

Addition of Milk Does not Affect the Absorption of Flavonols from Tea in Man

PETER C.H. HOLLMAN^{a,*}, KARIN H. VAN HET HOF^b, LILIAN B.M. TIJBURG^b and MARTIJN B. KATAN^c

^aState Institute for Quality Control of Agricultural Products (RIKILT) Bornsesteeg 45; 6708 PD, Wageningen; The Netherlands, ^bUnilever Research Vlaardingen P.O. Box 114; 3130 AC Vlaardingen; The Netherlands and ^cDivision of Human Nutrition and Epidemiology, Wageningen University, P.O. Box 8129; 6700 EV Wageningen; The Netherlands

Accepted for publication by Prof. B. Halliwell

(Received 13 July 2000)

Tea is a major source of flavonols, a subclass of antioxidant flavonoids present in plant foods which potentially are beneficial to human health. Milk added to tea, a frequent habit in the United Kingdom, could inhibit absorption of tea flavonoids, because proteins can bind flavonoids effectively. Eighteen healthy volunteers each consumed two out of four supplements during three days: black tea, black tea with milk, green tea and water. A cup of the supplement was consumed every 2 hours each day for a total of 8 cups a day. The supplements provided about 100 μmol quercetin glycosides and about 60 – 70 μmol kaempferol glycosides. Addition of milk to black tea (15 ml milk to 135 ml tea) did not change the area under the curve of the plasma concentration-time curve of quercetin or kaempferol. Plasma concentrations reached were about 50 nM quercetin and 30 – 45 nM kaempferol. We conclude that flavonols are absorbed from tea and that their bioavailability is not affected by addition of milk.

Keywords: flavonoids, flavonols, bioavailability, tea, milk, human study

INTRODUCTION

Flavonoids are antioxidants present in vegetables, fruits, tea and wine. Consumption of flavonoids might prevent lipid peroxidation and the formation of atherosclerotic plaques.^[1] Indeed, the intake of flavonols, a subclass of flavonoids, was inversely associated with cardiovascular disease in several though not all studies in man;^[2] a recent study from Wales in the United Kingdom was a notable exception.^[3] One explanation for the discrepancy with earlier studies could be poor absorption of flavonols from tea with milk, which is the major source of flavonols in the United Kingdom.

The plasma concentration of quercetin, a major species of flavonols, increases after consumption of quercetin-rich foods.^[1,4] However, absorption of quercetin from tea with milk, the major source of flavonols in the United Kingdom and several other countries, has not been studied

* Correspondence to: Peter C.H. Hollman State Institute for Quality Control of Agricultural Products (RIKILT) Bornsesteeg 45, 6708 PD, Wageningen, The Netherlands Tel: +31 317 475578 Fax: +31 317 417717 E-mail: p.c.h.hollman@rikilt.wag-ur.nl

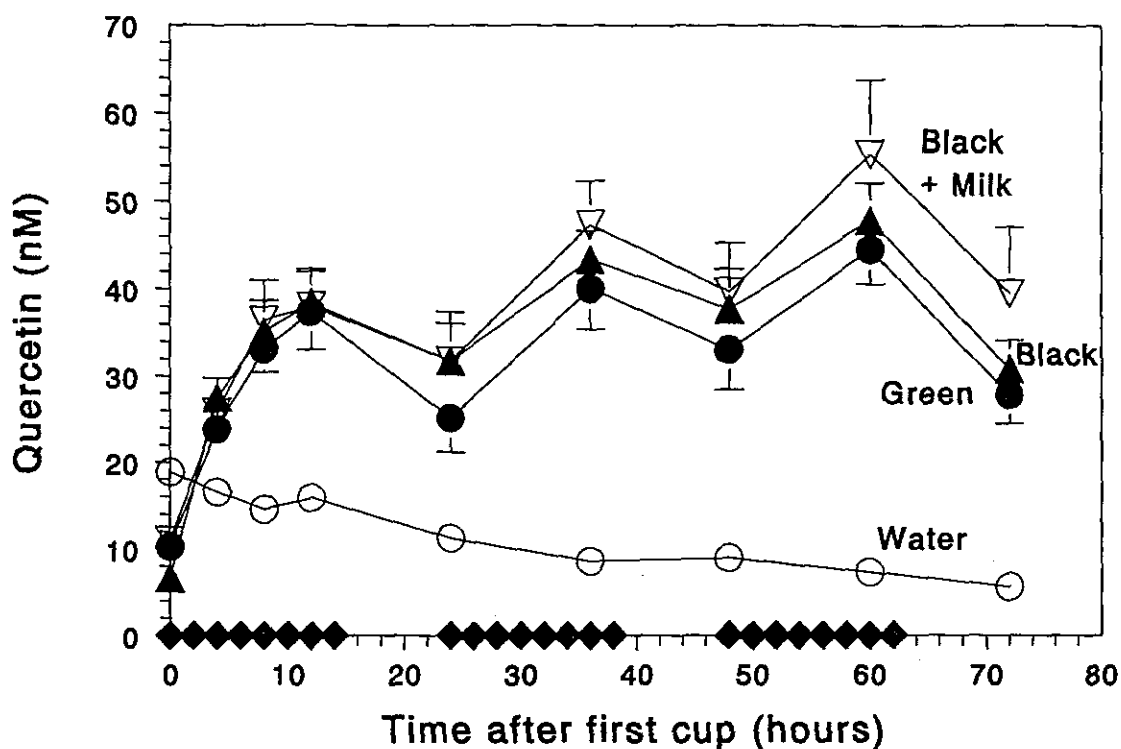


FIGURE 1 Quercetin concentration (mean \pm SD) in plasma of 9 subjects after consumption of black tea (\blacktriangle), black tea with milk (∇), green tea (\bullet) or water (\circ). \blacklozenge indicates the consumption of one cup of tea, or water. Subjects switched from their habitual diet to a low-quercetin background diet 1 day before $t = 0$

in depth. Proteins can bind flavonoids effectively; therefore milk could inhibit absorption of tea flavonoids. Reduced absorption of tea polyphenols was invoked to explain why consumption of black tea raised the plasma antioxidant capacity in volunteers while tea with milk did not.^[5] Here we report the effect of milk on the absorption of tea flavonols in humans.

SUBJECTS AND METHODS

Eighteen healthy volunteers each received two out of four possible treatments. These treatments were: black tea, black tea with milk, green tea,

and water. Allocation to treatments and treatment sequence was random. But it was stratified so that each treatment was tested in 9 volunteers. The two treatments of each subject were separated by 10 days in which they consumed ad-lib diets. One day before and during each 3-days treatment period, subjects abstained from flavonol-rich products; flavonol-low main meals were supplied. During each 3-days treatment period subjects consumed 1 cup containing 150 ml of tea or water every two hours between 8:00 and 22:00 every day for a total of 8 cups per day. For each cup of tea 0.5 g of tea extract (research blend, Thomas J Lipton Company, Englewood Cliffs, NJ) was dissolved in 150 ml of hot water.

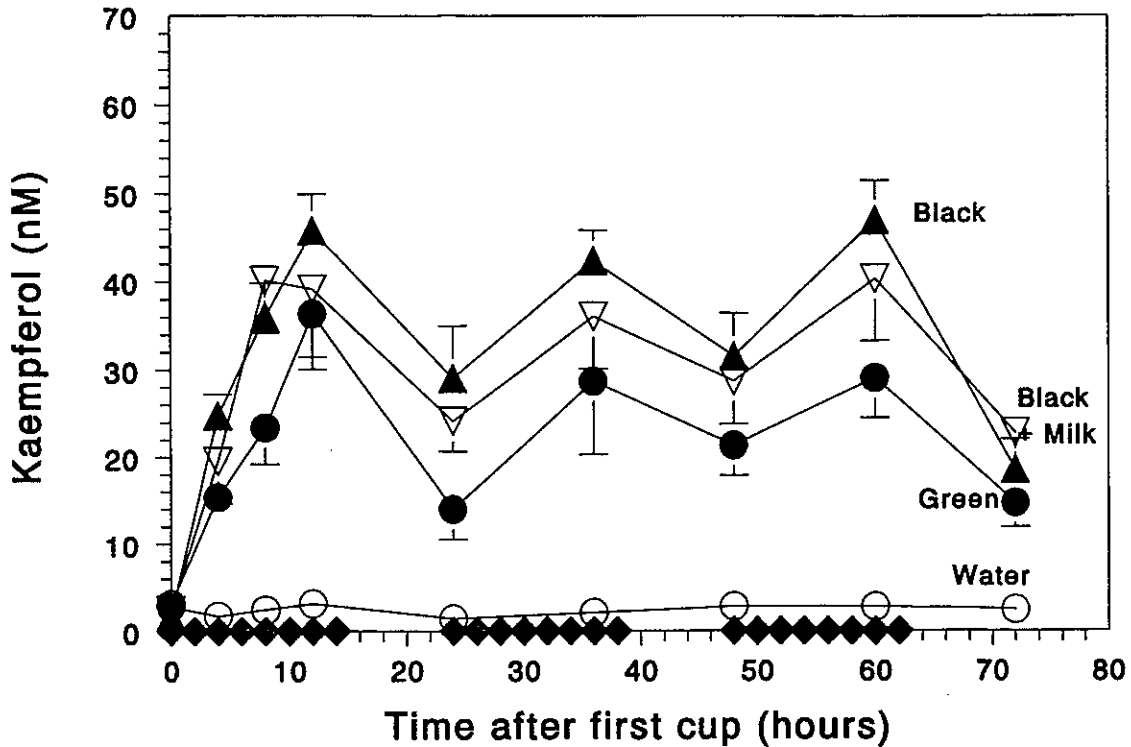


FIGURE 2 Kaempferol concentration (mean \pm SD) in plasma of 9 subjects after consumption of black tea (\blacktriangle), black tea with milk (∇), green tea (\bullet) or water (\circ). \blacklozenge indicates the consumption of one cup of tea, or water. Subjects switched from their habitual diet to a low-quercetin background diet 1 day before $t = 0$

For a cup of black tea with milk 0.5 g of the tea extract was dissolved in 135 ml of hot water and 15 ml semi-skimmed milk was added. Eight cups of black tea (4 g tea solids) provided 108 μmol of quercetin glycosides (equivalent to 32.5 mg as free quercetin) and 72 μmol of kaempferol glycosides. The green tea (4 g tea solids) provided 104 μmol of quercetin glycosides and 58 μmol of kaempferol glycosides per day.

Blood samples were taken into vacuum tubes containing EDTA, and plasma was prepared as described.^[4] Flavonol conjugates were hydrolyzed to the aglycone form with HCl/methanol and the aglycones were determined with HPLC and fluorescence detection after postcolumn derivatization with aluminum.^{[4],[6]}

RESULTS AND DISCUSSION

Addition of milk to black tea did not change the area under the curve of the plasma concentration-time curve of quercetin ($P > 0.5$; Figure 1) or kaempferol ($P > 0.5$; Figure 2). Plasma concentration-time curves after black tea were similar to those after green tea for quercetin (Figure 1) as well as for kaempferol (Figure 2). Thus, bioavailability of flavonols was independent of the addition of milk and of the type of tea. Quercetin, but not kaempferol, tended to accumulate in plasma: the maximum value of the plasma quercetin concentration at the end of the third day was clearly higher than that after the first day ($P < 0.05$). This agrees with the previously observed half-life of

24 h for quercetin in plasma.^[4] When subjects received water, plasma quercetin declined gradually as a result of the low-quercetin background diet and this rather long half-life (Figure 1). In contrast, kaempferol did not accumulate and plasma kaempferol already started at a very low level when subjects received water (Figure 2). These data suggest that the elimination half-life of kaempferol is much smaller than that of quercetin.

In a similar study, Van het Hof^[7] also found that the absorption of tea catechins was not impaired by addition of milk. Thus, poor absorption of flavonols or catechins from tea with milk cannot explain the discrepant results of the study from Wales,^[3] nor the failure to increase plasma antioxidant capacity.^[5]

We found that flavonols are absorbed from tea, and that their bioavailability is not affected by addition of milk. If flavonoids from tea have beneficial effects such effects will not be obviated by addition of milk to tea.

Acknowledgements

We thank Hilco van der Voet for statistical advice. This work was supported by the Founda-

tion for Nutrition and Health Research and the Netherlands Heart Foundation (94.128)

References

- [1] P. C. H. Hollman and M. B. Katan (1998) Absorption, metabolism, and bioavailability of flavonoids. In *Flavonoids in health & disease* (ed. Rice-Evans, C. and Packer, L.), Marcel Dekker Inc., New York, pp. 483–522.
- [2] M.B. Katan (1997). Flavonoids and heart disease. *The American Journal of Clinical Nutrition* 65, 1542–1543.
- [3] M.G.L. Hertog, P. M. Sweetnam, A. M. Fehily, P. C. Elwood, and D. Kromhout (1997). Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly Study. *The American Journal of Clinical Nutrition* 65, 1489–1494.
- [4] P.C.H. Hollman, J. M. P. van Trijp, M. N. C. P. Buysman, M. S. van der Gaag, M. J. B. Mengelers, J. H. M. de Vries, and M. B. Katan (1997). Relative bioavailability of the antioxidant quercetin from various foods in man. *FEBS Letters* 418, 152–156.
- [5] M. Serafini, A. Ghiselli, and A. Ferro-Luzzi (1996). *In vivo* antioxidant effect of green and black tea in man. *European Journal of Clinical Nutrition* 50, 28–32.
- [6] P.C.H. Hollman, J. M. P. van Trijp, and M. N. C. P. Buysman (1996). Fluorescence detection of flavonols in HPLC by postcolumn chelation with aluminum. *Analytical Chemistry* 68, 3511–3515.
- [7] K.H. van het Hof, G. A. A. Kivits, J. A. Weststrate, and L. B. M. Tjiburg (1998). Bioavailability of catechins from tea: the effect of milk. *European Journal of Clinical Nutrition* 52, 356–359.