Nutrition and Cancer

Dietary Folate and Selenium Affect Dimethylhydrazine-Induced Aberrant Crypt Formation, Global DNA Methylation and One-Carbon Metabolism in Rats

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ABSTRACT Several observations suggest a role for DNA methylation in cancer pathogenesis. Although both selenium and folate deficiency have been shown to cause global DNA hypomethylation and increased cancer susceptibility, the nutrients have different effects on one-carbon metabolism. Thus, the purpose of this study was to investigate the interactive effects of dietary selenium and folate. Weanling, Fischer-344 rats (n = 23/diet) were fed diets containing 0 or 2.0 mg selenium (as selenite)/kg and 0 or 2.0 mg folate/kg in a 2 × 2 factorial design. After 3 and 4 wk of a 12-wk experiment, 19 rats/diet were injected intraperitoneally with dimethylhydrazine (DMH, 25 mg/kg) and 4 rats/diet were administered saline. Selenium deficiency decreased (P < 0.05) colonic DNA methylation and the activities of liver DNA methyltransferase and betaine homocysteine methyltransferase and increased plasma glutathione concentrations. Folate deficiency increased (P < 0.05) the number of aberrant crypts per aberrant crypt foci, the concentration of colonic S-adenosylhomocysteine and the activity of liver cystathionine synthase. Selenium and folate interacted (P < 0.0001) to influence one-carbon metabolism and cancer susceptibility such that the number of aberrant crypts and the concentrations of plasma homocysteine and liver S-adenosylhomocysteine were the highest and the concentrations of plasma folate and liver S-adenosylmethionine and the activity of liver methionine synthase were the lowest in rats fed folate-deficient diets and supplemental selenium. These results suggest that selenium deprivation ameliorates some of the effects of folate deficiency, probably by shunting the buildup of homocysteine (as a result of folate deficiency) to glutathione. J. Nutr. 133: 2907–2914, 2003.

KEY WORDS: • selenium • folate • rats • DNA methylation • one-carbon metabolism

Colorectal cancer is the fourth most common type of cancer and the second most common cause of cancer deaths in the United States (1). Dietary factors are thought to play prominent roles in the causation of colorectal cancer. Several epidemiologic studies have suggested an inverse association between dietary folate and the risk for colorectal cancer (2–8). For example, Giovannucci et al. (2) showed that prolonged use of supplements containing folic acid significantly reduced the risk of developing colorectal cancer in the 89,000 participants in the Nurse’s Health Study. In animal studies, a folate-deficient diet increased the incidence of aberrant crypt foci and colonic adenomas, but only if the deficient diet was begun before the initiation/promotion phases of carcinogenesis (9–11). Aberrant crypt foci (ACF)3 are putative preneoplastic lesions that have been detected in human colon resections and in experimental animals treated with chemical carcinogens (12,13). Studies in humans have shown that colonic ACF are precursor lesions from which adenomas and adenocarcinomas will develop (14,15).

The main biochemical role of folate is the transfer of one-carbon moieties (16). Folate functions in DNA synthesis and repair, and in methylation, by providing metylene and formyl groups for the synthesis of thymidine and purines and methyl groups for the synthesis of S-adenosylmethionine (SAM). SAM is the methyl donor for DNA methylation reactions. DNA methylation is an important epigenetic mechanism exerting control on gene expression (17,18). In eukaryotic cells, only small regions of the genome containing repeat CpG residues (CpG islands) are methylated. These CpG islands are localized predominantly in the promoter regions of genes, and methylation is thought to control gene transcription (18). Several observations implicate a role for DNA methylation in cancer pathogenesis. Abnormal methylation patterns have been detected early in the development of cancer, including colorectal cancer (19–21). These changes consist mainly of global hypomethylation, regional DNA hypermethylation and overexpression of DNA methyltransferase I (22). Folate deficiency can cause hypomethylation within a highly conserved region of the p53 tumor suppressor gene,
where the majority of the genetic alterations implicated in the development of several neoplasms have occurred (21, 23, 24).

The nutrient selenium also affects colon cancer susceptibility and DNA methylation. Rats fed selenium-deficient diets had significantly hypomethylated liver and colon DNA compared with rats fed diets supplemented with selenite or selenomethionine (25). Selenium-deficient rats also formed more carcinogen-induced aberrant crypts (26, 27). Thus, alterations in DNA methylation may help explain the increased tumorigenesis associated with selenium deficiency.

Although both selenium and folate deficiency result in global DNA hypomethylation and increased cancer susceptibility, these nutrients influence one-carbon metabolism differently. Folate deficiency increases plasma homocysteine concentrations which decrease the nutritional value of dietary selenium and folate on aberrant crypt formation, DNA methylation and one carbon-metabolism. A second objective was to compare DNA methylation and one carbon-metabolism in the liver vs. the colon for a subgroup of rats.

**MATERIALS AND METHODS**

**Animals and diets.** Weanling male Fischer-344 rats (n = 92) were purchased from Sasco (Omaha, NE). All rats were housed individually in stainless steel wire-bottomed cages in a room with temperature and humidity. Rats had free access to demineralized water and purified diet. The basal diet was an amino acid-based diet formulated to be low in selenium and folate. The diets contained (on a per kg basis): 186.1 g amino acid mixture, 35 g selenium-deficient mineral mixture, 10 g folate-deficient vitamin mixture, 100 g corn oil, 50 g Alphacel (cornstarch). The vitamin and mineral mixtures had a composition of 2 factorial design, n = 8/diet, 4 administered DMH and 4 administered saline, the colon was opened longitudinally and the mucosa was scraped off with a microscope slide. All rats were purchased from Sasco (Omaha, NE). All rats were housed individually in stainless steel wire-bottomed cages in a room with controlled temperature and humidity. Rats had free access to demineralized water and purified diet. The basal diet was an amino acid-based diet formulated to be low in selenium and folate. These diets were considered to be either deficient or supplemented with 0.15 mg/kg diet as sodium selenite (by analysis, the diets contained 0.1 mg selenium/kg, respectively) and with 0 or 2 mg folate/kg as folic acid. No antibiotics were used so that only a moderate folate deficiency would be obtained. These diets were considered to be either deficient or supplemented in selenium and either deficient or adequate in folate because the recommendations for dietary selenium and folate in the AI/N-93 diet are 0.15 and 2.0 mg/kg diet, respectively (31). Supplementation rather than adequate dietary selenium was chosen to maximize potential differences between the dietary treatments. After 3 wk of consuming the experimental diets, 19 rats/diet were given two injections with saline. Rats consumed the same diets for an additional 1 wk to minimize potential differences between the dietary treatments. After 3 wk of consuming the experimental diets, 19 rats/diet were given two injections with saline. Rats consumed the same diets for an additional 8 wk.

This study was approved by the Animal Care Committee of the Grand Forks Human Nutrition Research Center. The rats were maintained in accordance with the guidelines for the care and use of laboratory animals.

**Sample collection.** Food was withheld overnight (at least 12 h) before rats were anesthetized with xylazine (Rompin, Mobay, Shawnee, KS) and ketamine (Ketaset, Aveco, Fort Dodge, IA) and killed by exsanguination. Blood was collected by cardiac puncture into syringes containing EDTA such that the final concentration was 1 g EDTA/L blood. Hematologic indices were determined with a Coulter S + IV hematology analyzer (Coulter Electronics, Hialeah, FL). For aberrant crypt analysis (n = 15/diet, all administered DMH), the colon and rectum were removed, flushed with 9 g/L NaCl, opened longitudinally and fixed flat between paper towels in 700 mL/L ethanol and stored at 4°C before analysis. For the remaining rats (n = 8/diet, 4 administered DMH and 4 administered saline), the colon was opened longitudinally and the mucosa was scraped off with a microscope slide. All rats were purchased from Sasco (Omaha, NE). All rats were housed individually in stainless steel wire-bottomed cages in a room with controlled temperature and humidity. Rats had free access to demineralized water and purified diet. The basal diet was an amino acid-based diet formulated to be low in selenium and folate. These diets were considered to be either deficient or supplemented with 0.15 mg selenium/kg, respectively) and with 0 or 2 mg folate/kg as folic acid. No antibiotics were used so that only a moderate folate deficiency would be obtained. These diets were considered to be either deficient or supplemented in selenium and either deficient or adequate in folate because the recommendations for dietary selenium and folate in the AI/N-93 diet are 0.15 and 2.0 mg/kg diet, respectively (31). Supplementation rather than adequate dietary selenium was chosen to maximize potential differences between the dietary treatments. After 3 wk of consuming the experimental diets, 19 rats/diet were given two injections with saline. Rats consumed the same diets for an additional 8 wk.

The activity of betaine-homocysteine methyltransferase (BHMT) was determined according to Finklestein and Mudd (34) as modified by Xue and Snowsell (35). The substrate [methyl-3H]betaine was prepared according to Xue and Snowsell (35). Liver was prepared by homogenization (1 g liver/4 mL buffer) in 0.04 mol/L potassium phosphate buffer, pH 7.4. The homogenate was centrifuged at 10,000 × g for 30 min. The supernatant was used for the assay. Methionine synthase (MS) activity was determined by the method of Sauer (36). For the MS assay, liver was prepared by homogenization (1 g liver/4 mL buffer) in 0.01 mol/L
Results

Rats fed the selenium-deficient diet gained less (P < 0.0001) body weight than rats fed the selenium-supplemented diet (314 ± 3 vs. 335 ± 3 g, respectively). Dietary folate and carcinogen treatment did not affect the body weight of the rats (data not shown). Only a moderate folate deficiency occurred as evidenced by the lack of an effect of folate on growth of the rats.

Dietary selenium and folate interacted to affect hematological indices and plasma folate and vitamin B-12 concentrations (P < 0.03; Table 1). Rats fed the diets containing adequate folate and supplemental selenium had the highest hematocrits, hemoglobin, plasma folate and plasma vitamin B-12 concentrations. In contrast, only dietary selenium affected plasma GSH concentrations (P < 0.0001; Table 1) and indicators of selenium status (Table 2). However, dimethylhydrazine treatment reduced liver selenium concentrations (P < 0.02; 28.4 ± 0.4 vs. 31.1 ± 0.9 mmol/kg in rats injected with dimethylhydrazine and PBS, respectively). This effect was significant only in the selenium-supplemented rats. Carcinogen treatment did not affect RBC or liver GSH peroxidase activity.

Dietary selenium and folate also interacted to affect the formation of aberrant crypts and aberrant crypt foci in the colon and rectum of rats (P < 0.0001; Fig. 1). Selenium supplementation significantly increased the number of aberrant crypts and the number of aberrant crypt foci when rats were fed folate-deficient, but not folate-adequate diets. Folate supplementation decreased the number of aberrant crypts per aberrant crypt foci (2.84 ± 0.07 vs. 2.99 ± 0.06 in rats fed a folate-adequate vs. a folate-deficient diet, respectively; P < 0.03). Neither dietary selenium nor an interaction of selenium and folate affected the number of aberrant crypts per aberrant crypt foci (data not shown).

Both supplemental selenium (P < 0.0001) and adequate dietary folate (P < 0.05) increased liver DNA methyltransferase activity (Table 3). Similarly, supplemental selenium (P < 0.02) increased colon DNA methyltransferase activity; however, a folate-adequate diet (P < 0.03) decreased colon DNA methyltransferase activity. Rats fed supplemental selenium and a folate-adequate diet had the highest level of liver and colon DNA methylation (Table 3) as indicated by lower
in vitro $^3$H-methyl incorporation. However, the effect of dietary selenium on DNA methylation was significant only in the colon.

Dietary selenium and folate had a very strong ($P < 0.0001$) interactive effect on plasma homocysteine concentrations (Fig. 2). Although folate deficiency increased plasma homocysteine concentrations ($P < 0.0001$), selenium deficiency decreased plasma homocysteine concentrations ($P < 0.0001$). This effect was most pronounced when rats were fed a folate-deficient diet and supplemental selenium.

Folate deficiency decreased SAM, increased SAH and decreased SAM/SAH ratios in the liver ($P < 0.0001$; Table 4), and these effects were partially ameliorated by a low selenium diet. However, dietary selenium did not affect these three variables when rats consumed a folate-adequate diet ($P < 0.0001$ for the folate $\times$ selenium interaction).

Folate deficiency did not depress SAM and increase SAH as much in the colon as it did in the liver (Table 5). In fact, only folate significantly affected colon SAH. However, similar to the results in the liver, the highest SAH, lowest SAM and lowest SAM/SAH ratio were observed in rats fed the folate-deficient diet supplemented with selenium. Although there was no significant correlation between liver SAM and colon SAM, liver and colon SAH and liver and colon SAM/SAH ratios were correlated ($r = 0.37$, $P < 0.04$ and $r = 0.35$, $P = 0.05$, respectively).

Selenium deficiency significantly decreased BHMT activity [1.14 $\pm$ 0.13 vs. 1.88 $\pm$ 0.13 nmol/(min $\cdot$ mg protein), respectively ($P < 0.0003$)] and tended to decrease liver methionine adenosyltransferase (MAT) activity [0.63 $\pm$ 0.02 vs. 0.70 $\pm$ 0.02 nmol/(min $\cdot$ mg protein), respectively ($P < 0.06$)] compared with rats fed supplemental selenium (Table 6). Although dietary folate did not affect liver BHMT or MAT activity, the effects of selenium deficiency on BHMT tended to be more pronounced when rats were fed a folate-deficient diet ($P < 0.06$, Table 6). In contrast, folate deficiency decreased liver MS activity ($P < 0.001$). Rats fed folate-deficient diets and supplemental selenium had lower ($P < 0.05$) MS activity that the other dietary groups. Folate deficiency also decreased liver CBS activity ($P < 0.005$), particularly in rats fed supplemental selenium. Rats injected with PBS had lower CBS activity ($P < 0.03$) than those injected with DMH. This difference was significant only in rats fed folate-deficient diets and supplemental selenium in which the CBS activity was 2.4 $\pm$ 0.7 vs. 4.1 $\pm$ 0.2 nmol/(min $\cdot$ mg protein) in rats injected with PBS vs. DMH, respectively. Plasma homocysteine concentrations were correlated with liver BHMT, MS and CBS

**TABLE 2**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Selenium concentration</th>
<th>Glutathione peroxidase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>Liver</td>
</tr>
<tr>
<td>Folate</td>
<td>Selenium</td>
<td>$\mu$mol/L</td>
</tr>
<tr>
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<td>0</td>
<td>0.11 $\pm$ 0.006</td>
</tr>
<tr>
<td>0 mg/kg</td>
<td>2</td>
<td>6.46 $\pm$ 0.27</td>
</tr>
<tr>
<td>2 mg/kg</td>
<td>0</td>
<td>0.11 $\pm$ 0.004</td>
</tr>
<tr>
<td>2 mg/kg</td>
<td>2</td>
<td>6.68 $\pm$ 0.27</td>
</tr>
</tbody>
</table>

Significant effects as determined by two-way ANOVA:

- Selenium
- Folate
- Selenium $\times$ Folate

$^1$ Values are means $\pm$ SEM, $n = 22$–23.

$^2$ NS, not significant, $P > 0.05$. 

**FIGURE 1** Effect of dietary selenium and folate on total number of aberrant crypts and aberrant crypt foci in the colon and rectum of rats treated with dimethylhydrazine and fed amino acid–based diets supplemented with 0 or 2 mg/kg folate and 0 or 2 mg selenium/kg as selinite in a $2 \times 2$ factorial design. Values are means $\pm$ SEM, $n = 15$. ANOVA: selenium $\times$ folate, $P < 0.0001$. Means without common letters differ, $P < 0.05$.

**FIGURE 2** Values are means $\pm$ SEM, $n = 15$. ANOVA: selenium $\times$ folate, $P < 0.0001$. Means without common letters differ, $P < 0.05$. 

Dietary selenium and folate had a very strong ($P < 0.0001$) interactive effect on plasma homocysteine concentrations (Fig. 2). Although folate deficiency increased plasma homocysteine concentrations ($P < 0.0001$), selenium deficiency decreased plasma homocysteine concentrations ($P < 0.0001$). This effect was most pronounced when rats were fed a folate-deficient diet and supplemental selenium.

Folate deficiency decreased SAM, increased SAH and decreased SAM/SAH ratios in the liver ($P < 0.0001$), and these effects were partially ameliorated by a low selenium diet. However, dietary selenium did not affect these three variables when rats consumed a folate-adequate diet ($P < 0.0001$ for the folate $\times$ selenium interaction).

Folate deficiency did not depress SAM and increase SAH as much in the colon as it did in the liver (Table 5). In fact, only folate significantly affected colon SAH. However, similar to the results in the liver, the highest SAH, lowest SAM and lowest SAM/SAH ratio were observed in rats fed the folate-deficient diet supplemented with selenium. Although there was no significant correlation between liver SAM and colon SAM, liver and colon SAH and liver and colon SAM/SAH ratios were correlated ($r = 0.37$, $P < 0.04$ and $r = 0.35$, $P = 0.05$, respectively).

Selenium deficiency significantly decreased BHMT activity [1.14 $\pm$ 0.13 vs. 1.88 $\pm$ 0.13 nmol/(min $\cdot$ mg protein), respectively ($P < 0.0003$)] and tended to decrease liver methionine adenosyltransferase (MAT) activity [0.63 $\pm$ 0.02 vs. 0.70 $\pm$ 0.02 nmol/(min $\cdot$ mg protein), respectively ($P < 0.06$)] compared with rats fed supplemental selenium (Table 6). Although dietary folate did not affect liver BHMT or MAT activity, the effects of selenium deficiency on BHMT tended to be more pronounced when rats were fed a folate-deficient diet ($P < 0.06$, Table 6). In contrast, folate deficiency decreased liver MS activity ($P < 0.001$). Rats fed folate-deficient diets and supplemental selenium had lower ($P < 0.05$) MS activity that the other dietary groups. Folate deficiency also decreased liver CBS activity ($P < 0.005$), particularly in rats fed supplemental selenium. Rats injected with PBS had lower CBS activity ($P < 0.03$) than those injected with DMH. This difference was significant only in rats fed folate-deficient diets and supplemental selenium in which the CBS activity was 2.4 $\pm$ 0.7 vs. 4.1 $\pm$ 0.2 nmol/(min $\cdot$ mg protein) in rats injected with PBS vs. DMH, respectively. Plasma homocysteine concentrations were correlated with liver BHMT, MS and CBS
enzyme activities (P < 0.0001; r = 0.68, −0.74 and −0.69, respectively).

**DISCUSSION**

The present study demonstrates that dietary selenium can modulate many of the adverse effects of folate deficiency including alterations in one-carbon metabolism and aberrant crypt formation. For example, when rats were fed the folate-deficient diet, there were large increases in plasma homocysteine concentrations that were somewhat ameliorated by a selenium-deficient diet. Hyperhomocysteinemia is a risk factor for cardiovascular disease and is hypothesized to be a risk factor for carcinogenesis (30).

Folate deficiency and selenium deficiency appear to have opposite effects on homocysteine metabolism. There are two major metabolic reactions involving homocysteine, i.e., remethylation and transsulfuration (Fig. 3). In remethylation, homocysteine is converted to methionine by acquiring a methyl group from either N-5-methyltetrahydrofolate or from betaine, catalyzed by the enzymes MS and BHMT, respectively. In the transsulfuration pathway, homocysteine condenses with serine to form cystathionine in an irreversible reaction catalyzed by CBS. The activities of all three liver enzymes involved in the metabolism of homocysteine were significantly correlated with plasma homocysteine concentrations.

Because MS is a folate-dependent enzyme, folate deficiency significantly decreased MS activity, resulting in less homocysteine being remethylated to methionine. However, MS activity was significantly lower in rats fed the diet deficient in folate but supplemented with selenium than in rats fed the diet deficient in both folate and selenium. SAH acts as a negative effector of MS (42), and rats fed the folate-deficient and selenium-supplemented diet had the highest liver SAH concentrations. As in our previous results (29), selenium deficiency significantly decreased BHMT activity. In the current

**TABLE 4**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Folate</th>
<th>Selenium</th>
<th>SAM</th>
<th>SAH</th>
<th>SAM/SAH</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg</td>
<td>µmol/g</td>
<td>nmol/g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>32.2 ± 1.9B</td>
<td>31.4 ± 1.7B</td>
<td>1.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>18.8 ± 0.9C</td>
<td>51.6 ± 2.0A</td>
<td>0.4 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>71.1 ± 1.5A</td>
<td>16.0 ± 0.7C</td>
<td>4.6 ± 0.2</td>
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</tr>
<tr>
<td>2</td>
<td>2</td>
<td>76.7 ± 2.0A</td>
<td>18.4 ± 0.6D</td>
<td>4.3 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

Significant effects as determined by two-way ANOVA:

1 Values are means ± SEM, n = 22–23. ANOVA: folate, P < 0.0001; selenium, P < 0.0001; folate × selenium, P < 0.0001. Means without common letters differ, P < 0.05.

2 NS, not significant, P > 0.05.
TABLE 5
Effect of feeding diets containing different concentrations of folate and selenium on colon S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH) and SAM/SAH ratio in rats

<table>
<thead>
<tr>
<th>Diet</th>
<th>Folate</th>
<th>Selenium</th>
<th>SAM</th>
<th>SAH</th>
<th>SAM/SAH</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg</td>
<td>nmol/g</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0</td>
<td>0</td>
<td>71.0 ± 8.0</td>
<td>6.9 ± 1.5</td>
<td>14.4 ± 3.5</td>
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</tr>
<tr>
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<td>7.5 ± 1.3</td>
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<tr>
<td>2</td>
<td>0</td>
<td>70.4 ± 8.1</td>
<td>5.5 ± 1.1</td>
<td>16.7 ± 3.5</td>
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</tr>
<tr>
<td>2</td>
<td>2</td>
<td>70.1 ± 5.9</td>
<td>4.3 ± 0.9</td>
<td>17.5 ± 3.9</td>
<td></td>
</tr>
</tbody>
</table>

Significant effects as determined by two-way ANOVA:

- Selenium: NS
- Folate: NS
- Selenium × Folate: NS

1 Values are means ± SEM, n = 8.
2 NS, not significant, P > 0.05.

The decreased homocysteine concentrations observed during selenium deficiency were most likely the result of increased transsulfuration of homocysteine. Hill and Burk (43) reported that γ-glutamylcysteine synthetase activity in selenium-deficient rat liver was twice that of controls, resulting in increased plasma GSH. In our study, we show that rats fed the selenium-deficient diet had significantly increased plasma GSH concentrations. This effect was most pronounced when rats were fed a folate-deficient diet. Selenium deficiency did tend to increase activity of liver CBS, the first enzyme in the transsulfuration pathway.

Interestingly, the activity of CBS was significantly lower in rats fed the folate-deficient, selenium-supplemented diets compared with the other dietary treatments. Decreased CBS activity contributed to the very high plasma homocysteine observed in rats fed this diet. This is also the group of rats with the highest liver SAH concentrations and the lowest SAM/SAH ratio. Similarly, CBS knockout mice have elevated plasma homocysteine and decreased liver SAM/SAH ratios compared with wild-type mice (44). Also, SAH concentrations in these mice were significantly higher in homozygous mutant mice than in wild-type mice in all tissues examined; however, SAM concentrations responded in a tissue-specific manner (44). In the current study, folate deficiency significantly increased colon SAH but did not affect colon SAM concentrations.

Despite the fact that folate deficiency significantly increased colonic SAH concentrations, folate did not affect colonic DNA methylation. Other investigators also observed

TABLE 6
Effect of feeding diets containing different concentrations of folate and selenium on liver betaine homocysteine methyltransferase (BHMT), methionine adenosyltransferase (MAT), methionine synthase (MS) and cystathionine synthase (CBS) in rats

<table>
<thead>
<tr>
<th>Diet</th>
<th>Folate</th>
<th>Selenium</th>
<th>BHMT (nmol/min · mg protein)</th>
<th>MAT (nmol/min · mg protein)</th>
<th>MS (nmol/min · mg protein)</th>
<th>CBS (nmol/min · mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0</td>
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<td>1.05 ± 0.15</td>
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<td>0.074 ± 0.004AB</td>
<td>4.7 ± 0.2</td>
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<tr>
<td>2</td>
<td>2</td>
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<td>0.67 ± 0.02</td>
<td>0.077 ± 0.006A</td>
<td>4.7 ± 0.1</td>
<td></td>
</tr>
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</table>

Significant effects as determined by two-way ANOVA:

- Selenium: 0.0003
- Folate: NS
- Selenium × Folate: NS

1 Values are means ± SEM, n = 7–8. Values in a column without a common letter differ, P ≤ 0.05.
2 NS, not significant, P > 0.05.
that folate deficiency does not induce significant genomic DNA hypomethylation in the colon despite increased SAH concentrations (45,46). This is a surprising finding considering that SAH is a potent inhibitor of most SAM-dependent methyltransferases including DNA methyltransferase (42) and that increased plasma and intracellular SAH levels were shown recently to be accurate predictors of genomic DNA hypomethylation (47,48). One explanation for the lack of correlation between colonic SAH and colonic genomic DNA methylation is that the range of changes in colonic mucosal SAH levels induced by dietary folate and selenium concentrations used in the present study is not sufficient to modulate colonic genomic DNA methylation (45). The present data do not rule out the possibility that the increases in SAH might have produced gene-specific hypomethylation of DNA (46) in the absence of genomic hypomethylation, as was observed previously in folate-deficient rats (23).

Another surprising result in the current study was the significantly increased liver and colon DNA methyltransferase activity when rats were fed a selenium-supplemented diet. The higher DNA methyltransferase activity was associated with increased colonic DNA methylation in selenium-supplemented rats. In contrast to the results in the current study, Fiala et al. (49) observed that sodium selenite, benzyl selenocyanate and 1,4-phenylenebis(methylene)selenocyanate (p-XSC) inhibited DNA methyltransferase extracted from nuclei of a human colonic carcinoma. p-XSC also inhibited the enzyme in HCT116 human carcinoma cells in culture at a concentration of 0–40 μmol/L. However, these studies investigated the effect of selenium on the activity of the purified enzyme in vitro at very high concentrations of selenium. The current study investigated the effect of selenium on the activity of the enzyme in vivo at physiologic concentrations of selenium. Future studies should investigate how and why selenium supplementation increases DNA methyltransferase activity.

In the current study, selenium deficiency significantly depressed global DNA methylation in the colon but not in the liver. Furthermore, the DNA was more hypomethylated in the colon than in the liver as evidenced by the higher incorporation of [3H-methyl] groups. This may be a result of the higher rate of cell proliferation in the colon than in the liver. Previous studies have suggested that hypomethylation may be a feature of proliferating cells because during DNA replication, the newly synthesized strand is not methylated. However, after cell replication, DNA methyltransferase recognizes the 5-methylcytosine at the CpG site on the parental strand as a signal to methylate the corresponding CpG site on the daughter strand (50). Rapidly proliferating cells have a relatively high proportion of hemimethylated sites in their DNA, and thus have relatively low total genomic DNA methylation, whereas differentiated cells have relatively stable methylation patterns (51).

In this study, dietary folate did not affect DNA methylation, and colonic DNA methylation was not associated with aberrant crypt formation. This is in contrast to current dogma, which suggests that low folate status may increase the risk of colon cancer through alterations in DNA methylation. However, colonic DNA methyltransferase activity was highest in rats fed folate-deficient diets and supplemental selenium (Table 3). This was also the group of rats with the highest number of aberrant crypts and aberrant crypt foci. Trasler et al. (52) observed that both DNA methyltransferase deficiency and folate deficiency can modulate intestinal tumor numbers in multiple intestinal neoplasia (Min) mice without concomitantly altering overall genomic DNA methylation. These results suggest that other mechanisms, in addition to changes in DNA methylation, are involved in the increased colon cancer susceptibility of folate-deficient animals. Examples might include induction of mutations and DNA damage.

Another interesting finding from the current study was the lack of correlation between liver and colon SAM, DNA methylation and DNA methyltransferase activity, thus suggesting that in future studies, these variables should be investigated in the tissue of interest rather than assuming that all tissues respond in the same manner.

In summary, results from the current study suggest that selenium deficiency and folate deficiency have opposite effects on plasma homocysteine concentrations. Selenium deficiency appears to ameliorate the large buildup of homocysteine as a result of folate deficiency and shunt it to GSH production. Future studies are required to evaluate whether the protective effect of selenium occurs between deficient and adequate or between adequate and supplemental selenium.

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LITERATURE CITED


