

ERYTHROCYTES AS MARKERS OF ZINC AND COPPER BIOAVAILABILITY: RESULTS OF A ZINC SUPPLEMENTATION TRIAL IN HEALTHY SUBJECTS

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The sensitivity of erythrocytes as markers of recent nutrient intake is limited by their long lifespan. However, if erythrocytes are fractionated according to age, the newly synthesised cells may reflect current nutrient availability. The aims of this study were two-fold. Firstly, to determine the effect of zinc supplements on the zinc concentration of newly synthesised erythrocytes and secondly, to investigate further the relationship between zinc and copper, given that high intakes of zinc can result in a decrease in copper status.

Six healthy female volunteers (age: 24.7 ± 2.1 years, BMI: 23.8 ± 0.8 kg/m², mean \pm SEM) were asked to supplement their habitual diet with 50 mg of elemental zinc (as capsules containing 220 mg zinc sulphate) per day for 12 days. Venous blood samples were obtained before and during supplementation at 4 day intervals. A subfraction of erythrocytes, representing newly synthesised cells (including reticulocytes), was isolated from each sample by centrifugation. Zinc concentrations were measured in this subfraction, in the unfractionated erythrocytes and in the plasma by atomic absorption spectrometry (Varian AA 575). Copper status was assessed by the marker enzyme, erythrocyte superoxide dismutase (SOD, E.C. 1.15.1.1). All erythrocyte values were expressed relative to the haemoglobin concentration.

Erythrocyte and plasma zinc concentrations tended to increase (3% and 9% respectively) however, erythrocyte SOD activity showed a significant 20% decrease following supplementation (see table).

		Initial	Day 12
Erythrocyte zinc concentration ($\mu\text{g/g Hb}$)	subfraction	37.5 ± 2.6	38.6 ± 2.4
	unfractionated	35.6 ± 3.4	36.5 ± 2.4
Erythrocyte SOD activity (U/g Hb)	subfraction	2337 ± 52^a	1847 ± 72^a
	unfractionated	2371 ± 168^b	1843 ± 76^b
Plasma zinc concentration ($\mu\text{mol/L}$)		18.3 ± 1.4	20.1 ± 1.0

Data expressed as mean \pm SEM for n=6. Values sharing a common superscript are significantly different ($P < 0.02$) using the paired Student's t test.

The subfraction of erythrocytes representing newly synthesised cells did not reflect changes in zinc intake. Our data lend support to theoretical kinetic models which predict that erythrocytes regulate strictly their intracellular zinc concentration and are unlikely to respond to dietary changes (Wastney et al 1986) unless the regulatory mechanism has deteriorated. However, a detrimental effect of increasing zinc intake on copper bioavailability is reflected by a reduction in the activity of erythrocyte SOD within 12 days of supplementation with a moderate amount of zinc (4xRDI). This reinforces the view that this enzyme is a sensitive marker of copper status (Samman and Roberts 1988).

Given the recent interest in zinc as an antioxidant, the aim of increasing the intake of zinc in the hope of promoting the cellular antioxidant potential must be balanced against the subsequent and rapid decrease in the activity of SOD. This enzyme functions as a scavenger of superoxide radicals and therefore has a role in preventing the generation of more reactive radical species. In the absence of quantitative data, this effect appears to be paradoxical in that an antioxidant in the diet may cause a rapid reduction in the cellular antioxidant potential.

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DISTRIBUTION OF ^{51}Cr EDTA IN AND ITS CLEARANCE FROM THE BODY OF MERINO SHEEP SELECTED FOR HIGHER CLEAN FLEECE WEIGHT

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It has been reported that selection of Romney sheep to increase greasy fleece weight increased creatinine clearance from plasma when their diet was a medium quality hay but not when it was a higher quality lucerne hay (Thomson et al. 1989). The authors inferred an effect of selection on renal function because creatinine clearance estimates glomerular filtration rate (GFR). We examine here GFR in Merino sheep, selected since 1960 for higher clean fleece weight, using ^{51}Cr EDTA as the clearance indicator (Stacy and Thorburn 1966).

Six 2-year old Merino ewes from each of an Armidale selection line (SA), a Cunnamulla line (SC) and a control line (US), all now maintained by CSIRO at Armidale, were given once daily one kg of either lucerne hay (LH) or mixed wheaten and lucerne hays (60/40; WL) in a crossover design. Blood samples were taken by jugular venipuncture at increasing intervals for 6 h after an i.v. dose of ^{51}Cr EDTA; ^{51}Cr was assayed in plasma and in urine collected for 72 h after the dose. From the ^{51}Cr EDTA dose, its urine recovery, plasma water content and the plasma ^{51}Cr concentration/time curve (which was described very well by the sum of three exponential components), its total clearance, renal clearance (GFR), distribution space and mean transit time were calculated by non-compartmental analysis (Ladegaard-Pedersen 1972).

	Selection line			Diet		Pooled SD
	SA	SC	US	LH	WL	
Liveweight (kg)	43.70	40.90	37.60	40.80	40.60	
Total clearance (g plasma $\text{min}^{-1}\text{kg}^{-3/4}$)	5.16	5.22	5.26	5.52 ^a	4.90 ^b	0.367
Renal clearance (g plasma $\text{min}^{-1}\text{kg}^{-3/4}$)	4.85	4.78	4.89	5.14 ^a	4.54 ^b	0.360
Distribution space (water % liveweight)	16.00	15.60	16.30	16.20	15.70	1.03
Mean transit time (min)	86.40	87.70	86.20	82.20 ^A	91.30 ^B	13.00
n =	12	12	12	18	18	

^a^b Means differ at $p < 0.001$; ^A^B Means differ at $P < 0.05$ (one-tail test)

There was no effect of selection on total clearance or GFR with either diet. Analysis of the combined data showed significantly lower total clearance and GFR ($P < 0.001$) and longer mean transit time ($P < 0.05$) when the medium quality diet was given to all sheep but there were no differences between the selection lines. The results do not support the findings of Thomson et al. (1989).

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RHEOLOGICAL PROPERTIES OF NOVEL AUSTRALIAN ACACIA GUMS

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Polysaccharides are used in the food industry as agents to increase the viscosity of processed foodstuffs. The polysaccharides can be readily obtained from seaweeds, seeds and exudates and are classed as gums.

Australia currently imports approximately 95% of the gum used in the food industry and this represents an annual cost of twenty million dollars. One that is widely used is gum arabic, an extract from the exudate of the African species *Acacia senegal*.

Australia has over one thousand species of Acacias, some of which produce seeds and exudates in quantities large enough to warrant a comparison of the rheological properties with those of recognised food grade gums.

The viscosity of four Australian Acacia gums was measured at various concentrations in 0.2M NaCl. Two samples of gum arabic were also examined for comparison. Intrinsic viscosity ($[\eta]$), critical concentration (C^*) and coil overlap ($C^*[\eta]$) parameter were calculated and used for characterisation (see table). The intrinsic viscosity represents the fractional increase in viscosity due to solute molecules at infinite dilution and as such is independent of concentration and solvent effects. The critical concentration and the coil overlap parameter are indicators of the effect that solute concentration has on the viscosity as a consequence of polymer-polymer interaction and network entanglement. These parameters give a measure of the hydrodynamic volume of the molecule (ie the volume of solvent swept by the molecule as it tumbles through space) and coupled with molecular weight determinations can give an indication of the conformation of the molecule.

Gum	$[\eta]$ (dL-1.g)	C^* (g.dL-1)	$C^*[\eta]$
Gum arabic (Sigma)	0.17	1.58	0.33
Gum arabic (Rowntree)	0.14	1.51	0.26
<i>Acacia pycnantha</i>	0.29	1.13	0.47
<i>Acacia spectabilis</i>	0.07	1.55	0.10
<i>Acacia baileyana</i>	0.07	1.74	0.08
<i>Acacia microbotyra</i>	0.05	1.90	0.07

Gum arabic is used in confectionary where "mouthfeel" (partly determined by viscosity) is important. The gum is also used to stabilise oil/water emulsions. This is achieved by the molecules covering the oil/water interface and thus the "volume" of the molecule is important in this function. The rheological data can not be used directly to predict the functional value of polysaccharides in foodstuffs; the information can only come from empirical testing. Nevertheless, comparison of rheological data can indicate similarities between polysaccharides. The data indicate that the Australian Acacia gums differ from gum arabic and are probably not ready substitutes. *A.pycnantha* gum may be suited to some uses of gum arabic where viscosity is the most important.