Original Communication

Marked Gender Difference in Plasma Total Homocysteine Concentrations in Indian Adults with low Vitamin B_{12}

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Abstract: Context: Plasma total homocysteine (tHcy) is higher in men than women. Objective: To explore the gender differences in tHcy in relation to determinants of one-carbon metabolism in Indian people with low B_{12} and adequate folate. Setting: The study took place in rural and urban areas of Pune, India. Design and participants: Participants were 441 men from the cross-sectional Coronary Risk of Insulin Sensitivity in Indian Subjects study (CRISIS) and premenopausal wives of 146 men (median ages 38 and 34 years, respectively). Main outcome measures: Gender difference in fasting tHcy in relation to plasma albumin and creatinine concentrations, lifestyle factors, diet and lean mass, plasma B_{12} and red cell folate (RCF) was assessed. Results: Prevalence of high tHcy (>15 μmol/L, median 14.4 μM) was 40 %, low B_{12} (<150 pmol/L, 114 pmol/L) 66 %, and low RCF (<283 nmol/L, 525 nmol/L) 8 %. Men had higher (1.8x) plasma tHcy concentrations (16.2 μmol/L) than women (9.5 μmol/L). Only 50 % of the gender difference was explained by age, lean mass, B_{12}, and RCF. The difference remained after controlling for other explanatory variables. Women with a tHcy of 9.3 μM had the same B_{12} concentration (129 pmol/L) as men with a tHcy of 15 μM; and for a tHcy of 10.0 μmol/L women had the same RCF concentration (533 nmol/L) as men with a tHcy of 15 μmol/L. Conclusions: Adult Indian women have markedly lower tHcy concentrations compared to men. This suggests a lower threshold for supplementation to improve reproductive and cardiovascular outcomes.

Key words: Homocysteine, B_{12}, folate, one-carbon metabolism

Introduction

Hyperhomocysteinemia is associated with cardiovascular [1] and neurological disorders [2]. In women elevated tHcy concentration is a risk factor for birth defects, neural tube defects, intrauterine growth retardation (IUGR), low birth weight (LBW), and neurocognitive abnormalities in the offspring [3,4]. Homocysteine concentration reflects nutritional status (vitamin B_{12}, folate, and other), renal function, genetic, and endocrine factors. Studies in and around Pune, India have shown that low vitamin B_{12} status (subclinical vitamin B_{12} deficiency) is common in men [5] and pregnant [6] and non-pregnant women [7], while folate status is adequate. The B_{12} deficiency is largely ascribed to vegetarianism and consumption of only a small amount of animal products. The reasons for this common dietary pattern include religious and personal beliefs, cultural practices and poverty [5, 8–12]. Indians have higher plasma tHcy concentrations compared to other ethnic groups [9, 11, 13]. Studies, mainly with European subjects, have reported lower plasma tHcy concentrations in women compared to men [13–19] but these populations, in general, were relatively low in folate and replete in B_{12}.

Gender differences in tHcy have not been extensively investigated in Indian people with a predominantly vegetarian diet. We investigated tHcy concentrations in men and women in the Coronary Risk of Insulin Sensitivity in Indian subjects (CRISIS) study and examined the possible contributors to the gender difference.

Methods

Subjects in this study were from the CRISIS study [5]. This was a population-based study of distribution of cardiovascular risk factors in rural and urban Indians [20]. We selected 441 men and wives of 146 of these men by multistage random sampling from rural (men 149, women 46), urban slum (men 142, women 50), and urban-middle class (men 150, women 50) residences in and around Pune. The study protocol was approved by the Ethics Committee of the King Edward Memorial Hospital and Research Center. All participants signed an informed consent.

Subjects were admitted overnight to a metabolic unit and were provided a standard vegetarian dinner. Demographic and socioeconomic characteristics were recorded. History of tobacco and alcohol use (never, past, and current), and use of medication including vitamin supplements was recorded. A 24-hour diet recall was used to assess intake of energy, carbohydrates, protein, and fats on an average day using nutritive values from a local and a national database [21, 22]. A food frequency questionnaire was used to assess the frequency of consumption of foods rich in vitamin B_{12} and folate: non-vegetarian foods (meat, chicken, fish, and eggs), milk and coffee, and green leafy vegetables (GLV). Standardized anthropometric measurements (height, weight, skinfolds) were performed. Body mass index (BMI) was calculated. Fat mass was calculated using Durnin's equation [23] and lean mass was derived.

Laboratory measurements

After an overnight fast, a sample of venous blood was collected into EDTA-vacutainers. Hematological measurements were performed on a Beckman Coulter Analyser (A^c.T diff^{TM}, Miami, Florida, USA). Plasma was separated. Plasma levels of albumin and creatinine were measured on an auto-analyzer (Hitachi 911, Hitachi Ltd., Tokyo, Japan) using standard methods. Plasma vitamin B_{12} and red cell folate (RCF) concentrations were measured with radioimmunoassay kits (Diagnostic Products Corporation, LA, USA). Plasma tHcy was measured by fluoropolarization immunoassay with the IMx system (Abbott Laboratories, IL, USA). The intra- and inter-assay coefficients of variation were less than 8 % for hematological and biochemical (tHcy, vitamin B_{12} and folate) parameters. Plasma insulin was measured using an in-house DELFIA assay (on Victor 2, Wallac,Turku, Finland). Glomerular filtration rate (eGFR) was estimated using the Modification of Diet in Renal Disease study formula [24].

Statistical methods

We used pooled data from three places of residence. Data are presented as median ($25–75^{th}$ centile) and percentages as appropriate. Variables (tHcy, vitamin B_{12} and folate) with skewed distribution were log-transformed to satisfy the assumption of normality. Gender differences were determined using analysis of variance. Comparison of the strength of associations between plasma tHcy and its risk factors was performed using generalized linear models to test the homogeneity of the regression slopes. If the inter-

Table I: Anthropometry, lifestyle factors and biochemistry of 441 men and 146 wives

	Men (441)	Men (146)	Women (146)	p1	p2
Anthropometry & body composition					
Age (years)	38.0 (34.0, 44.0)	38.0 (34.0, 43.0)	34.0 (29.0, 39.0)	0.000	0.000
Height (cm)	164.8 (161.1, 168.9)	166.5 (161.18, 169.6)	152.3 (149.5, 155.7)	0.000	0.000
Weight (kg)	59.6 (53.2, 68.7)	62.2 (54.9, 70.7)	48.9 (42.8, 56.7)	0.000	0.000
Fat mass (kg)	13.5 (8.9, 18.6)	14.6 (10.1, 18.8)	15.5 (10.7, 20.3)	0.000	0.000
Lean mass (kg)	45.5 (41.6, 49.2)	45.9 (42.7, 49.3)	33.5 (31.6, 36.3)	0.000	0.000
Lifestyle factors					
Smoking Current /Past (%)	30/18	30/16	0/0	0.000	0.000
Tobacco chewing; Current/Past (%)	50/2	45/3	3/1	0.000	0.000
Tobacco toothpaste; Current/Past (%)	30/8	24/8	30/5	0.53	0.34
Alcohol; Current/Past (%)	37/13	37/9	0/0		
Biochemistry					
Hemoglobin (g/dL)	14.1 (13.4, 14.7)	14.2 (13.7, 14.9)	11.4 (10.4, 12.2)	0.000	0.000
Anemia (Men < 13.5 Women < 12.0 g/dl)	111 (25.3 %)	27 (18.5 %)	96 (67.1 %)	0.000	0.000
Mean Corpuscular Volume (fL)	85.4 (82.2, 89.2)	85.5 (82.7, 89.7)	80.8 (75.0, 84.8)	0.001	0.000
Microcytosis (<80 fL)	64 (14.6 %)	18 (12.3 %)	60 (42.3 %)	0.000	0.000
Macrocytosis (>100 fL)	6 (1.4 %)	0 (0)	0 (0)		.
Insulin (pmol/L)	42.0 (27.1, 59.9)	43.5 (28.1, 60.0)	60.0 (46.7, 84.9)	0.000	0.000
Albumin (mg/dL)	4.1 (3.9, 4.2)	4.1 (3.9, 4.3)	3.9 (3.7, 4.0)	0.001	0.000
Creatinine (mg/dL)	1.0 (0.9, 1.1)	1.0 (0.9, 1.1)	0.9 (0.8, 0.9)	0.001	0.000
eGFR (mL/min)	86 (77, 96)	86 (77, 96)	76 (69, 86)	0.000	0.000
Homocysteine (μmol/L)	16.2 (12.5, 27.4)	17.3 (12.8, 29.4)	9.5 (6.9, 12.4)	0.001	0.001
(≥15 μmol/L)	251 (57.4 %)	87 (60.4 %)	21 (14.9 %)	0.000	0.000
Vitamin B_{12} (pmol/L)	109.7 (70.1, 176.1)	109.5 (67.7, 214.0)	127.3 (83.8, 190.5)	0.52	0.96
(< 150 pmol/L)	293 (67.0 %)	93 (65.0 %)	84 (60.9 %)	0.18	0.47
Red cell folate (nmol/L)	500.0 (401, 645)	530.0 (421, 647)	647 (498, 902)	0.009	0.0000
(<283 nmol/L)	34 (7.8 %)	10 (6.9 %)	10 (7.9 %)	0.96	0.74

Median (25^{th}–75^{th} centile), number (%), p1: for difference between men (n = 441) and women (n = 146), p2: for difference between men (n = 146) and women (n = 146); eGFR, estimated glomerular filtration rate.

action between gender and risk factor for tHcy was significant, it indicated that the associations were different in men and women. For the 146 couples, we used intraclass correlation coefficient and F-ratio to see the disparity of vitamin B_{12}, homocysteine and folate concentrations within family. A higher intraclass correlation indicates similarity.

The contribution of age, BMI, dietary intake, adiposity and central adiposity, circulating insulin, vitamin B_{12}, and folate concentrations, to the gender difference in tHcy concentrations was determined by multiple linear regression analysis using the indicator variable for gender. Contributions of plasma vitamin B_{12} and erythrocyte folate levels to hyperhomocysteinemia were calculated using Population Attributed Risk (PAR). SPSS version 16.0 for windows (SPSS Inc., Chicago, USA) was used for analysis.

Results

The participants, men and women, were apparently healthy. All women were premenopausal, none were pregnant, and none used oral contraceptives. One woman and 3 men took vitamin supplements. Women were younger, shorter, and lighter, and had a lower lean mass but higher fat percentage compared to men (Table I). None of the women smoked, but a third used tobacco toothpaste, and none consumed alcohol. A third of men smoked, half chewed tobacco, and a third drank alcohol regularly (Table I). Women had significantly lower hemoglobin, plasma albumin, and creatinine concentrations than men (all were within normal range). Six men and no women had macrocytic erythrocytes (Table I). Men had higher tHcy concentrations and a higher prevalence of hyperhomocysteinemia compared to

women. Plasma vitamin B_{12} concentrations and prevalence of low vitamin B_{12} status was similar in men and women, but RCF concentrations were significantly higher in women. Low folate status was 8 % in both (Table I). Low vitamin B_{12} status contributed 35 % to the PAR of hyperhomocysteinemia in men and 44 % in women, whereas low folate status contributed 15 % and 3 % respectively.

Women reported the daily consumption of fewer calories and grams of protein and fat than men (Table II). Frequent consumption of GLV, non-vegetarian foods, milk, and coffee were similar in men and women. Frequent consumption of non-vegetarian foods, milk, and coffee was directly associated with plasma vitamin B_{12} and inversely with tHcy concentrations in both men and women ($p < 0.001$ for both). Coffee consumption was directly associated with plasma tHcy concentrations only in women.

Shared environment

We studied familial resemblance of vitamin B_{12}, tHcy, and folate concentrations in 146 families where both spouses were studied. The intraclass correlation and F ratio for tHcy were ($r = -0.12$, $F = 0.78$, respectively) indicating low similarity ($p = 0.92$). For B_{12} the results were $r = 0.06$, $F = 1.13$, $p = 0.25$ and $r = 0.03$, $F = 1.05$, $p = 0.41$ for RCF.

The strength of associations between plasma tHcy concentrations and age, body composition, eGFR, serum albumin, plasma vitamin B_{12}, and RCF was similar in men and women (Table III).

Unadjusted plasma tHcy concentrations were (1.8x, 101 % difference) higher in men (Tables I and IV) than women. The difference persisted after adjusting for differences in age, lean mass, vitamin B_{12}, RCF, frequency of intake of non-vegetarian foods, milk, coffee, GLV, energy intake, quantity of protein, tobacco

Table II: Dietary intake of macronutrients (24-hour recall) and micronutrient-rich foods (food frequency questionnaire) of 441 men and 146 of their wives

	Men (441)	Men (146)	Women (146)	p1	p2
Macronutrient intake					
Energy (kcal/day)	2162 (1706, 2684)	2162 (1727, 2632)	1437 (1193, 1715)	0.000	0.000
Protein (g/day)	59 (46, 71)	59 (46, 74)	37 (30, 46)	0.000	0.000
Fat (g/day)	38 (27, 60)	38 (26, 58)	28 (19, 37)	0.000	0.000
Carbohydrate (g/day)	378 (300, 461)	372 (308, 474)	254 (213, 297)	0.000	0.000
Micronutrient consumption					
Frequency of GLV (%)					
Never	7	5	3	0.064	0.32
<2/week	17	16	12		
1, 2/day	21	24	19		
>2/day	55	55	66		
Frequency of non-veg consumption (%)					
Never	33	33	37	0.11	0.15
<2/week	27	21	26		
<1/day	36	41	29		
>1/day	4	5	8		
Frequency of milk consumption (%)					
Never	50	45	53	0.86	0.43
<2/week	17	20	14		
<1/day	5	9	6		
>1/day	28	26	27		
Frequency of coffee consumption (%)					
Never	65	62	72		
<2/week	22	27	22	0.09	0.13
<1/day	6	5	5		
>1/day	7	6	1		

GLV, green leafy vegeTable Intake; non-veg intake of non-vegetarian foods, eggs and meat.
Median (25^{th}–75^{th} centile), number (%), p1: for difference between men (n = 441) and women (n = 146).
p2: for difference between men (n = 146) and women (n = 146).

use and hemoglobin, insulin, creatinine, albumin concentrations and eGFR (Table IV).

The addition of insulin to the model did not change the gender difference. However, plasma tHcy concentrations were higher in men at any given plasma vitamin B_{12} and erythrocyte folate concentration ($p = 0.01$ for intercept, both) (Figure 1).

Women with a tHcy of 9.30 μmol/L had the same B_{12} concentration (129 pmol/L) as men with a tHcy of 15 μM; and for a tHcy of 10.0 μmol/L women had the same red cell folate concentration (533 nmol/L) as men with a tHcy of 15 μmol/L.

Discussion

The present study is the first detailed comparison of the relationship of homocysteine to vitamin B_{12} and red cell folate concentrations in predominantly vegetarian Indian men and women. For a wide range of B_{12} and folate concentrations, tHcy in men is consistently almost twice that of the women. Hyperhomocysteinemia is four times more common in men than women (58 % vs. 15 %). The gender difference in plasma tHcy persisted after adjusting for factors associated with higher tHcy concentrations including age, lean body mass, renal function (creatinine and eGFR), circulating vitamin B_{12} and folate concentrations, and diet and other lifestyle factors. The difference was of similar magnitude in 146 pairs of spouses, additionally adjusting for the "unmeasured" aspects of family environment such as passive smoking.

The magnitude of gender difference in tHcy in our study is substantially higher than that reported in other populations where folate and B_{12} status were not described [25–27] and where B_{12} is higher and folate lower than the present study [13–19] (Table IV). Our

Table III: Standardized regression slopes for determinants of tHcy and determinants (adjusted for age) in men and women and test for interaction

Determinant	Age-adjusted standardized β change in logged homocysteine for an increase of one standard deviation of predictor variable std β (95 % CI)				
	Men (441)	Men (146)	Women (146)	p1 homogeneity	p2 homogeneity
Age (years)	0.17 (0.08, 0.26) ***	0.17 (0.01, 0.33) ***	0.19 (0.03, 0.35) ***	0.86	0.80
Height (cm)	0.11 (0.02, 0.21) *	0.09 (–0.07, 0.26)	0.01 (–0.16, 0.17)	0.25	0.38
Weight (kg)	0.18 (0.09, 0.27) ***	0.17 (0.01, 0.33) *	0.13 (–0.04, 0.31)	0.60	0.63
Body mass index (kg/m^2)	0.15 (0.05, 0.24) **	0.15 (–0.01, 0.31)	0.12 (–0.05, 0.29)	0.47	0.50
Body fat (%)	0.18 (0.09, 0.28) ***	0.22 (0.05, 0.39) **	0.12 (–0.04, 0.33)	0.31	0.20
Fat mass (kg)	0.19 (0.09, 0.28) ***	0.21 (0.05, 0.38) **	0.12 (–0.05, 0.30)	0.34	0.24
Lean mass (kg)	0.15 (0.06, 0.24) **	0.11 (–0.06, 0.27)	0.10 (–0.06, 0.27)	0.86	0.92
Albumin (mg/dL)	0.07 (–0.03, 0.16)	0.06 (–0.10, 0.22)	–0.18 (–0.37, 0.01)	0.10	0.11
Creatinine (mg/dL)	0.07 (–0.02, 0.17)	0.13 (–0.03, 0.30)	0.15 (–0.02, 0.31)	0.47	0.99
eGFR (ml/min)	–0.09 (–0.19, 0.01)	–0.16 (–0.32, 0.01)	0.14 (–0.03, 0.32)	0.95	0.50
Vitamin B_{12} (pmol/L)	–0.27 (–0.36, –0.18)***	–0.34 (–0.49, –0.19)***	–0.13 (–0.30, 0.03)	0.15	0.29
Red cell folate (nmol/L)	–0.15 (–0.24, –0.05)**	–0.10 (–0.26, 0.06)	–0.32 (–0.49, –0.15)**	0.78	0.95

Values are standardized regression coefficients (95 % CI) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
p1-Homogeneity refers to significance of the interaction between men (441) and women (146).
p2-Homogeneity refers to significance of the interaction between men (146) and women (146).

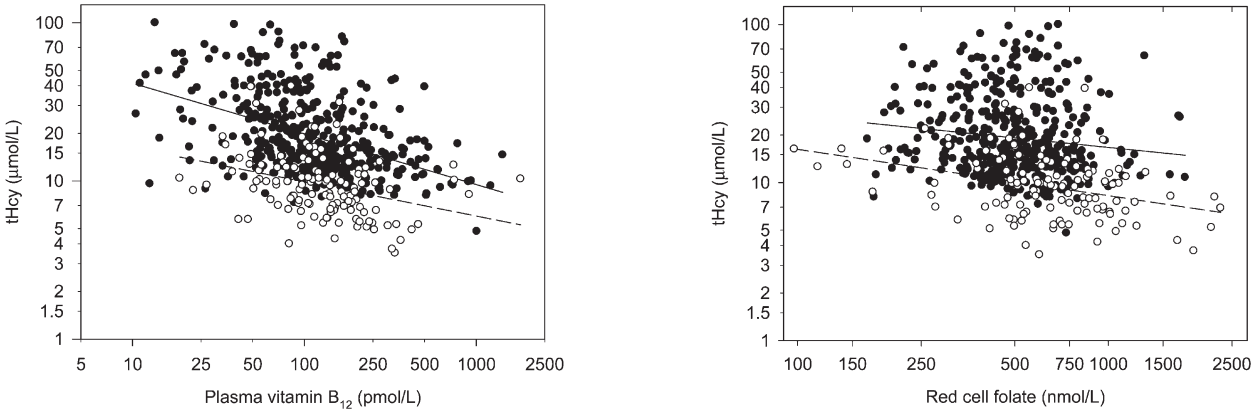

Figure 1: Relation between total plasma homocysteine (tHcy) and B_{12} concentrations (left hand graph) and red cell folate (right hand graph) of men (closed circles) and women (open circles). The linear regressions are: $\log_{10}$tHcy = -0.308$\log_{10}$ (B_{12}) + 1.912 (SEE = 0.22, r^2 = 20.1 % n = 441) and $\log_{10}$tHcy = -0.202$\log_{10}$(red cell folate) + 1.828 (SEE = 0.249, r^2 = 1.8 %, n = 446) for men (solid lines); $\log_{10}$tHcy = -0.217$\log_{10}$ (B_{12}) + 1.427 (SEE = 0.19, r^2 = 10.7 % n = 146) and $\log_{10}$tHcy = -0.263$\log_{10}$(red cell folate) + 1.715 (SEE = 0.192, r^2 = 10.1 %, n = 146) for women (dashed lines)

Table IV: Percentage difference in plasma total homocysteine concentrations of men and women

	% difference in plasma tHcy concentrations between men & women (95 % CI)	
	441 men and 146 women	146 men and 146 women
Unadjusted	101 (81, 123) **	112 (88, 139) **
Adjusted for age	87 (68, 109) **	97 (73, 124) **
Adjusted for age, lean mass	60 (38, 84) **	73 (42, 110) **
Adjusted for age, lean mass, eGFR, albumin	68 (43, 97) **	91 (54, 138) **
Adjusted for age, lean mass, eGFR, albumin vitamin B_{12}, folate	58 (34, 86)**	81 (45, 125) **
Adjusted for age, lean mass, eGFR, albumin, vitamin B_{12}, folate, macronutrient intake	55 (32, 83) **	82 (44, 130) **

Gender % difference (95 % CI) in concentrations of plasma homocysteine * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ linear regression using gender as independent predictor and adjustment for the listed predictors. Difference is represented by % due to log-normality of distributions and is calculated as (men -women)/men*100). Significance (*) indicates that the gender difference at that point is still significant. eGFR, estimated glomerular filtration rate; GLV green leafy vegetable intake; non-veg intake of non-vegetarian foods, eggs and meat.

study and the one of Singaporean Indians [13] report a higher ratio of tHcy men:women than the other studies which were predominantly European. Two Indian studies have also made a similar observation of a high ratio [28, 29]. In a small study [29] of staff members from the National Institute of Nutrition, Hyderabad, men had plasma tHcy concentrations 2.25 times higher than women (18 µmol vs. 8 µmol). In another study [28], conducted by our center, there was a substantial difference between men and women (29 µmol/L vs.15 µmol/L, ratio 1.9) before supplementation [28]. Migrant Indians in the United Kingdom showed a relatively smaller gender difference (16 µmol/L vs. 12 µmol/L, ratio 1.33) [9]. In other European (British and Dutch) [14, 16, 30] and American [31] studies the ratio ranged from 1.1 to 1.2 (Table V).

Gender differences in plasma tHcy may be related to characteristics such as sex hormone concentrations, lifestyle factors such as diet and smoking, and to sexual dimorphism in gene expression that contribute to differences in body composition. A study by Mudd and Poole [32] suggested that the difference between men and women may be related to the stoichiometric formation of Hcy and creatine or creatinine synthesis and the higher muscle mass in men. Brattstrom *et al.* [33] demonstrated that the gender difference was explained by plasma creatinine concentrations. Dierkes *et al.* [14] explained the gender difference in

Table V: Summary of studies that have measured plasma homocysteine, B_{12} and folate concentrations in men and women

Country	Ethnicity	Gender (n)	Age (y)	tHcy μmol/L	B_{12} pmol/L	Folate nmol/L	Ratio tHcy M/F	Reference
Singapore	Malays	men (104)	30–69	15.0 (13.8,16.1)	430.5 (395.0,460.0)	8.5 (7.5, 9.5)	1.20	[13]
		women (106)	30–69	12.5 (11.5, 13.5)	486.0 (449.8, 522.2)	10.8 (8.9, 12.6)		
	Chinese	men (136)	30–69	15.3 (14.3, 16.3)	371.1 (340.0, 402.3)	9.7 (8.8, 10.6)	1.25	
		women (132)	30–69	12.2 (11.3, 13.1)	373.7 (341.2, 406.1)	13.8 (12.1, 15.4)		
	Indian	men (129)	30–69	16.2 (15.1, 17.2)	352.5 (320.5, 384.4)	8.7 (7.8, 9.6)	1.41	
		women (119)	30–69	11.5 (10.5, 12.3)	350.7 (318.7, 385.0)	10.9 (9.2, 12.7)		
Germany	European	men (189)	40–64	10.8 (7.4, 17.6)	222 (131–399)	17.1 (10.1, 26.1)	1.20	[14]
		women (147)	35–64	9.0 (5.9, 14.1)	230 (125, 447)	16.4 (10.6, 30.0)		
Norway	European	men (1238)	47–50	10.9 ± 3.5	369.8 ± 142.9	7.2 ± 3.8	1.18	[16]
		women (1856)	47–50	9.2 ± 3.2	380.8 ±145.7	8.5 ± 6.2		
USA	European	men (871)	30–59	10.5 (10.3, 10.8)	307 (296, 318)	12.3 (11.7, 12.9)	1.18	[19]
		women (949)		8.9 (8.7, 9.1)	311 (300, 322)	14.1 (13.5, 14.8)		
Greece	Greek	boys (87)	11.6 ± 0.4	8.2 ± 2.6 *	397 ± 104	21.7 ± 10.8	1.04	[18]
		girls (99)		7.9 ± 1.6	427 ± 121	20.8 ± 6.4		
Australia	European	men (49)	18–32	9.67 (0.44) **	384.1 (14.24)	440.0 (20.46)	1.09	[15]
		women (57)		8.84 (0.33)**	283.0 (14.53)	363.8 (17.26)		
USA	American	men (1,764)	17–44	9.4	352.7	12.8	1.24	[17]
		women (1,751)	17–44 premenopausal	7.6	379.6	14.8		
		men (638)	55–69	10.7	336.6	19.3	1.14	
		women (562)	55–69 postmenopausal	9.4	370	19.9		
India	Indian	men (441)		16.2 (12.5,27.4)	109.7 (70.1–176.1)	500.0 (401–645)	1.82	Current
		men (146)		17.3 (12.8,29.4)	109.5 (67.7–214.0)	530.0 (421–647)		
		women (146)		9.5 (6.9,12.4)	127.3 (83.8–190.5)	647 (498–902)		

Mean (95 % confidence interval), * mean standard deviation, ** mean (standard error).

tHcy concentrations by plasma vitamin B_{12} (11 %), creatinine (11 %), folate (8 %), fat-free mass (5 %), estradiol (2 %), methylenetetrahydrofolate reductase (MTHFR) polymorphism (2 %), and plasma proteins (1 %). Insulin has recently [34] been shown to increase the accumulation of homocysteine by alteration in the expression of a key enzyme, cystathionine-β-synthase, involved in the transsulfuration pathway. In our study the gender difference in tHcy concentrations persisted after adjusting for age, lean mass, eGFR, albumin, B_{12}, folate, and insulin. Other explanations include a higher rate of methionine transamination in premenopausal women [35] and higher rates of remethylation and transmethylation of homocysteine in women [36]. The difference in the rates of remethylation remained significant even after adjusting for muscle mass, suggesting that in these subjects the difference in creatine or creatinine synthesis did not account for the higher tHcy concentrations in men [36].

The variant of the MTHFR gene that influences tHcy accumulation is MTHFR677T [37,38]. Although we have not looked at variants of the MTHFR gene in our study, data from Indian studies show a low frequency of MTHFR677T, and no gender difference in the frequency of this polymorphism has been documented. Moreover, MTHFR polymorphism effects are shown in folate-deficient states and most of our subjects were folate-sufficient with no difference in the prevalence of folate deficiency by gender.

An indirect approach to study the role of sex hormones and body composition in plasma tHcy concentrations is to examine tHcy by gender across the life course; at birth (cord blood), pre-pubertal, post-pubertal, young adults, and postmenopausal women. Hay *et al.* [39] reported similar plasma tHcy concentrations in boys and girls at birth and at six months of age. In a British study [30] of children aged 4–18 years, a significant gender difference was observed in children aged 15–18 years (8.5 μmol/L vs. 7.8 μmol/L), suggesting that the increase in circulating sex steroids at puberty is associated with the gender difference. Evidence for an age-related increase in the ratio, mainly for European populations, is summarized in Table V. The ratio is decreased at age 65+, when women are postmenopausal, but increased if the women are taking estrogens [17].

Similarly, treatment with estrogen and anti-androgens in male-to-female transsexuals decreased plasma tHcy concentrations significantly while treatment with androgens in female-to-male transsexuals was associated with increases in tHcy [40]. Thus, the difference in sex steroid milieu between men and women is an important contributor to the gender difference in tHcy. None of the women in our study were pregnant or postmenopausal and no use of contraceptives was reported.

Elevated homocysteine levels have been reported to be associated with a multitude of adverse health outcomes at various stages in life cycle such as recurrent miscarriages [41], low birth weight [3], cardiovascular morbidity [42, 43], and neurocognitive decline in elderly populations with Alzheimer's disease [44]. Hence prevention and reduction of hyperhomocysteinemia is increasingly important in improving health status but the need for vitamin supplementation will occur at a lower homocysteine concentration in women than in men.

The strength of our study is that it is a community-based study with a representative population, with precise measurements including many of important confounders. In addition to community base, we also had paired measurements in 146 wives, which controls for many unmeasured environmental variables and gives extra strength to our findings of the gender difference. A major limitation is the lack of measurement of sex hormones and genetic markers for one-carbon metabolism.

Adult Indian women have markedly lower tHcy concentrations compared to men. This suggests a lower threshold for supplementation to improve reproductive and cardiovascular outcomes.

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