0031-3998/89/2606-0633$02.00/0
PEDIATRIC RESEARCH

Vol. 26, No. 6, 1989
Printed in U.S.A.

Determination of the Production and Metabolic Clearance Rates of 1,25-Dihydroxyvitamin D_3 in the Pregnant Sheep and its Chronically Catheterized Fetus by Primed Infusion Technique

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ABSTRACT. Because little is known regarding the determinants of plasma 1,25-dihydroxyvitamin D_3(1,25(OH)$_2D_3$), or its fate in the fetus, we used a primed infusion technique, using physiologic amounts of high specific activity ^{3}H-1,25(OH)$_2D_3$ to study the *in vivo* production rate (PR) and metabolic clearance rate (MCR) of 1,25(OH)$_2D_3$ in chronically catheterized maternal and fetal sheep during the last month of gestation (term = 145 d). The fetal MCR of 1,25(OH)$_2D_3$ was calculated at steady state, achieved within 2 h, and was found to be 2.53 ± 0.19 mL/min (mean ± SEM) compared to the maternal value of 15.9 ± 0.94 mL/min. When expressed on a body wt basis, the fetal MCR of 1.22 ± 0.09 mL/min/kg was more than 4-fold higher than the corresponding maternal value of 0.27 ± 0.02 mL/min/kg. Measurement of endogenous plasma 1,25(OH)$_2D_3$ by RIA revealed mean fetal values of 89 ± 10 pg/mL compared to the maternal value of 65 ± 9 pg/mL. Fetal daily PR of 0.33 ± 0.024 μg/d was 22% of the maternal PR of 1.49 ± 0.11 μg/d. However, calculation of PR on a body wt basis revealed a fetal value of 0.159 ± 0.012 μg/d/kg that was more than 6-fold higher than the maternal value of 0.025 ± 0.002 μg/d/kg. Thus, fetal plasma concentrations of 1,25(OH)$_2D_3$ are sustained in the face of a high clearance rate of the hormone. The high MCR may be related to the high *in vivo* circulating concentrations of 1,25(OH)$_2D_3$, fetal to maternal placental transfer or target tissue uptake. In view of the high turnover of 1,25(OH)$_2D_3$, we suggest that this hormone has a biologic importance that warrants further investigation. (*Pediatr Res* 26: 633–638, 1989)

Abbreviations

PR, production rate
MCR, metabolic clearance rate
1,25(OH)$_2D_3$, 1,25-dihydroxyvitamin D_3
24,25(OH)$_2D_3$, 24,25-dihydroxyvitamin D_3
25OHD$_3$, 25-hydroxyvitamin D_3
PTH, parathyroid hormone

1,25(OH)$_2D_3$, the active metabolite of vitamin D_3, is well recognized as playing a central role in postnatal calcium homeostasis (1) via actions on the classic target organs, intestine, kidney, and bone. More recently, a number of reports have suggested that 1,25(OH)$_2D_3$ has wider physiologic functions related to the maturation and differentiation of the immune system (2, 3) and epidermal cells (4), as well as endocrine secretion (5, 6).

The renal synthesis of 1,25(OH)$_2D_3$ is tightly regulated according to the body's need for minerals. The dominant stimulators of 1,25(OH)$_2D_3$ synthesis are PTH, vitamin D deficiency, and hypophosphatemia (7–9), although other factors such as calcitonin, calcium, estrogens, thyroid hormones, prolactin, and growth hormone have been implicated as having a role under certain circumstances. Plasma concentrations of 1,25(OH)$_2D_3$ are elevated during periods of accelerated growth (10), pregnancy (11), lactation (12, 13), and during experimental dietary calcium or phosphorus restriction (14).

However, because plasma concentrations are the resultant of renal synthesis, target tissue uptake and catabolism and excretion, estimates of 1,25(OH)$_2D_3$ production based on plasma concentrations may be misleading if the rate of clearance is not known. Dietary calcium deficiency in pigs (14) leads to elevated plasma concentrations of 1,25(OH)$_2D_3$, as well as an enhanced MCR of 1,25(OH)$_2D_3$. The true renal response to a physiologic or experimental perturbation of mineral metabolism may be understimated if the clearance rate is not determined simultaneously.

Little is known regarding the determinants of plasma concentrations of 1,25(OH)$_2D_3$ during fetal life when mineral acquisition is high to meet the requirements of rapid skeletal growth. The fetus of the sheep, like that of other species, is hypercalcemic and hyperphosphatemic relative to the mother (15, 16) and several studies have demonstrated a fetal to maternal plasma concentration gradient for 1,25(OH)$_2D_3$ during pregnancy in this species (16–19). A similar fetal to maternal gradient for 1,25(OH)$_2D_3$ has been reported in one study of the cow (20) but not in another (21). Other species such as the human (22–23) and the pig (24, 25) demonstrate a maternal to fetal plasma gradient for total 1,25(OH)$_2D_3$.

Homogenates and isolated cells of the fetal kidney of several species have been shown to possess the capacity to metabolize 25OHD$_3$ to 1,25(OH)$_2D_3$ *in vitro* (26) and indirect studies in the human in which 1,25(OH)$_2D_3$ concentrations in the umbilical artery exceeded those in the umbilical vein (27) suggest that the human fetus synthesizes 1,25(OH)$_2D_3$ *in vivo*. The *in vivo* synthesis of 1,25(OH)$_2D_3$ has been confirmed directly in the sheep (16) where bilateral fetal nephrectomy resulted in markedly reduced plasma concentrations of 1,25(OH)$_2D_3$. Moreover,

Received April 5, 1989; accepted July 25, 1989.
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Supported in part by NIH Grants 5RO1AR36487-03, 5P50HD20148-03, Children's Hospital Research Foundation, and the Perinatal Research Institute.

$1,25(OH)_2D_3$ deficiency in this experimental situation was found to have a deleterious impact on fetal mineral homeostasis because marked fetal hypocalcemia and hyperphosphatemia developed (16, 28).

Because of the putative role of fetal $1,25(OH)_2D_3$ in fetal mineral homeostasis, we hypothesized that the actively growing and mineralizing fetus has a high requirement for $1,25(OH)_2D_3$. We therefore sought to develop a primed infusion method, using physiologic amounts of ^{3}H-$1,25(OH)_2D_3$, to study the PR and MCR of $1,25(OH)_2D_3$ in the pregnant sheep and its chronically catheterized fetus.

The results indicate that the circulating concentrations of $1,25(OH)_2D_3$ in the fetus are maintained despite an MCR that is 6-fold higher than the corresponding maternal value when expressed on a body wt basis. Thus, for a fetus that weighs approximately 3% of the maternal body wt, total fetal daily production of $1,25(OH)_2D_3$ represents approximately 30% of the maternal daily production rate of $1,25(OH)_2D_3$. We suggest that this high rate of $1,25(OH)_2D_3$ production in the fetus is indicative of a crucial role for this hormone during intrauterine development.

MATERIALS AND METHODS

Animals. In these studies, we used timed-dated pregnant sheep supplied by Morris & Co., Reisterstown, MD. Each sheep was maintained on a normal vitamin D-replete diet (Rumichow, Purina Ralston, St. Louis, MO) for 3 wk before the study with *ad libitum* access to water. All surgical and experimental procedures were performed in accordance with protocols that had been approved by the Institutional Animal Care and Use Committee of the University of Cincinnati. After surgery, animals were housed in individual stainless steel metabolic cages. Maternal animals were weighed before the experiment and fetal body wt was calculated using the nomogram of Huggett and Widdas (29).

Surgical procedure. Pregnant ewes (110 ± 2 d of gestation, term = 145 d) weighing between 50 and 60 kg were fasted for 48 h and water withheld for 24 h before surgery. Thirty min before surgery, the ewes were sedated with 5–10 mg of intravenous Valium (Lemmon Co., Sellersville, PA) and 250 mg intravenous of Thiopental, (Parke-Davis, Morris Plains, NJ). Subsequently, ewes received a hyperbaric spinal anesthesia induced with 12 mg Pontocaine hydrochloride (Breon Laboratories, NY). The ewe was placed in the supine position, restrained, and the abdomen and left flank cleansed and draped aseptically. After a midline abdominal incision, the hindlimb of the fetus was located by palpating the uterine horn. The uterine horn was then incised for 2–3 cm (taking care to avoid placental cotyledons) and the fetal hindlimb exteriorized to the level of the groin and wrapped in a warm saline sponge. All fetal surgery was performed after the administration of local infiltration of anesthesia (1% xylocaine). Through a 2-cm incision in the fetal groin, the femoral artery and vein were dissected free, tied distally, and cannulated with polyvinyl catheters. The catheters were secured and the fetal incision closed.

During the closure of the ewe's abdominal incision, the catheters were tunneled subcutaneously to the left flank, passed through a small skin incision, tethered to the skin, and placed in a canvas pouch. A 3–4 cm incision was then made in the ewe's left groin over the femoral artery. The femoral artery and vein were dissected free, ligated distally, and cannulated with a polyvinyl catheter to the level of the distal abdominal aorta and inferior vena cava, respectively. The catheters were secured and passed through a subcutaneous tunnel to the ewe's left flank pouch. All vascular catheters were flushed daily with heparinized saline solution (1000 U/mL for maternal; 400 U/mL for fetal). The stopcock for each catheter was wrapped in an alcohol-soaked sponge and placed in a tightly closed plastic bag within the canvas storage pouch. Postoperatively, all ewes were housed in stainless steel movable carts. They were given measured amounts of feed and had *ad libitum* access to water.

Radioisotope. High sp act 1α,25-dihyroxy-[26,27-methyl-^{3}H] cholecalciferol (^{3}H-$1,25(OH)_2D_3$:180 Ci/mmol) was purchased from Amersham, Arlington Heights, IL. It was repurified before use by HPLC system described below and stored in absolute ethanol. Aliquots were subsequently taken for use on the day of study and dried under vacuum.

Experimental protocol. Clearance studies were performed on a total of 10 fetal sheep and five pregnant sheep. In studies where clearances were determined on pregnant sheep and their fetus (n = 5), fetal studies preceded maternal studies by 3 d.

A 6-mL basal blood sample was taken for measurement of plasma $1,25(OH)_2D_3$ concentration by RIA before clearance measurements. After a bolus intravenous injection of ^{3}H-$1,25(OH)_2D_3$ averaging 90 ± 10 (SEM, n = 10) nCi/kg for fetuses and 7.22 ± 0.36 nCi/min/kg for mothers (n = 5), the label was infused at a constant rate of 293 ± 32 pCi/min/kg for fetuses and 17.3 ± 1.1 pCi/min/kg for mothers (see Table 1). The relative proportions of bolus to infusate were determined to be 40 and 60% of the total dose, respectively, and were similar to that previously used in postnatal pigs (14). Actual doses used were based on maternal and estimated fetal body wt and were calculated to be sufficient to obtain statistically significant radioactive counting rates using the volumes of plasma analyzed. The label was dissolved in 10% autologous plasma in 0.9% sterile saline. Fetal and maternal infusions were both carried out at a rate of 0.025 mL/min and were supplied by a syringe pump (Sage Instruments, Cambridge, MA). Fetal (1.1 mL) and maternal (5 mL) blood samples were taken at 60, 120, 150, 180, 200, 220, and 240 min after the start of the infusion. After separation of plasma, 0.5 and 2 mL of fetal and maternal plasma, respectively, were used for radioactivity determination.

Laboratory methods. Radioactivity in the plasma samples was determined by direct liquid scintillation counting (40 min/sample) on a TriCarb 460CD Spectrophotometer (Packard Instrument Co., Downers Grove, IL) and values were corrected for quenching using the external standard channels ratio. Counting efficiencies averaged 54%.

$1,25(OH)_2D_3$ was measured in fetal and maternal plasma samples (14). Briefly, lipophillic extracts of plasma were prepared by extraction with acetonitrile and subjected to preliminary purification (30) on Waters C18 reverse phase Sep-Pak cartridges (Waters, Milford, MA). Vitamin D metabolites were subsequently chromatographed on a 5-μ silica radial compression pak 5B equilibrated and eluted with hexane/2-propanol/methanol (92/4/4, vol/vol/vol) using a model 6000A pump at 2 mL/min and 200 psi pressure. Fractions eluting in the position of authentic $1,25(OH)_2D_3$ were collected, redissolved in ethanol, and subjected to RIA. The interassay coefficient of the assay was 20%.

A lipid extract of 0.5 mL plasma taken from a separate fetus 4 h after receiving 20 μCi ^{3}H-$1,25(OH)_2D_3$ as a bolus was prepared (31) before solid phase chromatography and HPLC as previously described. The chromatogram was extended for 40 min to identify metabolites that were less polar as well as more polar than $1,25(OH)_2D_3$. The identity of ^{3}H-activity was deter-

Table 1. *Gestational age, body wt, and doses of ^{3}H-$1,25(OH)_2D_3$ used in primed-infusion studies of fetal and maternal sheep*

	Fetal (n = 10)	Maternal (n = 5)
Gestational age (d)	127 ± 3	133 ± 5
Body wt (kg)	2.08 ± 0.07	58.7 ± 2.04
Bolus dose of ^{3}H-$1,25(OH)_2D_3$ (nCi/kg)	90 ± 10	7.22 ± 0.36
Infusion of ^{3}H-$1,25(OH)_2D_3$ (pCi/min/kg)	293 ± 32	17.3 ± 1.1