Dietary Methyl Donors Contribute to Whole-Body Protein Turnover and Protein Synthesis in Skeletal Muscle and the Jejunum in Neonatal Piglets

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Abstract

Background: The neonatal methionine requirement must consider not only the high demand for rapid tissue protein expansion but also the demands as the precursor for a suite of critical transmethylation reactions. However, methionine metabolism is inherently complex because upon transferring its methyl group during transmethylation, methionine can be reformed by the dietary methyl donors choline (via betaine) and folate.

Objective: We sought to determine whether dietary methyl donors contribute to methionine availability for protein synthesis in neonatal piglets.

Methods: Yucatan miniature piglets aged 4–8 d were fed a diet that provided 39 μg folate/(kg·d), 60 mg choline/(kg·d), and 238 mg betaine/(kg·d) [methyl-sufficient (MS); n = 8] or a diet devoid of these methyl precursors [methyl-deficient (MD); n = 8]. After 5 d, dietary methionine was reduced from 0.30 to 0.20 g/(kg·d) in both groups. On day 6, piglets received a constant [1-13C]phenylalanine infusion to measure whole-body protein kinetics, and on day 8 they received a constant [3H-methyl]methionine infusion to measure tissue-specific protein synthesis in skeletal muscle, the liver, and the jejunum.

Results: Whole-body phenylalanine flux, protein synthesis, and protein breakdown were 13%, 12%, and 22% lower, respectively, in the MD group than in the MS group (P < 0.05). Reduced whole-body protein synthesis in the MD piglets was attributed to 50% lower protein synthesis in skeletal muscle and the jejunum than in the MS piglets (P < 0.05). Furthermore, methionine availability in skeletal muscle was halved in piglets fed the MD diet (P < 0.05), and the specific radioactivity of methionine was doubled in the jejunum of MD piglets (P < 0.05), suggesting lower intestinal remethylation.

Conclusions: Dietary methyl donors can affect whole-body and tissue-specific protein synthesis in neonatal piglets and should be considered when determining the methionine requirement.

Keywords: 1-carbon metabolism, methionine requirement, isotope kinetics, protein metabolism, remethylation

Introduction

Despite considerable advances in determining the nutritional requirements of neonates, reduced postnatal growth is common in premature infants, partly because of inadequate protein intake. It is estimated that, compared with term infants, 40–95% of preterm infants are underweight at the time of hospital discharge (1). Being classified as underweight at discharge increases disease susceptibility later in life, similar to that of low-birthweight or intrauterine growth–restricted term infants. To ensure optimal early-life protein metabolism and growth rates, it is extremely important that indispensable amino acid requirements are clearly defined and met for neonates. In the case of methionine, this can be difficult because its availability depends not only on dietary concentrations but also on the availability of other nutrients that facilitate its resynthesis. Moreover, the respective rates of competing metabolic processes that consume methionine can also substantially affect its dietary requirement (2).

Methionine is an indispensable amino acid that is required for protein synthesis, and its adenosylated product S-adenosylmethionine plays a key role in whole-body metabolism...
because it is the primary biological methyl donor for the synthesis of >50 metabolites and methylation reactions (e.g., DNA and protein methylation) (3). Under normal metabolic conditions, and when methionine intakes are sufficient, the transfer of a methyl group from S-adenosylmethionine yields homocysteine, whose sulfur atom can be remethylated back to methionine by dietary sources of methyl groups from folate and choline (via betaine). In addition, homocysteine can be irreversibly oxidized to synthesize taurine and cysteine via transsulfuration. It is well established that the methionine requirement is considerably higher with limited dietary cysteine; indeed, dietary cysteine spares the methionine requirement by ~40% in neonatal piglets (4) and by ~55% in children (5). However, the extent to which the dietary methyl donors choline, betaine, and folate contribute to methionine availability for protein synthesis has to our knowledge not been determined.

In the 1940s, Du Vigneaud et al. (6) demonstrated that rats grow normally on methionine-deficient (MD) diets when homocysteine and choline (or betaine) were provided. Furthermore, we have recently shown in piglets that restricting dietary methyl donors (i.e., folate, choline, and betaine) dramatically altered the partitioning of methyl groups from methionine among transmethylation pathways (7). The aim of this study was to examine the role of dietary methyl donors on whole-body protein metabolism and protein synthesis in skeletal muscle, the jejunum, and the liver in piglets. Our primary hypothesis was that these remethylation precursors would spare methionine for protein synthesis in neonatal piglets.

Methods

Chemical reagents and isotopes. All chemicals and reagents were of the highest available purity and were obtained from Sigma-Aldrich, Fisher Scientific, or Alfa Aesar. Amino acids were from Ajinomoto, L-[1-13C]phenylalanine was obtained from Cambridge Isotope Laboratories, and L-[3H-methyl]methionine was obtained from American Radiolabeled Chemicals.

Piglets and surgical procedures. All animal handling procedures were approved by the Memorial University of Newfoundland Animal Care Committee in accordance with the Canadian Council on Animal Care. Sixteen Yucatan miniature piglets aged 4–8 d were removed from the sow in pairs (8 male, 8 female) on study day 0 and transported to an animal care facility. Surgical manipulations and housing conditions have been approved by the Memorial University of Newfoundland Animal Care Committee in accordance with the Canadian Council on Animal Care. Piglets and surgical procedures.

Dietary regimen. Diet was continuously infused with the use of peristaltic pumps on a body-weight basis. Piglets were adapted after surgery with a lipid-free elemental diet at 50% of the target rate delivered intravenously until the morning of day 1. This was followed by the same diet at 75% of the target rate delivered enterally (8) until the evening of day 1. On the evening of day 1, complete enteral feeding commenced with 11.3 mL elemental diet (kg·h) plus 0.4 mL lipid (kg·h) until the end of the study. All piglets were fed 1.1 MJ metabolizable energy (kg·d), and amino acids were infused at 16 g/kg·d. Unless otherwise noted, the complete diet composition was similar to Wykes et al. (9), except soybean oil (J.M. Smucker Company) was provided as the enteral lipid source because of the low concentrations of choline and betaine (10). From days 0 to 5, dietary methionine was fed at 120% [i.e., 0.30 g/(kg·d)] of the piglet requirement in the presence of excess cysteine [4].] On the evening of day 5, dietary methionine was restricted to ~80% of the piglet requirement [0.20 g/(kg·d)] and made isonitrogenous with alanine; this restricted methionine concentration ensured a response to the sparing effect by methyl donors. Piglets were fed this methionine-restricted diet for the remainder of the study (see Supplemental Table 1 for dietary amino acid composition and Supplemental Figure 1 for study overview).

To restrict dietary methyl precursors, 1 group of piglets (n = 8) was maintained on an MD diet (i.e., devoid of folate, choline, and betaine) for the duration of the study. A second group of piglets (n = 8) was fed the same diet made methyl-sufficient (MS) by providing 38 μg folate/(kg·d), 60 mg choline/(kg·d), and 238 mg betaine/(kg·d). Piglets were randomly assigned to the 2 diets, with sex balanced between groups. The dietary concentrations of folate and choline were based on the NRC requirements for pigs of this age (11). Dietary betaine provided an equimolar amount of methyl groups that would have been provided had dietary methionine been fed at requirement (4). All vitamins were provided to the MS piglets with a pediatric commercial solution (INFUVENT Pediatric; Baxter) at >110% of requirements (11). It should be noted that 1 μg cyanocobalamin/mL was provided to all piglets to ensure adequate methionine synthase activity; the purpose of the experiment was to restrict the dietary supply of labile methyl precursors and to determine how that affects flux through otherwise functional remethylation enzymes.

Gastric [1-13C]phenylalanine infusion. On the morning of day 6, piglets were placed into an airtight clear plastic chamber (30 x 30 x 90 cm) attached to a metabolic cart (Quibit Systems) that permitted sampling the rate of CO₂ production and enrichment during a 6-h gastric-primed (20 μmol/kg) constant [10 μmol/(kg·h)] infusion of [1-13C]phenylalanine (8) (Supplemental Figure 1). Briefly, exhaled 13CO₂ was collected into 1 M NaOH during the [1-13C]phenylalanine infusion, and blood was collected intermittently via the jugular catheter into heparinized tubes and immediately centrifuged for 10 min at 3000 x g. Extracted plasma was stored at −20°C. Piglets were returned to their metabolic cages after infusion and continued on their diet. The dietary intake of phenylalanine was unchanged during the infusion. Because of equipment failure, 1 set of piglets did not undergo this infusion protocol, so there was a total of 7 piglets for all phenylalanine isotope outcomes.

Gastric [3H-methyl]methionine infusion and tissue sampling. On the morning of study day 8, the piglets received a gastric infusion of [3H-methyl]methionine for 6 h (Supplemental Figure 1). Blood was sampled every 30 min into heparinized tubes and centrifuged at 3000 x g for 10 min, and plasma was frozen. Piglets were killed with the use of 3% isoflurane delivered with oxygen immediately after the final blood sample. Samples of the liver, biceps femoris (i.e., skeletal muscle), and proximal jejunum were rapidly removed, weighed, and freeze-clamped; the jejunum sample was flushed with ice-cold saline before freezing. Tissue samples remained at −30°C until analyzed. Because of a technical error before the [3H-methyl]methionine infusion, 1 MS piglet had to be excluded, so a total of 7 MS piglets were used for the outcomes from this infusion.

Plasma and tissue enrichment. The plasma enrichment of [1-13C]phenylalanine was measured with the use of pentafluorobenzyl bromide derivatives and detected by gas chromatography-mass spectrometry fitted with a DB-5MS column (0.25 mm x 30 m x 0.22 μm) (Agilent Technologies) (8, 12). The specific ions for phenylalanine were monitored (m/z = 434 and 435) in simulation mode.

The specific radioactivity (SRA) of free methionine in plasma and tissues and protein-bound methionine in skeletal muscle, the jejunum, and the liver were measured with the use of phenyl isothiocyanate derivatives and HPLC as previously described, with norleucine as the internal standard (13). Briefly, tissues were homogenized in perchloric acid and centrifuged at 5000 x g to precipitate tissue proteins. The supernatant was used to determine the SRA of tissue-free methionine, and the pellet was hydrolyzed for 24 h in 6 N HCl at 110°C to determine the SRA of methionine in tissue protein. For plasma, proteins were

Abbreviations used: DMG, dimethylglycine; dpm, disintegrations per minute; MD, methionine-deficient; MS, methionine-sufficient; SRA, specific radioactivity.
precipitated with 5 × 0.5% trifluoroacetic acid in methanol, and free methionine SRA was determined. The methionine peak was fractioned to
determine the radioactivity [disintegrations per minute (dpm)] to calculate SRA (dpm/nmol). All sample types were processed at the same time, and
a repeated standard containing methionine, phenylalanine, and
tyrosine was injected 10 times to assess intra-assay variability.

Plasma concentrations of betaine, choline, and dimethylglycine (DMG) were measured with the use of HPLC tandem MS (14, 15). Briefly, 50 μL plasma was deproteinized with 3× volume acetonitrile
containing internal standards of [3H1]betaine and [3H2]choline. The choline internal standard was used for DMG (14). The supernatant was
injected into a HPLC tandem MS with the use of an isotropic buffering system containing 15 mM 17.5% ammonium formate (pH 3.5) and
82.5% acetonitrile for 6 min at 0.6 mL/min. Compounds were detected with the use of the following m/z transitions: [3H1]betaine 129 → 68,
choline 118 → 59, [3H2]choline 113 → 69, choline 104 → 60, and DMG 104 → 58. Folate concentrations were quantified in plasma with
the use of an Architect i2000 automated immunoassay analyzer (Abbott Diagnostics). Plasma homocysteine and cysteine concentrations were
measured by fluorescence detection of ammonium 7-fluoro-2-oxa-1,3-
diazole-4-sulfonate thiol adducts (16).

Calculations. Plasma and breath enrichments were plotted over time
during the [1-13C]phenylalanine and [3H-methyl]methionine infusions,
and steady state was confirmed by linear regression. Plasma
[13C1]phenylalanine and breath 13CO2 enrichments were expressed as
mole percent excess, and plasma enrichment during the [1-13C]
methionine infusion was expressed as SRA (dpm/nmol). Whole-body
phenylalanine flux (QIG-Phe) was calculated from the [1-13C]phenylalanine
infusion (17, 18):

QIG-Phe = i(Et/Ephe) (1)

where i is the tracer infusion rate, Et is the enrichment of infused
phenylalanine, and Ephe is the mean phenylalanine enrichment in plasma
at steady state (P ≥ 0.05). The methionine flux (QIG-Met) was calculated
from the [3H-methyl]methionine infusion:

QIG-Met = i/SRAMet (2)

where i is the tracer infusion rate and SRAMet is the enrichment of
methionine in plasma at steady state.

Whole-body protein synthesis was calculated from the following:

QIG-Phe = S + Ox = B + I (3)

where S is the rate of protein synthesis, Ox is the rate of phenylalanine
oxidation, B is the rate of phenylalanine release from protein break-
down, and I is rate of phenylalanine intake (17, 18). Phenylalanine
oxidation ([μmol/kg h]) was calculated with the use of the 13CO2
production rate ([F13CO2] [μmol 13CO2/(kg h)]) corrected for phenylala-
nine flux (17, 18):

Ox = [(E13CO2/E13CO2) (44.6 μmol/cm3)]/(W/BRF) × [1/Ephe - 1/Et] (4)

where E13CO2 is the CO2 production rate (cm3/h), E13CO2 is 13CO2
enrichment in breath at steady state (mole percent error), W is piglet weight, and BRF is the bicarbonate retention factor (0.93) of fed piglets (19).

Net protein deposition (PD) was calculated as PD = S – B. Protein metabolism (PM) was expressed as PM = S + B (20).

The rate of tissue-specific protein synthesis (ks) was expressed for
skeletal muscle, the jejunum, and the liver by the following:

ks (%/d) = (SRAproduct/SRAPrecursor)/t × 100 (5)

where SRAproduct and SRAprecursor are expressed as dpm/μmol tissue-
bound methionine and dpm/μmol free methionine, respectively, and t is
the length of tracer incorporation expressed in days. Absolute synthesis rate (ASR) was calculated in the liver as follows:

ASR (g/d) = k × g protein in whole liver

Statistics. An unpaired t test was used to compare data between the MD
and MS piglets. Whole-body phenylalanine kinetic parameters were
correlated to tissue-specific protein synthesis with the use of linear regression
analysis. Normality was confirmed with the use of the Shapiro-Wilk
normality test. Statistics were calculated with the use of Prism version 3.0b.

Results

Piglet growth. Groups were not different with respect to sex, age,
and initial weight. Weight gain was not different between groups after
6 d (305 ± 72 g MD/kg compared with 284 ± 61 g MS/kg) or 8 d
(303 ± 66 g MD/kg compared with 320 ± 82 g MS/kg), and there
was no difference in growth rate from days 3 to 8 between groups, i.e.,
after methionine restriction (141 ± 50 g MD/kg compared with
153 ± 60 g MS/kg). The liver weights at the end of the study were
96.5 ± 14.2 g and 98.3 ± 16.2 g in the MD and MS piglets,
respectively.

Plasma concentrations of metabolites. MD piglets had lower plasma concentrations of folate (P = 0.0001), choline (P = 0.04),
betaine (P = 0.0001), and DMG (P = 0.0001) on day 8 but
higher homocysteine concentrations (P = 0.0001) (Table 1).

Isotopic steady state and flux. A steady state of isotopic
enrichment during the [1-13C]phenylalanine and [3H-methyl]
methionine infusions was achieved in plasma (and breath) by 4 h
for all piglets (Figure 1). The background was 15.0 ± 0.07 mol%
in plasma and 9.7 ± 0.02 mol% in breath. Phenylalanine flux was
13% lower in the MD piglets (P = 0.003) (Table 2). However,
methionine flux did not differ between groups (Table 3).

Whole-body protein dynamics. Whole-body protein synthesis
and breakdown was 12% (P = 0.01) and 22% lower (P = 0.01) in the MD piglets (Table 2), respectively; protein metabolism (synthesis + breakdown) was 17% lower during methyl
restriction (P = 0.01). However, phenylalanine oxidation and
protein deposition (synthesis – breakdown) did not change
during methyl restriction. Although plasma phenylalanine (P = 0.14) and tyrosine (P = 0.06) concentrations were not different
between groups on day 8, concentrations were highly variable
among all piglets. This variability seemed to be higher than usual.

1 Values are means ± SDs.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Plasma concentrations of methionine cycle intermediates and methyl precursors at the end of study day 8 in continuously fed MD and MS piglets1</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD</td>
<td>MS</td>
</tr>
<tr>
<td>Methionine, μM</td>
<td>50.0 ± 15.6</td>
</tr>
<tr>
<td>Homocysteine, μM</td>
<td>38.4 ± 10.2</td>
</tr>
<tr>
<td>Cysteine, μM</td>
<td>104 ± 25.5</td>
</tr>
<tr>
<td>Folate, ng/mL</td>
<td>245 ± 8.3</td>
</tr>
<tr>
<td>Choline, μM</td>
<td>2.2 ± 2.9</td>
</tr>
<tr>
<td>Betaine, μM</td>
<td>0.70 ± 0.41</td>
</tr>
<tr>
<td>DMG, μM</td>
<td>0.10 ± 0.010</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs, n = 8. DMG, dimethylglycine; MD, methyl-deficient; MS, methyl-sufficient.
for this herd of pigs and was likely caused by the metabolic stress from a deficient methionine diet, as observed previously (4). The intra-assay CV was 8% for phenylalanine and 9% for tyrosine.

**Methionine kinetics and tissue-specific protein synthesis.** Plasma methionine concentrations were not different between groups ($P = 0.10$) (Table 1). Tissue-free methionine concentrations and results of the [2H-methyl]methionine infusion are presented in Table 3. The free methionine concentration was ~50% lower in the skeletal muscle of the MD piglets ($P = 0.02$) but was not different between groups in the liver or jejunum. The SRA of free methionine was ~50% lower in the jejunum of the MD group ($P = 0.02$) but was not different between groups in the liver and skeletal muscle, although the latter tended to be lower ($P = 0.07$) (Table 3). Furthermore, protein synthesis was 60% lower in the jejunum ($P = 0.03$) and skeletal muscle ($P = 0.003$) during methyl restriction, but liver $k_s$ was unaffected by MD feeding.

**TABLE 2** Plasma phenylalanine and tyrosine concentrations and whole-body phenylalanine kinetics at steady state on study day 6 of continuously fed MD and MS piglets.

<table>
<thead>
<tr>
<th>Plasma amino acid, μM</th>
<th>MD</th>
<th>MS</th>
<th>$P$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe</td>
<td>$213 \pm 100$</td>
<td>$136 \pm 40.3$</td>
<td>0.14</td>
</tr>
<tr>
<td>Tyr</td>
<td>$51.2 \pm 40.0$</td>
<td>$145 \pm 100$</td>
<td>0.06</td>
</tr>
<tr>
<td>Steady-state rate, μmol Phe/(kg·h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q_{G-Phe}</td>
<td>$426 \pm 46.5$</td>
<td>$489 \pm 32.6$</td>
<td>0.003</td>
</tr>
<tr>
<td>Protein synthesis</td>
<td>$425 \pm 47.0$</td>
<td>$481 \pm 36.6$</td>
<td>0.03</td>
</tr>
<tr>
<td>Protein breakdown</td>
<td>$222 \pm 44.4$</td>
<td>$285 \pm 34.8$</td>
<td>0.01</td>
</tr>
<tr>
<td>Phenylalanine oxidation</td>
<td>$7.18 \pm 3.88$</td>
<td>$9.93 \pm 2.98$</td>
<td>0.15</td>
</tr>
<tr>
<td>Protein deposition</td>
<td>$201 \pm 4.33$</td>
<td>$198 \pm 3.20$</td>
<td>0.2</td>
</tr>
<tr>
<td>Protein metabolism (synthesis + breakdown)</td>
<td>$639 \pm 87.2$</td>
<td>$764 \pm 71.0$</td>
<td>0.01</td>
</tr>
</tbody>
</table>

$^1$Values are means ± SDs, $n = 7$. MD, methyl-deficient; MS, methyl-sufficient; Q_{G-Phe}, whole-body phenylalanine flux.

**Tracer comparisons.** Skeletal muscle protein synthesis correlated positively with whole-body protein synthesis ($r = 0.73; P = 0.005$) and protein metabolism ($r = 0.73; P = 0.005$) (Figure 2). Liver $k_s$ correlated negatively with whole-body protein deposition ($r = -0.63; P = 0.03$) and positively with [1-13C]phenylalanine oxidation ($r = 0.63; P = 0.03$) (Figure 2).

**Discussion**

The principal finding of this study was that dietary methyl donors are capable of affecting whole-body and tissue-specific protein dynamics in neonatal piglets. Indeed, the omission of dietary methyl donors had a dramatic effect on their plasma concentrations and reduced whole-body protein synthesis, breakdown, and metabolism as well as reduced tissue-specific protein synthesis in skeletal muscle and the jejunum. To our knowledge, this is the first study to show a methionine-sparing effect of these dietary methyl donors.

The lower whole-body protein synthesis and breakdown with methyl deficiency suggests that remethylation to methionine is an important contributor to protein homeostasis in growing piglets. The absence of dietary methyl donors for 7 d led to methyl deficiency, which was demonstrated by the diminished plasma concentrations of these metabolites; this dietary protocol has also been previously shown to lead to a lower whole-body remethylation flux rate (7). Although we did not observe differences in growth, these findings are of clinical importance because a reduction of ~60 μmol phenylalanine/(kg·h) in whole-body protein synthesis translates to ~6 g muscle/(kg·d) in neonatal piglets (230 μmol phenylalanine/g muscle) (21). However, reduced protein synthesis did not lead to growth changes, which might be partially explained by reduced protein breakdown during MD feeding. The acute plasticity of protein metabolism is concerning for piglets of this age, when tissues are being rapidly synthesized and degraded to expand (22, 23). Indeed, neonatal rates of protein synthesis are ~4-fold greater than protein intake at birth and at weaning (23). Acute or chronic MD feeding could have long-term consequences associated with protein restriction early in life (1, 24) and perturbed 1-carbon metabolism (25–27).

Among the most dramatic changes resulting from dietary methyl donor restriction was lower protein synthesis and free methionine in skeletal muscle. Therefore, whereas splanchnic methionine uptake was apparently unaffected by dietary methyl donors, the availability of methionine for protein synthesis was compromised during MD feeding, either because of lower remethylation or lower protein breakdown. This outcome is important in neonates because skeletal muscle is the site of most protein synthesis in the body that dictates growth and muscle function. The absolute values for skeletal muscle $k_s$ were ~2–3-fold higher than in other studies (28), but this could have resulted from [2H-methyl]methionine being used as the tracer in piglets fed a low methionine diet. The lower dietary methionine in our study might have induced remethylation several-fold (29), diluting the tissue-free methionine SRA. However, the response to methyl deficiency was similar regardless of the tracer used. Indeed, we observed significant positive correlations between whole-body protein synthesis and skeletal muscle $k_s$ (Figure 2). From birth to weaning, skeletal muscle mass increases 30% in humans and 60% in piglets (28, 30). In addition, in rat pups, skeletal muscle constitutes 30% of total biomass at birth, which rapidly increases to 45% at weaning (31–33). The consequences of perturbed postnatal growth are vast and include diminished...
size (34), neuronal deficits (35), and higher susceptibility to cardiovascular disease and obesity in later life (36, 37). Moreover, these findings in piglets are probably most applicable to low-birth-weight infants, who frequently experience acute protein restriction episodes (1) that would involve methionine restriction. Thus, ensuring adequate dietary supply of methyl donors could spare limited methionine for growth and transmethylation requirements.

Protein synthesis in the jejunum was also affected by methyl restriction, which is important because of the massive protein demands of the small intestine. Indeed, the gut completely regenerates itself every ~5–6 d (38), and although the gut comprises only 4–6% of total biomass, it contributes ~35% of whole-body protein turnover (39). Furthermore, the SRA of methionine was higher during MD feeding in the jejunum, which suggests less dilution of label, likely because of higher transmethylation or lower remethylation in the intestine. Indeed, the gut is an important site of transmethylation and remethylation in piglets, especially on first pass (40). Furthermore, because gut extracts only ~20% of dietary methionine (40), it is interesting that protein synthesis in the jejunum was lowered during methyl restriction as opposed to simply extracting more methionine to maintain protein synthesis. The ramifications of dysregulated intestinal protein synthesis are expected to be more severe in piglets of this age because the fractional rate of jejunal protein synthesis is higher in piglets aged 7 d than in littermates aged 26 d (28). Potential consequences of reduced jejunal protein synthesis are lower intestinal mass, absorptive capacity, and barrier integrity. Further investigations are warranted to determine whether dietary methyl donors can improve intestinal health and feeding practices.

Dietary methyl donors did not affect hepatic protein synthesis in methionine-restricted piglets. The conservation of liver protein synthesis during methyl restriction suggests that the liver exerts control over whole-body methionine partitioning and that methionine does not become available to the other tissues until protein demands in the liver are fulfilled. This hypothesis is supported by the observation that higher hepatic protein synthesis was associated with lower whole-body protein deposition (Figure 2); moreover, lower whole-body protein synthesis would lead to more amino acids being sent for disposal to the liver, which was associated with higher phenylalanine oxidation rates (Figure 2). However, unlike skeletal muscle and to a lesser extent the jejunum, the liver is an important site of protein secretion; indeed, the 10 most expressed transcripts in the liver code for secretory proteins (41). Therefore, it should be emphasized that our analysis may not have captured the complete effects of methyl restriction on hepatic protein metabolism. It is possible that methyl restriction affected the secretion of secretory proteins, which could have masked changes in the hepatic protein synthesis rate.

We used enteral infusion of tracers to trace the metabolic products of dietary amino acids, the metabolism of which includes first-pass metabolism by the gut and liver. This first-pass splanchnic metabolism is substantial for amino acids (42), representing 27% of dietary phenylalanine (43) and ~30% of dietary methionine (4, 40) in piglets. This enteral tracer protocol typically yields higher flux and oxidation rates than intravenous tracer infusions because of the added metabolism by splanchnic tissues (43, 44).

This study demonstrated the capacity of dietary methyl donors to spare methionine for protein synthesis in neonatal piglets. These findings have translational relevance for infants who commonly experience postnatal protein restriction and consequent growth restriction. Further studies are required to determine whether dietary methyl donors can lower whole-body methionine requirements and affect methionine availability for transmethylation (2, 45).

TABLE 3  Methionine kinetics parameters in tissues of continuously fed MD or MS piglets after a 6-h infusion of \([^{3}H\text{-methyl}]\) methionine on study day 8

<table>
<thead>
<tr>
<th></th>
<th>MD</th>
<th>MS</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>O(_{\text{A, Met}}), μmol Met/(kg·h)</td>
<td>153 ± 57.8</td>
<td>153 ± 51.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Liver-free Met, μmol/g</td>
<td>0.14 ± 0.051</td>
<td>0.16 ± 0.020</td>
<td>0.3</td>
</tr>
<tr>
<td>Muscle-free Met, μmol/g</td>
<td>0.10 ± 0.033</td>
<td>0.19 ± 0.089</td>
<td>0.02</td>
</tr>
<tr>
<td>Jejunum-free Met, μmol/g</td>
<td>0.24 ± 0.16</td>
<td>0.34 ± 0.13</td>
<td>0.3</td>
</tr>
<tr>
<td>Liver-free Met SRA, dpm/nmol</td>
<td>80.8 ± 26.7</td>
<td>76.7 ± 23.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Muscle-free Met SRA, dpm/nmol</td>
<td>44.0 ± 24.2</td>
<td>22.2 ± 14.5</td>
<td>0.07</td>
</tr>
<tr>
<td>Jejunum-free Met SRA, dpm/nmol</td>
<td>676 ± 261</td>
<td>342 ± 239</td>
<td>0.02</td>
</tr>
<tr>
<td>Liver ks, %/d</td>
<td>37.1 ± 18.9</td>
<td>48.5 ± 22.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Liver SRA, g/d</td>
<td>4.6 ± 2.6</td>
<td>6.3 ± 3.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Muscle ks, %/d</td>
<td>25.1 ± 15.0</td>
<td>59.7 ± 18.3</td>
<td>0.003</td>
</tr>
<tr>
<td>Jejunum ks, %/d</td>
<td>20.3 ± 10.0</td>
<td>49.4 ± 33.3</td>
<td>0.03</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs, n = 8 for MD and n = 7 for MS. ASR, absolute synthesis rate; dpm, disintegrations per minute; ks, fractional protein synthesis rate; MD, methyl-deficient; MS, methyl-sufficient; O\(_{\text{A, Met}}\), whole-body methionine flux; SRA, specific radioactivity.

FIGURE 2  Correlations between outcomes from the 2 tracer infusions in continuously fed methyl-deficient or methyl-sufficient piglets. The y axis represents parameters from \([^{1-13}C]\)phenylalanine infusion, and the x axis represents the rate of \([^{3}H\text{-methyl}]\)methionine incorporation into skeletal muscle (A) and the liver (B). Protein metabolism refers to synthesis + breakdown; protein deposition refers to synthesis – breakdown. Each symbol represents an individual piglet. ks, fractional protein synthesis rate.
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