Inflammation and Calcium Homeostasis: Potential Implications for the Transition Period

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Introduction

The vast majority of what we understand about calcium (Ca) homeostasis during the transition period was originally discovered by Dr. Ronald L. Horst (1949-2019) and his students/collaborators at the USDA National Animal Disease Center in Ames, IA. At lactation onset, dairy cows experience a marked increase (>65%; DeGaris and Lean, 2008) in Ca requirements to support colostrum and milk synthesis (Horst et al., 2005). The dairy industry has long hypothesized that the mammary gland's Ca demand is so extensive and acute that it often exceeds the homeostatic mechanisms (i.e., parathyroid hormone [PTH] and Vitamin D) employed to replenish it and as a result clinical or subclinical hypocalcemia (SCH) occurs (Horst et al., 2005; Goff, 2008). Implementing therapeutic and prophylactic strategies (i.e., pre-calving acidifying rations) has markedly reduced the incidence of clinical hypocalcemia (Charbonneau et al., 2006; Reinhardt et al., 2011), but SCH remains common. Some consider post-calving SCH as "pathological" and assume it causal in a myriad of seemingly unrelated negative health outcomes (ketosis, poor reproduction, displaced abomasum [DA], immune suppression, etc.; Caixeta et al., 2017; Rodríguez et al., 2017; Neves et al., 2018a,b). We suggest (outlined below) that periparturient immune activation contributes to clinical and SCH and the low circulating Ca is a consequence (a reflection) of inflammation rather than a predictor of future problems. In fact, we hypothesize that many post-calving undesirable phenotypes (reduced dry matter intake [DMI], hypocalcemia, elevated non-esterified fatty acids [NEFA], hyperketonemia) are a consequence of immune activation and not themselves causative of transition cow maladies.

Subclinical Hypocalcemia

Subclinical hypocalcemia remains a prevalent metabolic disorder afflicting ~25% of primiparous and ~50% of multiparous cows in the United States (Reinhardt et al., 2011). Although no overt symptoms accompany SCH, it has been loosely associated with poor gut motility, increased risk of DA, reduced production performance (i.e., milk yield and feed intake), increased susceptibility to infectious disease, impaired reproduction, and an overall higher culling risk (Curtis et al., 1983; Hansen et al., 2003; Seifi et al., 2011; Oetzel and Miller, 2012; Caixeta et al., 2017). Recent reports indicate that the severity of negative health outcomes observed in SCH cows appears dependent on the magnitude, persistency, and timing of SCH (Caixeta et al., 2017; McArt and Neves, 2019). For example, Caixeta et al. (2017) classified cases as either SCH or chronic SCH and observed more pronounced impairments on reproductive performance with chronic SCH. Similarly, McArt and Neves (2019) classified cows into 1 or 4 groups based on post-

calving Ca concentrations: normocalcemia (>2.15 mmol/L at 1 and 2 DIM), transient SCH (≤ 2.15 mmol/L at 1 DIM), persistent SCH (≤ 2.15 mmol/L at 1 and 2 DIM), or delayed SCH (> 2.15 mmol/L at 1 DIM and ≤ 2.15 mmol/L at 2 DIM). Cows experiencing transient SCH produced more milk and were no more likely to experience a negative health event when compared to normocalcemic cows, whereas the opposite (i.e., higher health risk and hindered productivity) was observed in cows experiencing either persistent or delayed SCH. Clearly not all cases of SCH are equivalent; in fact, transient hypocalcemia appears to be correlated with improved "health" and productivity and this may explain why inconsistencies exist in the relationship between SCH and reduced productivity and health (Martinez et al., 2012; Jawor et al., 2012; Gidd et al., 2015). However, it remains unclear why despite successful implementation of mitigation strategies, SCH remains prevalent, why SCH is associated with a myriad of seemingly unrelated disorders, and what underlying factors may be explaining the different "types" of SCH.

In addition to SCH, there are on-farm milk-fever situations that are biologically difficult to explain. For example, even while strictly adhering to a pre-calving calcium strategy, there remains a small percentage (~<1%) of cows that develop clinical hypocalcemia. Additionally, reasons for why a mid-lactation cow develops milk-fever are not obvious. Further, there appears to be an undecipherable seasonality component to clinical hypocalcemia in the southwest and western USA that coincides with the rainy season. Inarguably, there remain some aspects of Ca homeostasis that continue to evade discovery.

Inflammation in the Transition Period

Regardless of health status (Humblet et al., 2006), increased inflammatory biomarkers are observed in nearly all cows during the periparturient period (Ametaj et al., 2005; Humblet et al., 2006 Bionaz et al., 2007; Bertoni et al., 2008;; Mullins et al., 2012). The magnitude and persistency of the inflammatory response seems to be predictive of transition cow performance (Bertoni et al., 2008; Bradford et al., 2015; Trevisi and Minuti, 2018). During the weeks surrounding calving, cows are exposed to a myriad of stressors which may permit endotoxin entry into systemic circulation and thereby initiate an inflammatory response (Khafipour et al., 2009; Kvidera et al., 2017a; Proudfoot et al., 2018; Barragan et al., 2018; Koch et al., 2019). The frequency and severity of these inflammation-inducing insults presumably determines the level of inflammation that follows (Bertoni et al., 2008; Trevisi and Minuti, 2018). Common origins of endotoxin entry include the uterus (metritis) and mammary gland (mastitis). Additionally, we believe the gastrointestinal tract may contribute as many of the characteristic responses (rumen acidosis, decreased feed intake, and psychological stress) occurring during the transition period can compromise gut barrier function (see companion paper by Horst and Baumgard).

Although an overt inflammatory response is present around calving, numerous reports have described a reduction in immune competence during this time (Kehrli et al., 1989; Goff and Horst, 1997; Lacetera et al., 2005). Traditionally, hypocalcemia has been one of the primary factors considered responsible for periparturient immunosuppression

(Horst, 1997; Kimura et al., 2006), however, recent evidence suggests that the systemic inflammatory milieu may be mediating these effects (Heyland et al., 2006; Trevisi and Minuti, 2018). Furthermore, it was recently proposed that the immune system was not necessarily "suppressed," but merely dysregulated around calving (Trevisi and Minuti, 2018). Whether or not the immune incompetence frequently reported post-calving (Kehrli et al., 1989; Goff and Horst, 1997; Lacetera et al., 2005) is causative to future illnesses or is a consequence of prior immune stimulation needs further attention.

Inflammation and Metabolic Disorders

The periparturient period is associated with substantial metabolic changes involving normal homeorhetic adaptions to support milk production. Early lactation dairy cows enter a normal physiological state during which they are unable to consume enough nutrients to meet maintenance and milk production costs and typically enter into negative energy balance (NEB; Drackley, 1999; Baumgard et al., 2017). During NEB, cows mobilize NEFA in order to partition glucose for milk production in a homeorhetic strategy known as the "glucose sparing". Excessive NEFA mobilization and the affiliated increase in hepatic lipid uptake, triglyceride (TG) storage, and ketone body production has been traditionally believed to be the driving factor leading to ketosis and fatty liver (Grummer, 1993; Drackley, 1999). Until recently, this dogma has been well-accepted as ruminants are thought to have a poor capacity to export TG as very low density lipoproteins (Emery et al., 1992). However, increasing evidence suggests that chronic inflammation may be the driver of these disorders (Bertoni et al., 2006; Eckel and Ametaj, 2016) and this is supported by human, rodent, and ruminant literature which demonstrate effects of lipopolysaccharide (LPS) and inflammatory mediators on metabolism and hepatic lipid accumulation (Li et al., 2003; Barbuio et al., 2007; Endo et al., 2007; Bradford et al., 2009; Ilan et al., 2012; Ceccarelli et al., 2015). We and others have demonstrated that cows which develop ketosis and fatty liver postpartum had higher concentrations of LPS and acute phase proteins prior to diagnosis (Ohtsuka et al., 2001; Ametaj et al., 2005; Abuajamieh et al., 2016). Aside from the mechanistic changes on hepatic function, immune activation markedly reduces DMI (highly conserved response across species) which further increases NEFA mobilization and hepatic ketogenesis. Additional investigation is still needed to better elucidate the mechanisms by which LPS alters hepatic lipid handling and ketone synthesis and extra-hepatic ketone utilization.

Inflammation and Reproductive Function

Bacteria are ubiquitous within the postpartum uterus and pathogenic strains often persist leading to immune activation and consequently infertility (Sheldon and Dobson, 2004; Sheldon et al., 2019). Uterine infection has a variety of negative impacts on reproductive function including; a prolonged luteal phase (Peter and Bosu, 1988; Williams et al., 2008; Sheldon et al., 2009), disrupted ovarian steroidogenesis (Sheldon et al., 2009), and abnormal or delayed folliculogenesis after parturition (Huszenicza et al., 1999). Impaired reproductive functions are not isolated to infections which originate within the uterus. Mastitis, one of the most prevalent transition cow infections, disrupts follicular steroid concentrations and hinders oocyte maturation (Lavon et al., 2011; Asaf et al.,

2014). Furthermore, cows diagnosed with clinical mastitis prior to first service have an increased number of days to first service and days open (Barker et al., 1998). Administering LPS intravenously results in marked disruptions in hypothalamic and pituitary hormone release and ovarian responsiveness (Coleman et al., 1993; Battaglia et al., 2000) and induces abortion (Giri et al., 1990). Presumably, regardless of the origin, infection negatively influences immediate and future reproductive performance. The direct effects of endotoxin and inflammation on reproduction likely explain the associated effects that NEFA, ketones and calcium have with fertility (because immune activation also directly affects these metabolites; as described below).

Inflammation and Hypocalcemia

Impressively, immune activation was originally hypothesized by early investigators to be involved with milk-fever (Thomas, 1889; Hibbs, 1950), but until recently (Eckel and Ametaj, 2016) it has rarely been considered a contributing factor to hypocalcemia. Independent of the transition period, we and others have repeatedly observed a marked and unexplainable decrease in circulating calcium following LPS administration in lactating cows (Griel et al., 1975; Waldron et al., 2003; Kvidera et al., 2017b; Al-Qaisi et al., 2017; Horst et al., 2018a,b, 2019). Infection-induced hypocalcemia is a species conserved response occurring in humans (Cardenas-Rivero et al., 1989; Dias et al., 2013), calves (Tennant et al., 1973; Elsasser et al., 1996;), dogs (Holowaychuk et al., 2012), horses (Toribio et al., 2008), pigs (Carlstedt et al., 2000) and sheep (Naylor and Kronfeld, 1986). Additionally, hypocalcemia occurs in response to ruminal acidosis in dairy cows (Minuti et al., 2014). It is unlikely that cows (even those that are presumably "healthy") complete the transition period without experiencing at least one immune stimulating event and we are likely underestimating its contribution to postpartum hypocalcemia.

Traditional Dogmas

Long-standing tenets describe a causal role of hypocalcemia, increased NEFA, and hyperketonemia in the incidence of transition diseases and disorders (Figure 1). Hypocalcemia has traditionally been considered a gateway disorder leading to ketosis, mastitis, metritis, displaced abomasum, impaired reproduction, and decreased milk yield (Curtis et al., 1983; DeGaris and Lean, 2008; Goff, 2008; Martinez et al., 2012; Chapinal et al., 2012; Riberio et al., 2013; Neves et al., 2018a,b). The proposed mechanisms by which hypocalcemia leads to these ailments include impaired skeletal muscle strength and gastrointestinal motility (Goff, 2008; Oetzel, 2013; Miltenburg et al., 2016), decreased insulin secretion (Martinez et al., 2012, 2014), and the development of immunosuppression (Kimura et al., 2006). Similar to hypocalcemia, increased NEFA and hyperketonemia are presumed causative to illnesses such as DA, retained placenta, metritis, reduced lactation performance, poor reproduction, and an overall increased culling risk (Cameron et al., 1998; LeBlanc et al., 2005; Duffield et al., 2005; Quiroz-Rocha et al., 2009; Ospina et al., 2010; Chapinal et al., 2011; Huzzey et al., 2011). Additionally, elevated NEFA and ketones are thought to compromise immune function (Lacetera et al., 2004; Hammon et al., 2006; Scalia et al., 2006; Ster et al., 2012) and suppress feed intake

(Allen et al., 2009). Thus, the magnitude of changes in NEFA, BHB and Ca have traditionally thought to be predictors of future performance and problems.

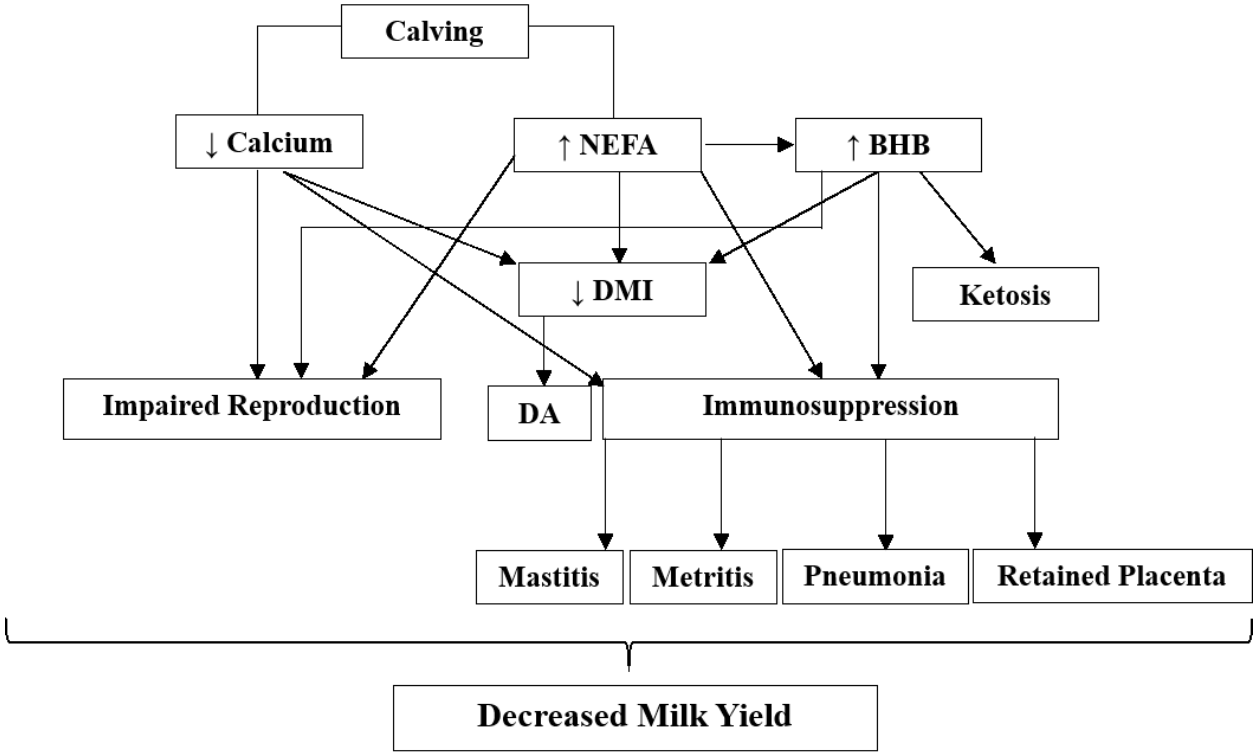

Figure 1. Traditional mechanisms by which hypocalcemia and increased NEFA and ketones are thought to cause poor transition cow health and performance.

Immune Activation: The Etiological Origin

Strong evidence has been generated connecting immune activation as the etiological origin of many of the metabolic and reproductive disorders traditionally observed within the transition period. Additionally, it is probable that immune activation is at least partially explaining the incidence of SCH in the postpartum period (Figure 2). It is intriguing to suggest that cases of delayed, persistent, and chronic SCH recently described by Caixeta et al. (2017) and McArt and Neves (2019) may be related to the severity of the periparturient inflammatory response. This hypothesis may explain why these cases of SCH are associated with reduced “health”, as these represent direct consequences of immune activation rather than being related or caused by decreased Ca. Regardless, these reports challenge the traditional dogmas surrounding hypocalcemia, elevated NEFA, and hyperketonemia and the sequence of transition cow disease.

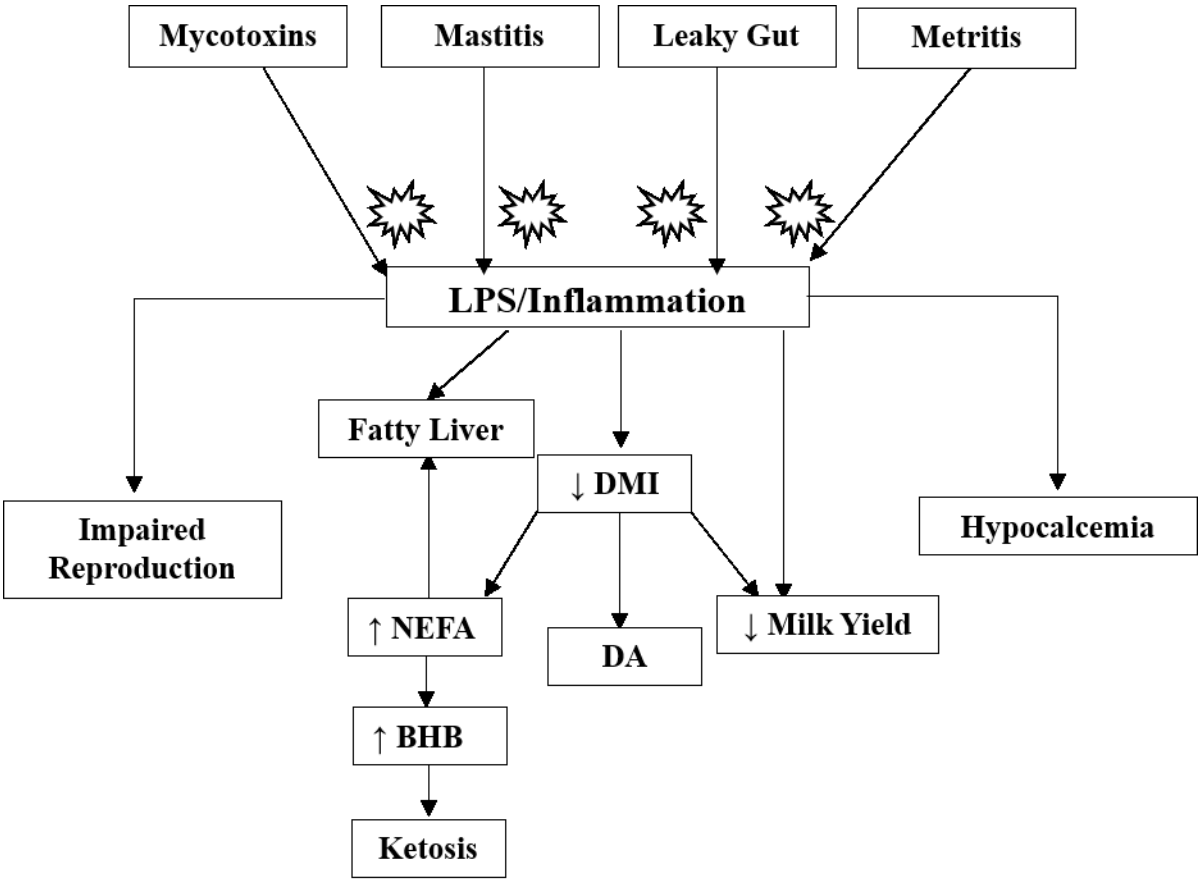

Figure 2. Potential downstream consequences of immune activation. In this model, decreased feed intake, hypocalcemia, excessive NEFA, hyperketonemia and hepatic lipidosis are not causative to poor transition cow performance and health, but rather a reflection of prior immune stimulation.

Calcium Administration following Immunoactivation

Although LPS-induced hypocalcemia is a commonly observed phenomenon, it remains poorly understood what role Ca plays in inflammation and why it acutely decreases. Recently, we investigated the effects of oral and i.v. Ca administration following an LPS challenge in lactating dairy cows (Al-Qaisi et al., 2017; Horst et al., 2018b). Administrating Ca (both orally and intravenously) successfully alleviated the magnitude of LPS-induced hypocalcemia (Figure 3A & B). Furthermore, utilizing a LPS-eucalcemic clamp technique we determined that the total Ca deficit was ~27 g during an acute (12 hour) and intense model of immune activation (Horst et al., 2018b).

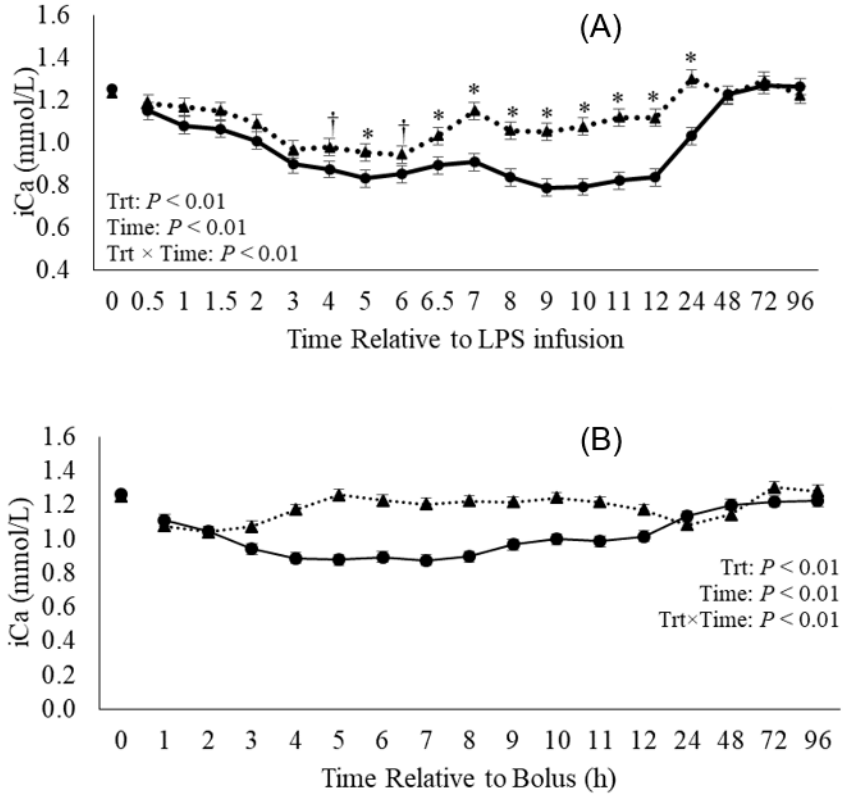

Figure 3. Ionized Ca concentrations from cows allowed to develop hypocalcemia (solid line) vs. cows administered oral (A) or intravenous (B) Ca (dashed line) following lipopolysaccharide infusion.

Although both models (oral and i.v. Ca) successfully lessened the degree of hypocalcemia, the impacts on productivity were markedly different. Administering oral Ca prior to and immediately following LPS administration improved milk yield and DMI when compared to cows allowed to become hypocalcemic (Al-Qaisi et al., 2017). In contrast, maintaining eucalcemia (via i.v. infusion) caused a more intense inflammatory response (i.e., increased acute phase proteins and rectal temperature) and impaired production performance (Horst et al., 2018b). Incidentally, LPS-induced severe hypocalcemia had no impact on neutrophil function nor did rescuing eucalcemia influence neutrophil function metrics (i.e., oxidative burst and myeloperoxidase activity; Horst et al., 2018b). Although it remains unclear why we observed conflicting results, it may be explained by the presence of both live yeast and Ca in the oral bolus. Yeast has previously been demonstrated to have immunomodulatory effects and benefit nutrient utilization, DMI, fermentation patterns, and lactation performance (Desnoyers et al., 2009; Broadway et al., 2015), therefore, we are unable to distinguish between the effects of live yeast vs. Ca in the oral bolus experiment. Another potential likely explanation for the conflicting results may be the route of administration. Intravenous Ca has recently been demonstrated to be detrimental to hormonal regulation of Ca when compared to oral boluses, and studies suggest it should not be utilized to treat subclinical cases (Wilms et al., 2019). Presumably, there are secondary signals associated with alimentary Ca absorption that

might explain why the oral Ca improved multiple productivity metrics following immunoactivation and the i.v. route did not.

Even though the results of the eucalcemic-clamp trial (Horst et al., 2018b) were surprising, they actually agree with the sepsis literature. Septic humans are typically hypocalcemic (Zaloga, 1992; Kelly and Levine, 2013) and early reports indicate that Ca administration to septic patients increased the incidence of organ failure and mortality (Malcolm et al., 1989). It is now hypothesized that sepsis-induced hypocalcemia serves as a protective strategy and should not be considered pathologic. Early investigators described a critical role of decreased blood Ca for optimal LPS detoxification via non-inflammatory routes (Figure 4; Skarnes and Chedid, 1964). In the absence of Ca, LPS aggregation is inhibited, a situation that allows LBP to transfer LPS monomers to cluster of differentiation 14 and eventually to acute-phase high density lipoproteins (ap-HDL) for biliary excretion. Formation of ap-HDL is mediated by SAA displacement of apolipoprotein from normal HDL (Skarnes and Chedid, 1964). This mechanism allows LPS to be detoxified with minimal leukocyte activation and thus less inflammation. In contrast, during eucalcemia the disaggregation of LPS monomers is inhibited (Skarnes and Chedid, 1964) and consequently, LPS is recognized by pro-inflammatory mechanisms; a scenario contributing to a hyper-inflammatory systemic response. Much remains unclear about why Ca decreases following immunoactivation, whether preventing it is beneficial, and where Ca is going during infection. These questions have direct implications to the periparturient inflamed dairy cow and to practical on-farm management and nutrition decisions.

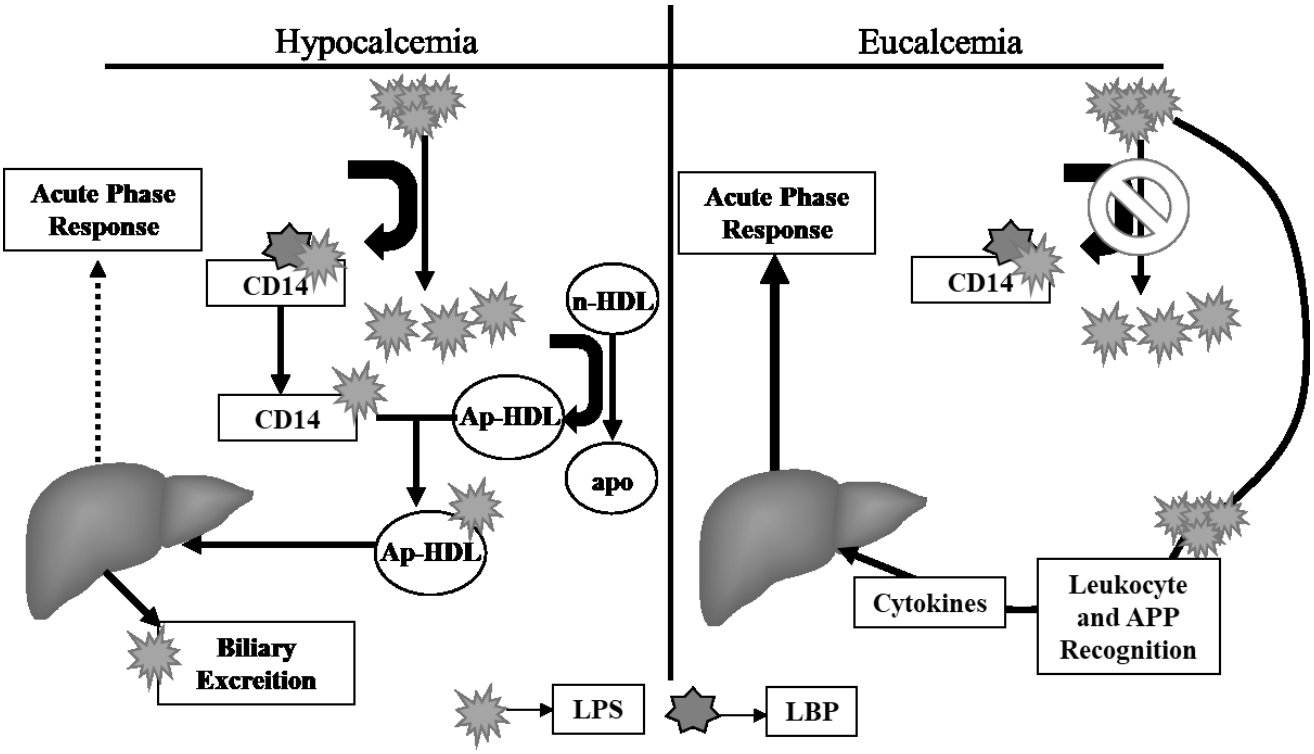

Figure 4. Calcium's proposed role in LPS detoxification.

Conclusion

Transient hypocalcemia remains a prevalent metabolic disorder afflicting dairy cows. Based upon the literature and our supporting evidence we suggest that SCH, along with the many disorders it is believed to be causal towards (i.e., ketosis, poor reproductive performance, metritis, etc.), can be explained (at least partially) by immune activation and the corresponding inflammatory response, a hypothesis which challenges several long standing dogmas in dairy science. More research is required to understand the mechanisms of infection induced-hypocalcemia and whether Ca administration (in particular the route of delivery) is beneficial or detrimental to SCH and future productivity. From a bigger picture perspective, we need a better understanding of whether or not these basic metabolites are “dangerous” (the reductionist theory) or are just reflective of prior immune insults.

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