Pregnancy impairs the counterregulatory response to insulin-induced hypoglycemia in the dog

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Connolly, Cynthia C., Lisa N. Aglione, Marta S. Smith, D. Brooks Lacy, and Mary Courtney Moore. Pregnancy impairs the counterregulatory response to insulin-induced hypoglycemia in the dog. Am J Physiol Endocrinol Metab 287: E480–E488, 2004. First published May 4, 2004; 10.1152/ajpendo.00529.2003.—The impact of pregnancy on the counterregulatory response to insulin-induced hypoglycemia was examined in six nonpregnant (NP) and six pregnant (P; 3rd trimester) conscious dogs by tracer and arteriovenous difference techniques. After basal sampling, insulin was infused intraportally at 30 pmol·kg⁻¹·min⁻¹ for 180 min. Insulin rose from 70 ± 15 to 1,586 ± 521 pmol/l and 27 ± 4 to 1,247 ± 61 pmol/l in the 3rd h in NP and P, respectively. Arterial glucose fell from 5.9 ± 0.2 to 2.3 ± 0.2 mmol/l in P. Glucose was infused in NP to equate the rate of fall of glucose and the steady-state concentrations in the groups (5.9 ± 0.2 to 2.3 ± 0.1 mmol/l in NP). Glucagon was 32 ± 6, 69 ± 11, and 48 ± 10 ng/l (basal and 1st and 3rd h) in NP, but the response was attenuated in P (34 ± 5, 46 ± 6, 41 ± 9 ng/l). Cortisol and epinephrine rose similarly in both groups, but norepinephrine rose more in NP (Δ3.01 ± 0.46 and Δ1.31 ± 0.13 nmol/l, P < 0.05). Net hepatic glucose output (NHGO: μmol·kg⁻¹·min⁻¹) increased from 10.6 ± 1.8 to 21.2 ± 3.3 in NP (3rd h) but did not increase in P (15.1 ± 1.5 to 15.3 ± 2.8 μmol·kg⁻¹·min⁻¹, P < 0.05 between groups). The glycogenolytic contribution to NHGO in NP increased from 5.8 ± 0.7 to 10.4 ± 2.5 μmol·kg⁻¹·min⁻¹ by 90 min but steadily declined in P. The increase in glycerol levels and the gluconeogenic contribution to NHGO were 50% less in P than in NP, but ketogenesis did not differ. The glucagon and norepinephrine responses to insulin-induced hypoglycemia are blunted in late pregnancy in the dog, impacting on the magnitude of the metabolic responses to the fall in glucose.

gluconeogenesis; glycogenolysis; ketogenesis; hepatic glucose production

INTENSIVE INSULIN THERAPY is recommended for pregnant women with type 1 diabetes (T1DM) to reduce the perinatal and obstetric complications that are associated with poorly controlled maternal diabetes (24, 36, 39, 40). This group of women is susceptible to more frequent and more severe hypoglycemic episodes than nonpregnant women receiving intensive insulin treatment (14, 38, 41). It is not clear why this occurs. The stringency with which blood glucose is controlled in pregnant women with diabetes may contribute to the problem. A reduced counterregulatory hormone response and a lowering of the glucose threshold(s) for its release are associated with intensive insulin treatment, compared with the treatment of patients with less strictly controlled glucose levels (1, 46). Recurrent episodes of hypoglycemia may also contribute to a reduced counterregulatory response (15). However, a few studies have indicated that a reduction in the counterregulatory hormone response may be intrinsic to pregnancy itself (18, 42, 43).

The role of the liver in the counterregulatory response to insulin-induced hypoglycemia in pregnancy remains incompletely understood. Investigation of hepatic glucose metabolism during pregnancy in the human is limited by the invasiveness of the techniques required to assess hepatic substrate metabolism thoroughly and the need to protect the fetus from experimental conditions that might cause it harm. Therefore, during insulin-induced hypoglycemia in a canine model of pregnancy (11), we examined the counterregulatory responses, including hormone and substrate release and hepatic gluconeogenesis and glycogenolysis.

METHODS

Animals and surgical procedures. Experiments were performed on 12 overnight-fasted (18 h), conscious, female mongrel dogs (21.1 ± 0.5 and 24.8 ± 0.8 kg in nonpregnant and pregnant, respectively), with diet and housing as previously described (11). The protocol was approved by the Vanderbilt University Animal Care and Use Committee. Six of the dogs were 7–8 wk pregnant (term = 9 wk) when studied. The other six dogs were not pregnant and had basal progesterone and estrogen levels throughout the time they were housed and studied.

Approximately 16 days before the experiment, splenic and jejunal vein infusion catheters were surgically inserted for intraportal infusions, and sampling catheters were inserted in the femoral artery, hepatic portal vein, and hepatic vein. Criteria for study were as previously described (11). On the morning of an experiment, the catheter ends were removed from the subcutaneous pockets of dogs under local anesthesia, the contents of the catheters were aspirated, and the catheters were flushed with saline. The dog was placed in a Pavlov harness. Intravenous access was established in the left and right cephalic veins.

Experimental design. Each experiment consisted of a 120-min tracer and dye equilibration period (−150 to −30 min), a 30-min basal sampling period (−30 to 0 min), and a 180-min experimental period (0 to 180 min). A primed (83.3 μCi), constant infusion (0.70 μCi/min) of [3-3H]glucose (New England Nuclear, Boston, MA) was begun at −150 min and continued throughout the experiment. Indocyanine green dye infusion (0.08 mg/min; Sigma, St. Louis, MO) began at −120 min and continued throughout the experiment. At 0 min, intraportal infusion of pork insulin (30 pmol·kg⁻¹·min⁻¹; Eli Lilly, Indianapolis, IN) was begun via the splenic and jejunal catheters. A 0.2-ml aliquot of blood was drawn every 5 min for measurement of plasma glucose. The glucose level was allowed to fall in the pregnant dogs. In the nonpregnant group, exogenous glucose was...
infused as needed via a saphenous vein, initially to maintain a similar rate of fall and then to keep the steady-state glucose level equivalent to that in the pregnant group (Table 1). Blood samples were collected from the artery, portal vein, and hepatic vein, as previously described (11).

At the end of the experimental period, the dogs were anesthetized and then euthanized 5 min later with an overdose of pentobarbital sodium. On necropsy, the positions of the catheter tips were verified to ensure proper placement.

Analytical procedures. Analyses have been described in detail previously (11) and included plasma concentrations of glucose, insulin, glucagon, cortisol, estrogen (data not shown), progesterone, prolactin (data not shown), epinephrine, norepinephrine, nonesterified fatty acids (NEFA); plasma $[^{3}H]glucose$ content; and blood concentrations of lactate, alanine, glycerol, β-hydroxybutyrate, acetoacetate, glutamine, glutamate, serine, glycine, and threonine.

Calculations. Total hepatic blood flow was assessed by measuring hepatic extraction of indocyanine green (11). The proportions of the hepatic blood supply provided by the hepatic artery and portal vein were assumed to be 20 and 80%, respectively. This distribution has since been confirmed for both the pregnant and nonpregnant dogs with ultrasonic flow probes (11).

Net hepatic substrate balance was calculated using the formula: $[H - (0.2 \times A + 0.8 \times P)] \times HF$, where $A$, $P$, and $H$ are the arterial, portal vein, and hepatic vein substrate concentrations, respectively, and $HF$ is the hepatic blood or plasma flow, as appropriate for the particular substrate. Whole blood glucose values were assumed to equal 73% of plasma values (11). Net 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, by use of the formula: $[(0.2 \times A + 0.8 \times P) - H] / (0.2 \times A + 0.8 \times P)$.

The rates of glucose appearance ($R_{a}$) and disappearance ($R_{d}$) were calculated by a two-compartment model with dog parameters (20, 35). Endogenous $R_{d}$ was the difference between total $R_{a}$ and the glucose infusion rate. The gluconeogenic rate from circulating precursors was estimated by using the arteriovenous difference technique and assuming that all gluconeogenic precursors taken up by the liver in a net sense represent the gluconeogenic flux ($GNG_{flux}$) within the liver. Briefly, net hepatic balances of the gluconeogenic precursors alanine, serine, threonine, glycine, glutamine, glutamate, lactate, and glycerol were summed. Net hepatic balance of pyruvate was assumed to be 10% of net hepatic lactate balance (48) and was included in the calculation. When any precursor that the liver can either consume or release in a net sense (such as lactate) exhibited net hepatic output, the calculation. When any precursor that the liver can either consume or release in a net sense (such as lactate) exhibited net hepatic output, the

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**Table 1. Exogenous glucose infusion rates in nonpregnant female dogs during hypoglycemia induced by continuous intraportal insulin infusion**

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
<th>105</th>
<th>120</th>
<th>135</th>
<th>150</th>
<th>165</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0</td>
<td>5.5</td>
<td>17.8</td>
<td>22.8</td>
<td>13.7</td>
<td>9.3</td>
<td>8.0</td>
<td>7.4</td>
<td>6.6</td>
<td>7.5</td>
<td>7.2</td>
</tr>
<tr>
<td>2</td>
<td>0.0</td>
<td>7.3</td>
<td>9.4</td>
<td>2.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>2.3</td>
<td>7.9</td>
<td>9.4</td>
<td>2.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>3.3</td>
<td>23.9</td>
<td>22.9</td>
<td>16.9</td>
<td>8.0</td>
<td>3.9</td>
<td>0.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>6.0</td>
<td>19.1</td>
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<td>6.4</td>
<td>1.1</td>
<td>0.0</td>
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<td>0.0</td>
<td>0.0</td>
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</tr>
<tr>
<td>6</td>
<td>3.5</td>
<td>3.7</td>
<td>3.8</td>
<td>2.9</td>
<td>1.6</td>
<td>0.2</td>
<td>0.0</td>
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<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Mean ± SE 2.5 ± 1.0 11.2 ± 3.7 12.0 ± 3.2 10.3 ± 3.8 5.2 ± 2.3 2.4 ± 1.7 1.4 ± 1.4 1.3 ± 1.3 1.8 ± 1.3 2.4 ± 1.7 2.7 ± 1.9

Values are in μmol·kg⁻¹·min⁻¹. Exogenous glucose was not infused at any time in pregnant dogs.
Arterial plasma progesterone concentrations were 0.1 ± 0.0 and 6.0 ± 1.1 ng/ml in the NP and P groups, respectively (P < 0.0001).

Glucose concentrations and hepatic blood flow. The plasma glucose level fell from 5.9 ± 0.2 to 2.3 ± 0.2 mmol/l in P. Infusion of exogenous glucose (Table 1) was required to maintain a similar rate of fall in NP (from 5.9 ± 0.2 to 2.3 ± 0.1 mmol/l).

Hepatic blood flow increased from 25.6 ± 2.9 to 34.6 ± 3.8 ml·kg⁻¹·min⁻¹ in the 3rd h in NP and from 23.4 ± 1.9 to 30.2 ± 2.3 ml·kg⁻¹·min⁻¹ in P [not significant (NS) between groups].

Glucose kinetics. In NP, NHGO initially fell when insulin infusion was begun (from 10.6 ± 1.5 μmol·kg⁻¹·min⁻¹ in the basal period to 7.8 ± 2.0 μmol·kg⁻¹·min⁻¹ at 15 min), but it rose above basal by 1 h (19.4 ± 2.5 μmol·kg⁻¹·min⁻¹; Fig. 1). It remained elevated for the remainder of the experiment (3rd h: 21.2 ± 3.3 μmol·kg⁻¹·min⁻¹). NHGO in P followed a similar pattern, falling initially (from 15.5 ± 1.6 to 9.3 ± 2.2 μmol·kg⁻¹·min⁻¹ at 15 min of insulin infusion) and then rising, but the rise was more sluggish than in NP, with NHGO averaging only 15.3 ± 2.8 μmol·kg⁻¹·min⁻¹ in the 3rd h (P < 0.05 vs. NP). Overall, the AUC of change in NHGO from basal was significantly greater in NP than in P (1.4 ± 0.2 vs. –0.3 ± 0.3 μmol·kg⁻¹·min⁻¹, respectively, P < 0.05).

The changes in tracer-determined glucose production (Rₚ) were similar to the changes in NHGO in both groups (Fig. 2). Rₚ rose from 12.4 ± 0.5 to 22.0 ± 1.2 μmol·kg⁻¹·min⁻¹ in the 3rd h in NP. In P, Rₚ fell from 22.8 ± 2.5 to 17.4 ± 1.6 μmol·kg⁻¹·min⁻¹ at 30 min but then increased to 23.8 ± 1.0 μmol·kg⁻¹·min⁻¹ in the 3rd h (P < 0.05 for group × time interaction). Tracer-determined glucose utilization (Rₜ) rose from 12.4 ± 0.4 to 24.4 ± 1.1 μmol·kg⁻¹·min⁻¹ in the 3rd h in NP. In P, Rₜ rose slightly from 23.1 ± 2.3 to 28.1 ± 2.4 μmol·kg⁻¹·min⁻¹ at 30 min but then fell to 24.1 ± 1.0 μmol·kg⁻¹·min⁻¹ in the 3rd h (P < 0.05 for group × time interaction). Tracer-determined glucose clearance increased from 2.1 ± 0.1 to 10.7 ± 0.7 and from 3.9 ± 0.3 to 10.7 ± 1.0 μmol·kg⁻¹·min⁻¹ in the 3rd h in NP and P, respectively (P < 0.05 for group × time interaction).

Arterial plasma counterregulatory hormone levels. Arterial plasma glucagon increased from 32 ± 6 to 69 ± 11 ng/l within 1 h of insulin infusion (P < 0.05) in the NP animals, falling to 48 ± 10 ng/l in the 3rd h (Fig. 3). In contrast, the glucagon level did not increase significantly in the P group (basal, 1-h, and 3-h levels of 34 ± 5, 46 ± 6, and 41 ± 9 ng/l, respectively). Arterial plasma epinephrine rose from 0.49 ± 0.30 to 24.4 ± 2.4 ng/l in the 3rd h in NP and P, respectively (NS between groups). The AUC of

![Fig. 1. Arterial plasma concentrations of insulin and glucose and net hepatic glucose output in nonpregnant and pregnant chronically catheterized, over-night-fasted conscious female dogs (n = 6 in each group) during the basal period (−30 to 0 min) and during hypoglycemia induced by continuous intraportal insulin infusion (0−180 min). Insets: areas under the curve (AUC) of change from basal, shown only when significant differences or strong tendencies toward differences exist. Data are means ± SE. *P < 0.05 between groups.](http://ajpendo.physiology.org/)

![Fig. 2. Tracer-determined endogenous glucose production (EndoRₚ), glucose utilization (Rₚ), and clearance. Insets: AUC of change from basal. Data are means ± SE. *P < 0.05 between groups.](http://ajpendo.physiology.org/)
the epinephrine response in P was ~82% of that in NP (P = 0.6). Arterial plasma norepinephrine increased from 1.20 ± 0.22 to 4.21 ± 0.61 nmol/l in NP (3rd h), but this increase was blunted in P (1.78 ± 0.24 to 3.10 ± 0.31 nmol/l). The AUC of change in norepinephrine from basal concentrations was 2.2-fold greater in NP than in P (P < 0.05). Arterial plasma cortisol rose similarly in both groups (from 50 ± 9 to 247 ± 36 and from 69 ± 13 to 225 ± 30 nmol/l in the 3rd h in NP and P, respectively; NS between groups).

Gluconeogenic precursor metabolism. The arterial blood lactate level rose from 541 ± 69 to 1.685 ± 339 μmol/l in the 3rd h in NP, with a sharp rise between 60 and 90 min after the start of insulin infusion (Fig. 4). In contrast, arterial blood lactate rose slowly in P (from 507 ± 82 to 941 ± 139 μmol/l). Net hepatic lactate uptake increased from 1.55 ± 2.64 to 15.49 ± 4.07 μmol·kg⁻¹·min⁻¹ in NP and from 4.04 ± 2.48 to 15.50 ± 2.30 μmol·kg⁻¹·min⁻¹ in P.

The arterial blood alanine level was lower (P < 0.05) in P than in NP during both the basal and the hypoglycemic periods, but it decreased similarly with insulin infusion in both groups (Table 2). Net hepatic alanine uptake was lower (P < 0.05) in P than in NP at all times, but net hepatic fractional extraction (FE) of alanine was the same in the two groups, increasing similarly (compared with basal) during the hypoglycemic period.

![Fig. 3. Arterial plasma concentrations of counterregulatory hormones glucose, epinephrine, norepinephrine, and cortisol. Insets: AUC of change from basal for hormones when significant differences existed between groups. Data are means ± SE. *P < 0.05 between groups.](image)

**Fig. 4.** Arterial blood lactate levels and net hepatic lactate balance. Inset: AUC of change from basal when these values were significantly different. Data are means ± SE. *P < 0.05 between groups.

**Table 2.** Gluconeogenic amino acid data: arterial blood concentrations, net hepatic uptakes, and net hepatic fractional extraction

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Nonpregnant Basal</th>
<th>Nonpregnant Hypo</th>
<th>Pregnant Basal</th>
<th>Pregnant Hypo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>340 ± 28</td>
<td>209 ± 37</td>
<td>216 ± 45*</td>
<td>114 ± 20*</td>
</tr>
<tr>
<td></td>
<td>2.76 ± 0.30</td>
<td>5.76 ± 0.66</td>
<td>1.61 ± 0.27*</td>
<td>2.26 ± 0.42*</td>
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<tr>
<td></td>
<td>0.27 ± 0.03</td>
<td>0.52 ± 0.05</td>
<td>0.28 ± 0.05</td>
<td>0.49 ± 0.04</td>
</tr>
<tr>
<td>Serine</td>
<td>160 ± 12</td>
<td>105 ± 10</td>
<td>130 ± 9*</td>
<td>82 ± 5*</td>
</tr>
<tr>
<td></td>
<td>1.04 ± 0.09</td>
<td>2.68 ± 0.26</td>
<td>0.40 ± 0.12*</td>
<td>0.70 ± 0.20*</td>
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<tr>
<td></td>
<td>0.22 ± 0.03</td>
<td>0.44 ± 0.04</td>
<td>0.12 ± 0.03*</td>
<td>0.26 ± 0.06*</td>
</tr>
<tr>
<td>Threonine</td>
<td>250 ± 21</td>
<td>166 ± 15</td>
<td>153 ± 18*</td>
<td>94 ± 7*</td>
</tr>
<tr>
<td></td>
<td>0.44 ± 0.19</td>
<td>1.24 ± 0.17</td>
<td>0.03 ± 0.14*</td>
<td>0.08 ± 0.20*</td>
</tr>
<tr>
<td></td>
<td>0.07 ± 0.03</td>
<td>0.16 ± 0.03</td>
<td>0.01 ± 0.04</td>
<td>0.02 ± 0.07*</td>
</tr>
<tr>
<td>Glycine</td>
<td>232 ± 19</td>
<td>122 ± 8</td>
<td>241 ± 5</td>
<td>141 ± 6*</td>
</tr>
<tr>
<td></td>
<td>1.51 ± 0.19</td>
<td>2.93 ± 0.27</td>
<td>0.87 ± 0.24</td>
<td>1.05 ± 0.38*</td>
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<tr>
<td></td>
<td>0.21 ± 0.03</td>
<td>0.42 ± 0.03</td>
<td>0.13 ± 0.03</td>
<td>0.22 ± 0.08*</td>
</tr>
<tr>
<td>Glutamine</td>
<td>891 ± 81</td>
<td>639 ± 52</td>
<td>739 ± 42</td>
<td>494 ± 28*</td>
</tr>
<tr>
<td></td>
<td>−0.40 ± 0.38</td>
<td>1.30 ± 0.79</td>
<td>−1.50 ± 0.84</td>
<td>−1.81 ± 0.48*</td>
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<tr>
<td></td>
<td>0.05 ± 0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>122 ± 26</td>
<td>136 ± 46</td>
<td>96 ± 19</td>
<td>85 ± 13</td>
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<td></td>
<td>0.48 ± 0.17</td>
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<td></td>
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<td>0.07 ± 0.06</td>
<td>0.06 ± 0.02</td>
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</tr>
</tbody>
</table>

Data are means ± SE; n = 6/group. Concentrations (conc) are in μmol/l, uptakes are in μmol·kg⁻¹·min⁻¹, and fractional extractions (FE) have no units. Basal values are means of 2 samples in the basal period (−30 to 0 min), and “hypo” values are means of 2 samples during the last 30 min (150–180 min) of hypoglycemia. *P < 0.05 between groups during the same time period; †a negative value indicates net hepatic output.
hypoglycemia. Alanine increased more in NP than in P during insulin-induced period. However, FE of all gluconeogenic amino acids except glutamine. Excluding glutamine, only for serine was FE of glutamine. Excluding glutamine, only for serine was released a small amount of glutamine in the control period in NP but switched to net glutamine uptake in the experimental period. In contrast, net hepatic output of glutamine was more substantial in P in the control period and did not change in response to insulin-induced hypoglycemia (P < 0.05). In a net sense, the liver released a small amount of glutamine in the control period in NP but switched to net glutamine uptake in the experimental period. In contrast, net hepatic output of glutamine was more substantial in P in the control period and did not change in response to insulin-induced hypoglycemia. Excluding glutamine, net hepatic uptake of the other gluconeogenic amino acids was greater in NP in the control period and increased to a greater extent than it did in the P dogs in response to insulin-induced hypoglycemia (P < 0.05). There was no net FE of glutamine. Excluding glutamine, only for serine was there a significant difference in net hepatic FE in the basal period. However, FE of all gluconeogenic amino acids except alanine increased more in NP than in P during insulin-induced hypoglycemia.

Arterial blood glycerol levels rose from 88 ± 14 to 370 ± 60 μmol/l in NP but did not increase as much in P (from 81 ± 12 to 226 ± 17 μmol/l; P < 0.05 between groups; Fig. 5). Net hepatic glycerol FE was similar in both groups (0.64 ± 0.04 to 0.71 ± 0.02 vs. 0.66 ± 0.05 to 0.72 ± 0.03 in the 3rd h in NP and P, respectively). Consequently, the increase in net hepatic glycerol uptake in response to insulin infusion was greater in NP (1.70 ± 0.27 to 10.63 ± 1.62 μmol·kg⁻¹·min⁻¹ in the 3rd h in NP vs. 1.20 ± 0.12 to 4.73 ± 0.38 μmol·kg⁻¹·min⁻¹ in P; P < 0.05).

Gluconeogenesis and glycogenolysis. The gluconeogenic and net glycogenolytic rates were calculated during the control period and the last 30 min of the experimental period, when all gluconeogenic precursors were measured. The gluconeogenic rate increased from 4.9 ± 1.2 to 20.4 ± 3.4 μmol·kg⁻¹·min⁻¹ in NP. The gluconeogenic rate in the basal period in P (4.8 ± 0.9 μmol·kg⁻¹·min⁻¹) was similar to that in NP, but the increase with hypoglycemia was reduced (to 12.2 ± 1.9 μmol·kg⁻¹·min⁻¹ in the last 30 min of the experimental period). The glycogenolytic contribution to NHGO decreased in both groups, from 5.9 ± 0.6 to 0.8 ± 1.0 μmol·kg⁻¹·min⁻¹ in NP and from 10.8 ± 1.4 (P < 0.05 vs. NP) to 2.3 ± 1.2 μmol·kg⁻¹·min⁻¹ in P.

NEFA metabolism. With insulin infusion, arterial plasma NEFA levels initially fell from a basal value of 988 ± 100 to a nadir of 617 ± 90 μmol/l in NP but then increased to a peak level of 1,700 ± 193 μmol/l at 1 h, thereafter gradually falling to 1,159 ± 158 μmol/l in the 3rd h (Fig. 6). NEFA levels also declined initially during insulin infusion in P (basal of 975 ± 156 to a nadir of 564 ± 83 μmol/l), rising to 1,089 ± 152 at 1 h and 1,064 ± 159 μmol/l in the 3rd h. Hepatic NEFA FE did not differ between the groups (0.16 ± 0.04 to 0.20 ± 0.05 and 0.21 ± 0.05 to 0.22 ± 0.04 in NP and P, respectively). Consequently, the AUC of net hepatic NEFA uptake during hypoglycemia was >2.5-fold greater (P < 0.05) in NP than in P.

Ketone body metabolism. Net hepatic ketone (sum of acetoacetate and β-hydroxybutyrate) output rose from 1.90 ± 0.66 to 3.07 ± 0.76 μmol·kg⁻¹·min⁻¹ in the 3rd h in NP (Fig. 7). Basal net hepatic ketone output tended to be greater in P than in NP (3.49 ± 1.00 μmol·kg⁻¹·min⁻¹, P = 0.2) but increased by a similar extent during the experimental period (to 5.63 ± 1.84 μmol·kg⁻¹·min⁻¹ in the 3rd h). As a consequence, arterial blood ketone levels rose from 92 ± 17 to 112 ± 11 and 186 ± 44 to 216 ± 61 μmol/l in the 3rd h in NP and P.

Fig. 5. Arterial blood glycerol and net hepatic glycerol uptake. Insets: AUC of change from basal. Data are means ± SE. *P < 0.05 between groups.

Fig. 6. Arterial plasma nonesterified fatty acids (NEFA) and net hepatic NEFA uptake. Insets: AUC of change from basal. Data are means ± SE. *P < 0.05 between groups.
respectively. Because of the variability of the responses, there were no significant differences in the ketone concentrations, the rate of hepatic output, or group × time interactions.

**DISCUSSION**

The hypoglycemia-induced increases in two of the major counterregulatory hormones, glucagon and norepinephrine, were attenuated in the pregnant dogs. Blunted glucagon responses to hypoglycemia have also been observed in late pregnancy in normal rats (43) and healthy women (42). The integrated hormonal response that stimulates hepatic glucose production and limits peripheral glucose utilization in response to insulin-induced hypoglycemia is initially led by a rise in glucagon, which rapidly but transiently stimulates glycogenolysis and accounts for the initial increase in hepatic glucose production (19, 21). The nonpregnant group demonstrated this pattern, with net hepatic glucose output increasing by 83% over basal within 60 min of the start of insulin infusion, and peaking after 90 min. The initial response was stimulation of glycogenolysis, but by the 3rd h of hypoglycemia, there was a marked enhancement in the gluconeogenic rate (Fig. 8). In the pregnant dogs, although basal glycogenolysis was greater than in NP, there was blunting of the glycogenolytic response to hypoglycemia. A reduction in glycogen stores is not likely to account for the blunting of glycogenolysis, because pregnant dogs given a mixed meal (liquid formula diet) stored more hepatic glycogen during the 6.5-h postprandial period than nonpregnant dogs (12). From these data, it can be calculated that, during the basal period, the livers in the pregnant dogs contained a mass of glycogen similar to that available in the nonpregnant dogs, even if glycogenolysis proceeded at a higher rate in pregnant than in nonpregnant groups throughout the postabsorptive period. Thus the failure of glucagon to rise appears to be responsible for the loss of the early glycogenic response that characterizes counterregulation in hypoglycemia (19, 21, 25).

The effect of a loss of the glucagon response to hypoglycemia was previously examined in nonpregnant conscious dogs receiving insulin at 5 mU·kg⁻¹·min⁻¹ (19). The rise in glucagon accounted for 62% of the increase in endogenous glucose production and 53% of the increase in NHGO over the 3-h period of hypoglycemia. Thus the poor glucagon response in pregnant dogs in the current study can account for at least one-half the difference between groups in endogenous glucose \( R_g \) and NHGO. The differential \( \alpha \)-cell response that we observed raises the question as to what mechanism is responsible for the failure of glucagon to respond normally in pregnancy. The increase in glucagon in insulin-induced hypoglycemia is in part neurally driven (27, 28), and the attenuated response in the pregnant dogs is consistent with a reduction of sympathetic pancreatic stimulation.

The increase in circulating norepinephrine in response to insulin-induced hypoglycemia results from spillover from nerve endings as well as release from the adrenal gland. Because the rise in epinephrine is also derived from the

![Intraportal Insulin Infusion](image-url)
adrenal, and this was not affected by pregnancy, the attenuated norepinephrine response probably represented a decrease in either overall sympathetic nervous activity or neural activity at a specific tissue. The attenuated lipolytic response (glycerol) in the pregnant group supports this contention, because stimulation of lipolysis in response to insulin-induced hypoglycemia is driven by neural detection of the low glucose level (2) and is largely mediated by activation of the sympathetic nervous system (9). It is unlikely that attenuation of circulating norepinephrine levels in the pregnant group had a differential impact directly on NHGO (13); instead, the effect was likely indirect, via peripheral release of gluconeogenic precursors. The arterial lactate level rose above basal only in the nonpregnant group. As with glycerol, this may have represented a differential sympathetic nervous stimulation of lactate release by the muscle (9, 13, 19, 21). In the basal period, blood levels and net hepatic uptake of alanine and the other gluconeogenic amino acids (with the exception of glutamine) were lower in the pregnant group, presumably reflecting either a decrease in peripheral release of amino acids overall or fetal utilization of amino acids. Although overall hepatic amino acid uptake tended to be lower in the pregnant group during the period of hypoglycemia, hepatic FE of alanine, serine, threonine, and glycine increased approximately twofold in both groups, suggesting that the reduction in net hepatic amino acid uptake was primarily due to the reduction in amino acid concentrations. In keeping with the reduction of net hepatic uptake of gluconeogenic precursors, GNG flux in the pregnant dogs was only ~60% that of the nonpregnant group. The increment in the gluconeogenic rate in the last hour of the experiment was reduced similarly (~50%).

The impact of the catecholamines to limit the fall in glucose by reducing peripheral Ra was unclear. The tracer data indicated that total Rd increased in the nonpregnant group but not in the pregnant group. Since the tracer method cannot distinguish between maternal and utero/placental/fetal Rd, we could not delineate how either maternal or fetal Rd changed with pregnancy. The tendency toward a difference in insulin concentrations in nonpregnant and pregnant groups is unlikely to explain the differences in the counterregulatory response observed in the two groups, however. At the level of insulin used in this study, there is no differential impact of variations in the insulin level on the glucagon response in nonpregnant dogs (16). Moreover, the deficient counterregulatory hormone response observed in this study is in agreement with data from previous studies in late pregnancy in the rat (43) and human (42).

Lipolysis was clearly reduced in the pregnant dogs, as indicated by the decreased glycerol levels and net hepatic glycerol uptake. The changes in NEFA tended to follow those of glycerol. The somewhat lower rate of NEFA uptake in the pregnant group did not appear to limit gluconeogenesis, since the gluconeogenic flux rate was nearly identical to the gluconeogenic contribution to NHGO in both groups. Ketogenesis was not lower in pregnant vs. nonpregnant dogs in response to insulin-induced hypoglycemia, even though factors that influence ketogenesis, such as glucagon (30), norepinephrine (32), and NEFA availability, were reduced in the pregnant dogs. Although insulin normally suppresses ketogenesis, even when the fatty acid supply is maintained (31), it appears that the increase in either epinephrine or sympathetic nervous activity might have interfered with that action and maintained ketone production in both groups.

In summary, pregnancy was associated with a deficient glucagon and norepinephrine response to insulin-induced hypoglycemia. The reduced norepinephrine and glycerol responses suggest a decreased activation of sympathetic nervous activity. The impact of the attenuated hormonal responses on stimulation of hepatic glucose production, by a direct stimulation of glycogenolysis and indirect effects on gluconeogenesis via a decrease in precursor supply, was quite marked. A similar lack of such responses in pregnant women with T1DM could play a critical role in the ability to reverse the fall in glucose in response to overinsulization, thereby contributing to the severity of hypoglycemic episodes.

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