lipid) and for dark chocolate consumed with milk (100 g plus 200 ml milk, corresponding to more than 30 g lipid). These differences may therefore reflect matrix-dependent effects on flavanol absorption.

A mechanistic link between the antioxidant properties of flavonoids *in vitro* and their biological activity *in vivo* is not properly established, partly because factors such as biological transformation and tissue/plasma concentrations are often not considered[6]. Measurements of plasma antioxidant capacity ideally need to be complemented with markers of cardiovascular function to assess the biological effect of flavanols on cardiovascular health *in vivo*.

We tested the possible inhibition by milk of the absorption of chocolate flavanols in 12 healthy, non-smoking volunteers whose blood-lipid profiles were normal and who were not taking any dietary supplement (the study was approved by the Internal Review Board of the University of California, Davis, and informed consent was obtained from all subjects). Participants consumed a cocoa beverage (containing 0.66 g cocoa solids, the equivalent of 5.44 mg monomeric flavanols and 15.65 mg proanthocyanidins, per kg body weight) prepared with either whole milk (3.25% lipid) or water (supplemented with carbohydrate and 3.25% lipid as a control). Both beverages delivered an equal amount of fluid (4.8 g per kg body weight).

The area under the curve (AUC) in plots of epicatechin plasma concentration against time (where plasma epicatechin was extracted[7] and analysed by high-performance liquid chromatography[8]) shows that there is no significant difference (analysis of variance (ANOVA), $P = 0.499$) in epicatechin concentration in plasma after the consumption of a milk-containing (mean AUC, $3{,}074.8 \pm 536.7$ ng ml^{-1} h^{-1}) or water-based (mean AUC, $2{,}580.5 \pm 655.4$ ng ml^{-1} h^{-1}) cocoa beverage under isocaloric and isolipidaemic conditions.

Bearing in mind that measurements of plasma antioxidant capacity must be interpreted with caution in evaluating flavonoid-mediated biological effects, we used the total antioxidant potential (TRAP) assay[9] as an independent test of the results of Serafini *et al.*[5], who measured the ferric-reducing antioxidant potential (FRAP). We found that consumption of a cocoa-containing beverage resulted in a statistically significant, milk-independent increase in plasma antioxidant capacity, as shown by comparing the baseline value (mean, 234.2 ± 12.47 nmol trolox (vitamin E equivalent) per ml) with the maximum values for the milk-containing (mean maximum, 291.4 ± 8.6 nmol trolox ml^{-1}; ANOVA, $P < 0.001$) and water-containing (mean maximum, 283.72 ± 21.6 nmol trolox ml^{-1}; ANOVA, $P < 0.001$) drinks.

Results based on AUC values for plasma antioxidant capacity plotted against time, which take into account temporal differences in absorption, also indicate that milk does not influence the cocoa-mediated increase in plasma antioxidant capacity (mean AUC_{milk}, $1{,}028.7 \pm 40.5$; mean AUC_{water}, $1{,}005.6 \pm 35.7$ nmol trolox equivalents ml^{-1} h^{-1}; *t*-test, $P \pm 0.369$). Furthermore, the consumption of a milk-containing cocoa beverage resulted in a 25–30% reduction in platelet-mediated haemostasis (R.R.H. and C.L.K., unpublished results), a functional marker that is related to cardiovascular health.

Our findings show that the presence of milk in cocoa products does not counteract the absorption and biological activity of monomeric flavanols from cocoa products, nor does it affect plasma antioxidant capacity. In the context of potential health benefits mediated by dietary flavanols, food-matrix-dependent, temporal effects on absorption must be taken into account when comparing different food formulations and processing methods in terms of the biological activity of their constituent compounds.

Hagen Schroeter*, Roberta R. Holt*, Timothy J. Orozco*, Harold H. Schmitz†, Carl L. Keen*‡

*Departments of *Nutrition and ‡Internal Medicine, University of California, Davis, California 95616, USA*

e-mail: clkeen@ucdavis.edu

†Analytical and Applied Sciences, Masterfoods USA, Hackettstown, New Jersey 07840, USA

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Serafini et al. *reply* — Our results indicate that there is an increase in total antioxidant capacity (TAC) and (–)epicatechin content of plasma in people who have consumed dark chocolate, and that these effects are reduced by the presence of milk[1]. Halliwell and Schroeter *et al.* raise issues that are central to the debate over the fate and potential protective effects of dietary antioxidants.

Schroeter *et al.* suggest that a matrix effect in our study may have delayed the absorption of antioxidants when chocolate was consumed with milk, and that this went unnoticed because our experiments were terminated after 4 h. However, this is unlikely for several reasons. All of the participants in our trials fasted overnight for 12 h and, after supplementation with chocolate/milk, no other product was consumed for the duration of the experiment. Individuals who ate dark chocolate showed a significant increase in plasma TAC after 1 h and in plasma (–)epicatechin for 4 h after ingestion (as measured by the area under the curve in Fig. 1a of ref. 1). In a pilot study, we also monitored plasma TAC for 6 h (results not shown) and found no increase following the consumption of either milk chocolate or dark chocolate with milk.

The study described by Schroeter *et al.* differs in key respects from ours. The authors used a chocolate beverage, rather than solid chocolate. The amount of milk consumed with the beverage is not stated but, on the basis of the lipid content (3% as opposed to 30%), it is substantially less than in our study, which could explain why they did not observe an inhibition of plasma TAC by milk. Another factor could be that Schroeter *et al.* measured plasma TAC by using the TRAP-luminol assay, which is unlike the FRAP assay that we used in that it measures radical-scavenging activity rather than reducing power, and as such determines a different feature of TAC[2].

We were careful to distinguish between TAC and (–)epicatechin absorption because the aim of our investigation was to evaluate the effect of food associations on plasma TAC, and not to identify the compounds responsible for the effect. We agree that the increase in plasma TAC cannot be explained solely on the basis of increased (–)epicatechin levels. Other chocolate polyphenols, such as procyanidins or their *in vivo* metabolites, could also be involved in increasing FRAP values. However, whatever the components responsible, they too are likely to be affected by milk, causing a reduction *in vivo* in the antioxidant properties of dark chocolate.

We measured urate concentrations in plasma under similar conditions (results not shown) and the results are commensurate with an involvement of urate in the redox network. However, as noted by Halliwell, the situation is not straightforward and we did not therefore speculate on the contribution of urate in this context.

TAC measurement is increasingly being used to monitor redox status *in vivo* as well as for epidemiological purposes[3]. Understanding the factors that regulate plasma TAC will help to clarify the interplay between plant-derived foods, plasma redox status and oxidative disease; the effect of food associations on TAC *in vivo* is just one, albeit complicated, variable.

Mauro Serafini*, Alan Crozier†

**Antioxidant Research Laboratory, Unit of Human Nutrition, National Institute for Food and Nutrition Research, 00178 Rome, Italy*

e-mail: serafini@inran.it

†Plant Products and Human Nutrition Group, Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK

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