDM within 3 weeks (9). The N dogs were in the anestrous state. The P and P-HFF dogs were studied at 7–8 weeks of gestation. The number of fetuses was 7.8 ± 0.6 in the P and 6.7 ± 0.8 in the P-HFF groups (P = 0.28).

Approximately 16 days before the experiment, sampling catheters were inserted in the femoral artery and the hepatic portal, left common hepatic and common iliac veins; infusion catheters were placed in jejunal and splenic veins for delivery of glucose and hormones into the portal circulation; and ultrasonic blood flow cuffs (Transonic Systems, Ithaca, NY) were positioned around the hepatic artery, portal vein, and external iliac vein as previously described (10,11). Body weight in the N dogs was stable between the days of surgery and experimentation, whereas the P and P-HFF dogs gained 1.5 ± 0.3 and 3.5 ± 0.5 kg, respectively ($P < 0.05$). At study, the N, P, and P-HFF groups weighed 22.5 ± 0.6, 23.0 ± 1.0, and 24.7 ± 0.7 kg, respectively.

Hyperinsulinemic-hyperglycemic clamps. Dogs were fasted 18 h before study to allow basal samples to be collected under postabsorptive conditions. Each experiment started with a primed (777 kBq) constant (7.8 kBq/min) peripheral venous (intravenous) infusion of [3-^{3}H]glucose. Studies consisted of three periods: equilibration (−130 to −30 min), basal (−30 to 0 min), and

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hyperinsulinemic-hyperglycemic clamp (0–240 min). During the clamp, somatostatin was delivered at 0.8 $\mu g \cdot kg^{-1} \cdot min^{-1}$ i.v., whereas insulin 7.2 ± 0.00, 9.7 ± 1.7, and 9.1 ± 0.2 $pmol \cdot kg^{-1} \cdot min^{-1}$ in N, P, and P-HFF dogs, respectively, and glucagon 0.57 $ng \cdot kg^{-1} \cdot min^{-1}$ were infused into the portal vein. A higher insulin infusion rate is required in pregnant versus nonpregnant women and dogs to achieve the same circulating concentrations because of the higher clearance and greater volume of distribution during pregnancy (8,12,13). The insulin infusion rates were derived from previous data (8,14) and adjusted as necessary based on the results of early experiments in each group. Glucose 21.7 ± 0.11 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ mixed with *p*-aminohippuric acid 0.4 $mg \cdot kg^{-1} \cdot min^{-1}$ was infused intraportally, and additional glucose was infused intravenously as needed to maintain the hepatic glucose load at twofold basal. At 240 min, hepatic biopsy specimens were obtained under pentobarbital anesthesia, and dogs were then killed.

Chemical analysis. Analysis of blood lactate, glycerol, and alanine levels; plasma glucose, insulin, glucagon, cortisol, and nonesterified fatty acid (NEFA) levels; specific radioactivity of [^{3}H]glucose; and hepatic glycogen were carried out as described previously (7,10,15,16).

Enzyme activities. Hepatic glycogen synthase (GS) activity was determined by measuring incorporation of [^{14}C]glucose from uridine diphosphate (UDP)-[^{14}C]glucose into glycogen at low (160 μmol/L) or high (6.7 mmol/L) glucose-6-phosphate (G6P) concentrations with low UDP-glucose (134 μmol/L) (17). GS total activity was measured using high G6P and high UDP-glucose (4.45 mmol/L) (18). Hepatic GP activity was measured by the method of Gilboe et al. (19). Activity ratios represent the activity measured at low G6P divided by that at high G6P for GS or in the absence of AMP divided by that in the presence of AMP for GP.

Western blotting. Procedures were as described previously (20). Antibodies specific for total and phosphorylated Akt (Ser473) and glycogen synthase kinase 3β (GSK3β) (Ser9) were purchased from Cell Signaling (Danvers, MA), and antibodies against PEPCK and glucokinase (GK) were gifts from D. K. Granner and M. Shiota, respectively (Vanderbilt School of Medicine, Nashville, TN). Test protein bands were quantified using ImageJ software (http://rsb.info.nih.gov/ij).

Calculations. Mixing of the glucose infusate in the hepatic circulation was evaluated by recovery of *p*-aminohippuric acid in the portal and hepatic vein blood, using predefined exclusion criteria (15). Two dogs in the P group and none in the other groups were excluded on the basis of inadequate mixing; thus, the data are reported for seven dogs per group.

The hepatic load (rate of substrate delivery to the liver), net hepatic substrate balance (NHB), net hepatic fractional substrate extraction, and hepatic sinusoidal hormone concentrations were calculated as described previously (21). Net hepatic balance of glucose was calculated with both a direct and an indirect method to minimize the impact of any error introduced by streaming of the glucose infusate in the portal vein (15). For substrates not infused intraportally, only the direct calculation was used. Hepatic balance data are expressed per 100 g liver to normalize for differences in maternal body composition. Livers weighed 541 ± 14, 552 ± 25, and 519 ± 26 g in N, P, and P-HFF dogs, respectively (P = 0.61). Hepatic sinusoidal hormone concentrations were calculated as those for hepatic load and divided by hepatic plasma flow. Hindlimb balance was the difference between substrate concentrations in the artery and the common iliac vein multiplied by external iliac artery blood flow (11).

The rate of glucose appearance was calculated with [^{3}H]glucose using a two-compartment model (22) with dog parameters (23). Direct pathway hepatic glycogen synthesis was estimated as the ^{3}H disintegrations per minute in hepatic glycogen divided by the inflowing [^{3}H]glucose-specific radioactivity. Net hepatic carbon retention, an index of glycogen synthesis, and nonhepatic glucose uptake (non-HGU) were calculated as previously described (24).

Statistical analysis. ANOVA with or without a repeated-measures design (depending on the variable assessed) was used to compare P versus N and P-HFF versus P, with post hoc analysis using Tukey test (Sigmastat; Systat, Richmond, CA). $P < 0.05$ was accepted as significant. Unless specified otherwise, data are reported as the mean ± SEM for the entire relevant period.

RESULTS

Plasma hormone concentrations. Arterial and hepatic sinusoidal insulin concentrations were lower in the P than in the N group during the basal period but no different in P-HFF versus P (Table 1). However, during the experimental period, the arterial and hepatic sinusoidal concentrations increased to similar levels in all groups (reaching concentrations equivalent to approximately four- and fivefold basal in N in the artery and the sinusoids, respectively).

TABLE 1
Plasma insulin, glucagon, and cortisol concentrations

Parameter and group	Basal period	Experimental period
Arterial insulin (pmol/L)		
N	40 ± 4	156 ± 10
P	10 ± 4*	148 ± 12
P-HFF	13 ± 5	168 ± 17
Hepatic sinusoidal insulin (pmol/L)		
N	126 ± 28	659 ± 85
P	21 ± 7*	617 ± 45
P-HFF	39 ± 10	750 ± 109
Arterial glucagon (ng/L)		
N	53 ± 5	49 ± 6
P	62 ± 13	48 ± 5
P-HFF	41 ± 9	37 ± 2
Hepatic sinusoidal glucagon (ng/L)		
N	59 ± 4	68 ± 9
P	66 ± 15	56 ± 3
P-HFF	54 ± 16	47 ± 3†
Arterial cortisol (nmol/L)		
N	96 ± 11	97 ± 19
P	173 ± 37	105 ± 15
P-HFF	95 ± 18	84 ± 5

Data are mean ± SEM (n = 7/group) of two sampling points in the basal period and eight during the clamp period, except that there were only two clamp period values for cortisol. *$P < 0.05$ for P vs. N. †$P < 0.05$ for P-HFF vs. P.

Arterial and hepatic sinusoidal glucagon concentrations remained near basal concentrations in all groups; the sinusoidal concentration was lower in P-HFF than in P during the experimental period (Table 1). Arterial plasma cortisol concentrations were not significantly different between N and P. They tended to be lower in P-HFF than in P (P = 0.12) (Table 1).

Hepatic blood flow. In all groups, portal blood flow declined ~20% in response to somatostatin infusion, and an increase in arterial blood flow partially compensated for the fall in portal flow (Table 2). Hepatic blood flow tended to be greater in P-HFF than in P during the experimental period (P = 0.2).

Glucose metabolism. The blood glucose concentrations were significantly higher in N than in P under basal conditions, whereas the glucose concentrations in P-HFF did not differ from those in P (Fig. 1*A*). During the

TABLE 2
Hepatic blood flow

Parameter and group	Basal period	Experimental period
Hepatic artery ($mL \cdot 100\ g\ liver^{-1} \cdot min^{-1}$)		
N	24 ± 2	32 ± 5
P	28 ± 2	34 ± 3
P-HFF	27 ± 4	35 ± 5
Portal vein ($mL \cdot 100\ g\ liver^{-1} \cdot min^{-1}$)		
N	116 ± 12	96 ± 8
P	124 ± 7	95 ± 4
P-HFF	123 ± 19	105 ± 15

Data are mean ± SEM (n = 7/group) of two sampling points in the basal period and eight during the clamp period. There were no significant differences between the N and P groups or P and P-HFF groups.

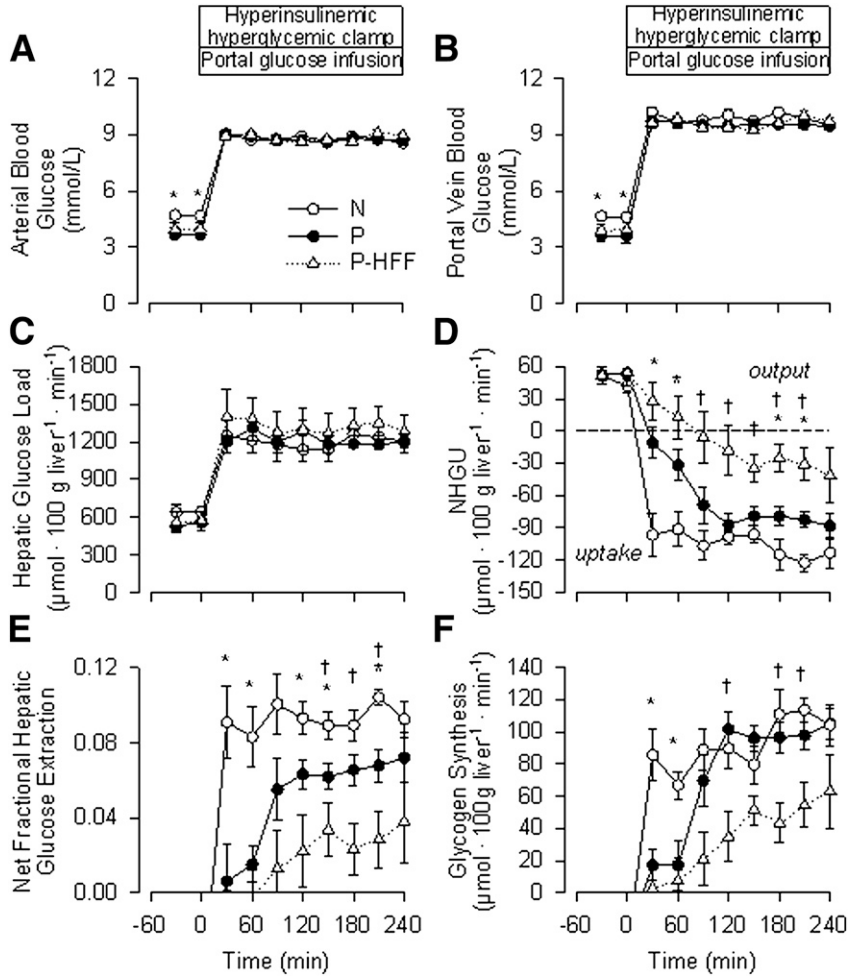

FIG. 1. Arterial (*A*) and portal vein (*B*) plasma glucose concentrations and hepatic glucose loads (*C*) in the N, P, and P-HFF groups were well matched during the hyperinsulinemic-hyperglycemic clamp period in the presence of the portal glucose signal. NHGU (*D*), fractional extraction (*E*), and net hepatic glycogen synthesis (carbon retention) (*F*) during the clamp period were greatest in the N group. $n = 7$ per group. *$P < 0.05$ for P vs. N; †$P < 0.05$ for P-HFF vs. P.

experimental period, the glucose concentrations (artery 8.8 ± 0.1, 8.7 ± 0.1, and 8.8 ± 0.1 mmol/L and portal vein 9.9 ± 0.1, 9.5 ± 0.1, and 9.6 ± 0.1 mmol/L in N, P, and P-HFF, respectively) (Fig. 1*A* and *B*) and the hepatic glucose loads were clamped at the same levels in all groups (Fig. 1*C*).

All groups exhibited net HGO during the basal period (Fig. 1*D*). With the onset of the experimental period, the N group switched rapidly to NHGU, and within 30 min, the rate had reached 96 ± 20 μmol·100 g liver^{-1}·min^{-1}, not significantly different from the maximal rate that occurred during the last hour (117 ± 11 μmol·100 g liver^{-1}·min^{-1}). The P group switched from net HGO to NHGU within 30 min, but the rate was very low (11 ± 14 μmol·100 g liver^{-1}·min^{-1}) and did not approach maximal until 90–120 min; during the last hour of study, it averaged 83 ± 8 μmol·100 g liver^{-1}·min^{-1} ($P < 0.05$ vs. N). The P-HFF group required 90 min to switch to NHGU, and the mean rate during the last hour of study was only 32 ± 17 μmol·100 g liver^{-1}·min^{-1} ($P < 0.05$ vs. P). If NHGU had been expressed per kilogram body weight, the rates during the last hour would have been 28 ± 2, 20 ± 2, and 7 ± 4 μmol·kg^{-1}·min^{-1} ($P < 0.05$ for P vs. N and $P < 0.005$ for P-HFF vs. P). Net hepatic fractional extraction followed a pattern similar to that of NHGU (Fig. 1*E*).

The rate of endogenous glucose appearance during the basal period was not significantly different between N and P or between P and P-HFF (63 ± 8, 65 ± 6, and 77 ± 8 μmol·100 g liver^{-1}·min^{-1} in N, P, and P-HFF, respectively), and it declined similarly to 33 ± 11, 35 ± 31, and 44 ± 21 μmol·100 g liver^{-1}·min^{-1} during the last hour of the experimental period. Glucose infusion rates did not differ among groups, although they reached a plateau at 51 ± 2 and 52 ± 6 μmol·kg^{-1}·min^{-1} during the last hour in P and P-HFF, respectively, but continued to rise in N (62 ± 8 μmol·kg^{-1}·min^{-1}, $P = 0.35$ vs. P) (Fig. 2*A*).

Non-HGU during the experimental period averaged 23 ± 4, 32 ± 2, and 38 ± 4 μmol·kg^{-1}·min^{-1} in N, P, and P-HFF, respectively (*P* NS for N vs. P and for P vs. P-HFF) (Fig. 2*B*). The rate of hindlimb glucose uptake did not differ in N and P, but the rate in P-HFF during the experimental period was double that in P ($P < 0.05$) (Fig. 2*C*). The hindlimb catheter failed to function in one dog in each group; thus, $n = 6$ per group for hindlimb data.

Lactate, alanine, glycerol, and NEFA metabolism. Arterial blood lactate concentrations rose in all groups at the onset of the experimental period (Fig. 3*A*). Under basal conditions, N exhibited net hepatic lactate output (NHLO), whereas P and P-HFF were in a state of net hepatic lactate uptake (NHLU) (Fig. 3*B*). The N group experienced an

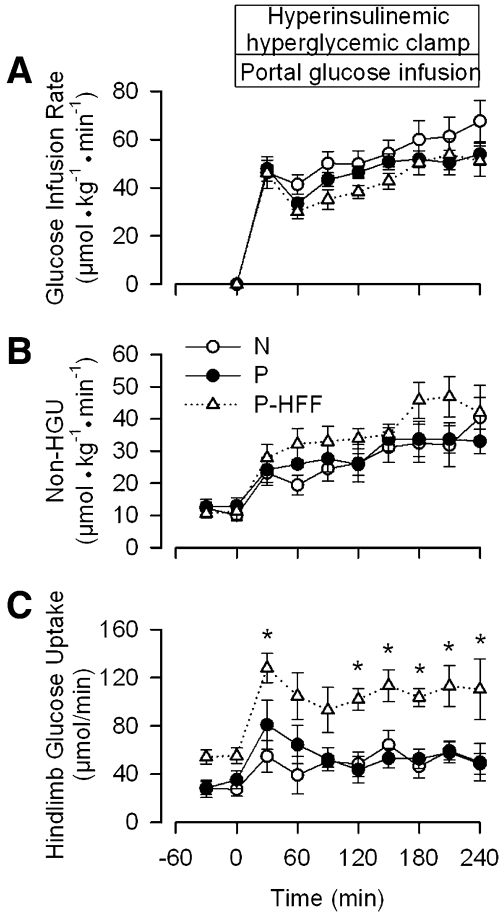

FIG. 2. Total (portal plus peripheral) glucose infusion rate (*A*), non-HGU (*B*), and hindlimb glucose uptake (*C*) during a hyperinsulinemic-hyperglycemic clamp in the presence of the portal glucose signal in the N, P, and P-HFF groups. n = 7 per group in *A* and *B*; n = 6 per group in *C*. *$P < 0.05$ for P-HFF vs. P.

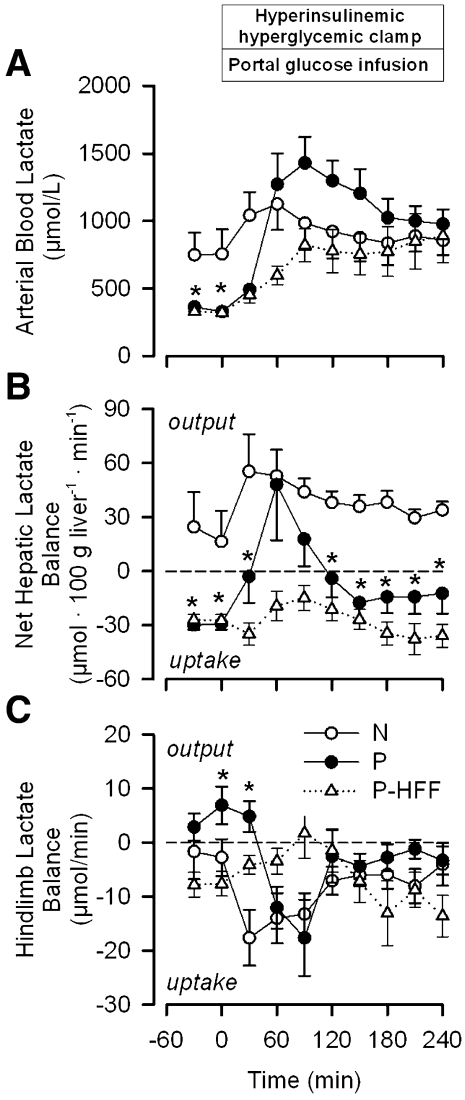

FIG. 3. Lactate concentrations (*A*) and hepatic (*B*) and hindlimb (*C*) balance data in the N, P, and P-HFF groups. n = 7 per group in *A* and *B*; n = 6 per group in *C*. *$P < 0.05$ for P vs. N.

increase in NHLO after the initiation of the experimental period but returned to basal rates within 90 min. In the P group, there was a transient peak of NHLO followed by a return to NHLU. The P-HFF group continued in NHLU throughout the experimental period. In N and P, hindlimb lactate uptake increased during the period of greatest NHLO.

Arterial alanine concentrations and net hepatic alanine uptakes were significantly lower in P than in N (Fig. 4*A* and *B*), but net hepatic fractional alanine extraction did not differ between groups (data not shown). Both P and P-HFF exhibited hindlimb alanine uptake during much of the clamp period, whereas hindlimb alanine output was present in N (Fig. 4*C*).

Basal arterial concentrations and net hepatic uptakes of glycerol were significantly higher in P than in N, but they fell in both groups during hyperinsulinemic hyperglycemia to values that were not significantly different between groups (Fig. 4*D* and *E*). The basal glycerol concentrations and hepatic glycerol uptakes were similar in P and P-HFF, but they tended to remain higher in P-HFF than in P throughout the clamp period (P = 0.17). Hindlimb glycerol release was suppressed during the clamp in all three groups (Fig. 4*F*). The basal NEFA concentrations and net hepatic NEFA uptakes were higher in P than in N, but they fell to levels not significantly different from those in N during the clamp, and P-HFF did not differ significantly from P (Fig. 4*G* and *H*).

Hepatic glycogen synthesis and tissue analyses. The hepatic glycogen concentrations at the end of study were significantly lower ($P < 0.05$) in P than in N (Fig. 5*A*). In P, hepatic glycogen synthesis calculated from net hepatic carbon retention initially lagged behind that in N (Fig. 1*F*), reflecting the delay in reaching maximal rates of NHGU. By 90 min, the rate in P was similar to that in N, but the overall area under the curve for the experimental period was modestly reduced (20%) in P versus N ($P < 0.05$) (Fig. 5*B*). Hepatic glycogen synthesis from [^{3}H]glucose did not differ between N and P. In P-HFF, hepatic glycogen concentrations, total glycogen synthetic rate, and glycogen synthesis from [^{3}H]glucose were reduced ~35% (P NS), 56% ($P < 0.05$), and 53% ($P < 0.05$), respectively, compared with P.

Hepatic GK protein did not differ between N and P, but it was reduced 45% in P-HFF compared with P ($P < 0.05$) (Fig. 5*C* and *J*). The catalytic activity of hepatic GK did not differ between N and P or between P and P-HFF (Fig. 5*D*).

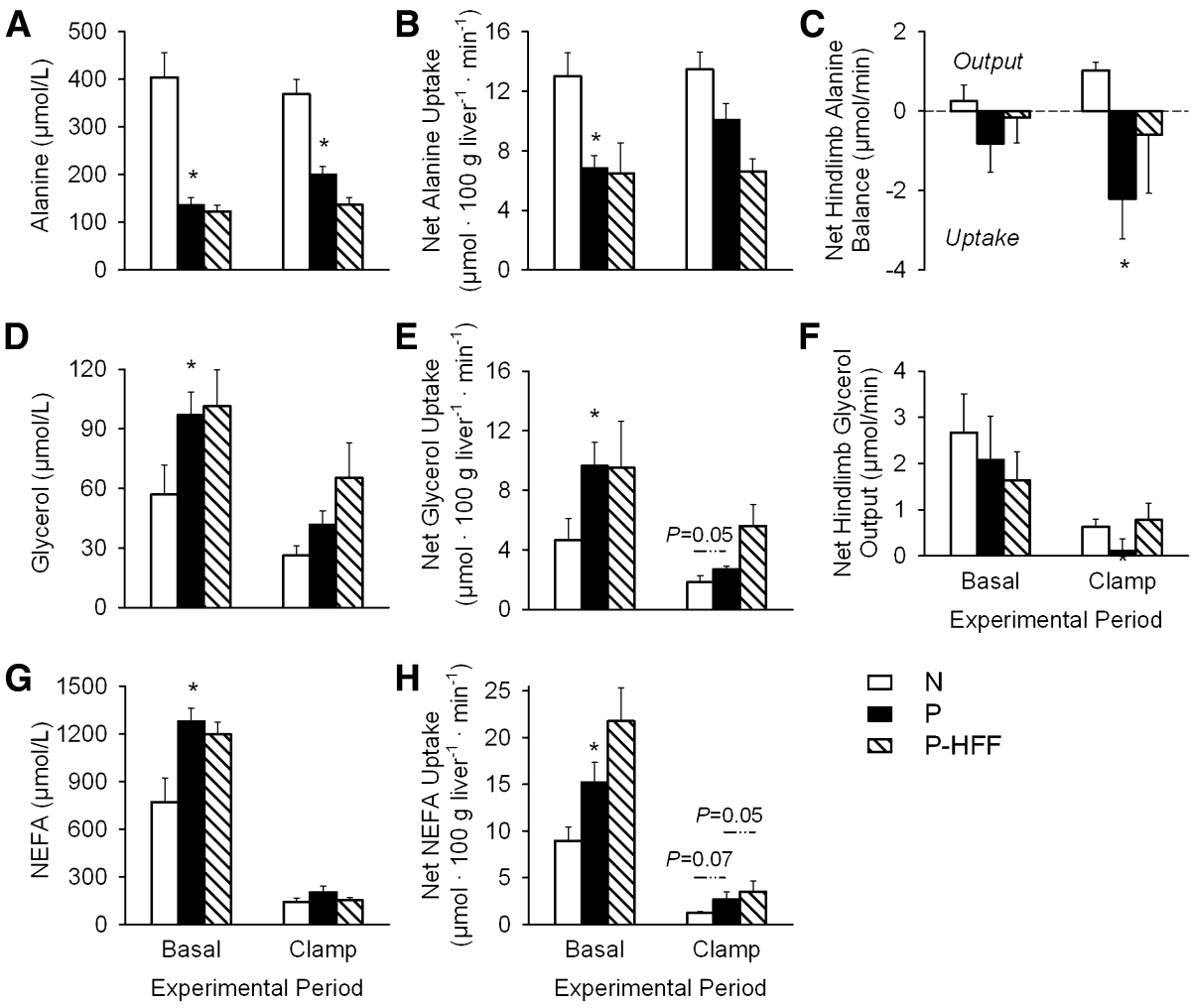

FIG. 4. Arterial alanine, glycerol, and NEFA concentrations (*A*, *D*, and *G*, respectively), net hepatic uptake rates (*B*, *E*, and *H*, respectively), and hindlimb alanine and glycerol balance data (*C* and *F*). Hindlimb NEFA balance was not assessed. *$P < 0.05$ for P vs. N.

Hepatic phosphorylated Akt, expressed relative to Akt (Fig. 5*E* and *K*), was similar in N and P but was ~20% lower in P-HFF than in P ($P = 0.12$). The phosphorylation of GSK3β did not differ significantly among groups (Fig. 5*F* and *K*). Hepatic PEPCK protein/actin was similar in N and P (1.00 ± 0.17 and 1.14 ± 0.10, respectively, $P = 0.44$), but was not evaluated in P-HFF (data not shown). The GS activity ratio did not differ in N and P but was reduced 75% in P-HFF ($P = 0.07$ vs. P) (Fig. 5*G*). The GP activity ratio in P was 1.7-fold greater than in N ($P < 0.05$) and 1.8-fold greater in P-HFF than in P ($P = 0.11$) (Fig. 5*H*), and the GS/GP ratio tended to be lower in P-HFF than in P ($P = 0.17$) (Fig. 5*I*).

DISCUSSION

Under normal circumstances, the liver plays an important role in the control of hyperglycemia after ingestion of a glucose load or a mixed meal because of its ability to switch rapidly from net HGO to NHGU and to store glucose as glycogen. The function of the maternal liver in pregnancy is thus of special interest because of the association of postload hyperglycemia (even if it does not meet the diagnostic criteria for diabetes) with adverse perinatal outcomes (25). The dog model makes possible quantification of NHGU through catheterization of the hepatic vasculature and, thus, is extremely useful in the assessment of postprandial metabolism (5,6). Fasting glucose and insulin concentrations tend to be lower in late-pregnant versus nonpregnant dogs, as observed in other polytocous species (4,26), which contrasts with findings in humans. On the other hand, changes in insulin sensitivity, response to insulin-induced hypoglycemia, and glucose tolerance in dogs are similar to those in pregnant women (7–9).

Normal pregnancy. In the presence of hyperinsulinemia, hyperglycemia, and portal glucose infusion, the livers of the P group shifted slowly to NHGU, and even during the last hour of the clamp, the rate of NHGU was lower than in the N group. Hepatic insulin sensitivity is maintained in late pregnancy in normal dogs (8), as it is in lean women (27), and thus, impairment of insulin sensitivity did not explain the liver's response. On the other hand, rate of glucose disappearance and the rates of glucose infusion, non-HGU, and hindlimb glucose uptake were remarkably similar in the N and P groups. Dogs in late pregnancy display whole-body and nonhepatic insulin resistance during a hyperinsulinemic-euglycemic clamp that is indistinguishable in degree to that observed in women during the third trimester (1,8). Thus, these findings of similar

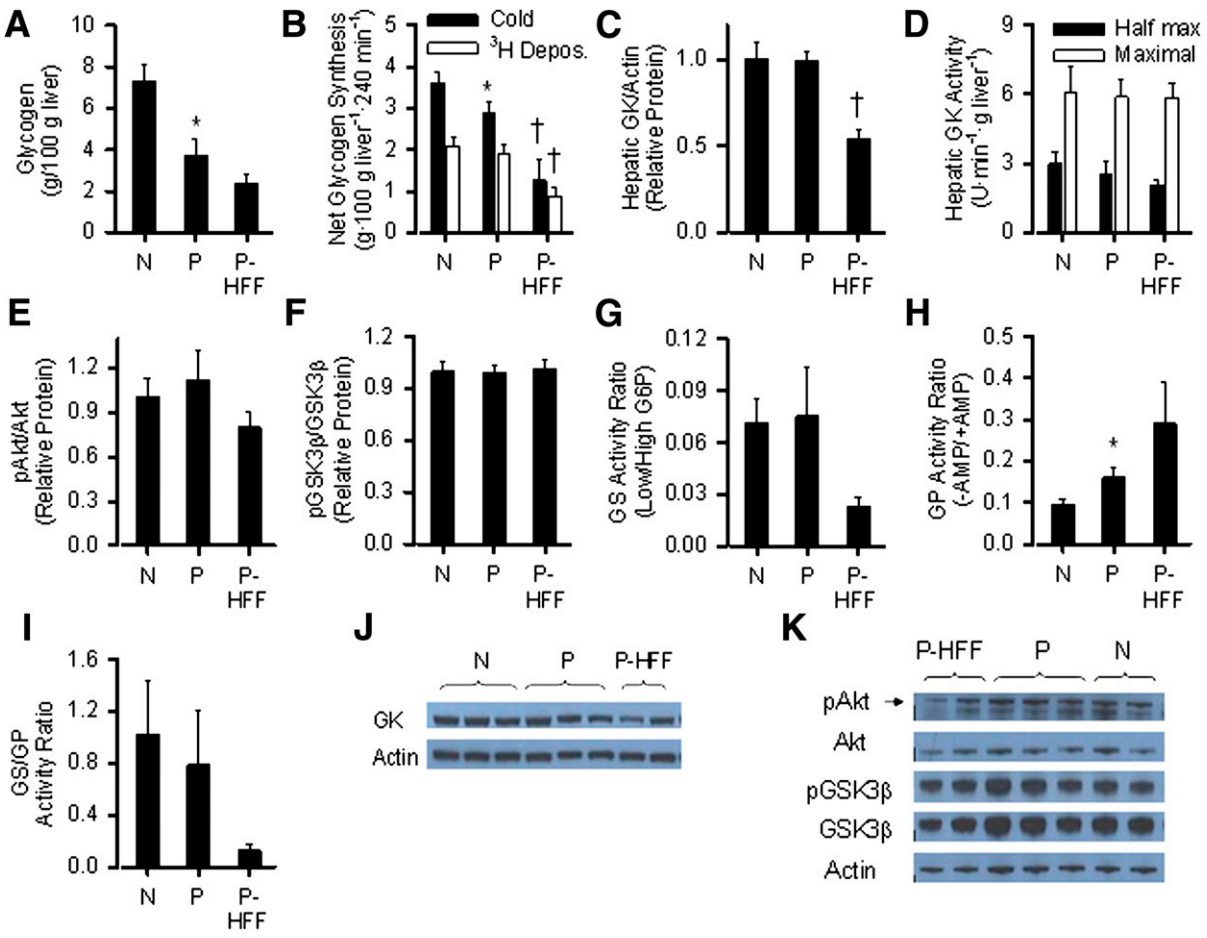

FIG. 5. Liver tissue analyses and glycogen synthesis. Glycogen concentrations at the end of study (*A*); net glycogen synthesis (*B*) (cold indicates the area under the curve of net hepatic carbon retention during the clamp, and ^{3}H depos. [deposition] indicates incorporation of ^{3}H from glucose into hepatic glycogen, i.e., synthesis through the direct pathway); GK protein expressed relative to β-actin (*C*); catalytic GK activity (*D*) at near half-maximal and maximal velocity (in the presence of 8 mmol/L [solid bars] and 100 mmol/L [open bars] glucose, respectively); Akt (*E*) and GSK3β (*F*) phosphorylation, expressed relative to total Akt and GSK3β protein, respectively; and activity ratios of GS (*G*), GP (*H*), and GS/GP (*I*) at the end of a 4-h hyperinsulinemic-hyperglycemic clamp in the presence of the portal signal. Also shown are representative Western blots (*J* and *K*). *n* = 7 per group. **P* < 0.05 for P vs. N; †*P* < 0.05 for P-HFF vs. P. p, phosphorylated. (A high-quality color representation of this figure is available in the online issue.)

whole-body and nonhepatic glucose disposal rates do not imply similar states of insulin sensitivity in the two groups; indeed, the conditions of study (hyperglycemia with portal glucose infusion) were not designed to evaluate insulin sensitivity. One explanation for the similarity in whole-body and nonhepatic glucose disposal in the N and P groups lies in the fact that non-HGU in the pregnant animal includes uptake by the uterus, placenta, and fetus, which is largely independent of maternal insulin concentration (28). In addition, the portal glucose signal in normal animals does not increase whole-body glucose disposal; instead, it directs the partitioning of the glucose load such that the enhancement of NHGU is accompanied by a reciprocal inhibition of non-HGU, particularly that of skeletal muscle (11).

The response to the portal glucose signal appears to be neurally mediated. Selective sympathetic hepatic denervation in the normal dog impairs both limbs of the response, reducing the enhancement of NHGU and preventing the suppression of non-HGU (29). There is no evidence of a global alteration of sympathetic signaling in pregnancy. Plasma norepinephrine (NE) concentrations are not significantly different in normal pregnant (third trimester) and nonpregnant women during the nocturnal period (considered the most stable period of NE release), although heart rate variability is reduced in pregnancy (30). Muscle sympathetic nerve activity is indistinguishable between normal pregnant and nonpregnant women (31), whereas the supine pressor test indicates increased sympathetic cardiovascular tone in normal women during late pregnancy (32). On the other hand, NE concentrations and turnover in retroperitoneal and perimetrial fat pads, liver, and heart are reduced in late pregnant (18 days) versus virgin rats (33,34). Moreover, pancreatic NE spillover, an index of sympathetic signaling, is markedly reduced during hypoglycemia in the pregnant dog (14). Thus, there are tissue-specific differences in neural tone during pregnancy, suggesting a mechanism for the blunted response to the glucose portal signal.

Hepatic GK catalyzes the rate-determining step in regulation of hepatic glucose metabolism (35). The present data indicate that hepatic GK protein expression and activity are unaltered in normal late pregnancy. However, short-term regulation of GK occurs through binding of the enzyme to its regulatory protein (GKRP), with activation resulting in dissociation of GK from GKRP and

translocation of the enzyme from the nucleus to the cytoplasm (35). We cannot rule out an alteration in GK translocation in pregnancy, which might contribute to the blunting of NHGU.

Hepatic glycogen concentrations in the P group were ~50% of those in the N group at the end of study, despite only a modest reduction in the rate of glycogen synthesis (net carbon retention) and virtually identical rates of direct glycogen synthesis. These findings, as well as the enhancement of GP activity in the P group, are consistent with previous observations that hepatic glycogen concentrations after an overnight fast are ~25% lower (5) and the rate of glycogenolysis is approximately twice as great (7) in pregnant versus nonpregnant dogs. The P group maintained a relatively high rate of glycogen synthesis (in spite of an increase in GP activity over that observed in the N group) apparently by also maintaining a high rate of GS activity. After a mixed meal, hepatic glycogen synthetic rates are several times higher in pregnant versus nonpregnant dogs (5) and rats (26). However, the postprandial increase in plasma glucose level is greater in pregnant animals, explaining at least part of the enhancement in the glycogen synthesis rates. To our knowledge, the current study is the first to compare NHGU and hepatic glycogen synthesis in pregnant and nonpregnant animals under conditions mimicking the postprandial state with the hormone and glucose concentrations clamped at the same concentrations in the two groups; thus, they reveal a previously unrecognized alteration in NHGU in normal pregnancy. The direct pathway (glucose → G6P → glycogen) accounted for ~55–60% of hepatic glycogen synthesis in both groups, similar to the proportion synthesized through the direct pathway after mixed-meal feeding in nonpregnant and pregnant dogs (5). NHGU in both the N and the P groups was sufficient to account for all glycogen synthesis; thus, it is possible that intrahepatic metabolism of glucose provided most of the precursors for glycogen synthesis through the indirect pathway.

Effect of the high-fat and -fructose diet in pregnancy. The further impairment of liver glucose uptake in the P-HFF group compared with the P group agrees with findings in male dogs fed a high-fat and -fructose diet similar to that used in the current study (36). The male dogs exhibited marked reduction of NHGU in response to hyperinsulinemia and hyperglycemia induced by peripheral glucose infusion, and the presence of the portal signal did not enhance NHGU. The male dogs also displayed a significant reduction in both hepatic GK protein and activity, which is in contrast to the P-HFF group, where only GK protein was reduced. The males had consumed the diet longer (4 vs. 3 weeks in P-HFF); thus, it is possible that the effects of the diet on GK activity are time dependent. However, it appears more likely that elevation of pregnancy-related hormones, such as estradiol, progesterone, or prolactin, contributed to the preservation of hepatic GK activity, as reported for pancreatic GK (37,38). Either estrogen or progesterone treatment of RIN 1046-38 rat insulinoma cells is associated with modestly increased GK mRNA expression but with a more significant increase in GK activity (38). Consistent with this, diabetic models (*db/db* mice [diabetic] and streptozotocin-treated rats) treated with phytoestrogens display enhanced hepatic GK activity (39–41). Data regarding hepatic GK activation during pregnancy are sparse and conflicting. Activity is increased (by as much as twice the rate in nonpregnant females) at 7, 16, and 21 days gestation in rats (42) but reduced as much as 50% during pregnancy in mice (43), with no information available regarding human liver GK activity during pregnancy. Regulation of hepatic GK expression and activity during pregnancy is an important question for further investigation.

The P-HFF dogs exhibited a significant reduction in GS activity and high GP activity in keeping with their impaired net glycogen synthesis and low terminal hepatic glycogen concentrations. That the P-HFF dogs exhibited actual enhancement of non-HGU and hindlimb glucose uptake in the presence of the portal glucose signal is consistent with the reciprocal nature of the signal (11). When liver glucose uptake is blunted, the peripheral tissues must play a larger role in glucose disposal.

Nonglucose metabolites. Both the P and the P-HFF dogs exhibited NHLU in the basal period, whereas the nonpregnant controls were in a state of NHLO. Because the shift from NHLO to NHLU is a marker of the progression from the fed to the fasted state, this reflects the accelerated fasting of pregnancy that has been noted in numerous species, including humans (44), dogs (45), and rats (46). The elevated glycerol and NEFA concentrations and NHGU in the pregnant animals during the basal period are also consistent with accelerated fasting. A state of advanced fasting is unlikely to explain the blunting of NHGU in the pregnant dogs, however, because the rate of NHGU in response to intraduodenal or portal glucose delivery in normal dogs is at least as great after a 36–42-h fast as it is following an 18-h fast (47,48).

Despite the presence of accelerated fasting, the rates of gluconeogenesis after an overnight fast do not differ between pregnant and nonpregnant dogs (7), and incorporation of labeled alanine or glycerol into plasma glucose or hepatic glycogen is indistinguishable in 24-h-fasted virgin and late-pregnant rats (49). Consistent with this, hepatic PEPCK protein was very similar in the N and P groups. Depletion of circulating gluconeogenic precursors (e.g., lactate and alanine [Figs. 3 and 4]) likely contributes to the lack of stimulation of gluconeogenesis in the pregnant animals.

Conclusion. The uniqueness of this study lies in the fact that it includes the most complete data available to date on hepatic glucose metabolism in pregnancy, including GK, GS, and GP activities, under conditions that stimulate NHGU. Thus, it provides a framework for better understanding of postprandial metabolism while underscoring that there is much yet to be done in characterizing the liver's role in glucose and meal disposition in both normal pregnancy and pregnancy complicated by IGT or GDM. The liver's response to hyperinsulinemia, hyperglycemia, and portal glucose delivery was delayed and blunted during the third trimester of normal pregnancy. Although this was associated with only a slight impairment of glycogen synthesis, it could contribute to postprandial hyperglycemia by reducing hepatic extraction of meal-related carbohydrates. Even among women who do not meet the criteria for GDM, a single elevated value on an oral glucose tolerance test is associated with adverse perinatal outcomes (25), underscoring the importance of normoglycemia during pregnancy. Moreover, in a diet-induced canine model of IGT/GDM, NHGU in the presence of hyperinsulinemia, hyperglycemia, and the glucose portal signal was suppressed even more than in normal pregnancy. The failure of the liver to respond appropriately to these factors known to stimulate NHGU likely exacerbates postprandial hyperglycemia, a hallmark of GDM.

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K.C.C. researched data, contributed to the interpretation and discussion of data, and reviewed and edited the manuscript. M.S.S. and B.F. researched data and read and approved the final manuscript. M.S. and J.M.I. researched data, contributed to the discussion, and reviewed and edited the manuscript. P.J.R. contributed to the discussion and reviewed and edited the manuscript. P.E.W. researched data and reviewed and edited the manuscript. M.C.M. designed and supervised the studies, researched and interpreted data, and wrote the manuscript. M.C.M. 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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