

Short Communication

Acute effects of tea on fasting and non-fasting plasma total homocysteine concentrations in human subjects

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Plasma total homocysteine concentrations (tHcy) are a putative risk factor for CVD. Tea is a rich dietary source of polyphenols and caffeine, both of which may raise tHcy. However, it is possible that much of any effect is transitory and may be influenced by the consumption of food. Our objective was to investigate the acute effect of tea, at a dose representative of ordinary population intakes, on tHcy and to determine whether consumption of a meal influences the magnitude of any effect. Measurements of tHcy were performed in twenty participants at baseline and 3.5 h after drinking three cups of black tea or hot water (consumed at time 0, 1.5 and 3 h) with and without a meal: a total of four treatments administered in random order. Drinking tea resulted in an acute increase in tHcy (0.30 (95 % CI 0.04, 0.56) $\mu\text{mol/l}$, $P=0.022$). The meal resulted in an acute decrease in tHcy (-0.42 (95 % CI -0.68 , -0.16) $\mu\text{mol/l}$, $P=0.002$). There was no interaction between tea and meal on tHcy ($P=0.40$); that is, the effect of tea on tHcy was not different in the fasting and non-fasting state. Our results suggest that drinking black tea can cause a small acute increase in tHcy and that this effect is not enhanced in the non-fasting state. Given that results of population studies have generally shown a negative association between tea intake and tHcy, the significance of these findings to CVD risk remains uncertain.

Homocysteine: Tea: Polyphenols: Caffeine

Introduction

Plasma total homocysteine concentrations (tHcy) provide a marker of CVD risk (Cesari *et al.* 2005). However, randomized controlled trials of tHcy-lowering therapy have yet to demonstrate a causal relationship and thus the importance of homocysteine as a risk factor remains uncertain (Kaul *et al.* 2006). The dietary precursor of homocysteine is the amino acid methionine, which when consumed as a supplement can raise tHcy acutely (Chambers *et al.* 1999; Ditscheid *et al.* 2005). However, the methionine load of the diet is not the main determinant of tHcy (Verhoef & de Groot, 2005). The metabolism of homocysteine is influenced by the intake of other dietary factors including polyhydroxylated phenolic compounds (polyphenols) (Olthof *et al.* 2001) and caffeine (Verhoef *et al.* 2002).

Coffee and tea are major dietary sources of polyphenols and caffeine. Regular consumption of coffee can raise tHcy (Grubben *et al.* 2000; Urgert *et al.* 2000), an effect due primarily to the polyphenol chlorogenic acid (Olthof *et al.* 2001) as well as caffeine (Verhoef *et al.* 2002). Regular consumption of a high dose of tea was also found to raise tHcy (Olthof *et al.* 2001), but a moderate intake of tea did not alter tHcy (Hodgson *et al.*

2003). Effects of regular consumption of coffee and tea, but not caffeine, to raise tHcy appear to be greater in the non-fasting compared with the fasting state (Olthof *et al.* 2001; Verhoef *et al.* 2002). It remains uncertain whether this is due to a transitory effect of polyphenols alone or whether there is interaction between polyphenols and other dietary components to enhance any increase in tHcy.

Our aim in the present study was to investigate the acute effect of tea on fasting and non-fasting tHcy. We wished to determine whether black tea, at a dose representative of ordinary population intakes, can cause a transitory increase in tHcy and whether consumption of a meal could influence the magnitude of any effect.

Methods

Participants

A total of twenty participants, aged between 45 and 70 years, who completed the study were recruited from the general population in response to media advertisements. Eligible participants had a history of documented coronary artery disease,

including stable angina pectoris, or coronary revascularization procedure or myocardial infarction more than 6 months prior to commencement of the present study. The exclusion criteria were: age >70 or <45 years; smoking; BMI >36 kg/m²; unstable angina, heart failure or arrhythmia; diabetes; a history of liver or kidney disease; premenopausal women and postmenopausal women taking hormone replacement therapy; alcohol intake >40 g/d; unwillingness to stop taking supplements, to stop drinking red wine or to limit tea and coffee intake to one cup/d for at least 4 weeks prior to commencement of the study and during the 4 week study period; regular tea intake of less than one cup/d. The Royal Perth Hospital Ethics Committee approved the project and all participants gave informed written consent to participate.

Experimental design

In a cross-over designed study, there was a total of four treatments. There was one treatment at each of four clinic visits, which were administered in random order, 1 week apart. The order of the visits was randomly assigned using computer-generated random numbers sealed in opaque envelopes. All measurements were performed at baseline and 3.5 h after drinking three cups of black tea or hot water with and without a meal. Participants were instructed to maintain their diet, physical activity and medication unchanged for 4 weeks prior to commencement of the study and for the 4 weeks during which the clinic visits were conducted. The clinic visits took place between 07.30 and 13.30 hours. The treatments included the following: (1) water and no meal; (2) black tea and no meal; (3) meal with water; (4) meal with black tea. One cup of water or one standard cup of black tea was provided at time 0, at 1.5 h and at 3 h. The meal was provided at time 0 and consumed over 0.5 h. Following >12 h overnight fast, a baseline blood sample was taken and a spot urine sample was collected, then a blood sample was taken at 3.5 h and a 5 h urine collection was performed.

The black tea was provided as an infusion of 2.2 g tea leaves in 250 ml boiled water. The tea was packaged into a tea bag, which was moved constantly in the hot water for 2 min. The control drink was the same volume (250 ml) of boiled water. Participants consumed the tea or water within 10 min of it being provided. Each cup of tea supplied approximately 50 mg caffeine and 300 mg polyphenols. The meal comprised a sausage, egg and bacon McMuffin[®] with two hash browns (McDonald's Corporation, Oak Brook, IL, USA), which supplied 3400 kJ, 33 g protein, 56 g carbohydrate, 50 g fat and 4 g dietary fibre. The meal was chosen to provide substantial amounts of energy as protein, carbohydrate and fat, but almost no polyphenols. No meal (continued fasting) was used as the control for this treatment.

Biochemistry

Plasma was prepared and immediately frozen at -80°C. The plasma total L-homocysteine was measured using a Fluorescence Polarization Immuno assay on an Abbott IMx Analyzer (Abbott Laboratories, Abbott Park, IL, USA). The inter-assay CV for homocysteine measurement was less than 5%. Plasma folate concentrations were measured using ion capture on the AxSYM Analyzer (Abbott Laboratories).

The inter-assay CV for folate measurement was less than 10%. Plasma vitamin B₁₂ concentrations were measured using a microparticle enzyme intrinsic factor assay on the Abbott AxSYM analyser (Abbott Laboratories). The inter-assay CV for vitamin B₁₂ measurement was less than 15%. A reversed-phase HPLC method was used to measure caffeine in tea and plasma. This has been previously described in detail (Hodgson *et al.* 2005). The minimum level of detection (per 50 µl plasma) was 0.2 ng (approximately 0.004 mg/l) and the intra-assay CV was 5.4%. 4-O-Methylgallic acid (4OMGA) concentrations were used as a marker of absorption and exposure to tea-derived polyphenolic compounds and of the degree of polyphenol O-methylation (Hodgson *et al.* 2000, 2003, 2006b). Concentrations of 4OMGA were measured in 1 ml urine samples, which had been frozen at -80°C prior to assaying, using a previously described method (Hodgson *et al.* 2000). Urinary creatinine was measured using routine laboratory methods within the Department of Clinical Biochemistry at Royal Perth Hospital. The minimum level of detection of 4OMGA (per ml) was 0.4 ng (approximately 0.2 nmol/mmol creatinine) and the intra-assay CV was 5.0%.

Statistical analysis

Statistical analyses were performed using SPSS 12.0 software (SPSS Inc., Chicago, IL, USA) or SAS 8.2 software (SAS Institute, Cary, NC, USA). Results are presented as means with 95% CI in the text and Table 1 or means with their standard errors in Fig. 1 and $P < 0.05$ was the level of significance. Spearman's rank correlation was used to assess the degree and direction of association between two variables. Treatment effects were analysed with random effects models using PROC MIXED. In the random effects models, participant was treated as the random effect and treatment (water or tea (TEA) and no meal or meal (MEAL)), period and treatment order as the fixed effects. An interaction term (TEA × MEAL) was also included in the models to determine whether the effects of drinking tea were different in the fasting and non-fasting states.

Results

The age and BMI of the participants were 62.1 (95% CI 59.1, 65.1) years and 28.1 (95% CI 26.5, 29.8) kg/m², respectively. There were seventeen men and three women who completed the present study. The tHcy, serum folate and vitamin B₁₂, plasma caffeine and urinary 4OMGA excretion at baseline and after each treatment are presented in Table 1.

For tHcy, there was no significant period or order effect. That is, the effects of treatment were not significantly different between periods or for the different orders in which treatments were administered. There was no significant interaction between TEA (water and tea) and MEAL (no meal and meal) on tHcy. That is, the effect of drinking tea on tHcy was not significantly different in the fasting and non-fasting state. In main effects analysis, drinking tea resulted in an acute increase in tHcy: 0.30 (95% CI 0.04, 0.56) µmol/l, $P = 0.022$. The meal resulted in an acute decrease in tHcy: -0.42 (95% CI -0.68, -0.16) µmol/l, $P = 0.002$. The mean tHcy for each factor (water and tea; no meal and meal) is presented in Fig. 1.

Table 1. Plasma total homocysteine (tHcy), serum folate, serum vitamin B₁₂ and plasma caffeine concentrations at baseline and at 3.5 h post baseline (post) and urinary excretion of 4-*O*-methylgallic acid (4OMGA) at baseline and for 5 h post for each treatment* (Mean values and 95% CI)

| Variable | Fasting | | | | Non-fasting | | | |
|--|---------|-------------|-------|-------------|-------------|-------------|-------|-------------|
| | Water | | Tea | | Water | | Tea | |
| | Mean | 95% CI | Mean | 95% CI | Mean | 95% CI | Mean | 95% CI |
| tHcy (μmol/l) | | | | | | | | |
| Baseline | 10.41 | 9.43, 11.38 | 10.63 | 9.56, 11.69 | 10.44 | 9.44, 11.44 | 10.46 | 9.34, 11.57 |
| Post | 10.26 | 9.25, 11.28 | 10.83 | 9.75, 11.90 | 9.98 | 8.98, 10.98 | 10.18 | 9.17, 11.19 |
| Serum folate (nmol/l) | | | | | | | | |
| Baseline | 28.6 | 19.9, 37.2 | 29.7 | 21.6, 37.8 | 29.8 | 20.8, 38.8 | 30.3 | 21.5, 39.1 |
| Post | 33.3 | 23.5, 43.0 | 34.5 | 25.0, 44.4 | 28.2 | 20.1, 36.2 | 27.5 | 20.5, 34.5 |
| Serum vitamin B ₁₂ (pmol/l) | | | | | | | | |
| Baseline | 315 | 265, 365 | 342 | 281, 402 | 332 | 278, 386 | 312 | 262, 362 |
| Post | 335 | 279, 391 | 361 | 301, 422 | 326 | 278, 373 | 315 | 263, 367 |
| Plasma caffeine (mg/l) | | | | | | | | |
| Baseline | 0.11 | -0.02, 0.23 | 0.06 | -0.03, 0.16 | 0.08 | -0.03, 0.18 | 0.12 | -0.07, 0.31 |
| Post | 0.07 | -0.03, 0.17 | 1.32 | 0.97, 1.67 | 0.02 | -0.02, 0.06 | 0.93 | 0.74, 1.11 |
| Urinary 4OMGA excretion (nmol/mmol creatinine) | | | | | | | | |
| Baseline | 6 | -2, 16 | 7 | 1, 12 | 13 | 1, 25 | 6 | 0, 12 |
| Post | 5 | -1, 11 | 618 | 419, 817 | 37 | -16, 89 | 462 | 344, 580 |

* All measurements were performed in twenty participants at baseline and after drinking three cups of black tea or hot water (consumed at time 0, 1.5 and 3 h) with and without a meal: a total of four treatments administered in random order.

For serum folate and vitamin B₁₂, there was no significant period or order effect and there was no significant interaction between TEA and MEAL on these variables. In main effects analysis, there was no effect of drinking tea on either serum folate (0.3 (95% CI -1.9, 2.5) nmol/l, $P=0.80$) or serum vitamin B₁₂ (6 (95% CI -10, 22) pmol/l, $P=0.47$). However, there was an effect of MEAL on both serum folate (-6.3 (95% CI -8.5, -4.1) nmol/l, $P<0.001$) and serum vitamin B₁₂ (-23 (05% CI -39, -7) pmol/l, $P=0.005$). This was due to an increase in the serum concentrations of these vitamins, from baseline to 3.5 h, during the fasting (no meal) treatment, rather than a decrease following the meal (Table 1).

The relationships of 4OMGA excretion, used as a marker of the degree of tea-derived polyphenol *O*-methylation, and plasma caffeine response with the tHcy response to tea was investigated within the two tea treatments. The change in tHcy was not significantly correlated with the change in 4OMGA (r 0.06, $P=0.75$) or plasma caffeine (r 0.26, $P=0.11$). In addition, in random effects models, the change

in 4OMGA ($P=0.42$) and the change in plasma caffeine ($P=0.37$) were not linearly related to the tHcy response.

Discussion

Our objective was to investigate the acute effect of tea on fasting and non-fasting tHcy. Drinking black tea resulted in a small increase in tHcy and the meal resulted in a small decrease in tHcy. The effect of tea on tHcy was not significantly different in the fasting and non-fasting states. We also explored the relationship between tea-derived polyphenol *O*-methylation and tHcy response to tea and observed no linear relationship.

Tea and coffee, and their major constituents, polyphenols and caffeine, can raise tHcy (Grubben *et al.* 2000; Urgert *et al.* 2000; Olthof *et al.* 2001; Verhoef *et al.* 2002). Polyphenols may cause increases in tHcy by acting as acceptors of methyl groups during the metabolism of methionine to homocysteine (Olthof *et al.* 2001; Hodgson *et al.* 2003). The demonstration that individual differences in the degree of polyphenol *O*-methylation were related to the effects of regular consumption of tea on tHcy is consistent with this hypothesis (Hodgson *et al.* 2003). The mechanism for the effect of caffeine is not known, but it has been suggested that caffeine may act as a vitamin B₆ antagonist (Verhoef *et al.* 2002).

A range of other dietary factors can also influence tHcy metabolism (Verhoef & de Groot, 2005). Thus, the overall effects of a polyphenol- and caffeine-containing beverage may be influenced by food. We found here that consumption of the meal did not influence the effect of tea on tHcy and that tea had an acute effect to raise tHcy. This result can be interpreted together with results of previous studies suggesting that effects of polyphenols to raise tHcy are enhanced in the non-fasting state (Olthof *et al.* 2001; Verhoef *et al.* 2002). Together, they suggest that a modest intake of tea will result in a small transitory effect to increase tHcy, which

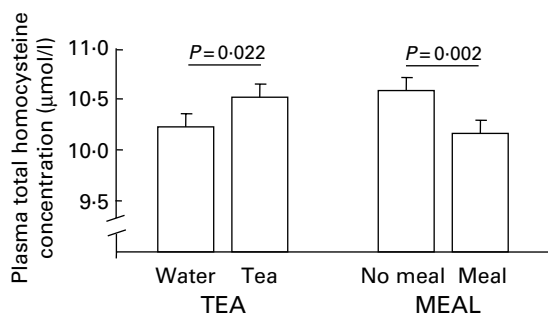


Fig. 1. Mean plasma total homocysteine concentration for each factor. Analysed using random effects models where participant was the random effect and treatment (water or tea (TEA) and no meal or meal (MEAL)) were fixed effects. Values are means with their standard errors. For details of subjects and procedures, see Methods.

may be primarily due to tea alone rather than any interaction with other dietary factors.

We have previously found that a moderate intake of tea, five cups per d for 4 weeks, did not alter fasting tHcy (Hodgson *et al.* 2003). Olthof *et al.* 2001 found that a high dose of quercetin, a polyphenol found in tea, did not significantly alter tHcy. Results of cross-sectional population studies have generally shown that a higher tea intake is associated with lower fasting tHcy (Nygard *et al.* 1997; de Bree *et al.* 2001; Jacques *et al.* 2001; Hodgson *et al.* 2006a). In addition, randomized controlled trials of tHcy-lowering therapy have failed to demonstrate benefit (Kaul *et al.* 2006). Therefore, it remains uncertain whether small acute increases in tHcy following the consumption of tea are clinically important in the long term.

Dietary protein provides methionine, the precursor for homocysteine. Although methionine can raise tHcy acutely (Chambers *et al.* 1999; Ditscheid *et al.* 2005), other dietary factors present in mixed meals may blunt any postprandial rise in tHcy or may lower tHcy (Ubbink *et al.* 1992; Verhoef *et al.* 2004). We found that the low polyphenol meal resulted in lower tHcy 3.5 h after consumption. The dietary factors that may have contributed to this decrease include choline and betaine (Olthof *et al.* 2003, 2005; Cho *et al.* 2006) and the amino acids serine and cystine (Verhoef *et al.* 2004). However, the effects of serine and cystine were demonstrated with high doses of these supplemented amino acids (Verhoef *et al.* 2004). Serum vitamin B₁₂ concentrations were not increased following the meal. However, the observed increases in serum vitamin B₁₂ and folate concentrations over time whilst continuing to fast are difficult to explain.

We have previously found a positive association between the change in 4OMGA and change in tHcy with regular ingestion of tea (Hodgson *et al.* 2003). These results suggested that individual differences in *O*-methylation of polyphenols may influence the ultimate effects of polyphenol-rich beverages on tHcy. We have also proposed that this may have additional clinical relevance if *O*-methylation alters the bioactivity of polyphenols (Hodgson *et al.* 2006b). In the present study, we found that there was no linear relationship between the change in 4OMGA and the change in tHcy, which does not provide further support for the proposed relevance of degree of polyphenol *O*-methylation. However, with only twenty participants, this is a small group in which to investigate the relationship.

In conclusion, we have shown that ingestion of tea can result in acute increases in tHcy, which are likely to be transitory. Regular ingestion of tea throughout the day may result in a small but significant increase in non-fasting tHcy. Consumption of the meal resulted in lower tHcy 3.5 h later, but it did not alter the magnitude of the tHcy-raising effect of tea. Given that results of population studies have generally shown a negative association between tea intake and tHcy, the significance of these findings to circulating tHcy over the longer term and to CVD risk remain uncertain.

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References

- Cesari M, Rossi GP, Sticchi D & Pessina AC (2005) Is homocysteine important as risk factor for coronary heart disease? *Nutr Metab Cardiovasc Dis* **15**, 140–147.
- Chambers JC, Obeid OA & Kooner JS (1999) Physiological increments in plasma homocysteine induce vascular endothelial dysfunction in normal human subjects. *Arterioscl Thromb Vasc Biol* **19**, 2922–2927.
- Cho E, Zeisel SH, Jacques P, Selhub J, Dougherty L, Colditz GA & Willett WC (2006) Dietary choline and betaine assessed by food-frequency questionnaire in relation to plasma total homocysteine concentration in the Framingham Offspring Study. *Am J Clin Nutr* **83**, 905–911.
- de Bree A, Verschuren WM, Blom HJ & Kromhout D (2001) Lifestyle factors and plasma homocysteine concentrations in a general population sample. *Am J Epidemiol* **154**, 150–154.
- Ditscheid B, Funfstuck R, Busch M, Schubert R, Gerth J & Jahreis G (2005) Effect of L-methionine supplementation on plasma homocysteine and other free amino acids: a placebo-controlled double-blind cross-over study. *Eur J Clin Nutr* **59**, 768–775.
- Grubben MJ, Boers GH, Blom HJ, Broekhuizen R, de Jong R, van Rijt L, de Ruijter E, Swinkels DW, Nagengast FM & Katan MB (2000) Unfiltered coffee increases plasma homocysteine concentrations in healthy volunteers: a randomized trial. *Am J Clin Nutr* **71**, 480–484.
- Hodgson JM, Burke V, Beilin LJ, Croft KD & Puddey IB (2003) Can black tea influence plasma total homocysteine concentrations? *Am J Clin Nutr* **77**, 907–911.
- Hodgson JM, Burke V & Puddey IB (2005) Acute effects of tea on fasting and postprandial vascular function and blood pressure in humans. *J Hypertens* **23**, 47–54.
- Hodgson JM, Devine A, Puddey IB, Beilby J & Prince RL (2006a) Drinking tea is associated with lower plasma total homocysteine in older women. *Asia Pacific J Clin Nutr* **15**, 253–258.
- Hodgson JM, Morton LW, Puddey IB, Beilin LJ & Croft KD (2000) Gallic acid metabolites are markers of black tea intake in humans. *J Agric Food Chem* **48**, 2276–2280.
- Hodgson JM, Puddey IB, Burke V & Croft KD (2006b) Is reversal of endothelial dysfunction by tea related to flavonoid metabolism? *Br J Nutr* **95**, 14–17.
- Jacques PF, Bostom AG, Wilson PW, Rich S, Rosenberg IH & Selhub J (2001) Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. *Am J Clin Nutr* **73**, 613–621.
- Kaul S, Zadeh AA & Shah PK (2006) Homocysteine hypothesis for atherothrombotic cardiovascular disease: not validated. *J Am Coll Cardiol* **48**, 914–923.
- Nygard O, Refsum H, Ueland PM, Stensvold I, Nordrehaug JE, Kvale G & Vollset SE (1997) Coffee consumption and plasma total homocysteine: The Hordaland Homocysteine Study. *Am J Clin Nutr* **65**, 136–143.
- Olthof MR, Brink EJ, Katan MB & Verhoef P (2005) Choline supplemented as phosphatidylcholine decreases fasting and post-methionine-loading plasma homocysteine concentrations in healthy men. *Am J Clin Nutr* **82**, 111–117.
- Olthof MR, Hollman PC, Zock PL & Katan MB (2001) Consumption of high doses of chlorogenic acid, present in coffee, or of black tea increases plasma total homocysteine concentrations in humans. *Am J Clin Nutr* **73**, 532–538.
- Olthof MR, van Vliet T, Boelsma E & Verhoef P (2003) Low dose betaine supplementation leads to immediate and long term lowering of plasma homocysteine in healthy men and women. *J Nutr* **133**, 4135–4138.
- Ubbink JB, Vermaak WJ, van der Merwe A & Becker PJ (1992) The effect of blood sample aging and food consumption on plasma total homocysteine levels. *Clin Chim Acta* **207**, 119–128.

- Urgert R, van Vliet T, Zock PL & Katan MB (2000) Heavy coffee consumption and plasma homocysteine: a randomized controlled trial in healthy volunteers. *Am J Clin Nutr* **72**, 1107–1110.
- Verhoef P & de Groot LC (2005) Dietary determinants of plasma homocysteine concentrations. *Seminars Vasc Med* **5**, 110–123.
- Verhoef P, Pasman WJ, Van Vliet T, Urgert R & Katan MB (2002) Contribution of caffeine to the homocysteine-raising effect of coffee: a randomized controlled trial in humans. *Am J Clin Nutr* **76**, 1244–1248.
- Verhoef P, Steenge GR, Boelsma E, van Vliet T, Olthof MR & Katan MB (2004) Dietary serine and cystine attenuate the homocysteine-raising effect of dietary methionine: a randomized crossover trial in humans. *Am J Clin Nutr* **80**, 674–679.