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Choline absorption and evaluation of bioavailability markers when supplementing choline to lactating dairy cows

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ABSTRACT

The metabolites of choline have a central role in many mammalian biological processes, and choline supplementation to the periparturient dairy cow improves hepatic lipid metabolism. However, variability in responses to choline supplementation has highlighted a lack of understanding of choline absorption in the lactating dairy cow. Our objective was to determine net choline absorption by measuring net portal fluxes of choline and choline metabolites in cows receiving either dietary supplements of rumen-protected choline (RPC) or abomasal delivery of choline (ADC). We also evaluated markers for choline bioavailability by examining relationships between net portal absorption of choline and choline metabolites in plasma and milk. Five late-lactation Holstein cows were used in a 5 × 5 Latin square design, with 5-d treatment periods and a 2-d interval between periods. Treatments were (1) control (0 g/d of choline), (2) 12.5 g/d of choline fed as RPC, (3) 25 g/d of choline fed as RPC, (4) 12.5 g/d of choline provided as ADC, and (5) 25 g/d of choline provided as ADC. At the end of each 5-d period, milk was sampled and 9 blood samples were collected simultaneously from an artery and portal vein at 30-min intervals. Plasma, milk, and feed ingredient concentrations of acetylcholine, betaine, free choline, glycerophosphocholine, lysophosphatidylcholine, phosphatidylcholine, phosphocholine, and sphingomyelin were quantified by hydrophilic interaction liquid chromatography-tandem mass spectrometry. With an increasing dose of ADC, the net portal flux of free choline increased and regression analysis indicated 61% net absorption of the infused dose. Among the choline metabolites, only concentrations of betaine, free choline, and phosphocholine increased in both arterial plasma (3.9, 1.9, and 0.4 times, respectively) and milk (2.5, 1.4, and 1.0 times, respectively) with 25 g/d of ADC relative to the control. For RPC, the net portal flux of free choline was low relative to ADC (13%), which was similar to the relative difference observed in the concentrations and yields of milk free choline and betaine (averaged 21%). When evaluating markers for choline bioavailability, betaine was the leading candidate. Betaine in plasma and milk (alone or in combination with phosphocholine) was strongly associated with net free choline portal flux (coefficient of determination ranging from 0.64 to 0.79). In summary, free choline supply to the lactating dairy cow increases only specific choline metabolites in plasma and milk, which can be potential markers for choline bioavailability.

Key words: choline, bioavailability, dairy cow, rumen protection

INTRODUCTION

Choline is an essential nutrient for optimal animal growth and performance and is transformed into various metabolite forms in cells (Figure 1). Phosphatidylcholine (**PC**), lysophosphatidylcholine (**LPC**), and sphingomyelin (**SM**) are lipid-soluble choline-containing metabolites that are key constituents of all cell membranes and have a central role in lipid metabolism and cell signaling (Jiang et al., 2014). Free choline (**Cho**) and choline metabolites, acetylcholine (**ACho**), betaine (**Bet**), glycerophosphocholine (**GPCho**), and phosphocholine (**PCho**), are water-soluble, with ACho serving as a primary neurotransmitter in the autonomic nervous system (Cheng et al., 1996). Mitochondrial oxidation of Cho forms Bet (Figure 1), which, along with GPCho, acts as organic osmolytes within cells (Eklund et al., 2005; Jiang et al., 2014). Further oxidation of

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Bet provides methyl groups for the conversion of homocysteine to methionine, the key step in one carbon metabolism in the cell (Eklund et al., 2005).

The most common clinical sign of choline deficiency across species is fatty liver, which arises from hepatic accumulation of triacylglycerol caused by insufficient PC for the synthesis and secretion of very low density lipoproteins (Jiang et al., 2014). For the dairy cow, the periparturient period is characterized by a high prevalence of moderate to severe fatty liver (Bobe et al., 2004), and supplementation of rumen-protected choline (**RPC**) was shown to reduce the extent of hepatic triacylglycerol accumulation and increase expression of genes involved in very low density lipoprotein transport (Zom et al., 2011; Goselink et al., 2013). Moreover, recent work with primary bovine hepatocytes reported increased very low density lipoprotein export when incubated with increasing concentrations of choline chloride (**CC**; McCourt et al., 2015).

Based on the importance of choline, dietary requirements have been established for the major production animals (i.e., poultry, swine, and fish); however, no choline requirement has been established for dairy cattle (NRC, 2001). When reviewing the importance of choline in dairy cattle, the NRC (2001) concluded that a requirement could not be established because of variable responses to choline supplementation in lactating dairy cows and that it would require more extensive feeding experiments than were available at the time of the publication. This variability may be partly explained by a focus on nonspecific biomarkers, such as milk yield and composition, rather than biomarkers related to the metabolic functions of choline. Choline shares many similar attributes to vitamins, for which it is more appropriate to define requirements based on specific biomarkers, such as enzyme activities and tissue concentrations (Combs, 2012). The metabolites of choline are of biological importance in the body, but

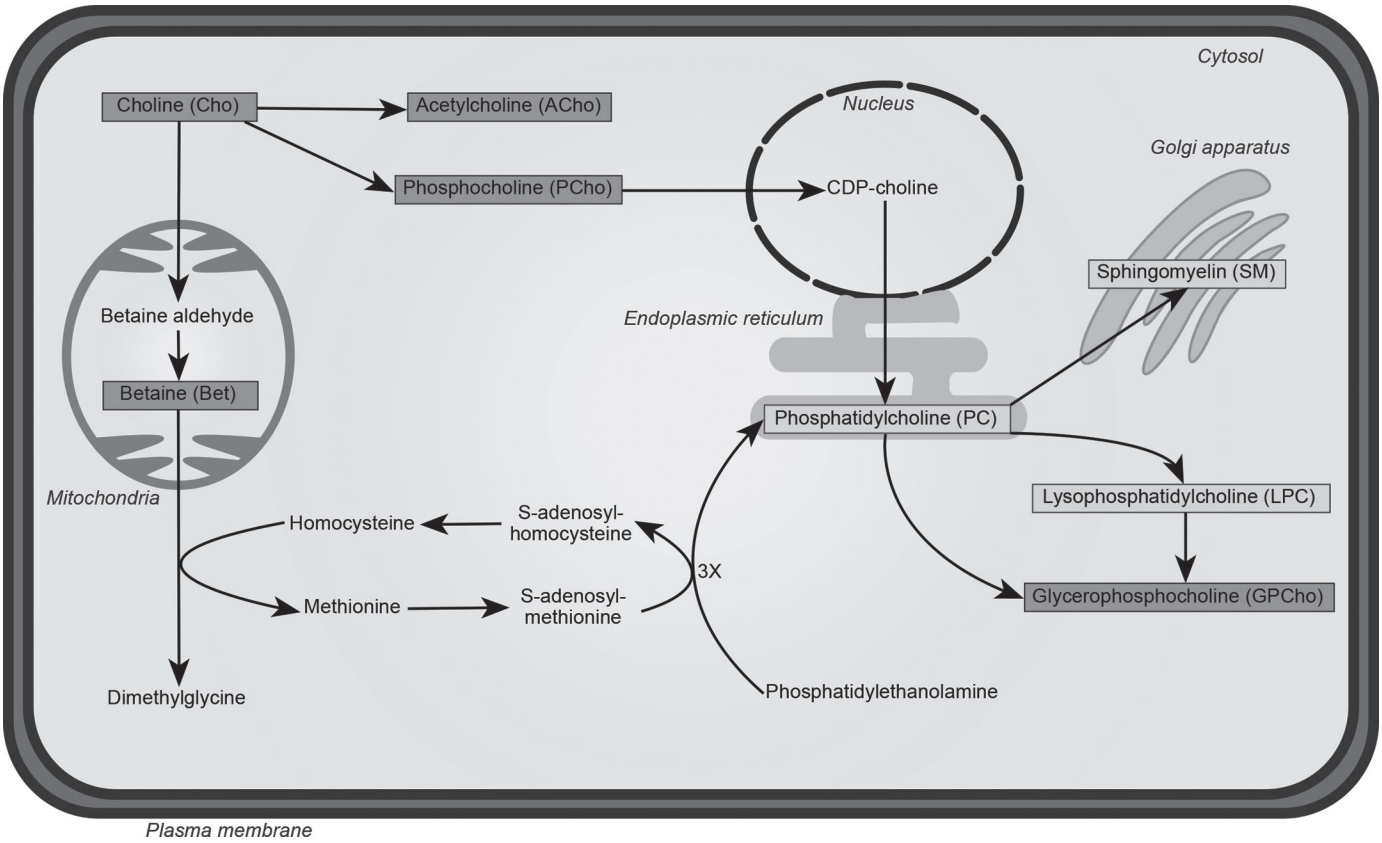

Figure 1. Metabolism of choline in mammalian cells. The site of each reaction is depicted by the location of its end product. The compounds shown in boxes were assayed in plasma and milk in the current experiment. Betaine (Bet), free choline (Cho), acetylcholine (ACho), glycerophosphocholine (GPCho), and phosphocholine (PCho) are all water-soluble, whereas lysophosphatidylcholine (LPC), phosphatidylcholine (PC), sphingomyelin (SM) are lipid soluble. The PCho and PC are formed from choline via the cytidine diphosphate (CDP) choline pathway in all cells; PC may also be formed by methylating phosphatidylethanolamine in a 3-step process via the phosphatidylethanolamine *N*-methyltransferase pathway. Color version available online.