SYMPOSIUM: CALCIUM METABOLISM AND UTILIZATION

Calcium and Vitamin D Metabolism in the Dairy Cow[1]

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ABSTRACT

Most dairy cows experience some degree of hypocalcemia during the periparturient period. There is, however, a subgroup of dairy cows that experience a breakdown in their ability to maintain plasma calcium and, consequently, suffer from severe hypocalcemia. This condition is also known as milk fever and usually occurs in cows in their third or greater lactation. The precise metabolic lesions responsible for the onset of milk fever have not yet been defined. Research has shown that milk fever is not the result of inadequate production of calcitropic hormones (parathyroid hormone and 1,25-dihydroxyvitamin D), but rather is more likely a result of inadequate receptor numbers or receptor dysfunction in the target cell of these hormones. This report reviews vitamin D and calcium metabolism, giving emphasis to 1,25-dihydroxyvitamin D receptor regulation and function as related to the periparturient dairy cow. The report also focuses on providing insights into nutritional (anionic diets) and endocrine strategies that have proved useful in milk fever management.

(Key words: calcium, vitamin D, parathyroid hormone, milk fever)

Abbreviation key: CaBP = calcium-binding protein, DCAB = dietary cation-anion balance, NAF = nuclear activation factor, PTH = parathyroid hormone, RAF = receptor auxiliary factor, RAR = retinoic acid receptor, RXR = retinoid X receptors, VDR = vitamin D receptor, VDRE = vitamin D response elements, $25\text{-}(OH)D_3$ = 25-hydroxycholecalciferol, $1,25\text{-}(OH)_2D$ = 1,25-dihydroxyvitamin D, $1,25\text{-}(OH)_2D_3$ = 1,25-dihydroxycholecalciferol, $1,24,25\text{-}(OH)_3D_2$ = 1,24,25-trihydroxyvitamin D_2, $24,26\text{-}(OH)_2D_2$ = 24,26-dihydroxyvitamin D_2, $1,24,26\text{-}(OH)_3D_2$ = 1,24,26-trihydroxyvitamin D_2.

INTRODUCTION

Calcium is required for the normal functioning of a wide variety of tissues and physiologic processes. Calcium is needed for bone formation, muscle contraction, nerve transmission, blood clotting, and as a second messenger regulating the actions of many hormones (30). In general, vertebrates maintain Ca with remarkable precision. An exception is the aged parturient dairy cow, which develops the metabolic disease, milk fever. The hypocalcemia of milk fever results from a breakdown in the homeostatic mechanisms needed to replenish Ca lost from the extracellular Ca pool at the initiation of lactation (30). Diet and bone are the primary sources of Ca in mammals and birds (11). The enhancement of intestinal Ca absorptive and bone Ca resorptive processes are under the influence of the Ca-regulating hormones, parathyroid hormone (PTH), which is secreted by the parathyroid glands, and 1,25-dihydroxyvitamin D [$1,25\text{-}(OH)_2D$], which is produced in the kidney (11). Because many endocrine disorders result from primary hormone deficiencies or excess, failure to secret PTH or $1,25\text{-}(OH)_2D$ was once hypothesized as the primary defect in cows with milk fever. However, these hypotheses were disproved when researchers found that PTH and $1,25\text{-}(OH)_2D$ were higher in blood of cows suffering from milk fever (33, 59). The cellular lesions involved in milk fever, therefore, still remain to be identified. This article reviews the known homeostatic controls involved in the regulation and maintenance of Ca, and empha-

sis is given to the biological basis and control of milk fever in dairy cows.

Vitamin D₂ and Vitamin D₃ Metabolism

Both vitamin D_2 and vitamin D_3 are used for supplementation of animal and human diets in the United States. Vitamin D_3 is the form of vitamin D that is synthesized by vertebrates (29), and vitamin D_2 is the major naturally occurring form of the vitamin in plants. Vitamin D_3 also occurs naturally in plants and may constitute as much as 1% of the total vitamin D in alfalfa (38). Whether or not vitamin D_3 occurs naturally in other plant species is presently unknown. Nocturnal herbivores, therefore, would have evolved with vitamin D_2 as their major (if not only) source of vitamin D. Vitamin D_3, however, would have served as the major vitamin D source in most diurnal species.

Figure 1 summarizes the major metabolic pathways for the metabolism of vitamin D_3. Activation of vitamin D_3 is initiated by C_{25} hydroxylation in the liver to form 25-hydroxycholecalciferol [25-(OH)D_3], which is the major circulating form of vitamin D and, in the normal cow, is present in the plasma at concentrations of 20 to 50 ng/ml. Concentrations of <5 ng/ml would be indicative of vitamin D deficiency, and concentrations of 200 to 300 ng/ml would indicate vitamin D toxicosis.
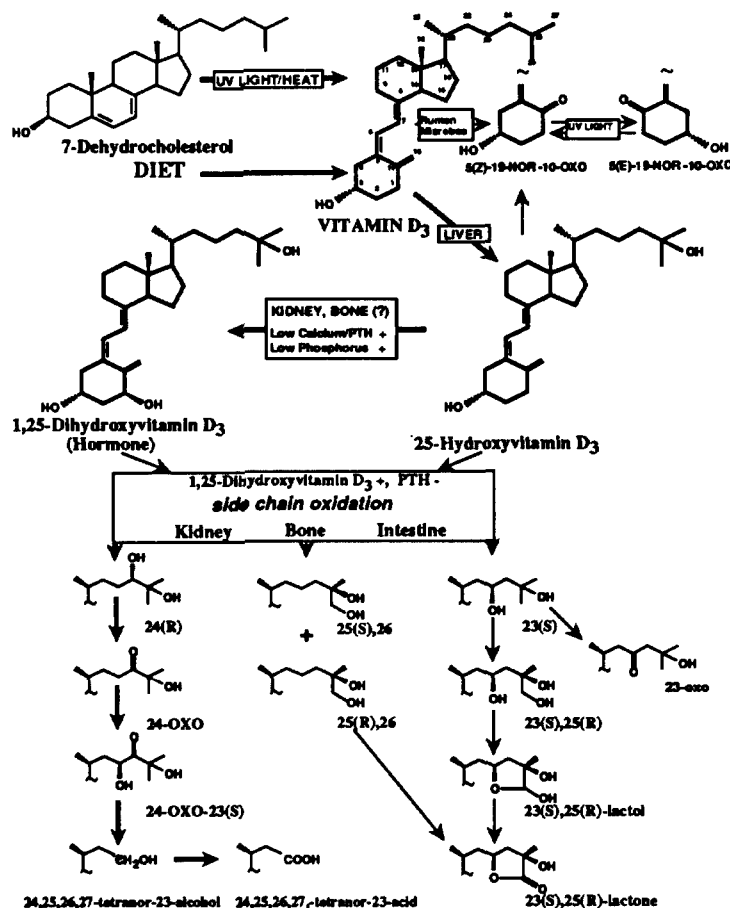

Figure 1. Pathways for the metabolism of vitamin D_3. The bold arrows indicate the major pathways for activation and catabolism. PTH = Parathyroid hormone.

The 25-(OH)D$_3$ is then 1α-hydroxylated in the kidney to form 1,25-dihydroxycholecalciferol [1,25-(OH)$_2$D$_3$], the active form of vitamin D$_3$. The control of the 1α-hydroxylation process is influenced by many factors (11). However, the protein hormone PTH is most active and most important in regulating the 1α-hydroxylase enzyme (11). The concentration of PTH in plasma is regulated mainly by plasma Ca. As Ca concentration in the plasma declines <10 mg/dl, the parathyroid glands are stimulated to produce PTH. In turn, PTH stimulates the activation of 25-(OH)D$_3$ by up-regulating 1α-hydroxylase enzyme in the kidney in the kidney to form 1,25-(OH)$_2$D.[3] If plasma Ca is >10 mg/dl, PTH secretion is depressed, and 1,25-(OH)$_2$D synthesis is depressed. In the adult nonpregnant, nonlactating cow, 1,25-(OH)$_2$D circulates in a range of 5 to 20 pg/ml. In late pregnancy, circulating 1,25-(OH)$_2$D may rise to a range of 20 to 50 pg/ml. During parturition and initiation of lactation, 1,25-(OH)$_2$D rises to values ranging from 100 to >300 pg/ml during severe cases of hypocalcemia (33).

Both 25-(OH)D$_3$ and 1,25-(OH)$_2$D$_3$ are subject to catabolic enzymes, which are present mainly in the intestine and kidney. Catabolism is initiated by C$_{24}$ oxidation (50, 82). The C$_{24}$-oxidized metabolites then act as substrates for further oxidation at C$_{23}$ (61, 62, 74), leading to cleavage between C$_{23}$ and C$_{24}$ (13, 73). Minor pathways of catabolism include C$_{26}$ oxidation and formation of 26,23-lactones (39, 83). Under the influence of vitamin D toxicity or if plasma 1,25-(OH)$_2$D concentrations are elevated by giving exogenous 1,25-(OH)$_2$D, the C$_{24}$ oxidative pathway of catabolism is up-regulated in both the intestine and kidney. However, if endogenous synthesis of 1,25-(OH)$_2$D is stimulated by a low Ca diet, the catabolic enzymes in the kidney are depressed, but these same enzymes are stimulated in the intestine (22). The difference in these tissue responses to exogenous and endogenous 1,25-(OH)$_2$D is that the kidney possesses receptors for PTH. High circulating PTH depresses C$_{24}$ oxidative enzymes in the kidney

and favors stimulation of the 1α-hydroxylase. The intestine, however, does not contain receptors for PTH. The C$_{24}$ oxidative enzymes in the intestine are, therefore, regulated mainly by 1,25-(OH)$_2$D.

As with vitamin D$_3$, the major physiologic pathway for activation of vitamin D$_2$ is initiated by hydroxylation at C$_{25}$ to form 25-(OH)D$_2$. However, the differences in side-chain chemistry between vitamin D$_2$ and vitamin D$_3$ offer opportunities for divergence in side-chain oxidation of the two sterols. For example, the 24-position in vitamin D$_2$ is a tertiary carbon, as is the 25-position in vitamin D$_2$ and vitamin D$_3$. In addition, the 24-position for vitamin D$_2$ is an allelic carbon, making it a far more reactive site than the corresponding position in vitamin D$_3$. On the basis of these chemical differences, 24-hydroxylation of vitamin D$_2$ may be a quantitatively significant pathway for further metabolism of vitamin D$_2$. In support of this argument is the recent finding of Horst et al. (34), who provided the first quantitative report evaluating the 24-hydroxylation of vitamin D$_2$ to form 24-(OH)D$_2$ as a relatively minor but intact pathway for vitamin D$_2$ activation. The 24-(OH)D$_2$ can then be 1α-hydroxylated to form 1,24-(OH)$_2$D$_2$, which, like 1,25-(OH)$_2$D$_3$, is active in target cells. Metabolism of 25-(OH)D$_2$ and 1,25-(OH)$_2$D$_2$ deviates somewhat from the classic vitamin D$_3$ pathways. For example, the 24-hydroxy derivatives of 25-(OH)$_2$D$_2$ and 1,24,25-trihydroxyvitamin D$_2$ [1,24,25-(OH)$_3$D$_2$], can be further hydroxylated at C$_{28}$ or C$_{26}$ (73). However, to date, neither 24-keto nor C$_{23}$ oxidized vitamin D$_2$ metabolites have been identified. The C$_{22}$ alkene and C$_{24}$ (S)-methyl groups in vitamin D$_2$ apparently preclude these classic side-chain oxidation reactions. Also, the compound 24-(OH)D$_2$ [and probably 1,24-(OH)$_2$D$_2$, as well] is a poor substrate for C$_{25}$ hydroxylation. Rather, these metabolites proceed through the C$_{26}$ hydroxylation pathway (49) to form 24,26-dihydroxyvitamin D$_2$ [24,26-(OH)$_2$D$_2$] and 1,24,26-trihydroxyvitamin D$_2$ [1,24,26-(OH)$_3$D$_2$] (Figure 2).

Vitamin D$_2$ and vitamin D$_3$ have similar biological activity in most mammals; however, birds and New World monkeys discriminate against vitamin D$_2$ in favor of vitamin D$_3$ (43, 87). Recent research, fostered by the discovery of sensitive analytical techniques and availability of high specific activity [^{3}H]-labeled vita-

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[3]Lack of subscript indicates either vitamin D$_2$ or vitamin D$_3$.

min D, indicates that differences exist in the metabolism of vitamin D_2 and vitamin D_3 in mammals also. Most notable was the apparent discrimination against vitamin D_2 by pigs and cows and the apparent preference for vitamin D_2 by rats (35). These same experiments and more recent studies (41) showed that chicks discriminate against vitamin D_2 as a result of enhanced clearance of vitamin D_2 metabolites from plasma.

Cellular Mechanism of Action of 1,25-(OH)₂D₃

The steroid hormone, 1,25-$(OH)_2D_3$, regulates Ca homeostasis, cell proliferation and differentiation, and immune cell function (80,

81). The 1,25-$(OH)_2D_3$ circulates in blood bound primarily to vitamin D-binding protein, and typically <5% of the hormone circulates in the free state. The free form of the hormone enters all cells freely because of its lipophilic nature. The hormone accumulates only in target tissues that possess intracellular receptors for 1,25-$(OH)_2D_3$ (VDR) (Figure 3). As with other receptors for steroid hormones, VDR exhibits high affinity for its ligand, 1,25-$(OH)_2D_3$. Characteristic of other members of the steroid-thyroid superfamily, VDR possesses two distinct functional domains: a hormone-binding domain in the COOH terminus and a zinc finger motif that makes up the DNA-binding domain in the NH_2-terminus.
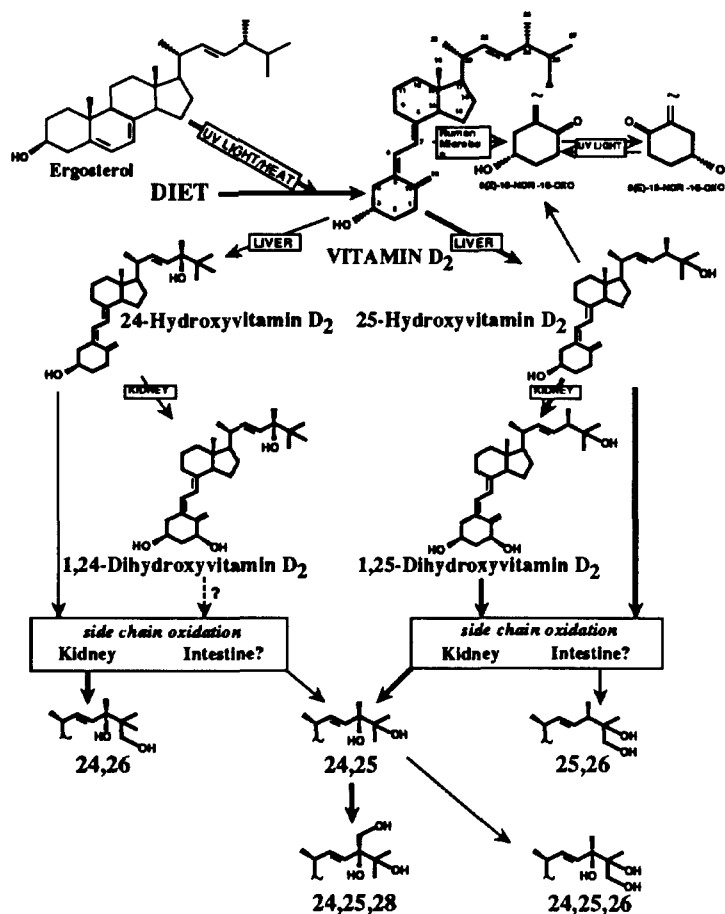

Figure 2. Pathways for the metabolism of vitamin D_2. The bold arrows represent the major pathway for activation and deactivation.
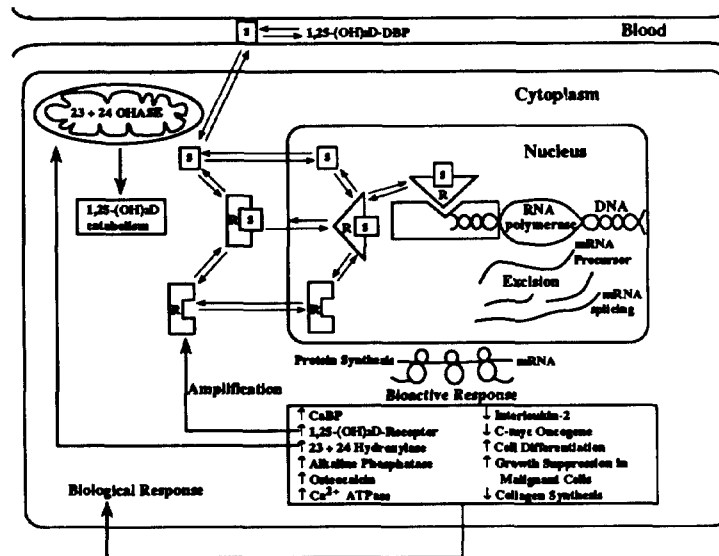
Figure 3. Metabolism of action of 1,25-dihydroxyvitamin D.

The binding of 1,25-(OH)₂D₃ to VDR in specific target tissues results in VDR binding to vitamin D response elements (VDRE) on genes regulated by 1,25-(OH)₂D₃. Early on, however, it was recognized that VDR itself was insufficient to bind tightly to VDRE (54, 55, 86). Several laboratories noted that the binding of VDR to VDRE required the presence of other nuclear proteins to form a tight complex with the VDRE. These other proteins were named nuclear activation factor (NAF) (53, 85) or receptor auxiliary factor (RAF) (55), depending on the lab that described it. Shortly after these observations, that an NAF or RAF was required for VDR to bind tightly to its VDRE, a family of proteins was identified and given the name retinoid X receptors (RXR) (57). These receptors were named orphan receptors for a short time because, although they resembled the retinoic acid receptor (RAR) family, they did not bind all-trans-retinoic acid (28, 53). Recently 9-cis-retinoic acid was discovered (28, 53) to be the cogent ligand for the RXR family. These RXR form heterodimers with many steroid hormone receptors, including VDR (48, 94, 95). Like VDR, both RAR and thyroid hormone receptor had been previously found to require an NAF-

like molecule to bind DNA (8). It now appears that RXR is the previously described NAF or RAF (94) and that RXR heterodimerizes with VDR, resulting in both greater binding to VDRE and enhanced 1,25-(OH)₂D₃-mediated gene transcription in vitro (48, 94, 95). Recent evidence suggests that regulation of VDR-responsive genes may be even more complex (8). At least two classes of VDRE appear to be on 1,25-(OH)₂D-regulated genes, genes that are activated by 1,25-(OH)₂D₃ and VDR alone, and those that are activated by VDR:RXR heterodimers. These interactions result in the specific regulation of protein synthesis required for maintenance of Ca homeostasis. Clearly, Ca homeostasis may be influenced not only by vitamin D [1,25(OH)₂D₃] but also may be controlled, in part, by metabolites of vitamin A (9-cis-retinoic acid). In future studies of the role of vitamin D in Ca homeostasis, it will be important to consider the role of 9-cis-retinoic acid and the factors that regulate its production.

Factors Regulating VDR Function

Regulation of cell VDR numbers is another important mechanism for modulating cellular responsiveness to 1,25-(OH)₂D₃ because the

biological activity of $1,25\text{-}(OH)_2D_3$ in cells is proportional to cell VDR number (9, 27). Several hormones (retinoic acid, glucocorticoids, and estrogen) have been shown to increase the synthesis of VDR (27), thus increasing cell responsiveness to $1,25\text{-}(OH)_2D_3$ in the specific tissues affected. One somewhat unique feature of VDR is VDR autoregulation by $1,25\text{-}(OH)_2D_3$ itself. The $1,25\text{-}(OH)_2D_3$ regulates VDR both quantitatively as $1,25\text{-}(OH)_2D_3$-dependent up-regulation and qualitatively via posttranslational phosphorylation of VDR (27). In cell culture, $1,25\text{-}(OH)_2D_3$ has been shown to up-regulate VDR numbers three- to four-fold (10, 76, 77). In vivo infusions of $1,25\text{-}(OH)_2D_3$ to rats results in increases in VDR concentrations of 30, 200, and 37% in intestine, kidney, and bone, respectively (21, 32, 60, 77). The dairy cow responds in a manner similar to that of the rat. Naito et al. (60) found significant increases in intestinal VDR of cows infused with $1,25\text{-}(OH)_2D_3$. These results may explain, in part, the mechanism by which exogenous vitamin D compounds help to prevent milk fever. However, the relationship of these observations to normal animal physiology remains unclear, because the $1,25\text{-}(OH)_2D_3$ was delivered exogenously, and the doses were pharmacological.

Tissue concentration of $1,25\text{-}(OH)_2D_3$ (hormone available for VDR binding) determines, in part, the biological response of a cow to a Ca crisis or stress. The higher a tissue's concentration of $1,25\text{-}(OH)_2D$, the greater is the biological response (9, 10, 27, 76, 78, 79). A caveat to this process is that the metabolism or tissue half-life of $1,25\text{-}(OH)_2D_3$ plays a role in determining the magnitude of a biological response to hormone (76, 78, 79). In addition to stimulating the synthesis of Ca-regulating proteins, $1,25\text{-}(OH)_2D_3$ stimulates the synthesis of enzymes responsible for $1,25\text{-}(OH)_2D_3$ inactivation. Therefore, it has been proposed (27, 37, 61, 82) that degradative enzymes, such as vitamin D-23 and -24 hydroxylases, may be important regulators of a cellular responsiveness to $1,25\text{-}(OH)_2D_3$ because of their ability to metabolize $1,25\text{-}(OH)_2D_3$ to inactive forms. We have recently shown this to be the case. The $1,25\text{-}(OH)_2D_3$ stimulation of VDR synthesis is paralleled by marked increases in these hydroxylases (76, 78). The net result is that

increased cell VDR numbers do not lead to increased cell responsiveness, because these enzymes can inactivate hormone as fast as it enters the cell, which leads to a decline in VDR binding and termination of the biological response to $1,25\text{-}(OH)_2D_3$ (76, 78, 79). True amplification of a biological response to $1,25\text{-}(OH)_2D_3$ via mediated up-regulation VDR is observed only if these enzymes are inhibited with vitamin D analogs or ketoconazole (76, 78, 79) or when VDR is up-regulated by hormones or physiological states (pregnancy or lactation) that do not also induce the degradation enzymes.

The physiological relevance of this VDR up-regulation was further questioned by Goff et al. (21). In contrast to previous studies, elevation of plasma $1,25\text{-}(OH)_2D_3$ naturally (endogenously) by dietary Ca restriction did not result in a $1,25\text{-}(OH)_2D_3$-mediated up-regulation of intestinal VDR, and renal VDR was down-regulated. Goff et al. (21) suggested that hyperparathyroidism, secondary to dietary Ca deficiency, might be responsible for the lack of $1,25\text{-}(OH)_2D_3$-mediated up-regulation in these rats. Further studies confirmed this hypothesis by demonstrating that PTH by itself could down-regulate VDR in vivo in rat osteosarcoma cells and that co-treatment of cells with PTH and $1,25\text{-}(OH)_2D_3$ or coinfusion of PTH and $1,25\text{-}(OH)_2D_3$ to rats blocked $1,25\text{-}(OH)_2D_3$-mediated up-regulation of VDR (77).

These data suggest that $1,25\text{-}(OH)_2D_3$-mediated up-regulation of VDR is relevant only during treatment with exogenous vitamin D compounds. In contrast, during a physiological stress such as milk fever (hypocalcemia), $1,25\text{-}(OH)_2D_3$ rises because of its normal physiological regulation by PTH. The $1,25\text{-}(OH)_2D_3$-induced metabolism of $1,25\text{-}(OH)_2D_3$ is, in part, prevented by PTH (77). As plasma Ca rises, PTH secretion declines, and $1,25\text{-}(OH)_2D_3$ induces its own metabolism. This increased target tissue metabolism of $1,25\text{-}(OH)_2D_3$, in conjunction with reduced production of $1,25\text{-}(OH)_2D_3$, leads to a termination of the biological response. We concluded that PTH is a potent regulator of VDR function.

This conclusion, that PTH plays an important role in VDR regulation and function, is supported further by the data of Jurutka et al.

(46). The mechanism of action of PTH is primarily through the activation of protein kinase A. They found that protein kinase A phosphorylated VDR and that this phosphorylation resulted in an attenuation of 1,25-(OH)$_2$D$_3$-induced transcriptional activity in vitro. Similarly, we (22) have shown that infusion of 1,25-(OH)$_2$D$_3$ alone increases the expression of renal 24-hydroxylase in rats. However, the putative activation of protein kinase A by PTH infusion with 1,25-(OH)$_2$D$_3$ blocks the expression of renal 24-hydroxylase, suggesting that protein kinase A activation is a potent regulator of VDR function in vivo.

The protein kinase C pathway also has been shown to result in the phosphorylation of VDR (42). Activation of this pathway by phorbol esters results in up-regulation of VDR (Reinhardt and Horst, 1993, unpublished data). Furthermore, activation of protein kinase C and 1,25-(OH)$_2$D$_3$ treatment results in a synergistic up-regulation of VDR. The effects of this transcriptional regulation remain to be determined in vitro and in vivo. One might speculate, however, that the protein kinase A and C pathways reciprocally regulate VDR-directed transcriptional regulation.

As described earlier, tissue VDR number regulates tissue responsiveness to a 1,25-(OH)$_2$D stimulus. Various physiological states, which are important to milk fever studies, have been examined for their effect on VDR.

Effect of Age on VDR

First lactation cows almost never develop milk fever. They may experience some degree of hypocalcemia during the first days of lactation, but their intestine and bone adapt rapidly to the Ca demands of lactation. As cows age, this adaptation process is slowed, resulting in moderate to severe hypocalcemia at parturition in most older cows. Intestinal Ca absorption efficiency decreases with age in the bovine (26). Studies in rats also indicate that older rats are less able to increase absorption efficiency in response to dietary Ca stress (31). Our laboratory has shown that intestinal 1,25-(OH)$_2$D receptor number declines with age in both the rat and the cow (32) (Table 1). Thus, the older cow is less able to respond to 1,25-(OH)$_2$D than the young cow and takes longer to adapt intestinal Ca absorption

TABLE 1. Unoccupied 1,25-dihydroxyvitamin D receptor number in intestinal tissue of young and old rats and cows.

	Rat (duodenum)		Cow (colon)	
	——— (fmol/mg of protein) ———			
	$\overline{X}$	SE	$\overline{X}$	SE
Young	670[a]	55	168[a]	15
Old	185	40	19	8

[a]Significantly different from old ($P < .05$).

mechanisms to meet lactational demands for Ca, which may explain, in part, why first lactation cows rarely develop severe hypocalcemia (32, 36).

Osteoblasts are the only type of bone cell to express the 1,25-(OH)$_2$D receptor protein. Our laboratory has demonstrated that bone from older rats contains fewer 1,25-(OH)$_2$D receptors than does bone from young rats (32). Osteoblast numbers decrease with age (56), which no doubt contributes to the loss of bone receptors with age. Bone accretion and remodeling also decrease with age, resulting in bone with less surface undergoing resorption by osteoclasts at any one time (69). Thus, with advancing age, fewer osteoblasts exist to respond to PTH or 1,25(OH)$_2$D stimulation, and fewer osteoclasts exist to respond to osteoblast resorption signals, which delays the ability of bone to contribute Ca to the plasma CA pool.

Pregnancy and Lactation Effects on VDR

Efficiency of intestinal Ca absorption is increased during pregnancy and lactation (25). In most species, pregnancy and lactation are accompanied by increased plasma 1,25-(OH)$_2$D concentrations to stimulate increased intestinal Ca absorption. Another factor mediating this increased Ca absorption is an increase in intestinal 1,25-(OH)$_2$D receptors during pregnancy and lactation (19, 36, 75) (Table 2). Preliminary evidence in our laboratory indicates that the up-regulation of receptors seen during pregnancy is controlled by some factor originating in the uterus, because pseudopregnancy (induced by hysterectomy on d 9 of pregnancy, leaving ovaries intact) failed to result in up-regulation of 1,25-(OH)$_2$D receptors. In addition, intestinal tissues are hypertrophied during

TABLE 2. Unoccupied 1,25-dihydroxyvitamin D receptor number in intestinal tissue of rats and cows in various physiologic states.

	Rat (duodenum)		Cow (colon)	
	(fmol/mg of protein)			
	$\overline{X}$	SE	$\overline{X}$	SE
Nonpregnant, nonlactating	343	57	19	8
Pregnant	610[a]	30	54[a]	13
Lactating	645[a]	32	76[a]	11

[a]Significantly different from nonpregnant, nonlactating animal ($P < .05$).

pregnancy and lactation, increasing the absorptive capacity of the gut (25).

Intestinal Ca Absorption

Calcium can be absorbed from the lumen of the intestine by passive diffusion between the intestinal epithelial cells (paracellular transport) and by active transport across the epithelial cells (6). Paracellular transport is strictly related to the concentration of Ca ions in the lumen of the gut. When ionized Ca concentration over the epithelial cells exceeds 6 mM, the Nernst equation would predict net flux of Ca into the extracellular fluid. Experimental studies suggest that, if animals are fed a high Ca diet, >50% of the Ca absorbed will be by the paracellular route (63).

Efficient absorption of dietary Ca, when dietary Ca is low or when demand is very high, occurs by active transport of Ca across the intestinal epithelial cells. This process requires 1,25-(OH)₂D. Calcium concentration in the lumen of the gut is about 1000-fold higher than within the epithelial cell; thus, Ca entry into the cell occurs readily. The 1,25-(OH)₂D may facilitate this diffusion (72), but this facilitation does not appear to be a rate-limiting step to Ca absorption. Calcium must than traverse the cell to the basal lateral side of the cell. This step is facilitated primarily by the Ca-binding protein (CaBP), which is cytosolic and dependent on vitamin D. Numerous studies suggest that the rate of transcellular Ca transport is directly correlated with the amount of CaBP in the cells (68). Bronner (6) has postulated that

the synthesis of CaBP is the rate-limiting step in transcellular Ca transport. Calcium, once transported to the basolateral membrane, is extruded from the cell against an ~1000-fold concentration gradient by Ca pumps dependent on Ca-Mg-ATPase (92). These pumps operate inside the cell at much less than their maximum velocity so that they are able to extrude all the Ca presented to the basal membrane, even in the state of vitamin D deficiency (16, 85). To prevent enzyme activity from becoming rate-limiting, 1,25-(OH)₂D stimulates activity of Ca-Mg-ATPase two to three times higher.

Ca Homeostasis and Milk Fever

A schematic representation of Ca homeostasis is presented in Figure 4. Maintenance of constant plasma Ca during lactation is a formidable challenge to the dairy cow. During the course of a lactation, a cow producing 9000 kg of milk secretes 11.07 kg of Ca and 8.56 kg of P into the milk. If dietary sources of Ca are assumed at only 38% availability and dietary P at 45% availability, this cow must consume, on average, 90 to 100 g of Ca and 60 to 70 g of P/d just to meet her needs for lactation (64). An additional 25 to 30 g of Ca and 15 to 20 g of P must be supplied for daily maintenance of the cow. Most cows are in negative Ca balance during the early weeks of lactation because more Ca leaves the body via milk, endogenous fecal loss, and urine than is absorbed from the diet, in part because the intestinal mechanisms for absorbing Ca are not fully adapted to lactation (70) and also because dry matter intake is less than favorable. To maintain normal plasma Ca, the negative Ca balance is met by resorption of bone Ca stores and absorption of Ca from intestine. Bone Ca mobilization is stimulated by a concerted effort of PTH and 1,25-(OH)₂D, but intestinal Ca absorption is controlled by 1,25-(OH)₂D alone. During the dry period, these mechanisms for replenishing plasma Ca are relatively inactive (70, 71). Thus, nearly all cows experience some degree of hypocalcemia during the first days after calving, and the intestine and bone adapt to lactation. The adaptation process begins with dramatic increases in the plasma concentrations of PTH and 1,25-(OH)₂D at the onset of hypocalcemia. About 24 h of 1,25-(OH)₂D

stimulation is required before intestinal Ca transport is increased significantly (5, 17, 40). Bone Ca resorption (recruitment and activation of osteoclasts) is not significantly increased until after about 48 h of PTH stimulation (20). In cows with milk fever, these adaptation processes can be even more prolonged. For those cows, the mammary drain of Ca causes extracellular and plasma Ca concentrations to fall, even to the point of eventual death, before adaptation of intestine and bone can occur. Intravenous Ca treatments (usually 8 to 11 g of Ca) are used to keep the cow alive long enough for adaptation to take place. Many theories have been proposed to explain why some cows develop milk fever. Failure to secrete either PTH or 1,25-(OH)₂D was once hypothesized as being the primary defect in milk fever cows. However, these hypotheses were discarded when circulating PTH and 1,25-(OH)₂D were found to be higher in the blood of cows with milk fever than in the blood of cows without (33, 59). Similarly, overproduction of calcitonin, a hormone that inhibits bone Ca resorption, was once thought to be a cause of milk fever, but has not been demonstrated (30, 58, 59).

Work in our laboratory (23), however, has shown that there is a subtype of milk fever (~20% of the milk fever cows) in which 1,25-(OH)₂D production is nonexistent or delayed. This syndrome was seen in cows that suffered relapses of milk fever (requiring i.v. Ca on more than a single occasion). All had been fed a high Ca (125 g Ca/d), high cation (+485 meq/kg) diet prepartum. In these relapsing cows, plasma 1,25-(OH)₂D concentrations did not increase as the cows became hypocalcemic. After 24 to 48 h of severe hypocalcemia, these cows eventually began to produce 1,25-(OH)₂D and recovered. Plasma PTH concentrations were as high or higher in these cows with relapsing milk fever as in those that had not relapsed. Because PTH should have stimulated renal 1,25-(OH)₂D production, the data suggest that kidneys of milk fever cows are temporarily refractory to PTH stimulation.

Dietary Control of Milk Fever

Because production of PTH and 1,25-(OH)₂D seems to be adequate in most cows with milk fever, current theories assert that intestine, bone, and kidney [target tissues of PTH and 1,25-(OH)₂] of milk fever cows have lost the ability to respond to these Ca-regulating hormones. For example, the ability of the cow to increase intestinal Ca absorption

Calcium Homeostasis
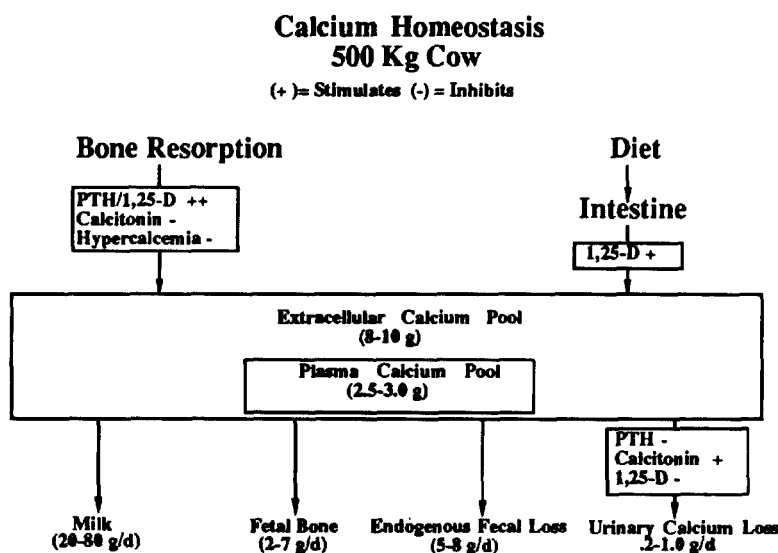**500 Kg Cow**
(+)= Stimulates   (-) = Inhibits

Figure 4. Calcium metabolism in the dairy cow. PTH = Parathyroid hormone; 1,25-D = 1,25-dihydroxyvitamin D.

and bone Ca resorption to levels that meet lactational Ca demands decreases with age, which (as discussed earlier) is probably a result of decreased intestinal and bone VDR with aging.

Dietary Ca of >100 g daily during the dry period is also associated with an increased incidence of milk fever (45). A 500-kg cow requires only about 31 g of Ca to meet daily maintenance and fetal Ca demands in late gestation. When a prepartum cow is fed a high Ca diet (>100 g of Ca/d), its daily requirement for Ca can be met almost entirely by passive absorption of dietary Ca. Active transport of Ca from the diet and bone Ca resorption mechanisms are homeostatically depressed and become quiescent (58, 70). As a result, at calving the cow is unable to use bone Ca stores or intestinal Ca mechanisms and is susceptible to severe hypocalcemia until these mechanisms can be activated, which may take several days. Low Ca diets (<20 g of Ca/d) fed during the last weeks of gestation, followed by a lactation diet that is high in Ca after parturition, dramatically reduce the incidence of milk fever (24). Intake of less dietary Ca than required places the cow in negative Ca balance, stimulating PTH and 1,25-$(OH)_2$D production and, therefore, the Ca homeostatic mechanisms before parturition. This change allows the cow to absorb Ca efficiently in the lactation diet and also to draw on bone Ca stores immediately after calving. The literature shows little benefit to be gained by decreases in dietary Ca from 100 to 60 g of Ca/d. The observed benefits of this type of dietary change are discussed in the next section.

Prepartal diets high in P (>80 g of PO_4/d) also increase the incidence of milk fever and hypocalcemia in general (1, 45, 47). Increased dietary P intake increases the P in the blood, which has a direct inhibitory effect on the renal enzymes that catalyze production of 1,25-$(OH)_2$D (88). This reduced production of 1,25-$(OH)_2$D further reduces intestinal Ca absorption mechanisms prepartum.

Dietary Cation-Anion Balance and Milk Fever

The theory and use of dietary cation-anion balance (DCAB) for prevention of milk fever has repeatedly been shown (3, 4, 12, 15, 18, 67). The most popular equation for use in

TABLE 3. Atomic weight, equivalent weight, and factor for conversion from percentage of diet to milliequivalents per kilogram of diet. Feed analysis reports usually present the ions S^{2-} and P^{3-} as elemental S and P.

Element	Atomic weight	Equivalent weight	Conversion factor
		(g)	
Na^+	23.0	23.00	435
K	39.1	39.10	256
Ca^{2+}	40.1	20.05	499
Mg^{2+}	24.3	12.20	823
Cl^-	35.5	35.50	282
S^{2-}	32.1	16.05	624
P^{3-}	31.0	10.33	968

calculating DCAB is the sum of the milliequivalents per kilogram of diet and dietary Na^+ and K minus the milliequivalents per kilogram of diet of S^{2-} and Cl^-. Table 3 summarizes the atomic and equivalent weights of these elements and the factors for converting percentage to milliequivalents per kilogram of diet. The more negative the DCAB, the more successful is the diet in preventing milk fever. Therefore, diets that are high in cations, especially Na^+, K, and Ca^{2+}, tend to induce milk fever, and diets that are relatively high in anions, primarily Cl^- and S^{2-}, can prevent milk fever (65, 80). Most prepartal diets based on forages are very high in cation content, particularly K. These fixed cations tend to keep the cow in a state of mild metabolic alkalosis, as evidenced by the relatively high pH of the urine of ruminants fed such diets (14). Addition of anions to the diet (or preferably reducing the cation content of the diet) reduces the alkalinity of the diet, reducing the metabolic alkalosis and perhaps initiating metabolic acidosis. Fredeen et al. (14) and LeClerc and Block (51) have demonstrated that addition of anions to the diet does not increase apparent intestinal absorption of Ca. Also, urinary Ca loss is greater in cows fed the high anion diets (14, 15). Based on these data, it would be difficult to understand how an anionic diet would be helpful in prevention of milk fever in cows.

However, studies of rats (2) and dogs (7) indicate that bone and perhaps renal tissues are refractory to the effects of PTH in the alkaline state, and the stimulatory effects of PTH are

enhanced during metabolic acidosis. Gaynor et al. (15) have shown that the acid-base balance of the cow can influence 1,25-(OH)₂D production, which is dependent on PTH, and influences the degree of hypocalcemia experienced at parturition. Cows fed a diet high in K and Na⁺ prior to parturition (inducing metabolic alkalosis) had lower plasma 1,25-(OH)₂D concentrations at parturition than did cows fed a diet high in Cl and SO₄ (inducing metabolic acidosis), despite more severe hypocalcemia in the cows on the alkalotic diet. Goff et al. (18) examined the plasma PTH and 1,25-(OH)₂D profiles of 47 Jersey cows fed either a high cation (+978 meq/kg) or high anion (−228 meq/kg) diet prior to parturition. The incidence of milk fever was reduced from 26 to 4% by the addition of anions to the diet. As the cows in this study developed hypocalcemia, all experienced an increase in plasma PTH concentrations, indicating that the parathyroid glands responded equally well to a decline in blood Ca in both groups of cows. However, the plasma 1,25-(OH)₂D response to hypocalcemia of cows fed the high cation diet was significantly reduced. Because PTH is normally responsible for stimulating the kidneys to produce 1,25-(OH)₂D, these data suggest that the kidneys were temporarily refractory to stimulation by PTH. Bone Ca resorption, which also requires stimulation by PTH, was also reduced in the cows fed the high cation diet, suggesting that bone tissues were also refractory to stimulation by PTH. Addition of anions to the diet restored the ability of these tissues to respond to PTH, preventing development of severe hypocalcemia. Vagg and Payne (91) and Block (4) also found that bones of ruminants fed highly anionic diets were capable of releasing more Ca than bones of cows fed highly cationic diets when Ca stressed.

Collectively, these data suggest that the underlying cause of milk fever is an inability of cow tissues to respond adequately to PTH, which causes poor production of the second Ca-regulating hormone, 1,25-(OH)₂D, and reduces ability to draw on bone Ca stores. The presumption is that metabolic alkalosis somehow disrupts the integrity of PTH receptors on target tissues. Anionic diets may work by increasing target tissue responsiveness to PTH, which controls renal 1α-hydroxylase and bone

Ca resorption, permitting the cow to adapt successfully to the Ca stress associated with the onset of lactation.

DCAB and Lactation Performance

Several studies have documented the benefits of anionic diets fed to cows during the dry period on performance in the subsequent lactation period. Block (4) and Beede et al. (3) found that addition of anions to the prepartal diet increased milk production by 486 and 327 kg/cow in the subsequent lactation. Beede et al. (3) also demonstrated a significant improvement in conception rate by 150 d postpartum in the cows fed the high anion diet. Anionic diets also reduced incidence of retained placenta (67). Cows fed the high anion diets prior to parturition also have reduced udder edema, perhaps as a result of the diuretic effect of the addition of these salts [(52); J. K. Miller, 1992, University of Tennessee, personal communication]. Anionic diets prepartum may enhance milk production and health in subsequent lactation, simply because hypocalcemia is decreased and the cow does not have the secondary problems associated with milk fever. The economic impact of subclinical hypocalcemia is difficult to assess. It seems likely that, if milk fever is associated with loss of muscle tone (for example, abomasum or teat sphincters) and ruminal stasis, subclinical hypocalcemia will be associated with these same problems to a lesser degree. The impact of subclinical hypocalcemia, which is much more common than milk fever, on herd health may be nearly as great as milk fever.

Additional studies have also been conducted to investigate the performance of lactating cows as affected by DCAB of the lactating ration. In a series of excellent papers (89, 90, 93), workers at the University of Kentucky have attempted to define the dietary DCAB most beneficial to milk production during lactation. Tucker et al. (89) found that milk production increased linearly as DCAB increased between −268 and +32 meq/kg, regardless of the ions used to manipulate the diet. In a second experiment, Tucker et al. (90) found that feed intake and milk production were reduced in lactating cows fed diets with DCAB <+150 meq/kg. Waterman et al. (93) found that the fat content of milk increased with increas-

ing DCAB between +10 and +157 meq/kg of diet. A review of the available literature shows that highly anionic diets (<+50 meq/kg diet) appear to be detrimental to lactation performance. Sanchez and Beede (84) found that peak lactational performance and peak dry matter intake were achieved when cows were fed diets with DCAB of approximately +225 meq/kg of diet. Their work also suggests that optimal Mg content of the lactating ration is .4%, and optimal Na content is .58%. Feed intake and milk production were adversely affected by diets with DCAB exceeding +450 meq/kg diet. The high rate of metabolism of the lactating cow (producing organic acids) may benefit from the neutralization of these acids by the addition of K or other cations in the diet.

Practical Use of DCAB Adjustment

The standard recommendation for prevention of milk fever has been to restrict Ca and P intake during the dry period, as discussed. However, Oetzel (65) provides evidence that, although the incidence of milk fever increases with increasing dietary Ca (up to ~1.25 of the diet), beyond 1.2% Ca in the diet, milk fever incidence is actually reduced. An earlier study by Oetzel et al. (67) and field experience suggests that cows fed a high anion diet prior to parturition may suffer more hypocalcemia if dietary Ca is restricted. Based on these observations, we recommend a Ca intake of 120 to 150 g/d. A controlled experiment testing the benefits of Ca restriction in cows fed the anionic diets remains to be conducted.

Addition of anions to the prepartal diet should be considered not only to prevent milk fever but also to prevent subclinical hypocalcemia, which may be responsible for problems such as retained placenta and displaced abomasum. Because alfalfa is a common ingredient in the lactation ration, there are advantages in dietary alfalfa fed prior to calving for rumen adaptation and the avoidance of forage switches at calving. Depending on the K content of the alfalfa (which varies tremendously), it may be possible to add anions to decrease the DCAB sufficiently to reduce hypocalcemia. Most of these forage-based diets have DCAB of +150 to +350 meq/kg, and it is generally thought that the DCAB should be reduced to ≤0 meq/kg to have an impact on the incidence of milk fever. Most successful studies have had DCAB between -100 and -200 meq/kg of diet. In our experience, addition of >300 meq of anion/kg of diet tends to reduce feed intake. Numerous studies suggest that any reduction in feed intake prior to parturition is likely to increase liver fat and, thus, increase the risk of ketosis, which essentially means that, if the prepartum DCAB is >+200 meq/kg of diet, it will be hard to use it successfully as the basis for a good anionic diet. Thus, addition of anionic salts into a diet that is causing milk fever will not always yield good results. Some diets just cannot be used prepartum! It may not be practical to use alfalfa that is very high in K in the dry period, because the quantity of anions required to acidify the diet is likely to cause inappetence. A switch to, or mixing with, corn silage or some other feedstuff (beet pulp is our choice) may be necessary.

The choice of salts is dictated by economics and palatability (66). As of this writing, $CaSO_4$ is about 2¢/equivalent, and $CaCl$ is about 4¢/equivalent. Ammonium chloride and $(NH_4)_2SO_4$ are ~5 and 3¢/equivalent, respectively. Magnesium sulfate, which is more palatable, is ~8 to 10¢/equivalent. Total cost of the added anions fed for ~3 wk is generally between $5 and $8 per cow.

As a rule of thumb, ammonium salts should not be used to supply all the anions to be added to the diet because these salts are not very palatable and present some risk of ammonia intoxication. Generally, a mixture of the ammonium salts with $CaSO_4$ or $MgSO_4$ is used to reduce DCAB of the diet. Usually, the use of these salts is most successful in total mixed rations, which mask the salt taste better. Pelleting of the salt mixture usually, but not always, increases the acceptability of the salts. The salt mixtures are generally fed for 3 to 5 wk prior to parturition. Whether shorter periods of supplementation would be effective is unknown.

The following represent practical recommendations for adjustment of the DCAB in dry cow rations:

1. The dry cow ration should be analyzed for Na, K, Cl, and S in addition to Ca, Mg, and P. (Table 3 allows rapid deter-

mination of DCAB.) Potassium content of forages can vary tremendously, depending on variety and fertilizer application rates. If DCAB is <+200 meq/kg of diet, step 2 should be followed; if not, low K feedstuffs should be substituted for part of the high K forage. Otherwise, gains made in prevention of milk fever will be offset by increased incidence of ketosis and displaced abomasum because of reduced feed intake.

2. Any added sources of cations should be removed. The primary source is $NaHCO_3$ added to the lead feed; $CaCO_3$ should also be removed. At this point, NaCl should not be in the diet, because the salty taste will only add to the unpalatability of a high anion diet;

3. Calcium sulfate should be added to the diet to achieve a dietary S of .45% until dietary Ca supplied is 140 g/d (~1.2 to 1.6% Ca), which slightly exceeds NRC (64) S recommendations but, in our experience, is well tolerated.

4. If needed, dietary S should be brought to .45% using $MgSO_4$. If still needed, Ca should be brought to 140 g/d (1.2 to 1.5% Ca) using $CaHPO_4$ (see step 6) or $CaCl_2$.

5. To reduce DCAB to −100 meq/kg of diet, NH_4Cl should be added.

6. Intake of P should be kept <60 g/d. Although addition of P to the diet would help acidify blood, elevated P in the blood inhibits the ability of the cow to produce the hormone 1,25-$(OH)_2$D, which may actually increase the risk of milk fever. For this reason, we do not advocate addition of P to the diet.

CONCLUSIONS

Significant advances have been made in the knowledge of Ca and vitamin D metabolism and the onset of milk fever in dairy cows. These advances have led to a better understanding of the mechanism of how low Ca diets and diet high in anions may work in milk fever prevention. Basic research has also shown that aging has a dramatic effect on the concentration of 1,25-$(OH)_2$D receptor in target tissues. Future research focusing on the regulation of the vitamin D receptor during the periparturient period is needed in order to determine how the biological response to 1,25-$(OH)_2$D may be enhanced.

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