Methionine and Serine Synergistically Suppress Hyperhomocysteinemia Induced by Choline Deficiency, but Not by Guanidinoacetic Acid, in Rats Fed a Low Casein Diet

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The effects of dietary supplementation with 0.5% methionine, 2.5% serine, or both on hyperhomocysteinemia induced by deprivation of dietary choline or by dietary addition of 0.5% guanidinoacetic acid (GAA) were investigated in rats fed a 10% casein diet. Hyperhomocysteinemia induced by choline deprivation was not suppressed by methionine alone and was only partially suppressed by serine alone, whereas it was completely suppressed by a combination of methionine and serine, suggesting a synergistic effect of methionine and serine. Fatty liver was also completely prevented by the combination of methionine and serine. Compared with methionine alone, the combination of methionine and serine decreased hepatic S-adenosylhomocysteine and homocysteine concentrations and increased hepatic betaine and serine concentrations and betaine-homocysteine S-methyltransferase activity. GAA-induced hyperhomocysteinemia was partially suppressed by methionine alone, but no interacting effect of methionine and serine was detected. In contrast, GAA-induced fatty liver was completely prevented by the combination of methionine and serine. These results indicate that a combination of methionine and serine is effective in suppressing both hyperhomocysteinemia and fatty liver induced by choline deprivation, and that methionine alone is effective in suppressing GAA-induced hyperhomocysteinemia partially.

Key words: methionine; serine; plasma homocysteine; choline deficiency; guanidinoacetic acid

Methionine prevents fatty liver due to phosphatidylcholine (PC) deficiency by stimulating PC synthesis via the phosphatidyethanolamine (PE) N-methylation pathway.1-3 In fact, choline deprivation does not cause fatty liver due to PC deficiency when diets contain relatively high levels of methionine.3 Homocysteine is a metabolite in the metabolism of methionine (Fig. 1).4 It is widely recognized that an elevated plasma homocysteine concentration is an independent risk factor for cardiovascular disease.5-7 Previously we found that deprivation of dietary choline caused hyperhomocysteinemia as well as fatty liver in rats fed a low (10%) casein diet (10C) or a moderate (25%) soybean protein diet (25S), but not in rats fed a moderate (25%) casein diet (25C).8 The resistivity of 25C against choline deprivation-induced hyperhomocysteinemia might be due to the higher methionine level of the diet, since a methionine content of 25C was higher than that of 10C or 25S. Choline deprivation-induced hyperhomocysteinemia is primarily attributable to a deficiency of betaine, a methyl-group donor for the re-methylation of homocysteine. We also found that hyperhomocysteinemia induced by choline deprivation in rats fed 25S was effectively suppressed by dietary supplementation with methionine at a level of 0.35%.9 On the other hand, dietary supplementation with methionine increased the plasma homocysteine concentration in a dose-dependent manner in rats fed 25C.9 Hence it is reasonable to assume that methionine has two opposing effects on the plasma homocysteine concentration, i.e., hypohomocysteinemic and hyperhomocysteinemic effects.

Methionine supplementation of choline-deprived 10C at a level of 0.5% to make the methionine level comparable to that of a 30% casein diet (30C) did not suppress hyperhomocysteinemia in rats, while choline-deprived 30C did not enhance the plasma homocysteine concentration (unpublished observation). This unexpected finding suggests that choline and methionine are not equivalent in preventing hyperhomocysteinemia associated with choline deficiency. Serine and its precursor glycine had suppressive effects on hyperhomocysteinemia induced by methionine supplementation in rats fed 25C, probably through stimulation of cystathionine formation.9 This interacting effect of methionine and serine or glycine might have nutritional significance in the metabolism of homocysteine. An elevated plasma homocysteine concentration due to supplementation at 10C with methionine at a level of 0.5% was significantly suppressed by concurrent supplementation with glycine and serine at levels of 0.32% and 0.94% respectively, to make these amino acid levels comparable to those of 30C, but little information is available about such an interaction effect, especially under conditions of choline deficiency. It was found that guanidinoacetic acid (GAA) caused hyperhomocysteinemia when added to...
The other ingredients of the diet were purchased from Wako, Casein, a mineral mixture (AIN-93G), a vitamin mixture (AIN-93), (Osaka, Japan) or Sigma-Aldrich, and were of analytical grade.

Materials and Methods

Choline bitartrate and GAA were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals, including l-methionine and l-serine, were purchased from Wako Pure Chemical Industries, (Osaka, Japan) or Sigma-Aldrich, and were of analytical grade. Casein, a mineral mixture (AIN-93G), a vitamin mixture (AIN-93), and cellulose powder were purchased from Oriental Yeast (Tokyo). The other ingredients of the diet were purchased from Wako.

Six-week-old male rats (120–140 g) of the Wistar strain were obtained from Japan SLC (Hamamatsu, Japan). They were housed individually in hanging stainless-steel wire cages in an isolated room obtained from Japan SLC (Hamamatsu, Japan). They were housed...
Fed the Experimental Diets (Experiments 1 and 2) to the levels in the rats fed 10C. Choline deprivation decreased hepatic betaine concentration significantly from 2.64 ± 0.08 (10C group) to 0.32 ± 0.02 μmol/g (10CCD group), together with hepatic BHMT and CBS activities (Fig. 4, panels A–D). Supplementation with methionine alone restored these enzyme activities to the levels in the rats fed 10C, but did not increase the hepatic betaine concentration. Supplementation with serine alone did not affect hepatic BHMT activity, whereas it restored hepatic CBS activity. Supplementation with methionine in combination with serine increased hepatic BHMT activity significantly to a level higher than that in the rats fed 10C and slightly but significantly increased the hepatic betaine concentration. The hepatic serine concentration was markedly decreased by supplementation with methionine alone, but was markedly increased by supplementation with serine alone, and remained at the same level to those in the rats fed 10C and 10CCD under supplementation with methionine in combination with serine (Fig. 4, panel D). Choline deprivation significantly increased the hepatic triglyceride concentration and white tissue was visible, indicating the development of fatty liver (Fig. 4, panel E). The increase in the hepatic triglyceride concentration was partially suppressed by supplementation with methionine alone, but was unaffected by supplementation with serine alone. In contrast, supplementation with methionine in combination with serine not only completely suppressed the increase in hepatic triglyceride concentration induced by choline deprivation but also significantly decreased hepatic triglyceride concentration to a level lower than that in the rats fed 10C.

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Table 1. Body Weight Gain, Food Intake, and Liver Weights of Rats Fed the Experimental Diets (Experiments 1 and 2)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Body weight gain</th>
<th>Food intake</th>
<th>Liver weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/10 d</td>
<td>% of body weight</td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10C</td>
<td>29.1 ± 2.2ª</td>
<td>151 ± 3ª</td>
<td>4.29 ± 0.06ª</td>
</tr>
<tr>
<td>10CCD</td>
<td>28.8 ± 2.1ª</td>
<td>152 ± 3ª</td>
<td>4.12 ± 0.06ª</td>
</tr>
<tr>
<td>10CCD + 0.5% l-Met</td>
<td>38.9 ± 1.0ªb</td>
<td>136 ± 3ªb</td>
<td>4.80 ± 0.06ª</td>
</tr>
<tr>
<td>10CCD + 2.5% l-Ser</td>
<td>29.6 ± 3.7ª</td>
<td>147 ± 5ª</td>
<td>4.17 ± 0.05ª</td>
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<tr>
<td>10CCD + 0.5% l-Met + 2.5% l-Ser</td>
<td>42.4 ± 2.5ª</td>
<td>131 ± 4ª</td>
<td>4.98 ± 0.11ª</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10C</td>
<td>31.9 ± 2.2ª</td>
<td>153 ± 3ª</td>
<td>3.86 ± 0.02ª</td>
</tr>
<tr>
<td>10CG</td>
<td>24.3 ± 1.2ª</td>
<td>143 ± 4ª</td>
<td>3.94 ± 0.03ª</td>
</tr>
<tr>
<td>10CG + 0.5% l-Met</td>
<td>47.5 ± 2.1ª</td>
<td>138 ± 2ª</td>
<td>4.46 ± 0.04ª</td>
</tr>
<tr>
<td>10CG + 2.5% l-Ser</td>
<td>31.2 ± 2.4ª</td>
<td>149 ± 2ªb</td>
<td>3.88 ± 0.02ª</td>
</tr>
<tr>
<td>10CG + 0.5% l-Met + 2.5% l-Ser</td>
<td>41.4 ± 1.7ª</td>
<td>126 ± 3ª</td>
<td>4.47 ± 0.03ª</td>
</tr>
</tbody>
</table>

*Values are mean ± SEM, n = 7. Values with different letters are significantly different at p < 0.05. 10C, 10% casein diet; 10CCD, choline-free 10C; 10CG, 10C + 0.5% guanidinoacetic acid.

Fig. 2. Plasma Homocysteine (A) and Cysteine (B) Concentrations in Rats Fed the Experimental Diets (Experiment 1).

Values are mean ± SEM, n = 7. Values in a panel without a common letter differ at p < 0.05. 10C, 10% casein diet; 10CCD, choline-deprived 10C; Cys, cysteine; Hcy, homocysteine; Met, methionine; Ser, serine. Experimental groups: 1, 10C; 2, 10CCD; 3, 10CCD + 0.5% Met; 4, 10CCD + 2.5% Ser; 5, 10CCD + 0.5% Met + 2.5% Ser.

decreased the hepatic betaine concentration significantly from 2.64 ± 0.08 (10C group) to 0.32 ± 0.02 μmol/g (10CCD group), together with hepatic BHMT and CBS activities (Fig. 4, panels A–D). Supplementation with methionine alone restored these enzyme activities to the levels in the rats fed 10C, but did not increase the hepatic betaine concentration. Supplementation with serine alone did not affect hepatic BHMT activity, whereas it restored hepatic CBS activity. Supplementation with methionine in combination with serine increased hepatic BHMT activity significantly to a level higher than that in the rats fed 10C and slightly but significantly increased the hepatic betaine concentration. The hepatic serine concentration was markedly decreased by supplementation with methionine alone, but was markedly increased by supplementation with serine alone, and remained at the same level to those in the rats fed 10C and 10CCD under supplementation with methionine in combination with serine (Fig. 4, panel D). Choline deprivation significantly increased the hepatic triglyceride concentration and white tissue was visible, indicating the development of fatty liver (Fig. 4, panel E). The increase in the hepatic triglyceride concentration was partially suppressed by supplementation with methionine alone, but was unaffected by supplementation with serine alone. In contrast, supplementation with methionine in combination with serine not only completely suppressed the increase in hepatic triglyceride concentration induced by choline deprivation but also significantly decreased hepatic triglyceride concentration to a level lower than that in the rats fed 10C.

Fig. 3. Hepatic Concentrations of S-Adenosylmethionine (A), S-Adenosylhomocysteine (B), Their Ratio (C), and Homocysteine (D) in the Rats Fed the Experimental Diets (Experiment 1).

Values are mean ± SEM, n = 7. Values in a panel without a common letter differ at p < 0.05. SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine. See the legend to Fig. 2 for further abbreviations.
Effect on guanidinoacetic acid-induced hyperhomocysteinemia (experiment 2)

The addition of GAA and supplementation with serine alone did not affect body weight gain, food intake, or relative liver weight (Table 1). Supplementation with methionine alone or in combination with serine significantly increased body weight gain and relative liver weight, whereas it significantly decreased or tended to decrease food intake. The addition of GAA increased the plasma homocysteine concentration markedly from $16.0 \pm 0.2$ (10C group) to $81.2 \pm 0.6 \mu\text{mol/L}$ (10CG group) (Fig. 5, panel A). GAA-induced hyperhomocysteinemia was significantly suppressed by supplementation with methionine alone or in combination with serine, but there was no difference between the effect of methionine alone and that of the combination of methionine and serine. Although supplementation with serine alone also significantly decreased the plasma homocysteine concentration, the effect was limited. The plasma cysteine concentration was significantly higher in the rats fed diets supplemented with methionine irrespective of simultaneous serine supplementation than in those fed the other diets (Fig. 5, panel B). The addition of GAA significantly decreased the hepatic SAM concentration and the SAM/SAH ratio and, conversely, increased hepatic SAH and homocysteine concentrations (Fig. 6, panels A–D). Supplementation with methionine alone and in combination with serine partially prevented the effects of GAA on the hepatic concentrations of methionine metabolites. In contrast, supplementation with serine alone did not affect these

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**Fig. 4.** Activities of Betaine-Homocysteine S-Methyltransferase (A) and Cystathionine $\beta$-Synthase (C), and the Concentrations of Betaine (B), Serine (D), and Triacylglyceride (E) in the Livers of Rats Fed the Experimental Diets (Experiment 1). Values are mean ± SEM, $n=8$. Values in a panel without a common letter differ at $p<0.05$. BHMT, betaine-homocysteine S-methyltransferase; CBS, cystathionine $\beta$-synthase. See the legend to Fig. 2 for further abbreviations.

**Fig. 5.** Plasma Homocysteine (A) and Cysteine (B) Concentrations in the Rats Fed the Experimental Diets (Experiment 2). Values are mean ± SEM, $n=7$. Values in a panel without a common letter differ at $p<0.05$. 10C, 10% casein diet; 10CG, 10C + 0.5% guanidinoacetic acid. See the legend to Fig. 2 for further abbreviations. Experimental groups: 1, 10C; 2, 10CG; 3, 10CG + 0.5% Met; 4, 10CG + 2.5% Ser; 5, 10CG + 0.5% Met + 2.5% Ser.

**Fig. 6.** Hepatic Concentrations of $S$-Adenosylmethionine (A), $S$-Adenosylhomocysteine (B), Their Ratio (C), and Homocysteine (D) in the Rats Fed the Experimental Diets (Experiment 2). Values are mean ± SEM, $n=7$. Values in a panel without a common letter differ at $p<0.05$. See the legends to Figs. 2 and 5 for abbreviations.
and this increase was completely prevented by supplementation with methionine in combination with serine, but not by supplementation with methionine alone or serine alone (Fig. 7, panel E).

**Discussion**

A choline of deprivation-induced hyperhomocysteinemia model

The choline deprivation characteristic of low-methionine diets induces hyperhomocysteinemia mainly due to betaine deficiency in the liver. The dietary methionine level affects choline status within the body, since methionine stimulates the synthesis of the choline moiety of PC via the PE N-methylation pathway.

This appears also to be the case for betaine status, because choline is metabolized to betaine. It is thought that the hepatic SAM concentration, which reflects the dietary methionine level, is critical in the PE N-methylation reaction. Hence it is expected that dietary methionine supplementation increases betaine supply in the liver and thereby suppresses the hyperhomocysteinemia associated with betaine deficiency, but the present study indicates that supplementation of choline-deprived 10C with methionine alone at a level of 0.5% did not have any suppressive effect on hyperhomocysteinemia. The methionine content of 10CCD + 0.5% methionine was comparable to that of 30C. In contrast to 10CCD + 0.5% methionine, choline-deprived 30C did not enhance plasma homocysteine concentration or cause the development of fatty liver (unpublished observation). These results suggest that the resistance of rats fed 30C to choline deprivation cannot be attributed solely to the higher methionine content of the diet. A major finding of experiment 1 was that supplementation with methionine in combination with serine completely suppressed hyperhomocysteinemia, although supplementation with serine alone also had a partial effect. This indicates that methionine and serine synergistically suppressed hyperhomocysteinemia.

Methionine has two opposing effects on the plasma homocysteine concentration. The hyperhomocysteinemic effect of methionine, usually observed when the rats were fed methionine-supplemented diets, appears to be due to increased homocysteine production, since methionine is the sole precursor of homocysteine. The hypohomocysteinemic effect of methionine, which was observed when the rats were fed choline-deprived 25S, appears to be due to an increased supply of betaine. On the other hand, the interacting or synergistic effect of methionine and serine on hyperhomocysteinemia might be explained by stimulation of homocysteine removal, mainly through increased cystathionine synthesis. The reaction of cystathionine synthesis is thought to be a critical step in the metabolism of homocysteine when dietary methionine levels are relatively high. Under such conditions, cystathionine synthesis appears to depend on serine supply rather than CBS activity. This assumption is confirmed by the fact that hyperhomocysteinemia caused by methionine supplementation was effectively suppressed by concurrent supplementation with serine or glycine without any increase in CBS activity. Furthermore, it has been found that methionine supplementation significantly...

![Fig. 7. Activities of Betaine-Homocysteine S-Methyltransferase (A) and Cystathionine β-Synthase (C), and the Concentrations of Betaine (B), Serine (D), and Triglyceride (E) in the Livers of Rats Fed the Experimental Diets (Experiment 2). Values are mean ± SEM, n = 7. Values in a panel without a common letter differ at p < 0.05. See the legends to Figs. 4 and 5 for abbreviations.](image-url)
decreases the hepatic serine concentration, suggesting that increases in serine consumption induced by methionine supplementation cannot be fully compensated for by serine synthesis within the body unless serine is provided exogenously. Also, in the present study supplementation of 10CCD with methionine alone decreased the hepatic serine concentration from $2.68 \pm 0.07$ to $0.31 \pm 0.02 \mu mol/g$, and this decrease was restored by concurrent supplementation with serine to $2.30 \pm 0.22 \mu mol/g$. Considering the reported $K_m$ value for serine in rat CBS, about $0.7 \text{mM}$, cystathionine synthesis might be reduced in rats fed 10CCD + 0.5% methionine and recovered in rats fed 10CCD + 0.5% methionine + 2.5% serine. Thus it is likely that one of the roles of supplementation with serine in combination with methionine is to stimulate cystathionine synthesis by supplying serine as a substrate for cystathionine synthesis, but not by increasing CBS activity, leading to cancellation of the plasma homocysteine-elevating effect of methionine. The results of the present study confirm that the resistance of the diet to choline deficiency is influenced not only by the dietary methionine level but also by dietary levels of other amino acids such as serine and glycine.

It should be noted that supplementation of 10CCD with methionine alone tended to increase the hepatic SAH concentration, whereas supplementation with methionine in combination with serine decreased the hepatic SAH concentration to the level of the rats fed 10C. The hepatic SAH concentration reflects the hepatic homocysteine concentration, since the reaction catalyzed by SAH hydrolase is reversible and favors the synthesis of SAH. The hepatic homocysteine concentration reflects homocysteine removal. Thus it is plausible to assume that the combination of methionine and serine affects not only the hepatic homocysteine concentration, but also the hepatic SAH concentration. This is important in considering PC synthesis via PE N-methylation, since SAH is known to be an inhibitor of various types of methyltransferase, including PE N-methyltransferase. It has been reported that the second and third methylation of PE were inhibited by SAH, with apparent $K_i$ values of 4.9 and 6.7 M, respectively, when assayed using partially purified rat liver enzyme. Although these in vitro kinetic data cannot be applied directly to the explanation of the in vivo phenomenon, it is possible that hepatic PC synthesis via PE N-methylation is depressed by supplementation with methionine alone through an increased SAH concentration, and is depressed by supplementation with a combination of methionine and serine through a suppressed SAH concentration. Hence we postulate that an increase in PC synthesis due to supplementation with methionine in combination with serine also contributes to suppression of hyperhomocysteinemia induced by choline deprivation.

It has been reported that the $K_m$ value for betaine was 120 M in rat liver purified BHMT and 48 M in rat liver semipurified BHMT. If these $K_m$ values are applicable to in vivo BHMT, hepatic BHMT might have been saturated with betaine even in the rats fed 10CCD, in which the hepatic betaine concentration was $0.32 \pm 0.02 \mu mol/g$, and the BHMT reaction might not have been enhanced by supplementation with methionine in combination with serine even though the betaine concentration was increased to $0.56 \pm 0.03 \mu mol/g$. The BHMT reaction is influenced both by BHMT activity and by the concentration of its substrate betaine. It has been found that hepatic BHMT activity is increased by dietary levels of substrate betaine and related compounds such as choline and methionine. This is in contrast to the case of CBS. Furthermore, it must be determined whether the hepatic betaine concentration is the cause, merely the result, or both of the BHMT reaction. The hepatic betaine concentration probably reflects both choline status within the body, which is determined by choline intake and PC synthesis via PE N-methylation, and the consumption of betaine by the BHMT reaction. Taking into consideration hepatic BHMT activity and the hepatic betaine concentration, it is apparent that supplementation of 10CCD with methionine in combination with serine strengthened the BHMT reaction.

The hepatic triglyceride concentration is one of the indices of PC status in the liver, since active synthesis of PC is required for the synthesis and secretion of very low density lipoprotein. Another major finding of experiment 1 is that fatty liver caused by choline deprivation was not fully suppressed by supplementation with methionine alone, but was completely suppressed by the combination of methionine and serine. A possible explanation of the different effects of methionine alone and the combination of methionine and serine is that PC synthesis via the PE N-methylation pathway in the rats fed 10CCD + 0.5% methionine was not fully stimulated because of the higher hepatic concentration of SAH. In contrast, it appears that supplemental serine in combination with methionine stimulates PC synthesis through suppression of the hepatic SAH concentration due to increased removal of homocysteine, and thereby prevents fatty liver.

**Model of GAA addition-induced hyperhomocysteinemia**

GAA is synthesized in the kidneys and metabolized to creatine with SAM as methyl-group donor, which is catalyzed by GAA N-methyltransferase in the liver. Stead et al. first reported that dietary addition of GAA increased the plasma homocysteine concentration in rats, and they postulated that GAA increases the plasma homocysteine concentration by accelerating the conversion of SAM to SAH and further to homocysteine due to compulsive metabolism of GAA. In support of this, we found that dietary addition of GAA significantly decreased the hepatic SAM concentration and increased the hepatic SAH and homocysteine concentrations in rats. In addition, we postulate that betaine deficiency might also contribute to GAA-induced hyperhomocysteinemia, based on the finding that dietary addition of GAA significantly decreases the hepatic betaine concentration. It has been estimated that a major portion (about 75%) of the methyl group of SAM is consumed to synthesize creatine from GAA in humans. Hence the GAA-induced hyperhomocysteinemia model appears to have physiological relevance. The present study indicates that hyperhomocysteinemia can be suppressed by supplementation with methionine alone, although the effect was limited. No additive or synergistic effect of methionine and serine on plasma homocysteine concentration was detected. This is in contrast to the case of the
model used in experiment 1. Consistently with our previous study, diet addition of GAA markedly decreased the hepatic betaine concentration, indicating that one of the mechanisms by which GAA induced hyperhomocysteinemia was a decrease in the hepatic betaine concentration even when the diet contained choline at a level of 0.1%. There are several possible mechanisms for the GAA-induced decrease in hepatic betaine concentration: (i) increased consumption of betaine due to acceleration of the methionine cycle by GAA loading, (ii) decreased synthesis of PC via the PE N-methylation pathway due to a decrease in the hepatic SAM concentration, inhibition by an increased SAH concentration, or both, and (iii) decreased synthesis of PC via the PE N-methylation pathway due to competition between PE N-methyltransferase and GAA N-methyltransferase for SAM. It appears that methionine supplementation partially restored PC synthesis through the latter two mechanisms, since the action of methionine supplementation in significantly increasing the hepatic SAM concentration and, conversely, decreasing the hepatic SAH concentration is favorable to restoration of decreased PC synthesis via the PE N-methylation pathway. The reason for the lack of an additive or synergistic effect of methionine and serine on plasma homocysteine concentration is currently unknown, but one possible reason is that the accelerated conversion of SAM to SAH by GAA, which enhances homocysteine production, has a greater effect on the plasma homocysteine concentration than does the increased conversion of homocysteine to cystathionine by serine. Previously, we found that GAA-induced hyperhomocysteinemia could be suppressed by raising the dietary casein level. The present study indicates the possibility that a higher methionine level contributes to the suppression of GAA-induced hyperhomocysteinemia by a high casein diet, although other mechanisms, e.g., increases in homocysteine-metabolizing enzymes, cannot be ignored.

The present study indicates that dietary addition of GAA caused fatty liver, suggesting that GAA addition brought about PC deficiency in the liver. It also indicates that GAA-induced fatty liver was completely prevented by supplementation with methionine in combination with serine, while supplementation with methionine alone did not suppress the development of fatty liver. It is difficult, however, to explain why the development of fatty liver was suppressed by the combination of methionine and serine but not by methionine alone, since some hepatic variables that affect PE N-methylation, such as SAM and SAH concentrations and the SAM/SAH ratio, did not differ between the rats fed the diet supplemented with methionine alone and the rats fed the diet supplemented with methionine in combination with serine.

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