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The effect of lutein- and zeaxanthin-rich foods *v.* supplements on macular pigment level and serological markers of endothelial activation, inflammation and oxidation: pilot studies in healthy volunteers

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Abstract

The aim of the present study was to compare the effect of lutein- and zeaxanthin-rich foods and supplements on macular pigment level (MPL) and serological markers of endothelial activation, inflammation and oxidation in healthy volunteers. We conducted two 8-week intervention studies. Study 1 (n 52) subjects were randomised to receive either carrot juice (a carotene-rich food) or spinach powder (a lutein- and zeaxanthin-rich food) for 8 weeks. Study 2 subjects (n 75) received supplements containing lutein and zeaxanthin, β-carotene, or placebo for 8 weeks in a randomised, double-blind, placebo-controlled trial. MPL, serum concentrations of lipid-soluble antioxidants, inter-cellular adhesion molecule 1, vascular cell adhesion molecule 1, C-reactive protein and F_2-isoprostane levels were assessed at baseline and post-intervention in both studies. In these intervention studies, no effects on MPL or markers of endothelial activation, inflammation or oxidation were observed. However, the change in serum lutein and zeaxanthin was associated or tended to be associated with the change in MPL in those receiving lutein- and zeaxanthin-rich foods (lutein r 0·40, P=0·05; zeaxanthin r 0·30, P=0·14) or the lutein and zeaxanthin supplement (lutein r 0·43, P=0·03; zeaxanthin r 0·22, P=0·28). In both studies, the change in MPL was associated with baseline MPL (food study r −0·54, P<0·001; supplement study r −0·40, P<0·001). We conclude that this 8-week supplementation with lutein and zeaxanthin, whether as foods or as supplements, had no significant effect on MPL or serological markers of endothelial activation, inflammation and oxidation in healthy volunteers, but may improve MPL in the highest serum responders and in those with initially low MPL.

Key words: Lutein: Zeaxanthin: β-Carotene: Macular pigment level: Inflammation: Oxidation

Age-related macular degeneration (AMD) is the leading cause of blindness in many developed countries, including the UK[1]. The role of diet in modulating AMD risk has received much attention; and higher intakes of carotenoids and other micronutrients have been associated with reduced risk of the disease[2–6], while a supplement containing an antioxidant 'cocktail' of vitamin C, vitamin E, β-carotene, Zn and Cu was shown to reduce the risk of advanced AMD by approximately 25 % in the Age-Related Eye Disease Study (AREDS)[7]. The macular pigment is made up of the xanthophylls, lutein, zeaxanthin and meso-zeaxanthin, and low macular pigment level (MPL) has been related to AMD risk[8]. Lutein and zeaxanthin have been shown to be potent antioxidants[9,10] and their location in the retina enables them to protect the lipid bio-layers. AMD is increasingly being understood in terms of inflammatory processes, as many of the genes known to increase the risk are involved in the complement system[11–13]. Dietary micronutrients have also been shown to be associated with inflammation. Carotenoid-rich foods have been shown to reduce C-reactive protein (CRP) concentrations, within the normal range of CRP in healthy humans[14], while serum levels of β-carotene have been shown to be inversely associated with inflammatory markers, including CRP, although it is not clear whether the carotenoids reduce inflammation or whether the inflammation suppresses carotenoid status[15]. Animal models have been used to show that lutein and zeaxanthin also have the capacity to reduce inflammation[16,17], although this is yet to be confirmed in studies involving human subjects.

Abbreviations: AMD, age-related macular degeneration; CRP, C-reactive protein; ICAM-1, inter-cellular adhesion molecule 1; MPL, macular pigment level; VCAM-1, vascular cell adhesion molecule 1.

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Therefore, carotenoids may contribute to AMD prevention by both increasing MPL and exerting an antioxidant and anti-inflammatory effect on the surrounding retinal tissue. Evidence for lutein and zeaxanthin contributing to AMD prevention has been shown in case–control studies[4] and cohort studies[2,18]. However, there have been few large intervention studies examining the effect of increased lutein and zeaxanthin intake on MPL and AMD progression.

Findings from intervention studies involving antioxidants have shown the potentially different effects of antioxidant-rich foods *v.* supplements. In a number of prospective cohort studies, those with the highest vitamin E levels were found to have a significantly lower risk of CHD[19,20]; however, supplementation trials involving vitamin E, either singly, or in combination with other antioxidants have yielded disappointing results, largely showing that they do not markedly reduce cardiovascular events or cancer[21,22]. It may be that isolated large single antioxidant supplement doses do not have significant health benefits, and that a fruit- and vegetable-rich diet may be more effective. Therefore, in the first study, a lutein- and zeaxanthin-rich food was compared to a β-carotene-rich food (food study) and, in the second study, a lutein and zeaxanthin supplement was compared with a β-carotene supplement or placebo (supplement study). We hypothesised that increased dietary intake of lutein and zeaxanthin, whether as foods or as supplements, would increase MPL in healthy volunteers and also reduce the markers of endothelial activation (inter-cellular adhesion molecule 1 (ICAM-1); vascular cell adhesion molecule (VCAM-1), inflammation (CRP) and oxidation (urinary isoprostanes). A β-carotene-rich food and supplement was included in the study design because of its previously documented association with inflammation[15].

Materials and methods

Ethics

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Research Ethics Committee of the School of Medicine and Dentistry, Queen's University Belfast. Written informed consent was obtained from all subjects/patients.

Subjects

Subjects were healthy male and female volunteers, recruited from the university staff and students, hospital staff and the general public. For each study, the protocol was explained in full to all subjects and written informed consent was obtained. Volunteers were screened and included in the study if they were male or female, and aged between 18 and 75 years, and excluded from the study if they suffered from age-related macular degeneration, any other eye disease affecting MPL assessment or if they were already taking carotenoid supplements.

At the start and end of the study, participants filled in a 4-d food diary (both 1 weekend day and 3 week-days). Energy and nutrient intakes were calculated by entering the dietary data onto an extensive computerised food analysis database based on UK food composition tables (WISP, Tinuviel Software, Warrington, UK).

Food study

This study was an 8-week randomised intervention study using dried spinach powder (a lutein- and zeaxanthin-rich food; Martin Speciality Foods, Belfast, UK) or carrot juice (a β-carotene-rich food; Schoenenberger Walther Pflanzensaftwerk GmbH, Magstadt, Germany).

Subjects were randomised, by random number generation, with a block design (block size = 4), to either dried spinach powder (10·4 g) daily or carrot juice (131 ml) daily for 8 weeks. Dried spinach powder (10·4 g) daily provided each volunteer with 15 mg of lutein and zeaxanthin (95 % lutein), and 131 ml of carrot juice provided each volunteer with 15 mg of β-carotene daily (amounts confirmed by food analysis; Craft[23]). The spinach powder also contained approximately 2 mg β-carotene/d. The pre- and post-supplementation clinic protocol followed is detailed in the 'Clinical protocol' subsection.

Supplement study

This study was an 8-week double-blind, placebo-controlled intervention study. Volunteers were randomised to either a mixed lutein and zeaxanthin supplement (containing 5 mg zeaxanthin and 10 mg lutein; Life Extension Foundation, Fort Lauderdale, FL, USA), a 15-mg β-carotene supplement (Holland & Barrett, Nuneaton, UK) or placebo (lactose powder in a gelatine capsule, supplied by Victoria Pharmaceuticals, Belfast, UK). Volunteers were randomised as previously described, but this time in blocks of nine subjects. The same pre- and post-supplementation clinic protocol was followed as detailed next in the 'Clinical protocol' subsection.

Clinical protocol for food and supplement studies

Volunteers were required to fast from 22.00 hours, the night before attending the clinic. Participant height, weight and blood pressure (Omron M5-I with standard cuff obtained from White Medical, Rugby, UK) were measured, a fasting

Table 1. Baseline characteristics of volunteers by intervention group in the food study

(Percentages, mean values and standard deviations)

	Spinach powder (*n* 25)		Carrot juice (*n* 27)		
	Mean	SD	Mean	SD	*P*
Sex (% male)	40·0		33·3		0·62
Age (years)	35·6	13·8	34·2	11·3	0·70
Systolic BP (mmHg)	119·4	11·5	127·0	19·8	0·10
Diastolic BP (mmHg)	76·8	8·6	79·7	11·1	0·29
BMI (kg/m^2)	24·0	3·7	24·1	4·0	0·94
Current smokers (%)	16·0		18·5		0·81
Drink alcohol (%)	92		74		0·09

BP, blood pressure.

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spot urine sample collected and a fasting blood sample (55 ml) taken. Subjects were also asked questions regarding their date of birth and other health-related habits including smoking, alcohol consumption and medication use, using a standard questionnaire.

Each subject's right pupil was dilated using a short-term dilator (1 % tropicamide), pupil size after dilation was measured and MPL was assessed by Raman spectroscopy[6]. The Raman spectrometer was obtained from Spectrotek LC, Salt Lake City, UT, USA. Resonance Raman spectroscopy involves a low-powered 1·0 mW argon laser spot (488 nm) being directed as a 1 mm diameter spot onto the macular retina for 0·25 s. The methodology exploits the spectral absorption characteristics of the macular pigment which absorbs light from about 400–500 nm, reaching a maximum absorption at approximately 460 nm. The laser resonantly excites the long conjugated carbon double and single bonds within the macular pigment producing two prominent stokes lines at 1159 and 1525 per cm, which correspond to those identified from lutein and zeaxanthin dissolved in tetrahydofuran. The backscattered light is collected by a fibre-optic collection bundle and the resultant Raman spectrograph analysed by a computer program. The peak height at the carotenoid carbon–carbon double-bond stretch frequency of 1525 per cm is quantified after subtraction of background fluorescence by the Windows-based computer software (Eye-C-Spec; Spectrotek, LC). The Raman signal intensity is expressed as photon counts. The manufacturers recommend that to overcome the errors introduced by misalignment or blink, the mean of the highest three of the five measurements recorded at any one sitting should be used for statistical analysis (Raman count). A previous study has reported 13·5 % as the CV for resonance Raman spectroscopy when carried out four times over a 2-week period[24].

Serum samples were kept in the dark for 1 h and separated by centrifugation. EDTA samples were stored for 1 h at 4°C and separated by centrifugation. Urine sample aliquots were frozen without the addition of a preservative. All samples, including urine samples, were stored at −80 °C until analysis (within 2 years). All baseline and post-supplementation samples were analysed together within each assay run.

Endpoint measurements

Inter-cellular adhesion molecule 1/vascular cell adhesion molecule 1. Analysis of ICAM-1 and VCAM-1 was carried out by ELISA (Immunodiagnostic Systems Limited, Tyne and Wear, UK). Intra- and inter-batch CV for both ICAM-1 and VCAM-1 was < 3 %.

C-reactive protein. CRP was assessed by latex-enhanced immunoturbidimetric assay (Randox Pharmaceuticals, Crumlin, UK) using an ILab 600 biochemical analyser (Instrumentation Laboratories Limited, Warrington, UK). The inter-assay CV was 1·7 % (n 10).

F_2-isoprostanes. Total urinary F_2-isoprostane concentrations were measured as an estimate of systemic oxidative stress using the method outlined by Roberts & Morrow[25]. Following extraction, derivatised spot urine samples were

Table 2. Baseline, post-intervention and change in endpoints by intervention group in the food study
(Mean values and standard deviations; mean values and 95 % confidence intervals; geometric means and interquartile ranges; geometric means and 95 % confidence intervals)

	Spinach powder (n 25)								Carrot juice (n 27)							
	Baseline			Post-intervention			Change			Baseline			Post-intervention			Change		
	Mean	SD	Interquartile range	Mean	SD	Interquartile range	Mean	95 % CI	P*	Mean	SD	Interquartile range	Mean	SD	Interquartile range	Mean	95 % CI	P*
MPL (Raman counts)	920	329		945	289		25	−63, 113	0·56	905	351		884	314		−20	−150, 110	0·75
Lutein (μmol/l)†	0·24		0·17–0·33	0·52		0·32–0·71	2·12	1·62, 3·02	<0·001	0·23		0·19–0·29	0·24		0·19–0·28	1·02	0·98, 1·16	0·57
Zeaxanthin (μmol/l)†	0·07		0·05–0·08	0·07		0·06–0·09	1·08	0·95, 1·24	0·05	0·07		0·05–0·09	0·07		0·06–0·07	1·04	0·89, 1·10	0·43
α-Carotene (μmol/l)†	0·09		0·05–0·14	0·08		0·05–0·14	0·87	0·65, 1·18	0·14	0·08		0·05–0·11	0·32		0·18–0·50	4·05	2·54, 7·96	<0·001
β-Carotene (μmol/l)†	0·34		0·16–0·68	0·35		0·18–0·67	1·03	0·82, 1·29	0·72	0·29		0·21–0·38	0·70		0·49–1·11	2·42	1·54, 3·88	<0·001
ICAM-1 (ng/ml)	697	341		707	367		10	−48, 68	0·73	743	422		728	443		−15	−50, 20	0·39
VCAM-1 (ng/ml)	807	261		821	287		14	−62, 91	0·71	704	217		715	213		12	−25, 48	0·53
CRP (mg/l)†	1·29		0·72–1·39	1·40		0·87–1·71	1·09	0·68, 1·74	0·71	1·31		0·85–1·81	1·33		0·77–1·87	1·01	0·91, 1·13	0·79
Isoprostanes (ng/ml creatinine)†	0·84		0·62–1·31	0·76		0·48–1·24	0·90	0·69, 1·17	0·43	0·87		0·53–1·23	0·77		0·58–1·07	0·89	0·64, 1·23	0·46

MPL, macular pigment level; ICAM-1, inter-cellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; CRP, C-reactive protein.
*P value for difference in each variable pre- and post-intervention assessed for spinach powder and carrot juice separately using a paired samples t test.
† Data for baseline and post-intervention presented as geometric mean, and for change as geometric mean of the post to pre ratio.

analysed by GC–MS using a Trace GC Ultra–DSQ II MS system (Thermo Fisher Scientific, Waltham, MA, USA). The inter-assay CV for this technique was < 12 %. Total F_2-isoprostane content was standardised for urinary creatinine. The latter was measured using an automated enzymatic method (Randox, Crumlin, Northern Ireland, UK) on an ILab 600 biochemical analyser (ILab 600; Instrumentation Laboratories).

Vitamin A, E and carotenoids. Levels of vitamin A, E and carotenoids in serum were assessed using HPLC with diode array detection following extraction into heptane[23]. Intra- and inter-batch CV were < 10 %.

Statistical analyses

All statistical analyses were carried out using SPSS for Windows version 14.0. (SPSS Inc., Chicago, IL, USA)

Continuous variables were checked for normality and were logarithmically transformed where necessary. Data are presented as means and standard deviations for normally distributed data, and geometric mean (interquartile range) for logarithmically transformed data. χ^2 tests were carried out to compare categorical variables between the intervention groups. Comparisons of baseline characteristics between two groups were made using the independent-samples *t* test.

Comparisons between pre- and post-values in the food study were made using paired samples *t* tests. Comparisons between three groups in the supplementation study were made using a one-way ANOVA followed by the Newman–Keuls multiple-range comparison test. Associations between two continuous variables were assessed using Pearson correlation coefficients. Analysis was conducted on an intention-to-treat basis.

Results

Food study

A total of fifty-seven volunteers (male and female) aged 22–62 years were recruited and fifty-two completed the study (the five dropouts were due to difficulty in consuming the intervention foods). Table 1 shows the baseline characteristics of the food study population by intervention group. There was no difference in any of the variables assessed between groups at baseline.

Dietary intake of the study population by the intervention group was assessed by the 4-d food diary, and was within usual population ranges. There was no difference in any of the main macro- and micronutrients assessed between groups at baseline, including carotene, and there was also no change in any of these variables during the intervention period (excluding food supplement; data not shown).

Table 2 shows that there was a significant increase in serum lutein ($P<0{\cdot}001$) and a trend towards an increase in serum zeaxanthin ($P=0{\cdot}05$) after supplementation in the spinach powder group. There were significant increases in both serum α- and β-carotene after supplementation ($P<0{\cdot}001$) with carrot juice. However, there was no change in MPL or in the markers of endothelial activation, inflammation or oxidation after supplementation with either spinach powder or carrot juice.

Supplement study

A total of seventy-five volunteers (male and female) aged 21–72 years were recruited and all seventy-five volunteers completed the study. Table 3 shows the baseline characteristics of the supplementation study population by the intervention group. There was no significant difference in any of the variables shown between the three groups at baseline.

Dietary intake of the study population by the intervention group was assessed by the 4-d food diary, and was within usual population ranges. There was no difference in any of the main macro- and micronutrients assessed between groups at baseline, including carotene, and there was also no difference in change in any of these variables assessed between groups during the intervention period (excluding supplement intake; data not shown).

Table 4 shows that there was a significantly larger increase in serum lutein and zeaxanthin in the lutein and zeaxanthin group, compared to the β-carotene and placebo groups ($P<0{\cdot}001$). There was also a significantly larger increase in serum β-carotene in the β-carotene group compared with the lutein and zeaxanthin, and placebo groups ($P<0{\cdot}001$). However, there was no difference in change in MPL or markers of endothelial activation, inflammation or oxidation between the intervention groups.

Further exploration of the data revealed that, although there was no significant effect of lutein and zeaxanthin supplementation or consumption of lutein- and zeaxanthin-rich

Table 3. Baseline characteristics of volunteers by intervention group in the supplement study
(Percentages, mean values and standard deviations)

	Lutein and zeaxanthin (*n* 25)		β-Carotene (*n* 25)		Placebo (*n* 25)		
	Mean	SD	Mean	SD	Mean	SD	*P*
Sex (% male)	44		32		48		0·49
Age (years)	36·6	10·6	36·6	11·9	39·6	14·2	0·61
Systolic BP (mmHg)	124·5	11·7	124·7	15·5	131·1	14·0	0·17
Diastolic BP (mmHg)	79·3	7·4	78·9	8·0	80·1	10·0	0·88
BMI (kg/m^2)	24·5	3·4		2·5	25·8	3·6	0·19
Current smokers (%)	16		20		12		0·74
Drink alcohol (%)	92		92		72		0·07

BP, blood pressure.

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Table 4. Baseline, post-intervention and change in endpoints by intervention group in the supplement study

(Mean values and standard deviations; mean values and 95 % confidence intervals; geometric means and interquartile ranges; geometric means and 95 % confidence intervals)

	Lutein and zeaxanthin (*n* 25)							
	Baseline			Post-intervention			Change	
	Mean	SD	Interquartile range	Mean	SD	Interquartile range	Mean	95 % CI
MPL (Raman counts)	849	351		817	317		−32	−142, 78
Lutein (μmol/l)†	0·20		0·15–0·29	0·50		0·40–0·67	257[a]	215, 308
Zeaxanthin (μmol/l)†	0·06		0·04–0·07	0·16		0·14–0·21	283[a]	231, 347
α-Carotene (μmol/l)†	0·08		0·06–0·13	0·08		0·07–0·12	111	94, 131
β-Carotene (μmol/l)†	0·30		0·19–0·42	0·35		0·25–0·52	117[a]	101, 135
ICAM-1 (ng/ml)	933	385		935	380		2	−78, 69
VCAM-1 (ng/ml)	812	224		809	239		−3	−34, 73
CRP (mg/l)†	1·30		0·78–2·20	1·26		0·81–2·02	93	68, 128
Isoprostanes (ng/ml creatinine)†	1·11		0·83–1·40	1·04		0·78–1·42	94	83, 106

	β-Carotene (*n* 25)							
	Baseline			Post-intervention			Change	
	Mean	SD	Interquartile range	Mean	SD	Interquartile range	Mean	95 % CI
MPL (Raman counts)	791	307		776	311		−16	−109, 77
Lutein (μmol/l)†	0·21		0·14–0·29	0·21		0·15–0·27	102[b]	93, 110
Zeaxanthin (μmol/l)†	0·06		0·05–0·09	0·06		0·05–0·08	100[b]	91, 110
α-Carotene (μmol/l)†	0·12		0·07–0·20	0·12		0·08–0·17	105	93, 120
β-Carotene (μmol/l)†	0·40		0·29–0·64	1·03		0·59–1·75	256[b]	195, 337
ICAM-1 (ng/ml)	907	356		902	310		−4	−57, 61
VCAM-1 (ng/ml)	818	220		838	232		20	−44, 37
CRP (mg/l)†	0·86		0·64–1·81	1·08		0·69–1·68	126	75, 211
Isoprostanes (ng/ml creatinine)†	1·00		0·72–1·49	0·99		0·80–1·43	98	77, 126

	Placebo (*n* 25)								
	Baseline			Post-intervention			Change		
	Mean	SD	Interquartile range	Mean	SD	Interquartile range	Mean	95 % CI	*P**
MPL (Raman counts)	702	329		688	323		−14	−94, 67	0·96
Lutein (μmol/l)†	0·23		0·18–0·29	0·21		0·17–0·25	92[b]	86, 97	<0·001
Zeaxanthin (μmol/l)†	0·06		0·04–0·07	0·05		0·04–0·06	92[b]	85, 99	<0·001
α-Carotene (μmol/l)†	0·08		0·07–0·11	0·09		0·06–0·13	106	90, 124	0·85
β-Carotene (μmol/l)†	0·29		0·19–0·47	0·28		0·17–0·49	98[a]	85–114	<0·001
ICAM-1 (ng/ml)	1088	408		1090	445		2	−40, 45	0·98
VCAM-1 (ng/ml)	816	233		812	239		−4	−51, 43	0·71
CRP (mg/l)†	1·34		0·62–2·65	1·70		0·71–3·20	127	68, 236	0·60
Isoprostanes (ng/ml creatinine)†	1·26		0·81–1·60	1·33		0·83–1·70	106	92, 121	0·60

MPL, macular pigment level; ICAM-1, inter-cellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; CRP, C-reactive protein.
[a,b] Superscripted letters indicate homogeneous subsets.
* *P*-value for difference in change in each variable between the intervention groups assessed using one-way ANOVA.
† Data for baseline and post-intervention presented as geometric mean, and for change as geometric mean of the post to pre ratio.

foods on MPL, the increase in serum lutein and zeaxanthin over the intervention period for each study was significantly associated (lutein – supplement study), or tended to be associated (lutein – food study and zeaxanthin – both studies) with the increase in MPL. This is shown in Fig. 1. In both studies, the change in MPL was associated with baseline MPL (all subjects; food study r −0·54, $P<0·001$; supplement study r −0·40, $P<0·001$).

Discussion

This study has assessed the effects of lutein- and zeaxanthin-rich foods and supplements on MPL, markers of inflammation and oxidation as biomarkers of AMD risk, in healthy subjects.

Previous observational studies have shown associations between lutein, zeaxanthin and MPL[26–28]. Due to these initial epidemiological findings suggesting that higher serum or

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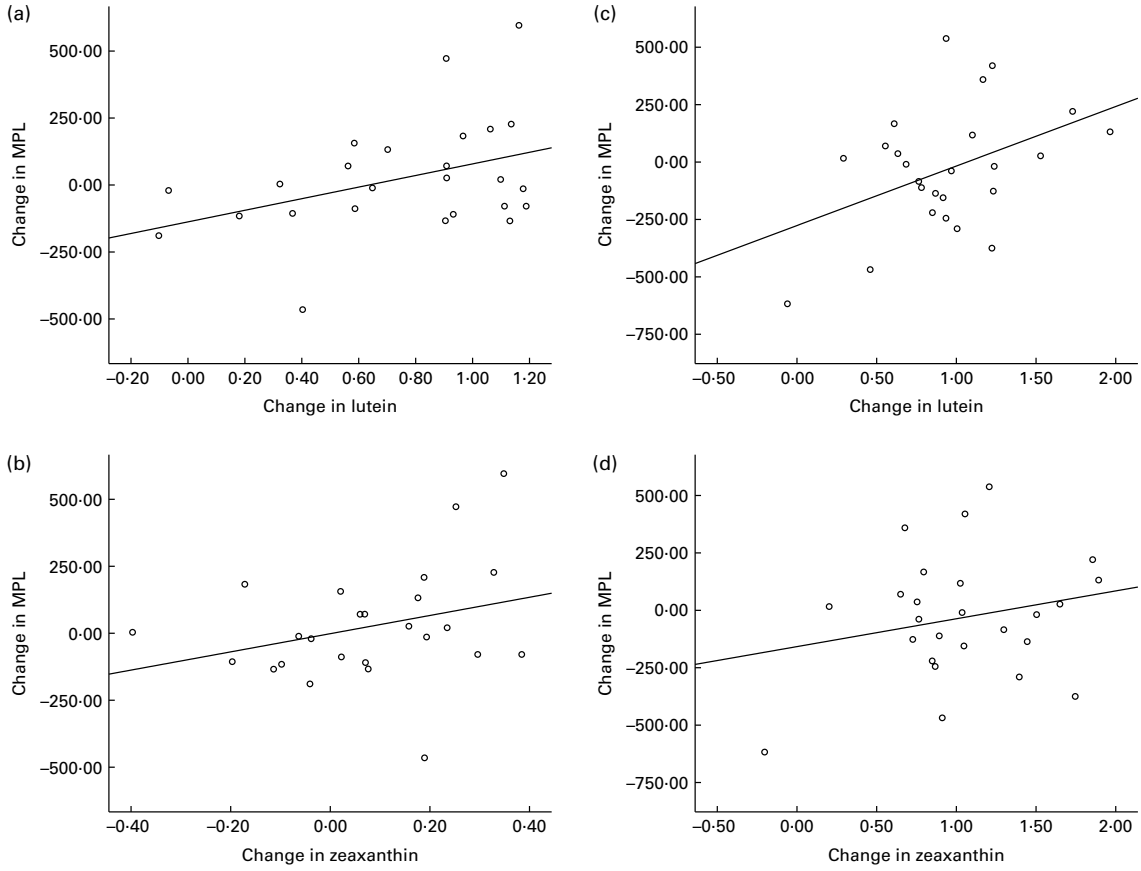

Fig. 1. Change in serum lutein and zeaxanthin *v.* change in macular pigment level (MPL) for the food study and supplement study (in the spinach powder group (food study) or lutein and zeaxanthin supplement group (supplement study). (a) Change in MPL *v.* change in lutein while consuming spinach powder (r 0·396, P=0·05; R^2 linear 0·156); (b) change in MPL *v.* change in zeaxanthin while consuming spinach powder (r 0·304, P=0·14; R^2 linear 0·092); (c) change in MPL *v.* change in lutein while on lutein and zeaxanthin supplementation (r 0·425, P=0·03; R^2 linear 0·181); (d) change in MPL *v.* change in zeaxanthin while on lutein and zeaxanthin supplementation (r 0·224, P 0·28; R^2 linear 0·05).

dietary intakes of lutein and zeaxanthin are associated with higher MPL, a number of intervention trials were undertaken to determine whether supplementation with these compounds increased MPL. These studies tended to be small, and just a few have been placebo-controlled[29–36]. They also used a variety of methods to measure MPL, each with their own inherent advantages and disadvantages[37]. We chose Raman spectroscopy as our method of measurement, which has been used successfully in both healthy subjects and AMD patients[6,24]. This technique is objective, sensitive, specific and fast, as well as being highly repeatable[24].

There are a number of possible reasons as to why no difference in MPL was noted in response to supplementation in this study. These were pilot studies, but a retrospective power calculation, using data from participants in the present studies revealed that, with the numbers of participants we recruited, we would have had 80% power to detect as statistically significant, a change of 354 in MPL in the food study, and a difference in MPL between the intervention groups of 215 in the supplement study, indicating that these studies were underpowered, and this should guide the design of future studies. However, there are a number of other study design considerations during the planning of further studies. First, the duration of supplementation may have been insufficient; Cardinault *et al.*[36] did not show a change after 35 d, and most of the studies that showed a significant response supplementation continued for at least 120 d. Secondly, and supported by the retrospective power calculation, the sensitivity of the Raman measurement may have been unable to show small changes, given that the population variation is large. Berendschot *et al.*[31] confirmed the influence of different measurement methodologies in a supplementation study that used both scanning laser ophthalmoscopy and spectral reflectance maps; 100% of participants showed an increase on the basis of the scanning laser ophthalmoscopy results, whereas only 50% showed a significant increase on the spectral reflectance maps, indicating the importance of the method of assessment of MPL.

It could also be that the findings in these studies are attributable to the volunteers being young (average age 35–38 years), health-aware (mostly from a hospital/research environment), and therefore already consuming a healthy diet with a high intake of lutein and zeaxanthin. Their MPL measurements may therefore have been initially normal, although a normal range for Raman measurements has yet to be formally defined, and the capacity for an increase in MPL through increased lutein and zeaxanthin consumption may have been minimal, particularly since the extent of the increase in the present

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study was inversely related to baseline MPL level. One of the few other studies to report Raman data that can be compared with our study reported similar MPL levels for a comparable age group (mean 1060 (SD 459) Raman counts)[24]. Also, doses of 15 mg of lutein and zeaxanthin in the spinach powder and lutein and zeaxanthin supplement may have been too low to affect what were already normal MPL in these healthy individuals, although others have shown an effect of similar doses[31,38–40].

Although there were no between-group differences in MPL response over the two studies, there was some evidence, within the groups on the lutein and zeaxanthin study arms, that the increase in serum lutein and zeaxanthin was associated with the increase in MPL, and the increase in MPL was associated with baseline MPL. Evidence of 'retinal non-response' is an interesting characteristic demonstrated in many of the previous supplementation studies, with the proportion of participants showing this feature varying widely. The reasons for this occurring are not known, though various conclusions can be drawn from looking at the previous studies[32,33,36,38–43]: it occurs in both healthy volunteers and those with retinal pathology[36,41], it can be dependent on the methodology used[31], the eccentricity at which the measurement is made[43] and is sometimes associated with baseline levels of MP and serum lutein and zeaxanthin[32,38,42] but not always[32,33,39,40]. Other factors such as BMI and baseline fruit and vegetable, or fat intake may also affect retinal response. The studies reported here were not adequately powered to explore this issue fully. Further larger studies are therefore required over a longer supplementation period and with careful consideration of dose to determine if lutein- and zeaxanthin-rich foods or supplements increase MPL and ultimately reduce AMD risk in healthy volunteers, and also to fully characterise the factors influencing retinal non-response.

Numerous antioxidant micronutrients and dietary patterns have been associated with inflammatory markers and endothelial activation markers and may have a role in ameliorating inflammation and endothelial activation in AMD patients[15,44–46]. However, in the present study, there was no change in CRP, ICAM-1 or VCAM-1 on lutein- and zeaxanthin- or β-carotene-rich foods or supplements. It may be because initial inflammatory or activation marker status was too low for these compounds to have an effect and these findings suggest that carotenoids may have a limited role, if any, in reducing inflammation or reducing endothelial activation in healthy volunteers. Carotenoid supplementation may only be beneficial in those with higher levels of inflammatory markers at baseline. These results also reveal no differential effects of foods *v.* supplements on inflammatory markers or markers of endothelial activation.

A number of studies have examined the effects of antioxidants on oxidative stress either in fruit and vegetable interventions, or as supplements[47–54]. Most[47–52], although not all[53,54], of these studies found a reduction in various measures of oxidative stress after supplementation. We, however, found no changes in isoprostanes in either the food or the supplement study. Again, it may be because the volunteers involved in the study were young, with supplementation unlikely to reduce what were already low levels of markers of oxidative stress at baseline.

Conclusion

Supplementation over an 8-week period with a food rich in lutein and zeaxanthin (spinach powder), or with a food rich in carotene (carrot juice), or with a combined lutein and zeaxanthin supplement, *v.* a β-carotene supplement or placebo, had no effect on MPL as assessed by Raman spectroscopy or markers of inflammation, endothelial activation or oxidation in healthy volunteers. However, the change in serum lutein and zeaxanthin was associated or tended to be associated with the change in MPL in the spinach powder and lutein and zeaxanthin supplement groups, while the change in MPL was associated with baseline MPL, suggesting that such interventions may improve MPL in the highest serum responders and in those with initially low MPL.

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