# REGULATION OF GLUCOSE PRODUCTION IN THE TRANSITION DAIRY COW

Barry J. Bradford
Department of Animal Sciences & Industry
Kansas State University

The transition from gestation to lactation dramatically increases requirements for energy, glucose, amino acids, and other nutrients in dairy cattle. The increased demand for glucose is especially challenging, because little glucose is absorbed from the ruminant gastrointestinal tract. Shulze et al. (1991) measured glucose production rates of 1.25 kg/day in late gestation cows and 2.68 kg/day at peak lactation; therefore, over the course of 8 weeks or less, glucose production by the dairy cow increases by at least 2-fold. The ability of a cow to successfully up-regulate gluconeogenesis in early lactation is critical to both avoid metabolic problems (e.g. ketosis) and to maximize peak milk production. Therefore, dairy nutritionists should consider whether their transition cow programs promote adequate glucose production.

## What controls the rate of glucose production?

Hepatic gluconeogenesis is a carefully-controlled process in monogastrics. While many hormones influence the pathway, insulin and glucagon are the two that are classically considered to have the most influence on glucose production. Insulin's control of this pathway is so critical that hepatic insulin resistance and the subsequent increase in hepatic glucose production is thought by many to be a key component of the pathogenesis of type 2 diabetes (Brown and Goldstein, 2008).

In contrast, the traditional view of bovine gluconeogenesis has been that the rate is controlled primarily by substrate availability. This is somewhat logical; the ruminant liver continuously produces glucose and thus is less dependent on hormonal cues to activate and de-activate the pathway. Early data in lactating cows, for example, showed that plasma glucose appearance actually increased after insulin infusion (Kronfeld and Raggi, 1964), suggesting that insulin effects on glucose uptake by peripheral tissue allowed for increased hepatic glucose release, and that insulin had little or no direct ability to suppress gluconeogenesis. In studies where energy intake was varied, hepatic glucose release was very tightly correlated with energy intake (Wieghart et al., 1986), suggesting that gluconeogenic rate may be determined largely by production of propionate, the primary gluconeogenic substrate, during ruminal fermentation.

Nevertheless, most of these experiments did not really apply to the modern dairy cow. On most farms, cows are offered ad libitum access to highly fermentable diets, and the fact that restricted feeding of a low-energy diet decreases glucose production has little bearing in this situation. Furthermore, many of the early studies attempted to investigate hormonal effects over very short periods of time. Such treatments may have been adequate to observe effects on enzyme activity (i.e. through effects on enzyme

phosphorylation) but often were too short to allow for hormonal effects on gene expression and subsequent changes in enzyme capacity.

In fact, recent findings suggest that our traditional focus on substrate regulation of gluconeogenic flux needs to be revisited, at least in the case of the dairy cow. A series of studies by Beitz and colleagues at Iowa State University have clearly demonstrated that repeated administration of glucagon increases plasma glucose concentration in early lactation cows (Hippen et al., 1999), most likely because of increased expression of pyruvate carboxylase (**PC**) and phosphoenolpyruvate carboxykinase (**PEPCK**), rate-determining enzymes in gluconeogenesis (Bobe et al., 2009). Because these responses occurred in the face of a mild decrease in feed intake, they suggest that glucose production is not always limited by substrate availability in transition cows.

Phlorizin is a compound that alters glucose metabolism not by influencing the liver directly but by blocking resorption of glucose in the kidneys, resulting in significant urinary excretion of glucose. Therefore, phlorizin provides a tool to test whether increased "pull" on gluconeogenesis can influence the capacity of this pathway. Indeed, phlorizin increased glucose production in growing steers (Veenhuizen et al., 1988) and also increased transcription of gluconeogenic genes in lactating cows (Bradford and Allen, 2005). In addition to this evidence that increased enzyme capacity can promote greater glucose production, there are hints that cofactors for certain reactions in the gluconeogenic pathway may also be limiting. Specifically, vitamin $B_{12}$ is a required cofactor for methylmalonyl-CoA mutase, a critical enzyme for gluconeogenesis from propionate, and a recent report suggests that vitamin $B_{12}$ may increase glucose production in early lactation (Fürll et al., 2010). Each of these examples points to the conclusion that substrate is not the primary factor limiting glucose production in a typical dairy cow.

Another factor complicates the question of whether substrate or pathway capacity ultimately controls glucose production: the two factors may be physiologically linked. One possibility is that volatile fatty acids, especially propionate, may promote expression of gluconeogenic genes. Donkin and colleagues (2009) recently demonstrated both in vitro and in preruminant calves that propionate can increase transcription of PEPCK and glucose-6-phosphatase (**G6Pase**). Although this mechanism may be less important in mature ruminants that are constantly exposed to absorbed propionate, it is possible that the dramatic restrictions in fermentable organic matter intake used in some studies not only decreased supply of gluconeogenic substrate, but also led to corresponding decreases in pathway capacity. A link that is more relevant to lactating cows is based on the hepatic oxidation theory (Allen et al., 2009). According to this theory, any oxidizable substrate that is supplied to the liver in excess of its need for anabolic pathways will promote satiety when it is oxidized and ATP is produced. This is particularly important for propionate, because its absorption can increase dramatically during meals and it is rapidly taken up by the liver. When propionate supply exceeds the capacity of gluconeogenesis to use it, it is oxidized, increasing the ATP charge of the hepatocyte. The hepatic oxidation theory predicts that this eventually generates a satiety signal to the brain via the hepatic vagus nerve. If this hypothesis is correct, then gluconeogenic capacity, to some extent, determines the feed

intake of the animal and therefore the substrate supply. Although substrate availability may be intimately linked with glucose production rate, this does not necessarily imply that substrate supply limits gluconeogenesis *per se*.

**Fatty liver alters gluconeogenic capacity**

A key reason to better understand control of gluconeogenic capacity is the possibility that decreased glucose production is central to the metabolic problems that many cows experience in early lactation. Several studies have found decreased capacity for gluconeogenesis in liver slices from cows with fatty liver (Mills et al., 1986, Veenhuizen et al., 1991). Such measurements are made ex vivo and are therefore independent of any differences in gluconeogenic substrate supply in cows with metabolic problems. Others have shown that induced fatty liver results in decreased activities of several rate-determining gluconeogenic enzymes (Rukkwamsuk et al., 1999, Murondoti et al., 2004). Additionally, prepartum overfeeding, which induces metabolic problems and lipid infiltration in the liver, significantly decreased expression of PC throughout the transition period (Loor et al., 2006).

Importantly, this decrease in glucose production that apparently accompanies fatty liver promotes further adipose tissue lipolysis, creating a positive feedback loop between adipose tissue and the liver (**Figure 1**). Decreased plasma glucose concentration promotes lipolysis both directly (Brockman, 1984) and indirectly, through decreased stimulation of insulin secretion. Additionally, because gluconeogenesis is an important sink for NADH produced through fatty acid oxidation, decreased gluconeogenesis leads to end-product inhibition of fatty acid oxidation and greater lipid accumulation (Hakimi et al., 2005). If not interrupted, this cycle leads to excessive adipose tissue lipolysis and hepatic fat accumulation, and is likely to result in clinical ketosis, fatty liver, and/or other associated disorders.

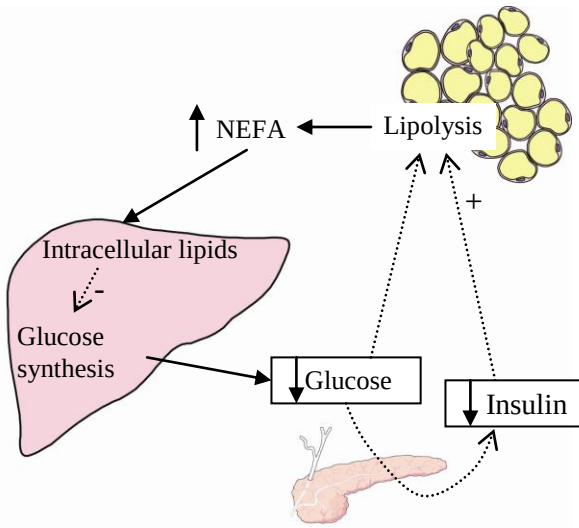


**Figure 1. Metabolic consequences of fatty liver in early lactation cows.** Liver lipid accumulation is associated with decreased capacity for glucose production. Low glucose production results in low plasma insulin concentration; both hypoglycemia and hypoinsulinemia promote lipolysis in adipose tissue. Elevated plasma NEFA is associated with depressed feed intake, high plasma ketone concentrations, and continued accumulation of lipids in the liver. This positive feedback loop makes it difficult for the cow to return to a state of positive energy balance.

The biochemical cause of decreased gluconeogenic capacity in cows with fatty liver is not clear. Cadórniga-Valiño and colleagues (1997) incubated bovine hepatocytes with high concentrations of non-esterified fatty acids (**NEFA**) for 48 hours, resulting in intracellular lipid content of 12.5%, typical of dairy cows with moderate fatty liver. This lipid infiltration subsequently decreased conversion of propionate to glucose by 24% (Cadórniga-Valiño et al., 1997). This effect was observed even when NEFA were removed from the media during the glucose production assay, meaning that this response was a carryover effect of prior NEFA exposure. The question remains, however: what process mediates this negative effect of lipids on glucoeneogenesis?

Inflammation is emerging as a process that is likely involved in the metabolic problems common in transition cows (Drackley, 1999). The metabolic effects of acute inflammation, as observed in septic shock, include adipose tissue mobilization, glycogenolysis, and hepatic lipid accumulation (Chiolero et al., 1997). The plasma glucose response to inflammation is often biphasic, with hyperglycemia consistently observed for a few hours after acute inflammation (Waldron et al., 2006), followed by long-term hypoglycemia in many cases (Kenison et al., 1991, Kushibiki et al., 2000). These observations are consistent with glycogenolysis (accounting for the initial hyperglycemia), coupled with impaired gluconeogenesis (explaining hypoglycemia after liver glycogen is depleted). If this interpretation is accurate, the effects of inflammation on gluconeogenesis are far more important than effects on glycogenolysis for disorders such as fatty liver that develop and resolve over the course of weeks rather than hours. Activation of inflammatory pathways likely alters metabolism through transcriptional effects. For example, endotoxin-induced mastitis caused systemic inflammation and resulted in decreased expression of gluconeogenic genes in liver (Jiang et al., 2008).

Elevated NEFA can promote inflammation via a number of different mechanisms. One key recent discovery is that hepatocytes are capable of producing inflammatory cytokines such as tumor necrosis factor α (**TNFα**). In fact, increasing NEFA concentration from 0.5 mM to 1 mM induced a 7-fold increase in TNFα mRNA abundance in a human hepatocyte cell line (Feldstein et al., 2004). This raises the possibility that elevated NEFA induce the production of inflammatory cytokines in hepatocytes, creating an autocrine loop to further activate inflammatory pathways and promote maladaptive changes in metabolism.

To test whether inflammation can directly alter gluconeogenesis, our lab injected lactating cows with TNFα once daily for 7 days, which was adequate to stimulate hepatic inflammation. In addition to doubling liver triglyceride content, our TNFα injection protocol also decreased expression of G6Pase and tended to decrease PEPCK expression (Bradford et al., 2009). Furthermore, TNFα numerically decreased glucose production rate by 18% compared to cows fed to the same level of intake, although this response was not statistically significant with 5 cows per treatment. Coupled with evidence that TNFα consistently decreases activity and mRNA abundance of gluconeogenic enzymes (Hill and McCallum, 1992, Metzger et al., 1997), these findings suggest that inflammation is at least one important mechanism by which elevated lipids can impair hepatic glucose production.

## Promoting glucose production

If glucose production in transition cows can in fact be impaired by inflammation, there are a number of nutritional and pharmacological strategies that may promote increased gluconeogenic capacity. First and foremost, avoiding cows with body condition scores >4 remains critical to a healthy transition to lactation. From the standpoint of this hypothesis, obese cows are known to mobilize more fatty acids after calving, resulting in greater elevation of plasma NEFA and potentially greater impairment of hepatic glucose production. However, transition cows may be further aided by increasing dietary antioxidants (e.g. vitamin E or non-nutritive chemical antioxidants), feeding rumen-bypass choline to promote clearance of lipid from the liver, or by employing strategies to limit lipolysis. Finally, although it is clear that glucose production is sometimes limited by gluconeogenic capacity, cows with suboptimal feed intake are more likely limited by substrate supply, and in these situations, drenching with propylene glycol remains an effective tool.

## Conclusions

Glucose production is a critical factor both for maintaining metabolic health and promoting high milk production in early lactation. Recent investigations suggest that fatty liver and associated disorders are problematic in part because they lead to decrease gluconeogenic capacity. The underlying mechanism for this impairment in glucose production is likely initiated by intracellular lipids in the liver, and inflammatory pathways appear to be involved. Strategies that limit accumulation of liver lipids or counteract their inflammatory potential may be effective means of supporting increased glucose production in transition cows.

## References


Allen, M. S., B. J. Bradford, and M. Oba. 2009. Board-Invited Review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. J. Anim Sci. 87(10):3317-3334.

Bobe, G., J. C. Velez, D. C. Beitz, and S. S. Donkin. 2009. Glucagon increases hepatic mRNA concentrations of ureagenic and gluconeogenic enzymes in early-lactation dairy cows. J. Dairy Sci. 92(10):5092-5099.

Bradford, B. J. and M. S. Allen. 2005. Phlorizin administration increases hepatic gluconeogenic enzyme mRNA abundance but not feed intake in late-lactation dairy cows. J. Nutr. 135(9):2206-2211.

Bradford, B. J., L. K. Mamedova, J. E. Minton, J. S. Drouillard, and B. J. Johnson. 2009. Daily injection of tumor necrosis factor-α increases hepatic triglycerides and alters transcript abundance of metabolic genes in lactating dairy cattle. J. Nutr. 139(8):1451-1456.

Brockman, R. P. 1984. Effect of glycemic changes on lipolysis in sheep in vivo. Metabolism. 33(4):329-331.

Brown, M. S. and J. L. Goldstein. 2008. Selective versus total insulin resistance: a pathogenic paradox. Cell Metab. 7(2):95-96.

Cadórniga-Valiño, C., R. R. Grummer, L. E. Armentano, S. S. Donkin, and S. J. Bertics. 1997. Effects of fatty acids and hormones on fatty acid metabolism and gluconeogenesis in bovine hepatocytes. J. Dairy Sci. 80(4):646-656.

Chiolero, R., J. P. Revelly, and L. Tappy. 1997. Energy metabolism in sepsis and injury. Nutrition 13(9 Suppl):45S-51S.

Donkin, S. S., E. Cedeño, and S. L. Koser. 2009. Effect of propionate infusion on hepatic PEPCK and glucose-6-phosphatase expression in neonatal Holstein calves. J. Dairy Sci. 92 (E-Suppl. 1):70-71 (Abstr.).

Drackley, J. K. 1999. ADSA foundation scholar award. Biology of dairy cows during the transition period: the final frontier? J. Dairy Sci. 82(11):2259-2273.

Feldstein, A. E., N. W. Werneburg, A. Canbay, M. E. Guicciardi, S. F. Bronk, R. Rydzewski, L. J. Burgart, and G. J. Gores. 2004. Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression via a lysosomal pathway. Hepatology 40(1):185-194.

Fürll, M., A. Deniz, B. Westphal, C. Illing, and P. D. Constable. 2010. Effect of multiple intravenous injections of butaphosphan and cyanocobalamin on the metabolism of periparturient dairy cows. J. Dairy Sci. 93(9):4155-4164.

Hakimi, P., M. Johnson, J. Yang, D. Lepage, R. Conlon, S. Kalhan, L. Reshef, S. Tilghman, and R. Hanson. 2005. Phosphoenolpyruvate carboxykinase and the critical role of cataplerosis in the control of hepatic metabolism. Nutr. Metab. 2(1):33.

Hill, M. R. and R. E. McCallum. 1992. Identification of tumor necrosis factor as a transcriptional regulator of the phosphoenolpyruvate carboxykinase gene following endotoxin treatment of mice. Infect. Immun. 60(10):4040-4050.

Hippen, A. R., P. She, J. W. Young, D. C. Beitz, G. L. Lindberg, L. F. Richardson, and R. W. Tucker. 1999. Alleviation of fatty liver in dairy cows with 14-day intravenous infusions of glucagon. J. Dairy Sci. 82(6):1139-1152.

Jiang, L., P. Sorensen, C. Rontved, L. Vels, and K. Ingvartsen. 2008. Gene expression profiling of liver from dairy cows treated intra-mammary with lipopolysaccharide. BMC Genomics 9(1):443.

Kenison, D. C., T. H. Elsasser, and R. Fayer. 1991. Tumor necrosis factor as a potential mediator of acute metabolic and hormonal responses to endotoxemia in calves. Am. J. Vet. Res. 52(8):1320-1326.

Kronfeld, D. S. and F. Raggi. 1964. Glucose kinetics in normal, fasting, and insulin-treated cows. Am J Physiol 206(1):109-112.

Kushibiki, S., K. Hodate, Y. Ueda, H. Shingu, Y. Mori, T. Itoh, and Y. Yokomizo. 2000. Administration of recombinant bovine tumor necrosis factor-alpha affects intermediary metabolism and insulin and growth hormone secretion in dairy heifers. J. Anim Sci. 78(8):2164-2171.

Loor, J. J., H. M. Dann, N. A. J. Guretzky, R. E. Everts, R. Oliveira, C. A. Green, N. B. Litherland, S. L. Rodriguez-Zas, H. A. Lewin, and J. K. Drackley. 2006. Plane of nutrition prepartum alters hepatic gene expression and function in dairy cows as assessed by longitudinal transcript and metabolic profiling. Physiol. Genomics 27(1):29-41.

Metzger, S., N. Begleibter, V. Barash, O. Drize, T. Peretz, E. Shiloni, and T. Chajek-Shaul. 1997. Tumor necrosis factor inhibits the transcriptional rate of glucose-6-phosphatase in vivo and in vitro. Metabolism. 46(5):579-583.

Mills, S. E., D. C. Beitz, and J. W. Young. 1986. Evidence for impaired metabolism in liver during induced lactation ketosis of dairy cows. J. Dairy Sci. 69(2):362-370.

Murondoti, A., R. Jorritsma, A. C. Beynen, T. Wensing, and M. J. Geelen. 2004. Activities of the enzymes of hepatic gluconeogenesis in periparturient dairy cows with induced fatty liver. J. Dairy Res. 71(2):129-134.

Rukkwamsuk, T., T. Wensing, and M. J. H. Geelen. 1999. Effect of fatty liver on hepatic gluconeogenesis in periparturient dairy cows. J. Dairy Sci. 82(3):500-505.

Schulze, E., H. Fuhrmann, E. S. Neitzel, W. W. Giese, and H. P. Sallmann. 1991. Glucose entry rate in dairy cattle as determined by stable isotope $^{13}C$-labelled glucose at different stages of reproduction. Comp Biochem Physiol B 100(1):167-171.

Veenhuizen, J. J., J. K. Drackley, M. J. Richard, T. P. Sanderson, L. D. Miller, and J. W. Young. 1991. Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. J. Dairy Sci. 74(12):4238-4253.

Veenhuizen, J. J., R. W. Russell, and J. W. Young. 1988. Kinetics of metabolism of glucose, propionate and $CO_2$ in steers as affected by injecting phlorizin and feeding propionate. J. Nutr. 118(11):1366-1375.

Waldron, M. R., A. E. Kulick, A. W. Bell, and T. R. Overton. 2006. Acute experimental mastitis is not causal toward the development of energy-related metabolic disorders in early postpartum dairy cows. J. Dairy Sci. 89(2):596-610.

Wieghart, M., R. Slepetis, J. M. Elliot, and D. F. Smith. 1986. Glucose absorption and hepatic gluconeogenesis in dairy cows fed diets varying in forage content. J. Nutr. 116(5):839-850.