This opal-type structure is an example of a three-dimensional 'photonic crystal'. Using the formula $\lambda_{max} = 2d \times 0.816\sqrt{(n^2 - \sin^2\theta)}$ as an approximation (for details, see www.icmm.csic.es/cefe/Infiltration/R6G/R6G_infill.htm), the wavelength of maximum reflectance, λ_{max}, for an angle of incidence θ, was calculated as 573 nm at $\theta = 20°$. The constant $0.816 = 2/3^{1/2}$ accounts for the spacing between the close-packed planes in units of sphere diameter; n represents the average refractive index in the system where point scatterers are arranged in planes, calculated as $(n_s + n_m)/2$, where n_s is the refractive index of the microspheres and is taken as 1.56, and n_m is the refractive index of the matrix (1.33) — that is, the refractive index of the chitinous material and 'water' that make up the beetle's exoskeleton[7,8], respectively.

This is a good match with the measured λ_{max} and indicates that the cause of the optical effect is indeed similar to that of opal[7]. Variations in θ do, however, cause differences in λ_{max}; the range of θ values of 0–70° equates to a range of λ_{max} from 590 nm to 448 nm. The invariant colour (yellow–green) seen from the whole animal is a result of global averaging of the different domains within each scale and juxtaposed scales; the domain structure thus creates omnidirectional colour, removing the iridescent effect.

The first photonic crystal revealed as such in an animal (in this case a polychaete)[9] has a two-dimensional structure. By contrast, the three-dimensional properties described here allow for a relatively omnidirectional optical effect, which is important to the behaviour of this weevil because it appears to be strongly coloured from every direction *in situ*. This could be useful for interspecific colour or pattern recognition.

Opal is notoriously difficult to manufacture in solid form. However, transmission electron micrographs of the weevil's opal-like structure reveal repeating patterns of light and dark areas within each microsphere, providing a clue to their molecular structure (which may be revealed by X-ray diffraction analysis) and production. These microspheres must be constructed by molecular self-assembly, a process that could potentially be reproduced[10] by the synthetic-opal industry. After all, the self-assembly technique of the abalone has been successfully copied in the manufacture of a nanocomposite coating that is analogous to the nacre of its shell[11].

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COMMUNICATIONS ARISING

Plasma antioxidants

Health benefits of eating chocolate?

In assessing whether or not a compound or food acts as an antioxidant *in vivo*, a conventional approach is to monitor biological markers of oxidative damage in response to the intervention[1]. Another is to measure the change in total plasma antioxidant capacity, as investigated by Serafini *et al.*[2] in relation to the consumption of chocolate in the presence and absence of milk. The implications of the authors' finding that eating chocolate causes an increase in total plasma antioxidant capacity, and the mechanism by which this is achieved, must also be considered — however, it should not be assumed that the effect is necessarily beneficial.

I calculate that the maximum plasma epicatechin concentration in the study of Serafini *et al.* is about 1 μM. Epicatechin metabolites are likely to be present at lower concentrations and to have reduced antioxidant activity compared with that of epicatechin itself, because of blocking of radical-scavenging hydroxyl groups by conjugation[3]. However, the total plasma antioxidant capacity (TPAC) measured by Serafini *et al.*[2] rose by up to 18%. When measured by the ferric-reducing antioxidant-potential (FRAP) used by the authors, TPAC is usually 0.6–1.6 mM (ref. 4), of which 18% would be 108–288 μM. The rise in TPAC is therefore so large that it is unlikely to be due in significant part to the antioxidant action of epicatechin and its metabolites, or of other phenolics in chocolate.

The FRAP activity of human plasma is mainly attributable to ascorbate, α-tocopherol, bilirubin and urate[4]. Given normal plasma levels of these substances[5], an increase in urate concentration is most likely to account for the results of Serafini *et al.*, because urate is present in plasma at much higher levels than those of other antioxidants, and chocolate is unlikely to contain much ascorbate. Although α-tocopherol may be present in chocolate, even a high intake of this vitamin can increase its concentration in plasma by only a few micromolar at most[5].

The mechanism and consequences of increased plasma urate levels following consumption of chocolate would be interesting to investigate, but should not necessarily be regarded as beneficial. Hyperuricaemia has been associated with stroke, cardiovascular and renal morbidity, and gout[5–8].

Evidence is mounting regarding the potential importance of the overall redox network in disease prevention. It is crucial to our understanding to elucidate the mechanisms by which TPAC is modulated, and the effect of food on this important parameter.

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Nutrition

Milk and absorption of dietary flavanols

Flavanol compounds in wine, cocoa products and tea can exert a cardioprotective effect, for example by influencing endothelial-cell function[1], antithrombic mechanisms[2] and blood pressure[3,4]. Serafini *et al.*[5] claim that consuming dark chocolate, but not milk chocolate or dark chocolate together with milk, increases the antioxidant capacity of human plasma, and suggest that interaction between milk proteins and chocolate flavonoids inhibits the *in vivo* antioxidant activity of chocolate and the absorption of epicatechin into the bloodstream. This inference could have implications beyond chocolate consumption if dairy products do indeed counteract the putative health benefits of dietary flavanols.

The results of Serafini *et al.*[5] are open to a different interpretation if the biological availability and subsequent activity of any compound depends on the varying nutritional and biophysical properties of the matrix in which it is ingested (that is, caloric background, lipid/water content, viscosity, density, extent of mastication). To compare the absorption of epicatechin for chocolate ingested in the presence and absence of milk, it is necessary to control for the composition of the matrix in which the flavanols are delivered.

Compared with values obtained after consumption of dark chocolate alone (100 g, of which about 30 g is lipid), Serafini *et al.* find a lower plasma antioxidant capacity, as well as a reduction in the area under the curve of a plot of plasma antioxidant activity for epicatechin against time, in the case of milk chocolate (200 g, of which about 60 g is

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.

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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.

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