

Plasma antioxidants from chocolate

Dark chocolate may offer its consumers health benefits the milk variety cannot match.

There is some speculation that dietary flavonoids from chocolate, in particular (–)epicatechin, may promote cardiovascular health as a result of direct antioxidant effects or through antithrombotic mechanisms^{1–3}. Here we show that consumption of plain, dark chocolate (Fig. 1) results in an increase in both the total antioxidant capacity and the (–)epicatechin content of blood plasma, but that these effects are markedly reduced when the chocolate is consumed with milk or if milk is incorporated as milk chocolate. Our findings indicate that milk may interfere with the absorption of antioxidants from chocolate *in vivo* and may therefore negate the potential health benefits that can be derived from eating moderate amounts of dark chocolate.

To determine the antioxidant content of different chocolate varieties, we took dark chocolate and milk chocolate prepared from the same batch of cocoa beans and defatted them twice with *n*-hexane before extracting them with a mixture of water, acetone and acetic acid (70.0:29.8:0.2 by volume). We measured their *in vitro* total antioxidant capacities using the ferric-reducing antioxidant potential (FRAP) assay⁴; FRAP values were 147.4 ± 4.5 and 78.3 ± 3.4 μmol

reduced iron per 100 g for dark and milk chocolate, respectively. Volunteers must therefore consume twice as much milk chocolate as dark chocolate to receive a similar intake of antioxidants.

We recruited 12 healthy volunteers (7 women and 5 men with an average age of 32.2 ± 1.0 years (range, 25–35 years). Subjects were non-smokers, had normal blood lipid levels, were taking no drugs or vitamin supplements, and had an average weight of 65.8 ± 3.1 kg (range, 46.0–86.0 kg) and body-mass index of 21.9 ± 0.4 kg m⁻² (range, 18.6–23.6 kg m⁻²). On different days, following a crossover experimental design, subjects consumed 100 g dark chocolate, 100 g dark chocolate with 200 ml full-fat milk, or 200 g milk chocolate (containing the equivalent of up to 40 ml milk).

One hour after subjects had ingested the chocolate, or chocolate and milk, we measured the total antioxidant capacity of their plasma by FRAP assay. Plasma antioxidant levels increased significantly after consumption of dark chocolate alone, from 100 ± 3.5% to 118.4 ± 3.5% (*t*-test, *P* < 0.001), returning to baseline values (95.4 ± 3.6%) after 4 h (Fig. 2a). There was no significant change in plasma FRAP values over the same period after ingestion of milk chocolate alone or of dark chocolate with milk (Fig. 2a).

The areas under the curves of (–)epicatechin plasma levels plotted against time⁵ were measured over the same 4-h period after ingestion for the three different conditions. Absorption of (–)epicatechin into the bloodstream after ingestion of chocolate was significantly less when the chocolate was accompanied by milk (–46.4 ± 4.1%; analysis of variance (ANOVA), *P* < 0.001) or if the chocolate itself contained milk (–69.1 ± 3.9%; ANOVA, *P* < 0.001; Fig. 2b).

Addition of milk, either during ingestion or in the manufacturing process, therefore inhibits the *in vivo* antioxidant activity of chocolate and the absorption into the bloodstream of (–)epicatechin. This inhibition



Figure 1 Stack of benefits? Unlike its milky counterpart, dark chocolate may provide more than just a treat for the tastebuds.

could be due to the formation of secondary bonds between chocolate flavonoids and milk proteins^{6,7}, which would reduce the biological accessibility of the flavonoids and therefore the chocolate's potential antioxidant properties *in vivo*.

Our findings highlight the possibility that the *in vivo* antioxidant activity of flavonoids could be impaired by other dietary constituents. Other food combinations may also counteract the absorption and protective effects of flavonoids. There is therefore a need to take into account dietary habits when designing studies to assess the association between flavonoid-rich foods, antioxidant activity and degenerative diseases.

Mauro Serafini*, **Rossana Bugianesi***, **Giuseppe Maiani***, **Silvia Valtuena***, **Simone De Santis***, **Alan Crozier†**

*Antioxidant Research Laboratory, Unit of Human Nutrition, National Institute for Food and Nutrition Research, Via Ardeatina 546, 00178 Rome, Italy

e-mail: serafini@inran.it

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

1. Rein, D. *et al.* *J. Nutr.* **130**, 2109S–2114S (2000).
2. Holt, R. R., Schramm, D. D., Keen, C. L., Lazarus, S. A. & Schmitz, H. H. *J. Am. Med. Assoc.* **287**, 2212–2213 (2002).
3. Steinberg, F. M., Bearden, M. N. & Keen, C. L. *J. Am. Diet. Assoc.* **103**, 215–223 (2003).
4. Benzie, I. F. F. & Strain, J. J. *Anal. Biochem.* **239**, 229–240 (1996).
5. Maiani, G., Serafini, M., Salucci, M., Azzini, E. & Ferro-Luzzi, A. *J. Chromatogr. B* **692**, 311–317 (1997).
6. Charlton, A. J. *et al.* *J. Agric. Food Chem.* **50**, 1593–1601 (2002).
7. Serafini, M., Ghiselli, A. & Ferro-Luzzi, A. *Eur. J. Clin. Nutr.* **50**, 28–32 (1996).

Competing financial interests: declared none.

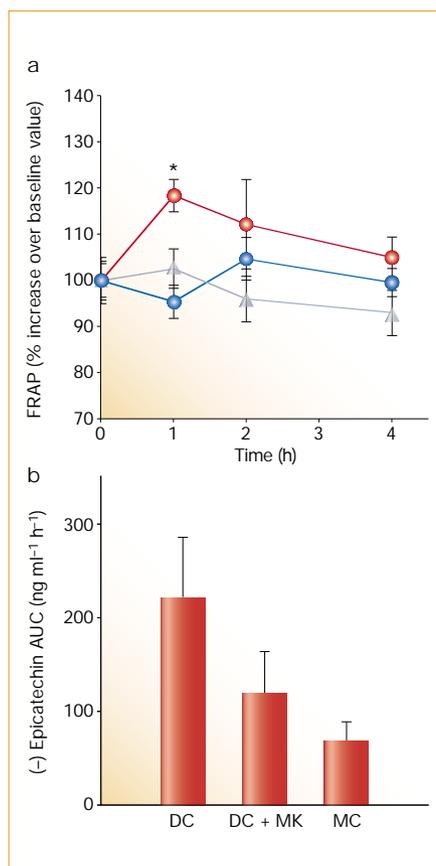


Figure 2 Effects of acute ingestion of 100 g dark chocolate (DC), 100 g dark chocolate with 200 ml milk (DC + MK) or 200 g milk chocolate (MC) on the total antioxidant capacity (TAC) and (–)epicatechin content of human plasma. **a**, Mean TAC of plasma samples at the indicated times after chocolate consumption, expressed as ferric-reducing antioxidant potential (FRAP)⁴. Values are mean percentage increases (± s.e.m.) relative to baseline values (*n* = 12). Red circles, DC; blue circles, DC + MK; grey triangles, MC. Asterisk denotes *P* < 0.001. **b**, Mean (–)epicatechin levels in plasma, expressed as the area under the curve in **a** (AUC, in ng ml⁻¹ h⁻¹) for the 4-h period after chocolate consumption. Values are significantly different from one another (see text).