Dietary Selenomethionine Increases Exon-Specific DNA Methylation of the p53 Gene in Rat Liver and Colon Mucosa1,2

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Abstract

The regulation of site-specific DNA methylation of tumor suppressor genes has been considered as a leading mechanism by which certain nutrients exert their anticancer property. This study was to investigate whether selenium (Se) affects the methylation of globular genomic DNA and the exon-specific p53 gene. Three groups of rats (n = 6–7/group) were fed the AIN-93G basal diet supplemented with 0 [Se deficient (D)], 0.15 [Se adequate (A)], or 4 mg [Se supranutritional (S)] (Se as L-selenomethionine)/kg diet for 104 d, respectively. Rats fed the A or S diet had greater plasma and liver glutathione peroxidase activity, liver thioredoxin reductase activity, and plasma homocysteine concentration than those fed the D diet. However, compared with the A diet, rats fed the S diet did not further increase these Se-dependent enzyme activities or homocysteine concentration. In contrast, Se concentrations in kidney, liver, gasticmucius muscle, and plasma were increased in a Se-dose–dependent manner. Interestingly, rats fed the S diet had significantly less global liver genomic DNA methylation than those fed the D diet. However, the S diet significantly increased the methylation of the p53 gene (exons 5–8) but not the β-actin gene (exons 2–3) DNA in liver and colon mucosa compared with those fed the D diet. Taken together, long-term Se consumption not only affects selenoprotein enzyme activities, homocysteine, tissue Se concentrations, and global genomic DNA methylation but also increases exon-specific DNA methylation of the p53 gene in a Se-dose–dependent manner in rat liver and colon mucosa. J. Nutr. 141: 1464–1468, 2011.

Introduction

Colon cancer is the third most common cancer globally and accounts for 130,000 new cancer cases and ~56,000 deaths each year in the United States, and it is estimated that one-half of the Western population can expect to develop at least one colorectal tumor by age 70 y (1). Epidemiological evidence indicates that selenium (Se) status is inversely associated with cancer risk, and results from intervention studies show that high-Se intakes effectively reduce the risk of mammary, prostate, lung, colon, and liver cancer (2–7). Interest in this area was stimulated by the landmark finding that supplementation of free-living people with Se-enriched brewer’s yeast with predominantly selenomethionine (SeMet) decreased the overall cancer morbidity by nearly 50% (5–8) but not the β-actin gene (exons 2–3) DNA in liver and colon mucosa compared with those fed the D diet. Taken together, long-term Se consumption not only affects selenoprotein enzyme activities, homocysteine, tissue Se concentrations, and global genomic DNA methylation but also increases exon-specific DNA methylation of the p53 gene in a Se-dose–dependent manner in rat liver and colon mucosa. J. Nutr. 141: 1464–1468, 2011.

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Increasing evidence indicates that aberrant epigenetic gene regulation may serve as a common mechanism underlying cancer development (13,14). It is thought that altered DNA methylation plays a critical role in the onset and development of colon cancer (1,15). Although all of the functions of DNA methylation are not

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3 Abbreviations used: A, selenium adequate; D, selenium deficient; GPx, glutathione peroxidase; S, selenium supranutritional; SELECT, Selenium and Vitamin E Cancer Prevention Trial; SeMet, selenomethionine; TRR, thioredoxin reductase.
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elucidated, it appears to be an important mechanism for modulating gene expression and for stabilizing areas of the genome (16,17). In colon cancer, several lines of evidence demonstrate that there is an association between DNA hypomethylation and colon carcinogenesis (18). The p53 tumor suppressor gene is commonly found to be mutated in colorectal cancer and is thought to play a critical role in the transition from dysplasia to invasive cancer (19). It has been documented that dimethylhydrazine, a colon-specific chemical carcinogen, which alters the 5’-adenosylmethionine concentration and DNA methyltransferase activity preceding the histological appearance of dysplasia and induces the exon-specific p53 hypomethylation resulting in DNA instability in rat colon (18). These effects are directly related to an increase in colon carcinogenesis (20). Our data indicated that dietary Se decreased dimethylhydrazine-induced aberrant crypt formation in rat colon and altered DNA methylation in rat colon and liver (21). In the present study, we therefore hypothesized that dietary Se may increase the exonic DNA methylation of the p53 gene in a gene-specific manner.

**Materials and Methods**

**Rats and diets.** Male weanling Sprague-Dawley rats (strain: SAS/VAF) \((n = 20)\) were obtained from Charles River. Rats were individually housed in stainless-steel cages with wire-mesh bottoms in an atmosphere of 50% relative humidity at 22°C with a 12-h-light/dark cycle. Rats were given free access to food and deionized water. A 30% Torula yeast-based diet formulated according to the AIN-93G formulation (22) was used as the basal diet in this study in which the yeast was used as the protein source. Torula yeast is relatively rich in Fe, P, K, Zn, Mn, and Mg, and a 30% yeast diet provides an adequate amount of these minerals for rodents (22). Thus, a mineral mix was prepared containing only those minerals needed to meet NRC recommendations (23) and those included in the AIN-93G formulation. The AIN-93G basal diet had an average Se content of 0.004 mg/kg, which was extremely low relative to the re-commendation of 0.004 mg/kg (22,23). All 20 rats were randomly assigned to 3 groups \((n = 6–7 each)\) fed the AIN-93G basal diet, which was supplemented with 0 [Se deficient (D)], 0.15 [Se adequate (A)], and 4 mg SeMet/kg [Se supranutritional (S)] diet for 104 d, respectively.

This study was approved by the Animal Use Committee of the Grand Forks Human Nutrition Research Center and the rats were maintained in accordance with the guidelines for the care and use of laboratory animals (24).

**Sample collection.** Rats were weighed weekly and food was withheld overnight before rats were anesthetized with a mixture of ketamine and xylazine. Whole blood, liver, colon mucosa, right gastrocnemius muscle, and kidneys were collected immediately and held at -80°C for enzyme and Se analyses. Liver, muscle, and kidneys were lyophilized prior to Se analysis.

**Se status.** Se concentrations in the plasma, liver, kidney, and muscle were determined by hydride-generating atomic absorption spectrometry according to a published procedure (12,25). Samples were prepared for analysis by predigestion in nitric acid and \(H_2O_2\), followed by high temperature ashing, while in the presence of \(MgNO_3\) as an aid to prevent Se volatilization.

**Enzyme assays and homocysteine analysis.** Glutathione peroxidase (GPx) activity was determined in whole blood and liver by the method of Lawrence and Burk (26) using \(H_2O_2\) as the substrate in the presence of azide. The activity in whole blood was expressed as units/mg hemoglobin and in liver as units/mg protein; 1 unit of activity was defined as the amount of enzyme required to oxidize 1.0 \(\mumol\) NADPH/min. Thioredoxin reductase (TRR) activity was determined in the liver as previously described (23,27). Homocysteine in plasma was determined by enzyme-linked immunosorbent bead assay (Immulite 1000 Homocysteine kit, Diagnostic Products).

![Figure 1](https://academic.oup.com/jn/article-abstract/141/8/1464/4630510)

**TABLE 1** Tissue Se and plasma homocysteine concentrations and GPx/TRR activities in rats fed a Se-deficient (D), Se-adequate (A), or Se-supranutritional (S) diet for 104 d\(^1\)

<table>
<thead>
<tr>
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<th>D</th>
<th>A</th>
<th>S</th>
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<tbody>
<tr>
<td>Kidney Se, (\mumol/g) tissue</td>
<td>4.50 ± 0.34(^a)</td>
<td>70.4 ± 1.86(^b)</td>
<td>295 ± 16.4(^a)</td>
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<tr>
<td>Liver Se, (\mumol/g) tissue</td>
<td>0.47 ± 0.03(^a)</td>
<td>40.2 ± 1.20(^b)</td>
<td>195 ± 6.87(^a)</td>
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<tr>
<td>Muscle Se, (\mumol/g) tissue</td>
<td>0.05 ± 0.03(^a)</td>
<td>7.50 ± 0.22(^b)</td>
<td>121 ± 2.38(^a)</td>
</tr>
<tr>
<td>Plasma Se, (\mumol/L)</td>
<td>0.16 ± 0.05(^a)</td>
<td>6.68 ± 0.26(^b)</td>
<td>12.3 ± 0.44(^a)</td>
</tr>
<tr>
<td>Plasma homocysteine, (\mumol/L)</td>
<td>3.2 ± 0.3(^a)</td>
<td>6.7 ± 0.3(^b)</td>
<td>6.0 ± 0.4(^a)</td>
</tr>
<tr>
<td>Blood GPx, U/mg hemoglobin</td>
<td>12.3 ± 1.2(^a)</td>
<td>851 ± 33(^b)</td>
<td>727 ± 35(^a)</td>
</tr>
<tr>
<td>Liver GPx, U/mg protein</td>
<td>14.9 ± 2.3(^a)</td>
<td>2888 ± 150(^b)</td>
<td>3216 ± 192(^a)</td>
</tr>
<tr>
<td>Liver TRR, U/mg protein</td>
<td>2.2 ± 0.1(^a)</td>
<td>7.9 ± 0.1(^b)</td>
<td>8.4 ± 0.3(^a)</td>
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</tbody>
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\(^1\) Values are means ± SEM, \(n = 6–7\). Means in a row without a common superscript letter differ, \(P < 0.05\).

**Genomic DNA methylation.** The methylated DNA was detected by antibody and ELISA with a DNA methylation quantification kit (Epigentek Group). DNA was extracted from liver, colon mucosa, and blood by using a genomic DNA isolation kit (Qiagen).

**Quantitative HpaII-PCR assay for gene-specific methylation.** Genomic DNA was isolated from rat liver, blood, and colon mucosa by using a genomic DNA isolation kit (Qiagen). The methylation status of the p53 (exons 5–8) and \(\beta\)-actin (exons 2–3) genes was assessed using a Quantitect SYBR Green RT-PCR kit (Qiagen) and an Applied Biosystem, 7300 Real-Time PCR System. This assay utilizes PCR primers flanking the HpaII cleavage sites (CCGG) within the genes, as previously described (18). HpaII, a restriction enzyme, does not cut CCGG if the internal cytosine is methylated (18). However, HpaII does cut at non-methylated CCGG sites and stops the progression of Taq polymerase during PCR amplification. Thus, quantitative real-time PCR product amplified over primer-defined exons after treatment with HpaII is directly proportional to the degree of methylation at CCGG sites. The relative extent of internal cytosine methylation at the CCGG sequences within the specific site of each DNA sample was assessed and normalized by comparative \(C_T\) method of HpaII-treated product with the respective non-HpaII-treated product of the p53/\(\beta\)-actin gene. The amount of HpaII-resistant (methylated) DNA was then expressed relative to that of rats fed the D diet. For the amount of methylated DNA in rats, a 1-fold of D change indicated no change, >1-fold of D change indicated an increase of DNA methylation status, and <1-fold of D change indicated a decrease of DNA methylation status. The specific PCR products were confirmed via 1.9% agarose gel electrophoresis.
Statistical analysis. Results are given as means ± SEM. Body weights were analyzed using 1-way ANOVA. Tissue Se, enzymes, and DNA data were analyzed by 1-way ANOVA using the Proc Mixed procedure in SAS version 9.2 (SAS Institute). Because of variance heterogeneity, separate variance estimates were computed for each group within the ANOVA. The ANOVA for the DNA data included experiment as a blocking factor. Tukey contrasts were used to compare individual group means. Differences of \( P < 0.05 \) were considered significant.

Results

Weight and Se concentration in tissue. Body weights of rats fed the D diet were less than those of rats fed the A diet. Body weight of the S group was intermediate and did not differ from the other 2 groups (Fig. 1).

Kidney, liver, muscle, and plasma Se concentrations were greater in rats fed the S diet than in those fed the A diet, which in turn were greater than in rats fed the D diet (Table 1).

GPx, TRR activities, and plasma homocysteine. Blood GPx, liver GPx/TRR activities, and plasma homocysteine concentrations were greater in rats fed the S or A diet than in rats fed the D diet, and these did not differ in those fed the S or A diet (Table 1).

Global genomic DNA methylation. Liver global genomic DNA methylation of rats fed the S diet was 57% less than that of those fed the D diet (Fig. 2). Liver global genomic DNA methylation of rats fed the A diet was intermediate and did not differ from the other 2 groups (Fig. 2). In contrast, the global genomic DNA methylation of rats fed the D, A, or S diet did not differ from one another in whole blood or colon mucosa.

Exon-specific DNA methylation. Exon-specific DNA methylation of the \( \beta \)-actin gene in blood, liver, and colon mucosa did not differ among the groups (Fig. 3A). However, exon-specific DNA methylation of the p53 gene in liver and colon mucosa of rats fed the S diet was 0.7- and 1.3-fold greater than that in rats fed the D diet, respectively (Fig. 3B). Values in the group fed the A diet were intermediate and did not differ from the other 2 groups.

**FIGURE 2** Global genomic DNA methylation in blood cells, liver and colon mucosa of rats fed a Se deficient (D), Se adequate (A), or Se supranutritional (S) diet for 104 d. Values are means ± SEM, \( n = 6–7 \). Means without a common letter differ, \( P < 0.05 \).

**FIGURE 3** Exon-specific DNA methylation of the \( \beta \)-actin (exons 2–3; A) and p53 (exons 5–8; B) genes in blood cells, liver, and colon mucosa of rats fed a Se deficient (D), Se adequate (A), or Se supranutritional (S) diet for 104 d. Values are means ± SEM, \( n = 6–7 \). Means without a common letter differ, \( P < 0.05 \).
Discussion

The current study shows that the activities of the selenoproteins GPx and TRR were significantly decreased by the D diet compared with the A and S diets; activities in rats fed the D diet did not differ from the A and S diets, consistent with previous studies (12,21,25,28,29). An explanation for the decreased plasma homocysteine concentrations in rats fed the D diet is that homocysteine is directed toward the transsulfuration pathway to increase glutathione production (14). In contrast to the above observation of GPx and TRR enzyme activity, the tissue Se concentrations in numerous tissue types (kidney, muscle, liver, plasma) were: the S diet group > A diet group > D diet group, with differences significant in each tissue. This notion further demonstrates that SeMet is an excellent form of Se to enhance general tissue Se storage, which may directly relate to the fact that SeMet can nonspecifically incorporate into the place of methionine during protein synthesis (30). In addition, there was significant growth retardation in rats fed the D diet compared with those fed the A diet, indicating a critical role of optimal Se amount in rat growth. Interestingly, the present data indicated that dietary SeMet significantly decreased global DNA methylation in liver. This observation was initially counterintuitive given the reported hypermethylation associated with Se supplementation in other rat experiments (14,21). However, consistent with our present data, a more recent report also showed that Se supplementation decreased the global methylation of liver DNA in a mouse model (31). There are several factors in these studies that may contribute to this seemingly inconsistent role of Se supplementation in global DNA methylation. First, the diet composition (other than Se) may play a role, because all of these studies used different basal diets. The present study used an AIN93G-based diet that contained higher amounts of linolenic acid and vitamins E and K compared with the AIN76A diet that was used in previous studies (14,21); a high-fat diet was used in the recent report that studied mice (31). Furthermore, several other studies that used an amino acid-based diet showed no difference in liver global DNA methylation (32,33).

Second, animal strain and species may be important, because Sprague-Dawley rats, Fisher-344 rats, and C57BL/6 mice were used in these studies (14,21,31,34). Thus, it is difficult to connect global genomic DNA methylation and its direct role in gene regulation.

At present, changes in global DNA methylation alone cannot explain the chemopreventive effect of the S diet, because there is little difference in DNA methylation between rats fed the A and S diets (14,21,28,29). Importantly, our present data on exon-specific DNA methylation of the p53 gene may provide an alternative explanation for the anticancer property of the S diet. The p53 tumor suppressor gene was selected for this study, because it is commonly found to be mutated or differentially methylated in colon cancer and other cancer types (18,19,35). It is well recognized that alterations in DNA methylation of certain tumor suppressor genes may be the leading mechanism by which anticancer nutrients exert their action (20,36). Although colon cancer is uniformly amenable to surgery if diagnosed at the early stages, advanced carcinomas are lethal, with metastases to the liver, which is the most common cause of death (37). Thus, our present finding that dietary SeMet induced exon-specific DNA hypermethylation of the p53 but not the β-actin gene in a Se dose-response manner is extremely important in both liver and colon mucosa and has several biological implications. It has been documented that breaks in genomic DNA within exons 5–8 of the p53 and exons 2–3 of the β-actin gene are associated with hypomethylation, and genomic DNA stability within this region is directly correlated with hypermethylation (38,39). Therefore, dietary SeMet may increase the genomic p53 DNA stability, which is critical to prevent DNA mutation, and may subsequently reduce the risk of colon cancer and its metastasis to the liver. This effect appears to be gene specific, because dietary SeMet did not affect the DNA methylation status of the housekeeping β-actin gene, under the same conditions. It is also important to know that exon-specific DNA methylation of the p53 gene in rats fed the S diet was significantly greater than that in rats fed the D diet (but not those fed the A diet). This observation further suggests that the failure of Se to reduce cancer risk in the SELECT trial may be due to the high-plasma Se concentration of the participants at baseline (7,9,10). In addition, our data may also explain in part the chemopreventive effect of the S diet in previous animal studies (12,28–30). The current reference daily intake is 55 μg/d for a healthy adult in the US (30), and the supranutritional dose will be 300–600 μg/d for a healthy U.S. adult if we extrapolate these experimental animal data (12,28–30). Thus, we think that these doses are physiologically achievable in humans.

In this study, we found that the A and S diets were equally effective at maintaining Se-enzyme activities and the plasma homocysteine concentration, but the S diet resulted in higher tissue Se concentrations than the A diet. In addition, rats fed the S diet had lower global genomic DNA methylation in liver than those fed the D diet. More importantly, our data demonstrated that exon-specific DNA methylation of the p53 gene but not the β-actin gene was dose dependently increased by dietary Se in rat liver and colon mucosa, which may relate to the anticancer action of dietary Se.

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Literature Cited


