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Original Article

Associations between S-adenosylmethionine, S-adenosylhomocysteine, and colorectal adenoma risk are modified by sex

Martha J Shrubsole[1,2,3,4], Conrad Wagner[5], Xiangzhu Zhu[1,2], Lifang Hou[6], Lioudmila V Loukachevitch[5], Reid M Ness[4,7], Wei Zheng[1,2,3,4]

[1]Division of Epidemiology, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN, USA; [2]Vanderbilt Epidemiology Center, Nashville, TN, USA; [3]Vanderbilt-Ingram Cancer Center, Nashville, TN, USA; [4]Geriatric, Research, Education and Clinical Center (GRECC), Department of Veterans Affairs, Tennessee Valley Healthcare System, Nashville, TN, USA; [5]Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN, USA; [6]Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, USA; [7]Division of Gastroenterology, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN, USA

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Abstract: Methionine metabolism is an important component of one-carbon metabolism. S-adenosylmethionine (SAM), the methyl donor for nearly all methylation reactions, is irreversibly converted to S-adenosylhomocysteine (SAH), an inhibitor of methyltransferases, some of which are key enzymes for methylation. Changes in DNA methylation are common in colorectal cancers. We evaluated plasma SAM and SAH with colorectal adenoma risk in a matched case-control study conducted among individuals undergoing routine colonoscopy. 216 cases were individually matched to polyp-free controls in a 1:1 ratio on age (± 5 years), sex, race (white/non-white), study site (academic medical center/VA hospital) and date of sample collection (± 60 days). Sex-specific quantiles were evaluated based on the control distribution due to vastly different metabolite levels by sex. Conditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs). Among males, both higher SAM (OR = 0.38, 95% CI: 0.18-0.77, p for trend = 0.007) and higher SAH (OR = 0.45, 95% CI: 0.22-0.91, p for trend = 0.02) were associated with statistically significantly decreased risks of colorectal adenoma in comparison to lowest plasma SAM or SAH tertile. Conversely, among females, both higher SAM and higher SAH were associated with increased risk of colorectal adenoma, which was statistically significant for SAH (OR = 5.18, 95% CI: 1.09-24.62, p for trend = 0.04). The difference in these associations between men and women was statistically significant ($p < 0.05$). The ratio of SAM/SAH was not associated with colorectal adenoma risk among males or females. These findings suggest SAM and SAH may be involved in the development of colorectal adenoma and the association may be modified by sex.

Keywords: Methionine, colorectal adenoma, S-adenosylmethionine, S-adenosylhomocysteine, epidemiology, biomarker, sex difference

Introduction

Methionine metabolism is a key component of one carbon metabolism. Methionine and its metabolic derivatives regulate the activity of most methyltransferases and the activity of several enzymes involved in one-carbon metabolism. It is integrally involved in most physiologic methylation reactions including DNA and RNA methylation [1]. S-adenosylmethionine (SAM or AdoMet), the direct metabolite of methionine, is the methyl donor for nearly all methylation reactions in the body. SAM is irreversibly converted to S-adenosylhomocysteine (SAH or AdoHcy), the metabolic precursor of homocysteine (Hcy). SAH is a potent inhibitor of all methyltransferases. In some studies, as the SAM: SAH ratio decreases, so too does the methylating capacity of the cell, thus the ratio has been proposed as a measure of the methylating capacity or potential [2] although this has not been supported by all studies [3]. A low ratio

has also been associated with increased risks of chronic kidney disease and cardiovascular disease [4, 5]. Given the role of DNA methylation during colorectal carcinogenesis [6], it is conceivable that SAM and SAH are involved in colorectal carcinogenesis.

In the few studies that have evaluated methionine intake and risk of colorectal cancer [7-15], intake has largely not been associated with risk. Very few studies have evaluated biological measures of methionine or its metabolites with colorectal neoplasia. Most studies of Hcy have been null [16-20], although there are some exceptions [21-23]. A large cross-sectional study found plasma methionine was associated with a reduced risk of distal colorectal adenoma [24]. Previous studies indicate SAH is a more sensitive biomarker than Hcy in predicting disease risk [4, 5, 25]. To our best knowledge, no study has evaluated SAM, SAH, or their ratio with colorectal cancer or adenoma. In this study, we evaluated the plasma levels of these markers with risk for colorectal adenoma using a colonoscopy-based, matched, case-control design.

Materials and methods

Study design

The Tennessee Colorectal Polyp Study is a large case-control study of colorectal adenoma. The study design has been described in detail previously [26]. Briefly, participants who received colonoscopy as part of routine care at Vanderbilt University or the Veterans Affairs Tennessee Valley Medical Center between 2003 and 2010 were approached for participation in the study. Eligible participants were aged 40-75 years, and had no prior histories of genetic colorectal cancer syndromes (e.g., hereditary nonpolyposis colorectal cancer or familial adenomatous polyposis), inflammatory bowel disease, adenomatous polyps, or any cancer other than nonmelanoma skin cancer. The study was approved by relevant committees for the use of human subjects in research.

Among 12,585 eligible persons, 7,621 (61%) provided written informed consent and participated in at least one component of the study including biological sample collection, and questionnaire data collection. Questionnaire data were collected using a self-administered semi-quantitative food frequency questionnaire (78%) and an interviewer-administered telephone questionnaire (84% of participants) which solicited information on personal and familial health history, physical activity and anthropometrics, smoking and alcohol drinking practices, meat consumption, reproductive history (females only), non-steroidal anti-inflammatory drug use, and demographic information. Participants and nonparticipants were similar with respect to age, sex, and study location. On the basis of the colonoscopy and pathology findings, participants were assigned as polyp-free controls, cases with adenomatous polyps or persons with other diagnoses. In order to be diagnosed as a control, the participant must have had a complete colonoscopy reaching the cecum and have been polyp-free at colonoscopy.

For the present study, controls were individually matched to randomly selected adenoma cases in a 1:1 ratio on age (± 5 years), sex, race (white/non-white), study site (academic medical center/VA hospital) and date of sample collection (± 60 days). The sample sizes were limited to 216 adenoma cases and 216 polyp-free controls due to funding constraints. Included were 159 male pairs and 57 female pairs.

Measurement of plasma methionine metabolites

Fasting blood samples were collected at colonoscopy. Blood was drawn into an EDTA tube and placed on ice. Whole blood was separated into plasma, buffy coats (white cells), and red blood cells. Samples were processed within 6 hours of collection and stored for future analyses in -80°C freezer. 5824 participants provided a blood sample (87.7% of participants recruited at colonoscopy). Samples with visible hemolysis were excluded from sample selection. Laboratory staff was blinded to the case-control status of plasma samples and the identity of quality control samples included in the study.

SAM and SAH were measured in plasma using a modified method we previously described [27]. Briefly, thawed samples were clarified by centrifugation at 14,000 x g for 10 min at 4°C to remove any precipitate. Samples were diluted with cold 0.1 M Na-phosphate buffer, pH 8.3 and then allowed to flow by gravity through

Table 1. Characteristics of cases and controls, Tennessee Colorectal Polyp Study, 2003-2010

Characteristic	Case (n = 216)	Control (n = 216)	*p*-value[a]
Matching Factors			
Age, mean ± SD	58.0 (6.8)	57.2 (6.9)	
White, %	97.2	97.2	
Male, %	73.6	73.6	
Academic Medical Center Recruitment, %	65.3	65.3	
Other Factors			
Indication for Colonoscopy, %			0.34
Screening	57.4	62.0	
Family History	14.8	9.3	
Diagnostic	19.4	19.9	
Other	8.3	8.8	
Education, %			0.78
Less than high school graduate	26.4	23.6	
High school graduate	27.3	28.2	
College graduate	22.2	20.8	
Graduate or professional school	24.1	27.3	
Household Income, %			0.01
0-29,999	31.9	20.1	
30,000-49,999	17.6	23.0	
50,000-74,999	13.3	12.4	
≥ 75,000	37.1	44.5	
Family History of Colorectal Neoplasia, %			0.45
No family history	72.4	77.6	
Only polyps	19.0	15.2	
Colorectal cancer	8.6	7.1	
Cigarette Smoking Status, %			0.02
Never smoker	35.6	44.7	
Former smoker	40.3	40.5	
Current smoker	24.1	14.9	
Multivitamin Use, %	46.9	59.2	0.02
Regular Alcohol Use, %	50.9	56.1	0.23
Regular NSAID Use, %	57.9	59.7	0.69
Height (m), mean (SD)	1.8 (0.1)	1.8 (0.1)	0.93
BMI (kg/m^2), mean (SD)	28.3 (5.6)	27.6 (5.0)	0.19
Metabolic Equivalent of Task (MET) – h/week/year in past 10 yrs, mean (SD)	18.3 (28.9)	18.1 (27.2)	0.97
Postmenopausal[b], %	66.7	71.9	0.47
Daily Total Energy Intake (kcal), mean (SD)	2355 (1195)	2196 (967)	0.38
Daily Red Meat Intake (g), mean (SD)	69.7 (62.6)	60.1 (78.8)	0.14

[a]*P*-value derived from ANOVA for continuous variables and conditional logistic regression models for categorical variables. [b]Among females only.

Varian Bond Elut 1 ml phenylboronic cartridges. Next, the samples were washed 4 times with 1 ml of the 0.1 M Na-phosphate buffer and 3 times with 1 ml of water. Adsorbed material was eluted with 1 ml of 4 N formic acid then collected into microcentrifuge tubes and dried. Dried samples were dissolved in 0.6 ml 0.12 M Na-borate buffer, pH 9.0 and filtered with 0.22 μm Spin-X Centrifuge Tube Filters (Costar). Samples were then isoindole derivatized and HPLC of the derivatized samples was used to measure SAM and SAH. Within-assay coefficients of variance (CV) are 5.7% for SAM and 7.1% for SAH. Between-assay CVs are 5.1% for SAM and 22.1% for SAH.

Statistical analysis

Baseline covariates that included known risk factors for colorectal adenoma or cancer and other characteristics of participants that had previously been identified as confounders in the parent study were compared between cases and controls to identify potential confounding factors. Factors which were associated with both case control status and with SAM or SAH level were treated as potential con-

Table 2. Sex-specific associations of plasma SAM, SAH, and their ratio with risk of colorectal adenoma, Tennessee Colorectal Polyp Study, 2003-2010

Analyte	Sex-Specific Analyte Level OR (95% CI)			p for trend	p for interaction[c]
	Tertile 1 (low)	Tertile 2	Tertile 3 (high)		
MALES					
SAM					
Median, nM	63.2	86.0	119.9		
Case/control	63/53	55/53	41/53		
OR (95% CI) Model[a]	1.00 (ref)	0.75 (0.42-1.35)	0.59 (0.33-1.06)	0.08	0.04
OR (95% CI) Model[b]	1.00 (ref)	0.53 (0.27-1.02)	0.38 (0.18-0.77)	0.007	
SAH					
Median, nM	19.7	27.5	36.6		
Case/control	70/53	45/53	44/53		
OR (95% CI) Model[a]	1.00 (ref)	0.59 (0.34-1.04)	0.53 (0.28-0.99)	0.04	0.02
OR (95% CI) Model[b]	1.00 (ref)	0.54 (0.29-1.01)	0.45 (0.22-0.91)	0.02	
SAM/SAH ratio					
Median	2.5	3.2	4.4		
Case/control	51/53	53/53	55/53		
OR (95% CI) Model[a]	1.00 (ref)	1.05 (0.58-1.89)	1.11 (0.60-2.06)	0.75	0.48
OR (95% CI) Model[b]	1.00 (ref)	1.16 (0.58-2.32)	1.06 (0.53-2.12)	0.89	
FEMALES					
SAM					
Median, nM	65.5	84.5	96.8		
Case/control	11/19	21/19	25/19		
OR (95% CI) Model[a]	1.00 (ref)	2.65 (0.84-8.40)	2.79 (0.89-8.82)	0.10	
OR (95% CI) Model[b]	1.00 (ref)	2.24 (0.65-7.79)	2.38 (0.66-8.60)	0.20	
SAH					
Median, nM	15.5	20.2	29.2		
Case/control	9/19	18/20	30/18		
OR (95% CI) Model[a]	1.00 (ref)	3.16 (0.87-11.65)	3.99 (1.12-14.24)	0.04	
OR (95% CI) Model[b]	1.00 (ref)	3.45 (0.77-15.40)	5.18 (1.09-24.62)	0.04	
SAM/SAH ratio					
Median	3.2	3.9	4.9		
Case/control	22/19	17/19	18/19		
OR (95% CI) Model[a]	1.00 (ref)	0.80 (0.27-2.35)	1.07 (0.36-3.19)	0.86	
OR (95% CI) Model[b]	1.00 (ref)	0.74 (0.20-2.74)	1.00 (0.27-3.84)	0.92	

[a]Adjusted for age (years). [b]Adjusted for age (years), body mass index (kg/m^2), cigarette smoking status (never, former, current), and income (0-$29,999, $30,000-$49,999, $50,000-$75,000, ≥ $75,000). [c]P for interaction comparing individual metabolite between males and females.

founding variables. Continuous variables were evaluated in ANOVA. Conditional logistic regression was used to analyze the association between characteristic of participants or SAM and SAH level and the risk of colorectal adenoma. Quantiles (median and tertiles) of metabolite levels were derived using the control distribution. Because of the large differences between males and females in metabolite levels, sex-specific quantiles are presented in all tables although models using common quantiles were also evaluated. Models evaluating SAM or SAH were adjusted for age (model 1) or for additional potential confounding factors (model 2) which are listed in the footnotes of relevant tables. Analyses were stratified by sex. Tests for trend were performed by entering the categorical variables as continuous variables in the model. Tests for multiplicative interaction were also performed. The above-mentioned statistical data analysis was performed with SAS 9.3 software (SAS Institute, Cary, NC). All of

Table 3. Joint association of plasma SAM and SAH with colorectal adenoma risk, Tennessee Colorectal Polyp Study, 2003-2010

	Low SAM Low SAH	Low SAM High SAH	High SAM Low SAH	High SAM High SAH	p for trend
Males[a]	1.00 (ref)	0.99 (0.47-2.09)	0.53 (0.21-1.33)	0.51 (0.26-1.00)	0.03
Females[a]	1.00 (ref)	1.17 (0.11-12.36)	3.42 (0.78-15.02)	2.11 (0.58-7.71)	0.14

[a]Adjusted for age (years), body mass index (kg/m^2), cigarette smoking status (never, former, current), and income (0-$29,999, $30,000-$49,999, $50,000-$75,000, ≥ $75,000).

the reported *P* values were two-tailed, and statistical significance was set at 0.05.

Results

Characteristics of cases and controls are presented in **Table 1**. Compared with controls, cases were more likely to have smoked cigarettes and to have lower household income. Although not statistically significant, cases also had higher red meat consumption and higher body mass index (BMI) than their matched controls.

Among controls, males (n = 159 pairs) had significantly higher SAH levels and significantly lower SAM/SAH ratio than females (n = 57 pairs) (p < 0.001 for both measures; results not shown in table). Thus, stratified analyses by sex were conducted. Sex-specific associations for SAM, SAH, and their ratio with risk for adenoma are presented in **Table 2**. Among males, both higher SAM (OR = 0.38, 95% CI: 0.18-0.77, p for trend = 0.007) and higher SAH (OR = 0.45, 95% CI: 0.22-0.91, p for trend = 0.02) were associated with statistically significantly decreased risks of colorectal adenoma in comparison to low plasma SAM or SAH. Conversely, among females, both higher SAM and higher SAH were associated with increased risk of colorectal adenoma, which was statistically significant for SAH (OR = 5.18, 95% CI: 1.09-24.62, p for trend = 0.04). The ratio of SAM/SAH was not associated with colorectal adenoma risk among males or females. For both SAM and SAH, statistically significant interactions between sex, analyte, and adenoma risk were observed (p = 0.04 and 0.02, respectively). The sex-specific associations with SAM or SAH level were similar, although slightly attenuated, when common quantiles were used for males and females (results not shown in table). In the subset of participants with dietary data, adjustment for multivitamin use or dietary intakes of folate, vitamin B6, and vitamin B12 did not appreciably alter point estimates and did not alter statistical significance (results not shown in table). Cigarette smoking history, multivitamin use, and hormone replacement therapy were evaluated as possible explanatory variables for the observed sex differences, however, there was no interaction between SAM or SAH and colorectal adenoma risk for these factors (results not shown in table).

The joint association between plasma SAM, plasma SAH, and colorectal adenoma risk is presented in **Table 3** using median cutpoints. In comparison to those with both the lowest SAM and lowest SAH levels, males with both high SAM and high SAH had an approximately 49% reduced risk of colorectal adenoma (OR = 0.51, 95% CI: 0.26-1.00, p for trend = 0.03). Among females, high SAM was associated with non-significant increased risk of colorectal adenoma in comparison to females with both low SAM and low SAH levels.

Discussion

In this study, the findings differed by sex. Similar to other studies [28], women had lower SAM levels than men. Higher plasma levels of SAM or SAH were associated with reduced risk for colorectal adenoma among men. Among women, higher plasma levels tended to be associated with increased risk of adenoma and this difference was statistically significant. The findings are biologically plausible. Women have been found to have higher rates of transmethylation of SAM and remethylation of Hcy [29] indicating that the utilization of methionine metabolites and associated pathways may differ between the sexes. Oral administration of estradiol to post-menopausal women did not alter SAM, SAH, or their ratio [30] indicating that estrogen, per se, may not explain sex differences. However, in mice, hypophysectomy removes sex differences in glycine n-methyltransferase (GNMT) expression, an enzyme that regulates SAM levels, suggesting a possible role for androgens [31] in one-carbon metabolism.

Female mice also have more remethylation of Hcy to methionine through the choline-betaine pathway than male mice [32]. Thus, although there are experimental studies which demonstrate sex differences in methionine metabolism, the mechanism(s) for their impact on colorectal adenoma risk is not known. Further studies are needed to confirm or refute the sex differences in colorectal carcinogenesis observed in this study.

SAM is the primary methyl donor for most methylation reactions, including methylation of DNA, a global and gene-specific event important in physiology and colorectal carcinogenesis, including CpG island methylator phenotype (CIMP) cancers [6]. In this study, a higher level of plasma SAM was associated with a 60% decreased adenoma risk among men which would support the theory that adequate SAM is necessary for maintenance of colorectal health. SAH which is closely related to SAM was also associated with a decreased risk of adenoma in men. SAH is the demethylated metabolite of SAM and the precursor to Hcy. SAH is an inhibitor of all SAM-mediated methyltransferases. A few small previous studies described the ratio of SAM:SAH as a marker of methylating potential and observed associations with disease status [4, 5]. However, there was no association between the ratio and risk for adenoma in this study.

Further, in joint analysis, the protective association with plasma levels appeared to be primarily due to SAM and not SAH. SAM is also an allosteric inhibitor of methylenetetetrahydrofolate reductase (MTHFR), which is the enzyme for conversion of 5,10-methyltetrahydrofolate (MTHF) to 5-MTHF. Therefore, it is possible that among men and in the context of ample availability of SAM, MTHFR activity is reduced and folate is available for nucleotide synthesis instead of for remethylation of Hcy. In theory, this could result in adequate availability of important substrates for methylation, DNA synthesis, and DNA repair and, thus, decrease risk of colorectal polyps. However, the mechanism is not clear and this finding needs to be confirmed in future studies.

It is possible that a single blood sample collected from study participants at the time of colonoscopy and after their use of a colon cleansing preparation may not be an adequate representation of long-term levels. To address this issue, we also measured SAM and SAH prior to colon cleansing preparation in 10 paired samples and the correlation between the two collection times was high ($r = 0.89$ for SAH, $r = 0.79$ for SAM) consistent with a previous study which included repeated measurements of SAM and SAH [28]. As in any case-control study, the possibility that the disease status affected levels of the metabolites cannot be refuted. However, our findings of dissimilar associations for both SAM and SAH in men and women also argue against our observations being due to measurement errors or reverse causation. Participants were recruited prior to diagnosis which decreases the likelihood of selection bias affecting the findings. Further, all participants underwent a complete colonoscopy which reduces the likelihood of misclassification of disease status.

In summary, higher plasma SAM and SAH were associated with a decreased risk of colorectal adenoma among men. Increased levels of SAM or SAH may be a risk factor among women. To our best knowledge, this is the first study to evaluate SAM or SAH with risk of colorectal neoplasms. Future prospective studies with larger sample sizes are needed to confirm these findings and to evaluate the potential effect modification by sex.

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Disclosure of conflict of interest

None to declare.

Address correspondence to: Martha J Shrubsole, Vanderbilt Epidemiology Center, Eighth Floor, Suite 800, 2525 West End Avenue, Nashville, TN 37203-1738. Tel: 615-936-0812; Fax: 615-936-8288; E-mail: martha.shrubsole@vanderbilt.edu

References

[1] Chiang PK, Gordon RK, Tal J, Zeng GC, Doctor BP, Pardhasaradhi K, McCann PP. S-Adenosylmethionine and methylation. FASEB J 1996; 10: 471-80.

[2] Caudill MA, Wang JC, Melnyk S, Pogribny IP, Jernigan S, Collins MD, Santos-Guzman J, Swendseid ME, Cogger EA, James SJ. Intracellular S-adenosylhomocysteine concentrations predict global DNA hypomethylation in tissues of methyl-deficient cystathionine beta-synthase heterozygous mice. J Nutr 2001; 131: 2811-8.

[3] Hübner U, Geisel J, Kirsch SH, Kruse V, Bodis M, Klein C, Herrmann W, Obeid R. Effect of 1 year B and D vitamin supplementation on LINE-1 repetitive element methylation in older subjects. Clin Chem Lab Med 2013; 51: 649-55.

[4] Kerins DM, Koury MJ, Capdevila A, Rana S, Wagner C. Plasma S-adenosylhomocysteine is a more sensitive indicator of cardiovascular disease than plasma homocysteine. Am J Clin Nutr 2001; 74: 723-9.

[5] Wagner C, Stone WJ, Koury MJ, Dupont WD, Kerins DM. S-adenosylhomocysteine is a more sensitive indicator of renal insufficiency than homocysteine. Nutrition Research 2004; 24: 487-94.

[6] Lao VV, Grad-y WM. Epigenetics and colorectal cancer. Nat Rev Gastroenterol Hepatol 2011; 8: 686-700.

[7] Razzak AA, Oxentenko AS, Vierkant RA, Tillmans LS, Wang AH, Weisenberger DJ, Laird PW, Lynch CF, Anderson KE, French AJ, Haile RW, Harnack LJ, Potter JD, Slager SL, Smyrk TC, Thibodeau SN, Cerhan JR, Limburg PJ. Associations between intake of folate and related micronutrients with molecularly defined colorectal cancer risks in the Iowa Women's Health Study. Nutr Cancer 2012; 64: 899-910.

[8] Terry P, Jain M, Miller AB, Howe GR, Rohan TE. Dietary intake of folic acid and colorectal cancer risk in a cohort of women. Int J Cancer 2002; 97: 864-7.

[9] Slattery ML, Curtin K, Anderson K, Ma KN, Edwards S, Leppert M, Potter J, Schaffer D, Samowitz WS. Associations between dietary intake and Ki-ras mutations in colon tumors: a population-based study. Cancer Res 2000; 60: 6935-41.

[10] Jiang Q, Chen K, Ma X, Li Q, Yu W, Shu G, Yao K. Diets, polymorphisms of methylenetetrahydrofolate reductase, and the susceptibility of colon cancer and rectal cancer. Cancer Detect Prev 2005; 29: 146-54.

[11] Kune G, Watson L. Colorectal cancer protective effects and the dietary micronutrients folate, methionine, vitamins B6, B12, C, E, selenium, and lycopene. Nutr Cancer 2006; 56: 11-21.

[12] Flood A, Caprario L, Chaterjee N, Lacey JV Jr, Schairer C, Schatzkin A. Folate, methionine, alcohol, and colorectal cancer in a prospective study of women in the United States. Cancer Causes Control 2002; 13: 551-61.

[13] La Vecchia C, Negri E, Pelucchi C, Franceschi S. Dietary folate and colorectal cancer. Int J Cancer 2002; 102: 545-7.

[14] Konings EJM, Goldbohm RA, Brants HAM, Saris WHM, van den Brandt PA. Intake of dietary folate vitamers and risk of colorectal carcinoma: results from The Netherlands Cohort Study. Cancer 2002; 95: 1421-33.

[15] Ishihara J, Otani T, Inoue M, Iwasaki M, Sasazuki S, Tsugane S. Low intake of vitamin B-6 is associated with increased risk of colorectal cancer in Japanese men. J Nutr 2007; 137: 1808-14.

[16] Levine AJ, Grau MV, Mott LA, Ueland PM, Baron JA. Baseline plasma total homocysteine and adenoma recurrence: results from a double blind randomized clinical trial of aspirin and folate supplementation. Cancer Epidemiol Biomarkers Prev 2010; 19: 2541-8.

[17] Weinstein SJ, Albanes D, Selhub J, Graubard B, Lim U, Taylor PR, Virtamo J, Stolzenberg-Solomon R. One-carbon metabolism biomarkers and risk of colon and rectal cancers. Cancer Epidemiol Biomarkers Prev 2008; 17: 3233-40.

[18] Kato I, Dnistrian AM, Schwartz M, Toniolo P, Koenig K, Shore RE, Akhmedkhanov A, Zeleniuch-Jacquotte A, Riboli E. Serum folate, homocysteine and colorectal cancer risk in women: a nested case-control study. Br J Cancer 1999; 79: 1917-22.

[19] Le Marchand L, White KK, Nomura AMY, Wilkens LR, Selhub JS, Tiirikainen M, Goodman MT, Murphy SP, Henderson BE, Kolonel LN. Plasma levels of B vitamins and colorectal cancer risk: the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev 2009; 18: 2195-201.

[20] Dahlin AM, Van Guelpen B, Hultdin J, Johansson I, Hallmans G, Palmqvist R. Plasma vitamin B12 concentrations and the risk of colorectal cancer: a nested case-referent study. Int J Cancer 2008; 122: 2057-61.

[21] Ulvik A, Vollset SE, Hansen S, Gislefoss R, Jellum E, Ueland PM. Colorectal cancer and the methylenetetrahydrofolate reductase 677C -> T and methionine synthase 2756A -> G polymorphisms: a study of 2,168 case-control pairs from the JANUS cohort. Cancer Epidemiol Biomarkers Prev 2004; 13: 2175-80.

[22] Martínez ME, Giovannucci E, Jiang R, Henning SM, Jacobs ET, Thompson P, Smith-Warner SA, Alberts DS. Folate fortification, plasma folate, homocysteine and colorectal adenoma recurrence. Int J Cancer 2006; 119: 1440-6.

[23] Bobe G, Murphy G, Rogers CJ, Hance KW, Albert PS, Laiyemo AO, Sansbury LB, Lanza E, Schatzkin A, Cross AJ. Serum adiponectin, leptin, C-peptide, homocysteine, and colorectal adenoma recurrence in the Polyp Prevention Trial. Cancer Epidemiol Biomarkers Prev 2010; 19: 1441-52.

[24] De Vogel S, Schneede J, Ueland PM, Vollset SE, Meyer K, Fredriksen A, Midttun Ø, Bjørge T, Kampman E, Bretthauer M, Hoff G. Biomarkers related to one-carbon metabolism as potential risk factors for distal colorectal adenomas. Cancer Epidemiol Biomarkers Prev 2011; 20: 1726-35.

[25] Jabs K, Koury MJ, Dupont WD, Wagner C. Relationship between plasma S-adenosylhomocysteine concentration and glomerular filtration rate in children. Metab Clin Exp 2006; 55: 252-7.

[26] Shrubsole MJ, Wu H, Ness RM, Shyr Y, Smalley WE, Zheng W. Alcohol drinking, cigarette smoking, and risk of colorectal adenomatous and hyperplastic polyps. Am J Epidemiol 2008; 167: 1050-8.

[27] Capdevila A, Wagner C. Measurement of plasma S-adenosylmethionine and S-adenosylhomocysteine as their fluorescent isoindoles. Anal Biochem 1998; 264: 180-4.

[28] Poirier LA, Wise CK, Delongchamp RR, Sinha R. Blood determinations of S-adenosylmethionine, S-adenosylhomocysteine, and homocysteine: correlations with diet. Cancer Epidemiol Biomarkers Prev 2001; 10: 649-55.

[29] Fukagawa NK, Martin JM, Wurthmann A, Prue AH, Ebenstein D, O'Rourke B. Sex-related differences in methionine metabolism and plasma homocysteine concentrations. Am J Clin Nutr. 2000; 72: 22-9.

[30] Smolders RGV, de Meer K, Kenemans P, Jakobs C, Kulik W, van der Mooren MJ. Oral estradiol decreases plasma homocysteine, vitamin B6, and albumin in postmenopausal women but does not change the whole-body homocysteine remethylation and transmethylation flux. J Clin Endocrinol Metab 2005; 90: 2218-24.

[31] Aida K, Tawata M, Negishi M, Onaya T. Mouse glycine N-methyltransferase is sexually dimorphic and regulated by growth hormone. Horm Metab Res 1997; 29: 646-9.

[32] Chew TW, Jiang X, Yan J, Wang W, Lusa AL, Carrier BJ, West AA, Malysheva OV, Brenna JT, Gregory JF 3rd, Caudill MA. Folate intake, MTHFR genotype, and sex modulate choline metabolism in mice. J Nutr 2011; 141: 1475-81.