Interaction among energy status, retinol-binding protein and intra-mammary infections in periparturient dairy cows

Pedram Rezamand, Independent dairy nutritionist
Mark A. McGuire, University of Idaho, and
Sheila M. Andrew, University of Connecticut

Summary

The risk of mastitis is greatest during the transition period with a cost to the dairy industry of greater than $1.7 billion each year. During the last 25 years, there has been improvement in mastitis prevention and control as well as a greater understanding of the effects of dietary nutrients on immune function. However, research on nutritional factors, except vitamin E and selenium, affecting immune function are limited for dairy cows. In particular, vitamin A and its retinoid metabolites and retinol binding protein (**RBP**), the carrier protein for retinol, may be important modulators of immune function. There is a need to investigate the metabolic challenges during the transition period in dairy cows and their effects on specific immune measures related primarily to mastitis. Such research efforts will create new knowledge of the effects of the retinoids (e.g., vitamin A and retinoic acid) and their interaction with protein and energy nutrition on immune system enhancement in dairy cows. Results potentially lead to improved management practices that reduce the risk of mastitis and to develop intervention strategies that can reduce the need for antibiotic use on dairy farms to treat infections.

Overview

The management of dairy cows during the transition period represents a challenge for dairy farmers (Drackley, 1999). The risk for metabolic disorders and diseases, such as mastitis, is great during the transition period for dairy cows and may be related to an impairment of immune function (Goff and Horst, 1997). The cost of mastitis is substantial and is estimated to be more than $1.7 billion each year in the United States alone. Several vitamins have been shown to be involved in some aspect of immune function. A number of studies reported a reduction in somatic cell count (**SCC**), and enhanced lymphocyte function, phagocytosis and *in vitro* intracellular killing by blood neutrophils against *Staphylococcus (**S.**) aureus* with increased dietary retinol and β-carotene (Daniel et al., 1986; Chew, 1987; Tjoelker et al., 1990). In some studies, however, no beneficial effects were reported (Oldham et al., 1991; Jukola et al., 1996). To our knowledge, no study has investigated the effect of RBP (the retinol transport system in circulation), when retinoid metabolism is altered, on the risk of mastitis. It is also not known how the interaction between retinoids and energy metabolism is affected by RBP status and how this affects immune function. Therefore, there is a need to better understand how alterations in retinoid metabolism and RBP status, due to a functional transition from a non-lactating to lactating state affects immune function, interacts with energy and relates to incidence and severity of a new intra-mammary infection (**IMI**).

The immune system is altered during the transition period

During the periparturient period, the immune system is suppressed (Goff and Horst, 1997). In particular, neutrophil and lymphocyte functions are altered or impaired during this time (Mallard et al., 1998). In a study by Saad et al. (1989), blood neutrophil phagocytosis activity increased before calving, fell sharply on the first day postpartum, and peaked 2 wk postpartum. Further, bovine lymphocytes are impaired in their ability to respond to mitogens and to produce antibodies during the periparturient period (Wells et al., 1977; Kehrli et al., 1989, 1990). Serum components of the bovine immune system, such as complement, conglutinin, (Kehrli et al., 1990; Stabel et al., 1991) and immunoglobulins (Ig) are also decreased at parturition (Kehrli et al., 1989, 1990; Detilleux et al., 1995), and lower IgG concentrations have been associated with increased

incidence of mastitis (Kehrli et al., 1989). Feed intake is significantly reduced during the final weeks before parturition (Grummer, 1995) and is accompanied by a decrease in phagocytosis and intracellular killing of pathogens by neutrophils (Hogan et al., 1992a), which may lead to increased risk of mastitis.

Vitamin A and β-Carotene during the transition period

The most abundant form of retinoids in blood circulation is retinol, a long-chain unsaturated alcohol form of vitamin A, which is an essential nutrient involved in growth, vision, immunity and epithelial cell differentiation and proliferation (Chew, 1987; Scherf et al., 1994; Van Merris et al., 2004a). Acid derivatives of vitamin A mediate specific effects on the immune system (Petcovich et al., 1987). In ruminant nutrition, among plant carotenoids, β-carotene is the major source of vitamin A and has the highest pro-vitamin A activity (Chew, 1993). In addition to its pro-vitamin A function, β-carotene acts as an oxidant scavenger protecting immune cells from oxidative damage (Chew, 1993).

In healthy cows, plasma concentrations of retinol and β-carotene gradually decrease as parturition approaches and then plasma concentrations of retinol normalize to the prepartum concentrations by wk 4 after parturition (Chew et al., 1982). Feeding lower quality forages during the dry period (Michal et al., 1994), reduced feed intake during the last 2 wk before parturition (Grummer, 1995), and increased secretion of lipid-soluble vitamins in colostrums and milk immediately following parturition (Weiss et al., 1990) are all associated with the temporal decreases in plasma concentrations of retinol and β-carotene. During the past 25 years, both observational as well as intervention studies have demonstrated evidence of a relationship between retinol and β-carotene status and mastitis.

Does feeding supplemental vitamin A and β-Carotene affect mastitis?

Initial intervention studies demonstrated that increasing dietary vitamin A and β-carotene intake resulted in a reduction in the incidence and severity of mastitis in dairy cows (Chew et al., 1982; Chew and Johnston, 1985; Dahlquist and Chew, 1985). The effects of feeding greater vitamin A prepartum on immune function during the transition period, however, have been equivocal. Cows fed a diet supplemented with vitamin A (173,000 IU/d) or β-carotene (at a concentration equivalent to 173,000 IU vitamin A/d) had lower milk SCC as compared with control cows (fed 53,000 IU vitamin A/ d) from 2 to 8 wk postpartum (Chew and Johnston, 1985). Feeding diets supplemented with vitamin A or β-carotene also enhanced lymphocyte proliferation in response to mitogens (Daniel et al., 1986) and increased phagocytosis and intracellular killing indices by milk polymorphonuclear (PMN) cells against *S. aureus* (Tjoelker et al., 1986).

Oldham et al. (1991), however, found no beneficial effects on immune function by feeding diets supplemented with vitamin A and β-carotene from the cessation of lactation through 6 wk postpartum. The lack of response may be explained by the fact that the control cows in that study had adequate plasma concentrations of β-carotene (10 mg/L; Weiss, 2002). A threshold of >3 mg/L of plasma β-carotene for optimum udder health has been suggested (Jukola et al., 1996). Tjoelker et al. (1990) determined that milk neutrophil phagocytosis decreased in cows fed either 53,000 or 21,300 IU vitamin A /d from 6 wk before dry off through 2 wk after dry off; however, cows fed 53,000 IU vitamin A plus 400 mg β-carotene /d (equivalent to 21,300 IU vitamin A/d) did not experience such a decrease in phagocytosis, indicating a role for β-carotene in immune cell function, independent of its pro-vitamin A activity.

A decrease in new IMI was observed when cows were fed diets supplemented with 53,000 IU vitamin A and 300 mg β-carotene/d compared with cows fed diets supplemented with vitamin A at 53,000 IU/d, or at greater concentrations, 173,000 IU/d (Dahlquist and Chew, 1985). An in vitro experiment, however, showed that retinol had no effect and β-carotene had a stimulatory effect on concanavalin-A induced mononuclear cell proliferation, suggesting that β-carotene, but not vitamin A, influenced immune cell proliferation (Daniel et al., 1991). The summation of available information resulted in a new recommendation (NRC, 2001) for increased (to 110 IU/kg of body weight) dietary

intake of vitamin A relative to the previous recommendation (NRC, 1989). No daily recommendation of supplemental dietary β-carotene has been established (Weiss, 2002).

What are the relationships of vitamin A and β-Carotene to mammary gland health and mastitis?

In an epidemiological study, LeBlanc et al., (2004) detected a significant inverse association between serum concentrations of retinol and mastitis during early lactation (first 30 d in milk); as serum concentrations of retinol increased the occurrence of mastitis was decreased. Our data (Rezamand et al., 2007) demonstrate that plasma concentrations of ß-carotene and retinol (Figures 1 and 2, respectively) were reduced at wk 1 or 2 postpartum. Temporal patterns of change in plasma concentrations of ß-carotene and retinol reported here are consistent with previous reports. Plasma concentrations of ß-carotene 2 wk before calving were greater for cows with a new IMI after calving compared with cows that did not develop a new IMI but this relationship was reversed postpartum (Figure 1). Cows without a new IMI had greater plasma concentrations of ß-carotene at wk 8 compared with cows with a new IMI during early lactation.

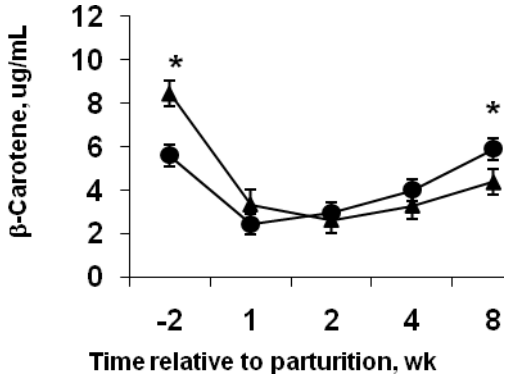

Figure 1. Least square means and SE of plasma concentrations of β-carotene (μg/mL) during the periparturient period for cows with (▲, n = 14) or without (●, n = 16) a new IMI during early lactation (IMI status × wk; $P = 0.003$).

Although a reduction in plasma concentrations of retinol was observed at wk 1 postpartum (Figure 2), there were no differences between cows with or without a new IMI during early lactation; either plasma concentrations of retinol were sufficiently high (≥ 1.0 ug/mL) to render a protective response or plasma concentrations of retinol during the transition period may not be a reliable indicator of vitamin A status, in relation to the mammary gland health and occurrence of an IMI.

Indeed, a recent study (Van Merris et al., 2004a) demonstrated that serum concentrations of albumin, retinol and 13-*cis* retinoic acid were reduced in nulliparous cows during experimentally induced *Escherichia coli* mastitis whereas concentrations of serum amyloid A and *all-trans* retinoic acid (at-RA; the active isomer of retinol) were increased. It was also reported that a one-unit increase in serum amyloid A, an indicator of acute phase response, was associated with a 1.12 unit decrease in serum retinol (Van Merris et al., 2004a), suggesting an associative relationship between retinol and acute phase response. This observation is supported by our observation, where a negative correlation between plasma haptoglobin and retinol was observed 1 wk postpartum ($P = 0.01$; Rezamand, 2006). In addition, a recent *in vitro* study (Van Merris et al., 2004b) showed that addition of either at-RA or 9-*cis* RA (~10 ng/mL) to bovine bone marrow cells stimulated granulocyte but inhibited monocyte formation. Studies in humans have shown that at-RA increased granulocyte formation which was associated with a reduction in monocytes formation (Collins, 2002).

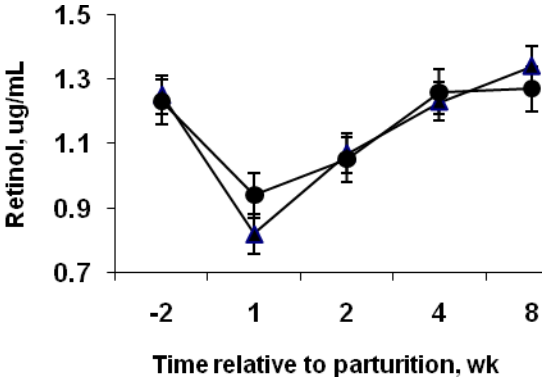

Figure 2. Least square means and SE of plasma concentrations of retinol (μg/mL) during the periparturient period for cows with (▲, n = 14) or without (●, n = 16) a new IMI during early lactation. Plasma concentration of retinol was reduced at wk 1 postpartum compared with that at 2 wk before parturition for cows with or without a new IMI ($P < 0.001$).

What is retinol binding protein (RBP)?

Although lipid-soluble vitamins such as vitamin E and β-carotene are transported through circulating lipoproteins, retinol is primarily transported by RBP from the liver to target organs (Heller, 1975a). Retinol binding-protein is a single polypeptide chain with a molecular weight of 21 kD (Heller, 1975b), and contains one binding site for retinol. Approximately 95 percent of plasma RBP is bound to transthyretin (TTR; Lindberg et al., 1999). Retinol binding-protein is primarily synthesized in the liver; however, others have shown that in addition, RBP, as well as TTR, are synthesized and secreted by extra-hepatic sources (Liu et al., 1990; Liu and Godkin, 1992; Ong et al., 1994). Retinol deficiency can reduce the secretion of RBP, which in turn results in decreased plasma RBP while increasing hepatic RBP (Goodman, 1980; Rask et al., 1980; Lespine et al. 1996). There may be, however, species-specific differences in relationship between retinol intake and RBP status.

Export proteins, such as RBP, are produced in lower concentrations when amino acid availability is limited (Lindberg et al., 1999). Our observations (Figure 3) and those of Lindberg et al. (1999) support the hypothesis that dietary protein intake alters RBP synthesis and export. Consequently, reduced RBP may limit retinol delivery and its availability to other tissues, most notably the immune system. It is therefore conceivable to hypothesize that the lack of immune response to vitamin A supplementation may be due to RBP deficiency resulting from an overall protein deficiency during the transition period (Rezamand et al., 2007). Studies using a rodent model have demonstrated that plasma RBP plays an important role in immune function. The inflammatory response of rats to lipopolysaccharide (LPS) from *Pseudomonas aeruginosa* for example, is associated with decreased plasma retinol and RBP concentrations. Rosales et al. (1996) suggested that decreased plasma retinol in the rat resulted from a reduction in RBP synthesis and secretion of the retinol-RBP complex when inflammation by LPS was induced. In agreement, our data demonstrate, for the first time, that cows with a new IMI during early lactation had a reduced plasma concentration of RBP as compared with cows that did not develop a new IMI (Figure 3). There was also a significant correlation between plasma concentrations of RBP and retinol, as expected. In addition, reduction in plasma RBP and retinol at wk 1 postpartum was mirrored by an increase in plasma concentrations of haptoglobin, regardless of the IMI status (Rezamand, 2006). Currently, there is no information available to explain whether bovine plasma RBP plays an immunomodulatory role when retinoid metabolism is altered during an IMI.

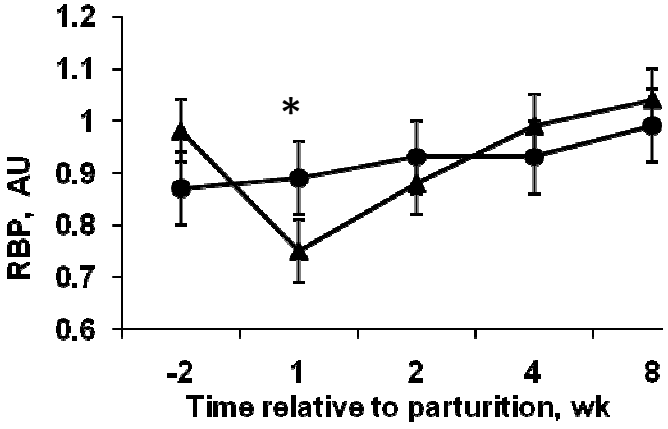

Figure 3. Least square means and SE of plasma concentrations of retinol binding protein (RBP, expressed in arbitrary unit; AU) for cows with (▲; n = 14) or without (●; n = 16) a new IMI during the periparturient period. Plasma concentration of RBP was reduced at wk 1 postpartum compared with that 2 wk before parturition for cows with a new IMI (time $P < 0.001$) and also compared with that of cows without a new IMI (IMI status; $P = 0.03$) during early lactation.

Inflammation and hyporetinemia are interrelated. A number of hypotheses have been set forward to explain hyporetinemia associated with inflammatory response including increased retinol output, increased requirements, likely due to an increased usage of the vitamin as an antioxidant, and reduction in retinol transfer from the liver to target organs. Although plasma retinol was reduced wk 1 postpartum, the lack of a difference between cows with or without a new IMI in our observation (Figure 2) contrasts with previous reports (Chew et al., 1982; Chew, 1987). Plasma RBP, however, was reduced in cows with a new IMI as compared with cows that did not develop a new IMI (Figure 3). This observation lends further support to the hypothesis that plasma retinol is not a reliable indicator of vitamin A status in relation to inflammation (Rosales and Rose, 1998a; 1998b).

Does altered retinoid metabolism affect bovine polymorphonuclear (PMN) function?

Bovine PMN are the primary line of defense in protecting the mammary gland against mastitis pathogens (Burvenich et al., 2007). In an *in vitro* study, superoxide anion release from activated neutrophils was reduced as retinol concentration increased; however, no reduction in cytotoxicity or scavenging of the free radicals was observed (Sharma et al., 1990). It is not fully understood how altered retinoid metabolism, due to increased dietary supplementation of vitamin A, affects bovine peripheral blood and milk cytokines and leukocyte profile and function. It is also not well understood whether the functional capacity of PMN during the periparturient period and in response to an IMI are affected by an alteration in retinoid metabolism. Dairy cows may be oxidatively stressed during the periparturient period (Bernabucci et al., 2005; Aitken et al., 2009), and oxidative stress and inflammation are interrelated (De Nigris et al., 2001). Increases in pro-inflammatory cytokines, observed during the periparturient period and accompanying IMI, can alter PMN survival and function. Addition of granulocyte/macrophage colony-stimulating factor (GM-CSF), a pro-inflammatory cytokine, increased human neutrophil survival (Brach et al., 1992; Colotta et al., 1992; Kobayashi et al., 2005) as well as human neutrophil functions such as phagocytosis and reactive species oxygen (ROS) production (Kobayashi et al., 2005). Excessive recruitment and activation of PMN to the site of inflammation, however, can cause further damage to the mammary gland in an uncontrolled inflammatory state. Therefore, resolution of inflammation requires a timely regulated clearance of PMN from mammary tissues (Paape et al., 2003).

Energy status is altered by supplemental dietary ionophores

Ionophores alter transport of ions across the bacterial cell wall specifically inhibiting growth of Gram positive bacteria. Monensin is a carboxylic polyether ionophore that is naturally produced by *Streptomyces cinnamonensis* and fed to cattle in the form of sodium salt (Duffield and Bagg, 2000). The specific effect of monensin on rumen microbial populations results in improved efficiencies of energy

metabolism, N metabolism, and reduced bloat and lactic acidosis (Schelling, 1984; Duffield et al., 2008a). The observed increase in efficiency of energy metabolism is related to an alteration in VFA production in the rumen such that propionate production is increased whereas molar percentages of butyrate and acetate are reduced (Schelling, 1984). Propionate, a major glucogenic precursor, accounts for 50 to 60% of total net hepatic glucose release in dairy cattle (Reynolds et al., 1988 and 2003). A meta-analysis of the impact of monensin on blood metabolites revealed that inclusion of monensin in dairy cattle diets results in significant reductions in blood non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), and acetoacetate, as well as reductions in short-chain fatty acids and stearic acid content of milk (Schelling, 1984; Duffield et al., 2008a and 2008b). Overall, these changes are considered to be related to increased hepatic gluconeogenic activities.

Pyruvate carboxylase (PC, EC 6.4.1.1) and phosphoenolpyruave carboxykinase (PEPCK, 4.1.1.32) are two key and potentially rate-limiting gluconeogenic enzymes that regulate hepatic gluconeogenesis in ruminants (Greenfield et al., 2000; Agca et al., 2002). Relative to the transition period, research has shown that bovine hepatic PC mRNA expression increased d 1 postpartum compared with prepartum abundances, and remained elevated for 4 wk postpartum before declining to prepartum levels at wk 8 (Greenfield et al., 2000). Whereas no increases at 4 or 2 wk before calving or at d 1 postpartum were observed, hepatic PEPCK mRNA abundance increased at 4 and 8 wk postpartum as compared with those of prepartum abundances (Greenfield et al., 2000). The delayed increase in hepatic expression of PEPCK mRNA, compared with elevated PC mRNA expression relative to parturition (Greenfield et al., 2000; Hartwell et al., 2001), may be related to a lag phase in increment of feed intake during early lactation. It has been shown that expression of cytosolic PEPCK mRNA is affected by concentrations of VFA in cultured rat hepatic cells (Massillon et al., 2003). Whereas hepatic PC mRNA expression increased d 1 postpartum relative to that at 4 and 2 wk prepartum, monensin had no effect on PC mRNA expression (Karcher et al., 2007). In addition, an increased cytosolic PEPCK mRNA abundance was observed at 2 wk prepartum and d 1 postpartum in response to feeding monensin during the last 4 wk prepartum (Karcher et al., 2007). Increased hepatic PEPCK mRNA abundance parallels enzyme activity (Agca et al., 2002). It has been postulated that increased hepatic PEPCK mRNA abundance postpartum, in part, may be due to elevated propionate production during that time period, which may be further amplified by feeding monensin prepartum (Karcher et al., 2007).

Relationship between retinoids, retinol-binding protein, and energy status

It has been shown that several enzymes involved in gluconeogenesis, including PEPCK, are regulated at the gene level by retinoid status (Shin et al., 2002). Specifically, retinol deficiency inhibits cytosolic PEPCK mRNA expression and this phenomenon can be reversed by addition of all-*trans* or 9-*cis* retinoic acid treatment (Shin et al., 2002). It has been suggested that whole body insulin sensitivity and thus hepatic glucose output may be regulated by expression of adipocyte GLUT4 (insulin-responsive facilitative glucose transporter) through serum RBP (Figure 4; Tamori et al., 2006; Yang et al., 2005). Although liver is the major source of RBP synthesis (Heller, 1975a), adipocytes are considered to be the second major source of RBP in rodents (Tsutsumi et al., 1992). Others have shown that RBP is also synthesized and secreted by bovine retinal pigment epithelium, placental membranes, and uterine tissues (Ong et al., 1994; Liu et al., 1990 and 1992). Retinol binding protein was thought to exclusively function as the transporter system for retinol from liver to target tissues; however, recent findings suggest circulating RBP affects whole body glucose metabolism. Obese children were found to have greater concentrations of serum RBP as compared with lean children and plasma RBP correlated positively with markers of inflammation (e.g., CRP and IL-6; Balagopal et al., 2007). In addition, a reduction in serum RBP was associated with decreased concentrations of these inflammatory markers (Balagopal et al., 2007). Fasting insulin and triacylglyceride (TAG) concentrations were correlated with the ration of serum RBP to retinol, indicating a relationship between RBP status and obesity (Aeberli et

al., 2007). More interestingly, elevated RBP concentrations were associated with non-alcoholic fatty liver in diabetic humans (Wu et al., 2008). A recent study also showed an inverse relationship between serum RBP with insulin sensitivity and non-oxidative glucose metabolism in both lean and obese women (Kowalska et al., 2008). Mice with adipocyte-specific GLUT4 knockout had an elevated expression of the gene encoding serum RBP (Yang et al., 2005). Serum concentrations of RBP are increased in several mouse and human models of obesity and insulin resistance (see **Figure 4**; Yang et al., 2005). Studies in humans have also demonstrated that severe negative energy balance results in a reduction in adipose tissue, adipose tissue mRNA expression of gene encoding RBP, and plasma levels of RBP4 (Vitkova et al., 2007). More importantly, it was shown that hepatic gene expression of PEPCK was increased when mice were injected with purified human RBP4 for 21 d and in hepatocytes cultured in the presence of exogenous RBP (Yang et al., 2005). Reduction of serum RBP through fenretinde (a synthetic retinoid designed for cancer therapy) resulted in improved insulin sensitivity in mice on high fat-diet (Yang et al., 2005).

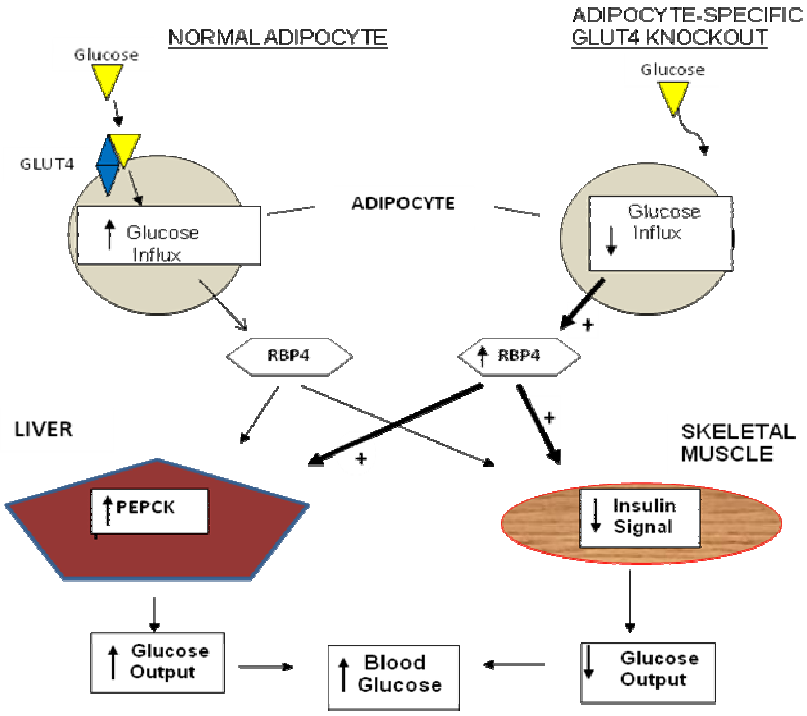

Figure 4. Glucose metabolism is regulated in skeletal muscle and liver through retinol-binding protein (RBP) by GLUT4 expression in adipose tissue under normal condition (a) or adipocytes lacking GLUT4 (adapted from Tamori et al., 2006).

During the transition period, dairy cows undergo substantial metabolic and physiological adaptations to support the transition from pregnant, non-lactating status to non-pregnant, lactating status. These adaptations include coordinated shifts in nutrient partitioning in order to meet the increased demand for energy and other nutrients for fetal growth and lactation (Bell, 1995; Drackley, 1999; Overton and Waldron, 2004). It has been estimated that there is a 3-fold increase in glucose requirement for the mammary gland 4 d postpartum compared with nutrient requirements of a 250-d fetus. Increased demand for glucose by the mammary gland to synthesize lactose is met through alterations in metabolism such as increased hepatic gluconeogenesis as well as a reduced rate of glucose oxidation by tissues other than the mammary gland (Reynolds et al., 2003; Bennink et al., 1972). Periparturient cows experience insulin resistance and this helps repartitioning glucogenic nutrients toward functions with higher priority including fetal growth, milk lactose synthesis, and to increase fatty acid mobilization from adipose tissue (Bell and Bauman, 1997). Although previous findings demonstrate an improved energy status by prepartum monensin feeding through elevated gluconeogenesis and hepatic PC and PEPCK expression, and that there exists a modulatory effect of RBP status on gluconeogenesis and

hepatic PEPCK, there is currently no information available in dairy cattle that RBP status and dietary ionophores may interact. It is also not known what the outcome(s) of this interaction may be, relative to energy metabolism, and if this may affect immune response to IMI causing pathogens.

Future Directions

Retinoid metabolism and retinol transport (i.e., RBP) affect immune function relevant to incidence and severity of a new IMI during early lactation. These factors are also affected by energy status. Thus, retinoids and energy status may interact to alter risk of disease in early lactation. The questions to be raised are whether hyporetinemia (low vitamin A) associated with a greater risk for mastitis can be explained by a decrease in retinol-RBP complex, whether altered retinoid metabolism, through dietary protein, can affect immune response to mastitis pathogens, and finally whether RBP status interacting with energy metabolism could affect overall energy status in periparturient cows that could alter the risk of new IMI.

A better understanding of nutritional factors that affect the interaction between RBP and retinoid metabolism during the periparturient period, elucidating effects of dietary vitamin A supplementation on acute phase response and certain aspects of immune function relevant to mammary gland health, and to understand how alterations in energy metabolism and the retinol-RBP complex affect the immune system during periparturient period are needed. A clearer understanding of mechanisms involved in nutrient metabolism and mammary gland health will potentially allow dairy and nutrition scientists to improve management practices and develop more practical intervention strategies to reduce antibiotic use on dairy farms.

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