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## *Short Communication*

# Long-term plant stanol and sterol ester-enriched functional food consumption, serum lutein/zeaxanthin concentration and macular pigment optical density

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Observational epidemiological studies have shown that low carotenoid intake and/or low carotenoid blood levels increase the risk of degenerative diseases like age-related macular degeneration. Functional foods enriched with plant sterol or stanol esters may lower serum concentrations of fat-soluble carotenoids. Theoretically, as a result the macular pigment optical density (MPOD), a marker for eye health, may change. We carried out a double-blind placebo-controlled human intervention trial with a duration of 18 months to evaluate the possible effects of plant stanol and sterol esters on serum lutein/zeaxanthin concentration in relation to the MPOD. Forty-seven subjects were randomly assigned to one of the three treatment groups: margarine without added plant sterols or stanols, plant sterol-enriched margarine, or plant stanol-enriched margarine. Serum cholesterol and lutein/zeaxanthine concentrations and the MPOD were evaluated at baseline and at study end. Changes in lipid-adjusted serum lutein/zeaxanthine concentrations between baseline and study end differed significantly between the three groups ($P=0{\cdot}001$). We found no differences in the MPOD between the three treatment groups, despite the differences in both absolute and cholesterol-standardized serum lutein/zeaxanthine concentrations. This shows that the observed reduction in serum carotenoid concentrations during 18 months consumption of these functional foods does not affect MPOD.

**Macular pigment optical density: Plant stanols: Plant sterols: Carotenoids**

Lutein, zeaxanthin and meso-zeaxanthin are the only carotenoids present in the macular pigment of the retina[1] and it is suggested that they could protect the retina by blue light filtering[1], thereby decreasing chances for photochemical light damage[2,3]. In addition, they are capable of scavenging free radicals[3]. An initial study by Seddon *et al.* [4] observed an inverse association between a diet with a high content of lutein and the prevalence of exudative age-related macular degeneration, the most common cause of irreversible, severe loss of vision among the elderly in the Western countries. A definite proof for a causal relationship however is still lacking[5]. Several studies addressed the possible role of macular pigment more explicitly by measuring the macular pigment optical density (MPOD) in patients with, or at risk of age-related macular degeneration, also with ambiguous results[6–10]. Macular pigment is entirely of dietary origin and it has been show that MPOD can be increased by a dietary modification[11] or by supplements[12–15]. Functional foods containing plant sterol or stanol esters may lower serum concentrations of fat-soluble carotenoids[16–18]. The relevance of these reductions on health is unknown, and only a few human intervention studies addressed the long-term effects of decreased carotenoid concentrations[17,19]. Therefore, we carried out a human intervention trial to evaluate the effects of plant stanol and sterol esters on serum lutein/zeaxanthin reduction and the possible change in MPOD as a consequence of this reduction.

## Methods

### *Subjects*

Subjects were recruited via local newspaper advertisements and posters in the university and hospital buildings. Inclusion criteria were: current treatment with a 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor (statin), age 18–65 years, BMI $\leq 32\,kg/m^2$, no proteinuria or glucosuria, diastolic blood pressure $\leq 95$ mm Hg and systolic blood pressure $\leq 200$ mm Hg. Using statins was an inclusion criteria since another purpose of the study was to analyse the long-term effects of plant sterol or stanol esters on serum lipoprotein metabolism and markers of endothelial dysfunction and vascular stiffness in

**Abbreviation:** MPOD, macular pigment optical density.

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patients on stable statin treatment[20]. Exclusion criteria were clinical manifestations of liver disorders, having diabetes mellitus type 2 or having had cardiovascular or cerebral events within a period of 6 months prior to the study. The Ethics Committee of the Maastricht University had approved the protocol and all subjects signed an informed consent.

### *Diets and design*

Subjects were asked to replace their own margarine or butter with the experimental 'light' margarines (40 % fat) we supplied. They were instructed to consume 30 g 'light' margarine per day, and to divide the margarine over at least two meals during the day. During a 5-week run-in period, all subjects used a control margarine without added plant sterols or stanols. At the end of the run-in period subjects were randomly allocated to one of the three experimental groups, stratified for sex and age. For the following 85 weeks, the first group continued with the control margarine, the second group with a plant sterol-enriched margarine (2·5 g plant sterols/d) and the third group with a plant stanol-enriched margarine (2·5 g plant stanols/d). Plant sterols and stanols were provided as fatty acid esters obtained by transesterification of free plant sterols and stanols with sunflower oil-based fatty acids (Unilever, Vlaardingen, The Netherlands) or rapeseed oil-based fatty acids (Raisio Group, Raisio, Finland), respectively. Neither the plant stanol ester nor sterol ester margarines contained lutein or zeaxanthin.

### *Measurements*

*Blood sampling*. Fasting blood samples were taken by venepuncture in weeks 4, 5, 89 and 90. Blood was sampled in 10 ml serum separator and EDTA tubes. Serum was obtained by centrifugation at 2000 ***g*** for 30 min at 4°C, minimally 1 h after blood sampling, and was used for analysis of cholesterol concentrations. EDTA plasma was obtained by centrifugation at 2000 ***g*** for 30 min at 4°C, and was used for analysing lutein/zeaxanthin concentrations. All samples were snap-frozen and stored in small aliquots directly after sampling at −80°C until further analysis.

*Cholesterol and lutein/zeaxanthin*. Cholesterol and lutein/zeaxanthin concentrations were determined as described[16]. Samples from one subject of weeks 4 and 5 as well as of weeks 89 and 90 were pooled before analysis and measured in the same analytical run to minimize the potential influence of factors other than the dietary intervention and as well as the intra-assay variation.

*Macular pigment optical density*. MPOD was determined at weeks 5 and 90 by a full spectral analysis[21] of light reflected at the fovea, measured with the Utrecht Foveal Reflection Analyser[22]. The subjects' pupils were dilated with 0·5 % tropicamide and 1 % phenilefrine.

### *Statistical analyses*

Since serum lutein/zeaxanthin depends on the amount of lipoprotein carriers in plasma[16] we present total cholesterol standardized (μmol/mmol cholesterol) lutein/zeaxanthin concentrations, to correct for changes in serum cholesterol concentrations. Overall differences in changes (week 90 − week 5) between groups were tested by ANOVA. In addition, to correct for possible influence of the baseline concentrations on the serum changes, we applied an ANCOVA with the end value as dependent, diet as fixed factor and baseline as covariate. To look for a possible association between the serum lutein/zeaxanthin and the MPOD we calculated the Pearson correlation coefficient. All data analysis was performed with the SPSS statistical software package version 14.0.0 (SPSS Inc., Chicago, IL, USA).

## Results

### *Recruitment and follow-up*

As described elsewhere, fifty-four subjects completed the study[20]. However, during the study six subjects changed their statin medication. Therefore, their data were not included in the analysis. One other subject showed a decrease in total cholesterol concentration of almost five standard deviations above the mean of all others. This subject was considered an outlier and also excluded from the analysis. Thus, data of forty-seven subjects was available for analysis (Table 1). MPOD was obtained at a different study site than all other measures. This caused some logistical problems, and MPOD was measure in only thirty-four subjects at both study visits (see also Table 1). Compliance was evaluated by evaluating changes in serum plant sterol and stanol concentrations throughout the study period as reported elsewhere[20].

### *Serum lipids and lutein/zeaxanthin*

The average total cholesterol concentration over the entire study period was lowered by 6·8 % or 0·38 mmol/l ($P=0·005$) and 8·8 % or 0·48 mmol/l ($P=0·001$) in the plant sterol ester and plant stanol ester groups as compared to the control group[20]. These reductions could almost entirely be ascribed to reductions in LDL-cholesterol, and were not significantly different between the plant sterol and plant stanol ester groups ($P=0·18$).

The reduction in cholesterol-standardized serum lutein/zeaxanthin concentrations was significantly larger in the sterol ester group as compared to the stanol ester group ($P<0·001$), whereas compared to the control the reduction in the plant sterol ester group nearly reached significance ($P=0·07$; Table 2). The rather unexpected mean increase in cholesterol-standardized serum lutein/zeaxanthin concentration in the stanol ester group was significantly different

**Table 1.** Characteristics of the forty-seven volunteers that were included in the study and of the thirty-four volunteers that had their macular pigment optical density determined twice*

(Mean values and standard deviations)

| | | Control | | Sterols | | Stanols | | |
|---|---|---|---|---|---|---|---|---|
| | *n* | Mean | SD | Mean | SD | Mean | SD | *P* |
| Men/women | 47 | 9/7 | | 10/6 | | 10/5 | | 0·83 |
| | 34 | 8/6 | | 8/4 | | 5/3 | | 0·88 |
| Age (years) | 47 | 60 | 7 | 60 | 7 | 59 | 8 | 0·87 |
| | 34 | 59 | 7 | 62 | 6 | 60 | 8 | 0·64 |

* For details of subjects and procedures, see Methods.

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**Table 2.** Mean lutein serum concentrations corrected for total cholesterol, and macular pigment optical density (MPOD) during the study*

(Mean values and standard deviations)

| | | Control | | Sterols | | Stanols | | |
|---|---|---|---|---|---|---|---|---|
| | Week | Mean | SD | Mean | SD | Mean | SD | P† |
| Lutein (μmol/mmol) | 5 | 0·059 | 0·016 | 0·074 | 0·021 | 0·062 | 0·018 | 0·064 |
| | 90 | 0·054 | 0·014 | 0·062 | 0·018 | 0·067 | 0·023 | 0·15 |
| | 90−5 | −0·004 | 0·007 | −0·013 | 0·016 | 0·005 | 0·012 | 0·001 |
| MPOD (*n* 34) | 5 | 0·43 | 0·08 | 0·51 | 0·18 | 0·47 | 0·08 | 0·27 |
| | 90 | 0·46 | 0·09 | 0·52 | 0·19 | 0·47 | 0·08 | 0·54 |
| | 90−5 | 0·029 | 0·051 | 0·011 | 0·063 | 0·028 | 0·081 | 0·76 |

* For details of subjects and procedures, see Methods.
† *P* values represent whether the differences between the three groups are significant.

($P=0·032$) from the change in lipid-adjusted serum lutein/zeaxanthin concentrations in the control group. In an ANCOVA analysis, the cholesterol-standardized serum lutein/zeaxanthin concentrations at study end were not only explained by differences in baseline concentrations ($P<0·001$), but there was also a significant contribution of the different diets ($P=0·017$).

### *Macular pigment optical density*

There were no differences between the MPOD at baseline and despite the differences in serum lutein/zeaxanthin concentrations in the three groups, there were no differences in MPOD changes between the groups ($P=0·76$).

At baseline, the MPOD did not correlate with the cholesterol-standardized serum lutein/zeaxanthin concentration ($r$ 0·20, $P=0·22$). If we stratified for gender we found a nearly significant positive correlation for men ($r$ 0·37, $P=0·080$) and a nearly significant negative correlation for women ($r$ − 0·47, $P=0·07$). Also, at baseline the MPOD for men of 0·50 (SD 0·14) was significantly higher compared with the MPOD for women of 0·41 (SD 0·05) ($P=0·008$), despite the almost equal cholesterol-standardized serum lutein/zeaxanthin concentrations of 0·066 (SD 0·18) for men and 0·063 (SD 0·022) for women ($P=0·58$). We found no correlation between the change in the MPOD and the change in lutein/zeaxanthin concentration.

## Discussion

It is well recognized that plant sterol and stanol esters consistently lower serum LDL-cholesterol concentrations. As a result, also concentrations of fat-soluble antioxidants are lowered. We have now shown in this 85-week intervention study that again lutein/zeaxanthin plasma concentrations were significantly lowered. Interestingly, the reduction in lutein/zeaxanthin was only observed in the plant sterol ester group and not in the plant stanol ester group, for which we do not have an explanation.

Macular pigment consists of lutein, zeaxanthin and mesozeaxanthin and could be directly affected by a change in serum lutein/zeaxanthin[11–15]. In comparison with the significant increases in MPOD in supplementation studies, the present study showed that significantly different changes in serum cholesterol-adjusted lutein/zeaxanthin between the three groups did not result in a significant difference between the groups in MPOD. This may be due to the small number of subjects that underwent MPOD measurements. On the other hand, the present results are in accordance with the results of Cooper *et al.*[23] and Broekmans *et al.*[19] who reported that consumption of a dietary fat replacer was not associated with reduced MPOD.

Despite the small numbers, at baseline the MPOD was significantly higher for men than for women, in line with results from other studies[24–27]. Also, the differences in the correlation between the MPOD and the cholesterol-standardized serum lutein/zeaxanthin concentration for men and women have been observed before[24,28,29].

In conclusion, we found a significantly lowered cholesterol-standardized lutein/zeaxanthin concentration after 85 weeks of consumption of margarines enriched with plant sterol or stanol esters. However, this did not translate into a change in MPOD.

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J. P. and R. P. M. planned the study. A. de J. and T. T. J. M. B. collected and analysed the data. T. T. J. M. B. drafted the article. J. P., R. P. M. and A. de J. reviewed and commented on the manuscript. There are no conflicts of interest.

## References

1. Davies NP & Morland AB (2004) Macular pigments: their characteristics and putative role. *Prog Retin Eye Res* **23**, 533–559.
2. Landrum JT, Bone RA & Kilburn MD (1997) The macular pigment: a possible role in protection from age-related macular degeneration. *Adv Pharmacol* **38**, 537–556.
3. Wu J, Seregard S & Algvere PV (2006) Photochemical damage of the retina. *Surv Ophthalmol* **51**, 461–481.
4. Seddon JM, Ajani UA, Sperduto RD, *et al.* (1994) Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye disease case-control study group. *JAMA* **272**, 1413–1420.
5. Trumbo PR & Ellwood KC (2006) Lutein and zeaxanthin intakes and risk of age-related macular degeneration and cataracts: an evaluation using the Food and Drug Administration's evidence-based review system for health claims. *Am J Clin Nutr* **84**, 971–974.

6. Beatty S, Murray IJ, Henson DB, *et al.* (2001) Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. *Invest Ophthalmol Vis Sci* **42**, 439–446.
7. Berendschot TTJM, Willemse-Assink JJM, Bastiaanse M, *et al.* (2002) Macular pigment and melanin in age-related maculopathy in a general population. *Invest Ophthalmol Vis Sci* **43**, 1928–1932.
8. Bone RA, Landrum JT, Mayne ST, *et al.* (2001) Macular pigment in donor eyes with and without AMD: a case-control study. *Invest Ophthalmol Vis Sci* **42**, 235–240.
9. Nolan JM, Stack J, O'Donovan O, *et al.* (2007) Risk factors for age-related maculopathy are associated with a relative lack of macular pigment. *Exp Eye Res* **84**, 61–74.
10. Kanis MJ, Berendschot TTJM & van Norren D (2007) Influence of macular pigment and melanin on incident early AMD in a white population. *Graefes Arch Clin Exp Ophthalmol* **245**, 767–773.
11. Hammond BR, Johnson EJ, Russell RM, *et al.* (1997) Dietary modification of human macular pigment density. *Invest Ophthalmol Vis Sci* **38**, 1795–1801.
12. Berendschot TTJM, Goldbohm RA, Klöpping WA, *et al.* (2000) Influence of lutein supplementation on macular pigment, assessed with two objective techniques. *Invest Ophthalmol Vis Sci* **41**, 3322–3326.
13. Bone RA, Landrum JT, Guerra LH, *et al.* (2003) Lutein and zeaxanthin dietary supplements raise macular pigment density and serum concentrations of these carotenoids in humans. *J Nutr* **133**, 992–998.
14. Trieschmann M, Beatty S, Nolan JM, *et al.* (2007) Changes in macular pigment optical density and serum concentrations of its constituent carotenoids following supplemental lutein and zeaxanthin: the LUNA study. *Exp Eye Res* **84**, 718–728.
15. Koh HH, Murray IJ, Nolan D, *et al.* (2004) Plasma and macular responses to lutein supplement in subjects with and without age-related maculopathy: a pilot study. *Exp Eye Res* **79**, 21–27.
16. Plat J & Mensink RP (2001) Effects of diets enriched with two different plant stanol ester mixtures on plasma ubiquinol-10 and fat-soluble antioxidant concentrations. *Metabolism* **50**, 520–529.
17. Neuhouser ML, Rock CL, Kristal AR, *et al.* (2006) Olestra is associated with slight reductions in serum carotenoids but does not markedly influence serum fat-soluble vitamin concentrations. *Am J Clin Nutr* **83**, 624–631.
18. Colgan HA, Floyd S, Noone EJ, *et al.* (2004) Increased intake of fruit and vegetables and a low-fat diet, with and without low-fat plant sterol-enriched spread consumption: effects on plasma lipoprotein and carotenoid metabolism. *J Hum Nutr Diet* **17**, 561–569.
19. Broekmans WMR, Klopping-Ketelaars IA, Weststrate JA, *et al.* (2003) Decreased carotenoid concentrations due to dietary sucrose polyesters do not affect possible markers of disease risk in humans. *J Nutr* **133**, 720–726.
20. de Jong A, Plat J, Lütjohann D, *et al.* (2008) Effects of long-term plant sterol or stanol ester consumption on lipid and lipoprotein metabolism in subjects on statin treatment. *Br J Nutr* **100**, 937–941.
21. Berendschot TTJM & van Norren D (2004) Objective determination of the macular pigment optical density using fundus reflectance spectroscopy. *Arch Biochem Biophys* **430**, 149–155.
22. Zagers NPA, van de KJ, Berendschot TTJM, *et al.* (2002) Simultaneous measurement of foveal spectral reflectance and cone-photoreceptor directionality. *Appl Opt* **41**, 4686–4696.
23. Cooper DA, Curran-Celentano J, Ciulla TA, *et al.* (2000) Olestra consumption is not associated with macular pigment optical density in a cross-sectional volunteer sample in Indianapolis. *J Nutr* **130**, 642–647.
24. Broekmans WMR, Berendschot TTJM, Klöpping WA, *et al.* (2002) Macular pigment density in relation to serum and adipose tissue concentrations of lutein and serum concentrations of zeaxanthin. *Am J Clin Nutr* **76**, 595–603.
25. Hammond BR, Curran-Celentano J, Judd S, *et al.* (1996) Sex differences in macular pigment optical density: relation to plasma carotenoid concentrations and dietary patterns. *Vision Res* **36**, 2001–2012.
26. Hammond BR & Caruso-Avery M (2000) Macular pigment optical density in a Southwestern sample. *Invest Ophthalmol Vis Sci* **41**, 1492–1497.
27. Ciulla TA, Curran-Celentano J, Cooper DA, *et al.* (2001) Macular pigment optical density in a midwestern sample. *Ophthalmology* **108**, 730–737.
28. Johnson EJ, Hammond BR, Yeum KJ, *et al.* (2000) Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density. *Am J Clin Nutr* **71**, 1555–1562.
29. Burke JD, Curran-Celentano J & Wenzel AJ (2005) Diet and serum carotenoid concentrations affect macular pigment optical density in adults 45 years and older. *J Nutr* **135**, 1208–1214.


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