Original Article

Drinking tea is associated with lower plasma total homocysteine in older women

Jonathan M Hodgson PhD[1], Amanda Devine PhD[2,3], Ian B Puddey MD[1], John Beilby PhD [4] and Richard L PrinceMD[2,3]

[1]*University of Western Australia School of Medicine and Pharmacology, and the Western Australian Institute for Medical Research (WAIMR)* [1]*Royal Perth Hospital Unit*
[2] *Sir Charles Gairdner Hospital Unit*
[3]*Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital*
[4]*Pathcentre and School of Pathology and Surgery, University of Western Australia, Perth, Western Australia, Australia.*

Dietary polyphenols are suggested to elevate plasma total homocysteine concentration (tHcy). Although tea is rich in polyphenols, it has been associated with lower tHcy, which may be due to its folate content. Our aims were to investigate relationships of tea intake and 4-O-methylgallic acid (4OMGA) – a biomarker of exposure to tea-derived polyphenols – with tHcy in older women. In a cross-sectional study of 232 women over 70 years of age, we measured tHcy, tea intake, 24 h urinary excretion of 4OMGA, and red cell folate. Tea intake and 4OMGA excretion were inversely related to tHcy. Tea intake (>2 cups) and 4OMGA excretion above the median were associated with lower tHcy by ~1mmol/L (P <0.01). Red cell folate was not associated with tea intake or 4OMGA excretion. The observed lower tHcy in women with higher tea intake is consistent in direction and magnitude with previous epidemiological studies, but any mechanisms remain unclear.

Key Words: tea, polyphenols, homocysteine, folate, women.

Introduction

An elevated total plasma homocysteine concentration (tHcy) may be causally related to an increased risk for cardiovascular disease.[1] Homocysteine is an intermediate in methionine metabolism, and its metabolism can be influenced by several dietary factors including folate, vitamin B_{12}, vitamin B_6, betaine, and poly-hydroxylated phenolic compounds (polyphenols).[2-4] Tea is a rich source of dietary polyphenols and contains caffeine.[5] Results of controlled intervention studies suggest that both polyphenols[4] and caffeine[6] can raise tHcy. In spite of this, results of cross-sectional population studies generally show that a higher intake of tea is associated with lower tHcy.[7-10] This association is often attenuated after adjustment for coffee intake.[7,9,10] A controlled intervention study using high doses of tea solids found that tea solids increased tHcy,[4] but a dose more representative of a usual tea intake did not alter tHcy.[2]

The proposed mechanism for a tHcy raising effect of dietary polyphenols involves polyphenols accepting methyl groups during metabolism of methionine to homocysteine.[2,4] Consistent with this hypothesis is the demonstration that the degree of methylation of tea-derived polyphenols, assessed by measuring 24 h urinary excretion of 4-O-methylgallic acid (4OMGA), was positively associated with the tHcy response to regular ingestion of tea.[2] The inverse association between tea and tHcy in populations is also consistent with the presence of other factors in tea, such as folate,[11] which could contribute to lower tHcy. In the present study we have used 24 h urinary excretion of 4OMGA[12] as a biomarker for tea-derived polyphenol exposure and methylation, and measured red cell folate to indicate folate status. Elderly women are at increased risk of cardiovascular disease. In a population based, unselected group of women over 70 years of age we have investigated the cross-sectional relationships between beverage intake and tHcy. The major aims of this study were to investigate relationships of tea intake and 4OMGA with tHcy.

Methods

Participants and design

The participants involved in this study were recruited to a 5 year, prospective, randomized, controlled trial of oral calcium supplements to prevent osteoporotic fractures in randomly selected women aged between 70 and 85 y. We present a cross-sectional analysis from a subset of these women. The women were recruited from the Western Australian general population of women aged over 70 y by mail using the electoral roll. A random selection of 24,800 women on the electoral roll (N = 33,366) was sent a letter inviting participation.

Correspondence address: Dr J Hodgson, School of Medicine & Pharmacology, PO Box X2213, Perth, WA 6001, Australia
Tel: 61 8 9224 0267; Fax: 61 8 9224 0246
E-mail: Jonathan.Hodgson@.uwa.edu.au
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5,586 women (22.5%) responded, and 1,510 women were willing to take part and were eligible. All participants were healthy and did not have medical conditions likely to influence 5 y survival. The first 1500 women were randomized into the study.[13] Participants involved in the study did not differ from the general population in health resource utilization.[14] At baseline every third woman was asked to provide a 24 h urine collection and information about food and beverage intake using an interviewer-administered 24 h dietary recall; a total of 281 women agreed. Complete dietary information and 24 h urine collections were obtained from 275 women. All data, including dietary intake, tHcy, and demographic and anthropometric factors, were available on a total of 232 women. Informed and written consent was obtained and the Human Rights Committee of the University of Western Australia approved the study.

Dietary assessment

An interviewer-administered 24 h dietary recall was used to collect food and beverage intake data during a clinic visit. Tea and coffee intake was assessed in cups. Tea intake included black tea and green tea, but not "herbal teas." Almost all tea consumed within this population was black tea with added milk. Almost all coffee consumed was instant (soluble) coffee. The food intake data were analysed to obtain nutrient intakes using Foodworks Professional (Xyris, Brisbane Australia) based on the Australian Food Composition Database (NUTTAB 95, Australian Government Nutrient Database, Canberra, Australia).

Biochemistry

The plasma total L-homocysteine (tHcy) was measured using a Fluorescence Polarization Immuno-assay on an Abbott IMx Analyzer (Abbott Laboratories, Abbott Park, IL). The inter-assay CV for homocysteine measurement was less than 5%. The tHcy includes free monomeric homocysteine, free dimeric homocysteine, protein bound forms and mixed dimeric low molecular mass forms. Red blood cell folate levels were determined using a chemiluminescence method on an ACS**:**180 Immuno-assay analyser (Chiron Diagnostics Corporation/Bayer, CA). The inter-assay CV for red cell folate measurement was less than 12%.

A 24 h urine sample was collected for the period corresponding to the dietary recall information. Urinary 4OMGA concentrations were used as a marker of tea-derived polyphenol intake[12] and metabolism.[2] 4-O-methylgallic acid was measured in urine samples using gas chromatography-mass spectrometry according to a previously described method.[12,14] The intra-assay variability was 5%.

Demographic and anthropometric factors

During a clinic visit all participants completed a questionnaire to collect information on age, smoking history, physical activity and residential postcode. Weight and height were measured, and the body mass index was calculated in kg/m^2. Smoking status was coded as non-smoker, ex-smoker or current smoker. For physical activity, the women were asked if they currently participated in any sports, recreation or regular physical activity. Those who answered 'yes' to this question were asked to list up to four activities and the duration in hours per week that they engaged in each activity. Activity levels for these women were calculated in kJ/d using published energy costs of listed activities. Women who answered 'no' to the activity question were classified as being sedentary and scored zero for activity.[15] Socioeconomic status was assessed using relative social disadvantage according to residential postcodes, and was divided into three levels: low, medium and high.

Statistical analysis

Statistical analyses were performed using SPSS 11.5 software (Chicago, IL, USA). Results are presented as mean (95% CI), and P <0.05 was the level of significance. The values for tHcy, tea intake, coffee intake, and 4OMGA excretion were not normally distributed. For tHcy and 4OMGA log-transformed values were used. Results for these variables are presented as geometric mean (95% CI). Log-transformations of tea and coffee intake could not be performed because many participants had zero values for these variables. Therefore, Spearman's rank correlation was used to assess univariate associations of tea and coffee intake with other variables. Pearson's correlation was used to assess univariate associations between normally distributed variables. Linear regression analysis was used to further investigate observed correlations, with adjustment for potential confounding factors. General linear models were used to assess differences in tHcy between participants with tea intake and 4OMGA excretion above and below the median.

Results

The characteristics of the study population are presented in Table 1. Most of the population drank at least one cup of tea (75%) or coffee (65%), but most (67%) had not consumed alcohol. About 44% of the population drank more than 2 cups of tea over the 24 h and about 16% drank more than 2 cups of coffee. Amongst those who had consumed alcohol the mean intake was 19.0 (16.4, 21.6) g. Tea intake was positively associated with urinary 4OMGA excretion (r=0.62, P<0.001). Tea and coffee intake were negatively associated (r=-0.46, P<0.001). Tea and coffee intake were not significantly associated with age, body mass index, smoking status, physical activity or social disadvantage. Significant but opposite associations were observed for both tea and coffee intake with energy intake (r=-0.13, P=0.05 and r=0.25, P<0.001, respectively). After adjustment for energy intake, significant but again opposite associations were found for tea and coffee intake with alcohol intake (r=-0.13, P=0.04 and r=0.17, P=0.01, respectively). 4-O-methylgallic acid excretion was not significantly associated with age, body mass index, smoking status, physical activity, social disadvantage, energy intake or nutrient intakes. Tea intake (r=-0.03, P=0.65) and 4OMGA excretion (r=-0.07, P=0.32) were not significantly associated with red cell folate. Plasma total homocysteine concentrations were positively associated with age (r=0.16, P=0.02) and body mass index (r = 0.20, P = 0.003), and negatively associated with red cell folate (r = -0.24, P<0.001).

Table 1. Characteristics of the 232 women involved in the study.

	Mean (%)	95%CI
Age (years)	75.0	74.6,75.3
Body mass index (kg/m^2)	27.1	26.5,27.7
Smoking status		
non-smokers	59	
ex-smokers	36	
current smokers	5	
Social disadvantage		
low	59	
medium	27	
high	14	
Physical activity (kJ/day)	608	532,683
Energy and nutrient intakes		
total energy (kJ)	6683	6434,6932
alcohol (g)	6.4	4.9,7.9
protein (g)	75.2	72.3,78.3
fat (g)	54.3	51.0,57.7
carbohydrate (g)	192.7	184.6,200.9
dietary fibre (g)	24.3	23.2,25.4
Tea (cups)	2.2	2.0,2.4
Coffee (cups)	1.2	1.0,1.4
4-O-Methylgallic acid excretion (μg/mmol creatinine)	50.9	39.6,65.4
Red cell folate (nmol/L)	406	388,424
Plasma total homocysteine (μmol/L)	11.2	10.8,11.7

There was no association between tHcy and alcohol, energy or any specific nutrient intakes. Coffee intake (r =-0.02, *P*=0.79) was not associated with tHcy. Tea intake (r=-0.13, *P*=0.05) and 4OMGA excretion (r = -0.20, *P* = 0.002) were negatively associated with tHcy. A dose-response rela-tionship was apparent across quintiles of tea intake (Fig. 1A; *P* = 0.07 for trend) and 4OMGA excretion (Fig. 1B; *P* = 0.01 for trend). These relationships were largely un-changed after adjustment for age, body mass index, energy intake, coffee intake and alcohol intake.

Participants were then divided according to median tea intake and median level of 4OMGA excretion. In comparison to a tea intake of <2 cups, an intake of >2 cups of tea was associated with significantly lower tHcy (11.9 μmol/L (95%CI:11.3,12.5) v 10.9μmol/L (95%CI: 10.4, 11.4) *P* = 0.01) (Fig. 2A). In comparison to 4OMGA excretion below the median, 4OMGA excretion above the median was also associated with significantly lower tHcy (11.9 μmol/L (95% CI:11.3,12.5) v 10.8 μmol/L (95%CI: 10.3,11.3) *P* = 0.004) (Fig. 2B). The magnitude of these observed differences was largely unchanged after adjustment for age, body mass index, energy intake, coffee intake and alcohol intake.

Discussion

In a cross-sectional study of women aged 70 to 85 years, we have found a negative association of both tea intake and a biomarker of exposure to tea-derived polyphenols with tHcy. A tea intake of ≥2 cups per day was associated with approximately a 1 μmol/L lower tHcy. A recent meta-analysis of studies carried out in healthy populations suggests that a decrease in homocysteine level of about 3 μmol/L is associated with a 19% lower risk of stroke and an 11% lower risk of ischemic heart disease.[16] Tea is a widely consumed beverage, and therefore, any physiological effects of drinking tea on cardiovascular disease risk could have a significant impact on population health. If tea can independently lower homocysteine, such an effect has immediate clinical relevance.

The relationship of tea intake and our biomarker, 4OMGA, with tHcy is consistent in direction and magnitude with previous population studies.[7-10] We have proposed that the folate in tea[11] may counteract any tHcy raising effect of tea polyphenols[4] and caffeine.[6] One cup of tea may provide 5 to 10% of a 200 μg/d recommended daily intake for folate.[11] However, the lack of association of tea intake or 4OMGA excretion with red cell folate concentrations would not support this suggestion. An alternative explanation is that the relationships may be confounded by coffee intake and dietary and lifestyle factors associated with beverage choice, such as energy and alcohol intake. We found an inverse association between tea and coffee intake, and observed significant

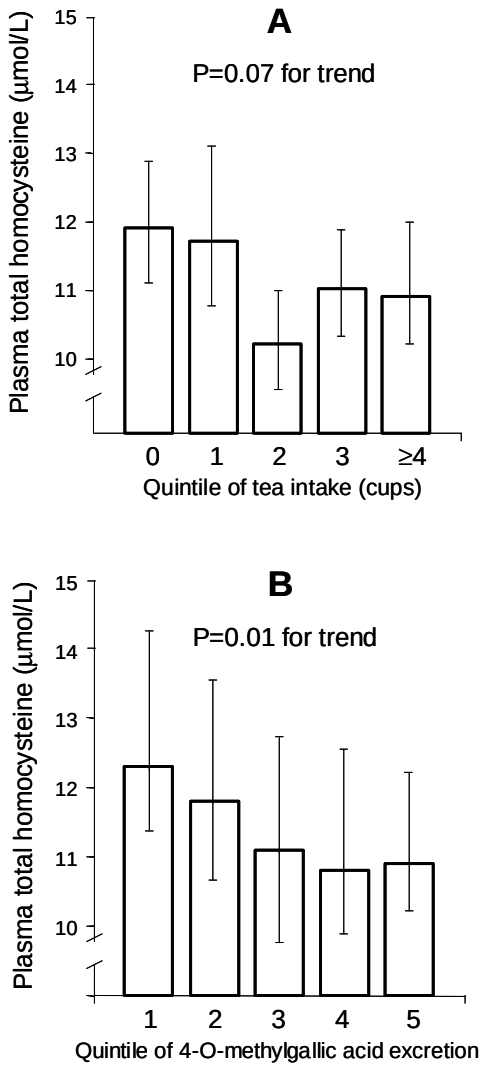

Figure 1. Mean plasma total homocysteine concentration according to quintiles of tea intake (A) and 4-O-methylgallic acid excretion (B) in a cross-sectional study of 232 women aged between 70 and 85 y (Results are geometric means and 95%CI).

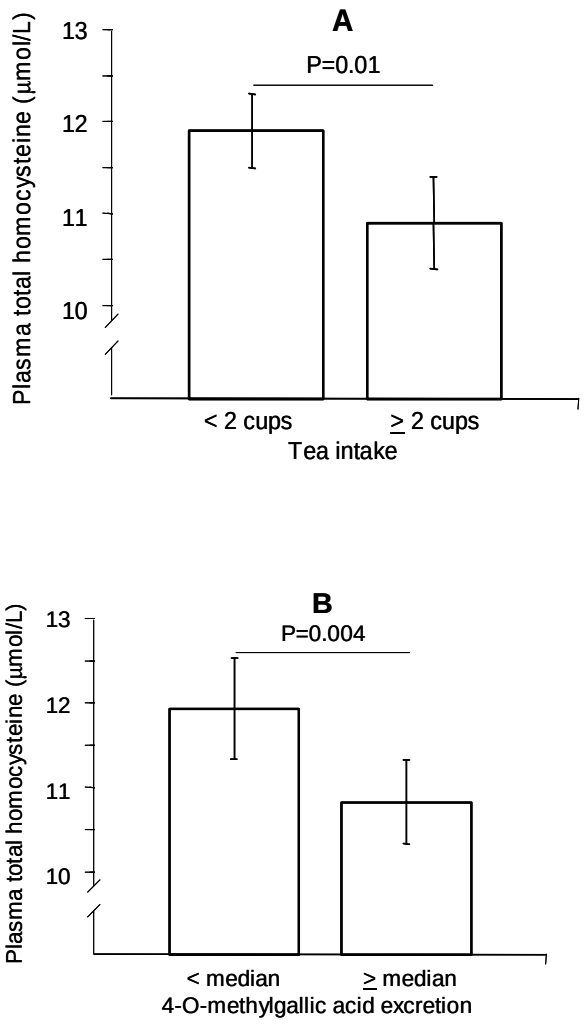

Figure 2. Relationships of tea intake (A) and 4-O-methylgallic acid excretion (B) with plasma total homocysteine concentration when the population of 232 women aged between 70 and 85 y was divided according to the median tea intake and 4-O-methylgallic acid excretion (Results are geometric means and 95%CI).

associations of tea intake with energy, alcohol and nutrient intakes which were in the opposite direction to those observed for coffee intake. However, adjustment for these potential confounders did not alter the interpretation of the observed associations. Therefore, potential mechanisms of any tHcy lowering effect of tea remain uncertain. We have previously shown that regular ingestion of 5 cups/d for 4 weeks did not alter tHcy in predominantly male volunteers.[2] These results suggest that short-term regular ingestion of tea does not significantly alter tHcy in men. The effects of regular ingestion of tea in women, and the longer-term impact of drinking tea in both men and women have not been investigated in controlled intervention studies.

In our previous study,[2] we also found that a higher degree of methylation of tea polyphenols to 4OMGA was associated with an increase in tHcy. It has been proposed that dietary polyphenols, including those found in tea and coffee, can contribute to elevations in tHcy.[2,4] Any tHcy-raising effect of tea polyphenols may be balanced by a tHcy lowering effect of some other constituent of tea. Given that we found a negative association between tea intake and tHcy, if tea-derived polyphenols do contribute to elevations in tHcy then we might have expected that the association of 4OMGA excretion with tHcy to be weaker or even positive. In fact, the negative association with tHcy was stronger for 4OMGA excretion. This result does not support the suggestion that: (1) tea-derived polyphenols elevate tHcy; (2) tea-derived polyphenols attenuate any tHcy lowering effect of other components of tea; or (3) any effect of tea on tHcy is related to metabolism (methylation) of tea polyphenols.

Our results are consistent with previous findings that 4OMGA excretion may be a good biomarker for tea intake.[12,17] There appear to be few other important dietary sources of 4OMGA within populations studied.[17] The observation of a stronger linear association with tHcy for 4OMGA excretion in comparison to tea intake suggests that this biochemical measurement may provide a better indication of exposure to tea than estimated cups. A range of factors may influence the precision in measurement of beverage exposure using cups as the estimate.[18]

The lack of a positive association between coffee intake and tHcy is not consistent with results of previous studies.[7-10,19-21] The lack of association may be explained by the low coffee intake within our population and imprecision in the estimation of coffee exposure. The mean coffee intake was 1.2 cups, and only 16% of participants drank more than 2 cups of coffee. The mean coffee intake in populations where positive associations have been found has often been between 2 and 5 cups per day.[7,10,19] A range of factors influence the precision in measurement of coffee exposure.[18] Important factors are likely to be the strength of the coffee, which can vary widely, and although most of the coffee used by the women in our study was instant coffee, the method used to prepare the coffee may also influence exposure.

The observed lower tHcy in women with higher tea intake (by about 1 µmol/L) is consistent in direction and magnitude with previous epidemiological studies. The strength of the association between tea intake and 4O MGA excretion, and the similarity of observed associations with tHcy indicate that 4OMGA is a reasonable biomarker of tea intake. The results of this study do not provide support for the hypotheses that folate in tea is responsible for lowering tHcy, or that methylation of dietary polyphenols can contribute to elevations in tHcy. Rather they suggest that some other constituent of tea may be responsible for a tHcy lowering effect.

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Jonathan M Hodgson PhD[1], Amanda Devine PhD[2,3], Ian B Puddey MD[1], John Beilby PhD [4] and Richard L PrinceMD[2,3]

[1]*University of Western Australia School of Medicine and Pharmacology, and the Western Australian Institute for Medical Research (WAIMR)* [1]*Royal Perth Hospital Unit*
[2] *Sir Charles Gairdner Hospital Unit*
[3]*Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital*
[4]*Pathcentre and School of Pathology and Surgery, University of Western Australia, Perth, Western Australia, Australia.*

饮茶与老年妇女血浆较低水平的总同型半胱氨酸相关性研究

饮食中的多酚类物质被认为可能提高血浆总同型半胱氨酸浓度(tHcy)。尽管茶中含有高浓度多酚物质，但它与降低 tHcy 浓度相关，这可能与它所含的叶酸有关。我们研究小组分析了饮茶、4-O-甲基没食子酸 (4OMGA)（一种摄入茶多酚物质的生物标志物）和老年妇女同型半胱氨酸浓度的相关性关系。在一份对 232 名 70 岁以上妇女的研究中，我们分析了 tHcy、茶摄入量、24 小时尿中 4OMGA 的排泄量和红细胞叶酸含量。结果表明，茶摄入量、尿 4OMGA 排出量和 tHcy 量成负相关。茶摄入量（>2 杯）和高于正常水平的 4OMGA 排出量与较低水平的 tHcy 相关(P <0.01)。红细胞叶酸量并不与茶摄入量和 4OMGA 排出量相关。在妇女人群中观察到的较低水平的同型半胱氨酸量和较高水平的茶摄入量与先前流行病学研究分析结果一致，但一些机理还并不明确。

关键词：茶、多酚、同型半胱氨酸、叶酸、妇女。