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Critical Review

Gluconeogenesis in Dairy Cows: The Secret of Making Sweet Milk from Sour Dough

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Summary

Gluconeogenesis is a crucial process to support glucose homeostasis when nutritional supply with glucose is insufficient. Because ingested carbohydrates are efficiently fermented to short-chain fatty acids in the rumen, ruminants are required to meet the largest part of their glucose demand by *de novo* genesis after weaning. The qualitative difference to nonruminant species is that propionate originating from ruminal metabolism is the major substrate for gluconeogenesis. Disposal of propionate into gluconeogenesis via propionyl-CoA carboxylase, methylmalonyl-CoA mutase, and the cytosolic form of phosphoenolpyruvate carboxykinase (PEPCK) has a high metabolic priority and continues even if glucose is exogenously supplied. Gluconeogenesis is regulated at the transcriptional and several posttranscriptional levels and is under hormonal control (primarily insulin, glucagon, and growth hormone). Transcriptional regulation is relevant for regulating precursor entry into gluconeogenesis (propionate, alanine and other amino acids, lactate, and glycerol). Promoters of the bovine pyruvate carboxylase (PC) and PEPCK genes are directly controlled by metabolic products. The final steps decisive for glucose release (fructose 1,6-bisphosphatase and glucose 6-phosphatase) appear to be highly dependent on posttranscriptional regulation according to actual glucose status. Glucogenic precursor entry, together with hepatic glycogen dynamics, is mostly sufficient to meet the needs for hepatic glucose output except in high-producing dairy cows during the transition from the dry period to peak lactation. Lactating cows adapt to the increased glucose requirement for lactose production by mobilization of endogenous glucogenic substrates and increased hepatic PC expression. If these adaptations fail, lipid metabolism may be altered leading to fatty liver and ketosis. Increasing feed intake and provision of glucogenic precursors from the diet are important to ameliorate these disturbances. An improved understanding of the complex mechanisms underlying gluconeogenesis may further improve our options to enhance the postpartum health status of dairy cows. © 2010 IUBMB
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Keywords cellular glucose metabolism; dairy cow; enzyme activity; gene expression; gluconeogenesis.

Abbreviations bST, bovine somatotropin; CREB, cAMP-response element binding; FBPase, fructose 1,6-bisphosphatase; G6-Pase, glucose 6-phosphatase; MCM, methylmalonyl-CoA mutase; NF-Y, nuclear factor-Y; OAA, oxaloacetate; PC, pyruvate carboxylase; PCoAC, propionyl-CoA carboxylase; PDV, portal-drained viscera; PEP, phosphoenolpyruvate; PEPCK, phosphoenolpyruvate carboxykinase; PEPCK-C, cytosolic isoform of PEPCK; PEPCK-M, mitochondrial isoform of PEPCK; PPAR, peroxisome proliferator-activated receptor; TBP, TATA-binding protein; TCA, tricarboxylic acid; UTR, untranslated region

INTRODUCTION

Glucose is a universal fuel that can be used in energy metabolism and synthesis pathways of all mammalian cell types (*1, 2*). Certain cell types and tissues like brain, erythrocytes, kidney medulla, and mammary tissue have an obligatory requirement for glucose as a substrate (*3*). Thereby, glucose is not only a universal fuel but also an essential fuel for all higher organisms, which needs to be permanently available at a sufficient level in the blood stream. Humans and most other nonruminant species have the privilege to be supplied by intestinal absorption with

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significant quantities of glucose. Their major challenge is to ensure sufficient storage of glucose in the form of glycogen during the absorptive phase of digestion and to mobilize from these stores during the postabsorptive phase (*4*). *De novo* generation of glucose by gluconeogenesis from noncarbohydrate precursors (*e.g.*, lactate, glycerol, and amino acids) supplements the exogenous supply of glucose (*4*). The importance of gluconeogenesis increases in times of low glucose supply (*e.g.*, starvation) or increased glucose demand (*e.g.*, exercise) (*5*). However, it is important to understand that glucose supply is not the only function of gluconeogenesis. A second function is to regulate the prevalence of glucogenic precursors in blood plasma, which is, for example, critical in the control of systemic lactic acid accumulation, *i.e.*, systemic lactic acidosis (*6*).

Gluconeogenesis in suckling (preruminating) ruminants has many similarities to that in monogastric species (*7*) although they already show phylogenetic features of carbohydrate metabolism that prepare them for later life (*e.g.*, a high capacity for gluconeogenesis from propionate and a relatively low suppressive action of insulin on gluconeogenesis) (*8, 9*). The situation changes dramatically when ruminants are weaned and start to ingest fiber carbohydrates that are not digestible by mammalian enzymes. Ingesta are then temporarily stored in a forestomach, mainly the rumen, to allow for microbial fermentation of fiber carbohydrates (*10*). Because soluble carbohydrates and starch are even more fermentable by microbes than fiber (*10*), glucose availability for direct absorption is low under most dietary settings (*11, 12*). Instead of glucose, short-chain fatty acids are released as the major end products of ruminal microbial fermentation. Of these, only propionate, valerate, and isobutyrate can serve as glucogenic precursor for net synthesis of glucose (*10, 13, 14*). Propionate is by far the most abundant of the three glucogenic acids (~15–40% of total ruminally released organic acids; refs. *10* and *15*) and by far the predominant substrate for gluconeogenesis in ruminants (*13, 14, 16*), which qualitatively distinguishes gluconeogenesis in ruminants from that in nonruminant species. As the production and absorption of propionate are highest after feeding, gluconeogenesis in ruminants is also highest after feeding and in periods of high energy intake. This is in sharp contrast to nonruminant species where gluconeogenesis is highest in the fasted state or in periods of low energy intake (*17*). The absence of an inhibitory effect of insulin on hepatic gluconeogenesis from propionate observed in hepatic tissues or cell cultures from adult (but not preruminating) cattle may explain part of this phylogenetic specialty (*8, 18*). It underlines that gluconeogenesis in adult ruminants has the important accessory function to prevent the negative consequences of systemic propionate accumulation, *e.g.*, depressed appetite (*19*) or propionic acidemia (*20*).

Amongst all ruminants, efficient gluconeogenesis is most important in high-producing dairy cows because it is the major pathway for maintaining adequate glucose supply for the mammary gland (*12*). The glucose-derived lactose output in milk may exceed the animal's glucose demand for maintenance several times during peak lactation (see next section). Therefore, this review will largely focus on current knowledge, critical issues, and future perspectives concerning gluconeogenesis in the lactating dairy cow. After a short quantitative analysis of the gluconeogenic demand, the critical control points for gluconeogenesis will be analyzed, *i.e.*, substrate supply, enzyme activity, and end-product feedback.

CONTRIBUTION OF GLUCONEOGENESIS TO TOTAL GLUCOSE REQUIREMENT

Glucose entry into the systemic circulation can be either via absorption from the gastrointestinal tract or via hepatic or renal gluconeogenesis. As regards absorption, the sodium-dependent glucose transporter, SGLT-1, and several isoforms of the facilitated glucose transporter family have been identified throughout the gastrointestinal tract in ruminants including the reticulum, rumen, omasum, duodenum, jejunum, ileum, and cecum (*21, 22*). Functionality of these transporters has been demonstrated in the forestomach (*22, 23*) and the small intestine (*24*); the latter being the most important site for glucose absorption under most dietary settings (*25*). However, only a few ordinary feedstuffs (*e.g.*, corn, sorghum, or sodium hydroxide-treated wheat grain) will provide digestible starch to the small intestine in quantitatively important amounts (*26, 27*). In many cases, the net absorption of glucose from portal-drained viscera (PDV) is negative, implying that PDV tissues metabolize more arterially supplied glucose than absorbed from the lumen. Also, first-pass metabolism in enterocytes might be responsible for ~30–40% of luminal glucose never reaching the blood (*28, 29*).

The rate of total glucose entry increases markedly following parturition by ~200–400 mmol/h, reaching ~700 mmol/h (~3 kg/day) during peak lactation at a milk yield level of ~40 kg/day (*29, 30*; Table 1 and Fig. 1). The glucose entry in top-performing cows producing 90 kg milk per day has been estimated at 7.4 kg/day of which 4.4 kg will end up as lactose in milk (*17*). This large increase in glucose entry cannot be accounted for by an increase in the absorption of glucose across the PDV (*30*). Although starch passing from the rumen into the intestine may reach up to 5 kg/day with extreme feeding strategies (*27*), limitations of intestinal starch hydrolysis and glucose absorption, as well as splanchnic metabolism, decrease the contribution of absorbed glucose to less than 5% of the net splanchnic glucose supply in most studies (*14, 30*). These data clearly show that ruminants have a large reliance on gluconeogenesis in terms of meeting the glucose requirement. The central organ for gluconeogenesis is the liver. Although we currently have no estimates on the relative organ contributions to gluconeogenesis in dairy cows, measurements in sheep suggest that the liver comprises >80% of the total gluconeogenic capacity (*36*).

CONTROL POINTS OF GLUCONEOGENESIS

Availability and Partitioning of Glucogenic Precursors

Quantitatively important substrates for hepatic gluconeogenesis in the order of hepatic uptake are propionate (60–74%),

Table 1
Glucose production measured by isotope tracer techniques in cows at different stages of lactation as well as milk yield level and dry matter intake

Days in milk (day)	Milk yield (kg/day)	Dry matter intake (kg)	Glucose production (mmol/h)	Study (number of cows)
21	35.9	15.8	552	Hammon, Görs, and Metges, unpublished, $n = 7$
60	40.3	22.5	575	Knowlton et al. (*31*), $n = 4$
63	37.1	21.3	675	Hammon, Görs, and Metges, unpublished, $n = 6$
89	24.9	15.6	502	Lemosquet et al. (*32*), $n = 4$
100	26.7	17.2	547	Hammon et al. (*33*), $n = 3$
100	15.3	–	394	Hammon et al. (*34*), $n = 10$
104	27.5	16.9	504	Bauman et al. (*35*), $n = 12$

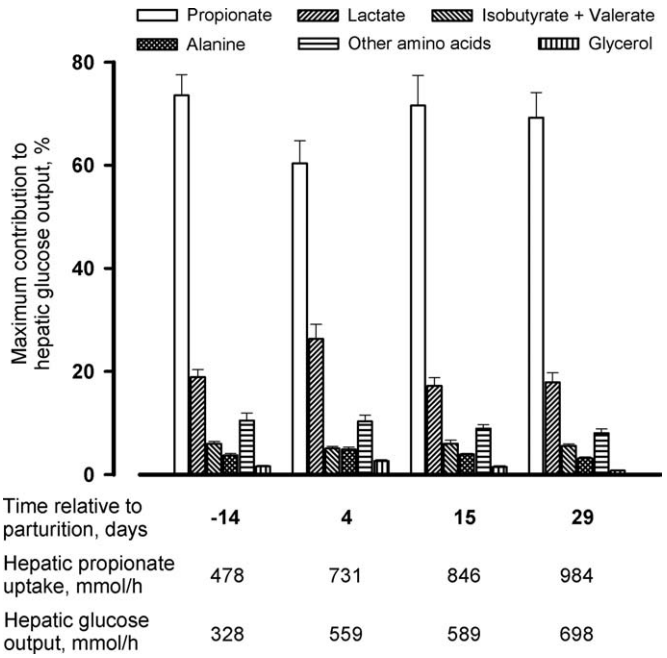

Figure 1. Maximum substrate contribution to hepatic glucose output in periparturient dairy cows. Data show that propionate is the quantitatively most important source of C3 carbon. However, hepatic glucose output increases faster than hepatic propionate uptake immediately after parturition. Thus, the relative importance of endogenous glucogenic precursors like alanine, lactate, and glycerol is higher after parturition (day 4) compared with observations before as well as longer after parturition. Each data point represents the mean of 10 observations ± SEM. Data were obtained from Larsen and Kristensen (*13*); Dalbach et al., unpublished; and Raun and Kristensen, unpublished.

L-lactate (16–26%), alanine (3–5%), valerate and isobutyrate (5–6%), glycerol (0.5–3%), and other amino acids (8–11%) (*13, 14*). The percentage values in the preceding sentence are estimates for the relative contribution of the different glucogenic substrates to net hepatic release of glucose. They are based on the maximum net supply of three-carbon units and cover substrates efficiently used for gluconeogenesis (propionate, L-lactate, alanine, and valerate) and the assumption that amino acids other than alanine will predominantly be used for hepatic protein synthesis and synthesis of other N-containing substances, *e.g.*, hippuric acid synthesized from glycine and serine. The contribution from endogenous glucogenic precursors such as glycerol and lactate increases during energy deficiency and associated lipid mobilization (*37*). On the other hand, the proportions of exogenous substrates used for gluconeogenesis depend on their absorption rates from the gastrointestinal tract. The net supply of glucogenic substrates by the PDV is largely modified by first-pass metabolism within the PDV. This reduces the hepatic supply of glucogenic amino acids (glutamine, asparagine, and proline) (*38*), valerate and to a lesser extent also propionate (*39, 40*).

One special feature of ruminants is that lactate is not only the dominating endogenous glucogenic substrate but may also be produced in significant amounts by ruminal microbes if very high amounts of starch are fed. This is often linked to health disturbances (ruminal lactic acidosis). When absorbed from the rumen, the D-isomer of lactic acid is metabolized much slower than the L-isomer by the host ruminant. Nonetheless, gluconeogenesis is one pathway to alleviate the build-up of deleterious D-lactic acid concentrations in blood plasma in such situations (*41–43*). Another special feature of the ruminant is that microbial enzymes in the rumen may increase the usability of certain glucogenic substrates that are not as easily utilizable by nonruminants. The most prominent of these is 1,2-propanediol, which is applied as a feed additive in ruminant nutrition (*16, 44*). Although 1,2-propanediol is only slowly metabolized by the liver (mainly to L-lactate via alcohol and aldehyde dehydrogenases), its intraruminal conversion to propanol and, to a lesser extent, also propanal provides more readily utilizable glucogenic substrates that finally account for more than half of the glucogenic potential of 1,2-propanediol (*44*).

Although the provision of glucogenic precursors from the PDV is an important prerequisite for hepatic glucose production, mesenteric vein infusions of alanine did not stimulate glucose production in an experiment using beef heifers (*45*). An increased hepatic uptake of alanine was balanced by a shift from net lactate uptake to net L-lactate release from the liver (*45*). Consequently, the hepatic supply of glucogenic precursors generally modifies their relative contribution to glucose production, but the total glucose production will also depend on compensatory changes especially on L-lactate use by liver compared with peripheral tissues.

A special situation exists for the cow in early lactation, which faces a severe shortness of glucogenic precursors. Early lactating cows greatly benefit from increases in exogenous glucogenic precursor supply from either increased feed intake, increased rates of propionate fermentation in the rumen (achieved especially by increased supply of starch, but also by adding ionophore antibiotics like monensin to the diet) or from a direct provision of feed additives with glucogenic potential (*e.g.*, 1,2-propanediol or glycerol) (*16*). The mammary use of glucose for lactose and NADPH synthesis as well as amino acids for milk protein synthesis increase tremendously right after parturition (*46*), which cannot be fully compensated by increased feed intake (*47*). The hepatic glucose output is approximately doubled between late pregnancy and 2 weeks after parturition where cows often show signs of glucose shortage as evidenced by decreased plasma concentrations of glucose and insulin (*14*), decreased concentrations of most essential amino acids in blood (*48*), and increased plasma glucagon concentrations (*49*). Mobilized body protein in the early phase of lactation is mainly directed to milk protein synthesis and not used to maintain normoglycemia (*13*). Thus, cows in the early postpartal period will increase the relative contribution of L-lactate, glycerol, and alanine (but not other amino acids) to total hepatic glucogenic substrate uptake (Fig. 1). Correspondingly, the relative contribution of propionate to glucose output is slightly decreased in very early lactation although both absolute hepatic propionate uptake and glucose output increase postpartum (Fig. 1).

Although exogenous glucose supply had no influence on hepatic amino acid uptake in the postpartum period (*13*), it decreased hepatic lactate uptake relative to glucose output from about 36% in control cows to 16% in glucose-infused cows (*29*). It is not very likely that the general increase in hepatic use of L-lactate after parturition is driven by enhanced hepatic pull as the increase in hepatic lactate uptake does not affect circulating concentrations of lactate (*29*; Raun and Kristensen, unpublished data). It appears, therefore, that the primary metabolic response to a low glucose status in dairy cattle is the increased release of glucogenic carbon from peripheral tissues. Effects of glucose status on glucose carbon return might involve C3 return from mammary pentose phosphate cycle as this pathway is of importance for NADPH generation for *de novo* fatty acid synthesis in the mammary gland. Lemosquet et al. (*50*) showed that mammary release of L-lactate was changed to mammary uptake with exogenous supply of glucogenic substrate (propionate and nonessential amino acids). Lactate release by skeletal muscle might as well contribute to the return of glucose carbon to the liver, whereas the PDV release of L-lactate seems primarily related to feed intake (*29*; Raun and Kristensen, unpublished data).

Glucocorticoids are hormones that may increase the availability of endogenous glucogenic precursors (*51*), but their therapeutic efficacy in the improvement of postpartum metabolic status of dairy cows has been inconsistent (*52*). Meanwhile, it has become clear that glucocorticoids influence neither the enzymes crucial for precursor entry into gluconeogenesis (*53, 54*) nor hepatic glucose output (*55*). The inconsistently observed improvement of postpartum metabolic status by application of glucocorticoids could thus be mediated via increased mobilization of endogenous glucogenic precursors, an effect that may vary significantly based on concurrent insulin status (*51, 56*). Future studies are needed to explore the applicability of this hypothesis in more detail.

Control of Precursor Entry Into Gluconeogenesis

The prominent role of propionate in ruminant gluconeogenesis requires a more complex view on precursor entry into gluconeogenesis and its regulation compared with monogastric species. A merging point for the entry of most glucogenic substrates into gluconeogenesis is mitochondrial oxaloacetate (OAA). However, propionate is converted through mitochondrial propionyl-CoA carboxylase (PCoAC), methylmalonyl-CoA mutase (MCM), and part of the tricarboxylic acid (TCA) cycle to OAA, whereas lactate and the major glucogenic amino acid, alanine, are initially converted to pyruvate in the cytosol before being converted to OAA by mitochondrial pyruvate carboxylase (PC). OAA can then be metabolized by phosphoenolpyruvate carboxykinase (PEPCK) to phosphoenolpyruvate (PEP) and further to glucose or serve as an acetyl-CoA acceptor in the TCA cycle (Fig. 2). The presence of PEPCK activity in the cytosol (PEPCK-C) and mitochondria (PEPCK-M) further extends the possibilities to regulate the precursor entry into gluconeogenesis. The stoichiometry of gluconeogenesis dictates that formation of phosphoenolpyruvate from propionate, pyruvate, and some amino acids requires the independent synthesis of NADH in the cytosol for the subsequent reduction of 1,3-diphosphoglycerate in gluconeogenesis. Accordingly, it has been proposed that PEPCK-C is required for gluconeogenesis from amino acids, and PEPCK-M is more suited for gluconeogenesis from lactate (*57*). If so, entry of lactate into gluconeogenesis can be regulated via PC and PEPCK-M activity, entry of amino acids via PC and PEPCK-C activity, and entry of propionate via PCoAC, MCM, and PEPCK-C activity.

The vitamin B12-dependent enzyme MCM is considered a control point of gluconeogenesis that appears to be amenable to prophylactic or therapeutic intervention. Supplementation with vitamin B12 has been shown to enhance gluconeogenesis from propionate in liver slices of sheep (*58*), and peripartal administration of vitamin B12 can have positive effects on the

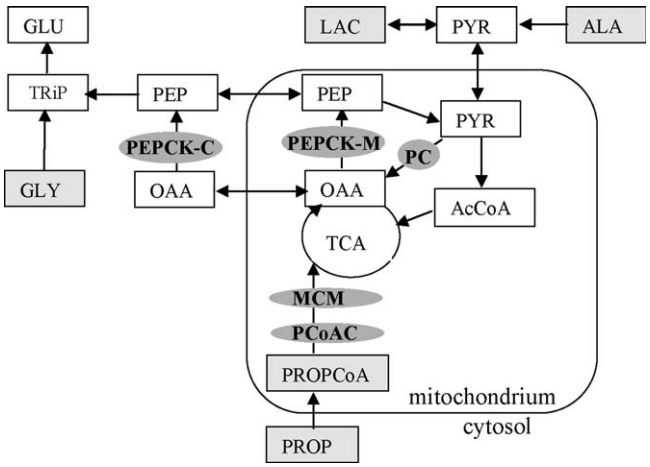

Figure 2. Control points for glucogenic precursor entry into gluconeogenesis in bovine liver. The relative entry rates of propionate, lactate, and alanine can be regulated via differential expression of pyruvate carboxylase (PC), propionyl-CoA carboxylase (PCoAC), and the cytosolic and mitochondrial isoforms of phosphoenolpyruvate carboxykinase (PEPCK-C and PEPCK-M). Entry of propionate can additionally be modified by increasing coenzyme availability (vitamin B12) for methylmalonyl-CoA mutase (MCM). Abbreviations: AcCoA, acetyl CoA; ALA, alanine; GLU, glucose; GLY, glycerol; LAC, lactate; OAA, oxalaoacetate; PEP, phosphoenolpyruvate; PROP, propionate; PROPCoA, propionyl CoA; PYR, pyruvate; TCA, tricarboxylic acid cycle; TRiP, triose phosphates.

metabolic status of high-yielding dairy cows (*59*). On the other hand, little is known about the transcriptional or translational regulation of bovine hepatic MCM and PCoAC. The few existing studies on this topic, however, indicate that at least PCoAC seems to be regulated in a coordinated fashion to PEPCK. Murondoti et al. (*60*) found coordinated depressions of PCoAC and PEPCK activities in cows with induced fatty liver, whereas Hammon et al. (unpublished) discovered coordinated changes in the mRNA expression of isoform A of PCoAC and PEPCK-C in transition dairy cows. These results support the view that changes in PEPCK activity have a high predictive value for glucose production from propionate even if the concurrent changes in PCoAC (and MCM) are not known (*61, 62*). Moreover, the bovine PEPCK gene promoter itself is positively regulated by propionate (*63*), constituting a feed-forward mechanism of substrate control for hepatic gluconeogenesis that is linked to the end products of rumen fermentation. Accordingly, increasing ruminal propionate production by feeding monensin prepartum induces hepatic PEPCK-C mRNA expression (*64*). Likewise, expression of PEPCK mRNA in dairy cattle is increased as feed intake is increased during lactation (*61*), which differs considerably from the response observed for PEPCK in monogastrics (*65*). The fact that feed restriction does not alter PEPCK mRNA abundance in dairy cows (*66*) or PEPCK activity in sheep (*67–69*) is in direct contrast with data from nonruminants, demonstrating that feed restriction leads to transcriptional induction of PEPCK-C activity (*65*).

Contrary to PEPCK, the mRNA abundance and activity of PC is elevated in response to the onset of calving (*61, 70–72*), which appears to be linked to a reduced insulin/glucagon ratio (*49, 72*). Further exploration of control of PC and PEPCK with feed restriction and bovine somatotropin (bST) administration indicates that PC, but not PEPCK, is elevated during feed restriction (*66*) and PEPCK, but not PC, is elevated with bST (*73*). These data highlight the uniqueness of the bovine species with regard to control of glucose metabolism. When these data are considered collectively, the unique features of the bovine indicate that PC expression plays a pivotal role in promoting the entry of endogenous precursors in gluconeogenesis when intake is compromised such as during the transition to calving, whereas PEPCK-C expression is linked to control of gluconeogenesis when feed intake is not constrained. This differential pattern of regulation is of vital importance because an upregulation of PEPCK activity without sufficient glucogenic precursor availability might drain OAA from the TCA cycle (Fig. 2). The consequence would be an impaired oxidation of acetyl-CoA and alternatively increased synthesis of ketones (*74, 75*). By contrast, increased PC activity in periods of negative energy balance allows pyruvate carbon to be channeled through OAA to maintain hepatic glucose output and simultaneously minimize ketogenesis. The direct allosteric activation of PC by acetyl-CoA (*3*) would serve to further augment the impact of increased PC mRNA expression in such situations. Postparturient dairy cows often experience some degree of ketosis and hepatic triglyceride accumulation (*52, 75*). Although the mechanisms leading to postparturient fatty liver and ketosis are not fully understood, it could be related in part to the drainage of OAA from the TCA cycle (*74*). Increases in PC mRNA, which coincides with increased clearance of lipid from liver in dairy cattle given glucagon infusions (*76, 77*), support a role for PC in this process.

Given the important role of PC for fine-tuning precursor entry into gluconeogenesis, a huge interest has emerged recently to discover the molecular basis of bovine PC regulation. PC mRNA from rat, human, mouse, and bovine has similar open reading frames (*78*) and thus produces the same functional protein. However, bovine PC appears to possess the greatest genomic complexity (Fig. 3). Six unique 5′ untranslated region (UTR) variants have been identified for the bovine (A through F) that are simultaneously expressed in bovine liver (*78*). These variants originate from three unique promoters in combination with alternative splicing (*81*). The proximal promoter (P1) of the bovine PC gene drives the expression of variants A, B, C, and F. The distal promoter (P3) drives the expression of variant D, and promoter 2 (P2) drives the expression of the E variant. Variants A and F show liver-specific expression (*78*), and variants A, D, and F are among the most abundantly expressed in liver at calving (*82*). These data point to a fine-tuning for control of PC that links promoter activation to metabolic status,

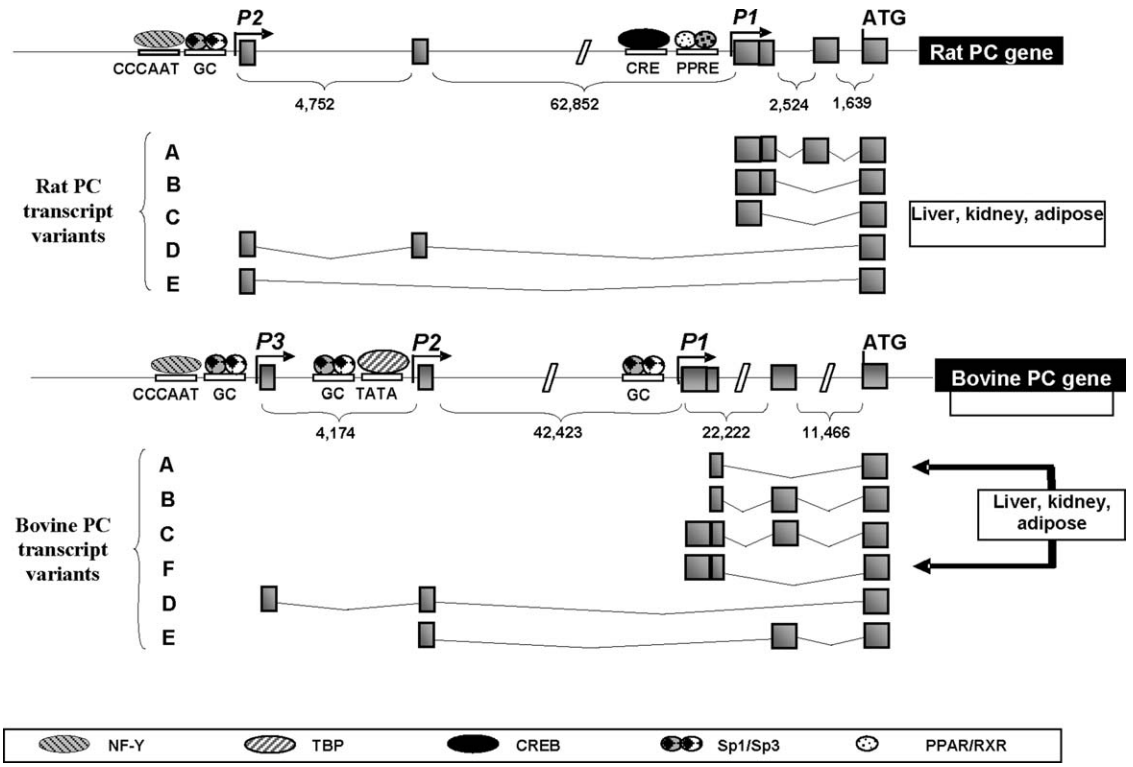

Figure 3. Comparison between rat and bovine pyruvate carboxylase (PC) genes and origin of 5′ untranslated region variants. Alternative promoters (P1, P2, and P3) are highlighted, exons are indicated by the shaded boxes, and the number of nucleotides between exons is given. The start of the coding sequence is indicated as ATG. Transcription from the P1 promoter in both species produces PC mRNA that is found exclusively in liver, kidney, and adipose tissue. Putative binding sites within promoters for transcription factors, *i.e.*, cAMP-response element-binding protein (CREB), peroxisome proliferator-activated receptor (PPAR), and Sp1/Sp3, are indicated. A confirmed CCAAT box binding site for nuclear factor-Y (NF-Y) is located upstream of P2 for nonruminant and a putative site exists in P3 for bovine. A putative site for TATA-binding protein (TBP) is located upstream of bovine P2. Models based on information from Jitrapakdee et al. (*79, 80*) and Hazelton et al. (*81*).

and recent work indicates a differential response for each promoter in the liver based on nutritional and environmental cues (*83, 84*). Although promoter elements that are essential for transcription have been characterized (*81*), future studies are needed to elucidate the physiologically relevant mechanisms that control the activity of the different PC promoters.

Glucose Status and Its Role for Gluconeogenesis

Glucose requirements and glucose status are critically dependent on lactation in dairy cattle, and the level of milk production is closely related to endogenous glucose production (*34, 85*; Table 1). Endogenous glucose production, in turn, appears to function very appropriately to support the genetic merit for milk production in most situations as surplus supply of glucose, especially by the intravenous route, rarely increases milk yield (*86*). The high capacity for gluconeogenesis is complemented by a high priority of the mammary gland for glucose utilization. During periods of high glucose demand for milk production, glucose utilization is primarily reduced in nonmammary tissues such as muscle and adipose tissue, and glucose oxidation is negatively correlated to the level of milk production (*34*). The high priority of the mammary gland for glucose utilization is supported by endocrine changes during peak lactation. Plasma insulin concentrations decrease after parturition, and stimulation of pancreatic insulin secretion by glucose infusion is lower in lactating than nonlactating cows (*34, 52*). A reduced insulin status after parturition reduces glucose uptake into insulin-sensitive organs (muscle and adipose tissue) and thus favors glucose uptake into the mammary gland, which is not affected by insulin action (*87*).

The high metabolic priority for hepatic gluconeogenesis in cattle is based on distinct phylogenetic adaptations to fermentation acid production from carbohydrates in their rumen. Cattle differ from most monogastric species in that they lack hepatic glucokinase (*88*). This enzyme is necessary for capturing excess glucose from blood plasma and accumulating it inside hepatocytes as glucose 6-phosphate for use in glycolysis, glycogen synthesis, and other synthesis pathways (*89*). The lack of glucokinase ensures not only low hepatic glycolysis but also entails that intracellular glucose 6-phosphate levels are primarily

replenished from gluconeogenesis, which may be assisted by glycogenolysis in times of glucose shortage. On the other hand, adult cattle have a very high activity of glucose 6-phosphatase (G6-Pase), the enzyme necessary for glucose release from hepatocytes (*88*). As a consequence, net glucose flux across the basolateral hepatocyte membrane is always outward driven, and the liver is always an organ of net glucose release in adult cattle (*17*). These evolutionary adaptations to a low glucose status imply that cattle respond to experimentally or therapeutically applied infusions of glucose predominantly with an increase in peripheral glucose utilization (especially in adipose and skeletal muscle tissues) (*86*), while the negative feedback on hepatic glucose output is more subtle and sometimes even missing in contrast to monogastric species (*29, 62, 86*). With an intravenous infusion route, extremely high glucose doses (~2.65 kg/day) were necessary to affect the activity of enzymes responsible for the exit of glucose from the gluconeogenetic pathway (fructose 1,6-bisphosphatase, FBPase) and the liver (G6-Pase). Most notably, these changes in FBPase and G6-Pase activities were not correlated with their mRNA abundance, indicating that the release of glucose from the gluconeogenesis pathway and from the liver is regulated to a large degree on a posttranscriptional level according to actual glucose status (*62*).

SUMMARY AND CONCLUSIONS

Gluconeogenesis in dairy cows differs quantitatively and qualitatively from that in monogastric species based on the huge and continuous requirement for *de novo* glucose synthesis and based on the primary use of propionate as substrate. Marginal effects of intravenous glucose infusion on lactation performance suggest that gluconeogenesis mostly functions appropriately to support milk production. In times of insufficient availability of glucogenic precursors (*i.e.*, during peak lactation), however, the high metabolic priority for gluconeogenesis may lead to drainage of OAA from the TCA cycle. Together with other contributing factors, this can cause severe disturbances in the utilization of acetyl-CoA from β-oxidation, leading to lipid metabolism disorders like fatty liver and ketosis. To minimize fatty liver and ketosis, feeding strategies aim at increasing the entry rate of propionate and glucogenic amino acids from the PDV, including measures to increase dry matter intake, to modify fermentation patterns or to directly add glucogenic precursors to the diet. Glucagon and glucocorticoid applications may be supportive measures to stimulate hepatic PC activity and glucogenic precursor supply, respectively; while supplementation with vitamin B12 may support the replenishment of OAA in the TCA from propionate via MCM. Future research should intend to complement our view on the specificities and complexities of the molecular events regulating gluconeogenesis. This includes the unique roles of PCoAC and MCM in the bovine, the mechanisms controlling PC transcription via different promoters, as well as the transcriptional and posttranscriptional events that finally determine hepatic glucose output.

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