

## A supplementation study in human subjects with a combination of *meso*-zeaxanthin, (3R,3'R)-zeaxanthin and (3R,3'R,6'R)-lutein

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We measured the blood uptake of *meso*-zeaxanthin (MZ) from a mixture of macular pigments since its bioavailability in man has not been studied. Volunteers (ten men and nine women) were recruited and received one capsule of Lutein Plus<sup>®</sup>/d. Blood was taken at baseline, day 10 and day 22. One capsule contained 10.8 mg lutein, 1.2 mg (3R,3'R)-zeaxanthin and 8.0 mg MZ. Plasma lutein and total zeaxanthin concentrations were quantified using isocratic liquid chromatography and the eluting xanthophyll fractions were collected and re-chromatographed on a chiral column to assess the proportion of MZ. Plasma concentrations per mg dose at day 22 suggested that (3R,3'R)-zeaxanthin (0.088 µmol/l per mg) was about 50% more actively retained by the body than lutein (0.056 µmol/l per mg) (although the difference was not significant in women) and 2.5–3.0 times more than MZ (0.026 µmol/l per mg). Concentrations of MZ at day 22 were 2.5 times higher in women than men. The plasma responses from lutein and (3R,3'R)-zeaxanthin in the Lutein Plus<sup>®</sup> were lower than literature values for the pure substances. That is, their uptake into plasma appeared to be slightly depressed by the presence of MZ. Plasma concentrations of β-carotene were depressed by about 50% at day 10 and about 35% at day 22. In conclusion, the lower plasma response to MZ compared with (3R,3'R)-zeaxanthin probably indicates that MZ is less well absorbed than (3R,3'R)-zeaxanthin but work with pure MZ will be needed to confirm that the lower plasma response was not due to the large amount of lutein in the Lutein Plus<sup>®</sup>.

### *meso*-Zeaxanthin: Zeaxanthin: Lutein: Supplementation study: Macular disease

*meso*-Zeaxanthin (MZ; (3R,3'S)-zeaxanthin) is one of the three principal xanthophyll pigments found in the retinal pigment epithelium of the eye<sup>(1,2)</sup>. The other two xanthophylls are lutein ((3R,3'R,6'R)-β-ε-carotene-3,3'-diol) and (3R,3'R)-zeaxanthin. These three *all-trans* carotenoids are the major pigments in the retina (Fig. 1)<sup>(2)</sup>. It has been suggested that lutein and zeaxanthin are present in the eye in roughly equal amounts but that the distribution is uneven. The two forms of zeaxanthin predominate in the macula while lutein is found principally in the more peripheral areas of the retina<sup>(2–4)</sup>.

There is a biological plausible rationale, which is supported by some scientific evidence, that the macular carotenoids may play a role in preventing age-related macular degeneration. The retina is highly active metabolically and has a higher blood flow than other tissues. High metabolic activity and the simultaneous presence of light and oxygen will generate reactive oxygen species, which, in the absence of suitable antioxidants, can damage PUFA that are rich in the photoreceptor outer segments<sup>(5,6)</sup>. The macula is especially exposed to high light intensity and the high concentration of xanthophylls may exert a protective role against oxidative damage<sup>(3)</sup>. The importance of xanthophylls in protecting the eye has been shown in macaque monkeys that were raised on a

carotenoid-free diet for 5 years<sup>(7)</sup>. There was a loss of macular pigment, more drusen and other indicators of photic damage. Others have shown that in zeaxanthin-treated quails there were fewer apoptotic rod and cone photoreceptor cells following light exposure than in the controls<sup>(8)</sup>.

MZ is an important component of the macular pigments but the major natural form of zeaxanthin found in the diet is the (3R,3'R)-form. MZ has only been reported in a few foods, namely, shrimp carapace, fish skin and turtle fat, where all three isomers of zeaxanthin were found<sup>(9)</sup>. As the latter foods are minor components of human diets, MZ has not been found to naturally occur in human blood<sup>(1)</sup>. The source of MZ in the eye is likely to be lutein. This was originally suggested on chemical grounds<sup>(1,4,10)</sup> but has now been confirmed in monkeys<sup>(11)</sup>. The mechanism of the conversion is not known. A binding protein that is specific for the two forms of zeaxanthin but not lutein has been isolated from human retinas<sup>(12)</sup>. The localisation of the binding protein suggests that its primary role is to take up zeaxanthin from the plasma. However, as MZ is not normally present in blood, it is possible that the binding protein may assist in the conversion of lutein to MZ<sup>(13)</sup>.

Age-related macular degeneration is a leading cause of visual impairment and blindness in industrialised countries<sup>(14)</sup>

**Abbreviation:** MZ, *meso*-zeaxanthin.

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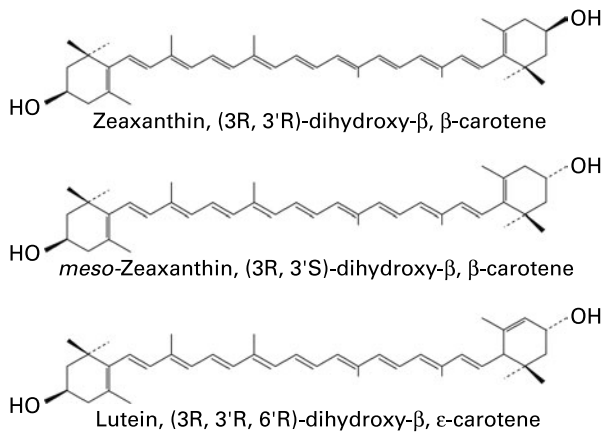


Fig. 1. Structures of the three principal xanthophyll carotenoids found in the macular pigment of the eye.

and there is evidence that the prevalence has been increasing over the last 50 years<sup>(15)</sup>. Changes in dietary intake may be a factor<sup>(16,17)</sup> and, although the consumption of fruit and vegetables is widely promoted, the intake of lutein by many people is less than 1 mg/d<sup>(18)</sup>. Dietary inadequacy of lutein or elevated demands for MZ may result in a functional deficiency of macular MZ. It is not known if MZ has any beneficial role as a nutritional supplement for the prevention of age-related macular degeneration but it was of interest to investigate the uptake of MZ into the blood of healthy volunteers. The present study reports blood concentrations of MZ at 10 and 22 d following the consumption (20 mg) of a mixed supplement of lutein, (3R,3'R)-zeaxanthin and MZ per d.

## Methods

### Supplementation study

Nineteen subjects (ten men, nine women) were recruited to a supplementation study to take one capsule of Lutein Plus<sup>®</sup> (Holland & Barrett, Nuneaton, UK) once per d with meals for 21 d. Men began the study in early November 2006 while the women started taking their capsules the day immediately after menstruation during late October to early December. Non-fasted, heparinised blood (10 ml) was collected at baseline and on days 10 and 22. Plasma was stored at  $-40^{\circ}\text{C}$ . Ethical approval for the present study was received from the University of Ulster Research Ethics Committee (application no. REC/06/56). Weights and heights were recorded after the study was completed.

### Measurement of lutein, zeaxanthin isomers and other carotenoids

Baseline plasma samples were measured in duplicate. Days 10 and 22 samples were measured singly. Separation and quantitation of the xanthophyll pigments was achieved using a two column procedure. Using a modification of a method described previously<sup>(19)</sup>, 0.5 ml plasma was mixed with 0.5 ml sodium dodecyl sulphate (10 mmol/l) and 1.0 ml internal standard (tocopheryl acetate 84  $\mu\text{mol/l}$  in ethanol). The solution was mixed and then extracted twice with 1.0 ml hexane (E Merck, Poole, Dorset, UK) and the supernatant extracts (0.7 and 1.0 ml

successively) combined, evaporated to dryness at  $40^{\circ}\text{C}$  and reconstituted in 0.5 ml acetonitrile–methanol (85:15 containing 0.1% triethylamine). Using the same solvent mixture at 1.5 ml/min, 0.1 ml of the final extract was chromatographed isocratically using a 3  $\mu\text{m}$  Ultracarb ODS column ( $0 \times 4.6$  mm, Phenomenex, UK) to quantify lutein and zeaxanthin. A further 0.1 ml of the extract was chromatographed on a 3  $\mu\text{m}$  Spherisorb ODS-2 column ( $100 \times 4.6$  mm) at 1.0 ml/min using a mobile phase of acetonitrile–methanol–dichloromethane (47:47:12, by vol.) to provide a rapid quantitation of  $\beta$ -carotene in 10 min. Elution was monitored in both systems using a photodiode-array detector (model 2996; Waters Ltd).

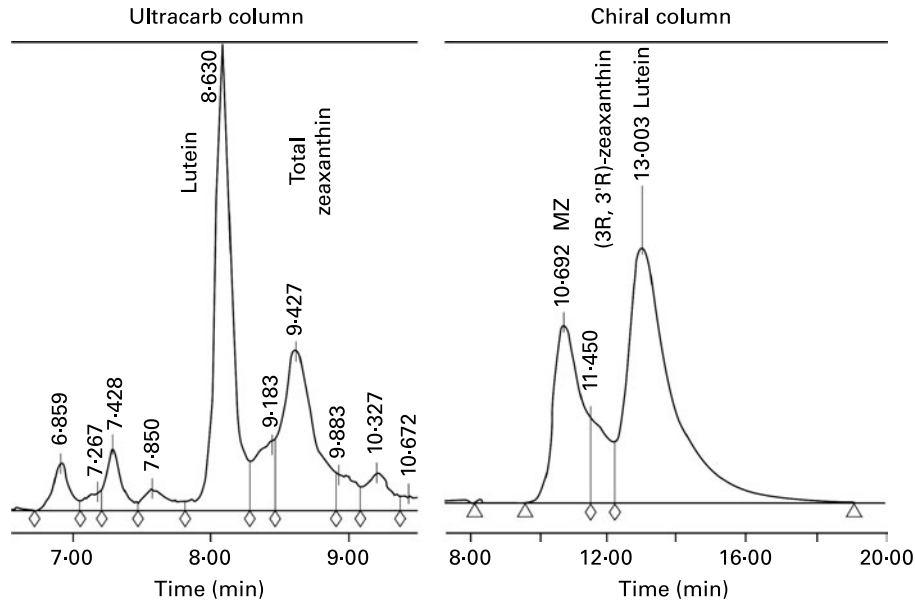
The Ultracarb system gave baseline separation of lutein and zeaxanthin at approximately 7.8 and 8.6 min to enable quantitation of the xanthophyll pigments. Eluent that coincided with the emergence of these peaks was collected from the waste line and evaporated to dryness under nitrogen. The combined lutein and zeaxanthin extract was then reconstituted in 0.1 ml hexane–isopropanol (90:10) and 50  $\mu\text{l}$  was chromatographed on a 10  $\mu\text{m}$  Chiralpak<sup>®</sup> AD column ( $250 \times 4.6$  mm; Chiral Technologies Europe, France) to determine the proportion of MZ in the zeaxanthin isomers using gradient elution at 0.8 ml/min starting with 90% hexane and 10% isopropyl alcohol and increasing to 95% hexane in a linear gradient over 30 min.

### Accuracy and precision

Identity of lutein (Sigma) and (3R,3'R)-zeaxanthin (DSM Nutritional Products, Basle, Switzerland) was confirmed with standards. Response factors from these standards were identical and we assumed the same value to calculate the concentrations of MZ. We used the same chiral chromatography described by Bone *et al.*<sup>(20)</sup> to identify the stereoisomers of zeaxanthin. Fig. 2 shows a typical chromatogram of the separation of lutein and zeaxanthin on the Ultracarb column and the subsequent separation of the stereoisomers from the zeaxanthin peak on the chiral column. We estimated the proportions of (3R,3'R)-zeaxanthin and MZ by dropping perpendicular lines to quantify the proportions present and, to maintain consistency, this was always done by one person (D. I. T.). Precision of xanthophyll measurements on the Ultracarb column was assessed from the SD of the differences between duplicates on the baseline data and the total recoveries for the capsule data. CV for lutein and zeaxanthin were 6.6 and 12.8% (baseline) and 5 and 7.5% (capsules) using data from the Ultracarb column and 9.9% for MZ using data from the capsule analysis from the Chiral column.

### The capsules

The capsule contents were described as containing 20 mg lutein, MZ and zeaxanthin in soya bean oil and the capsule shell was made from gelatine and glycerine containing yellow beeswax as thickener (Holland & Barrett Retail Ltd). The original source of the lutein was from a non-polar extract of marigold flowers (*Tagetes erecta*), which was then processed using alkaline hydrolysis to convert some of the lutein to MZ and the non-esterified carotenoids were purified by a patented procedure<sup>(21)</sup>. The nominal



**Fig. 2.** Chromatography of xanthophyll pigments in a plasma extract of a sample collected on day 22. In the Ultracarb system the peak elution times of lutein and total zeaxanthin were 8.6 and 9.4 min respectively. The total volume between 8.4 and 9.8 min was collected for rechromatography on the chiral column because of initial uncertainty of the identity of the substance eluting between the two main peaks.

proportions of *all-trans* lutein, MZ and (3R,3'R)-zeaxanthin in Lutein Plus<sup>®</sup> were 50:37:13 by weight (Jose Torres Quiroga, personal communication) but as the raw material varies so the product will also vary slightly and an analysis of the capsules used in the present study found the proportions of MZ and (3R,3'R)-zeaxanthin in Lutein Plus<sup>®</sup> to be 82 and 18% respectively. Analyses of the zeaxanthin stereoisomers by the manufacturer were done by liquid chromatography and chemical derivatisation<sup>(22)</sup> (Ricardo Montoya, personal communication).

#### *Analysis of the capsules using the methods described in this study*

The capsules were opened with fine scissors and the contents transferred by successive washings with AnalaR acetone to a 50 ml flask. The contents were thoroughly mixed and 1 ml was further diluted to 20 ml in more acetone. Aliquots of the second solution (0.2 ml) were saponified to remove the soya bean oil by addition of 0.15 ml (50 g/100 ml) aqueous potassium hydroxide and 0.25 ml internal standard echinenone in ethyl alcohol (80 µg/l) followed by incubation at 45°C for 45 min in an orbital mixing cabinet. The pigments were then extracted twice by successive additions of 1.0 and 1.5 ml hexane (containing 0.1 g/l butylated hydroxyl toluene (2,6-bis(1,1-dimethylethyl)-4-methylphenol)) and the extracts combined. As the alkaline/alcoholic residue was still yellow, it was evaporated to remove residual hexane and additional potassium hydroxide solution was added (0.15 ml, 50 g/100 ml) and 0.2 ml ethyl alcohol. Following a further period of saponification (45 min at 45°C), 1.0 ml hexane was added to extract any remaining pigment. Extracts were combined, evaporated to dryness at 40°C under nitrogen and reconstituted in acetonitrile-methanol (85:15 and 0.1% triethylamine) for chromatography on the Ultracarb and Chiral columns described earlier.

#### *Measurement of plasma cholesterol concentration*

Total plasma cholesterol concentrations were measured in the baseline samples using an Instrument Laboratory Analyser (ILab 650<sup>™</sup>, Milano, Italy) and IL Test<sup>™</sup> reagents (0018250540). Cholesterol and cholesterol ester (after reaction with cholesterol esterase) are oxidised to hydrogen peroxide, which reacts with 4-aminoantipyrine and phenol to produce a red colour that can be measured at 510 nm. The manufacturer's calibrator was used to standardise the assay. High and low controls conformed to expectations and within batch CV was less than 2%.

#### *Questionnaire*

A short questionnaire was administered to record any drugs or micronutrient supplements consumed by the volunteers, smoking history and exercise habits and any recent illnesses and general well-being. In the women, time of menstruation and name of oral contraceptive used was recorded. Frequency of intake of green, red/orange and yellow vegetables was recorded in four categories: daily; four to six times per week; one to three times per week; less than once per week. Fruit intake was similarly recorded.

#### *Statistical analyses*

Parametric tests were used as data were normally distributed. Repeated measures ANOVA was used for longitudinal data and paired *t* tests to assess differences within groups at different times. Independent *t* tests were used for measurements between the sexes. We used the Statistical Package for Social Sciences for these analyses (version 11.0 for Windows).

#### **Results**

Nineteen subjects were recruited from university staff and students and all completed the study. There were ten men and

nine women, aged 21 to 46 years, all were non-smokers. Women (mean 27 (SD 7) years) were slightly younger than the men (34 (SD 9) years,  $P=0.05$ ). Frequency of vegetable and fruit consumption by the men and women was almost the same. The median consumption of green vegetables was four to six times per week and median consumption of yellow and red vegetables was one to three times per week in both sexes. Only two subjects (one male, one female) consumed vegetables less than once per week. Men consumed fruit daily, while in the women it was slightly less often at four to six times per week. Mean baseline cholesterol concentrations were 4.81 (SD 0.75) and 4.77 (SD 1.01) mmol/l in women and men respectively and there were two men and one woman with values  $>5.5$  mmol/l. Mean weights were 59.6 (SD 9.39) and 80.2 (SD 11.01) kg and heights were 165.7 (SD 8.87) and 180.7 (SD 8.35) cm in women and men respectively.

Mean (SD) concentrations of plasma lutein, total zeaxanthin, the proportions of MZ and (3R,3'R)-zeaxanthin, and  $\beta$ -carotene were measured at baseline and days 10 and 22 following supplementation with Lutein Plus<sup>®</sup> for the combined sexes and men and women separately (Table 1). Although women tended to have the higher concentrations of these plasma carotenoids throughout the present study, there were no significant differences until the end, when lutein and total zeaxanthin concentrations were higher in the women ( $P=0.012$ ,  $P=0.001$  respectively). By day 22 the concentration of lutein, (3R,3'R)-zeaxanthin and MZ had increased in all volunteers.

The proportion of MZ in the total zeaxanthin concentration was measured in all subjects at days 10 and 22 and used to calculate the plasma concentration in the men and women separately. At day 10 there was no difference in the proportion

of MZ in the two sexes but, at day 22, the proportion in the women (63%) was significantly greater than that in the men (46%,  $P=0.027$ ). Thus, at day 10 there was no difference in the MZ concentration between men and women but, by day 22, the plasma MZ concentration in the women was almost three times higher than that in men (0.304 v. 0.124  $\mu\text{mol/l}$ ,  $P=0.001$ ). The baseline xanthophyll eluate for three subjects was also examined for the presence of MZ but none was detected (data not shown).

There was approximately a three-fold increase in the plasma concentration of (3R,3'R)-zeaxanthin over the duration of the study (Table 1) and there was no difference between the sexes. By day 10 the concentration had approximately doubled and was significantly different from baseline (men  $P=0.016$ ; women  $P=0.04$ ). However, although the mean concentration of (3R,3'R)-zeaxanthin appeared to continue to increase in both sexes, the differences between day 10 and 21 concentrations were not significant (men  $P=0.28$ ; women  $P=0.08$ ). Similar results were obtained for lutein, in that there was approximately a three-fold increase in both sexes over the 21 d. By day 21, however, the mean plasma lutein concentration in the women was significantly greater than that in the men and the increase in the women (mean 0.778 (SD 0.335)  $\mu\text{mol/l}$ ) was significantly greater than that in the men (0.454 (SD 0.151),  $P=0.013$ ).

According to the manufacturer's details, the capsules contained a lutein blend (20 mg) comprising 'lutein, MZ and zeaxanthin'. Three analyses done by ourselves found the total xanthophyll content to be 21, 22 and 23 mg and the average proportions of the three components were 54, 40 and 6% respectively. Table 2 shows the relative efficiencies of plasma uptake for the three xanthophylls assuming that the capsule contents were 20 mg and that dietary intake remained the

**Table 1.** Plasma lutein and zeaxanthin concentrations at baseline, day 10 and day 21 in the male and female volunteers supplemented with a combination of *meso*-zeaxanthin (MZ), (3R,3'R)-zeaxanthin and lutein\*

(Mean values and standard deviations)

	Subjects	Number	Baseline		Day 10		Day 21		<i>P</i> †
			Mean	SD	Mean	SD	Mean	SD	
Lutein ( $\mu\text{mol/l}$ )	All	19	0.275 <sup>a</sup>	0.125	0.734 <sup>b</sup>	0.373	0.882 <sup>b</sup>	0.326	Time† $<0.001$
	Women	9	0.303 <sup>a</sup>	0.166	0.884 <sup>b</sup>	0.0476	1.081 <sup>bx</sup>	0.343	Sex 0.024
	Men	10	0.25 <sup>a</sup>	0.074	0.599 <sup>b</sup>	0.186	0.703 <sup>by</sup>	0.180	
Total zeaxanthin ( $\mu\text{mol/l}$ )	All	19	0.054 <sup>a</sup>	0.024	0.238 <sup>b</sup>	0.03	0.369 <sup>c</sup>	0.145	Time $<0.001$
	Women	9	0.064 <sup>a</sup>	0.029	0.254 <sup>b</sup>	0.152	0.474 <sup>cx</sup>	0.122	Time $\times$ sex 0.002
	Men	10	0.045 <sup>a</sup>	0.013	0.225 <sup>b</sup>	0.117	0.273 <sup>by</sup>	0.085	Sex 0.008
MZ (% total zeaxanthin)	All	19			47.88	20.0	53.89	17.7	
	Women	9			49.5	21.2	63.07 <sup>x</sup>	15.36	
	Men	10			46.44	19.9	45.63 <sup>y</sup>	16.03	
(3R,3'R)-zeaxanthin ( $\mu\text{mol/l}$ )	All	19	0.054 <sup>a</sup>	0.024	0.118 <sup>b</sup>	0.063	0.160 <sup>b</sup>	0.068	Time $<0.001$
	Women	9	0.063 <sup>a</sup>	0.029	0.118 <sup>ab</sup>	0.056	0.171 <sup>b</sup>	0.073	
	Men	10	0.045 <sup>a</sup>	0.013	0.118 <sup>b</sup>	0.072	0.150 <sup>b</sup>	0.065	
MZ	All	19	–‡	–	0.121 <sup>a</sup>	0.097	0.209 <sup>b</sup>	0.128	Time 0.004
	Women	9	–‡	–	0.136 <sup>a</sup>	0.121	0.304 <sup>bx</sup>	0.122	Time $\times$ sex 0.015
	Men	10	–‡	–	0.107	0.074	0.124 <sup>y</sup>	0.052	Sex 0.007
$\beta$ -Carotene ( $\mu\text{mol/l}$ )	All	19	0.321 <sup>a</sup>	0.168	0.162 <sup>b</sup>	0.083	0.212 <sup>b</sup>	0.140	Time 0.005
	Women	9	0.323 <sup>a</sup>	0.188	0.147 <sup>b</sup>	0.072	0.222 <sup>ab</sup>	0.101	
	Men	10	0.32 <sup>a</sup>	0.158	0.178 <sup>b</sup>	0.093	0.202 <sup>ab</sup>	0.173	

<sup>a,b,c</sup> Mean values within a row with unlike superscript letters were significantly different ( $P<0.05$ , paired *t* test).

<sup>x,y</sup> Mean values with unlike superscript letters were significantly different from the means for the sexes for the specific nutrient ( $P<0.05$ , independent *t* test).

\* For details of subjects and procedures, see Methods.

† Repeated measures ANOVA to show significance of the main effects, time and sex.

‡ None found in baseline samples.

**Table 2.** Relative increases in plasma concentrations of lutein, meso-zeaxanthin (MZ) and 3R,3'R-zeaxanthin to doses of respective xanthophylls from Lutein Plus®\* (Mean values and standard deviations)

Xanthophyll	Change in plasma concentration of xanthophyll over 21 d (µmol/l)				Change in plasma concentration of xanthophyll relative to intake per d† (µmol/l per mg daily intake)				Change in plasma concentration corrected for blood volume (mg/person)‡									
	Women		Men		Women		Men		Women		Men		P§	Ratio				
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD						
Lutein	0.778	0.353	0.454	0.151	0.072 <sup>a</sup>	0.031	0.042 <sup>a</sup>	0.67	0.013	0.056 <sup>a</sup>	0.028	1.72	1.81	0.767	1.41	0.4	0.162	1.28
(3R,3'R)-zeaxanthin	0.107	0.08	0.105	0.058	0.069 <sup>ab</sup>	0.067	0.087 <sup>b</sup>	0.048	0.95	0.088 <sup>b</sup>	0.056	1.02	0.25	0.209	0.33	0.179	0.381	0.76
MZ	0.304	0.122	0.124	0.052	0.038 <sup>b</sup>	0.015	0.015	0.31	0.001	0.026 <sup>c</sup>	0.016	2.59	0.73	0.346	0.30	0.159	0.012	2.43

<sup>a,b,c</sup> Mean values within a column with unlike superscript letters were significantly different ( $P < 0.05$ , paired  $t$  test).

\* For details of subjects and procedures, see Methods.

† Calculated daily consumption of xanthophyll from one capsule of Lutein Plus® containing 20 mg/capsule comprising lutein, 3R,3'R-zeaxanthin and MZ (10.8, 1.2 and 8 mg or 54, 6 and 40 % respectively).

‡ Plasma concentration multiplied by the blood volume (70 ml/kg) and molecular weight of 568.9.

§ Differences between the sexes ( $t$  test).

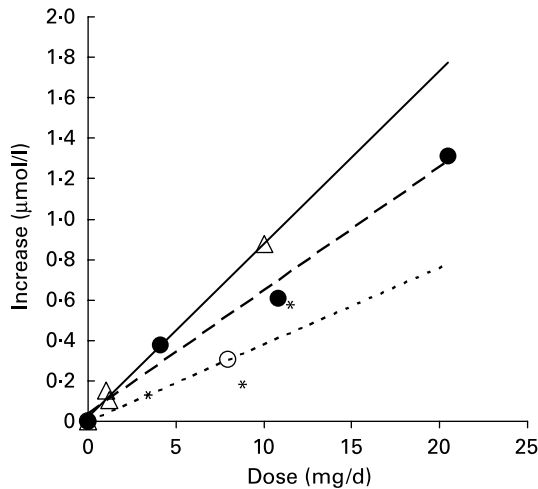
same during the 21 d. The data suggested that plasma concentrations of lutein (1.7 ×) and MZ (2.6 ×) increased more rapidly in women than men, while there was no difference between the sexes for (3R,3'R)-zeaxanthin.

The data in Table 2 also show the increases in the concentrations of the three carotenoids in the combined and separate sexes. (3R,3'R)-Zeaxanthin appears to be the most readily taken up of all three xanthophylls, although in women the relative increase in (3R,3'R)-zeaxanthin was not significantly greater than that of lutein ( $P = 0.065$ , paired  $t$  test). When results for men and women were combined, the relative increase in plasma (3R,3'R)-zeaxanthin concentration was approximately 50 % greater than lutein. In the case of zeaxanthin isomers, overall, the change in plasma concentration of (3R,3'R)-zeaxanthin relative to dose was 3.38 times greater (i.e. 0.088/0.026) than MZ (2.4 times in women and almost six times in men).

Baseline plasma cholesterol concentrations and body weight were examined to try and explain the differences in the increases in xanthophyll concentrations between the sexes. There were no significant relationships between cholesterol and any of the xanthophyll measurements (data not shown). There were, however, strong relationships between the increases in both lutein ( $r = 0.626$ ,  $P = 0.004$ ) and MZ ( $r = 0.512$ ,  $P = 0.025$ ) with weight but no relationship with zeaxanthin ( $r = 0.007$ ,  $P = 0.976$ ). Blood volumes and estimates of the approximate increased amount of xanthophyll in the blood per person at day 22 were therefore calculated (Table 2). Correcting for blood volume in this way removed the difference in lutein uptake between the sexes ( $P = 0.162$ ) but the difference in MZ uptake remained whether arithmetic ( $P = 0.012$ ) or logged ( $P = 0.015$ ) data were tested. There were, however, two MZ outliers in the women, which skewed the data. Nevertheless, the difference between the sexes still remained ( $P = 0.04$ ) even after the removal of these two subjects.

## Discussion

Previous work with both lutein<sup>(23)</sup> and zeaxanthin<sup>(24)</sup> supplements reported that plasma concentrations of both xanthophyll pigments had plateaued around days 18–20. Lutein was given at 4.1 and 20.5 mg/d and (3R,3'R)-zeaxanthin at 1 and 10 mg/d. In the present study the daily amounts of lutein and (3R,3'R)-zeaxanthin from Lutein Plus® were 10.8 and 1.2 mg/d respectively. In response to 10.8 mg lutein/d for 21 d, we found an increase of 0.607 µmol/l, while the previous study reported increases of 0.38 and 1.31 µmol/l in response to 4.1 and 20.5 mg/d. In response to 1.2 mg (3R,3'R)-zeaxanthin/d, we found an increase of 0.106 µmol/l while the previous workers obtained increases of 0.152 and 0.872 µmol/l in response to 1 and 10 mg (3R,3'R)-zeaxanthin per d. These data are shown in Fig. 3 and indicate that the individual responses of both lutein and (3R,3'R)-zeaxanthin in the mixture are slightly lower than those reported previously. Nevertheless, the responses do lie very close to the response lines of the respective xanthophylls when fed as almost pure substances<sup>(23,24)</sup>. These data also show the superior plasma response to (3R,3'R)-zeaxanthin over both lutein and MZ and it is also worth noting that, in spite of the small amount



**Fig. 3.** Plasma xanthophyll concentrations at plateau following 21 d supplementation with the doses shown. The increases in plasma concentrations are shown of lutein (●), (3R,3'R)-zeaxanthin (Δ) and meso-zeaxanthin (MZ; ○) following 21 d oral supplementation. \* Indicates the responses from xanthophylls in Lutein Plus® (1.2 mg (3R,3'R)-zeaxanthin, 10.8 mg lutein and 8 mg MZ). The other data on lutein are from Thurnham *et al.* (23) and on (3R,3'R)-zeaxanthin from Hartmann *et al.* (24). Response lines are forced through zero.

of zeaxanthin in the supplement, there were no non-responders with regard to any of the xanthophyll carotenoids supplied.

At plateau concentrations in plasma, it can probably be assumed that the amount absorbed is equivalent to the amount lost from the plasma due to tissue storage, metabolism, breakdown and losses etc of the individual carotenoids. As plateau concentrations increase with increases in dietary supplement, the plasma concentration at equilibrium is probably a proxy for the amount of xanthophyll absorbed at different doses. In the cases of lutein and (3R,3'R)-zeaxanthin, these substances are already in the diet and plasma concentrations increased in proportion to the additional supplement intake. Therefore, by expressing the plasma concentration at equilibrium as the response relative to the dietary intake, the results are proxy indicators of absorption and can be compared. The data suggest that (3R,3'R)-zeaxanthin is better absorbed than lutein and the results using the mixture of xanthophyll pigments in Lutein Plus® produced responses that were slightly lower but nevertheless similar to those produced by pure supplements of lutein and (3R,3'R)-zeaxanthin.

In the case of plasma MZ concentrations at 22 d, the plateau concentrations are lower than those of both (3R,3'R)-zeaxanthin and lutein. That is, it appears that the absorption of natural (3R,3'R)-zeaxanthin was on average 2.6 times better absorbed than MZ. A similarly poor response to MZ was obtained previously in two male adults who were given a similar supplement for 42 d and where there was a three-fold difference in the relative absorption of MZ to (3R,3'R)-zeaxanthin (20,25). Earlier comparative studies of the three zeaxanthin isomers in chickens also found that the absorption of MZ ((3R,3'S)-zeaxanthin) was only 40% of that of the other two zeaxanthin isomers (3R,3'R and 3S,3'S), although MZ was better absorbed from a racemic mixture (26). However, we should be cautious in interpreting the human data. In the previous studies (20,25), the intake of MZ and (3R,3'R)-zeaxanthin was 14.9 and 1.4 mg/d respectively and in the current study Lutein Plus® provided about 8 mg MZ and about

1.2 mg (3R,3'R)-zeaxanthin. That is, in both studies the relative amounts of MZ were much larger than the intake of (3R,3'R)-zeaxanthin and it has been shown previously with β-carotene that, while small amounts of β-carotene are almost fully absorbed and metabolised (27), absorption falls off rapidly as the dose increases (28).

It is possible that the superior plasma response to (3R,3'R)-zeaxanthin compared with MZ was due to the larger amount of MZ and lutein in Lutein Plus®. However, the xanthophylls may be absorbed differently from β-carotene since the plasma response to increasing amounts of both lutein (up to 20 mg/d (23)) and (3R,3'R)-zeaxanthin (up to 10 mg/d (24)) were still relatively high at the upper dose when fed to plateau at 18 d (Fig. 3). Nevertheless, the plasma response to 1 mg pure (3R,3'R)-zeaxanthin was 0.152 μmol/l (24), whereas the response was only 0.106 μmol/l following 1.2 mg (3R,3'R)-zeaxanthin in Lutein Plus® (0.088 μmol/l per mg) and the figure of 1.2 mg may be an underestimate. The chiral chromatography used to assess the proportions of MZ and (3R,3'R)-zeaxanthin did not provide baseline separation of the two stereoisomers in most cases. We found the relative proportions of these isomers in the capsules to be 87:13% but a separate assessment of the same batch using chemical derivatisation found 82:18% respectively (R Montoya, personal communication). Such figures would mean the plasma (3R,3'R)-zeaxanthin response from the supplement was even lower than 0.088 μmol/l per mg. Furthermore, if the content of (3R,3'R)-zeaxanthin in the capsule was underestimated then the amount of MZ may have been overestimated but a small discrepancy in the amount of MZ will not substantially alter the results from those shown in Table 2.

An additional factor that may have influenced the plasma response to MZ in Lutein Plus® may be the fact that there is almost no MZ in the human food chain and 21 d may not be long enough for the intake of MZ to equilibrate within the body. There is some evidence for this in the women receiving Lutein Plus®, since a comparison of days 10 and 22 results suggest that the plasma concentration of MZ is still rising. However, this was not the case in the men and the differences between days 10 and 22 were not significant in either sex. In addition, Bone *et al.* (20) gave their supplement for 40 d and they still found a three-fold difference in the relative increase in plasma concentration of MZ and (3R,3'R)-zeaxanthin. However, both their subjects were men.

While the absolute difference in proxy measures of absorption of the two zeaxanthin stereoisomers found in the current study may have been influenced by the different doses, comparisons between the sexes are more valid. MZ appeared to be absorbed 2.59 times more efficiently in women than in men. In previous studies there appeared to be a better uptake of the xanthophyll supplements in those with the poorer carotenoid status (20). However, previous carotenoid intake is unlikely to have influenced these data. If anything, plasma concentrations of both lutein and β-carotene at baseline were higher in the women than the men and the questionnaire data suggested that both groups had similar habits in their consumption of both vegetables and fruits. We (29) and others (30) have previously shown that inflammation can depress plasma carotenoid concentrations but there were only two occasions when there were raised acute-phase protein results and neither of these was associated with a

low carotenoid concentration (data not shown). Another possible factor that might influence carotenoid concentrations is body size as blood volume is directly related to weight. Correcting for weight removed the difference between the sexes in the case of the plasma response to lutein but the difference still remained in the case of MZ.

Finally, it is interesting to note that supplementation with Lutein Plus<sup>®</sup> depressed the plasma concentrations of  $\beta$ -carotene at days 10 and 22. Others have noted that lutein intake negatively affects the absorption of  $\beta$ -carotene<sup>(31)</sup>. Plasma  $\beta$ -carotene concentrations have a relatively rapid turnover time with a half-life of <12 d<sup>(32)</sup>. Hence, by day 10 in the Lutein Plus<sup>®</sup> study mean plasma  $\beta$ -carotene concentrations were depressed in both the women (54%) and the men (44%) and remained depressed at day 21 (women 31%, men 36%), although concentrations appeared to be starting to recover. How the body might adjust its  $\beta$ -carotene uptake in the face of the continuing high intake of xanthophyll pigments is not known.

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