

Original Article

One-carbon metabolism nutrient status and plasma S-adenosylmethionine concentrations in middle-aged and older Chinese in Singapore

Maki Inoue-Choi¹, Heather H Nelson^{1,2}, Kim Robien^{1,2}, Erland Arning³, Teodoro Bottiglieri³, Woon-Puay Koh⁴, Jian-Min Yuan^{1,2,5}

¹Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, Minnesota, United States; ²Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota, United States; ³Institute of Metabolic Disease, Baylor Research Institute, Dallas, Texas, United States; ⁴Saw Swee Hock School of Public Health, National University of Singapore, Singapore; ⁵Present affiliation and address: Cancer Control and Population Sciences, University of Pittsburgh Cancer Institute and Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, Pennsylvania, USA.

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Abstract: S-adenosylmethionine (SAM) is a primary methyl donor for the methylation of many molecules including DNA. DNA methylation is believed to play an important role in functions of cells and genes. Dietary, genetic and metabolic factors that influence systematic SAM levels are not fully understood. We conducted cross-sectional analysis to evaluate associations between plasma concentrations of one-carbon metabolism nutrients and metabolites and plasma SAM concentrations using healthy individuals within the Singapore Chinese Health Study. Plasma SAM, betaine, choline, folate, total homocysteine (Hcy), methionine, S-adenosylhomocysteine (SAH), vitamin B₆ and vitamin B₁₂ concentrations were quantified. Genotypes of methionine adenosyltransferases (*MAT1A*, *MAT2A* and *MAT2B*) were also determined. Linear regression and path analysis were performed to depict the directed dependencies in one-carbon metabolism. Age and body mass index were positively associated while cigarette smoking were inversely associated with plasma SAM concentrations. Plasma choline, methionine and SAH were positively and strongly associated with plasma SAM after adjustment for confounders. Plasma betaine and folate were positively associated with plasma SAM only in men. Men carrying the variant *MAT1A* genotypes had lower plasma SAM concentrations than men carrying the wild type genotype (p for gene x gender interaction = 0.02). This effect modification by gender was restricted to individuals with low plasma methionine. In conclusion, plasma choline, methionine and SAH were strongly associated with plasma SAM concentrations. The *MAT1A* genetic polymorphism may impact plasma SAM concentrations in men with low plasma methionine concentrations.

Keywords: One-carbon metabolism, plasma SAM, *MAT* genetic polymorphism, path analysis

Introduction

S-adenosylmethionine (SAM) is a key metabolite in one-carbon metabolism (OCM) (Figure 1). OCM consists of two major pathways – folate metabolism and the methylation cycle – which are connected via the methylation of homocysteine (Hcy) to methionine. Hcy is a sulfhydryl-containing amino acid derived from the metabolic demethylation of methionine. It can be remethylated back to methionine, which is subsequently converted to SAM to maintain methyl group supply for methylation reactions.

SAM provides methyl groups for nearly all biochemical reactions, including methylation of more than 80 molecules such as DNA, RNA, proteins, histones and neurotransmitters [1, 2]. After transfer of methyl groups to DNA methylation, SAM is converted to S-adenosylhomocysteine (SAH), a competitive inhibitor of DNA methylation [3]. DNA methylation influences gene expression, DNA integrity and stability, chromosomal modifications and mutations [4]. Aberrations in DNA methylation, at both gene-specific and global levels, play a crucial role in carcinogenesis [5, 6]. Global DNA

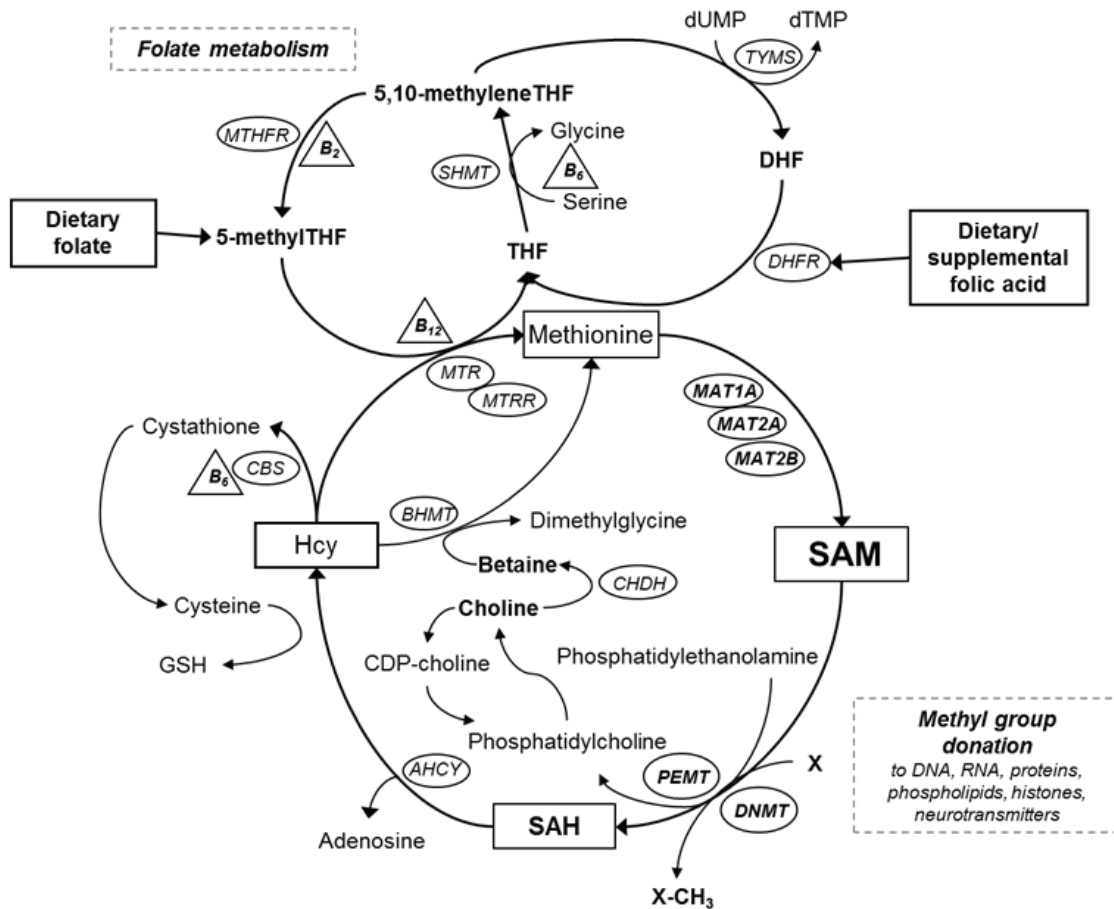


Figure 1. Overview of one-carbon metabolism and choline metabolism. AHCY = S-adenosylhomocysteine hydrolase; BHMT = betaine hydroxymethyltransferase; CBS = cystathione β -synthase; CDP-choline = cytidine diphosphocholine; CHDH = choline dehydrogenase; DHFR = dihydrofolate reductase; DNMT = DNA methyltransferase; GSH = glutathione; Hcy = homocysteine; MAT1A= methionine adenosyltransferase 1A; MAT2A = methionine adenosyltransferase 2A; MAT2B = methionine adenosyltransferase 2B; MTA = methylthioadenosine; MTHFR = methylenetetrahydrofolate reductase; MTR = methionine synthase; MTRR = methionine synthase reductase; PEMT = phosphatidylethanolamine methyltransferase; SAM = S-adenosylmethionine; SAH = S-adenosylhomocysteine; SHMT = serine hydroxymethyltransferase; THF = tetrahydrofolate; TYMS = thymidylate synthase; dTMP = deoxythymidine monophosphate; dUMP = deoxyuridine monophosphate

hypomethylation has been associated with many types of cancer including bladder, breast, colon, esophagus, head and neck, liver, lung, prostate and stomach [7, 8], and observed in cancer tissues, benign tumor tissues and normal tissues surrounding cancer tissues, indicating that global DNA hypomethylation may be an early event in carcinogenesis [9, 10].

Methionine is a precursor of SAM and can be derived from diet as well as the remethylation of Hcy in which a methyl group is transferred from either 5-methyl tetrahydrofolate (THF) or be-

taine, a choline derivative [11, 12]. In addition, vitamin B groups (vitamin B₂, B₆ and B₁₂) are cofactors for the remethylation of Hcy and other reactions [11]. Therefore, the availability of SAM, as a methyl donor for DNA methylation, depends on the availability of these nutrients involved in OCM. Nutritional studies have shown that low dietary intake of OCM nutrients resulted in decreased levels of SAM and increased SAH contents in the liver [13, 14].

In addition to nutritional intake, interindividual variation in OCM enzyme activity may impact

SAM availability. The biosynthesis of SAM from methionine is catalyzed by methionine adenosyltransferases (MATs). Three distinct forms of MATs have been identified: MAT I, MAT II and MAT III [15]. MAT I and MAT III are predominantly found in the liver and encoded by *MAT1A*. MAT II, encoded by *MAT2A* and *MAT2B*, can be found in the fetal liver and the tissues of many other organs. Differential expression of these genes has been shown to decrease hepatic and intracellular SAM concentrations and enhance global DNA hypomethylation and cancer cell growth *in vitro* [16-18]. In humans, deficiency of MAT I and MAT III, caused by mutations in the *MAT1A* gene, was associated with persistent hypermethioninemia [19]. A recent study showed that genetic polymorphisms of *MAT1A* were associated with DNA damage, but only among individuals with low plasma vitamin B₆ concentrations [20]. However, human data on genetic variation in MATs and plasma SAM concentrations are limited.

To date, human data describing associations between OCM nutrient/metabolite status and circulating SAM concentrations are limited. We therefore examined the associations between plasma concentrations of OCM nutrients and metabolites as well as *MAT* genotypes and plasma SAM concentrations among healthy adults.

Subjects and methods

Study population

The study subjects were selected from participants of the Singapore Chinese Health Study (SCHS), a population-based prospective cohort study of the role of diet and its interaction with genetic factors in the risk of cancer and other chronic diseases. The detailed study design and subject recruitment of the SCHS have been described elsewhere [11]. In brief, 63,257 Chinese men and women aged 45 – 74 years in Singapore were enrolled from April 1993 through December 1998. Eighty-five percent of eligible individuals participated in the study. At recruitment, information on demographic and lifestyle factors, medical history, family history of cancer and dietary intake was collected through in-person interviews. Usual dietary intake during the previous 12 months was assessed using a structured semi-quantitative food frequency questionnaire (FFQ) which was

specifically designed to assess the dietary habits of Chinese in Singapore and validated through a series of 24-h dietary recalls [21].

We collected biospecimen from a 3% random sample of the cohort participants between 1994 and 1999. A 20-mL blood sample was collected from the cohort participants. Immediately after blood draw, blood tubes were transported from the homes of the subjects to the laboratory at the National University of Singapore, where plasma was separated from whole blood within approximately 4 hours after blood draw. Eight straws of plasma, each with approximately 0.5 mL, were created and stored in a liquid nitrogen tank at -180°C until 2001, when they were moved to -80°C freezers for long-term storage. All samples were kept on dry ice during shipment.

The subjects of the present study were selected from 509 men and women who donated their blood samples in 1994 – 1999. This study was approved by the Institutional Review Boards at the National University of Singapore and the University of Minnesota.

Laboratory measurements

Plasma folate, vitamin B₆ and B₁₂ concentrations were determined at the National University of Singapore in 1996 – 1997. The methods for the assays were described previously [11]. All other plasma biomarker determinations were performed by the Institute of Metabolic Disease at Baylor Research Institute in Dallas, Texas. Total plasma homocysteine (Hcy) concentrations were determined by a rapid, isocratic high performance liquid chromatography (HPLC) coupled to a fluorescence detector [22].

We used stable-isotope dilution liquid chromatography-electrospray ionization (ESI) tandem mass spectrometry (LC-ESI-MS/MS) to determine SAM, SAH, methionine, choline and betaine in plasma. Calibrators and internal standards (²H₃-SAM, ²H₄-SAH, ²H₃-methionine, ²H₃-choline and ²H₃-betaine) were included in each analytical run for calibration. One-mM stock solutions of each standard were diluted in distilled water to perform a 5-point calibration curve (Table 1). Sample preparation utilized microcentrifugal filter units, Microcon YM-10, 10 kDa NMWL (Millipore, USA). Samples were prepared by the addition of 100 µL mobile

Table 1. Mass transitions and method statistics for the determination of plasma one-carbon metabolism nutrients and metabolites

Analyte	Analyte MRM (m/z)	Labeled Isotope	Labeled Isotope MRM (m/z)	Retention Time (minutes)	LOQ (nmol /L)	Calibration Curve	Inter-assay Precision	
							Level 1 (CV %)	Level 2 (CV %)
SAM	399→250	² H ₃ -SAM	402→250	7.1	5	400 - 25 nmol/L	9.2	6.2
SAH	385→136	² H ₄ -SAH	389→138	6.8	5	400 - 25 nmol/L	10.4	5.9
Betaine	118→59	² H ₃ -Bet	121→61	3.5	500	100 - 6.25 μmol/L	6.3	7.3
Choline	104→45	² H ₄ -Chol	108→49	4.9	500	100 - 6.25 μmol/L	6.9	6.7
Methionine	150→104	² H ₃ -Met	153→107	6.5	500	100 - 6.25 μmol/L	6.1	6.4

phase A containing 10 – 50 μmol/L labeled-isotope internal standards to 30 μl of standard or plasma followed by vortex. Microcentrifugal filter units were centrifuged for 20 min at 14800 x g at 4°C. Sample filtrate was transferred to a microtiter plate for analysis. Chromatographic separation was achieved on an EZ-faast 250 x 2.0mm 4μ AAA-MS analytical column (Phenomenex) maintained at 36°C at a flow of 250μL/min with a binary gradient with a total run time of 12 minutes. Solvents for HPLC were: (A) 4mM ammonium acetate, 0.1 % formic acid, 0.1 % heptafluorobutyric acid (pH=2.5); and (B) 100 % methanol and 0.1 % formic acid. The initial gradient condition was 75% A: 25% B and was increased in a linear fashion to 100% B in 6 minutes and held constant for 1 minute. At 7.1 minutes, the mobile phase was reset to initial conditions for 5 minutes. The flow from the column was delivered to the Turbo V source from the period of 3 to 8 minutes, otherwise the flow was diverted to waste. The compounds were detected by multiple reaction monitoring (MRM) using positive-ESI. Sample separation and injection was performed by a Shimadzu Prominence LC System interfaced with a 4000 Q TRAP® LC-MS/MS (ABSciex). All data were collected using Analyst software version 1.4.2. **Table 1** indicates the MRM transitions for each metabolite, internal standards used, retention times and performance (inter-assay precision, n=10) for this method.

SNP selection

We selected common single nucleotide polymorphisms (SNPs) of *MAT1A*, *MAT2A* and *MAT2B*. Given the relatively small study sample size, we

selected common SNPs with minor allele frequency (MAF) ≥20%. Using data from the International HapMap Project (Tagger Pairwise method, HapMap Data Rel 27 Phase II + III, Feb09, on NCBI B36 assembly, dbSNP b126 for the Han Chinese (CHB) population), a total of 3 tag SNPs and their proxies (in parentheses) were selected: rs2993763 and (rs4934028) for *MAT1A*, rs2289972 and (rs2028900) for *MAT2A*, and rs4869089 and (rs7733775) for *MAT2B*.

DNA extraction and Genotype determinations

DNA extraction and genotype determinations were performed by the University of Minnesota BioMedical Genomics Center (BMGC). DNA was extracted from buffy coats using a Qiagen Kit (Qiagen Inc., Valencia, CA). Genotype determinations were performed in multiplex using the Sequenom MALDI-TOF mass spectrometry system (Sequenom Inc., San Diego, CA). Positive and negative controls were included in each 96-well plate. A tag SNP of *MAT2B* (rs4869089) had a low call rate (<95%), and therefore its proxy SNP (rs7733775) was used in the analysis. In this study, we report a total of 3 SNPs – *MAT1A* rs2993763, *MAT2A* rs2289972 and *MAT2B* rs7733775. All 3 SNPs included in the analysis were in Hardy-Weinberg equilibrium ($p \geq 0.05$).

Statistical analysis

Of the 509 subjects, 14 subjects had missing values in plasma SAM concentrations and therefore were excluded. In addition, 19 subjects who had undetectable (n = 9) or extremely high values (n = 10) in all measured biomarkers, and 15 subjects who had missing

(n=14) or extremely high values (n=1) in serum creatinine concentrations were excluded. After these exclusions, 461 subjects were included in the analysis.

The distributions of plasma concentrations of all measured OCM nutrients and metabolites and serum creatinine concentrations were markedly skewed with fewer subjects having high values; therefore, logarithmically transformed values were used when included in the models as covariates to correct the departure from the normal distribution. Plasma concentrations of OCM nutrients and metabolites were compared by gender using multiple linear regression modeling adjusting for age at blood draw and serum creatinine concentrations. Analysis of covariance (ANCOVA) was used to examine the associations between selected demographic, lifestyle and genetic characteristics and plasma SAM concentrations. To test the hypothesis that plasma concentrations of OCM nutrients (betaine, choline, folate, methionine, vitamin B₆ and B₁₂) and OCM metabolites (SAH and Hcy) are associated with plasma SAM concentrations, multiple linear regression modeling was used, adjusting for gender, age at blood draw, body mass index (BMI), smoking status (current, past and never) and serum creatinine concentrations. Geometric means and corresponding 95% confidence intervals (CIs) of plasma SAM concentrations were presented for quartile groups of plasma OCM nutrient or metabolite concentrations. A trend across quartiles was tested using median values of quartile groups. To examine the associations between *MAT* genotypes and plasma SAM concentrations, multiple linear regression modeling was used with adjustment for age at blood draw, gender, BMI, smoking status, plasma methionine concentrations and serum creatinine concentrations. To evaluate interactions with gender and/or plasma methionine concentrations, we included interaction terms in the models, and also performed stratified analysis by gender and plasma methionine concentrations (\leq or $>$ the median (=23.4 $\mu\text{mol/L}$)).

To further depict the directed dependencies among a set of variables involved in the OCM pathway, we analyzed the data using a diagram-based approach of the path analysis [23]. We separated the associations between plasma choline or folate and plasma SAM concentrations. In the choline-SAM pathway and the folate

-SAM pathway, we further broke down the associations between plasma concentrations of choline or folate and plasma SAM concentrations into associations among intermediate molecules on each causal pathway. The final models for the choline-SAM pathway and the folate-SAM pathway were determined incorporating paths that showed statistically significant associations, controlling for age at blood draw, gender, BMI, smoking status and serum creatinine concentrations.

Linear regression analysis and path analysis were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC) and Mplus version 6.1 (Muthen & Muthen, Los Angeles, CA), respectively. All reported *p* values were two-sided, and significance was defined as *p* < 0.05. This study had 80% power to detect 7.1 nmol/L (= 0.39 x standard deviation (SD)) difference in plasma SAM concentrations between the lowest and highest quartiles of plasma OCM nutrient or metabolite concentrations and 12.6 nmol/L (= 0.7 x SD) difference in plasma SAM concentrations between homozygous wild type genotype and homozygous variant genotype of *MATs* assuming 20% MAFs.

Results

The study subjects consist of 204 men and 257 women. The mean age of study subjects was 58 y (SD, 7.8 y; range, 46 – 77 y) at blood draw. About 72% of the subjects had BMI below 24 and only 4% had BMI of 28 or above. While 41% of men had secondary school or higher education, 75% of women had no formal education or only primary school education. About 56% of men were previous or current smokers, whereas the majorities (95%) of women were never smokers. Alcohol intake was not common; 70% and 93% of men and women, respectively, reported no alcohol intake.

Table 2 shows geometric means of plasma SAM and other OCM nutrient/metabolite concentrations in men and women, separately. The median plasma SAM concentrations for all subjects was 63.3 nmol/L (interquartile range (IQR), 54.9 – 75.9 nmol/L). Serum creatinine concentrations were statistically significantly higher in men (geometric mean, 75.6 $\mu\text{mol/L}$; 95%CI, 73.6 – 77.7 $\mu\text{mol/L}$) than women (54.6 $\mu\text{mol/L}$; 53.3 – 55.9 $\mu\text{mol/L}$) (*p* < 0.0001). After adjustment for serum creatinine concentrations and

Table 2. Geometric means and 95% confidence intervals (CIs)^a of plasma one-carbon metabolism nutrients and metabolites^b by gender.

	Men (n = 204)	Women (n = 257)	p
SAM (nmol/L)	63.7 (61.1 – 66.4)	64.8 (61.6 – 66.2)	0.95
Betaine (µmol/L)	52.1 (49.9 – 54.4)	44.5 (42.9 – 46.2)	<0.0001
Choline (µmol/L)	12.9 (12.4 – 13.4)	11.9 (11.5 – 12.3)	0.006
Folate (nmol/L)	12.9 (12.0 – 13.8)	14.6 (13.8 – 15.5)	0.02
Hcy (µmol/L)	10.2 (9.7 – 10.6)	9.3 (9.0 – 9.7)	0.009
Methionine (µmol/L)	25.0 (23.9 – 26.1)	22.6 (21.7 – 23.4)	0.002
SAH (nmol/L)	26.5 (25.2 – 28.0)	29.2 (27.9 – 30.6)	0.02
Vitamin B ₆ (nmol/L)	29.4 (26.5 – 32.6)	36.2 (33.1 – 39.6)	0.009
Vitamin B ₁₂ (pmol/L)	313.5 (294.8 – 333.5)	336.4 (318.9 – 354.8)	0.13

^aAdjusted for age at blood draw and serum creatinine concentrations; ^bOne-carbon metabolism metabolites: SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; Hcy, total homocysteine.

age at blood draw, plasma SAM concentrations were comparable for men and women. Plasma concentrations of betaine, choline, Hcy and methionine were statistically significantly higher in men than women, whereas plasma concentrations of folate, SAH and vitamins B₆ were higher in women than men. Plasma vitamin B₁₂ concentrations did not differ by gender. Among OCM nutrients and metabolites, betaine, choline and methionine were positively correlated each other (Spearman partial correlation coefficients (r), 0.27 – 0.31; data not shown). Similarly, folate and vitamins B₆ and B₁₂ were positively correlated each other (r = 0.16 – 0.31). Hcy was inversely correlated with folate, betaine, and vitamins B₆ and B₁₂ (r = 0.12 – 0.32). Plasma SAM concentrations were positively correlated with plasma concentrations of choline, folate, methionine, SAH, and vitamin B₆ (r = 0.11 – 0.37).

Table 3 describes the associations between selected demographic, lifestyle and genetic characteristics of the study subjects and plasma SAM concentrations in men and women, separately. Higher plasma SAM concentrations were observed in women at older age and in men with high BMI. Current smokers had statistically significantly lower plasma SAM than never smokers in men. The difference in plasma SAM concentrations between current /past and never smokers in women was not statistically significant, most likely due to the very few smokers in women. Alcohol consumption and methylenetetrahydrofolate reductase (*MTHFR*) C677T genotype was not associated with plasma SAM concentrations.

Associations between plasma concentrations of each OCM nutrient or metabolite and plasma SAM concentrations in men, women and all study subjects from linear regression analyses are shown in **Table 4**. Higher plasma concentrations of choline, methionine and SAH were statistically significantly associated with higher plasma SAM concentrations in men and women. Plasma betaine and folate were positively associated with plasma SAM concentrations in men, but not in women. The positive association between plasma vitamin B₆ and SAM was borderline significant in both men and women combined (p for trend = 0.046). Plasma concentrations of Hcy and vitamin B₁₂ were not associated with plasma SAM concentrations in either men or women. We further examined the association between overall availability of methyl-containing nutrients in the choline (betaine)-mediated and the folate-mediated Hcy remethylation pathways and plasma SAM concentrations using a sum of scores for quartiles of plasma betaine, choline and methionine concentrations, or a sum of scores for quartiles of plasma folate and methionine concentrations, respectively: zero was assigned to the lowest quartile and 3 to the highest quartile of each biomarker. In the choline-mediated Hcy remethylation pathway, the geometric means of plasma SAM concentrations were 50.4 nmol/L (95%CI, 47.0 – 54.0 nmol/L) for subjects with the lowest summed score (0 to 1) and 70.0 nmol/L (95%CI, 65.5 – 74.7 nmol/L) for subjects with the highest summed score (8 to 9) in all subjects (p for trend <0.0001) (data not shown). Similarly, in the folate-mediated Hcy remethylation pathway, the geometric means of

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Table 3. Plasma SAM concentrations in men and women by selected demographic, lifestyle and genetic factors

	Men (n = 204)			Women (n = 257)		
	n	Geometric mean (95%CI) (nmol/L) ^a	<i>p</i> _{trend}	n	Geometric mean (95%CI) (nmol/L) ^a	<i>p</i> _{trend}
Age at blood draw						
<50	29	64.9 (59.1 – 71.3)	0.17	43	59.7 (55.3 – 64.5)	0.02
50 – 59	86	67.0 (63.5 – 70.8)		126	58.2 (55.6 – 60.8)	
60 – 69	72	67.2 (63.3 – 71.3)		58	68.0 (63.7 – 72.6)	
≥70	17	74.3 (65.8 – 84.1)		30	63.1 (57.4 – 69.4)	
BMI						
<20	27	63.1 (57.2 – 69.5)	0.02	41	59.0 (54.5 – 63.9)	0.21
20 – <24	119	66.5 (63.5 – 69.6)		147	60.7 (58.3 – 63.3)	
24 – <28	50	71.0 (66.2 – 76.3)		58	63.3 (59.3 – 67.7)	
≥28	8	73.5 (61.5 – 87.8)		11	62.0 (53.2 – 72.3)	
Level of education						
No formal school	26	68.8 (62.0 – 76.4)	0.26	96	60.2 (57.0 – 63.6)	0.59
Primary school	94	68.2 (64.7 – 71.9)		97	61.9 (58.8 – 65.2)	
Secondary school	58	67.0 (62.6 – 71.7)		54	60.4 (56.3 – 64.8)	
Some college or above	26	63.6 (57.4 – 70.5)		10	65.2 (55.5 – 76.6)	
Smoking status						
Never	90	70.9 (67.2 – 74.8)	0.004	243	61.2 (59.2 – 63.2)	0.75
Past	53	67.2 (62.6 – 72.1)		2	56.5 (39.4 – 80.9)	
Current	61	62.6 (58.8 – 66.7)		12	60.0 (51.7 – 69.7)	
Alcohol intake						
None	143	66.7 (63.9 – 69.6)	0.32	239	60.8 (58.9 – 62.9)	0.42
<7 drinks/week	45	68.3 (63.3 – 73.7)		14	65.6 (57.2 – 75.2)	
≥7 drinks/week	16	71.1 (62.6 – 80.7)		4	62.1 (48.2 – 80.0)	
MTHFR C677T						
CC	117	67.5 (64.4 – 70.8)	0.82	139	59.9 (57.4 – 62.5)	0.13
CT	69	67.7 (63.6 – 72.0)		89	61.5 (58.3 – 64.9)	
TT	15	65.7 (57.6 – 75.0)		22	65.7 (58.9 – 73.2)	

^aAdjusted for age at blood draw and serum creatinine concentrations.

plasma SAM concentrations were 56.2 nmol/L (95%CI, 53.3 – 59.2 nmol/L) for those with the lowest score (0 to 1) and 71.7 nmol/L (95%CI, 67.7 – 75.9 nmol/L) for those with the highest score (5 to 6) in all subjects (*p* for trend <0.0001). A similar dose-dependent positive association between the summed score and plasma SAM concentrations was observed in men and women in both pathways (*p* for trend <0.001).

We performed the path analysis for the choline (betaine)-mediated Hcy remethylation pathway for all subjects and the folate-mediated Hcy remethylation pathway in men and women separately, because the association between plasma folate and SAM was different in men and women in the regression analysis. Including plasma Hcy concentrations in the models did

not change the results; therefore Hcy was not included in the final path models. Plasma folate was not statistically significantly associated with plasma SAM through OCM or other unspecified mechanisms in men and women (data not shown). **Figure 2** graphically depicts a final path model for the choline (betaine)-mediated Hcy remethylation pathway. The total association between choline and SAM was statistically significant with a standardized path coefficient of 0.218 (*p* <0.0001). When associations among intermediate molecules were separated, the associations of choline with betaine, methionine or SAM, the association between betaine and methionine as well as the association between methionine and SAM were all statistically significantly positive. The direct path between betaine and SAM was not statistically significant and thus excluded in the final model. When sepa-

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Table 4. Geometric means and 95% confidence intervals (CIs)^a of plasma SAM concentrations (nmol/L) by quartiles of one-carbon metabolism nutrients and metabolites^b

	Quartiles of plasma concentrations				<i>p</i> _{trend}
	1 (lowest)	2	3	4 (highest)	
Betaine					
Men	63.0 (58.8 – 67.6)	65.9 (61.5 – 70.8)	67.7 (63.0 – 72.6)	70.9 (66.2 – 76.0)	0.02
Women	56.2 (48.9 – 64.6)	59.7 (51.7 – 69.0)	57.5 (50.0 – 66.1)	61.5 (53.2 – 71.0)	0.08
All subjects	60.7 (57.5 – 64.0)	60.2 (57.2 – 63.5)	61.6 (58.3 – 65.0)	65.1 (61.7 – 68.7)	0.03
Choline					
Men	59.5 (55.7 – 63.5)	63.4 (59.1 – 68.0)	74.0 (69.4 – 78.9)	70.7 (66.1 – 75.6)	<0.0001
Women	55.8 (48.5 – 64.2)	56.8 (49.3 – 65.4)	60.1 (52.3 – 69.1)	61.3 (53.0 – 71.0)	0.02
All subjects	57.5 (54.5 – 60.6)	59.3 (56.4 – 62.4)	65.8 (62.3 – 69.4)	65.4 (62.1 – 68.9)	<0.0001
Folate					
Men	64.8 (60.5 – 69.5)	63.8 (59.5 – 68.4)	67.1 (62.5 – 72.0)	72.1 (67.1 – 77.4)	0.02
Women	56.8 (49.2 – 65.6)	57.0 (49.5 – 65.7)	57.7 (49.8 – 66.9)	59.7 (51.9 – 68.8)	0.24
All subjects	60.2 (57.2 – 63.4)	61.1 (57.8 – 64.5)	61.8 (58.6 – 65.1)	64.8 (61.3 – 68.5)	0.03
Hcy					
Men	67.5 (62.8 – 72.4)	67.9 (63.4 – 72.6)	65.5 (61.2 – 70.0)	67.4 (62.7 – 72.5)	0.87
Women	57.8 (49.8 – 67.0)	55.8 (48.3 – 64.5)	59.3 (51.7 – 67.9)	57.2 (49.3 – 66.4)	0.92
All subjects	62.3 (58.9 – 65.9)	60.4 (57.3 – 63.7)	65.0 (61.6 – 68.6)	56.9 (56.9 – 63.4)	0.56
Methionine					
Men	56.9 (53.4 – 60.6)	64.1 (60.1 – 68.3)	71.5 (67.3 – 76.0)	77.1 (72.3 – 82.2)	<0.0001
Women	54.6 (48.0 – 62.2)	57.2 (49.7 – 65.8)	64.0 (55.7 – 73.5)	67.0 (58.2 – 77.2)	<0.0001
All subjects	55.5 (52.9 – 58.2)	59.0 (56.1 – 62.1)	65.9 (62.7 – 69.3)	70.8 (67.3 – 74.6)	<0.0001
SAH					
Men	60.7 (56.4 – 65.2)	63.9 (59.7 – 68.5)	72.9 (68.1 – 78.1)	70.3 (65.3 – 75.6)	0.006
Women	52.7 (45.9 – 60.5)	59.1 (51.3 – 67.9)	61.9 (53.8 – 71.0)	60.7 (52.6 – 69.9)	0.005
All subjects	55.7 (52.8 – 58.7)	61.3 (58.3 – 64.5)	65.6 (62.2 – 69.1)	65.2 (61.7 – 68.8)	<0.0001
Vitamin B₆					
Men	67.1 (62.5 – 72.1)	65.7 (61.3 – 70.3)	63.5 (59.3 – 68.1)	71.5 (66.5 – 76.9)	0.17
Women	57.3 (49.7 – 66.2)	55.2 (47.8 – 63.7)	59.1 (51.4 – 68.0)	59.6 (51.7 – 68.6)	0.19
All subjects	61.8 (58.8 – 65.1)	59.5 (56.4 – 62.8)	61.7 (58.4 – 65.1)	64.9 (61.4 – 68.6)	0.046
Vitamin B₁₂					
Men	68.3 (63.6 – 73.3)	64.0 (59.8 – 68.5)	65.4 (61.0 – 70.0)	70.3 (65.4 – 75.6)	0.41
Women	57.0 (49.2 – 66.0)	58.2 (50.4 – 67.2)	57.3 (49.8 – 66.0)	58.8 (51.1 – 67.7)	0.56
All subjects	60.9 (57.7 – 64.2)	61.0 (57.9 – 64.3)	62.1 (58.9 – 65.5)	63.5 (60.1 – 67.1)	0.19

^aAdjusted for age at blood draw, BMI, smoking status, serum creatinine concentrations and gender (for all subjects); ^bOne-carbon metabolism metabolites: SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; Hcy, total homocysteine.

rated, the association between choline and SAM through the OCM pathway and through unspecified mechanisms other than the OCM pathway were statistically significant; the standardized path coefficients were 0.105 and 0.114, respectively ($p < 0.01$ for both). We did not find any modifying effect of folate in the choline-SAM path model.

Overall, *MAT1A*, *MAT2A* and *MAT2B* genotypes were not associated with plasma SAM concentrations in both men and women combined

(**Table 5**). However, the *MAT1A*-SAM association was modified by gender and plasma methionine concentrations. Plasma SAM concentrations were lower in subjects carrying the variant allele of *MAT1A* rs2993763 only in men (p for trend = 0.047, p for a gene x gender interaction = 0.02). When plasma methionine was below the median (23.4 $\mu\text{mol/L}$), the *MAT1A* genotype was marginally statistically significantly associated with plasma SAM concentrations in men (p for trend = 0.05); men with the GA or AA genotype had 13% and 14% decreases in plasma

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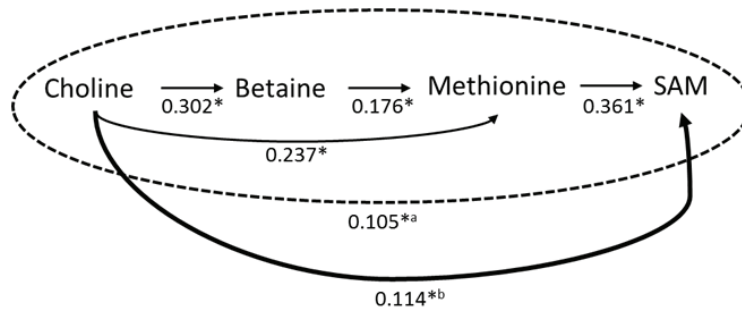


Figure 2. The final path analysis model for the choline-SAM pathway (figures are standardized regression coefficients for each path, adjusted for age at blood draw, gender, BMI, smoking status, and serum creatinine concentrations). * $p < 0.01$; ^aStandardized regression coefficient for the impact of choline on SAM through the one-carbon metabolism pathway. ^bStandardized regression coefficient for the impact of choline on SAM through unspecified pathways other than the one-carbon metabolism pathway. Total impact of choline on SAM (= a + b): standardized regression coefficient = 0.218 ($p < 0.0001$).

Table 5. Plasma SAM concentrations (nmol/L) by genotypes of *MAT1A*, *MAT2A* and *MAT2B*

	All subjects		Men		Women		$p_{\text{interaction}}^c$
	n^a	Geometric means (95% CIs) ^b	n^a	Geometric means (95% CIs) ^b	n^a	Geometric means (95% CIs) ^b	
<i>MAT1A</i> rs2993763							
GG	134	62.2 (59.4 – 65.1)	57	69.4 (65.5 – 73.4)	77	58.9 (51.7 – 67.2)	0.02
GA	230	63.0 (60.7 – 65.3)	102	67.2 (64.4 – 70.1)	128	61.6 (54.3 – 70.1)	
AA	94	61.8 (58.6 – 65.1)	44	63.5 (59.5 – 67.8)	50	63.5 (59.5 – 67.8)	
p_{trend}		0.88		0.047		0.17	
<i>MAT2A</i> rs2289972							
GG	228	62.7 (60.3 – 65.1)	100	67.4 (64.5 – 70.5)	128	61.3 (56.9 – 66.1)	0.72
GA	174	62.0 (59.5 – 64.6)	83	65.9 (62.8 – 69.1)	91	61.4 (56.6 – 66.7)	
AA	43	61.7 (57.2 – 66.4)	14	67.0 (59.5 – 75.5)	29	61.1 (54.6 – 68.3)	
p_{trend}		0.59		0.63		0.96	
<i>MAT2B</i> rs7733775							
GG	324	62.8 (60.7 – 65.0)	143	68.0 (65.6 – 70.6)	181	60.5 (53.4 – 68.6)	0.22
GA	118	62.6 (59.7 – 65.5)	53	65.0 (61.3 – 68.9)	65	61.8 (54.0 – 70.8)	
AA	10	62.6 (54.1 – 72.4)	5	63.1 (51.9 – 76.6)	5	62.7 (49.2 – 79.8)	
p_{trend}		0.87		0.16		0.51	

^aSubjects with missing data on a genotype of each SNP were excluded from an analysis; ^bAdjusted for age at blood draw, BMI, smoking status, serum creatinine concentrations, plasma methionine concentrations and gender (for all subjects); ^c p value for an interaction between *MAT* genotype and gender.

SAM, respectively, compared with men carrying the GG wild type genotype (Table 6). However, the *MAT1A* genotype was not associated with plasma SAM in men when plasma methionine concentrations were above the median. No such modulation of the *MAT1A*-SAM association by plasma methionine concentrations was observed in women. There was no modifying effect by plasma methionine concentrations or gender

on associations between the *MAT2A* or *MAT2B* genotypes and plasma SAM concentrations (data not shown).

Discussion

This is the first comprehensive study to examine the relationship between OCM nutrient and metabolite status and circulating SAM levels in a

Table 6. Geometric means and 95% confidence intervals (CIs) of plasma SAM concentrations and *MAT1A* rs2993763 genotype by plasma methionine concentrations and gender.

	Plasma methionine ≤23.4 μmol/L		Plasma methionine >23.4 μmol/L		p
	n ^a	Geometric means (nmol/L) ^b	n ^a	Geometric means (nmol/L) ^b	
Men					
GG	23	65.3 (59.5 – 71.6)	34	71.7 (66.4 – 77.5)	0.13
GA	40	57.0 (53.1 – 61.2)	62	74.9 (70.8 – 79.3)	<0.0001
AA	19	56.1 (50.6 – 62.3)	25	68.8 (62.9 – 75.4)	0.004
<i>p</i> _{trend}		0.05		0.66	0.18 ^c
Women					
GG	45	54.0 (47.0 – 62.1)	32	62.9 (53.9 – 73.5)	0.009
GA	75	57.0 (49.9 – 65.0)	53	66.2 (57.4 – 76.4)	0.001
AA	28	57.4 (49.1 – 67.0)	22	68.1 (57.9 – 80.1)	0.02
<i>p</i> _{trend}		0.35		0.10	0.86 ^c
<i>p</i> _{interaction}		0.06 ^d		0.14 ^d	0.48 ^e
All subjects					
GG	68	57.5 (54.0 – 61.1)	66	66.1 (62.0 – 70.5)	0.001
GA	115	57.0 (54.3 – 59.8)	115	69.2 (65.9 – 72.7)	<0.0001
AA	47	56.5 (52.5 – 60.8)	47	67.4 (62.6 – 72.5)	0.0005
<i>p</i> _{trend}		0.85		0.50	0.51 ^c

^aSubjects with missing data on a genotype of each SNP were excluded from an analysis; ^bAdjusted for age at blood draw, BMI, smoking status, serum creatinine concentrations and gender (for all subjects); ^c*p* value for an interaction between the *MAT1A* genotype and plasma methionine; ^d*p* value for an interaction between the *MAT1A* genotype and gender within plasma methionine groups; ^e*p* value for an interaction among the *MAT1A* genotype, plasma methionine, and gender in all subjects.

free-living human population. Plasma choline, methionine and SAH were strongly positively associated with plasma SAM concentrations among healthy Singapore Chinese men and women. Plasma folate and betaine concentrations were positively associated with plasma SAM concentrations only in men. Vitamin B₁₂ and B₆ had no or minimal positive associations with plasma SAM. In OCM, nutrients and metabolites in the methylation cycle may play a more important role than those in the folate cycle in determining plasma SAM concentrations.

We observed higher concentrations of plasma SAM in women at the age of 60 or higher. The animal study showed a decrease in tissue SAM levels in aging rats compared to adult counterparts because of increased utilization of SAM due to elevated activity of the enzyme, catechol-O-methyltransferase (COMT) [24]. On the other hand, Van Driel *et al.* reported lack of difference in plasma SAM concentrations by age among women of reproductive ages (20 – 48 y) [25]. The mean age of the women in our study was

57.5 y, and about 84% of the women were postmenopausal at the cohort baseline. The differences in age and menopausal status may explain these conflicting results. We also observed a positive association between BMI and plasma SAM concentrations only in men, while Van Driel *et al.* reported a positive association between BMI and plasma SAM concentrations among premenopausal women [25]. Future studies are required to unravel the association of age and BMI with plasma SAM concentrations.

SAM is synthesized from its precursor, methionine, via a process catalyzed by the MAT enzymes. In theory, the SAM pool can be affected by the availability of methionine and the activity level of MATs. Methionine is a sulfur-containing essential amino acid furnishing methyl groups and sulfur necessary for normal metabolism. Methionine can be derived from dietary sources such as fish, meats and nuts, as well as from Hcy remethylation which requires either 5-methyl THF or betaine (choline). Therefore, insufficient availability of these nutrients or dietary methionine may lead to low methionine and

SAM status. Although folate-mediated Hcy re-methylation is traditionally considered as a major pathway of the re-methylation of Hcy, it is estimated that approximately 60% of methyl groups are derived from choline in the liver [26]. Therefore, choline and betaine may play a more important role than folate in providing methyl groups for methylation of DNA and other molecules. The choline (betaine)-mediated Hcy re-methylation pathway may become even more dominant when the folate-mediated Hcy re-methylation pathway is impaired because these two pathways are interrelated. This interrelationship between folate and choline has been demonstrated by the reduction of choline stores following the administration of the folate-deficient diet in animal and human studies [27-30]. In the SCHS cohort, the average folate intake at baseline was 156 µg/day, which is significantly lower than the recommended daily intake (RDI) of 400 µg/day from the Institute of Medicine in the United States [31]. The low dietary folate intake in our study population could be one of the reasons for weak or no associations between plasma folate and SAM concentrations in the present study. The low folate status might have enhanced the association between plasma choline and SAM in this population.

In adults, MAT I and MAT III, encoded by *MAT1A*, are found in the liver, whereas MATII is encoded by *MAT2A* and *MAT2B* which can be found in many organs and tissues, including lymphocytes. Experimental studies showed that individuals with persistent hypermethioninemia had deficient MAT activity in the liver but normal MAT activity in red blood cells and lymphoid cells [32, 33], suggesting a critical role of hepatic MAT I and MAT III in methionine metabolism [17]. Our findings of the association between the *MAT1* genotype and plasma SAM concentrations, especially in the condition of low methionine status, are consistent with those results. The lack of the association between the *MAT1A* genotype and plasma SAM, when methionine is sufficient, suggests that high plasma methionine concentrations may overcome the reduced MAT enzyme activity level in individuals carrying the *MAT1A* variant genotypes. Therefore, a sufficient supply of methionine might be important for the maintenance of an adequate methyl pool for methylation reactions.

Besides via the methionine-mediated OCM path-

way, choline may play a critical role in determining plasma SAM concentrations via a non-methionine mediated OCM pathway. Choline can be acquired from diet, but also from the *de novo* biosynthesis via the methylation of phosphatidylethanolamine to phosphatidylcholine catalyzed by phosphatidylethanolamine methyltransferase (PEMT) (**Figure 1**). *De novo* choline biosynthesis might explain the lack of correlations between plasma concentrations of choline, betaine and methionine and dietary intake data in the current study (data not shown). Because PEMT consumes methyl groups from SAM to generate phosphatidylcholine, suboptimal choline intake may enhance the *de novo* biosynthesis of choline via the PEMT pathway that could reduce SAM concentrations. In the present study, we found a statistically significant positive association between plasma choline and SAM independent of the methionine-mediated OCM pathway. We speculate that this choline-SAM association via the non-methionine-mediated pathway may be in part through the choline biosynthesis pathway and may explain a stronger association of plasma choline than betaine with plasma SAM concentrations in the present study. Further studies are warranted to investigate this choline-SAM association.

After a methyl group is transferred to DNA catalyzed by DNA methyltransferases (DNMTs), SAM is converted to SAH, which is a competitive inhibitor of DNMTs. As expected, plasma SAH concentrations were statistically significantly positively associated with plasma SAM in the present study. SAH is subsequently converted to Hcy. We previously reported inverse associations between plasma Hcy and plasma folate, vitamin B₆ and B₁₂ in this Singapore Chinese population [11]. In the present study, plasma betaine concentrations were inversely associated with plasma Hcy but plasma choline was not. Hcy status is traditionally used as an indicator of OCM function in studies of health outcomes such as cardiovascular diseases, but health effects of Hcy on carcinogenesis are still not clear. In the present study, plasma Hcy concentrations were not associated with plasma SAM concentrations. Our result is consistent with the previous study reporting that plasma Hcy concentrations were highly correlated with plasma SAH, but not with plasma SAM among healthy adult females [34]. Lack of a correlation between plasma Hcy and SAM in the present study may be due to the fact that our study subjects were healthy individuals with normal

plasma Hcy concentrations. We may have observed a higher correlation between Hcy and SAM, if our study had included hyperhomocysteinemic individuals.

The present study is novel in that existing human data describing associations between OCM nutrient status and circulating SAM levels are limited. The simultaneous measurement of major compounds in the OCM pathway including SAM, SAH, Hcy, methionine, choline, betaine, folate, vitamins B₆ and B₁₂ allowed the comprehensive analysis for their effects on SAM. The inclusion of genetic variation in *MAT* sheds lights on the methionine-SAM association. In addition, the path analysis based on the understanding of the biological function of these OCM metabolites provides further insight on the OCM pathway in humans. Although the present study is a cross-sectional study, which does not allow us to determine cause-effect relationships, it would be extremely difficult to disentangle complicated pathways involving multiple molecules in OCM in an intervention study.

One concern of the present study is the possible degradation of SAM in stored plasma samples over time. The median plasma SAM concentrations in the present study (63.3 nmol/L) was somewhat lower than those found in populations in Europe (70 to 128 nmol/L) [25]. However, the wide range of plasma SAM in various studies (50 to 150 nmol/L) might be due to different study populations and different laboratory methods [34-40]. It should be noted that a reliable method to measure plasma SAM concentrations became only recently available. Furthermore, previously reported plasma SAM concentrations were mainly in whites; no reference data are available for Asian populations. In the present study population, we measured plasma Hcy concentrations in the same subjects at two different times in two different laboratories; one at the National University of Singapore in 1996 – 1997 and the other at Baylor Research Institute in 2010. High correlation between the two Hcy measurements ($r = 0.70$) indicates that the degradation of Hcy, and possibly SAM, might have been minimal during prolonged storage. Furthermore, degradation of plasma SAM would not result in the observed associations, given that it occurred in a non-differential manner.

In summary, the present study demonstrates that plasma choline, methionine and SAH were strongly associated with plasma SAM concentra-

tions. Plasma choline may be associated with plasma SAM through OCM (via betaine and methionine), but also through unspecified mechanisms other than OCM. The common genetic variant of *MAT1A* showed interactions with gender and plasma methionine on plasma SAM concentrations. Improving plasma methionine concentrations may increase plasma SAM concentrations in a subset of a population, depending on this *MAT1A* genotype and gender. Future studies are warranted to confirm our findings.

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Address correspondence to: Dr. Maki Inoue-Choi, 1300 S. Second Street, Suite 300, Minneapolis, MN 55454, United States Tel: +1 (612) 625-4542; Fax: +1 (612) 624-0315; E-mail: inou0021@umn.edu

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