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Short communication

Is reversal of endothelial dysfunction by tea related to flavonoid metabolism?

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Dietary flavonoids can improve endothelial function, but the response varies between individuals. Wide variability is also seen in flavonoid O-methylation, a major pathway of flavonoid metabolism. The O-methylation of flavonoids could alter activity, and thus influence any effect on endothelial function. The objective of the current analysis was to investigate whether variability in the endothelial function response to ingestion of tea, a rich source of flavonoids, is related to the degree of O-methylation of flavonoids. This relationship was investigated in two studies in which endothelium-dependent flow-mediated dilatation (FMD) of the brachial artery was assessed and urinary 4-*O*-methylgallic acid (4OMGA) excretion was used as a marker of the O-methylation of tea-derived flavonoids. In the first study, amongst participants consuming five cups of tea per day for 4 weeks, the degree of increase in 4OMGA excretion was inversely associated with the change in FMD responses (r −078, P=0·008). In the second study, there was a significant difference in the FMD responses to acute ingestion of three cups of tea between individuals with a low (<median) and high (>median) 4OMGA response (1·94 (SEM 0·79) % and −0·25 (SEM 0·53) %, respectively; P=0·03). That is, any improvement in FMD following ingestion of tea may be enhanced in individuals who O-methylate less of the absorbed flavonoids. The present results are consistent with the suggestion that differences in flavonoid metabolism may influence their effect on endothelial function. Thus, differences in flavonoid metabolism could be related to the level of benefit of dietary flavonoids on the risk of CVD.

Flavonoid: Tea: Endothelial function: Methylation: Catechol-*O*-methyltransferase

Tea is one of the richest dietary sources of flavonoids. Higher intake of tea and flavonoids has been associated with lower risk of CVD (Vita, 2005). Intervention trials in man consistently show that flavonoids can reverse endothelial dysfunction, assessed ultrasonographically by measurement of endothelium-dependent flow-mediated dilatation (FMD) of the brachial artery (Stein *et al.* 1999; Cuevas *et al.* 2000; Duffy *et al.* 2001; Hodgson *et al.* 2002; Heiss *et al.* 2003). Endothelial dysfunction appears to be important in the pathogenesis of CVD (Landmesser *et al.* 2004), so any improvement could reduce the risk of coronary events.

There can be considerable variability in the endothelial function response to dietary flavonoids (Stein *et al.* 1999; Hodgson *et al.* 2002; Heiss *et al.* 2003; Hodgson *et al.* 2005). In some people endothelial function returns to normal, while in others there is little or no change. This may be related to inter-individual differences in flavonoid metabolism. A pathway of flavonoid metabolism following absorption is O-methylation by catechol-*O*-methyltransferase (COMT; Lu *et al.* 2003). The activity of COMT can vary as much as 3-fold between individuals (Dawling *et al.* 2001). This could contribute to the variability in observed flavonoid methylation (Lee *et al.* 2002). The O-methylation of flavonoids reduces endothelial exposure to unaltered compounds and may alter vasodilator activity.

We have previously investigated the effect of black tea on endothelial function, assessed using FMD, in two studies (Hodgson *et al.* 2002, 2005). Urinary 4-*O*-methylgallic acid (4OMGA) excretion was used as a marker of the overall O-methylation of tea-derived flavonoids in both studies. Our objective with the current analysis was to investigate whether changes in endothelial function following chronic and acute ingestion of tea are related to the O-methylation of tea-derived flavonoids.

Methods

Study 1: participants and experimental design

The effects of regular ingestion of black tea on brachial artery vasodilator function were assessed in twenty-one individuals with mild hyperlipidaemia. Detailed methods and the primary outcomes of this study have been previously presented (Hodgson *et al.* 2002). Briefly, during a baseline 4-week

Abbreviations: COMT, catechol-*O*-methyltransferase; FMD, flow-mediated dilatation; GTN, glyceryl trinitrate; 4OMGA, 4-*O*-methylgallic acid.

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period all participants consumed five cups/d (250 ml each) of hot water. They were then randomized to five cup of black tea per day (n 10) or to continue with five cups/d of hot water (n 11) for 4 weeks. Each cup of tea was prepared using 2 g tea leaves in 250 ml hot water and consumed without additives such as milk and sugar. During the study, participants ceased intake of tea (except those assigned), coffee, red wine and chocolate beverages, and did not change usual food and alcohol intake, and physical activity. Measurements of brachial artery vasodilator function were measured in the morning after an overnight fast, and not having consumed tea in the morning prior to measurements being performed. Staff performing measurements were blinded to the drink being consumed. Urine samples (24 h) were collected at baseline and end of intervention for measurement of 4OMGA excretion. The project was approved by the Royal Perth Hospital Ethics Committee, and all subjects gave written informed consent.

Study 2: participants and experimental design

The acute effects of black tea on fasting and postprandial brachial artery vasodilator function were assessed in twenty individuals with a history of documented coronary artery disease. Detailed methods and the primary outcomes of the study have been previously presented (Hodgson *et al.* 2005). Briefly, participants attended our unit for four clinic visits during the study, 1 week apart on the same day of the week and at the same time of day. Tea and coffee intake was limited to one cup/d and red wine intake ceased, and participants maintained 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. One treatment was administered at each visit: a total of four treatments were administered in random order. Measurements of brachial artery vasodilator function were performed after an overnight fast at baseline and at 4 h after drinking three cups of black tea (2·2 g tea leaves in 250 ml, without additives) or hot water (consumed at time 0, 1·5 and 3 h) with and without a high-fat (50 g) meal (3400 kJ). Thus, treatments included: water alone (water), tea alone (tea), high-fat meal with water (water + meal) and high-fat meal with tea (tea + meal). Spot urine samples were provided at baseline, and a 5 h urine collection was then performed for measurement of 4OMGA excretion. The Royal Perth Hospital Ethics Committee approved the project, and all participants gave written informed consent.

Brachial artery vasodilator function

Brachial artery vasodilator function was assessed non-invasively by measurement of brachial artery dilatation using ultrasonography. The method has been described in detail elsewhere (Woodman *et al.* 2001). Briefly, endothelium-dependent FMD of the brachial artery was measured following an ischaemic stimulus. Endothelium-independent dilatation of the brachial artery was measured following administration of 400 μg sublingual glyceryl trinitrate (GTN) spray. The FMD and GTN-mediated dilatation responses were calculated as percentage change in brachial artery diameter from baseline. This method conforms to the guidelines set out for the ultrasound measurement of endothelium-dependent FMD of the brachial artery (Corretti *et al.* 2002).

Urinary 4-O-methylgallic acid measurement

4OMGA concentrations were used as a marker of the overall O-methylation of black tea-derived flavonoids (Hodgson *et al.* 2000, 2003). Concentrations of 4OMGA were measured in urine samples which had been frozen at −80°C using a previously described method (Hodgson *et al.* 2000). Briefly, following hydrolysis and extraction of phenolic acids, 4OMGA concentrations were quantified using GC–MS. Minimum level of detection (per ml) was 0·4 ng (≈0·2 nmol/mmol creatinine).

Statistics

Statistical analyses were performed using SPSS 12.0 software (Chicago, IL, USA) or SAS 8.2 software (SAS Institute, Cary, NC, USA). Results are presented as means with their standard errors, and $P<0·05$ was the level of significance. In study 1, Spearman's correlation coefficient (r) was used to determine the within-group degree and direction of linear association between change in 4OMGA excretion and change in FMD response. In study 2, the acute effects of ingestion of tea, and the potential impact of O-methylation, on FMD response were analysed with random effects models. Individuals were divided according to their O-methylation of tea-derived polyphenols. This was calculated as the creatinine-corrected 4OMGA concentration in the 5 h urine sample (after drinking three cups of black tea) minus the creatinine-corrected 4OMGA concentration in the spot urine sample. Individuals with 4OMGA excretion less than the median were classified as 'low' (n 10), and those with 4OMGA excretion above the median were classified as 'high' (n 10). The median was used to divide participants according to 4OMGA response instead of tertiles or quartiles because of the limitation of small numbers. In random effects models subject was treated as the random effect and treatment (tea or hot water control, and meal and no meal control) and degree of O-methylation (low or high) as the fixed effects. Two-way interactions were also included in the models.

Results

Study 1: effects of regular ingestion of black tea for 4 weeks

Regular ingestion of black tea resulted in a significant increase in FMD response ($P=0·008$) and 4OMGA excretion ($P<0·001$) relative to hot water, as described previously (Hodgson *et al.* 2002). Following regular ingestion of five cup of black tea per day for 4 weeks, there was a 3-fold difference in 4OMGA response between individuals, and the FMD responses ranged from −1·2 to 10·1 %. Within the group drinking black tea, the degree of increase in excretion of 4OMGA was inversely associated with the degree of improvement in FMD (r −0·78, $P=0·008$; Fig. 1). The GTN-mediated dilatation was not linearly related to the 4OMGA response to black tea.

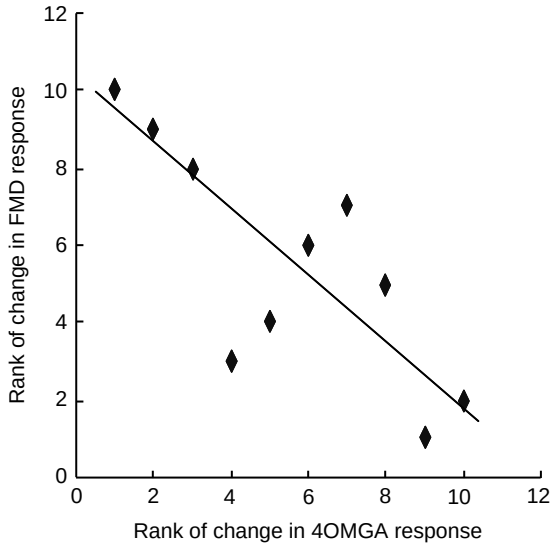

Fig. 1. Relationship between change in 4-*O*-methylgallic acid (4OMGA) excretion and change in flow-mediated dilatation (FMD) response among ten individuals drinking five cup of tea per day for 4 weeks. For details of procedures, see p. 15. Ranks of change in 4OMGA excretion and change in FMD response were used for calculation of the Spearman correlation coefficient (r −0·78; P=0·008).

Study 2: acute fasting and postprandial effects of black tea

Acutely, the increases in FMD response due to black tea in comparison to control did not reach significance, as described previously (Hodgson *et al.* 2005). Ingestion of black tea significantly increased 4OMGA excretion ($P<0·001$). The 4OMGA and FMD responses to ingestion of black tea varied between individuals. In the current analysis, we divided the group according to the median 4OMGA excretion in response to ingestion of three cups of tea. Individuals were classified as 'low' if 4OMGA excretion was less than the median, and 'high' if 4OMGA excretion was above the median. We found a significant interaction between treatment with tea (*v.* control) and 4OMGA excretion (low or high) for effects on change in FMD ($P=0·04$). That is, tea had a significantly different effect on FMD response according to whether 4OMGA excretion was low or high. There was a significant difference in the FMD responses to tea between individuals with a low and high 4OMGA response (1·94 (SEM 0·79) % and −0·25 (SEM 0·53) %, respectively; $P=0·03$). Thus, any improvement in FMD with acute ingestion of black tea appeared to be limited to individuals who excreted less 4OMGA. There was little change in FMD amongst individuals classed as high, who excreted more 4OMGA. There was no interaction between treatment with a meal (*v.* control) and 4OMGA excretion (low or high) for effects on change in FMD ($P=0·45$). The GTN-mediated dilatation was not related to the 4OMGA response.

Discussion

An important pathway for flavonoid metabolism involves O-methylation. We suggested that variability in the endothelial function response to tea-derived flavonoids might be related to inter-individual differences in O-methylation. We found that changes in endothelial function following chronic and acute ingestion of tea were related to the degree of O-methylation of flavonoids, assessed using urinary 4OMGA excretion.

Endothelial dysfunction appears to be important in the pathogenesis of CVD (Landmesser *et al.* 2004). Accumulating data suggest that flavonoids may reduce the risk of CVD by improving endothelial vasodilator function (Fitzpatrick *et al.* 1995; Stein *et al.* 1999; Cuevas *et al.* 2000; Duffy *et al.* 2001; Hodgson *et al.* 2002, 2005; Heiss *et al.* 2003). In controlled intervention trials chronic (Duffy *et al.* 2001; Hodgson *et al.* 2002) and acute (Duffy *et al.* 2001; Hodgson *et al.* 2005) ingestion of tea can improve endothelial function. Increased flavonoid intake in man from other dietary sources including red wine (Cuevas *et al.* 2000), grape juice (Stein *et al.* 1999) and cocoa (Heiss *et al.* 2003) have also been found to improve endothelial function. These effects may explain, at least in part, observed beneficial effects of dietary flavonoids and tea on the risk of CVD (Vita, 2005).

However, variability in the endothelial function response to dietary flavonoids may influence the magnitude of benefit from increased flavonoid intake. Wide variability in flavonoid methylation patterns has been seen in man (Lee *et al.* 2002). A more rapid and complete O-methylation of absorbed flavonoids may diminish or negate any reversal of endothelial dysfunction. More rapid O-methylation would result in reduced exposure of the endothelium to the original flavonoids. This may be important if O-methylated flavonoids lose the activity that is responsible for their vasodilator activity. There is evidence that antioxidant activity of O-methylated flavonoids is substantially reduced (Nanjo *et al.* 1996; Caccetta *et al.* 2000). In addition, when flavonoids act as acceptors of methyl groups, this may lead to elevations in plasma total homocysteine concentrations (Hodgson *et al.* 2003).

We suggest here that 4OMGA is a marker of flavonoid metabolism. An alternative explanation is that differences in uptake of the polyphenolic compounds in tea, including the flavonoids, are primarily responsible for the differences in 4OMGA response. However, if this were the situation, our results would suggest that FMD is improved more in those who absorb less flavonoids. This would seem unlikely given the available evidence linking flavonoids with benefit on FMD and CVD risk.

The main limitation of the current analyses is that neither study was designed specifically to investigate the suggestion that flavonoid metabolism is related to level of benefit on endothelial function. Our initial observation in study 1 was of an inverse association between degree of increase in 4OMGA excretion and degree of improvement in FMD (Fig. 1). We did not have data on the 4OMGA response to tea flavonoids in the control group, and consequently this observation was limited to an uncontrolled within-group analysis. The design of the second study allowed further investigation of the potential link in the controlled setting. Because this was a crossover study data on individual 4OMGA response to tea flavonoids was available for all participants.

We have shown here that the improvement in FMD with ingestion of black tea was significant only in those individuals with a low 4OMGA response. The present results are consistent with the suggestion that flavonoid metabolism can weaken their vasodilator activity *in vivo* and ultimately their effect on endothelial function. Thus, differences in flavonoid metabolism could be related to the level of benefit of dietary

flavonoids on the risk of CVD. Studies designed specifically to determine if O-methylation of flavonoids alters their biological activity and as a consequences influences their health effects are needed.

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