Alterations in basal glucose metabolism during late pregnancy in the conscious dog

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Alterations in basal glucose metabolism during late pregnancy in the conscious dog. Am J Physiol Endocrinol Metab 279: E1166–E1177, 2000.—We assessed basal glucose metabolism in 16 female nonpregnant (NP) and 16 late-pregnant (P) conscious, 18-h-fasted dogs that had catheters inserted into the hepatic and portal veins and femoral artery ~17 days before the experiment. Pregnancy resulted in lower arterial plasma insulin (11 ± 1 and 4 ± 1 μU/ml in NP and P, respectively), but plasma glucose (5.9 ± 0.1 and 5.6 ± 0.1 mg/dl in NP and P, respectively) and glucagon (39 ± 3 and 36 ± 2 pg/ml in NP and P, respectively) were not different. Net hepatic glucose output was greater in pregnancy (42.1 ± 3.1 and 56.7 ± 4.0 μmol·100 g liver−1·min−1 in NP and P, respectively), indicating that the increment in net hepatic glucose output resulted from an increase in the contribution of glycogenolytically derived glucose. However, total glycogenolysis was not altered in pregnancy. Ketogenesis was enhanced nearly threefold by pregnancy (6.9 ± 1.2 and 18.2 ± 3.4 μmol·100 g liver−1·min−1 in NP and P, respectively), despite equivalent net hepatic nonesterified fatty acid uptake. Thus late pregnancy in the dog is not accompanied by changes in the absolute rates of gluconeogenesis or glycogenolysis. Rather, repartitioning of the glucose released from glycogen is responsible for the increase in hepatic glucose production.

hepatic glucose production; gluconeogenesis; glycogenolysis; lipolysis; ketogenesis

As pregnancy progresses, the fetus grows more rapidly and its glucose requirements increase, necessitating changes in maternal glucregulation to meet maternal and uteroplacental-fetal glucose needs (2, 6, 14, 24, 29, 32, 35, 36, 40, 62, 63). Hepatic glucose production is increased in the basal state in pregnant women and rats (2, 6, 14, 35, 36, 53). This hepatic effect could be due to changes in insulin action at the liver, stimulation by pregnancy-associated hormones, or alteration(s) in the action of other glucoregulatory hormones, but the mechanism is unknown. Whether the increase in glucose release from the liver results from an increase in gluconeogenesis, or glycogenolysis also, has not been clarified (5, 35, 36, 53, 68, 74). In the human, an older study indicated that gluconeogenesis was not increased basally in late pregnancy (36), whereas a more recent study has suggested that changes in both gluconeogenesis and glycogenolysis contribute to the pregnancy-induced increment in hepatic glucose production (35). Other studies in which labeled precursor carbon was administered, in vivo (31) and in perfused rat liver preparations (46), have implied that the gluconeogenic potential of the liver is enhanced in late pregnancy. These studies did not actually reflect the basal state, however, since the subjects were loaded with unlabeled gluconeogenic precursor carbon as well. Conclusions regarding whether an increase in gluconeogenesis accounts for part of the pregnancy-associated increase in hepatic glucose production are difficult to draw, and the topic requires further study.

Alteration of the liver’s ability to release glucose is only one of the metabolic changes induced by pregnancy in the human (and other species). Changes occur in fat metabolism (3, 33, 38, 45, 49, 55), such that modest increases in circulating nonesterified fatty acid (NEFA) and glycerol levels and marked increases in triglyceride levels are characteristic of late pregnancy. Circulating ketone levels tend to be elevated as well (16, 31, 38, 54, 61). Insulin resistance at peripheral tissues is characteristic of pregnancy (29, 32, 38, 40, 41, 62, 63), although this is not evident in the basal state, given that basal glucose levels are unchanged or lower than in the nonpregnant state, despite accelerated hepatic glucose production and normal (4, 36, 48, 49) or slightly elevated (6, 13, 14) insulin levels in the human. Taken together, the changes in metabolism that ac-
company pregnancy allow the mother to provide for the growth of the fetus while meeting her own energy needs.

The lack of extensive study examining the mechanisms that control pregnancy-induced changes in glucose metabolism can probably be attributed to several causes. The study of carbohydrate metabolism in humans, and pregnant women in particular, is limited by the invasiveness of the techniques required to assess hepatic substrate metabolism thoroughly. In addition, protecting the fetus from experimental conditions that might cause it harm is of highest priority. These limitations necessitate the use of animal models of pregnancy to address many of the issues of metabolic regulation. Experiments using animal models have made important contributions to the study of glucose metabolism during pregnancy. However, the available animal models of pregnancy are not well suited to the assessment of maternal glucose metabolism. The small size and blood volume of animals such as rats, rabbits, and guinea pigs limit the ability to perform studies in which serial blood sampling is required to assess a number of metabolic parameters simultaneously. Studies using the sheep have greatly advanced knowledge of fetal and placental metabolism; however, the sheep is not an ideal model for studying the regulation of maternal carbohydrate metabolism, since it relies partly on fuels that are not normally used by the human and other nonruminants, and its fasting glucose levels are quite low.

These limitations led us to investigate the suitability of the dog as an animal model for studying the regulation of carbohydrate metabolism during pregnancy. This model is unique, because it allows us to assess changes in metabolic processes during pregnancy in a comprehensive manner by using approaches that could not be used in the pregnant woman. Surgical and experimental techniques are available that permit study of the chronically catheterized, conscious dog under nonstressful circumstances, eliminating anesthetic, surgical, and handling stressors that influence metabolism (24). In addition, the dog’s large size allows for thorough and simultaneous assessment of processes such as gluconeogenesis, glycogenolysis, lipolysis, and ketogenesis in one animal, since blood volume is not a limiting factor. Data gathered from the dog by use of these techniques are highly relevant to the human, since regulation of carbohydrate metabolism is quite similar in the dog and human (8). Finally, insulin resistance is thought to be a hallmark of canine pregnancy (12), just as it is in human pregnancy. The data presented here describe the changes in basal glucose metabolism that characterize pregnancy in this model.

METHODS

Animals and surgical procedures. Experiments were performed on 32 overnight-fasted (18 h), conscious female mongrel dogs [21.1 ± 0.5 and 24.8 ± 0.8 kg in nonpregnant (NP) and pregnant (P), respectively] that were fed a standard meat-and-chow diet (34% protein, 46% carbohydrate, 14.5% fat, and 5.5% fiber based on dry weight; Kal Kan meat, Kal Kan, Vernon, CA, and Purina Lab Canine Diet 5006, Purina Mills, St. Louis, MO) once daily; water was available ad libitum. The dogs were housed in a facility that met American Association for the Accreditation of Laboratory Animal Care guidelines, and the protocol was approved by the Vanderbilt University Medical Center Animal Care Committee.

Sixteen of the dogs were 7–8 wk pregnant (full term = 9 wk) when studied. The other 16 dogs were not pregnant and were in the anestrous state (basal progesterone and estrogen levels) throughout the time they were housed and studied.

Sixteen to 21 days before the experiment, each dog was placed under general anesthesia [thiopental sodium (Pentothal) and isoflurane gas] and a laparotomy was performed using standard sterile surgical techniques. A silicone rubber catheter (0.04 in. ID) was inserted into the hepatic portal vein and advanced so that its tip was positioned 4–5 cm into the common portal vein. A second catheter was inserted into the left common hepatic vein, which carries the largest volume of any of the hepatic veins (64), and the tip was placed 1.5 cm from its point of origin at the left lateral lobe. Another catheter was inserted into a vein leading into the portal drainage system and advanced 1 cm beyond the first site of coalescence with the common splenic vein. The splenic and jejunal catheters were used for saline infusion during the experiment. A small portion of the jejunalum was exposed, and a catheter (0.03 in. ID) was inserted into a mesenteric vessel and its tip was advanced 1 cm beyond the lymph nodes. The spleen was exteriorized, and another catheter was placed into a vein leading into the portal drainage system and advanced 1 cm beyond the first site of coalescence with the common splenic vein. The splenic and jejunal catheters were used for saline infusion during the experiment to allow these studies to be utilized as control experiments for future studies requiring splenic/jejunal infusions. Once inserted, the catheters were filled with heparinized saline (200 U/ml) and knotted. The muscular and subcutaneous layers were closed, with the catheter ends extending through the closures. The catheter ends were then placed in a subcutaneous pocket, and the skin layer was closed. A small incision was made in the left inguinal region, and a sampling catheter (0.04 in. ID) was placed into the femoral artery and advanced so that its tip was positioned beyond the branch of the common iliac arteries into the aorta. This catheter was filled with heparinized saline, knotted, and placed in a subcutaneous pocket, as described above. Arterial blood samples were obtained from this catheter during the experiment.

One to 2 days before each experiment, the leukocyte count and hematocrit were determined. Dogs were used for an experiment only if they met the following criteria: 1) leukocyte count <20,000/mm³, 2) hematocrit >35% for nonpregnant dogs and >29% for pregnant dogs, values consistent with the typical gestationally associated fall in hematocrit (20), 3) consumption of the entire daily food ration, and 4) normal stools.

On the morning of an experiment the catheter ends were removed from the subcutaneous pockets under local anesthesia (2% lidocaine, Abbott Laboratories, North Chicago, IL). The contents of the abdominal and femoral artery catheters were aspirated, and the catheters were flushed with saline. The dog was placed in a Pavlov harness. An Angiocath (20 gauge, Becton-Dickinson Vascular Access, Sandy, UT) was inserted percutaneously into a cephalic vein for infusion of indocyanine green and tracers.

Experimental design. Each experiment consisted of a 120-min tracer and dye equilibration period (−120 to 0 min) and a 30-min basal sampling period (0–30 min). A primed (41.7–83.3 mg/kg basal) bolus of each indocyanine green and tracer was infused i.v. through the sampling catheter at time 0. At 60 min, a tertiary primed bolus was infused into the mesenteric and portal veins. Arterial samples were obtained at 0–30 min (30-min basal period), 30–60 min, and 60–90 min (60-min tracer/dye equilibration period).
μCi), constant (0.35–0.70 μCi/min) infusion of [3-3H]glucose (New England Nuclear, Boston, MA) was begun at ~120 min and continued throughout the experiment. Infusions of indocyanine green (0.1 mg·m⁻²·min⁻¹; Becton-Dickinson Microbiology Systems, Cockeysville, MD) and [U-¹³C]alanine (0.42–0.67 μCi/min; NEN) were begun at ~120 min and continued throughout the experiment.

Blood samples were taken from the femoral artery at 7.5-min intervals and from the portal vein and hepatic vein catheter at 15-min intervals during the sampling period. The total amount of blood withdrawn from each dog during an entire experiment did not exceed 20% of its total blood volume. The volume withdrawn was replaced with double that volume of saline during the experiment. The arterial and portal vein blood samples were collected simultaneously. To allow accurate estimates of hepatic substrate balances, the hepatic vein blood sample was collected ~30 s after the arterial and portal samples to compensate for the transit time of glucose through the liver (27).

After the collection of baseline samples, all dogs were used immediately only for several other experimental protocols. At the end of the experiments, the dogs were anesthetized and then euthanized ~5 min later with an overdose of pentobarbital sodium. On autopsy, the positions of the catheter tips were verified to ensure proper placement, and the liver was removed and weighed.

Analytic procedures. Blood samples were treated as described below and, if not assayed immediately, were frozen at −70°C for later analyses. Three milliliters of whole blood were added to 60 μl of a solution containing 90 mg/ml EGTA and 60 mg/ml glutathione (pH 7.0) for later analysis of plasma epinephrine (interassay coefficient of variation (CV) = 11%) and norepinephrine (CV = 9%) by HPLC (51). The rest of the blood sample was placed in a tube containing potassium EDTA (1.6 mg EDTA/ml). One milliliter of whole blood was added to 3 ml of 4% (vol/vol) perchloric acid and centrifuged (3,000 rpm for 7 min). One milliliter of the supernatant was used for immediate spectrophotometric analysis of whole blood acetacetate (58). The remainder of the supernatant was frozen for later analysis of whole blood glutamine and glutamate (44) and whole blood lactate, alanine, glycine, and β-hydroxybutyrate (43). One milliliter of whole blood was added to 1 ml of 10% sulfosalicylic acid and centrifuged, and the supernatant was frozen for later analysis of whole blood glucose and glycogen (44) and whole blood lactate, alanine, glycine, and β-hydroxybutyrate (43). One milliliter of whole blood was added to 1 ml of 6% sulfosalicylic acid and centrifuged, and the supernatant was frozen for later analysis of [¹⁴C]glucose, [¹⁴C]lactate, and [¹⁴C]alanine using short-column ion-exchange chromatography (11). Determination of [³H]- and [¹⁴C]glucose by double-label, liquid scintillation counting was made from 1 ml of plasma using the Somogyi-Nelson deproteinization procedure, as previously described (9, 15, 71). One milliliter of plasma from the femoral artery and hepatic vein samples was used for spectrophotometric determination (805 nm) of indocyanine green immediately after the experiment. The remaining plasma was aliquoted and frozen. Immunoreactive plasma insulin was measured using a double-antibody RIA (52) (standard and ¹²⁵I-insulin from Linco Research, CV = 9%). Plasma cortisol was measured with the Clinical Assays Gamma Coat RIA kit (19) (CV = 11%, Incstar, Stillwater, MN). Plasma NEFA levels were determined by a colorimetric method (Wako Chemicals, Richmond, VA). Plasma levels of progesterone (59), estrogen (59), and prolactin (60) (Canine Prolactin Kit, Pituitary Hormones and Antisera Center, Harbor-UCLA Medical Center) were measured by RIA by the Diagnostic Laboratory, College of Veterinary Medicine, Cornell University (Ithaca, NY).

Calculations. The values reported in results are averages of the values obtained during the sampling period. Total hepatic blood flow was assessed by measuring hepatic extraction of indocyanine green, according to the method of Leevy et al. (39). The proportions of the hepatic blood supply provided by the hepatic artery and portal vein were assumed to be 20 and 80%, respectively, on the basis of the Doppler-determined blood flow from other studies done in the Vanderbilt Diabetes and Research Training Center. Since the complete balance of studies included here, Transonic flow probes have been implanted on the hepatic artery and portal vein in nonpregnant and pregnant dogs and have confirmed this distribution (unpublished observations; artery distribution of 20 and 19% in NP and P, respectively, n = 7 in each group).

Net hepatic substrate balance was calculated using the following formula

$$[H - (0.2 \cdot A + 0.8 \cdot P)] \cdot HF$$

where A, P, and H are the arterial, portal, and hepatic vein substrate concentrations, respectively, and HF is the hepatic blood or plasma flow, as appropriate for the particular substrate. With the use of this equation, net hepatic output of a substrate yields a positive value, while net uptake results in a negative value; however, most data are presented as positive values and labeled appropriately as net hepatic uptake or output. Although the dog does not gain much fat weight during pregnancy (12) and most of the weight gain is due to fetal/placental/uterine tissues, the data are expressed in relation to liver weight to yield a more precise estimate of substrate handling by the maternal liver, thereby avoiding the impact of potential differences in fat mass between the groups and the contribution of the uteroplacental-fetal mass. Whole blood glucose values were assumed to equal 73% of plasma values on the basis of extensive comparisons between whole blood and plasma glucose values done in our laboratory (1). Calculations utilizing plasma glucose values converted to blood glucose values yield results that are nearly identical to those utilizing blood glucose values. However, the variance is reduced because of the accuracy of plasma glucose arteriovenous differences, since analysis of plasma, unlike whole blood, does not require a deproteinization step.

Hepatic fractional extraction of substrate was calculated as the amount of substrate taken up by the liver relative to the amount provided to the liver as follows

$$\left(\frac{A - 0.2 \cdot A - 0.8 \cdot P}{0.2 \cdot A + 0.8 \cdot P}\right)$$

The conversion rate of circulating gluconeogenic precursors to glucose was calculated for each dog, using the arteriovenous difference technique (25), with the assumption that all precursors taken up by the liver in a net sense were completely converted to glucose. Net hepatic balances of the gluconeogenic precursors alanine, serine, threonine, glycine, glutamine, glutamate, lactate, and glyceral were measured. Net hepatic balance of pyruvate was assumed to be 10% of net hepatic lactate balance (70). The net hepatic uptake rates of the precursors were added together. When any precursor
that the liver can either consume or release in a net sense (such as lactate) exhibited net hepatic output, it was considered to have a zero uptake rate and to be a product, not a precursor. (Nevertheless, when net hepatic balance of the substrate was calculated, all values, whether negative or positive, were included.) For precursors that are only consumed by the liver, any value obtained that indicated net hepatic output (due to methodological limitations) remained in the calculation. Thus the group mean of net hepatic balance of a substrate such as lactate, that can be a product or a precursor, may not reflect the contribution of the precursor in a gluconeogenic sense. To calculate hepatic gluconeogenesis, the combined net hepatic precursor uptake rate was divided by 2 to account for the incorporation of two three-carbon precursors into a six-carbon glucose molecule. For the purposes of estimating glycogenolysis, this rate of gluconeogenesis from circulating precursors was assumed to be equivalent to the overall rate of gluconeogenesis from all sources. The validity of this assumption is discussed below.

The portion of net hepatic glucose output (NHGO) that is derived from glycogen does not necessarily represent total net glycogen breakdown, since glucose released from glycogen can enter other pathways (oxidation and glycolysis for lactate production). Thus the net rate of hepatic glycogenolysis (GLY) can be assessed as follows:

\[
\text{net hepatic GLY} = \text{NHLO} + \text{hepatic glucose oxidation} + \text{HGR} - \text{HGU} - \text{GNG}
\]

where NHLO is the rate of net hepatic lactate output (this is equal to 0 if there is net hepatic lactate uptake), hepatic glucose oxidation is assumed to be constant at 1.7 \(\mu\text{mol-kg}^{-1}\text{-min}^{-1}\) (28) (this is converted to \(\mu\text{mol/100 g liver wt}\) in the calculation for each individual dog, giving averages of 6.8 ± 0.2 and 6.4 ± 0.2 mg/100 g liver \(\text{-min}^{-1}\) in NP and P, respectively), HGR is total hepatic glucose release, HGU is total hepatic glucose uptake, and GNG is gluconeogenesis. HGU was not measured, but since HGR - HGU = NHGO, NHGO (which was measured) can be substituted into the equation. The rate of hepatic glucose oxidation in the dog remains unchanged in a variety of conditions (fasting, exercise, infection, hyperinsulinemia, and hyperglycemia) and, thus, is likely to remain unchanged by pregnancy as well. Indeed, a study in women demonstrated that glucose oxidation was unchanged by pregnancy when expressed on a weight-specific basis (2).

This method of assessing the overall glycolytic rate and the glycogenolytic contribution to NHGO is dependent on the ability of the arteriovenous method of calculating gluconeogenesis to yield an accurate value. The gluconeogenic rate obtained using this technique reflects gluconeogenesis from circulating precursors, and the assumption is made that the precursors are completely converted to glucose. It is conceivable that intrahepatic protein stores could contribute carbon to gluconeogenesis. This possibility has been tested (25) by simultaneously comparing the gluconeogenic rates obtained from the arteriovenous difference technique and an alternate method of measuring gluconeogenesis that has been described by Giaccari and Rossetti (23). The latter method utilizes HPLC techniques to determine hepatic UDP-glucose and phosphoenolpyruvate labeling from \(^3\text{H}\)glucose and \(^1\text{C}\)alanine. The gluconeogenic estimate derived from this method accounts for the contribution of circulating precursors and intrahepatic protein stores, although it misses the contribution from glycerol. The two methods yielded very similar estimates of the gluconeogenic rate when comparisons were made after an 18-h fast and more prolonged fasting (42 h). A chronic environment of high cortisol only modestly increased the apparent contribution of intrahepatic precursors to hepatic gluconeogenesis. Given the results of those studies, gluconeogenesis from an intrahepatic precursor pool is probably minimal in the pregnant dog, and its exclusion from the gluconeogenic calculation should have little impact on the conclusions drawn. The comparison (25) also indicated that the assumption that all circulating precursors consumed by the liver are converted to glucose is valid, meaning that this assumption would also have negligible impact on the gluconeogenic calculation. Moreover, this method accounts for the contribution of glycerol to gluconeogenesis and provides gluconeogenic information throughout the study. On the basis of the information from the studies comparing the gluconeogenic methods, we have assumed that the gluconeogenic rate calculated by the arteriovenous difference technique is a reliable estimate of the true gluconeogenic rate in the pregnant dog.

In addition to assessment of the gluconeogenic rate, a double-isotope technique was used as described previously (10) to assess the fraction of labeled alanine and lactate taken up by the liver that was incorporated into glucose. Briefly, \([U-1\text{C}]\)alanine was infused to provide a labeled substrate for gluconeogenesis, and \([3-\text{H}]\)glucose infusion was used to measure endogenous \([1\text{C}]\)glucose production. Conversion of \([1\text{C}]\)alanine to \([1\text{C}]\)glucose by the kidney is minimal under basal conditions; thus \([1\text{C}]\)glucose production results from hepatic gluconeogenesis almost exclusively (67). The fraction of labeled alanine and lactate converted to glucose was calculated by dividing the \([1\text{C}]\)glucose production rate by the rate of net uptake of \([1\text{C}]\)alanine and \([1\text{C}]\)lactate (in glucose equivalents) by the liver (derived from net hepatic balance equation above). If net hepatic \([1\text{C}]\)lactate production occurred, its net uptake was considered to be zero. The fraction converted is unlikely to result in a value of 1, since the labeled precursor enters the common oxaloacetate pool of the hepatocyte and is likely to become diluted, to yield a value that is <1. Thus infusion of a labeled gluconeogenic precursor and determination of its incorporation into glucose do not accurately reflect the gluconeogenic rate, since it can indicate a difference in an unidentified parameter (e.g., size of the oxaloacetate pool) in different conditions. The usefulness of this parameter lies not in the absolute values but in the differences between groups.

Statistical comparisons were made using \(t\)-tests (66). Values are means ± SE.

RESULTS

**Hormone levels and hepatic blood and plasma flow.** After an overnight fast, arterial plasma insulin and C-peptide levels were lower in the pregnant than in the nonpregnant group (Table 1). There were no differences in arterial plasma glucagon, cortisol, or epinephrine levels with pregnancy, but arterial plasma norepinephrine was ~50% greater in the pregnant group. Arterial plasma estrogen, progesterone, and prolactin levels were also elevated in the pregnant group.

Hepatic blood flow was not statistically different in the two groups (106.6 ± 6.1 and 91.5 ± 4.7 ml/100 g liver \(\text{-min}^{-1}\) in NP and P, respectively, \(P = 0.052\)).

**Glucose levels and kinetics.** Arterial plasma glucose was not significantly different between the two groups (5.9 ± 0.1 and 5.6 ± 0.1 mmol/l in NP and P, respectively; Fig. 1, Table 1). Net hepatic glucose output was greater during pregnancy (42.1 ± 3.1 and 56.7 ± 4.0

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Table 1. Glucose and hormone levels and liver weight in overnight-fasted female nonpregnant and pregnant dogs

<table>
<thead>
<tr>
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<th>Nonpregnant</th>
<th>Pregnant</th>
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<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>5.9 ± 0.1</td>
<td>5.6 ± 0.1</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>11 ± 1</td>
<td>4 ± 1*</td>
</tr>
<tr>
<td>C-peptide, pmol/l</td>
<td>0.17 ± 0.02</td>
<td>0.11 ± 0.02*</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>39 ± 3</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>Cortisol, µg/dl</td>
<td>2.2 ± 0.4</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>109 ± 19</td>
<td>81 ± 15</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>182 ± 19</td>
<td>269 ± 24*</td>
</tr>
<tr>
<td>Estrogen, pg/ml</td>
<td>11.4 ± 0.9</td>
<td>18.9 ± 2.1*</td>
</tr>
<tr>
<td>Progesterone, ng/ml</td>
<td>0.1 ± 0.0</td>
<td>6.3 ± 0.7*</td>
</tr>
<tr>
<td>Prolactin, ng/ml</td>
<td>1.1 ± 0.2</td>
<td>8.6 ± 1.3*</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>518 ± 14</td>
<td>649 ± 26*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 16 conscious, chronically catheterized dogs in each group. *P < 0.05 vs. nonpregnant.

μmol·100 g liver⁻¹·min⁻¹ in NP and P, respectively, P < 0.05; Fig. 1), consistent with the increase in tracer-determined glucose production (54.7 ± 2.3 and 80.8 ± 3.9 μmol·100 g liver⁻¹·min⁻¹ in NP and P, respectively, P < 0.05; Table 2). Tracer-determined glucose utilization and clearance rates were also elevated in the pregnant dogs.

Table 2. Tracer-determined rates of glucose production, utilization, and clearance in overnight-fasted female nonpregnant and pregnant dogs

<table>
<thead>
<tr>
<th></th>
<th>Nonpregnant</th>
<th>Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA, μmol·100 g liver⁻¹·min⁻¹</td>
<td>54.7 ± 2.3</td>
<td>80.8 ± 3.9†</td>
</tr>
<tr>
<td>RA, μmol·kg⁻¹·min⁻¹</td>
<td>13.4 ± 0.5</td>
<td>21.1 ± 1.0†</td>
</tr>
<tr>
<td>RD, μmol·kg⁻¹·min⁻¹</td>
<td>13.5 ± 0.5</td>
<td>21.7 ± 1.0†</td>
</tr>
<tr>
<td>Cl, ml·kg⁻¹·min⁻¹</td>
<td>2.31 ± 0.08</td>
<td>3.88 ± 0.15†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 16 conscious, chronically catheterized dogs in each group. RA, glucose production; RD, glucose utilization; Cl, glucose clearance. †Per kg of maternal body weight. *P < 0.05 vs. nonpregnant.

Glucose metabolism in pregnancy. The arterial blood lactate level was lower in the pregnant than in the nonpregnant dogs (643 ± 50 vs. 494 ± 35 μmol/l, P < 0.05; Fig. 2). Net hepatic lactate output was 1.70 ± 0.67 μmol·100 g liver⁻¹·min⁻¹ in the nonpregnant group. In contrast, the liver was a net consumer of lactate after an overnight fast in the pregnant group (21.38 ± 3.59 μmol·100 g liver⁻¹·min⁻¹, P < 0.05 vs. NP).

The arterial blood alanine level was lower in the pregnant than in the nonpregnant dogs (341 ± 21 vs. 194 ± 24 μmol/l, P < 0.05; Table 3). Hepatic fractional...
Table 3. Arterial blood levels, net hepatic balances, and fractional extractions of amino acids in overnight-fasted female nonpregnant and pregnant dogs

<table>
<thead>
<tr>
<th></th>
<th>Nonpregnant</th>
<th>Pregnant</th>
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</thead>
<tbody>
<tr>
<td>Alanine μmol/l</td>
<td>341 ± 21</td>
<td>194 ± 24*</td>
</tr>
<tr>
<td>NHB, μmol·100 g liver⁻¹·min⁻¹</td>
<td>-9.73 ± 0.84</td>
<td>-5.41 ± 0.53*</td>
</tr>
<tr>
<td>FE</td>
<td>0.26 ± 0.02</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>Serine μmol/l</td>
<td>155 ± 9</td>
<td>125 ± 6*</td>
</tr>
<tr>
<td>NHB, μmol·100 g liver⁻¹·min⁻¹</td>
<td>-5.33 ± 0.40</td>
<td>-1.62 ± 0.25*</td>
</tr>
<tr>
<td>FE</td>
<td>0.21 ± 0.02</td>
<td>0.15 ± 0.04</td>
</tr>
<tr>
<td>Threonine μmol/l</td>
<td>236 ± 15</td>
<td>163 ± 15*</td>
</tr>
<tr>
<td>NHB, μmol·100 g liver⁻¹·min⁻¹</td>
<td>-0.88 ± 0.53</td>
<td>-0.17 ± 0.27</td>
</tr>
<tr>
<td>FE</td>
<td>0.04 ± 0.03</td>
<td>0.02 ± 0.03</td>
</tr>
<tr>
<td>Glycine μmol/l</td>
<td>241 ± 16</td>
<td>229 ± 11</td>
</tr>
<tr>
<td>NHB, μmol·100 g liver⁻¹·min⁻¹</td>
<td>-5.34 ± 0.60</td>
<td>-3.20 ± 0.46*</td>
</tr>
<tr>
<td>FE</td>
<td>0.20 ± 0.02</td>
<td>0.16 ± 0.04</td>
</tr>
<tr>
<td>Glutamine μmol/l</td>
<td>928 ± 41</td>
<td>764 ± 23*</td>
</tr>
<tr>
<td>NHB, μmol·100 g liver⁻¹·min⁻¹</td>
<td>0.09 ± 1.19</td>
<td>6.46 ± 1.26*</td>
</tr>
<tr>
<td>Glutamate μmol/l</td>
<td>78 ± 13</td>
<td>84 ± 8</td>
</tr>
<tr>
<td>NHB, μmol·100 g liver⁻¹·min⁻¹</td>
<td>-0.72 ± 0.40</td>
<td>-0.42 ± 0.23</td>
</tr>
<tr>
<td>FE</td>
<td>0.00 ± 0.08</td>
<td>0.05 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 16 conscious, chronically catheterized dogs in each group. A positive value for net hepatic balance (NHB) indicates net output of the substrate by the liver; a negative value indicates net uptake. FE, fractional extraction. *P < 0.05 vs. nonpregnant.

The increment in NHGO was thus due to a greater contribution of glucose from glycogenolysis (21.5 ± 2.5 and 35.3 ± 3.8 μmol·100 g liver⁻¹·min⁻¹ in NP and P, respectively, P < 0.05). The overall rate of glycogenolysis was not significantly different between the two groups (36.4 ± 3.8 and 42.4 ± 4.0 μmol·100 g liver⁻¹·min⁻¹ in NP and P, respectively, P = 0.09). Thus a greater fraction of the glucose released from glycogen went to hepatic glucose production in the pregnant group (59 vs. 83%).

The fraction of [14C]alanine and [14C]lactate taken up by the liver and converted to [14C]glucose (see methods for a detailed description of this value) was greater in the pregnant than in the nonpregnant group (0.34 ± 0.05 and 0.77 ± 0.06, P < 0.05).

NEFA levels and net hepatic uptake. Arterial plasma NEFA levels (967 ± 68 and 1,094 ± 87 μmol/l in NP and P, respectively), hepatic NEFA fractional extraction (0.17 ± 0.02 and 0.21 ± 0.02 in NP and P, respectively), and net hepatic NEFA uptake (11.23 ± 1.56 and 14.23 ± 1.34 μmol·100 g liver⁻¹·min⁻¹ in NP and P, respectively) were similar in both groups (Fig. 3).

Glutamate was the only amino acid measured that did not exhibit a difference between the pregnant and nonpregnant group (Table 3). Arterial blood serine and threonine levels were lower in the pregnant group. There was a correspondingly lower rate of net hepatic uptake of serine, but threonine uptake by the liver was not different between the groups. The arterial blood glutamine level was also lower in the pregnant dogs, but net hepatic glutamine output was markedly elevated compared with the nonpregnant group. The arterial blood glycine level remained unchanged during pregnancy, despite a reduction in net hepatic glycine uptake.

Gluconeogenic parameters. The rate (see methods) of gluconeogenesis was not altered by pregnancy (20.6 ± 2.8 and 21.2 ± 1.8 μmol·100 g liver⁻¹·min⁻¹ in NP and P, respectively; Fig. 4). The increment in NHGO was thus due to a greater contribution of glucose from
Ketone body levels and net hepatic output. Arterial blood acetoacetate (76 ± 9 and 114 ± 9 μmol/l in NP and P, respectively) and β-hydroxybutyrate levels (23 ± 4 and 120 ± 32 μmol/l in NP and P, respectively) were significantly elevated in the pregnant dogs (Fig. 6). Likewise, the rates of net hepatic output of acetoacetate (2.96 ± 0.51 and 6.84 ± 0.97 μmol·100 g liver⁻¹·min⁻¹ in NP and P, respectively) and β-hydroxybutyrate (3.97 ± 0.70 and 11.26 ± 2.63 μmol·100 g liver⁻¹·min⁻¹ in NP and P, respectively) were increased during pregnancy.

DISCUSSION

It is well known that pregnancy is accompanied by significant alterations in glucose metabolism (2, 6, 14, 24, 29, 32, 35, 36, 40, 62, 63), yet the control mechanisms regulating these changes have not been well defined. Assessment of metabolism is limited in pregnant women by the invasive nature of the required methodology and in animal models by a variety of considerations, as discussed in the introduction. Our goal was to characterize a new animal model of pregnancy by thoroughly assessing hepatic glucose (gluconeogenesis and glycogenolysis), fat, and amino acid metabolism in the chronically catheterized, conscious, overnight-fasted (18-h) dog during late gestation.

The glucose level was not significantly decreased in the pregnant dogs, despite a 61% increase in tracer-determined glucose utilization and production have also been documented in other species (6, 12, 36, 62). The increase in net hepatic glucose output confirmed that an increase in glucose release from the liver (as opposed to the kidneys) was the primary source of the increment in glucose production.

The increment in NHGO could not be explained by a change in the rate of gluconeogenesis from circulating precursors. Nevertheless, pregnancy altered the profile of circulating gluconeogenic precursor availability. Most notably, when taken as a group average, there was net lactate release from the liver in the nonpregnant dogs but net lactate uptake in the pregnant group. In the subset of nonpregnant dogs that took up lactate, the rate of uptake was one-half that in the pregnant group (10.5 ± 4.0 vs. 22.8 ± 2.5 μmol·100 g liver⁻¹·min⁻¹). If net hepatic lactate uptake occurred at any time point, in pregnant or nonpregnant dogs, it was included in the gluconeogenic calculation (see METHODS for a detailed description of the technique).
There were no apparent differences in glycerol metabolism (5.6 ± 0.7 and 5.3 ± 0.5 μmol·100 g liver⁻¹·min⁻¹ in NP and P, respectively). Overall, the increase in hepatic lactate uptake equaled the decrease in gluconeogenic amino acid delivery to the liver in the pregnant group; therefore, if it is assumed that all precursors were converted to glucose, the rate of gluconeogenesis from circulating substrates was equivalent in the pregnant and nonpregnant groups. It is clear that gluconeogenic precursor availability did not limit gluconeogenesis, and thus the rise in hepatic glucose release in the pregnant dogs was a function of a liver event.

Methodological limitations and differences in experimental conditions probably explain the slight differences in conclusions made from our data and data of others regarding the rate of gluconeogenesis in the basal state in pregnancy. Administration of [¹³C]alanine to pregnant women resulted in less label incorporation in glucose in a study by Kalhan et al. (36), suggesting that gluconeogenic efficiency was decreased. More recently, however, Kalhan et al. (35) used the deuterated water method to assess gluconeogenesis and reported that the fractional contribution of gluconeogenesis to glucose production was unchanged in pregnancy. Since hepatic glucose production was modestly increased, the actual rate of gluconeogenesis was thus greater. This group explains the different results from the two studies as a function of limitations of the precursors used. However, in the more recent work (35) the subjects were studied after a slightly longer than usual, but metabolically important (49), length of fast, which resulted in mild hypoglycemia in the pregnant women. This may have evoked a modest counterregulatory response that could have contributed to the increase in gluconeogenesis (glucagon levels were not reported). Despite the minor differences in the conclusions of that study and the present study, it appears that, in general, gluconeogenesis is not dramatically altered by pregnancy in the basal, overnight-fasted state in the human or the dog.

The caveats of assessing gluconeogenesis using labeled gluconeogenic precursors have been discussed previously (35, 72). This approach cannot provide a quantitative measure of the gluconeogenic rate, and the assumptions that must be made regarding dilution of labeled precursor in intrahepatic pools limit the utility of methods (10) of estimating gluconeogenic efficiency. Nevertheless, the value of calculating the fraction of labeled precursor that was consumed by the liver and incorporated into glucose lies in the difference between groups. This parameter was markedly increased in the pregnant group (0.77 vs. 0.34), and yet the arteriovenous difference method indicated that there was no difference in the gluconeogenic rates in the pregnant and nonpregnant groups. The question thus arises as to how the liver could appear to be more gluconeogenic but not demonstrate an increase in glucose production from gluconeogenesis. The increased fraction of label in glucose suggests that there was an intrahepatic change in some aspect of the gluconeogenic process.

Circulating levels of four of the six gluconeogenic amino acids (serine, threonine, glutamine, and alanine) were lower in the pregnant group, while the rates of net hepatic uptake of serine, glycine, and alanine were significantly reduced. In contrast, glutamine output by the liver was markedly enhanced by pregnancy. The possibility that another gluconeogenic amino acid taken up by the liver contributed the carbon to glutamine synthesis, rather than gluconeogenesis, was not considered in the gluconeogenic calculation. If this were the case, the impact on the gluconeogenic calculation would be to reduce the rate by only −3 μmol·100 g liver⁻¹·min⁻¹, an amount insufficient to alter the conclusions drawn. Alternatively, the carbon for glutamine synthesis may have been derived from the breakdown of glycogen (50, 73). In either case, the net rate of glycogen breakdown would thus be greater than calculated. It is also possible that the hepatic output of glutamine was related to disposition of non gluconeogenic amino acids within the liver. In a net sense, then, liver consumption of gluconeogenic amino acid precursors was diminished by −50% in the pregnant dogs (20.4 ± 2.1 vs. 11.0 ± 1.2 μmol·100 g liver⁻¹·min⁻¹). Since steady state existed, this indicated that the supply of amino acids reaching the liver was reduced.
genic/glycolytic pathways in the pregnant dog. This possibility was, in fact, supported by the switch to lactate uptake in the pregnant dogs. Thus, although the load of amino acids delivered to the liver diminished in the pregnant group, this was offset by an intrahepatic change that pulled lactate into the liver, with a net result of no difference in total gluconeogenic precursor load to the liver in the two groups. It therefore appears that basal intrahepatic mechanisms in pregnancy could be geared to shunt precursors to glucosone synthesis, but the gluconeogenic rate is ultimately limited by the precursor load to the liver.

Given that gluconeogenesis from circulating precursors was unchanged in the pregnant dogs, the increment in glucose output must have resulted from an increase in the contribution of glycogenolytically derived glucose. Interestingly, this was not associated with a significant increase in the overall rate of glycogen breakdown. An increase in the rate would not have been unexpected given the lower insulin levels, since acute, complete insulin withdrawal in nonpregnant dogs causes glucose production to double, primarily because of increased glycogenolysis (7). The data indicated that the primary effect of pregnancy on glycogen metabolism in the basal state was to route the glucose released from glycogen through different pathways. In the pregnant group, the glucose left the cell as glucose, possibly as a result of the effect of lower insulin levels on glucose-6-phosphatase activity (42). Lactate was not released from the liver in the pregnant dogs, indicating that glucose did not flux through the glycolytic pathway in a net sense. In contrast, in the nonpregnant dogs a portion of the glucose released from glycogen not only left the cell as glucose but was also channeled into the glycolytic pathway for lactate production, as evidenced by the net hepatic lactate balance data. Thus the increment in hepatic glucose production during pregnancy in the dog is glycogenolytic in origin, but this occurs due to a change in postglycogenolytic partitioning of glucose, rather than a change in the net rate of glycogen breakdown per se. The mechanism for this is not known. Glucagon, cortisol, and epinephrine levels were unaffected by pregnancy, and norepinephrine was only slightly elevated, so these hormones were unlikely to affect liver glucose metabolism. Conceivably, the action of pregnancy-associated hormones or impaired hepatic insulin action could impact on hepatic glucose metabolism, but these possibilities await further study.

We do not consider it likely that an increase in the supply of gluconeogenic precursor within the liver itself contributed to the increment in hepatic glucose production. Recent work comparing the methodologies of Giaccheri and Rossetti (23) and Goldstein et al. (25) to assess gluconeogenesis indicated that intrahepatic gluconeogenic precursors provide only a minor contribution (<5%) to glucose release by the liver in dogs in the basal state (25; R. Goldstein, personal communication). Prolonged fasting did not affect the process, and the data indicated the possibility of only a modest stimulation in response to chronic cortisol administration. However, cortisol is not elevated in the pregnant dog, and there is no evidence that the sex steroids of pregnancy have an intrahepatic proteolytic effect. Thus we must assume that a change in intrahepatic gluconeogenic precursor metabolism does not contribute to the accelerated glucose release from the liver of the pregnant dog.

The concept of human pregnancy as a state of "accelerated starvation" (22), in which basal glucose levels are somewhat lower and ketone levels somewhat higher, appears to apply to pregnancy in the dog as well. This, in fact, probably explains the lower insulin levels in the pregnant group. Metzger et al. (49) showed that as fasting proceeds beyond the overnight-fasted state (for another 6 h), glucose levels fall in pregnant women, presumably due to the unremitting glucose needs of the fetus. Insulin levels fall as well, whereas glucose and insulin levels remain stable in nonpregnant women (49). While blood glucose was only slightly lower in the group of pregnant dogs, recent studies in dogs have shown that the β-cell is so sensitive to decrements in glucose that a fall in blood glucose of only 0.4 mmol/l can result in a 50% reduction in circulating insulin levels (21). Thus the lower insulin levels in the pregnant dogs suggest that the pregnant dog may be slightly further along in the switch to a fasting state than pregnant women, who generally have normal (4, 36, 48, 49) or elevated (6, 13, 14) insulin levels after an overnight fast. Although the glucose level was not reduced to as great an extent by pregnancy in the dog as it is in women, the dog adapts more quickly to a fasting state and does not experience a marked fall in glucose after a fast as long as 7 days (30). This ability to match the glucose production rate to the glucose utilization rate during fasting does not appear to be maintained in pregnant dogs, since in two pregnant dogs fasted for 42 h glucose dropped ~20 mg/dl. C-peptide levels were decreased by 35%, while insulin levels were decreased by a greater extent (64%) in the pregnant group, suggesting that pregnancy also may have caused an increase in insulin clearance, possibly due to placental degradation of the hormone (57).

The lipolytic parameters (glycerol and NEFA) were relatively unaffected by pregnancy, despite the lower insulin, although we could not assess whether there were changes within the adipocyte that were masked by offsetting changes in peripheral fat utilization. Acute insulin deficiency in nonpregnant dogs results in elevation of glycerol and NEFA levels (17; D. Edgerton, personal communication), despite inhibitory effects of the ensuing hyperglycemia on lipolysis (65). We cannot explain the failure of glycerol and NEFA to rise in response to the lower insulin in the pregnant group. It is interesting, however, that the most dramatic alteration in fat metabolism in pregnant women is elevation of circulating triglyceride levels (33, 55); in comparison, NEFA and glycerol levels are more moderately increased (3, 45, 49, 55). Triglyceride levels were measured in two pregnant and two nonpregnant dogs, and the data indicated that circulating triglycerides are...
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Elevated during gestation in the dog (70 vs. 41 mg/dl) as well.

Despite the lack of effect on hepatic NEFA uptake, ketogenesis was markedly elevated in the pregnant group. Acetoacetate and β-hydroxybutyrate levels rose due to a two- to threefold increase in net hepatic ketone production. It is not clear if the lower circulating insulin levels could have accelerated this process. Acute insulin deficiency (3 h) in the nonpregnant dog is insufficient to alter β-hydroxybutyrate production by the liver (26). In pregnant women, ketone levels are basal or elevated, despite the increased circulating insulin, suggesting that another factor must stimulate ketogenesis as well. Progesterone has been implicated in this process (37).

In summary, in the basal state, hepatic glucose production and glucose utilization are increased in late pregnancy in the dog. Interestingly, the increase in hepatic glucose release is not associated with a change in gluconeogenic flux or net glycogenolysis in the pregnant dog. Instead, there is a change in the partitioning of glucose once it is released from glycogen, such that the increment in hepatic glucose production is due to an increase in the contribution of glycogenolytically derived glucose, rather than a change in the contribution of gluconeogenesis. Further study is required to assess the mechanisms for these hepatic adaptations. Circulating basal gluconeogenic amino acid levels are reduced in the pregnant dog. Overnight-fasted ketone levels are lower in the overnight-fasted pregnant dog, while hepatic NEFA uptake is unchanged. Insulin levels are elevated by pregnancy, even though net gluconeogenic glucose production. It is not clear if the lower circulating insulin, insulin deficiency (3 h) in the nonpregnant dog is insufficient to stimulate ketogenesis in the hepatic glycogen repletion of diabetic rats. J Clin Invest 89: 26–45, 1992.


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