Choline Deprivation Induces Hyperhomocysteinemia in Rats Fed Low Methionine Diets

Minoru SETOUE, Seiya OHUCHI, Tatsuya MORITA and Kimio SUGIYAMA*

Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan
(Received May 8, 2008)

Summary To clarify the relationship between dietary choline level and plasma homocysteine concentration, the effects of choline deprivation on plasma homocysteine concentration and related variables were investigated in rats fed a standard (25%) casein (25C) diet or standard soybean protein (25S) diet. Using the 25S diet, the time-dependent effect of choline deprivation and the comparative effects of three kinds of lipotropes were also investigated. Feeding rats with the choline-deprived 25S diet for 10 d significantly increased plasma total homocysteine concentration to a level 2.68-times higher than that of the control group, whereas choline deprivation had no effect in rats fed the 25C diet. Increases in hepatic S-adenosylhomocysteine and homocysteine concentrations, decreases in hepatic betaine concentration and the activity of cystathionine β-synthase, but not betaine-homocysteine S-methyltransferase, and fatty liver also occurred in rats fed the choline-deprived 25S diet. Plasma homocysteine concentration increased when rats were fed the choline-deprived 25S diet for only 3 d, and the increase persisted up to 20 d. The hyperhomocysteinemia induced by choline deprivation was effectively suppressed by betaine or methionine supplementation. Choline deprivation caused hyperhomocysteinemia also in rats fed a choline-deprived low (10%) casein diet. The results indicate that choline deprivation can easily induce prominent hyperhomocysteinemia when rats are fed relatively low methionine diets such as a standard soybean protein diet and low casein diet, possibly through the suppression of homocysteine removal by both remethylation and cystathionine formation. This hyperhomocysteinemia might be a useful model for investigating the role of betaine in the regulation of plasma homocysteine concentration.

Key Words choline deficiency, homocysteine, betaine, soybean protein diet, low casein diet

Homocysteine is an intermediary metabolite in the metabolism of methionine (Fig. 1) and may be an independent risk factor for cardiovascular disease when elevated (1–3). Several factors are known to affect plasma homocysteine concentration, e.g., genetic factors, physiological and lifestyle determinants, nutritional or clinical conditions, and drugs (1–3). For instance, deficiencies of the vitamins such as folate, vitamin B-6 and vitamin B-12 result in elevation of plasma homocysteine concentration, since these vitamins are involved in the metabolism of homocysteine (1–3). The liver is the central organ of methionine metabolism, and hepatic homocysteine concentration is thought to reflect plasma homocysteine concentration to a considerable degree (4). In the liver, homocysteine concentration is affected by the rates of the following processes: (i) production of homocysteine from S-adenosylhomocysteine (SAH) or its precursor S-adenosylmethionine (SAM), (ii) remethylation of homocysteine to methionine, (iii) formation of cystathionine, and (iv) export of homocysteine into blood plasma. One strategy to lower plasma homocysteine is to increase homocysteine remethylation by providing either 5-methyltetrahydrofolate or betaine. Therefore, many attempts have been made to lower plasma homocysteine concentration by administering folate or betaine (5, 6). Unlike betaine, its precursor choline is thought to be an essential nutrient in higher animals, including humans (7). Choline is irreversibly oxidized to betaine aldehyde and then to betaine, although choline is also incorporated into choline-containing phospholipids, such as phosphatidylcholine (PC) and neurotransmitter acetylcholine (8). An important question is to what extent dietary choline level affects plasma homocysteine concentration, since endogenous betaine is solely derived from choline. A recent epidemiological study has shown that higher intakes of dietary choline, as well as betaine, were related to lower plasma total homocysteine concentrations independent of other determinants, including folate and B vitamins (9). Further, it has been shown that administration of choline or PC was effective in reducing plasma homocysteine concentration in humans (10, 11). On the other hand, several groups

*To whom correspondence should be addressed.
E-mail: acksugi@agr.shizuoka.ac.jp
have reported the effect of choline deficiency on plasma homocysteine concentration. Svardal et al. (12) showed that a choline-deficient diet alone did not affect serum homocysteine concentration in rats. In contrast, Varela-Moreiras et al. (13) showed that choline deprivation in the diet could significantly increase plasma homocysteine concentration in rats. Recently, da Costa et al. (14) showed that choline deprivation alone did not affect plasma homocysteine concentration in mice and humans, although it significantly increased plasma homocysteine concentration when animals were loaded with methionine. Thus, the results of previous studies on the effect of choline deficiency on plasma homocysteine concentration are not consistent. One of the reasons for the discrepancy in results of previous studies appears to be different methionine levels of the diets used, but this remains to be tested experimentally. Furthermore, the mechanism by which dietary choline level affects plasma homocysteine concentration has not yet been fully elucidated, although it can be deduced that dietary choline level might affect homocysteine metabolism through alteration of hepatic betaine concentration.

In this study, we investigated the effects of choline deprivation on plasma homocysteine concentration and other variables related to methionine metabolism in rats fed a standard (25%) casein (25C) diet or standard soybean protein (25S) diet, the 25C diet being relatively high in methionine and the 25S diet being relatively low in methionine. Using the 25S diet, the time-dependent effects of choline deprivation and the comparative effects of three kinds of lipotropes (choline, betaine and methionine) were investigated. In addition, we investigated the effect of choline deprivation on plasma homocysteine concentration in rats fed a low (10%) casein (10C) diet to confirm the relationship between the inductivity of hyperhomocysteinemia and dietary methionine level.

MATERIALS AND METHODS

Chemicals. Choline bitartrate and betaine were purchased from Sigma-Aldrich (St. Louis, MO) and L-methionine was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were purchased from Sigma-Aldrich or Wako and were of analytical grade. Milk casein was purchased from Nacalai Tesque, Inc. (Kyoto, Japan), and soybean protein isolate (SPI) was kindly supplied by Fuji Oil (Izumisano, Japan). The mineral mixture (AIN-93G), vitamin mixture (AIN-93), and cellulose powder were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan), and other ingredients of the diet were purchased from Wako.

Animals and diets. Six-week-old male rats (120–140 g) of the Wistar strain were obtained from Japan SLC, Inc. (Hamamatsu, Japan). They were individually housed in hanging stainless-steel wire cages kept in an isolated room at a controlled temperature (23–25˚C) and humidity (40–60%). Lighting was maintained on a 12-h cycle (lights on from 0700 to 1900 h). Before starting the experiments, all rats were acclimated to the facility for 5 or 6 d and given free access to water and a diet, which was the same as the 25C diet containing choline. Four separate experiments were conducted in this study. In Expt. 1, 25C and 25S diets with or without 0.1% choline were used. The composition of choline-containing control diets was as follows (g/100 g): casein or SPI, 25; corn starch, 43.25; sucrose, 20; corn oil, 5; mineral mixture (AIN-93G), 3.5; vitamin mixture (AIN-93), 1; choline bitartrate, 0.25; and cellulose, 2. Choline-deprived diets were prepared by increasing the content of corn starch. Rats were given free access to the experimental diets for 10 d. In Expt. 2, the 25S diets with or without 0.1% choline were used. Rats were previously given free access to the choline-containing 25S diet for 7 d, and six of them were killed on the final day (day 0) of the prefeeding. The remaining rats were divided into two groups: one group of rats was given free access to the same diet and the other group of rats was given free access to the choline-deprived 25S diet. The two groups of rats were killed after 3, 10 and 20 d. In Expt. 3, the 25S diet without choline was used as a choline-deficient control diet. Choline (0.25%), betaine (0.28%) or L-methionine (0.35%) was added to the control diet at the expense of starch. Rats were given free access to the experimental diets for 10 d. In Expt. 4, 10C diets with or without 0.1% choline were used. The 10C diets contained casein at a level of 10 g/100 g and the composition of other ingredients, except for corn starch, was the same as that of the 25C or 25S diet. Rats were given free access to the experimental diets for 10 d. The methionine contents of the 25C, 25S, and 10C diets were estimated to be approximately 0.63, 0.29, and 0.25%, respectively (15). The experimental plan of this study was approved by the Laboratory Ani-
nal Care Committee of the Faculty of Agriculture, Shizuoka University.

**Tissue collection and fractionation.** Rats were killed by decapitation to obtain blood and livers between 1000 and 1040 h without prior food deprivation. Blood plasma was separated from the heparinized whole blood by centrifugation at 2,000 × g for 15 min at 4˚C and stored at −80˚C until used for determination of concentrations of total homocysteine and cysteine. After the collection of blood, the whole liver was quickly removed, rinsed in ice-cold saline, blotted on filter paper, cut into three portions, weighed, and quickly frozen in liquid nitrogen and stored at −80˚C until used for determination of concentrations of methionine metabolites and enzyme activity. The homogenization of the liver and centrifugation of the homogenate for analyses of the concentrations of methionine metabolites and enzyme activity were conducted essentially according to the method described previously (16). For the determination of hepatic betaine concentration, one portion of the liver was homogenized in 4 volumes (vol/wt) of 0.3 m trichloroacetic acid solution, instead of 0.5 m perchloric acid solution. For the assay of hepatic triglyceride concentration, an aliquot of the liver homogenate was lyophilized, and total lipids were extracted by the method of Folch et al. (17).

**Biochemical analysis.** The concentrations of total homocysteine and cysteine in the plasma and liver were measured by HPLC essentially according to Durand et al. (18). The concentrations of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) in the liver were measured by HPLC essentially according to Cook et al. (19). The concentration of betaine in the liver was measured by HPLC according to Laryea et al. (20). The triglyceride concentration in the extract of liver homogenate was measured enzymatically by using a kit (Triglyceride G-Test, Wako). The activity of cystathionine β-synthase (CBS) in the liver was measured according to Mudd et al. (21) but using HPLC for the assay of the reaction product cystathionine according to Emanson et al. (22). The activity of betaine-homocysteine S-methyltransferase (BHMT) in the liver was measured according to Finkelstein and Mudd (23) but using HPLC for the assay of the reaction product N,N-dimethylglycine according Laryea et al. (20). The protein concentration was measured according to Lowry et al. (24) using bovine serum albumin as a standard.

**Statistical analysis.** Data are expressed as the mean ± SE. Data were analyzed by a one-way or two-way ANOVA, and the difference between mean values was tested by the Tukey test when the F value was significant (Expts. 1 and 3). Student’s t test was used when data of two corresponding groups were analyzed (Expts. 2 and 4). Statistical analysis was performed using the software Mac Toukei-Kaiseki ver 1.5 (Esumi, Tokyo, Japan). A p value of 0.05 or less was considered significant.

**RESULTS**

**Effect of choline deprivation and dietary protein type (Expt. 1)**

Choline deprivation did not affect the growth of rats fed 25C or 25S diets during the 10-d experimental...
period (Table 1). The relative liver weight was slightly increased by choline deprivation in rats fed the 25S diet. Plasma homocysteine concentration was significantly increased by choline deprivation in rats fed the 25S diet to a level 2.68-times higher than the level in the choline-fed control group: i.e., 12.5 (control) vs 33.5 (choline-deprived) μmol/L (Fig. 2A). In contrast, choline deprivation did not affect plasma homocysteine concentration in rats fed the 25C diet: i.e., 12.3 (control) vs 12.1 (choline-deprived) μmol/L. Plasma cysteine concentration, which was measured for comparison with homocysteine, was not affected by choline deprivation (Fig. 2B). Choline deprivation significantly decreased hepatic SAM concentration and SAM : SAH ratio regardless of the kind of diet (Fig. 2C and E). In contrast, choline deprivation significantly increased hepatic concentrations of SAH and homocysteine in rats fed the 25S diet but not in rats fed the 25C diet (Fig. 2D and F). The activity of CBS in the liver was significantly decreased by choline deprivation in rats fed the 25S diet but not in rats fed the 25C diet (Fig. 2D and F). The activity of BHMT in the liver was significantly decreased by choline deprivation in rats fed the 25S diet, whereas hepatic BHMT activity was unaffected by choline deprivation in both rats fed the 25C diet and rats fed the 25S diet (Fig. 3A and B). Choline deprivation significantly decreased hepatic betaine concentration from 3650 to 130 nmol/g in rats fed the 25S diet, although choline deprivation also tended to decrease hepatic betaine concentration from 880 to 435 nmol/g in rats fed the 25C diet (Fig. 3C). Liver triglyceride concentration was significantly increased by choline deprivation in rats fed the 25S diet but not in rats fed the 25C diet (Fig. 3D). Time-dependent effect of choline deprivation (Expt. 2)

Rats were fed the 25S diet with or without choline for up to 20 d to investigate the time-dependent effect of choline deprivation. There was no difference in the body weight gain of rats between the two groups during the 20-d experimental period. Feeding rats with the choline-deprived 25S diet for only 3 d led to a significantly higher plasma homocysteine concentration than that in rats fed the choline-containing 25S diet, and the concentration remained higher up to 20 d (Fig. 4A).
The time-dependent effects of choline deprivation on the concentrations of hepatic SAH and homocysteine were similar to the effect on plasma homocysteine concentration (Fig. 4C and E), whereas hepatic SAM concentration and SAM : SAH ratio were consistently decreased by choline deprivation (Fig. 4B and D). The activity of CBS in the liver was significantly lower in rats fed the choline-deprived 25% diet than in rats fed the choline-containing 25SCD diet on day 3 and day 10, although a significant difference was not detected on day 20 (Fig. 4F).

Effects of betaine and methionine on choline deficiency-induced hyperhomocysteinemia (Expt. 3)

The effects of supplementation with betaine or methionine on plasma homocysteine concentration and related variables were compared with the effect of choline. In this experiment, relatively high levels of choline (0.25%) and corresponding amounts of betaine (0.28%) were used to make the effects of these compounds clear. Methionine was added to the diet at a level of 0.35% to make the methionine content of the diet comparable to that of the 25C diet. All of the data are summarized in Table 2. There was no difference in the body weight gain of rats among the four experimental groups. The relative liver weight was slightly, but significantly, lower in rats fed 25SS diets supplemented with choline, betaine or methionine than in rats fed the choline-deprived control diet. Plasma homocysteine concentration was significantly decreased by dietary supplementation with choline, betaine or methionine, although the effect of methionine was slightly smaller than the effects of choline or betaine. Plasma cysteine concentration was significantly higher in rats fed the betaine- or methionine-supplemented diet than in rats fed the control diet. The SAM concentration and CBS activity in the liver were significantly increased by supplementation with choline, betaine or methionine to the same levels. The hepatic SAH concentration was significantly decreased and SAM : SAH ratio was significantly increased by choline, betaine or methionine, although the effect of methionine was smaller than the effects of the other compounds. The concentration of hepatic homocysteine was significantly decreased by choline, betaine or methionine to the same level.

Effect of choline deprivation in rats fed a low casein diet (Expt. 4)

The effect of choline deprivation on plasma homocysteine concentration and other variables in rats fed a low casein diet (Expt. 4). 1

The effects of supplementation with betaine or methionine on plasma homocysteine concentration and related variables were compared with the effect of choline. In this experiment, relatively high levels of choline (0.25%) and corresponding amounts of betaine (0.28%) were used to make the effects of these compounds clear. Methionine was added to the diet at a level of 0.35% to make the methionine content of the diet comparable to that of the 25C diet. All of the data are summarized in Table 2. There was no difference in the body weight gain of rats among the four experimental groups. The relative liver weight was slightly, but significantly, lower in rats fed 25SS diets supplemented with choline, betaine or methionine than in rats fed the choline-deprived control diet. Plasma homocysteine concentration was significantly decreased by dietary supplementation with choline, betaine or methionine, although the effect of methionine was slightly smaller than the effects of choline or betaine. Plasma cysteine concentration was significantly higher in rats fed the betaine- or methionine-supplemented diet than in rats fed the control diet. The SAM concentration and CBS activity in the liver were significantly increased by supplementation with choline, betaine or methionine to the same levels. The hepatic SAH concentration was significantly decreased and SAM : SAH ratio was significantly increased by choline, betaine or methionine, although the effect of methionine was smaller than the effects of the other compounds. The concentration of hepatic homocysteine was significantly decreased by choline, betaine or methionine to the same level.

Effect of choline deprivation in rats fed a low casein diet (Expt. 4)

The effect of choline deprivation on plasma homocysteine concentration and other variables in rats fed a low casein diet (Expt. 4). 1

Table 2. Effects of dietary supplementation with 0.25% choline, 0.28% betaine or 0.35% l-methionine on plasma homocysteine concentration and other variables in rats fed a choline-deprived soybean protein diet (Expt. 3). 1

<table>
<thead>
<tr>
<th>Diet</th>
<th>25SCD</th>
<th>+Cho</th>
<th>+Bet</th>
<th>+Met</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt. gain, g/10 d</td>
<td>47 ± 2</td>
<td>45 ± 1</td>
<td>49 ± 2</td>
<td>47 ± 1</td>
</tr>
<tr>
<td>Food intake, g/10 d</td>
<td>141 ± 4a</td>
<td>132 ± 4b</td>
<td>137 ± 5b</td>
<td>121 ± 3b</td>
</tr>
<tr>
<td>Liver wt., g/100 g body wt.</td>
<td>4.45 ± 0.04a</td>
<td>4.1 ± 0.06b</td>
<td>4.13 ± 0.05b</td>
<td>4.19 ± 0.06b</td>
</tr>
<tr>
<td>Plasma Homocysteine, μmol/L</td>
<td>33.5 ± 2.5a</td>
<td>7.3 ± 0.2c</td>
<td>7.7 ± 0.2c</td>
<td>13.7 ± 0.6b</td>
</tr>
<tr>
<td>Cysteine, μmol/L</td>
<td>118 ± 6c</td>
<td>132 ± 8bc</td>
<td>177 ± 8a</td>
<td>153 ± 4b</td>
</tr>
<tr>
<td>Liver SAM, nmol/g</td>
<td>30.5 ± 1.2b</td>
<td>70.0 ± 4.2a</td>
<td>76.6 ± 5.1a</td>
<td>85.1 ± 9.8a</td>
</tr>
<tr>
<td>SAH, nmol/g</td>
<td>21.3 ± 0.6a</td>
<td>10.9 ± 0.8c</td>
<td>13.2 ± 0.6c</td>
<td>16.6 ± 0.8b</td>
</tr>
<tr>
<td>SAM : SAH ratio</td>
<td>1.50 ± 0.09b</td>
<td>6.41 ± 0.58a</td>
<td>6.60 ± 0.47a</td>
<td>5.15 ± 0.43a</td>
</tr>
<tr>
<td>Homocysteine, nmol/g</td>
<td>8.3 ± 0.8a</td>
<td>3.4 ± 0.4b</td>
<td>3.7 ± 0.1b</td>
<td>3.3 ± 0.3b</td>
</tr>
<tr>
<td>CBS activity2</td>
<td>2.16 ± 0.09b</td>
<td>3.95 ± 0.12a</td>
<td>4.68 ± 0.36a</td>
<td>4.58 ± 0.22a</td>
</tr>
<tr>
<td>BHMT activity2</td>
<td>1.41 ± 0.09</td>
<td>1.3 ± 0.11</td>
<td>1.43 ± 0.12</td>
<td>0.99 ± 0.09</td>
</tr>
<tr>
<td>Betaine, μmol/g</td>
<td>0.12 ± 0.01c</td>
<td>9.81 ± 0.41b</td>
<td>13.3 ± 0.61a</td>
<td>0.26 ± 0.03b</td>
</tr>
</tbody>
</table>

1 Each value is the mean ± SE. n = 6 or 7. Values without a common superscript letter differ, p < 0.05. 25SCD, choline-deprived 25% soybean protein diet; Bet, betaine. See the legends of Figs. 2 and 3 for other abbreviations.

2 Expressed as nmol/(min·mg protein).

Table 3. Effects of choline deprivation on plasma homocysteine concentration and other variables in rats fed a low casein diet (Expt. 4). 1

<table>
<thead>
<tr>
<th>Diet</th>
<th>10C</th>
<th>10CCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt. gain, g/10 d</td>
<td>32 ± 2</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>Food intake, g/10 d</td>
<td>135 ± 5</td>
<td>153 ± 4</td>
</tr>
<tr>
<td>Liver wt., g/100 g body wt.</td>
<td>4.43 ± 0.08</td>
<td>4.2 ± 0.05*</td>
</tr>
<tr>
<td>Plasma Homocysteine, μmol/L</td>
<td>15.4 ± 0.5</td>
<td>34.3 ± 2.4**</td>
</tr>
<tr>
<td>Cysteine, μmol/L</td>
<td>101 ± 3</td>
<td>105 ± 2</td>
</tr>
<tr>
<td>Liver SAM, nmol/g</td>
<td>72.5 ± 4.5</td>
<td>55.1 ± 1.0**</td>
</tr>
<tr>
<td>SAH, nmol/g</td>
<td>5.7 ± 0.2</td>
<td>16.2 ± 2.0**</td>
</tr>
<tr>
<td>SAM : SAH ratio</td>
<td>13.2 ± 0.8</td>
<td>3.5 ± 0.4**</td>
</tr>
<tr>
<td>Homocysteine, nmol/g</td>
<td>1.5 ± 0.2</td>
<td>2.9 ± 0.2**</td>
</tr>
<tr>
<td>Betaine, μmol/g</td>
<td>2.34 ± 0.11</td>
<td>0.18 ± 0.01**</td>
</tr>
<tr>
<td>Triglyceride, μmol/g</td>
<td>20.1 ± 2.1</td>
<td>55.1 ± 4.6**</td>
</tr>
</tbody>
</table>

1 Each value is the mean ± SE. n = 6. 10C, 10% casein diet; 10CCD, choline-deprived 10C diet. See the legends of Figs. 2 and 3 for other abbreviations. *p < 0.05, **p < 0.01.
hcy concentration was investigated using rats fed a low casein diet to confirm the view that choline deprivation might induce hyperhomocysteinemia when rats are fed low methionine diets, irrespective of dietary protein source. Choline deprivation did not affect the body weight gain and significantly increased the food intake (Table 3). The relative liver weight was slightly decreased by choline deprivation. Plasma homocysteine concentration was significantly increased by choline deprivation to a level 2.23-times higher than the level in the choline-fed control group. Plasma cysteine concentration was unaffected by choline deprivation. Choline deprivation significantly decreased hepatic SAM concentration and SAM:SAH ratio and increased hepatic SAH and homocysteine concentrations. Choline deprivation significantly decreased hepatic betaine concentration and increased triglyceride concentration.

**DISCUSSION**

An important finding of this study is that choline deprivation led to hyperhomocysteinemia when rats were fed the 25S diet or 10C diet, but not the 25C diet, where the methionine contents of the 25S and 10C diets were lower than the methionine content of the 25C diet. These results suggest that differences in the methionine content of the diets used in previous studies (12–14) might explain the inconsistent results related to the effects of choline deprivation on plasma homocysteine concentration. For example, Svardal et al. (12) and da Costa et al. (14) used choline-deficient diets containing casein at a level of 20% with methionine content estimated to be approximately 0.52%, whereas the diet used by Varela-Moreiras et al. (13) contained methionine at a level of 0.2%. It is reasonable to consider that choline deprivation does not cause choline deficiency under the condition of higher levels of dietary methionine, since choline status within the body is determined not only by choline intake but also by methionine intake (25). Furthermore, it is confirmed that de novo synthesis of the choline moiety of PC can be made by phosphatidylethanolamine (PE) N-methylation using SAM derived from methionine and the PE N-methylation reaction is regulated by the hepatic SAM concentration, which reflects methionine intake and homocysteine remethylation, or SAM:SAH ratio rather than the enzyme mass of PE N-methyltransferase (26, 27). This indicates that diets containing higher levels of methionine resist choline deficiency. Consistent with this, the present study showed that choline deprivation of the 25C diet did not result in fatty liver, one of the symptoms of choline-deficiency. Thus, our results support the view that the combination of choline deprivation and low methionine diet brought about obvious choline deficiency and thereby induced hyperhomocysteinemia.

The results of the present study showing that hyperhomocysteinemia induced by choline deprivation and low methionine diet paralleled increases in hepatic SAH and homocysteine concentrations suggest that suppressed removal of homocysteine is associated with the hyperhomocysteinemia. Hence, there are at least two possible mechanisms for choline deficiency-induced hyperhomocysteinemia: suppressed remethylation of homocysteine and decreased formation of cystathionine. It is reasonable to assume that choline deficiency decreases betaine-dependent homocysteine remethylation that is catalyzed by BHMT. It has been shown that the activity of BHMT was influenced by some dietary conditions, e.g., dietary levels of choline, betaine and methionine (28, 29). In addition to the enzyme activity, the availability of betaine is also thought to affect the remethylation reaction catalyzed by BHMT. In the present study, hepatic BHMT activity was unaffected by choline deprivation (Fig. 3B). In contrast, hepatic betaine concentration was markedly decreased by choline deprivation to the level of 120–130 nmol/g in rats fed the 25S diet or to the level of 180 nmol/g in rats fed the 10C diet, while betaine concentration was still maintained at a relatively high level, 435 nmol/g, in rats fed the choline-deprived 25C diet. The response of hepatic betaine concentration to choline deprivation is essentially consistent with the results of previous studies (30–32). Lee et al. (33) reported that the Km value for betaine was 120 μM in rat liver purified BHMT, although a lower Km value (48 μM) was obtained by Finkelstein et al. (34) in an earlier study. When the data obtained by Lee et al. (33) are taken, the decreased betaine concentrations in rats fed the choline-deprived 25S diet appear to be lower than the saturable betaine concentration for BHMT, e.g., two-fold the Km value or more. Thus, it is likely that choline deprivation decreases betaine-dependent remethylation of homocysteine through a decrease in hepatic betaine concentration rather than alteration of hepatic BHMT activity, at least in rats fed the 25S diet.

It has been shown that the activity of CBS was affected by SAM concentration (35), although the enzyme activity was also regulated through gene expression in response to dietary protein level or cysteine supplementation (36, 37). In the present study, choline deprivation significantly decreased both hepatic SAM concentration and CBS activity in rats fed the 25S diet, while the enzyme activity was not affected by choline deprivation in rats fed the 25C diet. Therefore, it appears that decreased CBS activity may also favor the induction of hyperhomocysteinemia by choline deficiency in rats fed the 25S diet. On the other hand, it has been shown that choline deficiency caused secondary folate deficiency (38) and vice versa (39). Hence, the possibility that the decreased remethylation of homocysteine due to secondary folate deficiency is also involved in choline deficiency-induced hyperhomocysteinemia cannot be excluded.

As well as choline, betaine and methionine are lipotropic factors that stimulate PC synthesis and prevent the development of fatty liver (8, 27). In addition, a number of studies have shown that betaine administration was effective in decreasing plasma homocysteine concentration (6, 40). Therefore, we compared the effects of betaine and methionine with the effect of cho-
line on plasma homocysteine concentration and related variables in rats fed the choline-deprived 25S diet. The results clearly showed that choline deprivation-induced hyperhomocysteinemia could be effectively suppressed by three kinds of lipotrope, although the suppressive effect of methionine was slightly smaller than that of choline or betaine. Higher levels of dietary methionine might increase plasma homocysteine concentration, since methionine is the sole precursor of homocysteine. In contrast, the results of the present study showed that methionine could reduce plasma homocysteine concentration when added to the choline-deficient low-methionine diet at a relatively low level. Unlike our results, da Costa et al. (14) showed that a one-shot oral dose of methionine (100 mg/kg body wt) significantly increased plasma homocysteine concentration 2 h after methionine loading in mice and 4 h after methionine loading in humans fed choline-deficient diets. One of the reasons for discrepancy in the effect of methionine between the results by da Costa et al. (14) and the results of the present study might be the different administration method of methionine, one-shot administration vs dietary supplementation, in addition to the different methionine level of the diet. At any rate, our results demonstrate that methionine has two opposing properties, a hyperhomocysteinemic effect due to a precursor of homocysteine and a hypohomocysteinemic effect due to a lipotropic factor, with respect to plasma homocysteine concentration. The latter effect of methionine explains why deprivation of choline from the 25C diet did not increase plasma homocysteine concentration. The counteracting effects of lipotropes, including methionine, on hepatic concentrations of methionine metabolites and CBS activity suggest that homocysteine removal by both remethylation and cystathionine formation was stimulated by the lipotropes. This in turn supports the assumption described above that decreases in both remethylation and cystathionine formation might be associated with choline deficiency-induced hyperhomocysteinemia.

Several treatments for inducing experimental hyperhomocysteinemia have so far been reported, e.g., feeding diets supplemented with a homocysteine precursor such as methionine (16, 41) or homocyst(e)ine (41, 42), feeding diets supplemented with a methyl-group acceptor such as guanidinoacetic acid (43, 44) or nicotinic acid (45), and feeding vitamin-deficient diets (46, 47). In addition to these treatments, we propose that choline-deprived low methionine diets might be useful to induce experimental hyperhomocysteinemia, which is characterized as betaine deficiency-associated hyperhomocysteinemia. Another merit of this model is that hyperhomocysteinemia can be evoked by feeding the diet for a short period, e.g., only 3 d, as shown in Expt. 2.

Acknowledgments

This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Technology of Japan.

REFERENCES


