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Restriction of dietary methyl donors limits methionine availability and affects the partitioning of dietary methionine for creatine and phosphatidylcholine synthesis in the neonatal piglet☆

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Abstract

Methionine is required for protein synthesis and provides a methyl group for >50 critical transmethylation reactions including creatine and phosphatidylcholine synthesis as well as DNA and protein methylation. However, the availability of methionine depends on dietary sources as well as remethylation of demethylated methionine (i.e., homocysteine) by the dietary methyl donors folate and choline (via betaine). By restricting dietary methyl supply, we aimed to determine the extent that dietary methyl donors contribute to methionine availability for protein synthesis and transmethylation reactions in neonatal piglets. Piglets 4–8 days of age were fed a diet deficient (MD−) (n=8) or sufficient (MS+) (n=7) in folate, choline and betaine. After 5 days, dietary methionine was reduced to 80% of requirement in both groups to elicit a response. On day 8, animals were fed [^{3}H-methyl]methionine for 6 h to measure methionine partitioning into hepatic protein, phosphatidylcholine, creatine and DNA. MD− feeding reduced plasma choline, betaine and folate (P<.05) and increased homocysteine ~3-fold (P<.05). With MD− feeding, hepatic phosphatidylcholine synthesis was 60% higher (P<.05) at the expense of creatine synthesis, which was 30% lower during MD− feeding (P<.05); protein synthesis as well as DNA and protein methylation were unchanged. In the liver, ~30% of dietary label was traced to phosphatidylcholine and creatine together, with ~50% traced to methylation of proteins and ~20% incorporated in synthesized protein. Dietary methyl donors are integral to neonatal methionine requirements and can affect methionine availability for transmethylation pathways.

Keywords: 1-Carbon metabolism; Betaine; Choline; Folate; Remethylation; Transmethylation

1. Introduction

Methionine is an indispensable amino acid with a significant nonprotein requirement. Indeed, methionine is not only incorporated into protein but also in high demand for essential methylation reactions via the methionine cycle (Fig. 1). The cycle functions to transfer methyl groups in order to synthesize critical nutrients as well as to regulate gene expression. The partitioning of methionine between protein synthesis and the >50 transmethylation reactions [1] is of clinical importance, especially in neonates who not require methionine only for protein turnover and growth but also to provide substrate for rapidly expanding methylated product pools. However, the requirement and partitioning of dietary methionine for these transmethylation products is unknown.

Hepatic transmethylation is thought to utilize a significant portion of dietary methionine. Approximately 80% of dietary methionine is directed to the liver during first-pass [2], and liver is a major site of transmethylation in young pigs [2–4]. Methionine that is partitioned toward transmethylation is first adenylated to form *S*-adenosylmethionine (SAM), the primary biological methyl donor, which is demethylated to *S*-adenosylhomocysteine (SAH), which is in equilibrium with homocysteine [5]. It is thought that the vast majority of transmethylation occurs to synthesize creatine and phosphatidylcholine (PC) [6], as well as to methylate DNA and proteins. Recently, our group demonstrated that hepatic methionine partitioning toward transmethylation is readily altered by a portal infusion of the creatine precursor, guanidinoacetic acid (GAA) [3]. Moreover, piglets

Abbreviations: folate, 5-CH_3-tetrahydrofolate; DMG, dimethylglycine; DPM, disintegrations per minute; FSR, fractional synthetic rate; GAA, guanidinoacetic acid; K_s, fractional protein synthesis rate; MD−, methyl-deficient diet; MS+, methyl-sufficient diet; PC, phosphatidylcholine; PEMT, phosphatidylethanolamine methyltransferase; PITC, phenylisothiocyanate; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; SRA, specific radioactivity.

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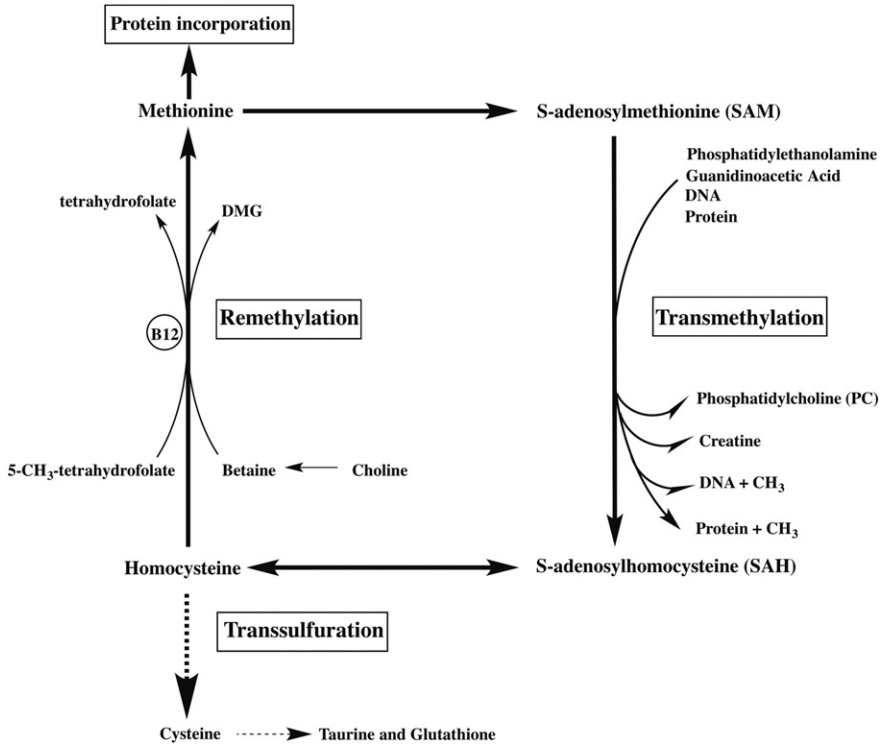

Fig. 1. A schematic of the methionine cycle highlighting the relevant metabolites and methionine products. 5-Methyltetrahydrofolate is referred to simply as folate throughout the manuscript. See text for pathway description.

experiencing intrauterine growth restriction exhibit evidence of dysregulated hepatic methionine partitioning [3,7], suggesting that growth rate has profound effects on methionine partitioning.

The process of remethylation complicates understanding of the priority and demands of transmethylation. Indeed, remethylation replenishes methionine upon transferring a methyl group to homocysteine from the dietary methyl donors, 5-CH_3-tetrahydrofolate (folate) and betaine. Folate regenerates methionine by donating a methyl group to homocysteine via methionine synthase. Betaine, which is an irreversible product of choline, remethylates homocysteine via betaine/homocysteine methyltransferase. The potential for these dietary methyl donors to affect methionine availability is great; indeed, ~25% of whole body methionine flux is derived from remethylation in piglets [8]. Moreover, when methionine is limiting, it is unclear whether protein synthesis or transmethylation is primarily affected and whether certain transmethylation pathways are spared. In this study, we hypothesized that restricting remethylation by omission of dietary methyl donors will limit methionine availability and affect partitioning among transmethylation pathways in the piglet model of the human neonate.

2. Materials and methods

2.1. Chemical reagents and isotopes

All chemicals and reagents were obtained from Sigma, Fisher Scientific or Alfa Aesar. Amino acids were from Ajinomoto, Co. [^{3}H-methyl]methionine was obtained from American Radiochemicals, Inc. [$^2H_9$-trimethyl] choline chloride and [$^2H_{11}$] betaine were obtained from Cambridge Isotope Laboratories.

2.2. Piglets and surgical procedures

All animal handling procedures were approved by the Institutional Animal Care Committee (Memorial University of Newfoundland) in accordance with the Canadian Council on Animal Care. On study day 0, 15 Yucatan miniature piglets were removed from the sow in pairs at 4–9 days of age (8 male, 7 female) and transported to the small animal care facility at the same institution. Surgical procedures and housing conditions have been described elsewhere [9,10]. Briefly, under anesthesia, piglets were fitted with gastric and jugular and femoral venous catheters for blood sampling. All dietary components with the exception of lipid were continuously provided using medical-grade, pressure-sensitive peristaltic pumps (Baxter Corporation); lipid was provided using a syringe pump.

2.3. Dietary regimen

All animals were fed an elemental diet that provided 1.0 MJ/(kg•day) of metabolizable energy and 15 g/(kg•day) of protein that, unless otherwise stated, was complete at >120% of requirements for piglets [11]. In order to restrict remethylation, one group of piglets (n=8) was maintained on a methyl-deficient (MD−) diet (i.e., devoid of the remethylation substrates folate, choline and betaine) starting on study day 0 after surgery. A second group of piglets (n=7) were made methyl sufficient (MS+) by providing folate (38 μg/kg•day), choline (60 mg/kg•day) and betaine (238 mg/kg•day) in the diet; the dietary concentrations of folate and choline were based on the National Research Council (NRC) requirements for pigs of this age [11] and the level of dietary betaine was chosen to provide an equimolar amount of methyl groups that would have been provided when dietary methionine was fed at the NRC requirement.

Dietary protein was provided to both groups of piglets as crystalline amino acids; from days 0 to 5, the dietary amino acid composition was above requirements and similar to other studies [9,10,12–14]. On the evening of day 5, dietary methionine was restricted to ~80% of the estimated requirement for piglets (0.73 g methionine/kg diet) [14]; diets remained isonitrogenous by providing an equimolar amount of alanine. Piglets were fed this methionine-restricted diet for the remainder of the study (days 5–8) (Supplementary Table 1). Methionine restriction was employed in order to detect a sparing effect of remethylation on methionine availability. In order to limit methionine diversion to trans-sulfuration, cysteine was provided in excess. Soybean oil was provided as the lipid source due to low concentrations of choline and betaine [15].

2.4. Enteral [^{3}H-methyl]methionine infusion and tissue collection

On the morning of study day 8, the animals received an enteral infusion of [^{3}H-methyl]methionine. The infusion was initiated with a priming dose of 11.1 kBq/kg (i.e., 0.3 μCi/kg) and continued at 11.1 kBq/(kg•h) for 6 h. Blood was sampled every 30 min for 6 h, transferred immediately to heparinized tubes, and centrifuged at 3000g for 5 min. The plasma fraction was frozen for later use. Immediately after the final blood sample was taken, animals were anesthetized using 3% isoflurane delivered with oxygen. The liver and a sample of biceps femoris muscle were rapidly excised, weighed and freeze clamped. Tissue samples remained at −80 °C until analyzed.