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In vitro mineral availability from digested tea: a rich dietary source of manganese

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Tea is potentially a rich source of some dietary metals and approximately 70 l are drunk *per capita* per year in the UK. In particular, tea may be an important source of Mn, since leaf tea contains 350–900 μg g^{-1} of this essential element. However, the leaching and bioavailability of Mn from tea have been little studied, so a recently developed *in vitro* assay was applied to compare the bioavailability of Mn from tea infusions with that of other major and trace essential elements. Analysis of tea infusions before digestion showed that 1.0 l contained 115% of the average daily dietary intake of Mn but < 6% of all other minerals. Samples of these infusions were incubated with human gastric juice (37 °C, 1 h) and some were then adjusted to pH 6.5 to simulate intestinal pH. All were centrifuged through ultrafilters with molecular mass cut-offs of 3, 10 and 30 kDa. The percentages of ultrafilterable (< 3 kDa) elements following simulated gastrointestinal digestion were (n = 3; mean ± s) Ca 47.7 ± 10.7, Cu 45.3 (n = 1), Fe < 5, Mg 66.4 ± 1.6, Mn 39.8 ± 11.4, K 40.3 ± 2.2, Na 100.0 ± 5.3 and Zn 33.7 ± 1.1. Hence the ultrafilterability of elements showed the general trend $M^+ > M^{2+} > M^{3+}$, which is probably the inverse of the order of their strengths of binding to tea polyphenols. However, Mn was the only element found in significant dietary amounts in tea, and under simulated intestinal conditions was still 40% bioavailable.

Keywords: *Tea; bioavailability; manganese; absorption; inductively coupled plasma optical emission spectrometry*

The tea drink is one of the most popular beverages in the world.[1] The average tea drink, or 'infusion', is prepared by seeping the dried leaf in near-boiling water, and contains little protein, vitamins, fibre or carbohydrate,[2] but may be a rich source of some essential dietary metals[2] and metal binding polyphenols.[3] From 1992 to 1994 the consumption of tea in the UK, for example, was approximately 70 l *per capita* per year,[4] and for many, therefore, tea drinking could be an important dietary source of some essential minerals. However, the contribution of tea drinking to mineral absorption is less certain because, even in the absence of other complicating dietary factors, the bioavailability of many of these metals with tea is uncertain. For example, tea drinking apparently enhances the solubilisation and absorption of copper from the gut, increasing storage of the metal in the liver,[5] but inhibits the absorption of both zinc and non-haem iron from the gut.[6] Furthermore, this latter effect is increased for iron, but not for zinc, by the addition of milk.[6] Indeed, tea contains only trace amounts of iron[1] but, at least in isolated studies, the infusion markedly decreases the dietary availability of iron from other sources.[7–9] In addition, although tea leaves and leaf infusions contain high levels of the toxic metal aluminium,[10] only a small proportion of it is available for absorption.[3] This poor bioavailability of iron and aluminium in the presence of tea has been attributed to the action of the polyphenols in tea, which avidly bind trivalent metals (Fe^{3+} and Al^{3+}) and prevent their intestinal absorption.[3,10]

Leaf tea contains about 600 (range 350–900) μg g^{-1} manganese.[11] Since about 3–3.5 g of leaf tea are used per 225 ml serving ('mug'), tea drinking could be a major dietary source of this element.[12] Manganese is an essential element that is incorporated into a number of metalloenzymes (Mn–metalloenzymes), of which the three primary ones in humans are liver pyruvate carboxylase, arginase and manganese-dependent mitochondrial superoxide dismutase (MnSOD).[13] Although in animal models of manganese deficiency other metal ions may often help maintain the activity of Mn–metalloenzymes, there is clearly a decrease in the activity of MnSOD and arginase.[13] In contrast, since high levels of dietary manganese could possibly be associated with long-term toxicity, setting a recommended dietary intake for this element has been difficult.[13] Hence the currently estimated safe and adequate daily dietary intake is 2–5 mg,[14] but in a recent review Greger[13] implied that ⩽4 mg d^{-1} may be prudent.

The bioavailability of manganese from tea has been little studied[11] and therefore in this work we applied an *in vitro* assay[3] to compare the potential bioavailability of manganese from tea infusions with that of other essential elements, namely calcium, copper, iron, magnesium, potassium, sodium and zinc. Both fresh tea infusions, and those incubated with normal human gastric juice to mimic digestion, were investigated.

Experimental

Samples of normal gastric juice (typically 10 ml) were collected as before[15] from patients undergoing routine upper gastrointestinal endoscopy and stored at −20 °C. Those from patients with endoscopically or histologically abnormal mucosa were subsequently discarded and the remaining normal samples (n = 19) were thawed and immediately pooled prior to analysis and incubation with tea.

Tea infusions and filtration studies were carried out using the method outlined by Powell *et al.*[3] Briefly, de-ionised water (Elga, High Wycombe, Buckinghamshire, UK) was freshly boiled in a standard electric kettle and 250 ml were added to a single tea bag, containing 3.1 g of black leaf tea (Tetley, Yorkshire, UK), in an acid-washed plastic container and incubated for 3 min. The resultant infusion was then cooled to room temperature and aliquots of the infusion, or aliquots diluted 1 + 1 with pooled gastric juice, were measured for pH and then incubated at 37 °C for 1 h. Both incubates and de-ionised water (to assess contamination) were centrifuged at 4000*g* for 90 min through pre-cleaned ultrafilters (Centricon microconcentrators, Amicon, Gloucestershire, UK) with molecular mass cut-offs of 3, 10 and 30 kDa. The ultrafilters were pre-cleaned as before[3] and according to the manufacturer's instructions with sodium hydroxide (0.1 M) and de-ionised water. Some aliquots of the gastric juice incubates were adjusted to pH 6.5 with sodium hydrogencarbonate (simulating pH conditions in the small bowel), incubated for 24 h (simulating maximum ileocolonic transit) and centrifuged through the cleaned ultrafilters. Species passing the 3 kDa ultrafilter were considered the most bioavailable as penetration

of the intestinal mucus layer and passive absorption are favoured by such smaller species.[16]

All of the above experiments were undertaken in triplicate using separate infusions. Prior to analysis samples of water and tea infusions were acidified to a final concentration of 0.11 mol l^{-1} nitric acid (Aristar grade, Merck, Poole, Dorset, UK) and the remaining samples (incubates) were diluted 1 + 1 with de-ionised water and similarly acidified. Analyses were carried out for the common, essential, major and trace cations, namely calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc, by inductively coupled plasma optical emission spectrometry (ICPOES) (Jobin-Yvon JY24, Instruments SA, Longjumeau, France). The analytical lines used for each element are given in Table 1. All analyses were performed in duplicate. Standards were prepared by dilution of 1000 mg l^{-1} standards of each element (Spectrosol grade, Merck, Poole, Dorset, UK). All results are given as means ± standard deviations.

Results

The elemental concentrations of the pooled gastric juice and de-ionised water used to make up the tea infusions are given in Table 1. Contamination from the ultrafilters is also shown; compared with the elemental levels of the digested tea samples and ultrafiltrates, only trace amounts of Ca, Fe, K, Mg, and Zn were observed. The pH values of the tea infusions and gastric juice-digested tea infusions were 4.8 ± 0.05 and 2.3 ± 0.1, respectively (n = 3).

Elemental analysis of the tea infusions before digestion (Table 2) showed that 1.0 l contained 4.6 ± 0.15 mg of manganese, or 115% of the average 4 mg daily dietary intake of the metal (see Introduction).[13,14] All the other minerals were present in 1.0 l of tea at < 6% of their average daily dietary intakes.[14]

The concentrations of metals in the gastric juice-digested tea and their ultrafilterabilities under simulated gastric conditions and at intestinal pH are shown in Fig. 1. In general, elements were more ultrafilterable at the acidic pH (2.3) rather than the more neutral pH (6.5). In all fractions, at both pH values, iron was below the limit of detection (2.3 μg l^{-1}), and potassium (not shown) was surprisingly poorly ultrafilterable for an M^+ species, *i.e.*, being 35.7% at pH 2.3 (not shown) and 40.3% at pH 6.5 (Table 3) for < 3 kDa. Otherwise, at the simulated

Table 1 Analytical wavelengths, limits of detection (LOD) and elemental concentrations of gastric juice and water

Element	Analytical line/nm	Gastric juice/ mg l^{-1}	De-ionised water (n = 2)/ mg l^{-1*}	De-ionised water following ultrafitration (n = 6)/mg $l^{-1*,\dagger}$
Ca	315.887	71.0	0.83–1.00	< 0.01–0.08
Cu	324.754	0.086	< 0.005	< 0.005
Fe	259.940	0.162	< 0.002	< 0.002–0.02
Mg	383.826	11.4	< 0.02	< 0.02–0.17
Mn	257.610	0.011	< 0.001	< 0.001–0.05
K	766.490	622	< 0.04	< 0.04–0.09
Na	588.995	1576	< 0.30	< 0.30
Zn	213.860	1.07	< 0.005–0.02	< 0.005–0.01

* Figures preceded by < are the elemental LODs. † To assess contamination from ultrafiltration.

Table 2 Concentrations of minerals in tea infusions and contributions to average daily dietary intakes

Element*	Average daily dietary intake/ mg d^{-1} (range)	Elemental concentrations of tea infusions/ mg l^{-1} (mean ± s)	% of average daily dietary intake from 1.0 l tea
Al	5 (2–10)	2.94 ± 0.13	58.8
Ca	1000 (800–1200)	4.01 ± 0.23	0.4
Cu	2.5 (2–3)	0.05 ± 0.003	2.0
Fe	15 (10–18)	0.006 ± 0.002	0.04
Mg	350 (300–400)	11.42 ± 0.37	3.3
Mn	4 (2–5)	4.60 ± 0.15	115
K	3800 (1900–5600)	220.6 ± 8.2	5.8
Na	2200 (1100–3300)	< 1.0	< 0.05
Zn	15	0.19 ± 0.08	1.3

* Al not measured here but value taken from Powell *et al.*[3] and average daily dietary intake from Powell and Thompson.[16] Average daily dietary intakes of other minerals are either the average recommended daily intakes (Ca, Fe, Mg and Zn) or average estimated safe and adequate daily dietary intakes (Cu, Mn, K, Na).[14] The level for manganese (≤4 mg d^{-1}) was also determined from Greger[13] (see Introduction).

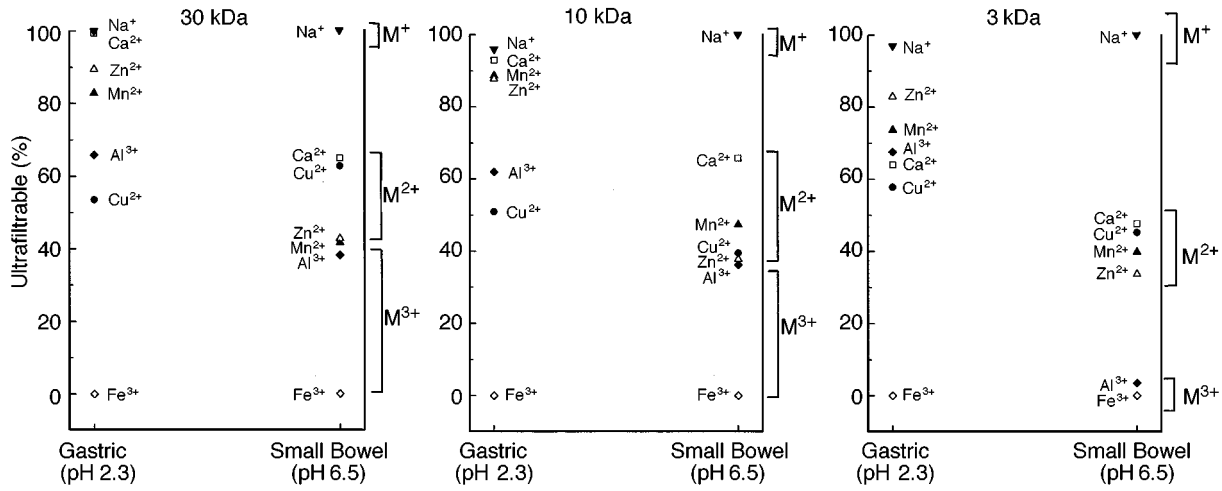

Fig. 1 Percentage of each element in the digested tea samples that passed 30 kDa, 10 kDa and 3 kDa membranes under simulated gastric conditions (pH 2.3) or small bowel pH (see Experimental). At the simulated intestinal pH (*i.e.*, small bowel, pH 6.5) ultrafilterability decreased in the general order $M^+ > M^{2+} > M^{3+}$, and the lowest molecular mass cut-off (3000 Da) gave the best discrimination. Iron was only 0–5% ultrafilterable, being undetected in any filtrate, and was arbitrarily indicated at zero. Elemental concentrations (mean ± s) of the gastric juice-digested tea infusions prior to ultrafiltration were Al, 1.47 ± 0.09, Ca, 32.46 ± 0.98, Cu, 0.07 ± 0.002, Fe, 0.09 ± 0.002, Mg, 11.25 ± 0.33, Mn, 2.18 ± 0.08, K, 462.9 ± 10.9, Na, 591.0 ± 18.9, Zn, 0.65 ± 0.04 mg l^{-1}.

intestinal pH (6.5), the ultrafilterability decreased in the order $M^+ > M^{2+} > M^{3+}$; the lowest molecular mass cut-off (3 kDa) gave the best discrimination (Fig. 1).

Hence the contribution of potentially bioavailable (< 3 kDa) elements from tea drinking to the average daily dietary intake could be calculated (Table 3); only that of manganese was significant, being 46% of the average daily dietary intake from 1.0 l of tea infusion, or 10% from a single 225 ml serving.

Discussion

The complex chemistry and physiology of the gastrointestinal lumen has been reviewed by us previously[17] and clearly in this study, as for all *in vitro* investigations, we simplified the *in vivo* situation. Nonetheless, human gastric juice was used as opposed to the usual, unphysiological, pepsin–hydrochloric acid mixture. Second, since polyphenols are present at high concentrations in tea,[1,12] and are barely absorbed from the gastrointestinal lumen,[3] any effects on mineral speciation observed *in vitro* may reasonably be anticipated *in vivo*.

As discussed previously,[3] the pH of the tea infusions in this study was slightly lower than that for normal tea infusions since they were prepared using de-ionised water rather than tap water; hence infusions may have been prepared that were marginally different to the normal dietary situation. However, for subsequent digestion, this slightly lower pH has little impact as tea infusions are normally exposed to the much more acidic gastric juice. The de-ionised water was contaminated with only relatively low levels of Ca (0.83–1.00 mg l^{-1}), presumably as particulates from the kettle, since this was much reduced following ultrafiltration (Table 1). The ultrafilters gave no significant contamination compared with the elemental levels of the tea digests or ultrafiltrates (Table 1). Concentrations of the elements in the pooled gastric juice sample were in general agreement with those reported previously.[15]

Iron was not detected (limit of detection 2.3 μg l^{-1}) in any ultrafiltrate of tea infusion or even the gastric juice-digested tea and was therefore < 5% ultrafiltrable for all fractions of gastric juice-digested tea. This lack of ultrafilterability for iron, even in the presence of low molecular mass ligands in normal gastric juice,[16,17] supports the finding that Fe^{3+} in tea is, under gastrointestinal conditions, exclusively bound to non-absorbed polyphenols, which represent 20% of the dry mass of black tea[1] and avidly bind M^{3+} species.[18]

Table 3 Availability of minerals in tea infusions and contributions to average daily dietary intakes

Element	% 'available element'* at intestinal pH	'Available element' from 1.0 l tea as % of average daily dietary intake
Al†	4.80 ± 3.0	2.82
Ca	47.7 ± 10.7	0.19
Cu	45.3	0.91
Fe	< 5	< 0.002
Mg	66.4 ± 1.6	2.2
Mn	39.8 ± 11.4	45.8
K	40.3 ± 2.2	2.34
Na	100 ± 5.3	< 0.05
Zn	33.7 ± 1.1	0.44

* 'Available element' is calculated as percentage of element in tea infusion that is ultrafiltrable (< 3 kDa) following gastric juice digestion and adjustment to pH 6.5. All $n = 3$ except for Cu ($n = 1$); mean ± *s*. Elemental levels of gastric juice digested tea are shown in Fig. 1. Average daily dietary intakes are given in Table 2. † Al not measured here but value taken from Powell *et al.*[3] and average daily dietary intake from Powell and Thompson.[16]

Only about a third of the potassium was ultrafiltrable at < 3 kDa in gastric juice digested tea at both pH 2.3 and 6.5. This is unusual, as simple monovalent non-hydrolytic ions such as K^+ are nearly completely absorbed in the small bowel,[17] being kinetically active and very weakly bound both to polyelectrolytes, such as gastrointestinal mucus,[16,17] and, theoretically, tea polyphenols; indeed, as expected, Na^+, even allowing for that added from sodium hydrogencarbonate, was 100% ultrafiltrable. The relatively high concentration of potassium in tea infusions (221 mg l^{-1}) and its low ultrafilterability suggest that the metal may be specifically incorporated within a binding ligand of the tea leaf; certainly synthetic cryptand ligands, for example, can accommodate M^+ cations in a complex that is stable even in aqueous conditions.[19]

The ultrafilterability of the other elements in this study showed the general trend, $M^+ > M^{2+} > M^{3+}$, which corresponds inversely to the strength of binding of these metals to both polyphenols[18] and gastrointestinal mucus.[17] Some soluble mucins would have been present in the gastric juice, but in much lower concentrations than the tea polyphenols, and it is the mucus gel layer, rather than the soluble mucins, that have particular affinity for metal cations.[17] Polyphenols are therefore the most likely strong metal binding agents of tea infusions.

Our data suggest that tea drinking may marginally decrease the availability of divalent metals, such as zinc and copper,[6] which are secreted into gastric juice[15] in addition to being ingested. However, in the Western diet, the balance of promoters and inhibitors is such that under real mixed dietary situations such effects are much less marked than in isolated studies[20] and so it is unlikely that tea significantly affects the absorption of essential divalent metals. Indeed, the absorption of Fe^{3+} ions, which is greatly inhibited by tea in isolated studies[7–9] as confirmed in this *in vitro* study, is much less affected in total dietary studies.[21]

In addition to these probable minor effects on metal absorption, we have also demonstrated that tea drinking is not a rich dietary source of essential metals in humans except, strikingly, manganese, with a single 225 ml serving ('mug') contributing approximately 25% of the average daily dietary intake of this metal. Furthermore, 40% of this (*i.e.*, 10% of the average daily dietary intake) is potentially in a bioavailable form in the small bowel. Manganese deficiency in humans is unusual although in the Western world, particularly in women, manganese intake is marginal and often less than adequate.[22,23] Manganese is an essential trace element and is bound to a number of essential enzymes, although for most of these the manganese ion can be replaced by other metal ions whilst maintaining the activity of the enzyme. However, mitochondrial superoxide dismutase is solely manganese dependent and its activity is suppressed by low manganese status.[22] Other consequences of manganese depletion may include degenerative bone changes and altered pancreatic function.[24] This *in vitro* study suggests that tea drinking is potentially an important dietary source of manganese and sometimes a major dietary source. Further studies need to be carried out *in vivo*. It would be interesting to assess manganese and superoxide dismutase status in tea drinkers and non-tea drinkers, and even to see whether excessive manganese intake/retention could occur with excessive tea drinking.

Conclusion

Tea is one of the most popular beverages in the world and is a rich dietary source of manganese. Under simulated intestinal conditions, a single serving of tea will contribute about 10% of the average daily dietary intake of the metal, in a potentially bioavailable form. For many people, tea drinking may be a major source of dietary manganese.

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