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Evidence for sex differences in the determinants of homocysteine concentrations

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Abstract

A high homocysteine phenotype, often accompanied by low folate, is associated with several pathologies including cardiovascular disease and birth defects. This phenotype appears to be influenced by both genetic and environmental factors, which may act in a sex-dependent manner. The present analyses were undertaken to identify the determinants of homocysteine concentrations in young men and women, and are based on data from a cohort of young, reproductive age (20–26 years old) individuals in Northern Ireland. Multivariate modeling indicated that homocysteine concentrations are associated with red blood cell (RBC) folate, vitamin B₁₂, *MTHFR* 677C>T genotype and smoking status in both males and females. However, the inter-relationships between these variables appear to differ between the sexes. Specifically, homocysteine levels in males were significantly associated with interactions between *MTHFR* 677C>T genotype and both RBC folate and smoking status. In contrast, homocysteine levels in females were significantly associated with interactions between smoking status and RBC folate. These results suggest that the characteristics of individuals who are at the highest risk for a high homocysteine phenotype differ for males and females. Among males, those with the *MTHFR* 677TT genotype appear to be at the highest risk and to be the most vulnerable to factors (e.g. smoking, low RBC folate) that are associated with homocysteine raising effects. Among females, smokers (regardless of *MTHFR* genotype) appear to be at the highest risk, and to be the most vulnerable to a single factor (i.e. RBC folate) that is associated with homocysteine raising effects.

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Introduction

A high homocysteine phenotype accompanied by low folate is associated with increased risk of several major human pathologies including cardiovascular disease [1,2],

birth defects such as spina bifida [3] and cleft palate [4], and other complications of pregnancy including spontaneous abortion, pre-eclampsia, prematurity and low birth weight [5]. Folate is required for nucleic acid biosynthesis and the methylation of lipids, hormones, DNA and proteins, while homocysteine is a key intermediate in the methylation cycle [5] and may itself be teratogenic [6,7]. As periconceptional folic acid supplementation is known to reduce the risk of spina bifida [3,8,9], the reciprocal quan-

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titative relationship between folate and homocysteine [10] is of particular importance to women of childbearing age.

Recognition that mild hyperhomocysteinemia is a cardiovascular risk factor/marker [1,2] has prompted considerable research into understanding the biochemical, environmental and genetic influences that have an impact on homocysteine concentrations. The variables known to be significantly associated with homocysteine levels include: vitamin status, in particular folate, riboflavin and vitamin B₁₂; age; sex; lifestyle, in particular smoking status; prescription drugs; renal function; and genetics, in particular the well-established influence of the 677C>T polymorphism in the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene [11].

It has previously been shown that the median plasma total homocysteine concentration is higher among men than women [12]. In addition, many studies have investigated the relationship between smoking and homocysteine levels. Although these studies have not been entirely consistent in their findings, the majority has reported a positive correlation between smoking and homocysteine levels [13,14]. In the Norwegian “Hordaland” study of almost 25,000 middle aged (40–42 years) and older (65–67 years) adults plasma total homocysteine concentration was positively correlated with the number of cigarettes smoked per day and the homocysteine raising effect of smoking appeared to be somewhat stronger in women and in the younger age group [12].

The *MTHFR* 677TT genotype confers a significant risk of hyperhomocysteinemia that is most pronounced in individuals with low folate [15,16] or low riboflavin [17] status. Interestingly, the *MTHFR* 677C>T polymorphism appears to have a sex-specific effect on homocysteine [18]. Men who are *MTHFR* 677TT homozygotes appear to be at higher risk of hyperhomocysteinemia than women with this genotype [19]. Furthermore, the impact of the *MTHFR* 677TT genotype on homocysteine may be largely restricted to male smokers and may have little influence on females regardless of smoking behavior [20].

Given the association between homocysteinemia and several disease phenotypes, identification of the factors that influence variation in homocysteine levels is of considerable public health interest. In this report we describe our investigation of the determinants of homocysteine concentrations in a cohort of young, reproductive age adults.

Population and methods

Study population

The Young Hearts Project (YH) is an ongoing longitudinal study designed to monitor cardiovascular disease risk factors in children and young adults living in Northern Ireland [21]. Briefly, a sample of 12 year old ($n = 509$) and 15 year old ($n = 506$) boys and girls were enrolled from post-primary schools in Northern Ireland between 1989 and 1990 (YH1). Between October 1997 and October 1999, all

YH subjects were invited to participate in a hospital-based screening evaluation (YH3) [22]. The participation rate for YH3, which was conducted when the subjects were between 20 and 26 years of age, was 48.2% ($n = 489$). Compared to non-respondents, YH3 subjects tended to be from families with higher socioeconomic status and to have lower body mass indices at the baseline (YH1) exam. In addition, male YH3 subjects were leaner and reported lower saturated fat intake at baseline relative to the male non-participants [23]. Ethical approval for each phase of the study was granted by the Research Ethics Committee of Queen's University Belfast. The current paper is based on self-reported data on smoking status, use of alcohol and multivitamin supplements, and fasting blood samples collected as part of YH3.

Laboratory methods

Homocysteine was measured by an established high performance liquid chromatography method [24]. Serum folate and vitamin B₁₂ were determined by time-resolved immunofluorescence on an AutoDelfia analyzer (Wallac, UK). Red blood cell (RBC) folate concentrations were determined by a microbiological assay [25], and are expressed as nanomoles per liter of packed RBCs. Creatinine was measured using an enzyme-based assay on an Ektachem analyzer (Johnson & Johnson). DNA was extracted [26] and the *MTHFR* 677C>T genotype [19,20] was determined as previously described.

Statistical methods

Descriptive analyses of the study variables were conducted using data from all study subjects, and within the subset of data provided by study subjects who had complete information for all variables of interest. These analyses were performed separately in data from male and female YH3 participants.

Using data from all study subjects, the Wilcoxon rank sum test and the chi-square test were used, respectively, to evaluate differences in continuous and categorical variables between males and females. As the distribution of RBC folate, vitamin B₁₂ and homocysteine are positively skewed, these analyses were conducted using log transformed values. All tests were two-tailed and P -values of <0.05 were considered to be statistically significant.

Simple and multiple linear regression analyses were conducted separately in data from males and females, using log transformed homocysteine values as the main outcome measure. These analyses were restricted to the subset of YH3 study participants who had complete data for all of the variables that were included in the regression analyses. Simple linear regression models were fitted to the data and the coefficient of determination (R^2) estimated from these models was used to assess the proportion of variation in homocysteine concentrations that was explained by each predictor variable. In addition,

the variable coefficients were used to estimate the direction and magnitude of the association between the variable and homocysteine levels. The significance of the association between each variable and homocysteine levels was assessed using the *t*-statistic.

Multiple regression models, including predictor variables and two-way interaction action terms, were also fitted to the data. In all multivariate models, the *MTHFR* CC and CT genotypes were combined to form a single category that was compared to the TT genotype. Adjusted R^2 values were used to compare nested models [27] and the difference in the adjusted R^2 between models was used to estimate the proportion of variation in homocysteine concentrations explained by the addition of a single predictor variable or interaction term to a model. An increase in the adjusted R^2 of 1% or more was considered of interest. The significance of individual predictor variables within a model was assessed using the *t*-statistic. Variables with P -value <0.05 were considered to be significantly related to homocysteine concentration, given all other variables in the model. All statistical analyses were carried out using SAS version 8.02 or 9.1.

Results

Characteristics of all YH3 study subjects and the subset of subjects with complete data for the variables of interest in this study are summarized, separately by sex, in Table 1. In the full cohort, median RBC folate, vitamin B₁₂ and creatinine concentrations were significantly lower in women than in men ($P < 0.01$). Median homocysteine levels were also lower in women than in men, but this difference was not statistically significant. There were also differences between the sexes in lifestyle variables that are known to have an impact on homocysteine. Specifically, males were more likely to report consuming alcohol than females ($P = 0.05$), and among those who reported the use of alcohol, intake was higher in males than females ($P < 0.01$). Although the proportion of males and females who reported that they smoked cigarettes was similar, male smokers reported significantly higher numbers of cigarettes smoked per day than did female smokers ($P = 0.04$). In addition, males were less likely than females to report the use of a multivitamin supplement, although this difference was not statistically significant. The characteristics of the subset of subjects with complete data for all of the study variables did not differ markedly from those of the full study cohort.

Given the observed differences in the biochemical and lifestyle variables between the sexes, all subsequent analyses were performed separately for males and females. In both males and females, RBC folate was significantly inversely related to, and explained the highest proportion of the variability in, homocysteine level ($R^2_{\text{male}} = 0.21$, $R^2_{\text{female}} = 0.26$, Table 2). Vitamin B₁₂ was also significantly and inversely related to, and explained the second highest proportion of the variation in, homocysteine concentration

in both sexes ($R^2_{\text{male}} = 0.17$, $R^2_{\text{female}} = 0.14$). Use of multivitamin supplements and genotype for the *MTHFR* 677C>T variant were also significantly associated with homocysteine in both sexes. These two variables each explained 3–5% of the variation in homocysteine concentration (Table 2). Smoking status accounted for approximately 7% of the variation in homocysteine concentration in females, but was not significantly associated with homocysteine concentrations in males. Creatinine and use of alcohol were not significantly associated with homocysteine levels in either sex, and accounted for 2% or less of its variation.

As a first step in the multivariate analyses, a model including the three variables that were significantly associated with homocysteine concentration in both sexes in the simple regression models (i.e. RBC folate, vitamin B₁₂ and *MTHFR* 677C>T genotype) was fitted to the data (Model 1). For both males and females, each of the individual predictor variables in Model 1 remained significantly associated with homocysteine ($P < 0.05$), and the model explained approximately 30% of the variation in homocysteine levels (Table 3). Models including two-way interaction terms between the variables in Model 1 were then fitted to the data (Table 3, Models 1a–1c). Among females, the inclusion of any one of these interaction terms did not markedly increase the proportion of variation in homocysteine concentration explained by the model (i.e. change in adjusted $R^2 < 0.01$) and none of the interaction terms were statistically significant. In contrast, among males, interaction terms between RBC folate and vitamin B₁₂ and between RBC folate and *MTHFR* genotype were both significantly related to homocysteine level ($P = 0.02$ and $P < 0.01$, respectively), and increased the proportion of variation explained by the model by approximately 2% and 8%, respectively. However, the model that included both interaction terms did not explain an increased proportion of the variation in homocysteine concentration, relative to the model that included only the interaction term for RBC folate and *MTHFR* genotype, and the interaction between RBC folate and *MTHFR* genotype was statistically significant ($P < 0.01$), whereas the interaction between RBC folate and vitamin B₁₂ was not ($P = 0.14$).

To determine whether the lifestyle variables are associated with homocysteine concentrations independent of the effects of RBC folate, vitamin B₁₂ and *MTHFR* genotype a second series of multiple regression models were fitted to the data. Given prior results indicating that in males, but not females, homocysteine is associated with an interaction between RBC folate and *MTHFR* genotype, the base model used for these analyses differed for males and females. In males, a model including RBC folate, vitamin B₁₂, *MTHFR* genotype and an interaction term between RBC folate and *MTHFR* genotype was fitted to the data (Table 3, Model 2M), and compared to models that included all of these terms plus a term for one of the lifestyle variables (Table 3, Models 2a–c). In the context of this model, smoking status was significantly related to homocysteine concentration ($P = 0.03$); its addition

Table 1
Characteristics of participants in the Young Hearts 3 study

Variable	Males		Females	
	Full dataset	Subset ^a	Full dataset	Subset ^a
<i>Biochemical variables</i>				
Homocysteine (μmol/l)				
<i>N</i>	229	160	191	149
Mean	10.2	10.1	9.8	9.6
Median (25th–75th percentile)	9.2 (7.6–11.0)	9.2 (7.6–10.8)	8.6 (7.3–10.9)	8.5 (7.2–10.6)
Red blood cell folate (nmol/l RBCs)				
<i>N</i>	198	160	182	149
Mean	747.1	734.1	626.4	629.8
Median (25th–75th percentile)	702.4 (541.1–888.7)	688.7 (530.1–880.8)	562.9 (428.7–752.0)	560.4 (428.2–746.3)
Serum vitamin B ₁₂ (pmol/l)				
<i>N</i>	200	160	166	149
Mean	304.6	307.8	244.0	241.4
Median (25th–75th percentile)	293.5 (211.5–370.5)	295.5 (213.5–370.5)	242.0 (173.0–300.0)	238.0 (171.0–297.0)
Creatinine (μmol/l)				
<i>N</i>	228	160	189	149
Mean	73.5	74.4	54.9	54.8
Median (25th–75th percentile)	73.0 (67.0–81.0)	73.5 (67.5–81.5)	55.0 (47.0–63.0)	55.0 (48.0–63.0)
<i>Lifestyle Variables</i>				
Current use of cigarettes (<i>N</i> , %)				
Yes	87 (37.5)	53 (33.1)	77 (38.9)	61 (40.9)
No	145 (62.5)	107 (66.9)	121 (61.1)	88 (59.1)
Cigarettes (per day) ^b				
<i>N</i>	87	53	77	61
Mean	12.4	13.5	10.0	10.0
Median (25th–75th percentile)	10 (7–20)	12 (10–20)	10 (5–15)	10 (5–11)
Current use of alcohol (<i>N</i> , %)				
Yes	199 (81.2)	137 (85.6)	154 (77.8)	116 (77.9)
No	46 (18.8)	23 (14.4)	44 (22.2)	33 (22.1)
Alcohol (g/day) ^c				
<i>N</i>	199	137	154	116
Mean	53.4	53.8	19.8	19.4
Median (25th–75th percentile)	43.0 (27.0–69.0)	43.0 (27.0–66.0)	16.0 (8.0–25.0)	16.5 (9.0–24.5)
Current use of multivitamin supplements (<i>N</i> , %)				
Yes	46 (19.7)	33 (20.6)	51 (25.8)	44 (29.5)
No	188 (80.3)	127 (79.4)	147 (74.2)	105 (70.5)
<i>Genotypes</i>				
<i>MTHFR</i> 677C>T (<i>N</i> , %)				
CC	102 (45.3)	73 (45.6)	81 (41.8)	63 (42.3)
CT	99 (44.0)	70 (43.8)	80 (41.2)	63 (42.3)
TT	24 (10.7)	17 (10.6)	33 (17.0)	23 (15.4)

^a Subset limited to data from study subjects with complete information for all of the study variables.

^b Cigarettes smoked per day among smokers.

^c Alcohol consumed per day among alcohol users.

increased by 1.5% the proportion of variation in homocysteine explained (Model 2a). In contrast, neither alcohol use ($P = 0.56$) nor multivitamin use ($P = 0.52$) was significantly related to homocysteine concentration, and the addition of either variable did not appreciably influence the proportion of homocysteine variation explained by the model (Table 3, Models 2b and 2c, respectively). Among females, a model including only RBC folate, vitamin B₁₂, and *MTHFR* genotype was fitted to the data

(Table 3, Model 2f). In the context of this model, the association between smoking status and homocysteine concentration was of borderline significance ($P = 0.07$), and the addition of this variable increased the proportion of variation in homocysteine explained by the model by only 1.0% (Model 2a). In contrast, neither alcohol use ($P = 0.17$) nor multivitamin use ($P = 0.66$) was related to homocysteine concentration, and the addition of either variable did not appreciably influence the proportion of homocysteine var-

Table 2

Proportion of the variation (R^2) in homocysteine concentration explained by selected biochemical and behavioral factors in Young Hearts 3 study participants

Predictor variables	Males		Females	
	R^2 (P -value) ^a	Variable coefficient (SE) ^b	R^2 (P -value) ^a	Variable coefficient (SE) ^b
Red blood cell folate (nmol/l)	0.21 (<0.01)	−0.0002 (0.00004)	0.26 (<0.01)	−0.0002 (0.00003)
Serum vitamin B ₁₂ (pmol/l)	0.17 (<0.01)	−0.0005 (0.00008)	0.14 (<0.01)	−0.0006 (0.0001)
Creatinine (μmol/l)	0.004 (0.43)	0.0007 (0.0009)	0.02 (0.07)	0.0019 (0.0011)
Current use of cigarettes (yes/no)	0.005 (0.39)	−0.0211 (0.0246)	0.07 (<0.01)	0.0850 (0.0250)
Current use of alcohol (yes/no)	0.001 (0.26)	0.0374 (0.0330)	0.02 (0.10)	0.0545 (0.0305)
Current use of multivitamin supplements (yes/no)	0.03 (0.03)	0.0619 (0.0283)	0.04 (0.01)	0.0706 (0.0274)
<i>MTHFR</i> 677C>T	0.05 (0.83, 0.01) ^c		0.05 (0.28, 0.01)	
CT		−0.0053 (0.0240)		0.0296 (0.0272)
TT		0.1046 (0.0387)		0.1042 (0.0372)

^a P -value for test of hypothesis of no linear association between predictor variable and homocysteine concentration.

^b Estimated regression coefficients and standard errors, variable coefficients estimate change in homocysteine concentration per unit change in the predictor variable.

^c P -value from comparison of *MTHFR* CC and CT genotypes, P -value for comparison of *MTHFR* CC and TT genotypes.

Table 3

Summary of linear regression modeling of homocysteine concentrations in Young Hearts 3 study participants

Model ^a	Variables ^b	Males		Females	
		Adjusted R^2	Change in adjusted R^2	Adjusted R^2	Change in adjusted R^2
1	Males and females: Folate + B₁₂ + <i>MTHFR</i>	0.317	—	0.334	—
1a	+folate-B ₁₂ interaction	0.335	0.018	0.338	0.004
1b	+folate- <i>MTHFR</i> interaction	0.397	0.080	0.329	−0.005
1c	+B ₁₂ - <i>MTHFR</i> interaction	0.317	0.000	0.330	−0.004
2	Males: Folate + B₁₂ + <i>MTHFR</i>+Folate-<i>MTHFR</i> interaction	0.397	—		
	Females: Folate + B₁₂ + <i>MTHFR</i>			0.334	—
2a	+smoking	0.412	0.015	0.344	0.010
2b	+alcohol	0.394	−0.003	0.338	0.004
2c	+multivitamin supplement	0.395	−0.002	0.330	−0.004
2d	+creatinine	0.399	0.002	0.330	−0.004
3	Males: Folate+ B₁₂ + <i>MTHFR</i>+Folate-<i>MTHFR</i> interaction+Smoking	0.412	—		
	Females: Folate+B₁₂ + <i>MTHFR</i>+Smoking			0.344	—
3a	+smoking-folate interaction	0.408	−0.004	0.362	0.018
3b	+smoking-B ₁₂ interaction	0.409	−0.003	0.344	0.000
3c	+smoking- <i>MTHFR</i> interaction	0.422	0.010	0.340	−0.004
4	Males: Folate+ B₁₂ + <i>MTHFR</i>+Folate-<i>MTHFR</i> interaction+Supplement	0.395	—		
	Females: Folate+B₁₂ + <i>MTHFR</i>+Supplement			0.330	—
4a	+supplement-folate interaction	0.396	0.001	0.326	−0.004
4b	+supplement-B ₁₂ interaction	0.393	−0.002	0.330	0.000
4c	+supplement- <i>MTHFR</i> interaction	0.403	0.008	0.325	−0.005
5	Males: Folate+ B₁₂ + <i>MTHFR</i>+Folate-<i>MTHFR</i> interaction+Alcohol	0.394	—		
	Females: Folate+B₁₂ + <i>MTHFR</i>+Alcohol			0.338	—
5a	+alcohol-folate interaction	0.390	−0.004	0.340	0.002
5b	+alcohol-B ₁₂ interaction	0.395	0.001	0.334	−0.004
5c	+alcohol- <i>MTHFR</i> interaction	0.392	−0.002	0.337	−0.001
6	Males: Folate+ B₁₂ + <i>MTHFR</i>+Folate-<i>MTHFR</i> interaction+Creatinine	0.399	—		
	Females: Folate+B₁₂ + <i>MTHFR</i>+Creatinine			0.330	—
6a	+creatinine-folate interaction	0.395	0.004	0.326	−0.004
6b	+creatinine-B ₁₂ interaction	0.398	−0.001	0.334	0.004
6c	+creatinine- <i>MTHFR</i> interaction	0.395	−0.004	0.326	−0.004

^a Change in adjusted R^2 was assessed relative to the first model (indicated in bold) within each group. It is of note that the adjusted R^2 need not increase when a variable is added to a model and that the change in the adjusted R^2 value upon addition of a variable to the model can be negative, indicating that the additional variable does not contribute any explanatory power to the model.

^b In all analyses the *MTHFR* CC and CT genotypes were combined and compared to the TT genotype.

iation explained by the model (Table 3, Models 2b and 2c, respectively).

To evaluate whether the associations between homocysteine and RBC folate, vitamin B₁₂ and *MTHFR* genotype might be modified by lifestyle or other factors (i.e. kidney function indexed by creatinine), several additional multiple regression models were fitted to the data (Table 3, Models 3–6). These analyses indicated that, in males, an interaction between smoking and *MTHFR* genotype ($P = 0.05$) and, in females, an interaction between smoking and RBC folate ($P = 0.02$), were significantly related to homocysteine concentration and increased the proportion of variation in homocysteine that could be explained by the model by 1–2% (Table 3, Model 3c in males and 3a in females). No other interaction term was significantly associated with homocysteine concentration, or with an appreciable increase in the proportion of variation in homocysteine explained by the model (Table 3, Models 4–6).

Hence, in these data, homocysteine concentration in males is best predicted by a model that includes RBC folate, vitamin B₁₂, *MTHFR* genotype, smoking status and interactions between *MTHFR* genotype and smoking status and *MTHFR* genotype and RBC folate. This model explains approximately 42% of the variation in homocysteine in males, and each of the terms in the models is statistically significant. Homocysteine concentrations in females are best predicted by a model that includes RBC folate, vitamin B₁₂, *MTHFR* genotype, smoking status and an interaction between RBC folate and smoking status. This model explains approximately 36% of the variation in homocysteine in females, and each of the terms in the model is statistically significant.

To illustrate the interactions suggested by the regression analyses, predicted homocysteine values were calculated from the best multiple regression model for males and females (Table 4). For example, to illustrate the predicted impact of the interaction between *MTHFR* and smoking, consider males with low folate and low B₁₂ (Table 1, row 1). Among such individuals, homocysteine is predicted to

be 33% higher in non-smokers with the *MTHFR* TT genotype relative to those with the CC or CT genotypes, and 82% higher in smokers with the TT genotype relative to those with the CC or CT genotype (Table 4, row 1). In general, for a given set of risk factors, the predicted homocysteine values for females are lower than the corresponding estimate for males (Table 4). The notable exception to this occurs in the subgroup of smokers with the *MTHFR* CC or CT genotype. In this subgroup, the predicted homocysteine levels for females are comparable to, or higher than those of males. Further, although predicted homocysteine levels in female smokers with the TT genotype are lower than those of their male counterparts (i.e. male smokers with the TT genotype), they are increased relative to female smokers with the CC or CT genotype. These findings suggest that, among young females, smoking eliminates the widely recognized sex difference in homocysteine concentration, and may expose females, regardless of the *MTHFR* 677C>T genotype, to excess morbidity and mortality from homocysteine-related diseases.

Discussion

These analyses confirm previously reported associations between homocysteine and folate, vitamin B₁₂, *MTHFR* 677C>T genotype and smoking status, and differences in homocysteine levels between males and females in a relatively unique cohort of young, reproductive age adults. In both males and females, folate and vitamin B₁₂ explained the highest proportion of the variation in homocysteine concentration. In addition, for both males and females, *MTHFR* 677C>T genotype and smoking status and interactions involving these variables were also significantly associated with homocysteine concentration, although these variables tended to explain a smaller proportion of the variation in homocysteine than folate and vitamin B₁₂.

Interestingly, although the same set of variables (i.e. RBC folate, vitamin B₁₂, *MTHFR* 677C>T genotype and smoking status) were identified in both males and females,

Table 4
Predicted homocysteine values for males and females^a

Sex	RBC folate ^b	Vitamin B ₁₂ ^b	Non-smoker		Smoker	
			CC or CT	TT	CC or CT	TT
Male	430	170	11.9	15.8	10.5	19.1
	430	300	10.7	14.1	9.4	17.1
	750	170	10.8	10.2	9.5	12.4
	750	300	9.7	9.2	8.5	11.1
Female	430	170	9.6	10.7	11.2	12.6
	430	300	8.5	9.6	10.0	11.2
	750	170	8.6	9.7	9.0	10.1
	750	300	7.7	8.6	8.0	9.0

^a Multiple regression model used to predict homocysteine concentrations in males: $\log_{\text{hcy}} = 1.19491 - (0.00013 \times \text{folate}) - (0.00036 \times \text{B}_{12}) + (0.31561 \times \text{MTHFR}) - (0.00045 \times \text{folate} \times \text{MTHFR}) - (0.05347 \times \text{smoking}) + (0.13689 \times \text{MTHFR} \times \text{smoking})$ Multiple regression model used to predict homocysteine concentrations in females: $\log_{\text{hcy}} = 1.10731 - (0.00014 \times \text{folate}) - (0.00038 \times \text{B}_{12}) + (0.05046 \times \text{MTHFR}) + (0.13281 \times \text{smoking}) - (0.00015 \times \text{smoking} \times \text{folate})$.

^b Values correspond to sex-specific 25th and 75th percentiles in the subset of female study subjects used in the multiple regression model.

the inter-relationships among these variables appeared to differ between the sexes. In males, the associations between homocysteine concentration and both RBC folate and smoking status were influenced by *MTHFR* genotype. In contrast, among females, the association between homocysteine concentration and both RBC folate and smoking appeared to be independent of *MTHFR* genotype, but there was evidence that homocysteine concentration was influenced by an interaction between RBC folate and smoking. These findings suggest that in young, reproductive age males, those with the *MTHFR* TT genotype are particularly vulnerable to factors that have a homocysteine raising effect, such as smoking and low folate status. The situation for young reproductive age females appears to be somewhat different. Specifically, young females who smoke appear to reduce or eliminate the protective effect of female gender on homocysteine levels, and to be particularly vulnerable to the homocysteine raising effects of low folate status (Table 4).

One possible explanation of the observed relationship between smoking status and homocysteine in females is suggested by epidemiological studies indicating that smoking interferes with estrogen metabolism and that women who smoke are relatively estrogen-deficient [28]. As it has been previously suggested that estrogen decreases plasma homocysteine levels [29], a hormonal mechanism may underly our observations. The precise mechanism by which estrogen could affect homocysteine metabolism is not known. However, it has been proposed that estrogen influences methionine catabolism [30,31], and thus may influence homocysteine levels via this component of the pathway. Another possible mechanism by which estrogen might influence homocysteine concentration has been suggested by Brown et al. [20] who postulated that estrogen-dependent changes in NOS3 activity influence folate levels [32].

In conclusion, our analyses of data from a cohort of young, reproductive age adults indicate that the predictors of homocysteine levels in males and females are similar, but that the inter-relationships between these predictive factors may differ. These differences indicate that the subgroups of males and females who are at highest risk for hyperhomocysteinemia are distinct. In males, individuals with the *MTHFR* 677TT genotype appear to be at the highest risk and to be the most vulnerable to factors (e.g. smoking, low folate) that are associated with homocysteine raising effects. In females, smokers (regardless of *MTHFR* genotype) appear to be at the highest risk, and to be the most vulnerable to other factors (e.g. low folate) that are associated with homocysteine raising effects.

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