Hepatic methyl metabolism: influencing success during the transition to lactation

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SUMMARY

- Adipose tissue mobilization during negative energy balance results in increased hepatic NEFA uptake.
- NEFA can be completely oxidized to energy, incompletely oxidized to ketones, or esterified to triglycerides for storage or export as VLDL.
- VLDL export from ruminant livers is limited, primarily because of limited phosphatidylcholine.
- Use of a cell culture models confirms that increasing choline concentrations can increase VLDL export from hepatocytes. Increasing choline concentrations also tended to reduce oxidative stress associated with a fatty acid challenge.
- Choline can be used to donate a methyl group for methionine regeneration and may have supported gluconeogenesis in hepatocytes.
- Increasing concentrations of methionine decreased the need for endogenous regeneration of methionine and may have supported fatty acid oxidation within hepatocytes. Increasing concentrations of methionine did not change VLDL export or oxidative stress.
- The lack of interaction between methionine and choline in cell culture models supports separate mechanistic roles for methionine and choline within the hepatocyte.

INTRODUCTION

The transition to lactation period is characterized by negative energy balance (NEB) which reflects decreased feed intake and increased energy and glucose demands associated with lactation. During NEB, stored body fat is mobilized in an attempt to compensate for the energy deficit and transported to the liver in the form of nonesterified fatty acids (NEFA) and glycerol. While the mobilized NEFA provide critical fuel sources during the transition to lactation period, inability of the liver to metabolize them can lead to ketosis and fatty liver which have negative effects on productivity and animal health.

HEPATIC UPTAKE AND METABOLISM OF NEFA

During periods of NEB, triglycerides (TG) are mobilized from adipose stores and are transported to the liver to aid in alleviating NEB (Dole, 1956; Gordon and Cherkes, 1956). Hepatic update of NEFA is reflective of blood flow and blood NEFA concentration, both of which are increased after

calving. It has been well characterized that blood NEFA concentration increases after calving, reflective of adipose tissue mobilization, and can increase to 1 mmol/L or greater (Grummer et al., 1993; Reynolds et al., 2003). Additionally, blood flow nearly doubles from one week precalving to one and a half weeks postcalving (Reynolds et al., 2003), increasing exposure of the liver to nutrients and metabolites, including NEFA. Glycerol can be used as a gluconeogenic precursor after hepatic uptake. Conversely, NEFA are β-oxidized to acetyl-CoA units with four possible fates: complete oxidation through the TCA cycle, incomplete oxidation through ketogenesis to ketones, TG synthesis and packaging as very-low density lipoprotein for export from the liver (minimal in ruminant animals), or TG synthesis for storage as liver lipids (reviewed by Grummer, 1993). When available acetyl-CoA exceeds the capacity of the TCA cycle, there are increases in production of ketones and deposition of TG, leading to the onset of ketosis and fatty liver syndrome (White, 2015). The progression of these disorders is the response to poor adaptation to the challenges associated with the transition to lactation period.

During this period of NEFA mobilization, the capacity of the liver to completely oxidize fatty acids to energy is only limitedly increased (Grum et al., 1996) and thus, more acetyl-CoA are metabolized through the alternative pathways including ketogenesis and synthesis of TG for storage or export. Capacity of the liver to synthesize TG from acetyl-CoA is increased by 188% at +1 vs. -21 days relative to calving, highlighting the capacity of the liver to store fatty acids that cannot be immediately oxidized (Grum et al., 1996). Accumulation of liver lipids during early lactation can be as high as 500 g/d and it is predicted that 60% of dairy cows have severe or clinical fatty liver, defined as a liver lipid content greater than 10% on wet weight basis (Drackley, 1999; Bobe et al., 2004).

VLDL EXPORT

Just as in nonruminants, export of very low density lipoproteins (VLDL) can prevent accumulation of fat within the liver and can allow for transport of lipid fuel sources to other tissues, including the mammary gland. Although the capacity of the liver to synthesize TG is increased during the transition to lactation, the ability of the ruminant liver to export TG as VLDL is not proportionately high. Components of VLDL includes TG, apolipoproteins (ApoB and ApoE, specifically), cholesterol, and phosphatidylcholine and have been well studied in nonruminant models. Generation of phosphatidylcholine can either be de novo (methylation of phosphotidylethanolamine) or dietary (choline) and depletion of methyl donors from rodent diets significantly increases liver TG accumulation (Rinella et al., 2008; Cole et al., 2012). In ruminants, the component limiting VLDL export is phosphatidylcholine. Supplementation of dairy cows with rumen-protected choline reduces liver TG concentrations during the transition to lactation period (Zom et al., 2011; Goselink et al., 2013). Examination of genes involved in fatty acid transport and VLDL assembly are increased in cows supplemented with rumen-protected choline, suggesting that the decreased liver TG accumulation is due to increased VLDL export (Goselink et al., 2013). Less is know about the interaction of the two pathways to generate phosphatidylcholine in ruminants.

METHYL DONOR METABOLISM

Methyl donors, including choline, methionine, betaine, and folate, are essential for DNA methylation, prevention of oxidative stress, energy metabolism, and protein synthesis; however, because of rumen fermentation, lactating ruminants are deficient in methyl donors (Pinotti et al., 2002). While the role of methyl donors has been extensively studied in nonruminants, less is understood regarding their action and mechanism in ruminants. In order to elucidate the mechanism of methyl donor metabolism, a bovine primary hepatocyte cell culture model was used to examine the role of two methyl donors, choline and methionine, in hepatic metabolism. Cells were exposed to increasing doses of choline and methionine in the absence or presence of a fatty acid cocktail designed to mimic the profile of fatty acids in circulation at calving (Chandler et al., 2015).

Given that methionine is a required amino acid essential for body and milk protein synthesis, regeneration of methionine is a vital role of methyl donors within liver cells. Increasing concentrations of methionine decreased endogenous regeneration of methionine suggesting that endogenous methionine regeneration is a hepatic priority when methionine concentrations are low (Chandler et al., 2015). Increasing choline concentrations increased methionine regeneration, suggesting that choline may serve a role as a methyl donor for methionine regeneration (Chandler et al., 2015).

Examination of pathways that support fatty acid oxidation via TCA cycle oxidation, and glucose production via gluconeogenesis also indicated specific regulatory roles of choline and methionine within the liver cells. The balance of carbon flux through these two cycles is influenced by the activity of the rate limiting enzymes, pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK). Supplementation with choline tended to increase both these genes, suggesting that more carbon may be used to generate glucose (Chandler et al., 2016). Conversely, supplementation with methionine increased PEPCK, without altering PC, which may suggest that more carbon were oxidized via the TCA cycle (Chandler et al., 2016).

Quantification of VLDL in ruminants is difficult due to differences in lipid profiles of the VLDL between ruminants and nonruminants. An antibody-based assay was validated and used to quantify VLDL secreted into the cell culture media in cells exposed to choline and methionine in the presence of a fatty acid challenge. Increasing choline concentrations increased VLDL export from the hepatocytes (McCourt et al., 2015). No change in VLDL export was observed as methionine concentrations were increased. This was supported by no differences in PEMT, the enzyme that catalyzes the methylation of phosphatidylethanolamine to phosphatidylcholine (Chandler et al., 2015).

Oxidation of fatty acids is critical for energy production in the liver; however, it also results in oxidative stress within the cells. Given this relationship, accumulation of reactive oxygen species (ROS) were examined in the cell culture model described above. Increasing concentrations of

choline, but not methionine, tended to decrease ROS released into the cell culture media (Chandler et al., 2015).

CONCLUSIONS

Negative energy balance and adipose tissue mobilization are well-characterized hallmarks of the transition to lactation period in dairy cows. The ability of the liver to metabolize NEFA and glycerol are not only essential to meeting the demands of lactation, but to avoiding metabolic disorders. Recent attention to methyl donors and their role in maintaining hepatic health and optimizing hepatic function has necessitated a better understanding of their mechanism in the liver. Use of cell culture models aid in understanding specific mechanisms and suggests a biological priority for methyl donor use. The lack of interaction between methionine and choline in cell culture models supports separate roles for methionine and choline within the hepatocyte. It is clear that the requirement for methionine needs to be met, either by dietary sources or by endogenous regeneration. Choline can provide methyl groups for regenerating methionine, but is also involved in increasing VLDL export and may decrease oxidative stress.

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