Increases in plasma lycopene concentrations change the antioxidant activity of the plasma as measured by ORAC but has no effect on two other *ex vivo* total plasma antioxidant assays

JM Fielding[1], R Stockmann[2], D Li[3], AJ Sinclair[1]

[1]*Dept of Food Science, RMIT University, Melbourne, VIC, 3001*
[2]*Food Science Australia, Werribee, VIC, 3030*
[3]*Dept of Food Science, Hangzhou University of Commerce, Hangzhou, China, 310035*

Antioxidants have been suggested to have a role in the prevention of cardiovascular disease and some cancers. Quantifying the action of antioxidants or the amount of oxidative stress of cells and tissues, before and after a dose is becoming of increasing interest in medical research. Methods have been developed to quantify the antioxidant capacity of total and fractionated plasma, such as the FRAP (ferric reducing antioxidant power), ORAC (oxygen radical absorbance capacity) and TBARS method (thiobarbituric acid reactive substances). This study investigated the effect of the addition of lycopene to total plasma, at biologically relevant concentrations, and the resultant *ex vivo* plasma antioxidant activity or production of pro-oxidants.

Lycopene (98% *trans* isomer, Hoffmann LaRoche, Switzerland; dissolved in DCM then nitrogen evaporated) was added to a pooled sample of human plasma (n=12), obtained at fasting, to give plasma concentrations between 0.28 and 1.87 µmol/L. The antioxidant capacity was measured by ORAC, a singlet oxygen assay (SOA) and lipid peroxidation was measured by TBARS, after 1 h and 24 h of incubation of the plasma at 37°C and 5% CO_2 (n = 6 at six different concentrations of lycopene). There was no change in the *ex vivo* antioxidant capacity or lipid peroxidation of the plasma at the 1 h and 24 h periods measured by TBARS (P = 0.179 and P = 0.369, respectively) and SOA (P = 0.338 and 0.311, respectively) at increasing lycopene concentrations in plasma. However, the ORAC assay showed a dose-dependant increase in the antioxidant capacity after 1 h (P = 0.002) of incubation but not after 24 h (P = 0.207).

Analysis	Incubation period (h)	R^2	Regression equation
ORAC	1	0.9216	y = 45.908x + 2384.7
ORAC	24	0.3600	y = 40.234 + 5274.1
TBARS	1	0.3983	y = -0.0041x + 1.4903
TBARS	24	0.0012	y = -5E-05x + 0.9254
SOA	1	0.2275	y = 0.0002x + 0.1222
SOA	24	0.2515	y = -0.0002x + 0.1144

We speculate that the lack of effect at 24 h was due to isomerisation of *trans* to *cis* lycopene under the incubation conditions, the failure of the exogenously added lycopene to partition into the appropriate plasma lipoprotein fractions (eg LDL) or the loss of other antioxidants in the plasma.

This data shows that addition of *trans*-lycopene to plasma across the range which could be encountered physiologically, leads to an increased antioxidant capacity but that there was no effect of the increased lycopene on lipid peroxidation or singlet oxygen quenching. This research suggests that some *ex vivo* antioxidant capacity assays may not be sufficiently sensitive for the prediction of antioxidant action *in vivo*.

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