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Kinetics of Splanchnic Progesterone Metabolism in Ewes Fed Two Levels of Nutrition

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ABSTRACT: The objective of this study was to determine whether chronic nutritional treatment influences progesterone \( (P_4) \) metabolism by splanchnic tissues and to develop a mathematical model of \( P_4 \) metabolism in ewes. Five ovariectomized (ovx), multiparous ewes were assigned to a high feed intake and five multiparous ovx ewes were assigned to a low feed intake. The ewes with high feed intake received daily ME intakes of \( 99 \) kcal/BW kg\(^{-7.5}\), and the ewes with low intake received daily ME intakes of \( 63 \) kcal/BW kg\(^{-7.5}\). Catheters were placed surgically in the abdominal aorta, the portal vein, a branch of the hepatic vein, and a mesenteric vein. Blood and plasma flows across visceral organs were determined by marker dilution (p-aminohippuric acid), and \( P_4 \) was determined with a RIA. The net splanchnic \( P_4 \) flux and oxygen (\( O_2 \)) consumption were determined during five rates of \( P_4 \) infusion into the jugular vein (112, 224, 449, 897, and 1,795 \( \mu \)g/h). Splanchnic \( O_2 \) consumption was greater \( (P = .05) \) in the ewes with high feed intake. Net splanchnic \( P_4 \) flux did not differ \( (P > .10) \) between nutritional treatments. The correlation between net splanchnic \( P_4 \) flux and \( O_2 \) consumption did not differ from zero \( (P = .69) \). Net splanchnic \( P_4 \) flux was related linearly to plasma arterial \( P_4 \) concentration. Splanchnic tissue clearance rate was 18% of the infusion rate. The behavior of the \( P_4 \) model indicates that whole-body \( P_4 \) metabolism is the sum of first-order kinetic reactions. The data indicate that splanchnic clearance of \( P_4 \) is not affected by nutritional status.

Key Words: Steroids, Liver, Portal-Drained Viscera

Introduction

Ewes maintained at heavier weights have more lambs than those that are maintained at lighter weights (Coop, 1966). Thomas et al. (1987) proposed that hepatic mixed-function oxidase activity increases with increased nutrition, which results in a decrease in progesterone \( (P_4) \) concentration and subsequently a decrease in inhibition at the hypothalamic-pituitary axis. During pregnancy, circulating \( P_4 \) concentrations decrease in ewes maintained on higher planes of nutrition (Cumming et al., 1971), supporting the hypothesis that the rate of \( P_4 \) metabolism can be increased through nutrition. In vivo studies indicate that increased feed intake increases liver oxygen consumption and hepatic blood flow (Burrin et al., 1989), and they indicate that splanchnic tissues are a primary site of \( P_4 \) metabolism (Bedford et al., 1974; Freetly and Ferrell, 1994). Thus, increased nutrition may affect circulating \( P_4 \) concentrations by increasing \( P_4 \) delivery to the liver and increasing hepatic \( P_4 \) metabolism.

Materials and Methods

Multiparous, polled Dorset ewes were housed in individual pens (1.17 m\(^2\)). Room temperature was kept at 20°C with a light:dark cycle of 14:10 h. Ten ewes weighing (mean ± SE) 62.2 ± .9 kg and in optimum body condition for breeding were assigned to one of two nutritional treatments. Five ewes were placed on a high feed intake, which amounted to daily ME intakes of 99 kcal/ BW kg\(^{-7.5}\) (100% of NRC, 1985, for maintenance). Five ewes were assigned to a low intake and received 63 kcal/BW kg\(^{-7.5}\) of ME daily (66% of NRC, 1985, for maintenance). Ewes were fed a pelleted diet (61.1% corn cobs, 20.0% corn gluten feed, 11.8% soybean meal [44% CP], 6.7% corn, .36% limestone, .005% zinc sulfate as dry matter; calculated ME = 2.00 Mcal/kg). Ewes were adapted to the environment and diets for 285 d before surgery.

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Ewes were ovariectomized, and catheters were surgically placed in the portal vein, a branch of the hepatic vein, a mesenteric vein, and the abdominal aorta, using techniques previously described (Ferrell et al., 1992). Ewes were fed daily at 1600, and feed refusal from the previous day was determined at feeding.

Within nutritional treatment, there were five collection periods (14-d intervals) and five infusion levels. Sampling was blocked so that all collection intervals had all infusion levels, and all ewes received all infusion levels.

Data were analyzed with least squares procedures using the General Linear Models procedures (SAS, 1989). Differences between nutritional treatment means were analyzed with a linear model in which nutritional treatment, ewe, and collection period were discrete effects and infusion rate was continuous. The model was nutritional treatment, ewe nested within treatment error term. Nutritional treatment differences were tested with the ewe nested within treatment error term.

Blood sample collection began 21 d after surgery. Seventeen hours before sampling, a catheter was placed in the right external jugular vein, filled with heparinized saline, and capped. Two and one-half hours before sampling, 160 mL of blood was collected aseptically from the jugular venous catheter in heparinized syringes. The blood was centrifuged, and plasma was harvested aseptically. Infusates were made by adding 1 mL of P4 dissolved in ethanol to 59 mL of plasma and agitating the mixture in a sealed, sterile bottle for 20 min. The final progesterone plasma infusate concentrations were 9.3, 18.7, 37.4, 74.8, and 149.6 µg/mL. Two hours before sampling, ewes were transferred to metabolism crates. Beginning 90 min before blood sampling, P4 was infused into the jugular venous catheter at .2 mL/min with a screw-driven pump. The target P4 infusion rates were 112, 224, 449, 897, and 1,795 µg/h. Sixty minutes before blood sampling, at 1100, 15 mL of a 5% solution of neutralized p-aminohippuric acid (PAH) was administered through the mesenteric venous catheter. Para-aminohippuric acid (5%) was infused continuously, at .8 mL/min for 2.25 h following the priming dose with a screw-driven pump. Samples (5 mL) of blood were drawn into heparinized syringes from the aortal, portal venous, and hepatic venous catheters beginning 60 min after infusion began. Blood samples were collected at 10-min intervals for a total of seven sets of samples (arterial, portal venous, hepatic venous).

Blood samples for PAH analyses were diluted 1:4 (vol:vol) with deionized water. All blood and plasma samples were analyzed for PAH with automated procedures (Technicon Industrial Systems, 1972 No. 216-72T, Tarrytown, NY). Blood PAH was determined within 2 h of collection, and plasma samples were frozen and assayed at a later date. Blood and plasma flows were calculated as described by Burrin et al. (1989). Blood and plasma flows were calculated as the mean of the seven replicate samples taken from each vessel.

On the first, fourth, and seventh set of samples, an additional 1.0 mL of blood was drawn into heparinized syringes and analyzed immediately for hemoglobin and percentage of oxygen (O2) saturation of hemoglobin (Hemoximeter, Model OSM-2, Radiometer America, Westlake, OH). Oxygen concentrations were calculated as described by Burrin et al. (1989), and O2 consumption was calculated as the mean of the three replicates.

Plasma P4 concentrations were determined in the fourth set of samples using a RIA, which included heptane extraction described previously by Maurer and Echternkamp (1982). The intra- and interassay CV were 14 and 7%, respectively. The lower limit of detection was 80 pg/mL. The net flux of metabolites across the portal-drained viscera (PDV) and liver were calculated by multiplying the concentration difference between vessels by the flow rate as described by Burrin et al. (1989), where flow rates were the mean flow rates of the replicates.

Results

One ewe on the low nutrition treatment was removed from the study due to feed refusal and not bled in periods four and five (infusion rates of 897 and 1,795 µg/h). One ewe on the high nutrition treatment was not bled in period five (infusion rate of 224 µg/h) due to catheter patency failure, and a second ewe on the high nutrition treatment was removed from the study due to feed refusal and was not bled in periods three, four, or five (infusion rates of 224, 449, and 897 µg/h).

By design, body weights between nutritional treatments differed (P = .0005). Ewes on the low nutrition treatment weighed 55.1 ± .2 kg, and ewes on the high nutrition treatment weighed 68.2 ± .2 kg.

The rates of blood flow in the splanchnic tissues tended to be greater in the high nutrition group than in the low nutrition group, but plasma flow did not differ with nutritional treatment (Table 1). Splanchnic O2 consumption was greater (P = .05) in the high nutrition group than in the low nutrition group. Portal-drained viscera O2 consumption in the low nutrition group was 97.8 ± 4.4 mmol/h and did not differ (P = .17) from that in the high nutrition group (111.3 ± 4.9 mmol/h). Hepatic O2 consumption in the low nutrition group was 104.5 ± 6.0 mmol/h and differed (P = .06) from that in the high nutrition group (150.5 ± 6.6 mmol/h). The correlations between PDV O2 consumption and PDV P4 net flux (P = .56; Figure 1) and hepatic O2 consumption and hepatic P4 net flux (P = .98; Figure 2) did not differ from zero.
Hepatic $P_4$ ($P = .64$) and PDV $P_4$ ($P = .57$) net flux did not differ between nutritional treatments. Splanchnic $P_4$ net flux increased with increasing arterial plasma $P_4$ concentration (Figure 3). The linear regression of splanchnic $P_4$ net flux on arterial plasma $P_4$ concentration had a coefficient of 7.70, an intercept of $-20.36$, and a $r^2$ of .88. Portal-drained viscera $P_4$ net flux increased with increasing arterial plasma $P_4$ concentration (Figure 4). The linear regression of PDV $P_4$ net flux on arterial plasma $P_4$ concentration had a coefficient of 35.06, an intercept of $-20.88$, and a $r^2$ of .66. Progesterone metabolism by the peripheral body (micrograms per hour) was determined as the difference between infusion rate and splanchnic tissue flux and was related linearly to arterial plasma $P_4$ concentration (Figure 5). The linear regression of peripheral body $P_4$ net flux on arterial plasma $P_4$ concentration had a coefficient of 430.28, an intercept of $-64.93$, and a $r^2$ of .57. Actual infusion rates ranged between 30.6 and 2,849 $\mu$g/h. Plasma arterial concentration increased linearly with increased infusion rate (Figure 6). The linear regression of arterial plasma $P_4$ concentration on infusion rate had a coefficient of .0013 $\pm$ .0001, an intercept of .7312 $\pm$ .1641, and a $r^2$ of .66.

**Discussion**

Hepatic venous blood and plasma flows were similar to those previously reported for ewes (Bergman et al., 1970; Freely and Ferrell, 1991, 1994). In the present study, hepatic venous blood flow tended to be greater, and splanchnic $O_2$ consumption was greater in the high-nutrition treatment, indicating that the feed intake assignments were successful in achieving a divergence in splanchnic metabolism.

Arterial plasma $P_4$ concentrations were within the normal physiological range during all $P_4$ infusion rates. Low concentrations (.3 ng/mL of arterial plasma) were similar to jugular venous plasma concentrations shortly after ovulation (Stabenfeldt et al., 1969), and high concentrations (5.1 ng/mL of arterial plasma) were similar to jugular venous plasma concentrations in pregnant ewes 15 d prepartum (Kitts et al., 1983).

In the current study, rates of $P_4$ metabolism by splanchnic tissue did not differ between nutritional treatments. The lack of correlation between $P_4$ metabolism and $O_2$ consumption by splanchnic tissues indicates that $P_4$ metabolism by the splanchnic tissue does not increase with an increase in tissue metabolic rate. Perhaps liver functions related to detoxification and metabolism of potential harmful metabolites will occur independently of delivery rate and thus are not

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**Table 1. Least squares means for blood and plasma flow (L/h)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hepatic arterial Mean ± SE</th>
<th>Portal venous Mean ± SE</th>
<th>Hepatic venous Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood flow</td>
<td>Lowa</td>
<td>20.1 ± 2.1</td>
<td>98.3 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>Higha</td>
<td>32.5 ± 1.9</td>
<td>100.1 ± 2.8</td>
</tr>
<tr>
<td>Plasma flow</td>
<td>Low</td>
<td>17.7 ± 1.8</td>
<td>69.0 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>23.2 ± 2.0</td>
<td>70.0 ± 2.2</td>
</tr>
</tbody>
</table>

*aLow nutrition treatment ewes received 63 kcal of ME/BW kg$^{.75}$ daily (n = 5). High nutrition treatment ewes received 99 kcal of ME/BW kg$^{.75}$ daily (n = 5).

Treatment means within vessel differed (P < .10).

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**Figure 1.** The relationship between oxygen consumption and net progesterone uptake in the portal-drained viscera (PDV). The linear regression had a coefficient of .04 ± .07, an intercept of 103.2 ± 5.2, and $r^2$ of .008.

**Figure 2.** The relationship between hepatic oxygen consumption and net hepatic progesterone uptake. The linear regression had a coefficient of .004 ± .13, and intercept 124.1 ± 10.2, and $r^2$ of .0002.
The relationship between net splanchnic tissue progesterone uptake and arterial plasma concentration in ewes receiving a jugular venous infusion of progesterone. The linear regression had a coefficient of $67.70 \pm 3.91$, an intercept of $-20.36 \pm 8.66$, and a $r^2$ of .88.

Net splanchnic P4 flux expressed as a fraction of infusion rate (.18) is less than hepatic blood flow expressed as a fraction of cardiac output (.36), indicating that other tissues have a greater rate of P4 uptake. Cardiac output was calculated as a function of metabolic body size (15.65 [L/h]-kg$^{-75}$; Hales and Fawcett, 1993). In this study, P4 uptake by the peripheral body accounted for 82% of the whole-body uptake, which is similar to the 73% estimated by Bedford et al. (1974) in isotope infusion studies.

In the current study, whole-body P4 metabolism can be described with the following mathematical model:

\[
\begin{align*}
U_{\text{splanchnic}} &= A_{\text{splanchnic}} + B_{\text{splanchnic}} \\
&\times ([P4]_{\text{arterial plasma}}) \\
U_{\text{PDV}} &= A_{\text{PDV}} + B_{\text{PDV}} \\
&\times ([P4]_{\text{arterial plasma}}) \\
U_{\text{peripheral}} &= A_{\text{peripheral}} + B_{\text{peripheral}} \\
&\times ([P4]_{\text{arterial plasma}}) \\
U_{\text{liver}} &= U_{\text{splanchnic}} - U_{\text{PDV}} \\
U_{\text{secretion}} &= U_{\text{splanchnic}} + U_{\text{peripheral}}
\end{align*}
\]

where $U$ = net uptake of progesterone by the tissue, $A$ = progesterone uptake by the tissue when P4 arterial plasma concentration equals zero, and $B$ = the coefficient that relates net P4 uptake to arterial plasma P4 concentration. Regression coefficients developed for Figures 3, 4, and 5 were used to parameterize Equations [1] to [3]. Hepatic net flux in the model is calculated as the difference between splanchnic and PDV net flux rates. Calculating hepatic P4 flux as the difference allows application of the model in nonsurgically prepared sheep from which portal venous or hepatic venous blood is not available. Using the model to simulate hepatic flux in the current data set resulted in predicted hepatic flux rates that were in close agreement with measured hepatic flux rates.

The linear form was selected for Equations [1] to [3]. Higher-order polynomial and nonlinear equations were fit to the data in Figures 3 to 5, but these equation forms were not significantly different from...
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Hypothesis that blood/plasma concentration is related linearly to secretion.

Figure 6. The relationship between jugular venous infusion of progesterone and arterial plasma progesterone concentration. The linear regression had a coefficient of .0013 ± .0001, and intercept of .7312 ± .1641, and a r² of .66.

the linear form fit with a single coefficient. Because net splanchnic P₄ flux did not differ between nutritional treatments, data from both treatments were combined in the regression analysis. As a consequence of the selection of the linear form for Equations [1] to [3], liver P₄ metabolism (Equation [4]) and P₄ secretion rate (Equation [5]) are related linearly to plasma arterial P₄ concentration (Figure 7). General behavior of the model was evaluated with the data reported by Bedford et al. (1972). When whole-body secretion rates (micrograms/minute) reported by Bedford et al. (1972) are regressed on jugular blood concentration (nanograms per milliliter), the data fit a linear model (mean ± SE; slope = 4.74 ± .25; intercept = −.12 ± 4.6; R² = .93), supporting the hypothesis that blood/plasma concentration is related linearly to secretion.

Conclusion

The net P₄ flux across the PDV and liver is not influenced by aerobic metabolic activity of the tissue, indicating that P₄ metabolism by splanchnic tissues is not influenced by nutrition. The rate at which splanchnic tissues metabolize P₄ is related linearly to arterial P₄ concentration.

Implications

Net splanchnic tissue uptake accounts for 18% of the whole-body progesterone uptake. Progesterone uptake by the splanchnic tissues does not change when blood flow to and oxygen consumption by splanchnic tissues are reduced by feed restriction. These findings indicate that changes in progesterone concentration following changes in feed intake are not the result of alteration in progesterone metabolism by the portal-drained viscera and liver.

Literature Cited


Figure 7. The relationship between simulated hepatic tissue progesterone uptake (—) or simulated progesterone secretion rate (−−) and arterial plasma progesterone concentration.


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