Insulin action during late pregnancy in the conscious dog

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Connolly, Cynthia C., Lisa N. Aglione, Marta S. Smith, D. Brooks Lacy, and Mary Courtney Moore. Insulin action during late pregnancy in the conscious dog. Am J Physiol Endocrinol Metab 286: E909–E915, 2004. First published January 28, 2004; 10.1152/ajpendo.00143.2003.—Our aim was to assess the magnitude of peripheral insulin resistance and whether changes in hepatic insulin action were evident in a canine model of late (3rd trimester) pregnancy. A 3-h hyperinsulinemic (5 mU·kg⁻¹·min⁻¹) euglycemic clamp was conducted using conscious, 18-h-fasted pregnant (P; n = 6) and nonpregnant (NP; n = 6) female dogs in which catheters for intraportal insulin infusion and assessment of hepatic substrate balances were implanted ~17 days before experimentation. Arterial plasma insulin rose from 11 ± 2 to 192 ± 24 and 4 ± 2 to 178 ± 5 µU/ml in the 3rd h in NP and P, respectively. Glucagon fell equivalently in both groups. Basal net hepatic glucose output was lower in NP (1.9 ± 0.1 vs. 2.4 ± 0.2 mg·kg⁻¹·min⁻¹, P < 0.05). Hyperinsulinemia completely suppressed hepatic glucose release in both groups (−0.4 ± 0.2 and −0.1 ± 0.2 mg·kg⁻¹·min⁻¹ in NP and P, respectively). More exogenous glucose was required to maintain euglycemia in NP (15.2 ± 1.3 vs. 11.5 ± 1.1 mg·kg⁻¹·min⁻¹, P < 0.05). Nonesterified fatty acids fell similarly in both groups. Net hepatic gluconeogenic amino acid uptake with high insulin did not differ in NP and P. Peripheral insulin action is markedly impaired in this canine model of pregnancy, whereas hepatic glucose production is completely suppressed by high circulating insulin levels.

The techniques necessary to make thorough assessments of hepatic substrate metabolism are too invasive for use in pregnant women. We recently described a conscious canine model of pregnancy (8) that employs chronic catheterization techniques to make such assessments. We showed that this canine model has basal characteristics similar to those of pregnant women, indicating that it would be useful for studying the regulation of metabolic changes during pregnancy. The aims of the present study were to further our examination of the dog as a model for human pregnancy by quantifying the magnitude of whole body insulin resistance and examining insulin-sensitive metabolic processes in this model.

METHODS

Animals and surgical procedures. Experiments were performed on overnight-fasted (18 h), conscious adult female mongrel dogs, six nonpregnant (NP group, 22.9 ± 1.3 kg) and six pregnant (P group, 22.8 ± 1.1 kg). The pregnant dogs were 7–8 wk pregnant (term = 9 wk) when studied. Diet was as previously described (8). The protocol was approved by the Vanderbilt Institutional Animal Care and Use Committee, and the animals were cared for according to Association for Assessment and Accreditation of Laboratory Animal Care guidelines. Data from the control period only was included in a larger set of data described previously (8).

Sixteen to 21 days before experiment, blood sampling catheters were inserted into the portal vein, left common hepatic vein, and femoral artery, and intraportal hormone infusion catheters were inserted into jejunal and splenic veins, and their ends were placed in subcutaneous pockets, as described in detail previously (8). Ultrasonic flow probes (Transonic Systems, Ithaca, NY) were placed around the hepatic artery and hepatic portal vein (8). Criteria for inclusion of a dog in an experiment and experimental preparation were as previously described (8).

Experimental design. Each experiment consisted of a 120-min 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). Infusion of indocyanine green (ICG) dye (0.08 mg/min; Sigma, St. Louis, MO) and [3-³H]glucose was begun at ~120 min and continued throughout the experiment. At 0 min, intraportal infusion of insulin (5 mU·kg⁻¹·min⁻¹) was begun and continued throughout the experiment. Arterial plasma glucose was measured every 5 min, and exogenous glucose was infused as needed to maintain euglycemia for the duration of the experiment.

Collection and immediate processing of blood samples have been described previously (8). At the end of the experimental period, the dogs were anesthetized and then euthanized 5 min later with an overdose of pentobarbital sodium.

Analytical procedures. Parameters measured included hematocrit and plasma glucose, insulin, glucagon, cortisol, estrogen, progesterone, prolactin, epinephrine, norepinephrine, and nonesterified fatty acids (NEFA), as well as blood concentrations of lactate, glycerol,

LATE PREGNANCY IS ACCOMPANIED by alterations in many metabolic processes, involving multiple maternal tissues, that allow the mother to balance her own nutritional needs with the increasing requirements of utero-placental-fetal tissues (14, 21). In the basal state, hepatic glucose production and ketogenesis are increased, indicating altered basal liver function, and fat and protein metabolism are clearly affected (1, 6–8, 14, 21, 23, 24). Pregnancy is accompanied by the development of marked whole body insulin resistance in a variety of species (2, 4, 5, 18, 20, 26, 27, 32, 33). Use of the hyperinsulinemic euglycemic clamp technique in pregnant women revealed a marked reduction in the glucose infusion rate (up to ~24%) required to maintain euglycemia compared with nonpregnant controls, even with pharmacological elevation of insulin to levels of ~600 µU/ml (33). Similar results obtained in clamp studies utilizing very high insulin levels in the pregnant rat (26) and rabbit (18) specifically revealed the significant attenuation of insulin’s ability to stimulate glucose uptake in peripheral tissues in pregnancy.


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β-hydroxybutyrate, acetoacetate, alanine, glutamine, glutamate, serine, threonine and glycine. All sample analyses have been described in detail previously (8).

Calculations. Total hepatic blood flow was assessed by two methods, hepatic extraction of ICG and ultrasonic probes (see Ref. 8). For ICG measurements, the proportions derived from the hepatic artery and portal vein were assumed to be 20 and 80%, respectively, and were confirmed for both the pregnant and nonpregnant dogs by use of ultrasonic flow probes. All calculations reported in results utilize the ultrasonic flow data because they do not require any assumptions about the distribution of flow. Calculations performed with the ICG and ultrasonic flow data do not differ significantly.

Net hepatic substrate balance and hepatic fractional extraction of substrate were calculated as described previously (8). The 3H-labeled hepatic glucose balance was divided by the inflowing plasma [3H]glucose specific activity (dpm/mg glucose) to yield the unidirectional hepatic glucose uptake (HGU). Unidirectional hepatic glucose release (HGR) was the difference between net hepatic glucose balance and HGU. The rate of glucose appearance (Ra) and disappearance (Rd) was calculated with a two-compartment model using dog parameters (12, 28). Endogenous glucose Ra (EndoRa) was the difference between the total glucose Ra and the glucose infusion rate.

The gluconeogenic (GNG) rate from circulating precursors was estimated using the arteriovenous difference technique as described in detail previously (8). This technique makes the assumption that all GNG precursors (alanine, serine, threonine, glycine, glutamine, glutamate, lactate, and glycerol) taken up by the liver in a net sense represent the GNG flux (GNGflux) within the liver. Net hepatic balance of pyruvate was assumed to be 10% of net hepatic lactate balance (38). On the basis of GNG comparisons (16) made simultaneously using the arteriovenous difference technique and the method of Giaccari and Rossetti (15), it does appear that the arteriovenous difference technique gives a reliable estimate of overall GNGflux (16). Net hepatic glycogenolysis (GLY) is then calculated as HGR − HGU + NHLO + GO − GNGmax, where NHLO is the rate of net hepatic lactate output (equal to 0 if there is net uptake), and GO is glucose oxidation [assumed to be constant at 0.2 mg·kg⁻¹·min⁻¹, based on our previous findings during mixed-meal feeding of pregnant and nonpregnant dogs (9)]. GLY and GNG both contribute to the glucose 6-phosphate pool from which glucose leaves the hepatocyte. Thus the portion of net hepatic glucose output (NHGO) derived from GNG can be expressed as the fraction of GNG that contributes to that pool multiplied by NHGO, according to formula GNG = [GNGmax/ (GNGmax + net GLY)] × NHGO. The contribution of GLY to NHGO is then NHGO − GNG. Use of these calculations assumes that oxidation of GNG substrates remains constant and does not differ between groups.

Statistical comparisons were made using two-way ANOVA with a repeated-measures design. Specific time points were examined for significance by using contrasts solved by univariate repeated measures. Unless otherwise indicated, the data reported in results are the averages of the three values obtained during the 30-min basal sampling period and the three values obtained during the last hour of the experimental period. All data are expressed as means ± SE.

**RESULTS**

Hormone levels and hepatic blood and plasma flow. In response to intraportal insulin infusion, arterial plasma insulin rose similarly in the NP and P dogs (from 11 ± 2 to 192 ± 24 and 4 ± 2 to 178 ± 5 μU/ml, respectively; Fig. 1). Arterial plasma glucagon fell similarly (from 40 ± 2 to 26 ± 3 and 39 ± 3 to 30 ± 2 pg/ml in NP and P, respectively). There were no differences between the groups in arterial plasma cortisol or epinephrine (Table 1). Basal arterial plasma norepinephrine was lower in NP compared with P, but there was no statistical difference between the groups in the experimental period. Basal estrogen, progesterone, and prolactin levels were 11.9 ± 1.6 pg/ml, 0.2 ± 0.1 ng/ml, and 1.7 ± 0.5 ng/ml, respectively, in NP, and 22.8 ± 3.3 pg/ml, 8.7 ± 1.3 ng/ml, and 10.4 ± 1.8 ng/ml in P, and did not change with insulin infusion.

There were no changes in hepatic blood (27.2 ± 2.4 to 26.2 ± 1.2 ml·kg⁻¹·min⁻¹ in NP and 23.8 ± 1.6 to 22.5 ± 2.1 ml·kg⁻¹·min⁻¹ in P) or plasma flow (16.7 ± 1.4 to 17.6 ± 0.8 ml·kg⁻¹·min⁻¹ in NP and 16.9 ± 1.1 to 16.0 ± 1.5 ml·kg⁻¹·min⁻¹ in P) in either group throughout the experiment, nor were there differences in either parameter between the groups. 

Glucose metabolism. Euglycemia was maintained in both groups during insulin infusion (from 107 ± 2 to 102 ± 3 and

*Table 1. Arterial plasma epinephrine, norepinephrine, and cortisol concentrations in NP female and P dogs during a hyperinsulinemic euglycemic clamp*
INSULIN ACTION IN PREGNANCY

Fig. 2. Arterial plasma glucose levels, net hepatic glucose balance, and exogenous glucose infusion. Although the mean basal values for net hepatic glucose balance and individual points in the experimental period were statistically different, the change from basal over time was the same between the groups. *P < 0.05 between groups.

98 ± 6 to 100 ± 2 mg/dl in NP and P, respectively; Fig. 2). Basal NHGO was lower in NP (1.9 ± 0.1 vs. 2.4 ± 0.2 mg·kg⁻¹·min⁻¹; P < 0.05). In response to insulin, NHGO was completely suppressed in both groups (−0.4 ± 0.2 and −0.1 ± 0.2 mg·kg⁻¹·min⁻¹ in NP and P, respectively). The glucose infusion rate required to maintain euglycemia in the 3rd h was reduced by 24% in P (15.2 ± 3 vs. 11.5 ± 1.1 mg·kg⁻¹·min⁻¹; P < 0.05).

Basal glucose EndoRa was significantly greater (difference between groups 1.0 ± 0.1 mg·kg⁻¹·min⁻¹; P < 0.05; Fig. 3) in P than in NP, and the rates were also significantly different during the last hour of study (difference between groups 1.9 ± 0.4 mg·kg⁻¹·min⁻¹; P < 0.05). However, the suppression of glucose EndoRa from basal during hyperinsulinemic euglycemia was not significantly different in the two groups. Tracer-determined HGU did not differ between groups either in the basal period (0.06 ± 0.05 and 0.16 ± 0.11 mg·kg⁻¹·min⁻¹ in NP and P, respectively) or the last hour of the experimental period (0.47 ± 0.17 and 0.26 ± 0.13 mg·kg⁻¹·min⁻¹ in NP and P, respectively). HGR was significantly less in the basal period in NP than in P (1.93 ± 0.11 vs. 2.58 ± 0.16 mg·kg⁻¹·min⁻¹; P < 0.05), but the two groups exhibited similar rates during the last hour (0.02 ± 0.19 and 0.16 ± 0.17 mg·kg⁻¹·min⁻¹ in NP and P, respectively), demonstrating that HGR was suppressible in pregnant dogs with these high insulin concentrations.

Lactate, alanine, and glycerol metabolism. Basal arterial blood lactate was similar in both groups (585 ± 69 vs. 472 ± 17 μmol/l in NP and P, respectively; Fig. 4). With insulin infusion, lactate rose (750 ± 73 vs. 976 ± 111 μmol/l in NP and P, respectively), and was not significantly different between groups. The change over time, from basal to the last hour of the experimental period, however, was significantly less in NP than in P (Δ165 ± 91 and 504 ± 102 μmol/l, respectively, P < 0.05). Basal net hepatic lactate uptake was less in NP (−1.5 ± 3.3 vs. −7.4 ± 0.9 μmol·kg⁻¹·min⁻¹; P < 0.05). With insulin infusion, the liver switched to net hepatic lactate output in NP, whereas net hepatic lactate uptake was reduced in P. In the 3rd h, both groups exhibited net hepatic lactate uptake (−0.6 ± 0.7 vs. −6.7 ± 1.3 μmol·kg⁻¹·min⁻¹ in NP and P, respectively). There were no differences between the groups in the changes from their own basal rates in net hepatic lactate balance over time.

Basal arterial blood alanine was higher in NP (309 ± 43 vs. 134 ± 21 μmol/l; P < 0.05; Table 2). In response to insulin infusion, blood alanine fell in NP but remained higher than in P (P < 0.05 between groups), which remained constant. Net hepatic alanine uptake remained unchanged in both groups but was lower (P < 0.05) in P throughout the experiment. Hepatic alanine fractional extraction was similar in the groups throughout the experiment.

Arterial blood glycerol fell from 82 ± 12 to 41 ± 12 and 101 ± 13 to 79 ± 7 μmol/l in NP and P, respectively (both groups in the changes from their own basal rates in net hepatic lactate balance over time.

Fig. 3. Endogenous glucose rate of appearance (EndoRa), glucose rate of disappearance (Rd), and glucose clearance. Although absolute rates of EndoRa were higher in the pregnant group during both the basal period and the last 90 min of the experimental period, the suppression of EndoRa from basal during insulin infusion did not differ in the two groups. *P < 0.05 between groups.

AJP-Endocrinol Metab • VOL. 286 • JUNE 2004 • www.ajpendo.org
Net hepatic glycerol fractional extraction (0.57 groups but did change differently over time in the two groups. 

\[
\text{fi and P, respectively) did not change significantly over time, and the area under the curve of each group's change from its own basal rates was the same between groups. *P < 0.05 between groups.}
\]

\[P < 0.05; \text{Table 3). Glycerol was not different between the groups but did change differently over time in the two groups. Net hepatic glycerol fractional extraction (0.57 ± 0.06 to 0.67 ± 0.03 and 0.69 ± 0.03 to 0.66 ± 0.02 in NP and P, respectively) and net hepatic glycerol uptake (1.3 ± 0.3 to 0.8 ± 0.2 and 1.7 ± 0.2 to 1.2 ± 0.2 \text{mmol-kg}^{-1}\text{-min}^{-1} \text{in NP and P, respectively) did not change significantly in either group during the experiment.}
\]

\[GNG \text{ amino acid metabolism. Basal levels of serine, threonine, glycine, glutamine, and glutamate were similar in NP and P (Table 2; serine not shown). However, when these amino acids were summed, there was a slight but significant difference in the levels in the basal period (} P = 0.049). Due to differences in hepatic glucose balance, in the basal state there was net hepatic uptake of these five GNG amino acids in NP (−2.1 ± 0.9 \text{mmol-kg}^{-1}\text{-min}^{-1}), whereas there was net hepatic output in P (0.3 ± 0.7 \text{mmol-kg}^{-1}\text{-min}^{-1}). During the last hour of insulin infusion, there was net hepatic uptake in both groups (−0.6 ± 1.3 vs. −0.4 ± 0.6 \text{mmol-kg}^{-1}\text{-min}^{-1} \text{in NP and P, respectively; not different between groups).}
\]

\[GNG \text{ and GLY. The contribution of GNG to NHGO (see methods) did not differ between the groups in the basal period (0.8 ± 0.2 and 1.0 ± 0.1 \text{mmol-kg}^{-1}\text{-min}^{-1} \text{in NP and P, respectively). The contribution of GLY to NHGO (not total GLY) was therefore 1.0 ± 0.2 and 1.3 ± 0.2 \text{mmol-kg}^{-1}\text{-min}^{-1} \text{in NP and P, respectively.}}
\]

\[\text{in NP and P, respectively. Basal GNG}_{\text{flu}} \text{ within the liver was 1.0 ± 0.2 and 1.1 ± 0.1 \text{mg-kg}^{-1}\text{-min}^{-1} \text{in NP and P, respectively. With insulin administration, GNG}_{\text{flu}} \text{ (directed in a net sense to glycogen, because NHGO was suppressed) decreased in the last hour of the experimental period in NP compared with P (0.6 ± 0.1 vs. 1.2 ± 0.2 \text{mg-kg}^{-1}\text{-min}^{-1}, } P < 0.05).}
\]

**NEFA metabolism.** Arterial plasma NEFA levels fell similarly in the two groups in response to insulin (from 1.082 ± 132 to 121 ± 28 and from 1.216 ± 173 to 175 ± 38 \text{pmol/l} \text{in NP and P, respectively; Table 3). Net hepatic NEFA uptake was lower basally in NP (2.7 ± 0.5 vs. 4.3 ± 0.1 \text{pmol-kg}^{-1}\text{-min}^{-1}, } P < 0.05) but fell to similar low levels with insulin infusion (0.1 ± 0.1 vs. 0.4 ± 0.1 \text{pmol-kg}^{-1}\text{-min}^{-1} \text{in NP and P, respectively). Basal hepatic NEFA fractional extraction was lower in NP and also tended to be lower in the experimental period (from 0.15 ± 0.02 to 0.01 ± 0.04 in NP vs. 0.21 ± 0.02 to 0.15 ± 0.04 in P; see figure legend for details).**

**Ketone metabolism.** Basal arterial blood ketone levels were lower in NP (99 ± 13 vs. 322 ± 90 \text{pmol/l}; } P < 0.05), but the two groups fell to similar values in response to insulin infusion (71 ± 10 vs. 43 ± 6 \text{pmol/l} \text{in NP and P, respectively; Table 3). The same pattern was observed for net hepatic ketone output (basal values of 2.0 ± 0.5 vs. 6.7 ± 1.6 \text{pmol-kg}^{-1}\text{-min}^{-1} \text{in NP and P, respectively, } P < 0.05, \text{ and 3rd-h values of 0.5 ± 0.1 vs. 0.4 ± 0.06 in NP and P, respectively).}

**DISCUSSION**

Elevation of circulating arterial insulin levels to almost 200 \text{μU/ml} \text{(~40-fold basal) completely inhibited the liver from releasing glucose in the pregnant group. Thus the reduction in insulin action observed in the pregnant dogs in this study was clearly due to peripheral tissue insulin resistance. The ability of insulin to retain its suppressive action on hepatic glucose release in the pregnant group under the conditions created in NP and P, respectively.}

### Table 2. Arterial blood concentrations, NHB, and FE of gluconeogenic amino acids in NP female and P dogs

<table>
<thead>
<tr>
<th>Levels</th>
<th>Nonpregnant</th>
<th>Experimental</th>
<th>Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>309±43</td>
<td>162±16</td>
<td>134±21*</td>
</tr>
<tr>
<td>NHB</td>
<td>−2.23±0.15</td>
<td>−2.06±0.28</td>
<td>−1.17±0.16*</td>
</tr>
<tr>
<td>FE</td>
<td>0.28±0.06</td>
<td>0.43±0.06</td>
<td>0.30±0.03</td>
</tr>
<tr>
<td>Threonine</td>
<td>182±23</td>
<td>75±10</td>
<td>159±32</td>
</tr>
<tr>
<td>NHB</td>
<td>−0.19±0.24</td>
<td>−0.22±0.18</td>
<td>−0.16±0.14</td>
</tr>
<tr>
<td>FE</td>
<td>0.03±0.06</td>
<td>0.09±0.08</td>
<td>0.06±0.06</td>
</tr>
<tr>
<td>Glycine</td>
<td>216±36</td>
<td>96±14</td>
<td>223±29</td>
</tr>
<tr>
<td>NHB</td>
<td>−1.09±0.27</td>
<td>−0.99±0.27</td>
<td>−0.86±0.15</td>
</tr>
<tr>
<td>FE</td>
<td>0.20±0.05</td>
<td>0.31±0.07</td>
<td>0.21±0.09</td>
</tr>
<tr>
<td>Glutamine</td>
<td>902±73</td>
<td>539±52</td>
<td>768±46</td>
</tr>
<tr>
<td>NHB</td>
<td>−0.05±0.87</td>
<td>0.98±0.64</td>
<td>1.86±0.46</td>
</tr>
<tr>
<td>FE</td>
<td>0.01±0.04</td>
<td>−0.06±0.04</td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>58±16</td>
<td>49±20</td>
<td>82±13</td>
</tr>
<tr>
<td>NHB</td>
<td>−0.06±0.16</td>
<td>0.07±0.41</td>
<td>−0.11±0.13</td>
</tr>
<tr>
<td>FE</td>
<td>0.06±0.06</td>
<td>0.15±0.03</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 6/group. Amino acid concentrations are in \text{μmol/l} \text{, net hepatic balance (NHGO) in \text{μmol-kg}^{-1}\text{-min}^{-1} \text{; net hepatic fractional extraction (FE) in no units. Basal period values are the means of 3 sampling times between 30 to 0 min, and experimental values are the means of the 3 samples in the last hour (120–180 min) of hyperinsulinemic euglycemia. Negative value indicates net hepatic uptake, *P < 0.05 vs. NP during the same period.}
Arterial blood glycerol and ketones (acetoacetate and β-hydroxybutyrate) and plasma NEFA concentrations, NHB, and FE in NP female and P dogs

<table>
<thead>
<tr>
<th></th>
<th>Nonpregnant</th>
<th>Experimental</th>
<th>Pregnant</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Concentration, μmol/l</td>
<td>82±12</td>
<td>41±12</td>
<td>101±13</td>
<td>79±7*</td>
</tr>
<tr>
<td>NHB, μmol·kg⁻¹·min⁻¹</td>
<td>−1.25±0.32</td>
<td>−0.80±0.20</td>
<td>−1.66±0.24</td>
<td>−1.20±0.17</td>
</tr>
<tr>
<td>FE</td>
<td>0.57±0.06</td>
<td>0.67±0.03</td>
<td>0.69±0.03</td>
<td>0.66±0.02</td>
</tr>
<tr>
<td>NEFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration, μmol/l</td>
<td>1.082±132</td>
<td>121±28</td>
<td>1.216±173</td>
<td>175±38</td>
</tr>
<tr>
<td>NHB, μmol·kg⁻¹·min⁻¹</td>
<td>−2.68±0.50</td>
<td>−0.06±0.11</td>
<td>−4.28±0.14*</td>
<td>−0.38±0.11*</td>
</tr>
<tr>
<td>FE</td>
<td>0.15±0.02</td>
<td>0.01±0.04</td>
<td>0.21±0.02</td>
<td>0.15±0.04*</td>
</tr>
<tr>
<td>Ketone</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Concentration, μmol/l</td>
<td>99±13</td>
<td>71±10</td>
<td>322±90*</td>
<td>43±6*</td>
</tr>
<tr>
<td>NHB, μmol·kg⁻¹·min⁻¹</td>
<td>1.95±0.48</td>
<td>0.48±0.08</td>
<td>6.72±1.55*</td>
<td>0.40±0.06</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 6/group. NEFA, nonesterified fatty acids. Basal period values are the means of the 3 samples in the last hour (120–180 min) of hyperinsulinemic euglycemia. Negative value indicates net hepatic uptake. Ketones are the sum of β-hydroxybutyrate and acetoacetate. No FE are given for ketones because there was net hepatic production. *P < 0.05 vs. NP during the same period.

This study is consistent with its effect in pregnant women (7). However, the insulin levels used in the present study to assess the magnitude of peripheral insulin resistance may have masked pregnancy-induced alterations in hepatic insulin action, since the liver of the nonpregnant dog (and the human as well) is quite sensitive to slight increases in insulin (31, 36). Thus the possibility that alterations in hepatic insulin action in the pregnant dog or pregnant woman occur but are subtler than in other species needs to be explored in further detail using small elevations in insulin.

The elevation of insulin caused a similar dramatic reduction in the arterial plasma level of NEFA in both groups, likely an effect of insulin on lipolysis and NEFA reesterification in adipose tissue. Net hepatic NEFA uptake remained significantly higher in P than in NP during hyperinsulinemia, but even in the P group the rate was less than 1% of that in the basal state. Ketone production was equivalently reduced in both the pregnant and nonpregnant groups despite being elevated threefold basally in the pregnant group. The marked reduction in fatty acid delivery to and uptake by the liver in both groups could have masked any impairment that might exist in insulin’s ability to inhibit hepatic ketone production in the pregnant dog. Future studies may address the effect of lower doses of insulin on hepatic ketogenesis to determine whether there is a differential response in the suppression of this process in pregnancy.

A decrease in alanine fractional extraction would have been expected due to the decrease that occurred in glucagon levels in both groups. Instead, alanine fractional extraction rose in both groups, indicating that insulin had an effect on the liver to counter the influence of the fall in glucagon, and this was not altered by pregnancy. The alanine concentration was lower in the basal period in the pregnant group; but interestingly, the level did not fall in response to hyperinsulinemia as it did in the nonpregnant group. Because this was not due to a difference in the liver, peripheral tissues in the pregnant group continued to release alanine at the same rate despite the elevation in insulin. This could possibly represent a resistance to insulin suppression of proteolysis in the pregnant group or perhaps reflect a lower availability of glucose for the glycolytic pathway and possible conversion of lactate to alanine. In contrast to alanine, there appeared to be no notable differential effects of hyperinsulinemia on the levels of the other gluconeogenic amino acids.

During the basal period, the liver was a net consumer of lactate in the pregnant group, as we have previously observed (8), but net lactate balance was essentially zero in the nonpregnant group. The shift from net hepatic lactate release to uptake is an indicator of the transition from the fed to the fasted state in the dog (29), as in the human (3, 39). This raises the question whether the pregnant group should have been studied after a shorter period of fasting than the nonpregnant group in order for the groups to start from a more similar metabolic state. However, we chose to consider the “accelerated starvation” of pregnancy (19) as a factor that influences the response of the pregnant dog (and woman) to hyperinsulinemia rather than try to compensate for it. The difference in basal net hepatic lactate balance does not mean that the pregnant animals have been postabsorptive for a longer time period than the nonpregnant ones. In fact the opposite is true because pregnant dogs require longer to absorb a mixed meal than nonpregnant dogs do (9). It is intriguing that, in the pregnant group, the arterial lactate levels continued to rise, even though there was a greater rate of hepatic uptake of lactate and a lower glucose utilization rate in peripheral tissues compared with the nonpregnant group. This suggests that not only is the total amount of glucose entering muscle and/or adipose tissue lower in pregnancy when insulin is elevated but that storage of glucose is altered, since a greater proportion of the glucose entering the cell leaves as lactate. This possibility is not surprising, since muscle glycogen synthesis is reduced in the insulin-resistant state accompanying type 2 diabetes (35). This may be related to the previously mentioned deficits in insulin signaling during late pregnancy (34).

Basal glucose EndoRa and hepatic glucose release were significantly greater in P than in NP, in agreement with findings in the human (23), and EndoRa in P remained significantly elevated over the rate in NP. The major impact of insulin on hepatic glucose production was clearly an inhibitory effect on glycogenolysis, since gluconeogenesis from circulating precursors did not decrease in response to insulin in the pregnant dogs. Even though the gluconeogenic rate remained elevated compared with the nonpregnant dogs, it is not surprising that this had no effect on hepatic glucose production, since even a
small elevation in insulin has recently been shown to elicit its direct effect via inhibition of glycogenolysis at the liver (13) and its indirect effect via a decrease in precursor availability (36). If insulin has any differential effect on glycogenolysis in pregnancy, the much higher insulin levels used in these studies could have masked such an effect. Interestingly, hepatic glucose release in the pregnant dogs was virtually completely suppressed by hyperinsulinemia, and this accounted for all of the suppression of glucose EndoR, suggesting that renal glucose production might be resistant to insulin suppression in pregnancy. In vitro data from the fasted rat demonstrate that renal gluconeogenesis is enhanced in late pregnancy (40).

The data from this study make it clear that the profound peripheral insulin resistance associated with pregnancy in other species also characterizes the pregnant dog. The reduced peripheral insulin action in the pregnant dog during hyperinsulinemia was verified by the initial attenuation of insulin-stimulated glucose utilization compared with the nonpregnant group. The increase in glucose $R_a$ was sluggish in the pregnant compared with the nonpregnant group, but by the last hour, glucose $R_a$ in the two groups did not differ significantly. The area under the curve (AUC) of the change in glucose $R_d$ (calculated as change from each group’s basal rate) was only 65% as large in P as in NP ($P = 0.003$). The AUC of the rate of exogenous glucose infusion required to maintain euglycemia, another indicator of the magnitude of insulin resistance, was reduced $\sim 30\%$ in P dogs compared with NP ($P = 0.02$). These measures are, in fact, likely to be an underestimate of maternal peripheral insulin resistance because it comprises glucose utilization rates by both maternal and utero-placental-fetal tissues. The increase in glucose utilization in response to hyperinsulinemia is essentially a function of maternal tissues, since fetal glucose utilization does not appear to increase in response to maternal hyperinsulinemic euglycemia (27), and it is unclear whether placental glucose utilization increases under this condition. Assuming that placental tissue does respond to the elevation in insulin, its contribution to overall glucose utilization would nevertheless be small relative to maternal tissue glucose utilization (26). Thus the ability of insulin to stimulate glucose utilization by maternal tissues is better reflected by the increment in glucose utilization over the basal rate (which at steady state is the rate of net hepatic glucose output). In the pregnant dogs, this increment ($9.1 \text{ mg kg}^{-1}\text{min}^{-1}$) increased by only two-thirds that which was observed in nonpregnant dogs ($13.3 \text{ mg kg}^{-1}\text{min}^{-1}$). This relationship held true even when the increment was expressed in milligrams per minute to avoid the contribution of uteroplacental-fetal weight to total maternal body weight.

The present study did not assess which nonhepatic tissues contributed to the observed insulin resistance. The involvement of both skeletal muscle and adipose tissue has been implicated from studies conducted in individual tissues of pregnant rats (20, 27), and certainly in other conditions of insulin resistance (35). It was unclear whether lipolysis (as indicated by glycerol) was suppressed appropriately by insulin in the pregnant group, but it remains possible that insulin’s separate action on glucose uptake by fat tissue was impaired. Likewise, the difference in glucose utilization in peripheral tissues in the two groups could not be explained by a differential change in the circulating NEFA level. The lactate and alanine data implicate the involvement of muscle insulin resistance in this study, although the peripheral provision of lactate could stem from fat as well (10). Indeed, although muscle is considered to be the largest contributor to insulin resistance in type 2 diabetes (25), largely by virtue of its greater mass, the possibility that adipose tissue also makes a contribution has recently been considered, given data obtained from transgenic studies in which GLUT4 specifically from adipose tissue in mice was either overexpressed or knocked out (22, 37).

In the present study, several hormones that affect either glucose production or utilization, even in the presence of elevated insulin, were measured throughout the study to determine whether there were differences that might have explained the altered insulin action in the pregnant group. However, glucagon (30), epinephrine, norepinephrine (11), and cortisol changed similarly in both groups. Thus the peripheral insulin resistance exhibited by the pregnant dogs must have been due to a factor(s) other than a differential effect of insulin on the hormones measured.

In summary, the marked insulin resistance that is common in late pregnancy among other species is characteristic of the pregnant dog as well. The profound peripheral resistance to insulin action is evident from the attenuated glucose utilization in the pregnant group, as well as from differences in alanine and lactate metabolism. These findings are specific to late pregnancy; insulin sensitivity during early stages of pregnancy is likely to differ from that in the latter portion (17). Hepatic glucose production is suppressible by high insulin levels in this pregnant canine model, in agreement with data available in the human. Whether hepatic insensitivity to insulin would be evident at lower, more physiological, doses of insulin requires further study. The presence of some degree of hepatic insulin resistance is implied by the presence of higher basal hepatic glucose release, despite higher insulin concentrations. The similarity in changes in insulin action induced by pregnancy in the dog and human, along with our previous observations that many of the changes in basal carbohydrate metabolism are common to both (8), indicates that the pregnant dog is a relevant model of human pregnancy and will be useful for studying alterations in the regulation of carbohydrate metabolism during pregnancy. This report not only demonstrates that the dog, like the human, exhibits substantial insulin resistance during pregnancy but also provides the most detailed data available to date on hepatic handling of gluconeogenic precursors during pregnancy. These data provide further confirmation (8) that the dog will provide a useful model for further study of metabolic alterations of pregnancy, allowing the use of invasive techniques not possible in humans.

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GRANTS

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