A 100-Year Review: Metabolic health indicators and management of dairy cattle¹

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ABSTRACT

Our aim in this *Journal of Dairy Science* centennial review is to describe the evolution of focus on metabolic indicators, from discovery and description to evaluation at the individual cow and subsequently herd levels, over the past 100 yr. Furthermore, we discuss current and future technologies that will be used in the dairy industry to utilize these indicators widely going forward. Knowledge of chemical changes in various fluids (e.g., blood, urine, and milk) accompanying numerous metabolic disease states in the dairy cow has existed since almost the beginning of the *Journal of Dairy Science* 100 yr ago. However, only during the last 25 yr have these metabolic indicators been developed into useful tools for cow- and herd-level monitoring for disease and management. From the 1920s through the 1940s, our understanding of the changes in blood chemistry accompanying milk fever and ketosis increased, as did our understanding of the underlying biology. In the 1950s and 1960s, workers studying ketosis and energy metabolism began to evaluate changes in lipid metabolism reflected by concentrations of circulating nonesterified fatty acids; furthermore, initial development occurred for on-farm tests of milk ketones. During the 1970s, blood metabolic profiling was applied to dairy farms but found to be of varied and limited usefulness. The turning point occurred when large epidemiologic studies of periparturient cow disease were pioneered in the United States, Canada, and Europe in the 1980s; these studies further solidified our understanding of risk factors and epidemiological interrelationships among disease, production, and reproduction. In the early 1990s, scientists first incorporated indicators of metabolic health into large observational studies and determined important epidemiological relationships between these indicators and outcomes of interest. This

field of study blossomed during the 2000s as several research groups conducted multiple investigations into metabolic indicators related to energy metabolism and began to develop cow-level thresholds and herd-level alarms for use in monitoring and management. This work was accompanied by additional studies to validate point-of-care instruments that could be used to implement these strategies at the cow and herd levels. Work in the 2000s continued to identify and evaluate other physiological indicators of inflammation and oxidative stress; however, these have yet to be incorporated into large-scale cohort studies. Finally, use of technology (e.g., activity monitoring, cow-monitoring collars and tags, milk-based analysis using Fourier transform infrared spectroscopy) continues to receive significant attention going forward to eventually allow for real-time and automatic monitoring of metabolic indicators and improved health and herd management on dairy farms. **Key words:** transition cow, technology, metabolic disease

INTRODUCTION

Recognition that clinical diseases and disorders are accompanied by chemical changes in blood, urine, or milk of dairy cattle has existed for almost 100 yr. Remarkably, only during the past 25 yr or so have we begun to realize the value of analytes measured in these fluids for both cow- and herd-level disease detection and monitoring. This realization, combined with advances in technology that enable practical measurement at the cow and herd levels through on-farm tests, has created a rapidly growing appetite among dairy farm managers, veterinary practitioners, and other herd consultants for this type of information. Continued gains of knowledge related to the chemistry of fluids that are readily measurable on farm and their relationships with disease and other economically important outcomes related to milk yield and reproductive performance, along with the rapid advancement of technologies that will enable real-time, automated measurement, will continue to revolutionize how we approach the monitoring of metabolic function and health in dairy cattle.

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In this *Journal of Dairy Science* (JDS) centennial review, we describe the evolution of knowledge of metabolic health indicators from the early days of discovery, description, and eventual use in individual cow medicine to the determination—beginning in the 1990s and rapidly expanding through the 2000s—that these indicators are associated more broadly with cow- and herd-level disease, milk production, and reproduction. Furthermore, we review the progression of focus from clinical to subclinical disease detection and monitoring at the herd level and how use of these metabolic health indicators has been an integral part of both the research and eventual on-farm application of programs targeting subclinical disease. We present what we think are the key milestones in Appendix Table A1. Concurrent with research on the biology and epidemiology of subclinical disease and metabolic dysfunction has been progress on the development and validation of technologies that can be applied on farm; we describe the state of our knowledge in this rapidly evolving area. Finally, we provide our perspectives on the development and use of metabolic health indicators in the management of dairy cattle going forward. We focus primarily on contributions made within JDS; however, given the nature of this topic, we also refer to selected works in other journals as appropriate.

THE EARLY DAYS: THE FIRST 75 YEARS

Evidence in the scientific literature that clinical health disorders such as milk fever and ketosis were related to changes in blood and urine chemistry began appearing in the 1920s and 1930s. Various scientists and veterinary practitioners (e.g., Hayden and Scholl, 1923; Dryerre and Greig, 1925; Little and Wright, 1925) determined that cows with milk fever had decreased blood Ca concentrations and hypothesized that dysfunction in the regulation of Ca metabolism in the cow caused milk fever. Others (e.g., Stinson, 1928; Sampson et al., 1933; Lormore, 1934; Boddie, 1935) reported that concentrations of acetone in blood and urine were elevated dramatically in cows diagnosed with ketosis, although causative and predisposing factors were not well recognized at the time. This early work provided the basis for what was to come with more focused work on these chemical changes in blood and urine.

Metabolic Indicators and Hypocalcemia

The first reports in JDS on blood Ca and P and relationships with milk fever appeared during the 1930s. Initially, these papers largely characterized “normal” concentration ranges (later commonly referred to as reference intervals) of these minerals (Anderson et al.,

1930; Palmer et al., 1930; Palmer and Eckles, 1930) and provided some initial characterization of how nutrition and management factors may affect them. Wilson and Hart (1932) assessed the dynamics of Ca and P during the immediate periparturient period in both healthy cows and cows diagnosed with milk fever and suggested that blood samples should be collected at multiple time points relative to calving to adequately characterize the decrease at parturition and recovery of blood Ca concentrations postcalving that occurred even in normal cows; furthermore, patterns of blood P concentrations were much more variable than blood Ca concentrations during the same period.

With the exception of additional reports (Kennedy et al., 1939; Vanlandingham et al., 1942) related to concentrations of Ca and P in blood and various factors affecting them related to age and stage of development, further studies of the relationships of blood chemistry with milk fever were not reported in JDS until the 1950s. Blosser and Smith (1950a) measured serum Ca and citric acid concentrations in both healthy cows and cows with milk fever and determined that citric acid and Ca concentrations generally followed the same pattern in normally calving cows and cows with milk fever; however, concentrations of citric acid were elevated during the immediate prepartum period in cows that subsequently developed milk fever. They determined that cows that developed milk fever had increased urinary excretion of citric acid during the immediate prepartum period and few other changes in urine chemistry other than increased excretion of Mg from d 3 to 16 prepartum. These cows also had increased serum Mg accompanying milk fever during the postpartum period (Blosser and Smith, 1950b). Van Soest and Blosser (1954) noted that cows with milk fever had elevated concentrations of blood glucose and strongly negative correlations between blood glucose and P concentrations.

By this time, milk fever was a well-recognized disorder and the linkage between milk fever and blood Ca concentrations was well established; therefore, scientific focus turned to the mechanisms underpinning Ca metabolism in the cow (e.g., Mayer et al., 1969; Ramberg et al., 1970; Ramberg et al., 1976; Horst et al., 1977) and management strategies to prevent milk fever (reviewed by Horst et al., 1997). Few additional reports relating to the specific use of blood Ca and related analytes as indicators of Ca metabolism appeared in JDS until Ballantine and Herbein (1991) evaluated the relationships of ionized (more metabolically active) and total Ca and determined that the relationships between the two varied by time relative to parturition; the proportion of total Ca in the ionized form was highest during the early postpartum period. Furthermore, Szenci

et al. (1994) determined that ionized Ca concentrations were more stable than total Ca concentrations during the periparturient period. We provide more discussion on testing technologies for Ca status and perspectives going forward in a subsequent section of this review.

Metabolic Indicators and Hyperketonemia

The first reports of the relationships of ketosis with blood and urine chemistry in JDS emerged from a series of studies conducted by workers at the University of Connecticut and published in the early 1940s. Knodt et al. (1942a) measured concentrations of ketone bodies (acetone, acetoacetate, and BHB) in blood and urine of cows and reported variation in ketone body concentrations both within day related to feeding time and across various parts of the year as cows moved from pasture to hay to grass silage feeding. In other work, they determined relationships of blood and urine ketone bodies with time relative to parturition and compared concentrations of ketone bodies in blood and urine, noting a correlation (but with *r* values ranging from 0.33 to 0.51) for concentrations of BHB, acetone, and acetoacetic acid and total acetone bodies in blood and urine (Knodt et al., 1942b). This group conducted numerous other studies (e.g., Knodt et al., 1942c; Shaw et al., 1942; Shaw, 1943) during this period to examine the dynamics of ketone body concentrations in growing animals and the quantitative utilization of ketone bodies related to milk fat synthesis and oxidative metabolism in the cow as well as the onset of ketosis. Furthermore, they noted that cows with complicated ketosis, particularly during the later stages of ketosis, also had elevated concentrations of free fatty acids in blood (Saarinen and Shaw, 1950).

During the late 1950s and 1960s, research groups at the University of Wisconsin and Michigan State University renewed this focus on ketosis and lipid metabolism in the periparturient dairy cow, with additional emphasis on strategies that could be used at the farm level to detect and potentially treat ketotic cows. Schultz and Myers (1959) evaluated a milk test for ketosis that could be used at the farm level, largely over concern that the urine tests available at the time resulted in false positives. Their evaluation suggested high correlations between blood and milk levels related to acetoacetic acid and acetone and lower correlations between blood and milk BHB but high specificity and sensitivity between blood and milk when assessed using total ketone body concentrations. Subsequently, Emery et al. (1964) used a milk ketone test on 2 dairy farms to detect ketosis followed by assignment of cows detected with ketosis to control or propylene glycol treatment groups; they reported that treatment decreased milk

ketones in both herds and increased milk production in one of the herds.

Radloff et al. (1966) conducted a series of studies to extend the information previously determined on blood ketones to also include and evaluate relationships with blood glucose and nonesterified fatty acids (NEFA). They measured sharply decreased circulating NEFA concentrations following feeding, particularly in higher producing cows, and large increases in circulating NEFA following fasting in goats, particularly in lactating compared with nonlactating animals. Finally, they characterized a pronounced increase in plasma NEFA concentrations near the time of parturition followed by decreasing concentrations for the first 5 to 6 wk of lactation. Correlations of blood ketones were much stronger with blood glucose than with plasma NEFA; in their study, there was little relationship of plasma NEFA with milk yield and a low correlation (~ 0.10) of blood ketones and plasma NEFA. In further work focused on ketotic cows (Radloff and Schultz, 1967), they determined much higher positive correlations (~ 0.85) between blood ketones and plasma NEFA following the onset of ketosis.

These groups continued to evaluate testing approaches for ketones and their application. Emery et al. (1968) determined that use of a milk test that would detect acetone plus acetoacetic acid identified 68% of the ketotic cows before the onset of clinical symptoms and claimed that the test used by Schultz and Myers (1959) would have detected only 41% of these cases; furthermore, 75% of the cows detected as ketotic developed another disease within 14 d after the positive test. Menahan et al. (1967) sought to further refine understanding of the relationships between various components of ketone bodies within blood and urine of goats experimentally induced to create varying degrees of hyperketonemia and hypoglycemia. They determined that BHB was the predominant ketone body at low concentrations but that the ratio of BHB to acetoacetate plus acetone decreased as total ketone bodies increased, suggesting greater relative concentrations of the latter at higher total ketone body concentrations. Despite this potential variation in the relationship between BHB and other ketone bodies of interest, BHB remains the sole blood ketone typically evaluated.

Blood Proteins as Metabolic Indicators

The first reports of changes in blood proteins in periparturient cows in JDS appeared in the 1950s following several papers published during the 1940s in *Journal of Biological Chemistry* focused on the relationships between blood proteins in the cow and transfer to the calf through colostrum. Larson and Kendall (1957) mea-

sured changes in various blood proteins during the dry period and continuing into lactation and determined that concentrations of total serum proteins decreased beginning about 4 wk before calving and were lowest at calving before increasing during lactation. Changes in several of the globulin fractions (i.e., β_2 and γ_1) were largely responsible for the decrease, as serum albumin concentrations and concentrations of other globulin fractions (i.e., β_1 and γ_2) were similar across the time period. Reports of changes in blood proteins and other indicators related to protein metabolism were absent from JDS until Blum et al. (1985) described changes in plasma 3-methylhistidine as a marker of protein breakdown and its relationships with other blood indicators during the periparturient period and lactation. They determined that plasma 3-methylhistidine concentrations increased sharply after calving, peaking during wk 1 postcalving and then decreasing until wk 5 postcalving. The spike in 3-methylhistidine coincided with lower concentrations of insulin, thyroid hormones, glucose, protein, and urea and increased concentrations of NEFA. Although we generally have an understanding of the dynamics of protein mobilization in the periparturient dairy cow, we still lack full understanding of the regulation thereof as well as dietary and management factors that affect it.

Metabolic Profile and Its Application

Following several papers in veterinary journals (e.g., Payne et al., 1970; Blowey, 1975; Parker and Blowey, 1976) in which results from blood metabolic profiles in dairy herds were reported and related to herd veterinary investigation or herd status, papers describing blood metabolic profiles, including the Compton metabolic profile and others, began appearing in JDS. Lee et al. (1978) reported means and variances for several blood analytes, including macrominerals, various indicators related to protein metabolism, and glucose in 5 normal and 25 “problem” herds in Illinois. Blood was collected from a representative sample of cows in high-producing, low-producing, and dry cow groups; although not reported, we suspect that most or all of the lactating cows sampled were in established lactation and likely not in the immediate postpartum period. The researchers determined that there were strong relationships of herd, production, stage of lactation, and season with several of the analytes and that several of the analytes were related to intake of certain nutrients; however, there were no clear patterns between normal and problem herds. Interestingly, they concluded that “concentrations of metabolites are of almost no practical use for individual cows because of the extreme variations

in diet required to generate abnormal concentrations of blood metabolites.”

Adams et al. (1978) published an excellent review based on results from blood chemistries from 750 problem herds submitted to the Penn State Large Animal Diagnostic Laboratory beginning in 1968 as well as an in-depth study of 15 high-producing herds with good health and reproduction. They noted statistically significant but low correlations of some blood indicators with nutrient intake along with very inconsistent patterns for herds varying in disease and reproductive status. They provided an excellent overall discussion of the limitations of interpretation of metabolic profiles, including the determination of reference ranges and the appropriate determination of deviations of indicators from normal, and concluded that metabolic profiles could be a useful adjunct in investigations of problem herds but that there was also great “potential for misuse.”

Several additional studies published in JDS in the 1980s focused on the application of blood metabolic profiles. Jones et al. (1982) studied profiles from 30 herds in Virginia varying in production level and concluded that metabolic profile testing was of limited value in assessing problems or nutritional deficiencies in herds. Kronfeld et al. (1982) analyzed blood profiles from 395 cows in 21 herds in Pennsylvania and suggested that distributions of values for many of the blood indicators did not fit normal distributions and that this nonnormality should be considered when developing ranges for interpretation. Furthermore, multiple regression analyses involving multiple ration characteristics improved correlations with blood analytes. These authors were more optimistic than previous authors about the potential value of metabolic profiling given these considerations.

Metabolic Indicators Related to Oxidative Metabolism

During the 1970s, papers began appearing in JDS on the determination of status of vitamins and trace minerals related to oxidative metabolism and immune function in dairy cattle. Schingoethe et al. (1978) assessed vitamin E concentrations of milk from cows fed only stored feed year round and from cows pastured during the summer. They determined that concentrations of vitamin E in milk were increased when cows were fed pasture but did not affect blood cell composition, hemoglobin and related indicators, serum glutamic oxalacetic transaminase, services per conception, calving interval, or retained placenta. However, the number of cows in the study was limited (20 cows per treatment

at the beginning and 12 at the end of the 4-yr study). Maus et al. (1980) reported that increasing dietary Se intake from basal sources and supplemental sodium selenite in the range from 2 to 6 mg/d increased both plasma and milk Se concentrations; however, further increases in Se intake did not further increase plasma or milk Se. Although vitamin E and Se are commonly thought to interrelate, Van Saun et al. (1989) measured vitamin E (α -tocopherol) and Se concentrations in serum from paired dams and fetuses during pregnancy and determined minimal interactions between these nutrients in both the dams and the fetuses.

Chew et al. (1982) determined that concentrations of plasma vitamin A, β -carotene, and total vitamin A equivalent were inversely related to California mastitis test scores indicative of mastitis. In a subsequent study, the same group determined that plasma concentrations of vitamin A and β -carotene decreased sharply during the prepartum period and were lowest during the first week postpartum; vitamin A concentrations were lower postpartum and, curiously, β -carotene concentrations were higher prepartum in cows that developed mastitis during the postpartum period (Johnston and Chew, 1984).

THE TURNING POINT

In the mid-1980s, researchers at Cornell University reported for the first time in JDS the results of large-scale observational studies involving commercial dairy farms and evaluating risk factors for and interrelationships among various periparturient diseases and disorders (Curtis et al., 1984, 1985). At about the same time, Canadian researchers (e.g., Dohoo et al., 1983, 1984; Dohoo and Martin, 1984) published an extensive series of papers from their own large-scale observational study of commercial dairy farms focused on disease, production, and culling in dairy cows. In addition, researchers in Sweden (Andersson and Emanuelson, 1985) published the results of a large observational study focused on hyperketonemia using concentrations of milk acetone to measure hyperketonemia, and Markusfeld (1986) published a large observational study focused on the epidemiological risk factors for displaced abomasum and relationships with other disorders in Israeli dairy herds. Until this time, much of our knowledge regarding risk factors for periparturient disease and relationships between diseases or metabolic indicators and production and reproduction had generally come from much smaller studies (e.g., Schingoethe et al., 1978; Larson et al., 1980) or observations stemming from randomized controlled trials evaluating various dietary treatments (e.g., dry period energy levels investigated by Coppock et al., 1972). Results from these and other

(e.g., Rowlands and Lucey, 1986; Gröhn et al., 1989; Detilleux et al., 1994) large-scale epidemiologic studies of periparturient health, production, and reproduction truly provided the foundation on which our modern understanding of the relationships among these in the periparturient dairy cow is built and facilitated the generation of innumerable testable hypotheses that we continue to evaluate today.

In the early 1990s, work continued to further refine our understanding of the relationships of various metabolic indicators with production, DMI, and energy balance of dairy cattle. In a comparatively small study involving 14 multiparous cows, Lean et al. (1992) investigated concurrent and temporal relationships of serum BHB, NEFA, glucose, cholesterol, milk yield, DMI, and predicted energy balance during the postpartum period using repeated measurements and sampling for all variables. They determined positive associations between BHB and milk yield; a similar relationship between NEFA and BHB; and negative correlations between DMI and BHB, energy balance and BHB, and glucose and BHB.

In our opinion, the study that truly integrated metabolic health indicators within the context of large-scale rigorous analytical studies of periparturient health, production, and reproduction and laid the groundwork for the approach that has been used so successfully by many other research groups, including ours, was conducted by researchers at Michigan State University in the early to mid 1990s and published in part in JDS (Cameron et al., 1998) and as a master's thesis (Dyk, 1995). They enrolled a total of 104 high-producing commercial dairy farms in Michigan, visited each farm 4 times within a 6-wk period, assigned BCS to cows at each visit, collected blood samples from each cow for subsequent analysis of plasma NEFA beginning 5 wk before the expected calving date until calving, and collected detailed health, nutrition, and management information from each farm. They conducted their statistical analyses at both the cow and herd levels. Among other findings, they determined that elevated prepartum plasma NEFA, high BCS, poor feed bunk management, and higher energy content of the prepartum rations were important risk factors for displaced abomasum (Cameron et al., 1998). Furthermore, they determined that elevated prepartum plasma NEFA concentrations were associated with many (dystocia, retained placenta, ketosis, displaced abomasum, and mastitis) but not all (milk fever) periparturient disorders.

In addition to the work above expanding the use of metabolic health indicators related to energy metabolism into large-scale observational studies, researchers interested in oxidative metabolism and immune func-

tion began to integrate these indicators into larger scale studies. Weiss et al. (1990) enrolled 9 herds in a year-long study relating selenium, vitamin E, and SCC. They analyzed blood samples collected from a subset of cows from each herd between 60 d prepartum and 60 d postpartum for plasma Se, α -tocopherol, and glutathione peroxidase and determined that bulk tank SCC and herd mean plasma Se concentrations were correlated negatively. Jukola et al. (1996) evaluated relationships of clinical mastitis, bacterial infections, fertility treatments, and fertility with serum concentrations of vitamin E, vitamin A, and β -carotene and whole-blood Se and glutathione peroxidase in 511 cows across 30 dairy herds in Finland. They determined that cows with higher concentrations of whole-blood Se had decreased mammary infections, but with a low coefficient of determination between serum vitamin A and SCC. However, the DIM for sampling was not well described in their paper, which makes further interpretation difficult.

EXPANDING USE OF METABOLIC HEALTH INDICATORS THROUGH EPIDEMIOLOGY OF PERIPARTURIENT COW- AND HERD-LEVEL OUTCOMES

Energy- and Macromineral-Related Indicators

The first rigorous epidemiologic study beginning to define cow-level cut-points of metabolite concentrations and their association with postpartum health events was conducted by scientists at the University of Guelph and published in 2005 (LeBlanc et al., 2005). In 20 predominantly tie stall herds, the researchers explored several metabolites (e.g., NEFA, cholesterol, BHB, glucose, urea, Ca, and P) and their association with left displaced abomasum (LDA) in 1,044 cows in which the incidence risk of LDA was 5.1%. This work indicated that cows with an NEFA concentration >0.5 mEq/L 4 to 10 d prepartum had 3.6 greater odds of developing an LDA and that cows with a postpartum BHB concentration $>1,200$ μ mol/L had 8 times greater odds of developing an LDA compared with cows with lower concentrations, whereas Ca concentration was not associated with LDA. These results were similar to those of a future Guelph study from a different set of predominately tie stall-housed, component-fed herds ($n = 25$) in Ontario in which the investigators (Duffield et al., 2009) also noted a strong association [odds ratio (OR) = 2.6] with LDA in cows with BHB concentrations $>1,200$ μ mol/L in wk 1 postpartum and negative effects on DHIA test milk yields.

In a series of prospective cohort studies in the northeastern United States by Ospina et al. (2010a,b),

Cornell investigators examined (1) the NEFA and BHB concentration cut-points that are associated with a higher risk of postpartum diseases, higher risk of poorer reproductive performance, and lower milk production; (2) the magnitudes of these risks; and (3) the frequency of elevated NEFA and BHB concentrations in predominately free stall-housed, TMR-fed herds. In 100 herds (average size = 840 cows) and 2,758 cows, they found strong associations between elevations in these metabolites and unwanted subsequent events in otherwise apparently healthy cows. Those cows with NEFA concentrations >0.3 mEq/L between 2 and 14 d prepartum had 2 times higher risk of any postpartum metabolic disease (LDA, metritis, or clinical ketosis), were $>15\%$ less likely to be pregnant by 70 d after the voluntary waiting period, and made approximately 680 kg less 305-d milk than those with lower concentrations. Those cows with NEFA concentrations >0.6 mEq/L between 3 and 14 d postpartum had >4 times higher risk of postpartum diseases (LDA, metritis, or clinical ketosis) and were $>15\%$ less likely to be pregnant by 70 d after the voluntary waiting period; multiparous but not primiparous cows made approximately 500 kg less 305-d milk than those with lower concentrations. Those cows with BHB concentrations >1.0 mmol/L between 3 and 14 d postpartum had >4 times higher risk of postpartum diseases (e.g., LDA, metritis, clinical ketosis) and were $>15\%$ less likely to be pregnant by 70 d after the voluntary waiting period; the cows but not the heifers made approximately 390 kg less 305-d milk than those with lower concentrations. The prevalence of elevated NEFA concentrations—about 25% of the cows and almost 50% of the heifers—was high during the prepartum period. More than 25% of the cows and heifers sampled had elevated NEFA or BHB concentrations during the postpartum period.

Ospina et al. (2010c) then set out to define herd alarm levels to identify when a herd is at risk of poor health, performance, or reproduction as a result of the transition period so that management opportunities can be better identified. Herd alarm levels can be defined as the proportion of statistically sampled animals above cow-level thresholds that are associated with herd-level effects on health, reproduction, and milk production. Ospina et al. (2010c) showed that when $>15\%$ of the sampled animals are above the cow-level NEFA and BHB cut-points there is an association with increased metabolic disease incidence, decreased reproductive performance, and lower milk yield in the herd. Indeed, there is a dose-response effect of increasing metabolic disease incidence when the proportion of animals with elevated NEFA or BHB increases (Ospina et al., 2013).

In a similar series of prospective cohort studies asking related questions, researchers led by the group at the

University of Guelph (Chapinal et al., 2011, 2012a,b) corroborated the findings of Ospina et al. (2013) in a large geographical range including a total of 55 herds from Ontario, New York, Minnesota, Wisconsin, California, and the southeastern United States. In general, with similar thresholds, elevated concentrations of NEFA and BHB were associated with a higher incidence of retained placenta, LDA, and metritis. Further, they showed an association with decreased milk yield but did not observe the same effect on reproduction. At the herd level they also showed an association of the proportion of transition cows above cow-level cut-points with metabolic disease, reproduction, and milk production, albeit at a slightly higher herd alarm level (usually >25%, depending on the outcome).

A comprehensive cohort study by Wolfgang Heuvelink's productive group from Germany (Suthar et al., 2013) showed similar risk for disease on 528 dairy farms from 10 European countries when studying hyperketonemia. In addition, they found an association between lameness and hyperketonemia. A notable recommendation from this study, based on the results from this study and others, was that a universal single threshold of 1.2 mmol of BHB/L for hyperketonemia was practical for farm management and monitoring.

McArt et al. (2012b) intensively examined the ecology of hyperketonemia in 4 high-producing large herds by testing BHB concentrations 6 times between 3 and 16 DIM. Doing so allowed for measurement of the incidence of this condition, which was on average 43%, for the first time in commercial herds; the majority of the new cases of hyperketonemia occurred within the first 7 DIM. Furthermore, the negative effect of hyperketonemia on LDA, culling, conception to first AI, and milk production was found to be much more profound when it first occurred in the first week after parturition compared with the second week. This intensive prospective cohort study established that the prevalence of hyperketonemia was generally half of the incidence. Again, the magnitude of the effect of hyperketonemia on metabolic disease and milk production was similar to that in the aforementioned studies. A randomized controlled trial also showed the value of oral propylene glycol therapy for cows with subclinical ketosis (McArt et al., 2011, 2012a) and described the cost and benefit of routine screening and treatment for hyperketonemia (McArt et al., 2014).

With the intent of preventing hyperketonemia, risk factors and nutritional management have been studied in the United States (McArt et al., 2013; Mann et al., 2015) and the Netherlands (Vanholder et al., 2015), among other places. These studies found that feeding a controlled-energy diet in the dry period greatly decreased the risk for hyperketonemia in the early

postpartum period without negatively affecting milk production compared with feeding cows well above ME requirements (Mann et al., 2015). Consistent and strong risk factors for postpartum hyperketonemia across studies are increasing parity, elevated concentrations of prepartum NEFA, and higher BCS. Hyperketonemia costs approximately US\$290 per case (McArt et al., 2015) or about Can\$200 per case (Gohary et al., 2016) and has a worldwide average prevalence of >20% (i.e., incidence of >40%); therefore, it is likely cost effective to allocate some resources to prevent hyperketonemia.

Ostergaard and Larsen (2000) investigated the relationship between early postpartum blood Ca and milk yield in 153 cows from 27 Danish dairy herds and did not observe an association with fat- and protein-corrected milk yield at any period in the lactation. This mirrors the lack of association noted previously by LeBlanc et al. (2005) between Ca and LDA. However, the series of studies by Chapinal et al. (2011, 2012a,b) previously described also examined the effect of Ca. They observed a 3.1 greater odds of subsequent LDA when serum Ca concentration in the first week postpartum was <2.2 mmol/L as well as a negative effect on first test DHIA milk production and pregnancy at first AI. Further investigating the association of immediate postpartum Ca with subsequent health and production events in the concurrent lactation, Chamberlin et al. (2013) defined hypocalcemia as <1.0 mmol/L of whole-blood ionized Ca. Although they observed an interesting association between hypocalcemia and higher NEFA concentrations as well as greater hepatic fat percentage, they did not find a relationship between milk production or common postpartum diseases like Ostergaard and Larsen (2000) and LeBlanc et al. (2005). However, this study was likely underpowered to detect differences in disease risk. An interesting and often overlooked finding in this study is that the dynamics of whole-blood ionized Ca and plasma total Ca were very similar, suggesting that measuring either is suitable. In an attempt to determine a cow-level cut-point for Ca concentration, Martinez et al. (2012) used receiver operator characteristic curves to find the threshold that had an association with subsequent metritis. In that study, cows with serum Ca <2.15 mmol/L from 0 to 3 d postpartum had an increased incidence of metritis and puerperal metritis compared with normocalcemic cows in one herd with a high incidence of metritis. This cut-point was similar to the 2.2 mmol/L noted by Chapinal et al. (2011) and mentioned previously.

The Guelph group was again the first to examine the relationship between BHB and subsequent reproductive performance (Walsh et al., 2007). In this study, they noted that cows with serum BHB concentrations >1,000 μ mol/L in the first week postpartum and those

with concentrations $>1,400 \mu\text{mol/L}$ in the second week postpartum were 20% less likely to be pregnant at first insemination than those cows with lower concentrations. These cut-points are the same as or similar to those found by Ospina et al. (2010b) and Chapinal et al. (2012b), who also studied other outcomes. Looking for some of the reasons for poorer reproductive performance in cows with hyperketonemia, Dubuc et al. (2010) studied some of the risk factors for uterine diseases such as metritis and cytological (subclinical) endometritis. They noted that hyperketonemic cows had greater odds ($\text{OR} = 1.4$) of cytological endometritis, as did cows with higher concentrations of haptoglobin ($\text{OR} = 1.59$). In a prospective cohort study of 779 cows from 38 herds, Cheong et al. (2011) found that multiparous cows with ketosis had a 5.6 greater odds of subclinical endometritis and suggested that it is likely the result of impaired uterine immune function due to negative energy balance. This concept was echoed by the University of Missouri group (Garverick et al., 2013), who noted that nutritional status during the early postpartum period, as indicated by blood NEFA and glucose concentrations, might affect subsequent fertility by a mechanism that is independent from interval to first ovulation. In support of this, Galvão et al. (2010) determined that, in addition to having higher circulating NEFA and BHB, diagnosis of metritis or cytological endometritis was preceded by or concurrent with decreased concentrations of glycogen in neutrophils harvested from the same animals.

Work from other groups has further investigated these relationships between indicators of metabolic health and reproductive diseases. Giuliodori et al. (2013) suggested that high prepartum NEFA and high postpartum BHB concentrations increased risk for clinical endometritis in Argentina. However, Burke et al. (2010) determined that concentrations of NEFA and BHBA in grazing cattle were not different between cows that did or did not develop cytological endometritis, although plasma albumin concentrations were lower and plasma glutamate dehydrogenase and aspartate aminotransferase were higher in cows that had cytological endometritis.

Energy-related analytes also are related to other infectious disease in the periparturient cow. Using a data set of 634 lactations from 317 cows in Denmark, Moyes et al. (2009) determined that cows with clinical mastitis during early lactation had higher NEFA and BHB concentrations compared with healthy cows and that cows with clinical mastitis during wk 1 postcalving had higher circulating glucose concentrations during the week before parturition. From wk 2 to 13 of lactation, aspartate aminotransferase activities were higher in cows developing clinical mastitis than in healthy cows. In a series of elegant experiments in which they induced

hyperketonemia, Bruckmaier's group in Switzerland suggested that the immune suppression observed during spontaneous hyperketonemia (and its association with increased susceptibility to mastitis) is likely directly caused by elevated concentration of BHB (Zarrin et al., 2014). However, Ster et al. (2012) reported that in vitro addition of NEFA, but not BHB, decreased proliferation and oxidative burst of immune cells. Further mechanistic work is needed to determine the effects of NEFA and BHB on immunity in transition cows.

In addition to relationships with health, reproduction, and milk yield, concentrations of energy-related analytes and Ca are related to risk of herd removal. Seifi et al. (2011) compiled a data set consisting of 849 cows across 16 herds and determined that elevated concentrations of BHB during the postpartum period were associated with increased odds of displaced abomasum, clinical ketosis, and culling by 60 d postpartum. Elevated postpartum NEFA was associated with increased clinical ketosis and culling, and lower serum Ca during wk 1 and 2 was associated with increased culling by 60 d postpartum. Roberts et al. (2012) pooled data from several of the aforementioned studies and found that high concentrations of NEFA and BHB and low concentrations of Ca during the periparturient period were associated with increased risk of culling during the first 60 d postpartum.

Ingvarsen and colleagues (Ingvarsen et al., 2003; Ingvarsen, 2006) postulated that markers or indicators that reflect physiological imbalance would be better than individual biomarkers for assessing periparturient cow status. Moyes et al. (2013) used a large data set (634 lactations from 317 cows, as described above) to evaluate a physiological index comprising plasma concentrations of NEFA, BHB, and glucose weighted with coefficients within week and compare it with calculated energy balance and the individual biomarkers. They reported that cows with a higher physiological imbalance prepartum had greater risk of many postpartum diseases; furthermore, the calculated index and NEFA during the prepartum period were better indicators of postpartum disease than were calculated energy balance, BHB, or glucose. Finally, the authors clearly articulated the need for automated, real-time information based on milk measurements that would accurately reflect physiological imbalance in the periparturient cow.

Blood Proteins

Limited data are available that characterize changes in major circulating proteins during the periparturient period, and relatively little is known about their sensitivity and specificity as potential diagnostic tools. Piccione et al. (2011) measured serum protein fractions

in cows during the late prepartum and early postpartum periods and determined that serum total protein concentrations decreased from the prepartum period to wk 1 postpartum and that decreased concentrations of globulins largely were responsible for the decline in total protein concentrations. Concentrations of serum albumin were relatively stable during the periparturient period but increased slightly in the sample collected at or around the time of calving. Cows with elevated inflammatory response during the postcalving period had decreased serum albumin concentrations and changes in a variety of other serum and plasma components (Bossaert et al., 2012; Trevisi et al., 2012). Consistent with this, Burke et al. (2010) reported that pasture-fed cows diagnosed with endometritis during early lactation had decreased concentrations of plasma albumin and a lower albumin:globulin ratio than cows without endometritis. Furthermore, Rezamand et al. (2007) reported that cows with new IMI postcalving had lower concentrations of plasma albumin.

Although it appears that serum protein or protein fractions may be associated with aspects of health at the cow level, the sensitivity and specificity of using them as markers of herd-level opportunities is not known. Furthermore, responses of these to dietary factors or management have not been well characterized. Cozzi et al. (2011) measured concentrations of a variety of blood-based markers in 740 Holstein cows in 33 dairy herds. They reported significant herd variance components for albumin as well as parity and season of production effects on total protein and globulin; however, detailed study of diets or management practices was not conducted in their survey.

Overall, it appears that changes in plasma protein fractions, particularly albumin, may be associated with health and reproduction in cows. Furthermore, herd-level variance suggests the potential for evaluation of these as metabolic indicators; however, large data sets will be required to assess the robustness (e.g., sensitivity and specificity) of these markers for cow- or herd-level evaluation.

Vitamin-Related Indicators and Disease

Several research groups continued to explore relationships between vitamin status and disease during this time period. LeBlanc et al. (2004) measured serum concentrations of α -tocopherol, β -carotene, and retinol during wk -1 and +1 relative to calving in 1,057 cows on 20 farms. They determined that increased α -tocopherol concentrations during the last week prepartum were associated with decreased risk of retained placenta and that increased retinol concentrations during the last week prepartum were associated with

decreased risk of clinical mastitis during early lactation. More recently, using a much smaller number of cows but more frequent blood sampling during the periparturient period, Qu et al. (2014) determined that cows that went on to have retained placenta and other diseases had lower serum α -tocopherol concentrations during the prepartum period along with higher NEFA and BHB. The same group also reported that lower serum α -tocopherol concentrations preceded displaced abomasum and that cows with displaced abomasum had lower serum cholesterol and higher NEFA, BHB, haptoglobin, and serum amyloid A concentrations during the postpartum period (Qu et al., 2013).

Putative Indicators of Oxidative Stress and Inflammation

During the early 2000s, Italian researchers moved past the prior work focused on measuring concentrations of vitamins or trace elements in blood or tissues as indicators of antioxidant status and started to evaluate indicators of oxidative stress more directly. Bernabucci et al. (2002) reported that periparturient cows subjected to moderate heat stress had increased activity of glutathione peroxidase and superoxide dismutase and concentrations of intracellular thiols in erythrocytes compared with spring-calving cows; however, differences in these indicators were not significant when measured in plasma. In subsequent work, they demonstrated that cows with higher BCS prepartum and greater BCS loss postcalving had higher concentrations of reactive oxygen metabolites, thiobarbituric acid reactive substances, and thiol groups and lower plasma superoxide dismutase activity and erythrocyte thiol groups postpartum, collectively suggestive of increased oxidative stress in cows of higher BCS and with greater BCS loss postcalving concurrent with higher NEFA and BHB concentrations postpartum in the same group (Bernabucci et al., 2005). More recently, Politis et al. (2012) reported that concentrations of α -tocopherol in cows receiving high dietary levels of vitamin E (3,000 IU/d) during the dry period were inversely related with concentrations of serum reactive oxygen metabolites and thiol groups at dry-off and calving; however, these markers of oxidative stress were not related to subsequent development of mastitis.

At about the same time, another research group in Italy began exploring relationships of indicators related to inflammation with metabolism. Bionaz et al. (2007) evaluated relationships of plasma paraoxonase as a marker of liver function. They determined that cows with lower plasma paraoxonase concentrations postpartum also had the lowest concentrations of negative acute phase proteins and the highest concentrations of

positive acute phase proteins such as haptoglobin and oxidative stress indicators such as reactive oxygen metabolites. Further, cows with low plasma paraoxonase produced about 10 kg/d less milk during the first 30 d postpartum compared with cows with the highest concentrations of paraoxonase in plasma. Interestingly, there were no clear relationships with plasma NEFA or BHB, suggesting that inflammatory status can be altered without direct associations with indicators of energy metabolism. Bertoni et al. (2008) proposed a liver activity index based on an aggregate of plasma concentrations of albumin, total cholesterol as a proxy for lipoproteins, and retinol-binding protein and determined that cows with lower liver activity index had higher plasma concentrations of haptoglobin and globulin during the first week postpartum and lower milk yield in early lactation. Cows with lower liver activity index also had more health disorders postpartum and generally poorer reproductive performance, although the final data set was quite small ($n = 77$ cows) for evaluating reproductive and health outcomes.

Circulating acute phase proteins and relationships with health, milk yield, and reproduction continued to receive focus during the late 2000s. Huzzey et al. (2009) reported that cows subsequently diagnosed as healthy, mildly metritic, or severely metritic based on clinical signs had increasing serum haptoglobin concentrations before the diagnosis. Stengårde et al. (2010) found that cows with displaced abomasum had elevated concentrations of serum haptoglobin in addition to elevated plasma concentrations of NEFA and BHB. Huzzey et al. (2011) determined that prepartum plasma haptoglobin had only modest relationships with postpartum health events; however, cows with increased plasma haptoglobin concentrations during wk 1 postpartum had subsequently decreased milk yield and lower risk of pregnancy by 150 DIM (Huzzey et al., 2015). Although circulating haptoglobin concentrations were related to clinical metritis as described above and were reported to be related to increased risk for purulent vaginal discharge and cytological endometritis (Dubuc et al., 2010), other work suggested that there was no difference in plasma haptoglobin concentrations during the postpartum period for cows that subsequently developed cytological endometritis (Yasui et al., 2014). Recently, Pohl et al. (2015) reported that serum haptoglobin concentrations during the early postpartum period were higher in primiparous cows than in multiparous cows and that serum concentrations of haptoglobin were higher in multiparous but not primiparous cows following assisted calving or retained placenta and were positively associated with metritis across all parities. Further, serum haptoglobin was positively as-

sociated with BHB but was not related to serum NEFA concentrations.

All of the studies above that have focused on indicators of oxidative stress and inflammation, with the exception of Huzzey et al. (2011, 2015), Politis et al. (2012), and Pohl et al. (2015), were conducted using small (generally 100 cows or less) data sets, and studies that had larger numbers of cows were conducted in 1 or 2 dairy herds. To date, we lack the type of information for these indicators that has been gleaned from large-scale observational studies described above that related NEFA and BHB to outcomes across many herds with many cows. These types of studies would potentially allow these indicators to advance to become useful in cow- and herd-level assessment and decision making.

CURRENT AND FUTURE TECHNOLOGIES FOR MEASUREMENT OF METABOLIC HEALTH INDICATORS

Blood

Hypocalcemia. Calcium status can be measured directly using either total Ca or ionized Ca concentrations in blood. Although ionized Ca is considered to be the more biologically active fraction and recent work (Leno et al., 2017) suggests that ionized Ca concentrations are more consistently related to neutrophil function in vitro than total Ca, research also suggests that the relationship between ionized Ca and total Ca varies in the days after calving (Ballantine and Herbein, 1991; Leno et al., 2017) and thus cannot be used interchangeably as indicators of Ca status. Notably, the large observational studies referenced above relating Ca status with postpartum outcomes related to disease, milk production, and reproduction all used total Ca as their indicator, and ionized Ca has received limited evaluation as a predictor of these outcomes.

Technologies for measurement of total Ca have not progressed far in the past few decades, and benchtop analyzers remain the gold standard. On-farm tests for ionized Ca, although available, are expensive and have not been validated for use with bovine blood with the exception of the i-STAT analyzer (Abbott Laboratories, Abbott Park, IL) which was evaluated using only 24 samples (Peiró et al., 2010). Due to the difficulty of handling samples to ensure accurate results and cost, ionized Ca is not commonly measured in research settings and is used even less for individual animal treatment or farm management decisions. However, a recent abstract suggested that technologies for cost-effective on-farm measurement of ionized Ca are in development (Neves et al., 2015).

The historical clinical impression of cold ears as an indicator of hypocalcemia was recently evaluated by Venjakob et al. (2016) using an infrared thermometer to determine ear skin temperature. Although cows with clinical hypocalcemia had lower ear temperatures than cows with subclinical hypocalcemia, ambient temperature was a major confounder. Thus, ear temperature alone could not be recommended for accurate diagnosis of subclinical hypocalcemia.

Hyperketonemia. The use of milk powders and milk and urine dip strips for the detection of ketosis gave way to more precise technologies in the late 2000s, when JDS published the validation of a handheld point-of-care blood BHB meter for use as a cow-side test (Iwersen et al., 2009). Laboratory validation of the Precision Xtra meter (Abbott Laboratories) provided 100% sensitivity and 100% specificity against serum BHB determined photometrically at a cut-point of 1.4 mmol/L, and subsequent work with 35 veterinary practices showed that the meter was an accurate and valuable tool for on-farm use to determine blood BHB concentration using cut-points of 1.2 or 1.4 mmol/L. The availability of such an accurate and economic tool allowed for huge advancements in our knowledge of hyperketonemia as mentioned above. Recently, several other handheld BHB meters have been validated for on-farm use in whole blood (Iwersen et al., 2013; Bach et al., 2016). These meters have become the norm for routine monitoring of herd-level hyperketonemia prevalence and individual animal testing for hyperketonemia treatment decisions; some have been validated for use in identifying elevated prepartum BHB concentrations as well (Tatone et al., 2015).

Alongside publication of handheld BHB meter performance, numerous reports have provided information regarding the preferred type, timing, and location of sample collection. Iwersen et al. (2013) and Pineda and Cardoso (2015) showed that plasma and serum samples can be accurately used with handheld BHB meters (some requiring adjusted thresholds) for classification of hyperketonemia. Use of these meters for on-farm whole-blood or stored plasma or serum BHB determination is thus an attractive and accurate method of BHB measurement for many researchers.

Although storage temperature of the meters and testing strips has not been found to confound results with tested devices, the temperature of blood samples has an important effect on the results: the difference between meter BHB and laboratory BHB concentrations decreases as blood temperature increases (Iwersen et al., 2013). Thus, samples should be as close to body temperature as possible to ensure accurate results. Work from Mahrt et al. (2014) showed that blood samples can be collected at any time of day for precise

measurement of BHB concentration in continuously fed dairy cows; however, the jugular vein or coccygeal vessels should be used for blood collection because blood BHB concentrations in the mammary vein are quite a bit lower. Other European colleagues (Kanz et al., 2015; Süss et al., 2016) have found that capillary measurement of blood BHB concentrations using a lancet device in the skin of the exterior vulva or ear is an acceptable and minimally invasive method for classifying cows with hyperketonemia.

Point-of-care meters have also been examined for cow-side determination of blood glucose concentration. Wittrock et al. (2013) found a strong correlation between blood glucose measured on the Precision Extra meter and serum samples run on a chemical analyzer; however, the prevalence of hypoglycemic samples was low and the standard deviation of difference in glucose concentrations was high, which may limit the meter's utility for classification of hypoglycemia. Additional work suggests using adjusted cattle-specific algorithms for both blood and plasma with a recommendation for plasma as the measured analyte (Megahed et al., 2015). Further investigation is thus warranted to optimize the use of point-of-care meters for cow-side measurement of blood glucose as well as to determine the health and production implications of blood glucose concentrations in early-lactation dairy cattle.

NEFA and Haptoglobin. Although there are numerous reports in JDS regarding the association of elevated pre- and postpartum serum NEFA concentrations with subsequent negative disease, production, and reproduction effects at both the individual animal and herd levels (Ospina et al., 2010a,b,c; Chapinal et al., 2011, 2012a,b), there remains no rapid cow-side test for this important energy metabolite. Thus, measurement of NEFA concentrations is currently evaluated either to monitor herd-level prevalence of excessive negative energy balance or for research projects, with samples being sent through a veterinary diagnostic laboratory or run in research laboratories. Both options are still expensive on a per-sample basis, and future development of a rapid, inexpensive cow-side test for blood NEFA concentrations will allow extension of our knowledge to routine use on commercial dairies.

Similar to NEFA, an increase in peripartum haptoglobin has been implicated as an indicator of early-lactation disease such as metritis and has been associated with reduced milk yield and risk of pregnancy (Huzzey et al., 2009, 2011, 2015; Pohl et al., 2015); however, no rapid cow-side test is currently available that limits the utility of this measurement on an individual animal- or herd-level basis. Serum concentrations of haptoglobin are correlated with their milk concentrations (Hiss et al., 2009); however, more comprehensive analysis is

needed before milk concentrations could be considered as a proxy for blood. Future development of a simple and economic testing method to determine blood haptoglobin concentration will allow further investigation into the use of this acute phase protein as a predictor of early-lactation disease, milk production, and reproduction.

Application of Metabolomics. The emerging area of metabolomics offers additional promise to enhance or move beyond the biomarkers of choice at the present time. Hailemariam et al. (2014) used a targeted quantitative metabolomics approach using plasma collected from 12 periparturient cows and assessed about 120 metabolites in plasma from each of 6 healthy and 6 diseased cows. They further subjected these data to principal component analysis and identified several compounds related to fatty acid metabolism (carnitine, propionyl carnitine, and lysophosphatidylcholine acyl C14:0 along with 2 other forms of plasma phosphatidylcholine) that were related to disease in this small data set. Rico et al. (2015) assessed changes in plasma sphingolipids such as ceramides as potential biomarkers of insulin resistance in periparturient dairy cows and determined that specific ceramides were positively correlated with NEFA and inversely correlated with insulin sensitivity. Furthermore, the authors noted marked changes in the plasma sphingolipidome during the periparturient period. We are confident that studies such as these focused on various aspects of metabolomics will provide additional biomarkers for further development into potential indicators for use in practical monitoring and management in the future.

Milk

Current measurement and monitoring of metabolic health using milk parameters rests on the assessment of prediction of the degree of or adaptation to negative energy balance. In the late 1990s and early 2000s, JDS published numerous reports regarding cow-side measurement of ketones in milk using dip strips and powders in relation to prediction of diseases such as LDA as well as their association with blood BHB (Geishauser et al., 1997, 1998, 2000; Enjalbert et al., 2001; Carrier et al., 2004). Their generally poor sensitivity, even with good specificity, has increased widespread adoption of cow-side blood BHB testing methods for disease diagnosis, as discussed above.

Investigation of in-line milk measurement for ketosis monitoring and diagnosis began in the late 1990s concurrent with cow-side milk tests. Duffield et al. (1997) published results in *Canadian Veterinary Journal* on a large study using 1,333 cows from 93 Canadian dairies to assess the association of test-day milk fat and

protein percentages with elevated serum BHB (≥ 1.2 mmol/L). They found that although both test-day fat and protein percentage were associated with elevated serum BHB, neither of these measurements alone or combined provided an accurate screening method. Even at an optimal protein:fat ratio cut-point of 0.75, the sensitivity and specificity of detection of elevated serum BHB were only 58 and 69%, respectively.

Furthering the evaluation of in-line techniques for analysis of metabolic health, the use of infrared spectroscopy as a screening tool for ketosis—an improvement over previously used wet chemistry methods—was first described in JDS by Hansen (1999). Although direct measurement of milk BHB using fluorometric methods was found to be straightforward even with the opacity issues with milk (Larsen and Nielsen, 2005), the lack of this type of measurement system in laboratories handling large quantities of milk samples most likely prevented its widespread adoption. Fourier transform infrared spectroscopy (FTIR) was already in place for measurement of milk fat, protein, lactose, and urea and thus provided a fast and inexpensive method of estimating milk acetone. First reports of the accuracy of mid-infrared FTIR for predicting elevated milk acetone concentrations compared with gold standard milk detection methods showed that it had potential as a screening method, with results varying based on the prevalence of elevated milk acetone in the tested population (Hansen, 1999; Heuer et al., 2001). This line of research has continued with further investigation as a ketosis screening method using FTIR with additional analysis of milk BHB as well as other milk biomarkers such as citrate (de Roos et al., 2007; Grelet et al., 2016). These analytes, and specifically the combination of milk BHB and acetone, increased the accuracy of FTIR predictions for detection of ketosis compared with older milk measurement methods. Similarly, biological models have been reported that use additional animal-specific data (e.g., parity, BCS, DIM, milk yield) along with milk BHB concentrations to predict the risk of ketosis and the suggested time to retesting of milk (Nielsen et al., 2005); however, none of these studies using milk BHB examined the correlation of these measurements with on-farm diagnosis of hyperketonemia.

In 2010, following advancements in cow-side blood BHB measurement accuracy, JDS published a report comparing the milk ketone concentrations determined through FTIR of 69 high-producing dairy cows with blood BHB determination (van Kneegsel et al., 2010). This study determined that FTIR measurement of milk acetone or milk BHB provided a higher sensitivity for detecting hyperketonemia (blood BHB ≥ 1.2 mmol/L) compared with a milk fat:protein ratio at 80, 80, and 66%, respectively. However, all 3 methods of classifica-

tion had specificities of approximately 70% compared with blood BHB, which allowed for a high number of false-positive diagnoses for hyperketonemia using any of these methods.

Recent studies in JDS have aimed at exploring the association of milk components taken at monthly DHIA tests with blood BHB concentrations as well as optimizing milk BHB thresholds for prediction of hyperketonemia. Using blood BHB data from 163 cows in 37 herds in Canada with flow-injection analysis for milk BHB and acetone and FTIR analysis for milk fat and protein, Denis-Robichaud et al. (2014) found good correlation of blood BHB with milk BHB and acetone ($r = 0.89$ and 0.73 , respectively) but poor correlation of blood BHB with fat percentage, protein percentage, and fat:protein ratio ($r = 0.21$, 0.04 , and 0.71 , respectively). They found that the optimal threshold for prediction of hyperketonemia was milk BHB ≥ 0.20 mmol/L and milk acetone ≥ 0.08 mmol/L, which provided sensitivities of 84 and 87%, respectively, and specificities of 96 and 95%, respectively. The first study reporting the association of elevated milk BHB with production was published by Santschi et al. (2016) using 498,310 milk samples from Holstein cows in 4,242 herds in Canada between 3 and 35 DIM on monthly DHI test. Cows with elevated milk BHB (≥ 0.20 mmol/L) produced 2.3 kg/d less milk and had lower protein yield and concentration, higher fat yield and concentration, and higher SCC than cows with milk BHB < 0.20 mmol/L. A limitation of the current literature evaluating production outcomes based on milk BHB or acetone is that studies have focused on testing during routine DHI tests. Although commercial systems such as Herd Navigator (DeLaval, Kansas City, MO) and Afilab (Afimilk Ltd., Kibbutz Afikim, Israel) make in-line milk measurements available for use in commercial dairies, individual animal health and production outcomes based on these technologies are not yet known. It is likely that systems that monitor milk BHB or other components on a continuous basis have better prediction accuracy; however, this idea needs to be investigated further.

Fourier transform infrared spectroscopy has good potential for measuring ketone bodies and other milk components such as fatty acid profiles (Ferrand-Calmels et al., 2014). However, a review by De Marchi et al. (2014) emphasized the variability of reporting results depending on the reference method used and robustness of equation model development. These factors can vary greatly between studies due to the variation in biology under different study conditions (e.g., breed, herd location, diet, and stage of lactation), and the authors highlighted the importance of data exchange between commercial and research groups to maximize future advancement in this area. Use of FTIR to assess energy

balance in early lactation has also been explored (Friggens et al., 2007; Løvendahl et al., 2010; McParland and Berry, 2016), with initial promise for use with additional information regarding individual animal physical traits and adjustment for type of production system. Additional methods of milk metabolomic analysis, including nuclear magnetic resonance spectroscopy, have recently been investigated (Klein et al., 2010), and we expect further exploration of methods for rapid analysis of milk constituents to continue with vast improvement in the near future.

In an attempt to improve prediction of individual animal early-lactation metabolic disease, multiple studies in the last decade of JDS have looked to milk to identify cows with elevated NEFA or hyperketonemia. The first of these was a case-control study from Van Haelst et al. (2008) investigating the milk fatty acid profiles of 8 healthy and 8 hyperketonemic (plasma BHB ≥ 1.2 mmol/L) cows; they found that hyperketonemic cows had elevated levels of C18:1 *cis*-9 in milk fat 2 wk before diagnosis. A larger data set using 92 early-lactation Holstein-Friesian cows from a research herd in the Netherlands found a 50% increased risk of plasma NEFA ≥ 0.6 mmol/L in cows with at least 240 g/kg of C18:1 *cis*-9 in milk fat; however, sensitivity for this method was just under 50% (Jorjong et al., 2014). This group also reported that a milk fat ratio of C18:1 *cis*-9 to C15:0 > 40 was present in 70% of hyperketonemic animals (Jorjong et al., 2015). Mann et al. (2016) investigated the diagnostic value of milk fatty acids and fatty acid ratios for correct classification of 84 multiparous cows in a research herd in New York State as having high NEFA or BHB concentrations. They found that although several fatty acid concentrations were associated with elevated NEFA and BHB, correct classification was only moderate; thus, they could not recommend this measurement method over direct blood detection methods. Both Jorjong et al. (2014, 2015) and Mann et al. (2016) stated that practical use of this information most likely requires routine analysis of milk fatty acids and repeated sampling of an individual animal; however, current technology does not support a method of on-farm milk fatty acid determination that is rapid and cost effective.

Ongoing work in this area attempts to predict blood BHB and NEFA concentrations using various milk components (e.g., BHB, acetone, fat, protein) in conjunction with cow-specific characteristics such as parity, DIM, and breed (Chandler et al., 2015). A potential complication with this comparison is that even when samples are taken repeatedly, the nature of milk sample collection (foremilk vs. composite), timing of sample collection relative to feeding or time of day, and milking frequency can affect milk components. As blood

concentrations of energy metabolites are taken at a single point in time, the association of this timing with milk components of interest, which are some combination of concentration over 8 to 12 h, needs further investigation. In addition, the correlation between milk components of interest specific to certain disease processes (e.g., hyperketonemia and milk BHB) is likely nonlinear and need to be explored further.

Rumination Collars and Tags

Rumination collars were first validated in JDS as an accurate method of determining rumination (Schirrmann et al., 2009). The reported electronic rumination monitoring system, HR-Tag (SCR Engineers Ltd., Netanya, Israel), was highly correlated ($r = 0.93$) with direct observation by a human over fifty-one 2-h periods in 27 Holstein cows. Schirrmann et al. (2013) continued to describe rumination times across the periparturient period in 11 Holstein cows. They found that rumination time decreased in the 24-h period before calving by an average of approximately 1 h (15%) compared with the baseline rumination time of 426 min/d in the 3 to 4 d preceding calving. Total rumination time in the 24 h after calving decreased by an average of a little more than 2 h (31%) compared with baseline but returned to baseline rumination times between 24 and 48 h after calving. When 2-h time periods were considered, rumination time declined in the 4 h before parturition, showing potential for this measurement system to identify cows nearing calving.

In the past 3 yr, multiple studies have investigated the use of rumination time as a tool for predicting early-lactation disease, with the number of enrolled cows in each trial increasing with time. Calamari et al. (2014) retrospectively assigned 23 Italian Friesian cows to a high or low rumination group using data from 3 through 6 DIM. They found that 90% of cows with low total rumination times during this period were diagnosed with clinical disease in early lactation compared with only 42% of cows with high total rumination times. Milk yield was also greater by an average of almost 8 kg/d for those cows with high compared with low total rumination times in the first month of lactation. In 296 Holstein cows on 1 dairy farm in Wisconsin, delivery of a stillborn was associated with a reduced rumination time of approximately 60 min/d for both the pre- and postpartum periods, and delivery of twins similarly reduced rumination time by approximately 60 min/d compared with delivery of a singleton (Liboreiro et al., 2015). This group also found that the rumination time of cows diagnosed with metritis was 30 min/d less than that of healthy herdmates. In 64 Holstein dairy cows on 1 farm in Canada, Schirrmann et al. (2016)

examined rumination time from 10 d before until 21 d after calving and found that cows with postpartum hyperketonemia (BHB ≥ 1.2 mmol/L) spent 14% less time ruminating during the prepartum period than healthy cows.

In 2016, JDS published multiple reports regarding the association of rumination time and disease on a large-scale basis in commercial dairies, focusing on differences in rumination time between healthy and sick cows as well as the predictive capabilities of the system. In a study using 339 Holstein cows from 4 commercial herds in Canada, Kaufman et al. (2016) evaluated the association of rumination time with postpartum hyperketonemia. They found no difference in rumination time from 2 wk before until 4 wk after calving between primiparous healthy cows, hyperketonemic cows, and hyperketonemic cows with additional early-lactation diseases. However, multiparous healthy cows, on average, ruminated 25 and 44 min/d more than hyperketonemic cows and hyperketonemic cows with additional early-lactation diseases, respectively. The largest difference in rumination time in multiparous cows in these groups was seen in the weeks before and 2 wk after parturition. This study showed increased odds of a cow being hyperketonemic or hyperketonemic with additional early-lactation disease if it had reduced rumination time in the week immediately before calving or the week immediately following calving, respectively, which suggests that rumination monitoring across the periparturient period might contribute to identification of early-lactation disease in individual animals.

In a large observational field trial in 1,121 Holstein cows on 1 commercial dairy in New York State, Stangaferro et al. (2016a,b,c) evaluated the performance of an automated health-monitoring collar system, which used a combination of rumination time and physical activity, in detecting cows with displaced abomasum, hyperketonemia, indigestion, mastitis, or metritis and described the time from system alert to diagnosis of disease by farm employees as well as the patterns of rumination and activity surrounding disease diagnosis. The sensitivity of the system for disease alert, assuming a positive system identification from -5 to 2 d after diagnosis of disease by farm personnel, was 93, 91, 89, 58, and 55% for displaced abomasum, hyperketonemia, indigestion, mastitis, and metritis, respectively, with an increase in effectiveness for cows with severe cases of mastitis or metritis. When all diseases of interest were combined, the overall sensitivity of the alert system was 59%; specificity was 98%; positive and negative predictive values were 58 and 98%, respectively; and accuracy was 96%. Of note is that this study was conducted on a single dairy. As the use of rumination and activity collars becomes more widespread in the dairy industry,

our knowledge of within- and between-herd variation in these parameters and their association with disease prediction will improve.

WHERE DO WE GO FROM HERE?

Multiple articles in this review discuss the key issues surrounding maintenance of metabolic health throughout the periparturient period, notably transition and periparturient nutrition and housing management. Other articles in this review discuss selection and effect of genetic traits in reducing disease and improving milk production. These areas should be our focus for preventing early-lactation metabolic disorders. However, the effect of genetic, nutritional, and management strategies on periparturient cow well-being cannot be assessed without appropriate tools for measuring metabolic health at both the individual and herd levels.

Future emphasis in this area will likely continue to be on measurement of constituents in blood or milk that have strong associations with economically important outcomes related to metabolic health in early lactation, such as disease occurrence, milk yield, and reproductive performance. To this end, accurate and precise cow-side or inline tests for these constituents are necessary to further explore the epidemiology of metabolic health during the periparturient period in large field-based studies.

Further advances in technology will allow for real-time measurement and automatic monitoring of metabolic health, providing us with immediate feedback and information on effectiveness of herd-level feeding and management strategies. The ability to directly measure or estimate biologically important items in milk or to develop prediction models for blood concentrations using milk analyses and cow-level information will improve our detection of disease with minimal disruption to farm and cow routines. Integration of these forthcoming discoveries with epidemiological evidence for herd-level strategies to optimize cow health will greatly enhance our knowledge and ability to improve the metabolic health of dairy cattle throughout the periparturient period and lead not only to better detection of opportunities but also to better and more specific and actionable cow- and herd-level recommendations.

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our appreciation for their many contributions. Furthermore, we are excited for our graduate students and contemporary scientists because we think that there will continue to be rapid advancement in this area in the years to come that will revolutionize how we manage dairy cattle on commercial farms.

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APPENDIX

Table A1. Timeline of advances in the understanding, development, and use of metabolic indicators in management of dairy cattle (the reader is referred to the text for citation of specific studies and dates)

Date	Milestone
Mid 1920s	Milk fever is characterized by decreased concentrations of blood Ca.
Late 1920s	Concentrations of acetone are determined to be elevated in urine and blood of cows with ketosis.
1930s	Dynamics of blood Ca and P in healthy cows and those with milk fever are characterized.
1940s	Rapid advances are made in understanding changes in blood and urine chemistry with ketosis and underlying physiology.
1950s	First on-farm milk tests for ketosis are developed and evaluated.
1950s	Changes in blood proteins and relationships to colostrogenesis are determined.
1960s	Ketosis research is extended to lipid metabolism and circulating nonesterified fatty acid concentrations; on-farm milk ketone tests are further refined.
1970s	Blood metabolic profiling is applied to herds and herd investigations with varying results.
1980s	Epidemiological approaches are first applied to determine risk factors for and interrelationships of periparturient disorders.
Mid 1990s	Nonesterified fatty acids are the first metabolic indicator to be incorporated into large-scale observational studies of periparturient health, production, and reproduction.
Late 1990s	Infrared testing of milk ketones is evaluated with a focus on milk acetone.
Mid 2000s	First epidemiological studies to rigorously define cow-level thresholds of metabolite concentrations and postpartum health are conducted.
Mid to late 2000s	Metabolic indicators related to inflammation and oxidative stress are characterized.
Late 2000s	Cow-level thresholds and herd alarm levels for nonesterified fatty acids and BHB are determined.
Late 2000s	Point of care meter for blood BHB is validated, enabling low cost and convenient on-farm measurement.
Early 2010s	Prevalence and implications of subclinical hypocalcemia are revisited.
Early 2010s	Ecology and economics of hyperketonemia are determined.
Early 2010s	Milk ketone testing is expanded to milk BHB using Fourier transform infrared spectroscopy.
Early to mid 2010s	Further evaluation is performed on rumination collars and other electronic monitoring of cow health.
Mid 2010s	Interest in using milk-based constituents to assess and predict metabolic indicators increases.