J. Dairy Sci. 84(E. Suppl.):E100-E112

Adaptations of Glucose and Long-Chain Fatty Acid Metabolism in Liver of Dairy Cows During the Periparturient Period

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ABSTRACT

Tremendous metabolic and endocrine adjustments must be made as dairy cows move from late gestation to early lactation. Requirements for glucose and metabolizable energy increase two- to threefold from 21 d before to 21 d after parturition. The liver must adapt quickly to provide the increased glucose needed to support high milk production, and to process the flood of nonesterified fatty acids taken up from extensive mobilization of adipose triglycerides. While the end results of these adaptations are well known, much less is known about the cellular and molecular mechanisms underpinning hepatic adaptation to lactation. Increases in metabolic activity per gram of liver tissue, not just increased liver mass, are responsible for increased metabolism. Compared with activities present at 21 d before parturition, the capacity of liver tissue isolated at 1 d postpartum to convert alanine (an important glucogenic amino acid) to glucose increases more on a percentage basis than does gluconeogenic capacity from propionate. Likewise, hepatic abundance of mRNA for pyruvate carboxylase increases around calving, whereas mRNA for phosphoenolpyruvate carboxykinase does not. These changes in gluconeogenic enzymes suggest that amino acids from body and feed protein may be critically important sources of glucose for peripartal cows. Hepatic tissue from cows 1 d postpartum has greater rates of palmitate esterification, total and peroxisomal beta-oxidation of palmitate, and activity of mitochondrial carnitine palmitoyltransferase than hepatic tissue from the same cows 21 d prepartum. Prepartal nutrition has been shown to modulate some of these metabolic adaptations in the liver. Effects of hormones and cytokines that mediate adaptive responses to environmental and infectious stressors (or the lack of "cow comfort") have not been investigated. Techniques of modern biochemistry promise to further our understanding of the mechanisms of metabolic adaptation during the peripartal period, and to quantify the effects of nutrition and environment during pre- and postpartum periods on hepatic glucose and lipid metabolism.

Key words: liver metabolism, transition cows, glucose, fatty acids.

Abbreviation key: apo = apolipoprotein, **CoA** = coenzyme A, **LCFA** = long-chain fatty acids, **MTP** = microsomal triglyceride transfer protein, **PC** = pyruvate carboxylase, **PEPCK** = phosphoenolpyruvate carboxykinase, **PPAR** = peroxisomal proliferator-activated receptor, **TG** = triglyceride, **VLDL** = very low-density lipoproteins.

Received July 26, 2000.
Accepted November 6, 2000.
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INTRODUCTION

The importance of a successful transition from late pregnancy to early lactation is unequivocal. Health problems during the periparturient period can easily erase the entire profit potential for an individual cow in that lactation (Drackley, 1999). Less well-documented, yet perhaps equally important, are the potential losses in peak milk yield and lactation persistency that are believed to result from suboptimal transitions. Interest in nutrition and management of dairy cows during the transition period has burgeoned during the last decade as researchers and field nutritionists have recognized the importance of this critical 6-wk period.

The liver sits at the crossroads of metabolism and plays a key role in coordination of nutrient fluxes in support of pregnancy and lactation. Indeed, the liver has been ascribed the ability to "sense the fuel needs of all of the other tissues in the body and respond by adjusting its metabolism accordingly" (Seifter and Englard, 1994). The rapid adaptation of key metabolic pathways in the liver to support lactation is central to the ability of cows to make an uneventful transition. Although the qualitative changes in metabolism that occur during the transition from pregnancy to lactation are well known and represent the concept of homeorhesis (Bauman and Currie, 1980), much less is known about the cellular and molecular nature of these adaptations. A number of reviews on various aspects of this subject are available (Bell, 1995; Bell et al., 2000; Drackley, 1999; Goff and Horst, 1997; Grummer, 1993, 1995; Ingvartsen and Andersen, 2000; Rukkwamsuk et al., 1999b). This review is not comprehensive and will not duplicate those efforts. Rather, we will focus on periparturient changes in metabolic capacity of hepatic carbohydrate and long-chain fatty acid (**LCFA**) metabolism, emphasizing recent contributions in this area. Where data are not available for periparturient cows, speculations are made based on data from other species that may lead to future research in dairy cows.

GENERAL FEATURES OF HEPATIC ADAPTATION TO LACTATION

Bell (1995) presented data and estimated nutrient supply for cows during the first week after parturition. As discussed previously (Drackley, 1999), the deficits in intakes of compounds supplying net energy and metabolizable protein pre-

sent a challenge even for healthy cows. Arguably the most acute challenges are presented by the sudden increases in demand for calcium and glucose at the initiation of milk synthesis. Regulation of calcium metabolism around parturition has been reviewed elsewhere (Horst et al., 1994, 1997) and will not be considered further here.

Calculations presented in detail elsewhere (Overton, 1998) show that estimated glucose demand in Holstein cows is 1000 to 1100 g/d during the last 21 d of gestation, but increases sharply after parturition to approximately 2500 g/d at 21 d postpartum. Most of this increased glucose requirement must be met via hepatic gluconeogenesis. Reynolds et al. (2000a) recently provided a preliminary report of splanchnic tissue metabolism during the periparturient period. Measured glucose release from liver was 1356 g/d at 11 d prepartum and 2760 g/d at 11 d postpartum. The net flux of nutrients removed or provided by the liver in vivo depends on the liver mass, rates of blood flow in arterial and portal supplies, rates of transfer across the cell membrane (determined by transporters or concentration gradients), and intracellular rates of metabolism per unit mass of liver tissue.

Until recently, little was known about changes in liver mass or blood flow during the periparturient period. Bell (1995) reported that fractional protein synthetic rate in liver was substantially increased in cows at d 6 postpartum compared with 9 d prepartum, suggesting liver hypertrophy during the periparturient period. In contrast, Reynolds et al. (2000b) measured visceral tissue mass in 36 Holstein cows slaughtered in late gestation and early lactation. Liver mass was 9.0, 8.8, 8.8, and 9.6 kg at -21, -7, 10, and 22 d relative to parturition, respectively. The DMI for the corresponding time points were 11.5, 12.3, 11.6, and 16.0 kg/d. Thus, a 38% increase in DMI resulted in only a 9% increase in liver mass. These data suggest that changes in liver mass over the transition period are relatively modest and can account for only a fraction of increased metabolism.

Hepatic blood flow is known to increase as digestible energy intake increases (Huntington, 1990). Consequently, the increasing DMI after parturition would be expected to increase hepatic blood flow. Reynolds et al. (2000a) determined that hepatic blood flow in cows increased 84% from 11 d prepartum (1140 L/h) to 11 d postpartum (2099 L/h), whereas DMI increased 44% (9.8 vs. 14.1 kg/d). Oxygen utilization by the liver increased 95%, from 1619 mmol/h at -11 d to 3159 mmol/h at 11 d. If liver mass is assumed to be 8.8 kg and not to change between -11 and 11 d relative to parturition (Reynolds et al., 2000b), the daily metabolic activity per gram of liver tissue nearly doubles, from 4.4 mmol O_2/g at 11 d prepartum to 8.6 mmol of O_2/g of liver at 11 d postpartum. Blood flow responds to metabolic demands of the tissue (Ganong, 1977), so that increased blood flow likely is a response to, and not a cause of, greater metabolic activity. Clearly, the liver's metabolic workload increases very quickly after parturition. Because DMI lags the increased nutrient demands for milk production, increased substrate delivery can account for only a portion of the increased metabolic activity. Key metabolic pathways also must be upregulated.

PERIPARTURIENT ADAPTATIONS IN HEPATIC GLUCOSE METABOLISM

Quantitative Aspects

Estimates of the supply of glucose relative to demand for glucose can be calculated based on the approaches of Bell (1995) as modified by Overton (1998). Results of such calculations (Figure 1) show that demand for glucose increases sharply at calving. Estimates of total splanchnic glucose supply predicted from digestible energy intake exceed demand in late pregnancy but are less than calculated demand by as much as 500 g/d after calving. Calculation of glucose supply from digestible energy intake would largely reflect glucose produced from ruminal propionate, which would be limited by the low DMI around and after parturition. That glucose supply calculated from digestible energy intake exceeded estimated demand before parturition may be associated with increased glycogen synthesis in liver and muscle as well as increased

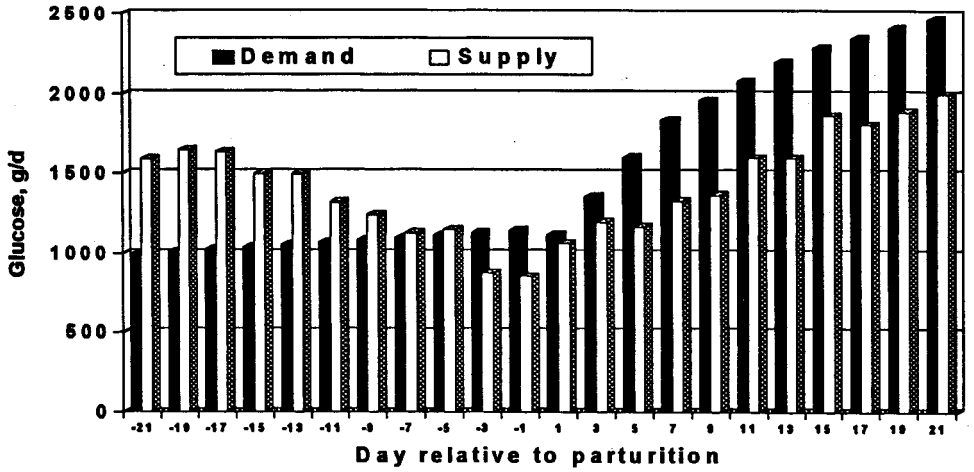

Figure 1. Estimated whole-body glucose demand compared with total splanchnic supply of glucose during the periparturient period. Cows were fed a moderate-grain diet for ad libitum intake during the dry period. Details of calculations are given in Overton (1998). Adapted from Overton (1998), based on data from Douglas et al. (1998) and Overton et al. (1998).

use of glucose for synthesis of $NADPH_2$ and glycerol to support lipogenesis; cows in this group gained liver glycogen and body condition during the dry period (Douglas et al., 1998). Bennink et al. (1972) showed that cows after parturition had decreased whole-body oxidation of glucose and increased glucose entry from a given quantity of feed compared with measurements prepartum. Glucose conservation likely is driven by the increased concentration of somatotropin that occurs around parturition (Grum et al., 1996; Simmons et al., 1994), given that administration of exogenous somatotropin increases glucose irreversible loss rate and decreases whole-body glucose oxidation (Bauman et al., 1988). However, calculation of glucose supply from digestible energy intake obviously underpredicts actual glucose availability to the cow during this time. Glucose output by the liver of transition cows measured in a recent experiment (Reynolds et al., 2000a) closely matches glucose demand calculated for the same cows. At 11 d postpartum when milk production was 36.3 kg/d, hepatic glucose output was 2760 g/d; whole-animal glucose demand calculated as described by Overton (1998) would be 2729 g/d. At 22 d postpartum, milk production was 41.9 kg/d and hepatic glucose output had increased to 3283 g/d; the estimated glucose requirement would be 3121 g/d.

The discrepancy of nearly 500 g/d between predicted glucose from digestible energy intake and estimated glucose demands must be made up by increased gluconeogenesis from intestinally absorbed amino acids and from endogenous substrates such as amino acids, lactate, and glycerol. Maximal contributions to gluconeogenesis have been estimated to be from 32 to 73% for propionate, 10 to 30% for amino acids, about 15% for lactate, and only small amounts from glycerol (Seal and Reynolds, 1993). Steinhour and Bauman (1988) utilized nonpregnant, nonlactating ewes fed at maintenance intake and found that propionate contributed 95% of "new" glucose carbon after mathematical correction for recycling of carbon. However, these estimates have largely been derived from well-fed ruminants; relative contribution of substrates could differ in periparturient cows with high nutrient demands and insufficient nutrient intakes.

Gluconeogenesis from Propionate

Propionate produced during ruminal and hindgut fermentation is quantitatively the most important substrate for gluconeogenesis. Perhaps not surprisingly, the capacity of liver to convert propionate to glucose seems to be responsive to propionate supply. For example, propionate contributed 43.3% of the carbon for gluconeogenesis in steers fed a control diet, but increasing propionate supply by feeding sodium propionate increased its contribution to 67.1% (Veenhuizen et al., 1988). In lactating dairy cows, Aiello et al. (1984) determined that the rate of conversion of [1-^{14}C]propionate to glucose was greater in liver slices from cows fed a high concentrate diet than in liver slices from cows fed a high-forage diet. Additionally, starvation of sheep (Lomax et al., 1986) and severe feed restriction in goats (Armentano et al., 1991) decreased capacity for conversion of propionate to glucose in isolated hepatocytes.

In agreement with these observations, recent data from our laboratory suggest that metabolism of propionate by liver is modulated during the transition period. Overton et al. (1998) measured gluconeogenic capacity in liver slices from cows on four of the five treatment groups used by Douglas et al. (1998). These four treatment groups constituted a 2 × 2 factorial arrangement of diet composition (moderate grain vs. high fat, low NSC) and intake level (ad libitum vs. restricted) during the dry period. Overton et al. (1998) found that propionate conversion to glucose by liver slices was 19 and 29% greater at d 1 and 21 postpartum, respectively, than at d 21 prepartum. Data from this experiment were utilized to determine whether the capacity of liver slices to convert propionate to glucose was correlated with NE_L intake (corrected for fat) during the periparturient period and early lactation (Table 1). Correlations at 21 d prepartum and 65 d postpartum were not significant; however, at 1 and 21 d postpartum, the capacity of liver slices to convert propionate to glucose was correlated with fat-free NE_L intake. Furthermore, the efficiency of conversion of propionate to glucose, defined as the ratio of conversion of radiolabel to glucose compared with conversion to CO_2 (Knapp et al., 1992), was increased at 21 d postpartum compared with 21 d prepartum and 1 d postpartum, suggesting that homeorhetic controls may result in adaptation of liver during early lactation to more efficiently utilize propionate for gluconeogenesis. In sum, these findings suggest that propionate supply and capacity of liver to utilize propionate for gluconeogenesis are linked closely during times of negative energy balance, but plentiful nutrient supply and positive energy balance may modulate this relationship.

Other data indirectly support the idea that gluconeogenic capacity of liver tissue increases after calving. Aiello et al. (1984) found that rates of gluconeogenesis from [1-^{14}C]-propionate in liver slices were threefold greater at d 30 of lactation than in slices from the same cows at 90 and 180 d of lactation. Likewise, rates of glucose synthesis from propionate were greater in hepatocytes isolated from lactating goats than in those from nonlactating goats (Aiello and Armentano, 1987).

Table 1. Correlations[a] between capacity of liver slices to convert propionate or alanine to glucose and NE_L intake corrected for fat[b] during the periparturient period and early lactation.

	Correlation (r) between fat-free NE_L intake and:	
Day relative to parturition	[1-^{14}C]Propionate to glucose	[1-^{14}C]Alanine to glucose
–21	NS[c]	NS
+1	0.48	NS
+21	0.62	NS
+65	NS	NS

[a] $P < 0.05$.

[b] Intakes of NE_L during the 24-h period preceding liver biopsy were corrected for the energetic value of fat (NRC, 1989). Data are from Douglas et al. (1998), Overton et al. (1998) and Overton, Douglas, and Drackley, 2000, unpublished.

[c] NS = Not significant ($P < 0.05$).

Gluconeogenesis from Amino Acids

Although not the primary gluconeogenic substrate under most conditions, amino acids make a sizable contribution to ruminant gluconeogenesis. All amino acids except for leucine and lysine, which are completely ketogenic, can make a net contribution to glucose synthesis, but alanine and glutamine typically make the greatest contribution. Together, alanine and glutamine account for 40 to 60% of the glucogenic potential of all the amino acids (Bergman and Heitmann, 1978). Alanine is

released in the greatest amount of any amino acid by the portal-drained viscera, and is removed by the liver in even greater amounts because of release of alanine by peripheral tissues involved in interorgan amino acid exchange (Bergman, 1986; Wolff et al., 1972).

Similar to propionate, available data indicate that the contribution of amino acids to glucose may be at least partly dependent upon their supply. Lindsay and Williams (1971) infused casein into the abomasum of sheep fed a number of different diets and measured a 40% increase in glucose entry rate. Intra-abomasal infusions of hydrolyzed casein stimulated glucose entry rate in sheep fed alfalfa (Judson and Leng, 1973). Reilly and Ford (1971) intravenously infused mixtures of [U-^{14}C]-labeled amino acids to determine the contributions of amino acids to gluconeogenesis as affected by dietary protein intake. They found that total glucose production was correlated positively with daily protein intake and that rate of glucose production from amino acids was correlated with amino acid entry rate. Excess amino acids are catabolized, primarily in the liver, and the nitrogen is excreted as urea. Therefore, utilization of amino acids for gluconeogenesis may indeed be partially dependent on their supply to the liver.

Danfaer et al. (1995) infused either propionate or amino acids into the mesenteric vein of goats fed alfalfa hay-based diets and, although total glucose flux was not affected by type of infusion, propionate contributed 62% of the glucose synthesized when propionate was infused and 24% when amino acids were infused. Accordingly, the contribution of amino acids to gluconeogenesis increased from 19% when propionate was infused to 36% when amino acids were infused. Infusion of propionate into the mesenteric vein of dairy cows (Casse et al., 1994) resulted in little difference in propionate flux across splanchnic tissues, but increased the release of alanine and somewhat decreased (304 vs. 240 mmol/h; SEM = 24.5) the release of urea by total splanchnic tissues, suggesting that changes in substrate availability affect utilization of individual glucose precursors.

Amino acids may play a particularly significant role as gluconeogenesis increases during the early postpartal period (Bell et al., 2000). Bell (1995) postulated that skeletal muscle serves as a labile pool of amino acids that is mobilized to support increased gluconeogenesis during the transition period. In support of this hypothesis, Overton et al. (1998) used alanine as an indicator of gluconeogenesis from amino acids and found that propionate conversion to glucose at 1 and 21 d postpartum was 119 and 129% of that at 21 d prepartum, but that alanine conversion to glucose at 1 and 21 d postpartum was 198 and 150% of that at 21 d prepartum. Prepartum nutrition had little effect on conversion of alanine to glucose, in contrast to results for propionate; furthermore, intake of NE_L was not correlated with conversion of alanine to glucose (Table 1). Similar results were obtained with hepatocytes isolated from wethers offered a constant amount of feed and administered phlorizin to increase urinary glucose loss; conversion of alanine to glucose was 285% of controls, whereas conversion of propionate to glucose was 166% of controls (Overton et al., 1999).

The substantial relative changes in capacity to convert alanine to glucose compared with changes in propionate metabolism suggest that pyruvate carboxylase (**PC**) activity was increased. Increases in PC activity would effectively channel carbon into glucose from substrates that are converted to pyruvate, including lactate and the amino acids alanine, cysteine, glycine, serine, and threonine. In support of this possibility, Greenfield et al. (2000) found that abundance of mRNA for PC in liver increased 7.5-fold at 1 d postpartum compared with values at -28 d, and then decreased to prepartum values by d 28 postpartum. Although PC activity was not determined on all samples, the enzyme activity was linearly related to mRNA abundance (R^2 = 0.89). In contrast, the mRNA for phosphoenolpyruvate carboxykinase (**PEPCK**) increased more slowly and only by about 50% in samples at 28 d postpartum compared with prepartal values (Greenfield et al., 2000), further supporting results of Overton et al. (1998) in liver slices. Because mitochondrial PEPCK, which is not responsive to hormones or physiological state, may account for over 60% of glucose formation in ruminant hepatocytes (Aiello and Armentano, 1987), PEPCK may not place the same restriction on gluconeogenesis in ruminants as in nonruminants (Greenfield et al., 2000).

Increased capacity for conversion of glucogenic amino acids to glucose might result in more intestinally absorbed amino acids being converted to glucose. Recent data also suggest that adaptations may occur in skeletal muscle, skin, and visceral tissues to supply additional amino acids for gluconeogenesis during the periparturient period (Bell et al., 2000). The ratio of 3-methyl histidine to creatinine in urine, used as an index of the rate of skeletal muscle protein degradation, increased by over threefold by 3 d after calving and then decreased to a new plateau by d 7 to 10 that was approximately twice that of prepartal values (Overton, 1998; Overton et al., 1998). These data support similar findings reported earlier by Simmons et al. (1994) and indicate that muscle protein pools undergo substantial mobilization during the first few days of lactation.

Gluconeogenesis from Lactate and Glycerol

Increased PC activity also should increase conversion of lactate to glucose. However, lactate utilization for gluconeogenesis primarily represents recycling of carbon because most circulating lactate is formed either during catabolism of glucose by peripheral tissues or by partial catabolism of propionate by visceral epithelial tissues. Although 44% of glucose carbon was contributed by lactate when steers were fed high concentrate diets (Krehbiel et al., 1992), lactate should not be produced in appreciable quantities during ruminal fermentation of typical diets fed to cows during the transition period (Nocek, 1997). Limited evidence suggests that lactate may make a greater contribution to gluconeogenesis during late gestation compared with early lactation because of release of lactate by the gravid uterus and muscle during late gestation (Bell, 1995). Baird et al. (1983) determined that the percentage of total glucose recycling and percentage contribution of lactate to glucose flux were lower during early lactation than during late pregnancy. Mills et al. (1986) reported that rates of conversion of lactate to glucose in bovine liver slices did not differ during the periparturient period.

Glycerol released from adipose tissue during lipid mobilization also represents recycling of glucose, but this recycling occurs over the course of a lactation cycle, not on a minute-to-minute basis as for lactate. Consequently, glycerol may be an important gluconeogenic precursor as the cow adapts to lactation. During extensive mobilization of adipose triglyceride (**TG**) of approximately 3.2 kg/d, glycerol may provide maxi-

mally 15 to 20% of the glucose demand at 4 d postpartum (Bell, 1995). Obviously, glycerol supply and its potential contribution to gluconeogenesis is entirely dependent on the amount of adipose tissue mobilized at any given time during the periparturient period. As rates of body fat mobilization moderate with time after parturition to values more typical of early lactation (0.5 to 1.0 kg/d), glycerol maximally would account for only 2 to 5% of total glucose demand. Accordingly, dietary circumstances that increase or decrease the amount of adipose tissue mobilized will increase or decrease the contribution of glycerol to gluconeogenesis during early lactation.

Endocrine Regulation of Gluconeogenesis

Hormones that signal for the increases in gluconeogenesis in the liver of periparturient dairy cows may include insulin, glucagon, somatotropin, and cortisol. Endocrine control of gluconeogenesis has recently been reviewed (Danfaer et al., 1995; Donkin, 1999). The concentration of somatotropin increases markedly at calving (Grum et al., 1996; Simmons et al., 1994). Insulin peaks sharply at parturition (Kunz et al., 1985) but is lower postpartum than prepartum (Grum et al., 1996; Kunz et al., 1985), whereas glucagon remains essentially unchanged (de Boer et al., 1986). Pocius and Herbein (1986) determined that conversion of [1-^{14}C]propionate to glucose and CO_2 was increased in liver slices from lactating cows treated with somatotropin. Conversion of [1-^{14}C]alanine to glucose and CO_2 in liver slices from these cows was not affected by somatotropin treatment, and conversion of [1-^{14}C]alanine to glucose was approximately 25% that of [1-^{14}C]propionate. Similarly, Knapp et al. (1992) found that the rate of conversion of [1-^{14}C]propionate to glucose was approximately doubled in liver slices from cows administered somatotropin compared with controls. Rates of conversion of [1-^{14}C]alanine, [4-^{14}C]aspartate, and [U-^{14}C]glutamate to glucose in liver slices were much lower than that of [1-^{14}C]propionate and were not affected by somatotropin administration in vivo (Knapp et al., 1992). In contrast, Liesman et al. (1995) found that conversion of [1-^{14}C] propionate to glucose in liver slices from cows administered either bovine growth hormone-releasing factor or somatotropin was unchanged compared with controls; however, they utilized a complete media containing multiple substrates for gluconeogenesis, which possibly masked treatment effects on conversion of propionate to glucose.

Glucagon affects gluconeogenesis directly in ruminants. Glucagon stimulated conversion of propionate to glucose in cultured hepatocytes from calves (Donkin et al., 1995) and isolated hepatocytes from both nonlactating and lactating sheep (Faulkner and Pollock, 1990). Brockman and Bergman (1975) demonstrated that intravenous infusion of glucagon into sheep increased glucose synthesis rate with an unchanged proportion of glucose derived from amino acids. Moreover, the proportion of alanine converted to glucose increased and accounted for most of the increased use of amino acids for gluconeogenesis (Brockman and Bergman, 1975). The utilization of alanine by extrahepatic tissues decreased, while release of alanine from extrahepatic tissues was unchanged, indicating that decreased muscle protein synthesis and net catabolism of muscle protein supported the increased glucose production (Brockman and Bergman, 1975). Furthermore, infusion of glucagon into the portal circulation stimulated net hepatic uptake of alanine, glycine, glutamine, arginine, asparagine, threonine, serine, and lactate (Brockman et al., 1975). Interestingly, glucagon infusion into cows during early lactation increased hepatic concentrations of mRNA for PC (She et al., 1999), which could act to shunt more amino acid carbon to gluconeogenesis as discussed earlier.

Conversely, insulin decreases gluconeogenesis. Intravenous infusion of insulin into sheep decreased glucose synthesis rate (Brockman, 1990; Brockman and Laarveld, 1986), but increased the proportion of glucose derived from propionate (Brockman, 1990). This could have resulted in part from the potential for increased uptake of amino acids by peripheral tissues caused by insulin, and thereby decreased amino acid availability for glucose synthesis. In support of this hypothesis, infusion of insulin with simultaneous infusion of glucose at rates appropriate to maintain euglycemia decreased hepatic removal of pyruvate, alanine, lactate, glutamine, and glycerol (Brockman, 1985). Likewise, addition of insulin did not alter conversion of propionate to glucose in monolayer cultures of hepatocytes isolated from ruminating calves (Donkin and Armentano, 1995).

Insulin has been shown to potently counteract the effects of glucagon. Chronic insulin treatment in cultured bovine hepatocytes decreased the ability of glucagon to stimulate gluconeogenesis from lactate (Donkin et al., 1997). Infusion of glucagon into lactating cows resulted in increased secretion of insulin (She et al., 1999); the authors concluded that glucagon alone would stimulate mRNA for PEPCK but that the simultaneous stimulation of insulin blocked the stimulatory effect of glucagon and actually resulted in decreased abundance of PEPCK mRNA. Variation in the concentrations of insulin relative to those of glucagon, which remains more constant, likely plays a key role in modulating gluconeogenesis. The primary effect of insulin is to decrease hepatic uptake of lactate, alanine, glutamine, and glycerol and to decrease conversion of lactate (or other substrates entering via pyruvate) to glucose (Donkin, 1999). Consequently, the increased somatotropin concentrations and the low insulin-to-glucagon ratio after parturition should favor increased gluconeogenesis from propionate, and the low insulin-to-glucagon ratio also would promote increased gluconeogenesis from substrates entering at pyruvate, such as alanine and lactate.

Glucocorticoids also generally promote gluconeogenic processes, although in many cases their effects appear paradoxical. For example, administration of glucocorticoids to lactating and nonlactating cows increased concentrations of glucose in plasma and increased liver concentrations of alanine (Baird and Heitzman, 1970). These changes were accompanied by decreased activity of pyruvate carboxylase, increased activity of glucose-6-phosphatase, and unchanged activity of fructose-1,6-diphosphatase in liver of treated lactating cows compared with their controls. Reilly and Black (1973) infused cortisol into the jugular veins of adrenalectomized sheep and demonstrated increased concentrations of plasma glucose, which resulted from decreased whole-body oxidation of glucose and increased fractional incorporation of carbon from [U-^{14}C]alanine into glucose. Cortisol concentrations begin to increase by the last 3 d before parturition, peak sharply around parturition, and normally decrease to near prepartum concentrations during the first 3 to 5 d of lactation in dairy cows (Goff et al., 1989; Patel et al., 1996). Whether this usually short-lived cortisol secretion plays a role in the increased gluconeogenesis in the early postpartal period is unknown.

From data discussed earlier (Overton et al., 1998; Greenfield et al., 2000), it is evident that hepatic capacity for gluconeogenesis is altered by 1 d after calving. Similar to many other homeorhetic adaptations (Bell, 1995; Grummer, 1995; McNamara, 1991), these adaptations in gluconeogenesis likely begin before parturition at some point in the late dry period. The concept of increasing energy and starch intake during the last 21 d before parturition has been widely accepted and adopted as a method to increase insulin and suppress NEFA release from adipose tissue around parturition, in turn decreasing hepatic TG accumulation (Dann et al., 1999; Minor et al., 1998; VandeHaar et al., 1999). Although this practice could provide increased substrate for gluconeogenesis, the effects on gluconeogenic capacity may be more problematic. On one hand, increased propionate production and digestible energy intake should promote increased capacity for gluconeogenesis from propionate (Overton et al., 1998). On the other hand, increased insulin could block the increase in capacity to synthesize glucose from lactate and alanine. Although data of Overton et al. (1998) showed no effect of prepartum diet on gluconeogenic capacity from alanine, diets were fed from dry-off through parturition. Effects of typical close-up or "steam-up" diets fed for a shorter time are unknown.

PERIPARTURIENT ALTERATIONS IN HEPATIC FATTY ACID METABOLISM

Quantitative Aspects

In contrast to the service function provided by the liver in synthesis of glucose for other tissues, hepatic metabolism of LCFA is of direct importance to the liver itself in provision of energy. Similar to glucose metabolism, however, the liver also performs a service function by converting LCFA to water-soluble fuels (ketone bodies) that can be used by many peripheral tissues. Research concerning periparturient fatty liver has been extensive over the last two decades (Drackley, 1999; Grummer, 1993; Roberts et al., 1981); however, this focus may have obscured more important aspects of the role of hepatic metabolism of LCFA in providing energy for the liver and other tissues.

The primary carbon sources oxidized to provide energy for the liver's functions in dairy cows are not well defined, but are believed to be mainly LCFA, lactate, butyrate, valerate and the branched-chain VFA, and acetyl-coenzyme A (**CoA**) produced from catabolism of amino acids. Data for net flux of substrates across the liver are not yet available for cows during the transition period. Reynolds et al. (1988) measured a net uptake of NEFA by liver of cows at wk 4 postpartum of 60.8 mmol/h, with an arterial NEFA concentration of 328 μM. With the regression equation of Pullen et al. (1989) that relates plasma NEFA concentration to NEFA entry rate, the predicted whole-body entry rate for the cows studied by Reynolds et al. (1988) would be 236.9 mmol/h. These estimates indicate that the liver takes up about 26% of whole-body NEFA flux, which is similar to estimates made with other data (Emery et al., 1992). The calculated contribution of liver to whole-body uptake of NEFA is in the range of values for the percentage of cardiac output flowing through the liver (24 to 38%; Huntington et al., 1990), suggesting that there is no preferential direction of NEFA to the liver at the expense of other tissues.

Calculations from the data set of Reynolds et al. (1988) for cows at wk 4 of lactation indicate that uptake of NEFA by liver could supply from 20 to >60% of O_2 utilization associated with ATP formation, depending on what portion of the NEFA were completely oxidized versus those converted to ketone bodies. These estimates suggest that most of the hepatic NEFA uptake in this situation would be needed for ATP generation. Uptake of butyrate, valerate, the branched-chain VFA, and lactate could supply the remainder without the need for substantial contribution from amino acid catabolism. Whether the demands of liver for oxidizable substrate are signaled to the central nervous system and whether these signals contribute directly to enhanced lipolysis in adipose tissue to meet those demands has not been adequately investigated in dairy cows.

Conversely, imbalances between hepatic uptake of NEFA and hepatic ATP demand may be an important factor in the rapid development of fat infiltration at parturition (Vazquez-Añon et al., 1994). Increased lipolysis triggered by hormonal changes, catecholamine release, and increased activity of the sympathetic nervous system around calving results in large increases in NEFA concentration at parturition (Grum et al., 1996; Simmons et al., 1994; Vazquez-Añon et al., 1994), often exceeding 1000 μM. If the relationships between NEFA concentration and NEFA entry rate determined by Pullen et al. (1989) for cows at 30 d of lactation hold true for periparturient cows, a NEFA concentration of 1000 μM would equate to a NEFA entry rate of 13.45 mol of NEFA daily. If the liver takes up 25% of entry rate as described above, and the average molecular weight of NEFA (approximately 75% C_{18}, 25% C_{16}; Bitman et al., 1984) is 276 g/mol, NEFA uptake would be 140 mmol/h or 928 g/d. The hepatic uptake of NEFA in cows at wk 4 of lactation producing 31.9 kg of milk/d was 60.8 mmol/h (Reynolds et al., 1988). Energy expenditure (oxygen use) of the liver at calving presumably would be similar to that in late gestation, so that the amount of NEFA needed to supply energy requirements likely would be substantially less than 60.8 mmol/h. Even if it is assumed, in this example, that the rate of NEFA oxidation is 60.8 mmol/h in the cow around calving, 79.2 mmol/h (140 to 60.8) or 525 g/d of NEFA would be available for esterification. Thus, 583 g of TG (90% fatty acid) could be produced in the liver in one day. If weight of the liver is 8.8 kg (Reynolds et al., 2000b), hepatic TG content could increase by 6.6 percentage units per day (wet weight basis).

Ketogenesis

In many nonruminant species, hepatic conversion of NEFA to ketone bodies is considered a strategy to spare glucose during times of deficit (Seifter and Englard, 1994). Although oxidative use of glucose generally is lower in dairy cows than in nonruminant animals, similar adaptive processes may occur in dairy cows during the transition period that further decrease oxidation of glucose. For example, Bennink et al. (1972) reported that glucose oxidation decreased markedly at 10 d after calving compared with 7 d before calving. Ketone bodies can be oxidized by the heart, kidney, skeletal muscle, mammary gland, and gastrointestinal tract of ruminants (Heitmann et al., 1987) and can serve as substrates for mammary fatty acid synthesis. Therefore, increased ketogenesis during the transition period may be an additional strategy to compensate for insufficient intake of glucose precursors.

According to the classical principles of respiratory control, substrate oxidation and ATP synthesis proceed only as

fast as needed to supply ATP for endergonic reactions within the cell. Consequently, mitochondrial β-oxidation and ketogenesis from high rates of NEFA uptake could continue only at the rate dictated by cellular demands for ATP. This concept has been challenged, however, by demonstration of "reverse electron transport" in isolated liver cells from rats (Berry et al., 1983). These researchers argued that oxidation of acetyl-CoA to CO_2 was preferentially coupled to ATP synthesis, but production of acetyl-CoA by β-oxidation is obligatorily linked to reverse electron transport. This process was theorized to involve cycling of reducing equivalents at the flavin-linked step of β-oxidation through reduction of acetoacetate to β-hydroxybutyrate, which then is re-oxidized in the electron transport chain to acetoacetate with the subsequent dissipation of energy as heat. Thus, ketogenesis was proposed as a thermogenic process, in which LCFA could continue to be metabolized to ketone bodies without being subject to limitations by ATP turnover. Such a process in ruminants would help to explain why oxidation rates continue to increase in bovine hepatocytes as media NEFA concentration is increased (Cadórniga-Valiño et al., 1997). However, support for this theory has not materialized, and the in vivo significance is unclear even in rats (Berry et al., 1983). Furthermore, because the ratio of acetoacetate to β-hydroxybutyrate increases as hepatic ketogenesis increases (Heitmann et al., 1987), it seems unlikely that this mechanism could be operative in ruminants. Other pathways, such as the glutamate dehydrogenase reaction, could be involved (Berry et al., 1983).

Recently, an inducible uncoupling protein, UCP-2, has been identified in tissues other than brown adipose tissue of rodents (Ricquier and Bouillaud, 2000). This protein has been postulated to be involved in dissipation of energy during active ketogenesis, but data in confirmation are not yet available. Exploration of these and similar processes in periparturient dairy cows could lead to improved understanding of the mechanisms of ketogenesis and lactation ketosis.

Fatty Acid Oxidation

The pathways of LCFA metabolism and their corresponding regulation in the liver of dairy cows have been the subject of numerous recent reviews (Drackley, 1999; Emery et al., 1992; Grummer, 1993, 1995; Hocquette and Bauchart, 1999). As described above, the liver of dairy cows at parturition is faced with a markedly increased uptake of NEFA mobilized from adipose tissue. Some degree of lipid infiltration of the liver during the periparturient period seems to accompany the adaptations to lactation under most circumstances (Grum et al., 1996; Minor et al., 1998; Roberts et al., 1981; Vazquez-Añon et al., 1994). That the liver adapts its metabolic capacity apart from changes in substrate delivery has been demonstrated with in vitro data from liver slices (Drackley et al., 1991b). Total capacity for palmitate metabolism seemed to follow energy balance and physiological state; palmitate utilization (nmol/[h × g wet weight]) was 547, 477, and 430 for nonlactating cows, cows in early lactation (mean 38 d), and nonlactating cows starved for 7 d, respectively. Furthermore, partitioning of palmitate metabolism also varied with physiological state. The proportion of total palmitate uptake that was oxidized increased inversely with energy balance (23.6, 40.7, and 66.3% for nonlactating, early lactating, and nonlactating-starved, respectively), whereas the proportion esterified decreased (76.2, 59.3, and 33.9%, respectively). Comparable longitudinal studies, in which rates of oxidation and esterification were measured in liver slices from cows during the transition period, have not been conducted.

Total oxidation of palmitate by liver homogenates was about 12% greater at 1 d postpartum than at 21 d prepartum (Grum et al., 1996). Total activity of carnitine palmitoyltransferase (CPT), as determined in mitochondria prepared from previously frozen liver tissue, was 49% greater at 1 d postpartum than at 21 d prepartum (Dann et al., 2000). Activity at 21 d postpartum still was 27% greater, but CPT activity had returned to prepartum values by d 65 postpartum. Because freezing disrupts mitochondrial membranes, this activity likely represented both CPT-1, which is the regulatory enzyme, and CPT-2, as well as some contribution from microsomal carnitine acyltransferase (Zammit, 1999a). Thus, the amount of CPT present for import of NEFA into mitochondria seems to be increased by d 1 postpartum. The hepatic concentration of carnitine, the second substrate for CPT-1, also is greatly increased around parturition (Grum et al., 1996). Increased carnitine in liver probably is a result of increased mobilization around parturition of skeletal muscle protein (Bell et al., 2000), which releases the precursor of carnitine, trimethyllysine.

Likely of more importance, however, is the regulation of the increased CPT-1 activity, including changes in malonyl-CoA concentration and sensitivity of CPT-1 to malonyl-CoA inhibition. Malonyl-CoA concentration is responsive to changes in insulin and glucagon in ruminants (Brindle et al., 1985; Knapp et al., 1990), increasing when insulin increases and vice versa. In rodent models, decreases in the sensitivity of CPT-1 to inhibition by malonyl-CoA follow decreases in insulin, which serve to amplify the signal and increase transport of NEFA into the mitochondria (Zammit, 1996, 1999a). Possible changes in sensitivity of bovine CPT-1 to malonyl-CoA during the periparturient period have not been reported. Evidence for a functional regulatory role for CPT-1 in ruminants has been presented by others (Chow and Jesse, 1992; Jesse et al., 1986).

Hepatic ketogenesis is regulated by 1) substrate (NEFA) supply to the liver, 2) the activity of CPT-1 to promote entry of fatty acyl-CoA into mitochondria for acetyl-CoA production, and 3) the intramitochondrial activity of 3-hydroxy-3-methylglutaryl-CoA synthase (HMG-CoA synthase), which is the regulatory step in conversion of acetyl-CoA to ketone bodies (Hegardt, 1999). HMG-CoA synthase is inactivated via succinylation by succinyl-CoA, and so serves as a sensor of anaplerotic carbon supply into the citric acid cycle. In dairy cows, therefore, abundant supply of propionate should increase the pool size of succinyl-CoA in mitochondria, thereby inhibiting HMG-CoA synthase. The reverse is true also; decreased propionate supply should decrease succinyl-CoA concentration in the mitochondria, allowing desuccinylation of HMG-CoA synthase and its subsequent activation. As pointed out by Zammit (1990), this mechanism likely is unaffected by carbon supply through pyruvate, allowing substantial rates of ketogenesis during active gluconeogenesis from lactate or alanine. Changes in HMG-CoA synthase expression or activity during the transition period have not been determined.

One theory of ketosis development that persists today is that high rates of gluconeogenesis during the negative energy balance of the periparturient period enhance ketogenesis because the removal of carbon from the mitochondrial citric acid

cycle for cytosolic glucose synthesis depletes oxaloacetate in the mitochondria (Krebs, 1966). According to this idea, insufficient oxaloacetate would be available to combine with acetyl-CoA from NEFA oxidation for combustion in the citric acid cycle; consequently, the "excess" acetyl-CoA is "diverted" to ketone body synthesis. In light of the increased understanding of regulation of mitochondrial β-oxidation and ketogenesis, this theory is untenable for several reasons. First, the rate of β-oxidation of NEFA essentially is controlled by CPT-1 in ruminants (Brindle et al., 1985), as discussed earlier. Second, oxaloacetate most likely is maintained at low concentrations in mitochondria. This is primarily attributable to the high NADH/NAD ratio during NEFA oxidation, which promotes efflux of carbon as malate to the cytosol (Zammit, 1990). Third, β-oxidation of NEFA produces acetyl-CoA, which is an activator of PC that can maintain oxaloacetate concentrations in the mitochondria (Chow and Jesse, 1992). Fourth, the high NADH/NAD ratios during NEFA oxidation, rather than deficiencies of oxaloacetate for citrate formation, inhibit the dehydrogenase reactions of the citric acid cycle (Mayes, 1996). Finally, low succinyl-CoA concentrations in the mitochondria activate HMG-CoA synthase (Hegardt, 1999) as described earlier.

An alternate pathway of β-oxidation in liver is found within the peroxisomes. Assumed to be an auxiliary pathway to mitochondrial β-oxidation, the peroxisomal pathway may be induced during times of increased hepatocellular influx of NEFA. Grum et al. (1994) found that peroxisomal β-oxidation represented nearly 50% of the total capacity for the initial cycle of β-oxidation of palmitate in liver homogenates from dairy cows, which was much higher than values found in the same experiment for retired female breeder rats (26%). Using the same methodology, Piot et al. (1998) subsequently studied peroxisomal β-oxidation in tissues from growing rats, calves, and growing bulls and found no differences between species in the proportion of peroxisomal versus mitochondrial β-oxidation. However, differences in age, sex, and hormonal exposure between the lactating (or previously lactating) female animals used by Grum et al. (1994) and the young growing male animals used by Piot et al. (1998) might explain the difference in results between studies. Grum et al. (1996) noted a 12% increase in peroxisomal β-oxidation at d 1 postpartum compared with rates at 21 d prepartum for cows fed control or high-grain diets prepartum. However, peroxisomal β-oxidation was 36% greater at 21 d prepartum for cows fed a high-fat diet during the dry period; this advantage compared with cows fed control or high grain diets persisted through 21 d postpartum. Peroxisomal β-oxidation was negatively correlated ($r = -0.41$) with hepatic TG content at 1 d postpartum (Grum et al., 1996). Induction of peroxisomal β-oxidation by dietary fat or nutrient restriction (Douglas et al., 1998) may help to deal with the excess uptake of NEFA around parturition by providing an oxidative pathway that is not subject to regulation by CPT-1.

Triglyceride Synthesis and Secretion

Rates of esterification of palmitate in liver slices from cows during the periparturient period were measured by Grum et al. (1996). Esterification rates at 1 d after parturition in cows fed control or high-grain diets during the dry period were 188% of rates at 21 d prepartum; rates at 21 d postpartum were 124% of prepartum values. In contrast, cows fed a high-fat diet during the dry period had a smaller increase (7%) in esterification activity at 1 d postpartum. Changes in esterification capacity were positively correlated ($r = 0.44$) with increased TG concentrations in liver at d 1 postpartum. The enzymatic basis for these changes is not known, although Van Den Top et al. (1995) reported increased activities of phosphatidate phosphohydrolase and diacylglycerol acyltransferase but not of glycerolphosphate acyltransferase in liver of cows after parturition compared with their activities prepartum. In a second experiment, Van Den Top et al. (1996) found no differences in activities of phosphatidate phosphohydrolase and diacylglycerol acyltransferase between overfed cows that developed fatty liver and control cows, but activity of glycerolphosphate acyltransferase was lower in cows that developed fatty liver. However, uptake of NEFA by liver of these cows probably exceeded hepatic demands for ATP synthesis, in which case the liver would need either to dissipate energy as heat as discussed earlier or esterify the excess NEFA. Enzymatic activities in the esterification pathway probably did not limit flux through the pathway in this case.

Although rates of TG formation in ruminants are similar to those in nonruminants (Kleppe et al., 1988), secretion of TG in very low-density lipoproteins (**VLDL**) is much lower in ruminants (Graulet et al., 1998; Kleppe et al., 1988; Pullen et al., 1989). Consequently, high rates of NEFA influx to the liver in excess of demands or capacities for oxidation lead to increased TG synthesis and accumulation in periparturient dairy cows. Much attention has been given to the mechanisms that might explain the low hepatic rates of synthesis and secretion of VLDL (Bauchart et al., 1996; Grummer, 1993; Hocquette and Bauchart, 1999). Low concentrations of apolipoprotein (**apo**) B in plasma and liver have been reported for cows in early lactation and in association with fatty liver (Gruffat et al., 1997; Marcos et al., 1990). However, given the high rates of NEFA uptake by the liver and the low rates of VLDL secretion, large increases in secretion rates likely would be needed to prevent the rapid accumulation of TG in periparturient cows.

Both in vitro and in vivo studies have suggested that newly synthesized TG enters cytosolic storage pools, but TG in newly synthesized VLDL originates from a smaller pool within the microsomal compartment (Zammit, 1996, 1999a, 1999b). This secretory pool is highly associated with the rate of de novo lipogenesis, which is low in cattle (Emery et al., 1992; Grummer, 1993). Transfer of TG from the cytosolic pool to the secretory compartment evidently requires hydrolysis at least to the level of diglyceride, which then can be transferred into the microsomal compartment and re-acylated to form TG (Zammit, 1999a, 1999b). A lipase that may be responsible for the lipolysis of stored cytosolic TG has recently been identified and characterized in rat liver (Lehner et al., 1999).

Recent studies have suggested that the size of the microsomal secretory pool of TG is much smaller in calf liver than in rat liver (Graulet et al., 1998). Rates of apo B synthesis were similar between calf and rat liver, but secreted apo B was much less in calf liver, suggesting that the synthesized apo B was degraded before secretion (Gruffat-Mouty et al., 1999). This finding strongly suggests that low plasma or liver concentrations of apo B (Gruffat et al., 1997; Marcos et al., 1990) are secondary to an as-yet unidentified factor that limits lipid accretion into secretory VLDL. Gruffat-Mouty et al. (1999) suggested that microsomal TG transfer protein (**MTP**), which

is responsible for transfer of TG into the growing VLDL particle, might be deficient or inactive in ruminant liver. However, Bremmer et al. (1999) reported substantial MTP activity in bovine liver. Furthermore, no relationship existed between measured MTP activity and reported VLDL export rates among a variety of species. In a subsequent study, Bremmer et al. (2000) found no relationship between degree of fatty liver and activity of MTP in dairy cows.

Endocrine Control of Hepatic Fatty Acid Metabolism

Hormonal regulation of hepatic LCFA metabolism has been discussed (Bell, 1995; Grummer, 1993; Hocquette and Bauchart et al., 1999). In general, LCFA metabolism in the liver of dairy cows seems to be less responsive to hormonal control than is metabolism in laboratory species (Cadórniga-Valiño et al., 1997). The most pronounced effects of hormones are on the supply of NEFA to the liver, rather than on intracellular disposal of NEFA. Insulin increased the proportion of total oleate uptake by cultured calf hepatocytes that was esterified, which is consistent with the known action of insulin to enhance esterification (Zammit, 1996). Insulin decreased CPT-1 activity in isolated sheep hepatocytes (Chow and Jesse, 1992), but did not affect oxidation of oleate in cultured calf hepatocytes (Cadórniga-Valiño et al., 1997). Recently, exogenous glucagon administered to dairy cows in early lacatation was shown to promote clearance of stored TG and prevent further hepatic TG accumulation (Hippen et al., 1999).

Similar to changes in glucose metabolism, most of the changes noted in hepatic capacity for LCFA metabolism occurred by 1 d postpartum, suggesting that adaptations began prepartum. The possibility that pathways of hepatic LCFA metabolism can be altered by nutritional management during the dry period (Douglas et al., 1998; Grum et al., 1996) is intriguing. Little is known about nutrient or hormonal effects on expression of genes for enzymes of LCFA metabolism in dairy cows. Nuclear transcription factors such as peroxisomal proliferator-activated receptors (**PPAR**) and hepatocyte nuclear factor-4 likely are involved in induction or repression of transcription of regulatory genes in bovine liver as in other species (Desvergne and Wahli, 1999), but this remains to be investigated.

Membrane Fatty Acid Composition

An aspect of LCFA metabolism during the periparturient period not related directly to energy derivation is the change in fatty acid composition of glyceride pools within the liver, including TG and cell membranes. Alteration of cell membrane composition could affect membrane fluidity and membrane transport of substrates or products (Peck, 1994). Such changes could directly impact regulation of LCFA and glucose metabolism in liver. For example, alteration of the fatty acid composition of hepatic mitochondrial membranes affects the sensitivity of CPT-1 to malonyl-CoA inhibition (Power et al., 1994). Alteration of dietary fat and thus hepatic membrane fatty acid composition affects the ability of glucagon to stimulate adenyl cyclase activity (Dax et al., 1990). Alteration of membrane fatty acid composition changes the fatty acids that are mobilized from phospholipids for formation of prostaglandins and other molecular signals in many species (Peck, 1994), including ruminants (Ashes et al., 1995). These topics have received little attention in periparturient dairy cows.

Hepatic lipid composition could be altered by prepartum diets or by the extensive mobilization of body fat around parturition. Rukkwamsuk et al. (1999a) measured fatty acid composition of total liver lipid in control cows and those with fatty liver induced by overfeeding. They found that in cows with fatty liver, content of $C_{18:1}$ increased and $C_{18:2}$ decreased relative to prepartum values. However, this information is difficult to interpret because of the different fate of TG versus phospholipid fatty acids. We (J. Rehage, G. N. Douglas, A. D. Beaulieu, and J. K. Drackley, unpublished data, 2000) have recently obtained preliminary evidence that the LCFA composition of both TG and phospholipids changes during the periparturient period and that these changes may be affected by prepartum diet. This area would seem to be of considerable potential importance for understanding regulation of liver and whole-body functions in periparturient cows.

INTERRELATIONSHIPS BETWEEN GLUCOSE AND FATTY ACID METABOLISM

Although the periparturient adaptations in metabolism of glucose and fatty acids have been discussed separately here, and often are studied in isolation, the challenge in understanding the biology of transition cows is to integrate information on all metabolic systems. Hepatic metabolism of glucose and LCFA are linked closely, as is metabolism of amino acids, which is not discussed in this review. The relative abundance of gluconeogenic substrates modulates LCFA metabolism; propionate is a powerful inhibitor of β-oxidation (Armentano et al., 1991; Drackley et al., 1991a, 1991b; Jesse et al., 1986). Possible mechanisms for this inhibition have been reviewed by others (Emery et al., 1992; Grummer, 1993; Zammit, 1990). Given the central importance of propionate in ruminant intermediary metabolism, the relative abundance of propionate likely is signaled to the liver through a combination of several mechanisms that in turn regulate LCFA metabolism.

Oxidation of NEFA and other substrates provides ATP needed for gluconeogenesis. Inhibition of LCFA oxidation decreases gluconeogenesis from propionate (Chow and Jesse, 1992), although the mechanism for this effect remains unknown, as well from pyruvate and lactate (Chow and Jesse, 1992). Oxidation of NEFA is necessary for stimulated gluconeogenesis from lactate or pyruvate, at least in part by provision of the activator of PC, acetyl-CoA (Chow and Jesse, 1992). In vitro studies also have shown that pyruvate, lactate, and alanine stimulate palmitate oxidation by as much as 34% (Drackley et al., 1991a), suggesting that increased availability of these substrates during the periparturient period could stimulate LCFA oxidation to drive their metabolism. Because nearly all β-hydroxybutyrate dehydrogenase is cytosolic in ruminants (Koundakjian and Snoswell, 1970), acetoacetate produced in ketogenesis is exported from the mitochondria as acetoacetate. This export appears to be in exchange for pyruvate (Zammit, 1990); thus, ketogenesis may help to drive gluconeogenesis from lactate or alanine during the periparturient period by enhancing mitochondrial uptake of pyruvate for conversion to oxaloacetate (Zammit, 1990). Taken together, these findings indicate that ketogenesis and gluconeogenesis from alanine, lactate, or pyruvate may be mutually supportive processes during the periparturient period.

The effects of intracellular TG accumulation during the periparturient period on gluconeogenesis remain controversial. Rukkwamsuk et al. (1999c) reported that activity of PEPCK was decreased in overfed cows that developed fatty liver post-

partum. Studies with cultured calf hepatocytes showed that previous accumulation of TG did not affect gluconeogenesis from propionate (Strang et al., 1998). However, a previous study from the same laboratory (Cadórniga-Valiño et al., 1997) yielded opposite results. From data for cows during the periparturient period (Overton et al., 1998), we calculated that capacities for conversion of propionate and alanine to glucose in liver slices were not correlated significantly with hepatic triglyceride content.

Cellular lipid accumulation dramatically decreased ureagenesis (Strang et al., 1998), and increased hepatic lipid accumulation in vivo was correlated with blood ammonia concentrations (Zhu et al., 2000). Increased ammonia decreases gluconeogenesis from propionate but not from alanine (Overton et al., 1999); therefore, fatty liver may indirectly compromise gluconeogenic capacity. The ability of liver to maintain glycogen concentrations in the face of increasing cellular TG accumulation may be critical to maintaining liver function. Drackley et al. (1992) reported that the ratio of TG to glycogen in liver might be a predictor of susceptibility to an induced ketosis. Subsequent research (Greenfield et al., 2000; Smith et al., 1997) has provided direct or indirect support for this idea.

EFFECTS OF ENVIRONMENT AND IMMUNE ACTIVATION

The transition period in dairy cows is characterized by some degree of stress, if stress is defined as the impacts of external stimuli (physiological, environmental, psychological) that challenge homeostasis (Moberg, 1985). Cows face a major challenge in maintaining homeostasis during the sudden and marked increase of nutrient requirements for milk production at a time when feed intake, and thus nutrient supply, lags far behind. The peripartal metabolic constraints imposed by decreased DMI, coupled with the immunosuppression that occurs during this time (Mallard et al., 1998) and other stressors associated with parturition and lactogenesis, no doubt contribute to the high incidence of infectious diseases and metabolic disorders encountered during the transition period. Changes in maximal activities of key pathways of gluconeogenesis and LCFA metabolism discussed earlier imply changes in gene expression, an area that has been scarcely investigated in domestic animals in general and in transition dairy cows in particular. While many of these changes probably represent the concept of homeorhesis in adaptation to lactation (Bauman and Currie, 1980), these responses may be modulated by additional stressors encountered during the periparturient period.

Mechanisms that sense the presence of stressors and result in changes in gene expression have been elucidated in laboratory animals. For example, in rodents, many changes in enzyme activities induced by high-fat diets or starvation are mediated by interaction of LCFA or their metabolites with PPAR. Activation of PPAR by LCFA, eicosanoids, or xenobiotics leads to binding of the PPAR to specific response areas of target genes, resulting in activation or repression of gene expression (Desvergne and Wahli, 1999). Peroxisome proliferator response elements have been identified in the promoter regions of a number of genes that encode lipid-metabolizing enzymes found in peroxisomes, mitochondria, microsomes, and cytosol of hepatocytes. Activation of PPARα leads to a coordinated induction of enzymes involved in plasma transport, intracellular trafficking, and metabolism of fatty acids. In rodents, nutritional and environmental stresses lead to increased expression of PPARα and subsequent increases of target gene expression (Desvergne and Wahli, 1999; Kroetz et al., 1998; Lemberger et al., 1996) similar to those observed in transition dairy cows. Thus, cytokines (such as tumor necrosis factor α and interleukins 1 and 6), acting in an endocrine or autocrine/paracrine manner (Hotamisligil et al., 1993) and neuroendocrine hormones released during times of stress may impact expression of enzymes controlling metabolism of glucose and lipids by the liver and other organs.

The emerging field of psychoneuroimmunology recognizes that the immune system and the neuroendocrine system are tightly and intimately linked (Neveu, 1997). Furthermore, activation of the immune system can impact central controls of metabolism and behavior. In turn, metabolic control mechanisms can impact the ability of the immune system to respond appropriately to infectious challenges (Johnson et al., 1997). Additionally, stressors can activate the immune system without the presence of infectious challenge (Faith et al., 1999). The potential roles of stress and immune challenge during the transition period in determining metabolic changes, postpartum feed intake (Ingvartsen and Andersen, 2000), and incidence of health disorders would seem to be of enormous importance, and demand extensive investigation.

To what extent are fatty liver and other signs of less-than-optimal transition success in dairy cows attributable to environmental stressors or subclinical infectious challenges? Variable success in the field with a wide range of researched nutritional programs (e.g., both increased nutrient intake [Dann et al., 1999; Minor et al., 1998; VandeHaar et al., 1999] and restricted nutrient intake [Douglas et al., 1998]) has created confusion about optimal management. In contrast, the likely importance of the poorly defined idea of "cow comfort" (e.g., proper ventilation, comfortable stall and facility design, good footing, protection from temperature extremes, minimizing social disruptions) in determining transition success is almost universally accepted without clear, substantiating data. From such field experiences one could argue that responses to infectious and environmental stresses might be more important than the nutritional program per se in determining metabolic adaptations to lactation and transition success. Well-designed scientific investigation of the interactions of environmental stressors and nutrition clearly could reveal important new insights into metabolic adaptations of dairy cows during the transition period.

IMPLICATIONS AND FUTURE PROSPECTS

From the data reviewed, we conclude that hepatic tissue undergoes adaptations during the periparturient period that enable increased rates of metabolism of glucose and LCFA. These adaptations involve increases in activity of metabolic pathways, not merely increased liver size or delivery of substrate. Because the metabolic activities already were altered by d 1 postpartum, the adaptive processes likely begin before parturition. Increases in tissue capacity to convert propionate to glucose were subtle and seemed to follow predicted propionate supply; such changes might be expected from a teleologic standpoint given that ruminant liver is in a constant state of gluconeogenesis and that propionate is the primary substrate. The more marked increase in capacity to convert alanine to glucose around the time of parturition is an adaptation to attempt to ensure adequate glucose production in the face of insufficient propionate supply during the first few days of lacta-

tion. Changes in capacities for metabolism of LCFA also can be viewed as adaptations to deal with an enhanced supply of mobilized NEFA during the periparturient period. Application of techniques of molecular biology to the periparturient period should speed progress in understanding these adaptations, because many likely involve increased transcription and translation of genes for regulatory enzymes, as shown for gluconeogenesis by Greenfield et al. (2000).

Understanding hepatic adaptations remains an important component of predicting nutrient needs of transition cows and designing management systems to minimize health disorders. At a minimum, the changing nature of liver metabolic activities emphasizes the importance of careful attention to cows during the transition period. The evidence implicating the importance of glucogenic amino acids as glucose precursors during the immediate peripartal period highlights the need to quantify supply of metabolizable protein and its utilization in transition cows. More exciting, however, are prospects of being able to tailor delivery of specific nutrients at critical times to maximize metabolic adaptation and disease resistance of periparturient cows. The demonstrations that nutritional management during the dry period can impact hepatic capacities for glucose and LCFA metabolism, decrease lipid accumulation in the liver, and alter postpartum DMI (Douglas et al., 1998; Grum et al., 1996; Overton et al., 1998) are evidence that this may be an achievable goal. Exploration of the complex interactions among environmental stressors, the immune and neuroendocrine systems, behavior, and nutrition during the periparturient period perhaps offers the most potential for true advancement in this field.

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