Hepatic and Renal Betaine-Homocysteine Methyltransferase Activity in Pigs as Affected by Dietary Intakes of Sulfur Amino Acids, Choline, and Betaine

Jason L. Emmert*,2, Douglas M. Webel*, Robert R. Biehl†, Margaret A. Griffiths‡, Linda S. Garrow†, Timothy A. Garrow†, and David H. Baker*,3

Departments of *Animal Sciences and †Food Science and Human Nutrition and Division of Nutritional Sciences, University of Illinois, Urbana 61801

ABSTRACT: In Exp. 1, young pigs were fed a basal diet containing .17% methionine (Met) (.14% digestible Met), and .48% cystine (.38% digestible cystine) for 14 d (34 to 48 d of age). Treatment additions were .25% DL-Met, .34% betaine, .30% choline, or .25% DL-Met and .34% betaine. Methionine, but not betaine or choline supplementation, increased (P < .05) weight gain and feed efficiency. Hepatic betaine-homocysteine methyltransferase (BHMT) activity was increased (P < .05) by betaine and choline supplementation but was not affected by Met deficiency. Renal BHMT activity was increased (P < .05) by Met deficiency and was further increased (P < .05) by betaine supplementation. In Exp. 2, 10-kg pigs were fed the basal diet from Exp. 1 supplemented with enough DL-Met to bring the total Met to .24% (.20% digestible Met). Treatment additions consisted of .20% DL-Met or .34% betaine, and diets were fed for 16 d (34 to 50 d of age). Feed efficiency increased (P < .05) in response to Met, but not to betaine, supplementation. Hepatic BHMT activity increased (P < .05) in response to betaine and Met, but no changes in renal BHMT activity occurred. Although statistically significant changes in hepatic and renal BHMT activity occurred in both experiments, the magnitude of the responses was probably not physiologically important. Therefore, in contrast to previous findings with rats and chicks, it does not seem that hepatic and renal BHMT activity in pigs is influenced substantially by Met deficiency, or by surfeit levels of choline or betaine.

Key Words: Methionine, Choline, Betaine, Pigs


Introduction

When elevated above normal levels, homocysteine (HCY)4, a metabolite formed from methionine (Met) in the transsulfuration pathway, has been shown to be an independent risk factor for the development of vascular disease in humans (Ueland and Refsum, 1989). Betaine-homocysteine methyltransferase (BHMT) catalyzes the conversion of HCY to Met by facilitating the transfer of a methyl group from betaine, thereby playing a critical role in maintaining normal physiological levels of Met and HCY. Although primarily a hepatic enzyme, some BHMT activity is present in kidneys of some species (Finkelstein et al., 1971; Finkelstein and Martin, 1984; Cao et al., 1995). The importance of BHMT in Met and HCY metabolism is evident, but data regarding the influence of changes in dietary levels of sulfur amino acids, choline, and betaine on BHMT activity are sparse.

Our objective was to evaluate the effects of varying levels of Met, choline, and betaine on hepatic and renal BHMT activity in pigs, which may be more similar to humans metabolically than are rats and chicks, from which most previous information regarding BHMT activity has been obtained. We evaluated the effects of either severe or moderate Met deficiency, with or without betaine or choline supplementation, on BHMT activity in liver and kidney.

Materials and Methods

All procedures were approved by the University of Illinois Laboratory Animal Care Advisory Committee. Water and experimental diets were freely available,
and diets were formulated to meet or exceed NRC (1988) recommendations for all nutrients, with the exception of Met. All additions to experimental diets were made at the expense of cornstarch.

Experiments were conducted with crossbred Pig Improvement Company (PIC) pigs (University of Illinois Swine Research Center). Pigs were housed in an environmentally controlled nursery following weaning at 21 d of age, and pens (1.2 m²) contained a woven-wire floor, a self-feeder, and a nipple waterer. Following a 13-d postweaning adjustment period during which a phase-1 starter diet was fed, pigs were deprived of feed for 12 h, weighed (average initial weight = 8 kg in Exp. 1 and 10 kg in Exp. 2), and assigned to uniform blocks based on ancestry and body weight. Pigs were then allotted randomly from within blocks to pens and treatment diets (Table 1), which were fed for 14 d in Exp. 1 and 16 d in Exp. 2. Each experiment had three pens of two pigs per dietary treatment. At assay termination, pigs were killed by exsanguination, and livers and kidneys were immediately removed, frozen in liquid nitrogen, and stored at −80°C for determination of BHMT activity.

The basal diet contained, by calculation, 18% CP, .17% Met, .48% cystine, and .08% choline. On the basis of digestibility estimates of Southern (1991), we assumed that the apparent digestibility of Met and cystine in the basal diet was 81 and 79%, respectively. Hence, the basal diet contained an estimated .14% digestible Met and .38% digestible cystine. Based on the Met requirement estimate of .29% for pigs between 5 and 20 kg (Chung and Baker, 1992), the basal diet was judged to be severely deficient in Met but superadequate in cystine. A small addition of supplemental choline chloride (134 mg/kg) was designed to bring total dietary choline to .08%. The least significant difference multiple-comparison procedure (Carmer and Walker, 1985) was used to evaluate potential differences among treatment means.

### Determination of Hepatic and Renal BHMT Activity

Activity of BHMT was measured in duplicate in crude extracts of liver and kidney cortex as previously described (Garrow, 1996), the only exception being that liver and kidney samples were homogenized in five volumes of buffer. Initial-rate measurements were made in all assays, and saturating levels of substrates were used: 2 mM betaine (1 μCi) and 5 mM DL-HCY. No more than 5% of the limiting substrates was consumed in any assay. Measurement of total protein in crude liver and kidney extracts was conducted using the method of Bradford (1976) with reagents from Bio-Rad Laboratories (Hercules, CA). As measured, a unit of BHMT activity was defined as nanomoles of product formed per hour.

**Experiment 1.** This experiment was conducted to determine the effects of severe Met deficiency, alone or in combination with excess choline or betaine, on the activity of hepatic and renal BHMT. Treatment additions to the Met-deficient basal diet (Table 1) consisted of .25% DL-Met, .34% betaine, .30% choline, .38% digestible cystine, and .06% available choline. The activity of hepatic and renal BHMT. The basal diet supplemented with DL-Met to achieve a level of .24% total Met (.32% digestible Met). Treatment additions to the Met-deficient basal diet (Table 1) consisted of .25% DL-Met, .34% betaine, .30% choline, .38% digestible cystine, and .06% available choline. The activity of hepatic and renal BHMT. The basal diet supplemented with DL-Met to achieve a level of .24% total Met (.32% digestible Met). Treatment additions to the Met-deficient basal diet (Table 1) consisted of .25% DL-Met, .34% betaine, .30% choline, .38% digestible cystine, and .06% available choline.

**Experiment 2.** This experiment was conducted to determine the effects of marginal Met deficiency on the activity of hepatic and renal BHMT. The basal diet from Exp. 1 (Table 1) was used but was supplemented with DL-Met to achieve a level of .24% total Met (.32% digestible Met). Treatment additions to this basal diet consisted of .20% DL-Met or .34% betaine.

### Results and Discussion

Homocysteine is a metabolite normally formed from Met in the transsulfuration pathway, but substantial elevations of plasma HCY can result from genetic
abnormalities such as severe deficiencies of cystathionine β-synthase (CBS) or 5,10-methylenetetrahydrofolate reductase (Mudd et al., 1964, 1972). Deficiencies of folate (Kang et al., 1987), vitamin B₁₂ (Brattstrom et al., 1988), and vitamin B₆ (Ueland and Refsum, 1989) can also elevate plasma HCY, as do renal insufficiency and psoriasis (Ueland and Refsum, 1989). The fact that plasma HCY, even when elevated only slightly above normal levels, is associated with the development of various types of vascular disease (Ueland and Refsum, 1989), stresses the importance of exploring and developing effective dietary means of normalizing this metabolite.

Treatment for elevated plasma HCY levels is typically designed to address the specific condition causing the elevation. Approximately 50% of CBS-deficient patients exhibit marked decreases in plasma HCY upon supplementation with vitamin B₆ (Mudd et al., 1985), and supplemental folate or betaine has been successful in patients with other disorders (Smolin et al., 1981; Wilcken et al., 1983; Berlow et al., 1989). In some cases, plasma HCY is lowered but remains higher than normal (Wilcken et al., 1983; Allen et al., 1993), in which case risk remains for increased susceptibility to vascular disease. It seems appropriate, then, to explore means of increasing the efficacy of treatment for HCY elevation, possibly by increasing the activity of enzymes capable of metabolizing HCY.

Information regarding the influence of dietary changes in sulfur amino acids, choline, or betaine on BHMT activity is limited. Most data have been collected using rats as the experimental animal. Table 2. Effect of severe methionine deficiency in the presence of choline or betaine on performance and hepatic and renal betaine-homocysteine methyltransferase (BHMT) activity in young pigs (Exp. 1)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Daily gain, g</th>
<th>Gain:feed, g/kg</th>
<th>BHMT activity, units/mg²</th>
<th>Pooled SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Basal (B)</td>
<td>75x</td>
<td>191x</td>
<td>40.6x</td>
<td>17.0x</td>
</tr>
<tr>
<td>2. B + 25% DL-Met</td>
<td>143x</td>
<td>259x</td>
<td>41.3x</td>
<td>14.9x</td>
</tr>
<tr>
<td>3. B + 34% betaine</td>
<td>99x</td>
<td>234x</td>
<td>57.3x</td>
<td>21.2x</td>
</tr>
<tr>
<td>4. B + 30% choline</td>
<td>121x</td>
<td>260x</td>
<td>55.4x</td>
<td>19.2x</td>
</tr>
<tr>
<td>5. As 2 + 34% betaine</td>
<td>424x</td>
<td>548x</td>
<td>47.2xx</td>
<td>16.0x</td>
</tr>
</tbody>
</table>

aData represent means of three pens, each containing two pigs averaging 8 kg at the start of the feeding trial, which was carried out for 14 d.

BHMT activity reported as units (nmol product formed per h) per mg protein.

The methionine-deficient basal diet contained 18% CP, .17% total methionine (.14% digestible methionine), .48% cystine (.38% digestible cystine), and .06% available choline.

Table 3. Effect of marginal methionine deficiency in the presence of betaine on performance and hepatic and renal betaine-homocysteine methyltransferase (BHMT) activity in young pigs (Exp. 2)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Daily gain, g</th>
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<th>BHMT activity, units/mg²</th>
<th>Pooled SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Basal (B)</td>
<td>536</td>
<td>577x</td>
<td>51.6x</td>
<td>13.3</td>
</tr>
<tr>
<td>2. B +.20% DL-Met</td>
<td>574</td>
<td>619w</td>
<td>64.0w</td>
<td>16.0</td>
</tr>
<tr>
<td>3. B +.34% betaine</td>
<td>576</td>
<td>567x</td>
<td>67.0w</td>
<td>16.4</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>43</td>
<td>12</td>
<td>1.7</td>
<td>2.3</td>
</tr>
</tbody>
</table>

aData represent means of three pens, each containing two pigs averaging 10 kg at the start of the feeding trial, which was carried out for 16 d.

BHMT activity reported as units (nmol product formed per h) per mg protein.

The methionine-deficient basal diet contained 18% CP, .24% total methionine (.20% digestible methionine), .48% cystine (.38% digestible cystine), and .06% available choline.

Pooled SEM 43 12 1.7 2.3

BHMT activity, units/mg²

Data means within columns with different superscript letters differ significantly (P < .05).

Table 2. Effect of severe methionine deficiency in the presence of choline or betaine on performance and hepatic and renal betaine-homocysteine methyltransferase (BHMT) activity in young pigs (Exp. 1)

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(Finkelstein et al., 1982a,b, 1983; Park et al., 1997), but rats practice coprophagy. Limited information is also available from studies with chicks (Emmert et al., 1996). Interpretation of previous data with regard to human nutrition and health must be made with caution, due to numerous metabolic differences among species. Specifically, rats and chicks have a very high activity of hepatic choline oxidase (Sidransky and Farber, 1960), an enzyme that oxidizes choline to betaine for subsequent use as a substrate for BHMT. In contrast, pigs exhibit a much lower activity of hepatic choline oxidase, similar to levels in humans (T. A. Garrow, unpublished data). Therefore, it was of interest to determine whether BHMT in liver and kidney of pigs would respond to dietary changes in Met, choline, and betaine.

Weight gain, feed efficiency, and BHMT activities of pigs in Exp. 1 are shown in Table 2. Weight gain and feed efficiency of pigs fed the basal diet, containing only .14% digestible Met, was severely reduced (P < .05) relative to those of pigs fed the adequate level of Met. Growth traits were not significantly affected (P > .05) by adding surfeit choline or betaine to the Met-deficient or Met-adequate diet. Specific activity of hepatic BHMT was not affected (P > .05) by Met deficiency but was increased (P < .05) by addition of surfeit choline or betaine to the Met deficient, but not to the Met-adequate, diet. In kidney, BHMT specific activity was higher (P < .05) in pigs fed the Met-deficient basal diet than in those fed the Met-adequate control diet. The specific activity of BHMT was further elevated (P < .05) by addition of surfeit betaine, but not by addition of choline.

In Exp. 2 (Table 3), pigs fed the low-Met diet (.20% digestible Met) had lower feed efficiencies (P < .05) than pigs fed the Met-supplemented diet, indicating
that the diet was marginally deficient in Met. No improvement in weight gain or feed efficiency (P > .05) occurred upon supplementing the Met-deficient diet with surfeit betaine. In contrast to Exp. 1, hepatic BHMT activity in Exp. 2 was reduced (P < .05) in pigs fed the diet marginally deficient in Met relative to pigs supplemented with Met or betaine. In kidney, BHMT activity was again substantially lower than that observed in liver, and no treatment effects were observed.

Although the results of Exp. 1 and Exp. 2 seem contradictory, the minimal magnitude of the responses, though statistically significant, bring into question the physiological importance of these responses. Had renal BHMT activity of pigs fed the Met-adequate diet in Exp. 1 been as high as renal activity of those fed the Met-adequate diet in Exp. 2, treatment differences in Exp. 1 would have been minimal. Thus, it seems unlikely that hepatic or renal BHMT activity in pigs is substantially influenced by dietary Met level or by the addition of surfeit choline or betaine.

The lack of increase in hepatic BHMT activity under Met-deficient conditions was unexpected. Previously, Met deficiency increased hepatic BHMT activity in rats (Finkelstein et al., 1982a; Park et al., 1997) and chicks (Emmert et al., 1996), leading to the hypothesis that in these species, BHMT activity increases under Met-deficient conditions and conserves Met. Research has shown that increases in BHMT activity in rats fed Met-deficient diets are associated with increases in BHMT mRNA (Park et al., 1997). Based on our results, pigs do not seem able to respond to Met-deficient conditions by increasing the activity of hepatic or renal BHMT.

Although hepatic BHMT activity of pigs fed our Met-deficient diets was elevated (P < .05) by the addition of surfeit betaine or choline, the response was of minimal magnitude, and therefore of questionable physiological importance. Our results are in contrast to previous observations in which excess choline or betaine addition to diets devoid of Met or choline led to markedly increased hepatic BHMT activity in rats (Finkelstein et al., 1982a, 1983; Park et al., 1997). In chicks, excess choline or betaine added to Met-deficient diets also increased hepatic BHMT activity (Emmert et al., 1996).

It is interesting to note that the specific activity of hepatic BHMT in our pigs fed a Met-adequate diet containing no substantial excesses of other nutrients was higher than that reported for either rats or chicks fed similar, completely adequate diets (Finkelstein et al., 1982a, 1983; Emmert et al., 1996; Park et al., 1997). It may be possible that pigs have a higher baseline activity of BHMT in liver, making it unnecessary to increase activity under the conditions that typically elevate BHMT activity in other species. This hypothesis is supported by the renal BHMT activity observed in both experiments, which was higher than values previously reported for rats (Finkelstein et al., 1971) or chicks (Emmert et al., 1996).

Implications

Previous work with rats and chicks has shown that hepatic betaine-homocysteine methyltransferase activity is substantially elevated under methionine-deficient conditions and is further elevated by supplementation with surfeit choline or betaine. The results herein with pigs show only moderate elevations in enzymatic activity due to choline or betaine supplementation. Under conditions of adequate dietary methionine, pigs seem to have a higher activity of betaine-homocysteine methyltransferase in the liver and kidney than do rats or chicks.

Literature Cited


