Special Article

# An appraisal of trials investigating the effects on macular pigment optical density of lutein and zeaxanthin dietary interventions: a narrative review.

Naomi Fitzpatrick, Veronique Chachay, Joanna Bowtell, Sarah Jackman, Sandra Capra, Angela Shore, and David Briskey

Affiliation: N. Fitzpatrick, V. Chachay, S. Capra, and D. Briskey are with the School of Human Movement and Nutrition Sciences, Faculty of Health and Behavioural Sciences, The University of Queensland, Saint Lucia, Queensland, 4067, Australia. J. Bowtell and S. Jackman are with Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, EX1 2LU, United Kingdom. A. Shore is with the School of Medicine, College of Medicine and Health, University of Exeter, Exeter EX1 2HZ, United Kingdom.

Correspondence: Naomi Fitzpatrick, Connell building, Blair Drive, School of Human Movement and Nutrition Sciences, The University of Queensland, Saint Lucia, Queensland, 4072. Email: n.fitzpatrick@uq.edu.au; Phone: +61-7-3365-6240

Abstract: Lutein and zeaxanthin (L/Z), xanthophylls obtained from the diet, are deposited in the macula of the eye. The macular concentration of L/Z is quantifiable as macular pigment optical density (MPOD). The aim of this review was to critically appraise the effect on MPOD of increasing L/Z intake by dietary intervention in adults. Pubmed, Cochrane Library, Web of Science, and Cinahl were searched up to April 2020. Ten studies investigating populations with and without age-related macular degeneration were included. MPOD increased significantly in two of the eight controlled studies. Studies varied largely in the prescribed dietary L/Z dosage, duration, and participant characteristics. No relationships between types of dietary L/Z

interventions and MPOD response could be determined. Limited monitoring of habitual dietary L/Z intake was identified as a major limitation of all ten studies. Habitual dietary L/Z intake should be closely monitored in future studies to account for their effects on MPOD response to dietary L/Z interventions.

Keywords: lutein; zeaxanthin; macular pigment optical density; macula; dietary intervention

## Introduction

Lutein (L), zeaxanthin (Z) and meso-zeaxanthin (MZ) are three xanthophylls, known as the macular pigments, that accumulate in the macula. The macula is part of the retina responsible for visual detail and colour vision. Thus, macular damage, as seen in age-related macular degeneration (AMD), can result in visual impairment or loss. [1] The macular pigments may play a role in optimising vision, such as visual acuity, [2] contrast sensitivity, [3] photostress recovery, [4] glare reduction, [4] and visual processing speed. [5] Additionally, the macular pigments are proposed to maintain macular health through two main mechanisms. Firstly, the macular pigments have direct and indirect antioxidant activity as demonstrated from *in vitro* studies using adult retinal pigment epithelial cell line cultures, and animal retinas dissected post-mortem. [6-11] Secondly, the macular pigments are photosensitive molecules and absorb blue visible light (400-500 nm). [12] Blue light is high energy and can stimulate the production of damaging singlet oxygen species in other macular photosensitive molecules. [12] The absorbance range of post-mortem human macular pigment samples has been shown to be between 430 nm and 490 nm, with peak absorption at approximately 460 nm. [13] The positioning and orientation of the macular pigments within the macula cell layers allow blue light absorption before it reaches other photosensitive molecules. Thus, it has been proposed that the macular pigments reduce the production of damaging singlet oxygen species in the macula. [12]

Macular lutein and zeaxanthin (L/Z) must be acquired through dietary intake, as they are not synthesized endogenously. Meanwhile, MZ is synthesised endogenously as a product of L isomerization in the retina. [14] Despite the required acquisition of L/Z from the diet and implications in macular health, a recommended dietary intake has not yet been established. However, the status of 'bioactive compounds' has been suggested. [15] The National Institutes of Health Office of Dietary Supplements defines bioactive compounds as "Bioactive food components are constituents in foods and dietary supplements, other than those needed to meet basic nutritional needs, which are responsible for changes in health status." [16] Traditionally, dietary recommendations have been developed for bioactive compounds deemed to be essential or conditionally essential through a deficiency-repletion model, and apply to protein, vitamins and minerals. [17] Ranard et al.[15] argued that L/Z meet the nine criteria recently proposed by Lupton et al.[18] to determine if a bioactive compound has the depth of evidence relating to essentiality in health to be considered for intake recommendations. [15,18] To date, determination of an intake recommendation has been limited by the paucity of clinical data about the effects of L/Z dietary intake (as opposed to supplemental intake) on macular concentrations and health.

The concentration of the L/Z/MZ within the macula, or macular pigment optical density (MPOD), is used as a surrogate marker of macular health. [19] MPOD can be measured through a number of methods, one of which is heterochromatic flicker photometry (HFP). [20] MPOD was identified as a potential marker of macular health in a number of cross-sectional studies. These studies observed MPOD to be significantly lower in eyes of individuals with AMD compared to healthy controls. [19,21-23] Despite the association between lower MPOD and AMD, MPOD thresholds representing 'optimal' or 'adequate' macular health for a specific age-group have not been determined. Additionally, the magnitude of MPOD change that is clinically or functionally meaningful is unclear. The lack of clarity surrounding MPOD values may partly

be due to the difficulty in comparing values obtained from the different measurement methods. [20] However, a higher MPOD is generally perceived to be associated with better macular health. [19]

L/Z/MZ supplementation studies have consistently shown to result in increased MPOD. A 2016 meta-analysis that pooled results from 20 randomised controlled trials (RCTs) investigating the effects of L/Z/MZ supplementation in adults with or without AMD found a significant increase in MPOD. [24] The pooled results from nine RCTs in populations with AMD (n = 938, 50 years of age and above) showed that supplementation with L, Z and/or MZ increased MPOD by 0.07 optical density units (ODU) compared with placebo. Additionally, the dose-response relationship in this population indicated that MPOD increased by 0.005 ODU for each additional 1 mg / day in L/Z/MZ supplementation. [24] Comparatively, the results of eleven pooled RCTs including healthy populations (n = 826, 18 years and above) showed that supplementation increased MPOD by 0.09 ODU compared with placebo. The dose-response relationship in healthy populations indicated that MPOD increased by 0.004 ODU for each additional 1 mg / day in L/Z/MZ supplementation. [24] Furthermore, a significant negative correlation was observed between baseline MPOD values and the degree of MPOD change with supplementation ($r = -0.71$, $p < 0.001$) [24], suggesting supplementation to be more effective when baseline MPOD values are lower.

In comparison to supplementation trials, there is less clarity with regard to the effects on MPOD of increasing L/Z intake through wholefoods. Understanding the impact of dietary interventions on MPOD is of interest to inform future research for the purpose of prevention of AMD. The aim of this narrative review was therefore to critically appraise reports from interventions that investigated the effect of increased dietary L/Z intake on MPOD in adults.

## Materials and Methods

The method for this review involved a systematic search with defined inclusion and exclusion criteria, data extraction, quality appraisal of all studies, and synthesis of study findings by narrative review. [25]

Inclusion criteria were: primary research papers published in English, full text availability, an intervention arm in adults increasing dietary L/Z intake through wholefood consumption, and measurement of MPOD as an outcome. A dietary intervention was deemed ineligible when the L/Z food product was prescribed in a highly concentrated form, i.e. freeze-dried powder, or liquid concentrate. No restrictions were placed on study design or year of publication. Four databases were searched up to April 2020: Pubmed, Cochrane Library, Web of Science, Cinahl. Search terms included; "retina*" OR "retinal pigment*" OR "macula lutea" OR "macular pigment" OR "macular pigment density" OR "macular pigment optical density") AND ("lutein" OR "zeaxanthin" OR "xanthophyll*" OR "macular xanthophylls" OR "macular pigments") AND ("diet* intake" OR "diet therapy" OR "dietary intervention" OR "diet supplement*" OR "dietary supplement*")). Titles and abstracts of 251 papers identified in the search were screened for eligibility. Full texts were reviewed to decide on inclusion, and references were screened for any potentially relevant articles that may have been missed through electronic search methods. The literature selection process is outlined in a flow chart (Figure 1[26]) adapted from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses. [26]

Quality appraisal of selected articles was performed using the Academy of Nutrition and Dietetics Quality Criteria Checklist (ANDQCC) for primary research. The ANDQCC contains four questions regarding the relevance of research, and ten questions relating to the validity of the research. The tool evaluates the quality of reporting of inclusion/exclusion criteria, the

quality of data collection and analysis, the generalizability of results, and identifies bias in order to grade the quality of the evidence. [27] One reviewer extracted information from included studies through identification of the factors of interest including: study design, study duration, subject characteristics, dietary intervention characteristics, dietary intake measures utilized, and MPOD outcomes.

## Results

### *Study characteristics*

Ten studies met the inclusion criteria and were published between 1997 and 2020. Study characteristics and outcomes are summarised in Table 1[28-37]. The ten studies included 613 (62% female) adults participants aged 18 to 92 years, with study sample sizes ranging from 13 to 114 participants. There were seven RCTs, [28-30,32,33,35,36] one single-blind non-randomised controlled trial, [34] one open label intervention, [38] and one cross-over study. [31] All studies measured MPOD by HFP. Specific inclusion criteria across the ten studies included AMD status, sex, age, body mass index (BMI), and habitual dietary L/Z intake. For the purpose of this review, habitual dietary intake refers to dietary L/Z intake outside of the intervention food consumption. Eight studies were conducted in healthy individuals, [28-32,34,36,38] and two in individuals with early AMD. [33,35] One study investigated exclusively female participants, [30] and three studies only included individuals 50 years or older. [28,31,33] Two studies included individuals with a BMI of 30 kg/m$^2$ or less, and one study a BMI 25 kg/m$^2$ or more. Lastly, only one study considered habitual dietary L/Z intake as part of the recruitment inclusion criteria. [28] Scott et al.[28] used a three-question tool to screen for intake low in L rich foods. Only participants consuming less than three serves per week of leafy vegetables, broccoli and/or eggs were included in the study. [28]

Seven studies met the criteria to receive a positive quality rating based on the ANDQCC for primary research, [28,29,31-33,35,36] and three studies a neutral rating. [30,34,38] One study did not provide adequate information regarding the selection and characteristics of participants. [38] One study did not clearly outline how participant group assignment occurred, and reported that mean baseline MPOD was significantly different between all three groups ($p < 0.05$). [30] Seven studies reported attrition rates, and rates ranged between 3% and 36%. [28,29,31-34,38] Reasons for attrition included dislike of intervention food, or gastrointestinal discomfort. [28,31,33] Furthermore, poor adherence to intervention protocol resulted in data exclusion at the time of analysis in one study. [29]

All studies provided adequate detail regarding the intervention prescription and utilised an appropriate tool to measure the primary outcome of interest, MPOD. [39] However, intervention adherence was monitored only in six studies, [28-31,34,38] and data reported only for two studies. [28,29] In these two studies, participants' dietary intervention adherence was greater than 90%. [28,29] Methods to monitor adherence included diet diaries and food frequency questionnaires in four studies, [28,29,31,38] return of empty food containers in two studies, [30,31] dietitian-administered interviews in two studies, [28,31] and supervision during food consumption by a study investigator in one study. [34] Habitual dietary intake was a secondary outcome that was assessed and reported in only four studies. [28,31,34,38] Eight studies reported clear and appropriate statistical methods. [28,29,31-35,38] Two of the RCTs did not report between-group analyses, and only considered change over time within group. [30,36]

The dietary interventions involved provision of a one or two specific foods without change to the overall habitual dietary pattern, termed *prescriptive dietary intake* hereinafter. As summarised in Table 1, for the nine studies that reported the intervention dosage of L/Z/MZ,

176 the median dose was 0.98 mg/day (range = 0.26–17.58 mg/day). One study reported the
177 L/Z/MZ dosage as a combined value, [29] all other studies reported dosage of L, Z, and/or MZ
178 individually. The frequency of consumption was daily in seven studies, [28,29,31-33,35,38] six days
179 weekly in one study, [30] and 5 days weekly in two studies. [34,36] The intervention food was
180 avocado (two studies) (0.5–0.7 mg/day L/Z), [28,29] egg (five studies) (0.26–1.88 mg/day L/Z),
181 [30-34] goji berries (17.58 mg/day L/Z), [35] spinach (3–4.32 mg/day L), [36] or a combination of
182 spinach and corn (11.8 mg/day L/Z) in the ten studies. [38]
183
184 Eight of the ten studies included a control group. The control intervention included isocaloric
185 amount of potato (0 mg L),[28] isocaloric meal without avocado (0.16–0.21 mg L/Z), [29]
186 continuation of habitual diet, [32,35,36] prescription of a sugar capsule (0 mg L/Z), [30] buttermilk
187 drink (0 mg L/Z), [33] or non-xanthophyll enriched egg as control in the xanthophyll enriched
188 egg study. [34] Xanthophyll concentration in enriched and control eggs were monitored but
189 values not reported. [34]
190
191 *Effects of dietary interventions on macular pigment optical density*
192 Only two of the eight controlled studies reported a statistically significant increase in MPOD
193 between the intervention and control groups, as seen in Table 1. [33,35] Of these two studies, the
194 first study reported a 16% MPOD increase after 12-months ($p < 0.05$), [33] and the second study
195 reported a 20% MPOD increase after three months ($p = 0.007$). [35] Both of these studies were
196 in adults with early AMD aged 50 years or above, with sample sizes greater than 100. The
197 other five controlled trials either reported no significant differences between groups, [28,29,32,34]
198 or did not report performing between-group analyses. [30,36] One of the two trials without a
199 control group reported a significant MPOD increase from baseline by 14 weeks ($p < 0.05$),
200 absolute values were not reported. [38] Across the eight controlled studies, no significant changes

201 in MPOD in the control group were observed except in one avocado based trial. In this trial, a
202 significant MPOD increase of 17% from baseline was reported at the halfway point of the
203 intervention in the control group receiving potato (0 mg L). However, statistical significance
204 was not maintained by the end of the study. [28] No changes in habitual dietary intake were
205 reported for the control group, as monitored by dietitian-administered interviews.
206
207 *Effects of dietary interventions on blood lutein and zeaxanthin concentrations*
208 Blood concentration of L was measured in all studies, Z in nine studies, [28,30-36,38] and MZ in
209 one study,[34] as seen in Table 1.
210
211 Only three of the eight controlled studies reported a significant increase in blood L response
212 compared control. [29,32,34] Interestingly, no significant MPOD changes were observed in these
213 three studies. A significant increase from baseline in mean blood L concentration ranging from
214 22% to 126% was observed within the intervention groups in nine studies. [28-34,36,38] A
215 significant increase was also observed in the control groups in two studies. [28,34] In the first
216 study, a 15% increase from baseline was observed at six months ($p = 0.03$). [28] This control
217 group was provided meals containing 0 mg L/Z and requested to make no other dietary
218 changes. In the second study, a 31% increase from baseline was observed at eight weeks in the
219 control group ($p = 0.007$). [34] This control group were provided a normal egg containing L/Z
220 and requested to make no other dietary changes. Meanwhile, the intervention group in this
221 study received egg enriched with L and MZ.
222
223 Three of the eight controlled studies reported significant increases in blood Z concentration
224 compared to the control. [32,34,35] A significant MPOD increase was observed in only one of these
225 three studies [35]. A significant increase from baseline in mean blood Z concentration ranging

from 36% to 337% was observed in the intervention groups in six studies. [30-35] Of note, significant increase from baseline in mean blood Z concentrations was also observed in the control groups of two studies. [28,34] In the first of the two studies, a 20% increase from baseline was observed at six months ($p = 0.004$). [28] In the second study, a 41% increase from baseline was observed at eight weeks ($p = 0.009$). [34] These two control groups were two of the three control groups that also reported significant blood L changes.

One study monitored blood MZ, and MZ was not detectable at baseline for either the control or intervention group. [34] At eight weeks, blood MZ was significantly increased compared to the control group which observed no change ($p < 0.001$). [34]

*Dietary intake measurement*

Habitual dietary intake was assessed and reported in only four of the ten studies, and assessed using different tools as seen in Table 1. [28,31,34,38] Scott et al.[28] used two types of measures: a 132-item semi-quantitative food frequency questionnaire (FFQ) with a recall timeframe of 12 months, and dietitian-administered interviews. [28] The FFQ was not specifically validated to quantify L/Z dietary intake. It was administered at baseline and the mean daily L/Z dietary intake was calculated from a food composition analysis software (Nutrition Data System for Research software (version 2016). The mean L/Z consumption for the intervention and control groups were not significantly different ($3.0 \pm 3.1$ mg/ day and $2.8 \pm 2.7$ mg/day respectively). The dietitian-administered interviews were conducted monthly to monitor maintenance of dietary habits. No significant change in habitual dietary intake was identified, but details of the interview questions were not reported. [28] In the study by Vishwanathan et al.[31] a 7-day diet diary was completed once by participants during each study phase. Total L/Z intake was not quantified, but the diaries were reviewed for intake of foods known to contain 'substantial'

amounts of L/Z. Whilst the criteria for 'substantial' was not defined, the intake of spinach, broccoli and corn were monitored. Intake of these three foods were reported to contribute approximately 0.3 mg/day during the study phases. [31] In the study by Kelly et al.[34], a dietary screening tool (DST) was used at baseline to infer whether habitual dietary L/Z intake was high or low. [34] The DST estimates overall dietary quality graded in three categories based on adherence to the American Dietary Guidelines. The 'at-risk' DST category has been correlated with lower serum L/Z concentration, when compared to the 'possible risk' or 'not-at-risk' categories. [40] The DST does not however quantitatively estimate L/Z intake. In the study by Hammond et al.[38], dietary intake was measured at baseline with the Health Habits and History Questionnaire, developed from the American National Health and Nutrition Examination Survey II data. [41] The Health Habits and History Questionnaire is not validated to specifically quantify L/Z dietary intake. Participants' L/Z intake was calculated from the questionnaire data using a food composition database, but values were not reported. [38] Therefore, only one of the ten studies quantified and reported baseline habitual L/Z dietary intake. [28] None of the studies quantitatively monitored and reported habitual dietary L/Z intake over the study duration.

## Discussion

This narrative review aimed to critically appraise reports from interventions that investigated the effect of increased dietary L/Z intake on MPOD in adults. A varied MPOD response was observed. The reason for this variation is difficult to determine due to substantial heterogeneity between studies, and limited monitoring of habitual dietary L/Z intake. Only two of the eight controlled studies reported significant increases in MPOD in the intervention group. [33,35] Of these two studies, only one also observed significant change in blood Z concentrations. [35] The other studies observed significant changes in blood L/Z/MZ concentrations, but without significant MPOD change. Heterogeneity in trial design and participant characteristics between

studies may explain the inconsistences between study results, and inform future study design. Identified heterogeneity between the studies included the variety of prescribed intervention foods, L/Z dosage, intervention duration, and differences in participant characteristics such as age, sex, AMD status, body composition, baseline MPOD and habitual dietary L/Z intake.

*Influence of participant characteristics on macular pigment optical density response*

Participant habitual dietary lutein and zeaxanthin intake.

A quantitative value for habitual L/Z dietary intake was reported at baseline in only one of the ten studies, [28] and measured but not reported in two studies. [31,38] The importance of quantitively monitoring habitual dietary L/Z intake is highlighted in the study by Scott et al.[28] The baseline intake of the intervention and control group was reported to be 3.0 ± 3.1 mg/day and 2.8 ± 2.7 mg/day respectively. [28] Following baseline, a significant MPOD increase from baseline of 17% was reported at three months in the control group. [28] This MPOD change was not maintained at six months, but serum L/Z was significantly elevated. Of note, no changes in dietary intake were reported, and intake was monitored by dietitian-administered interviews for which question details were not reported. Thus, the potential impact of change to habitual dietary intake, such as due to seasonal variation in available foods, cannot be quantitatively determined. The high baseline inter-individual variability also highlights the need for quantitative measurement of habitual L/Z dietary intake to determine whether the amount of L/Z prescribed as part of a dietary intervention is a small, moderate or large change relative to a participant's habitual intake. In the study by Scott et al.[28] the variable baseline dietary L/Z intake of the intervention group (3.0 ± 3.1 mg/day) meant the prescribed intervention of 0.5 mg/day of L was highly variable in how much it increased participants' total L/Z intake. [28] Thus, quantitative estimation of habitual L/Z intake is critical to measure over the whole study duration when considering the high inter-individual variability reported at baseline, the MPOD

change observed in the control group, and lack of significant MPOD change observed between the intervention and control group. Furthermore, the lack of continuous quantitative measurement is a substantial limiting factor when interpreting the MPOD response observed.

The importance of monitoring habitual dietary L/Z intake over the study duration is demonstrated again in the cross-over trial from Vishwanathan et al.[31] In this study, the three foods (broccoli, spinach and corn) analysed from 7-day diet diaries performed once during each study phase contributed 0.3 mg/day of L/Z in each phase. [31] The 0.3 mg/day of L/Z provided the equivalent of 33% of the phase 1 egg dosage (0.9 mg/day), and 16% of the phase 2 egg dosage (1.88 mg/day). Relative to the intervention L/Z dose prescribed, dietary L/Z intake from just three foods were measured to contribute a substantial amount of the total L/Z being consumed by participants. As a factor that may influence MPOD outcomes, measurement of total habitual L/Z intake, not just from three foods, is therefore critical to consider when interpreting the MPOD response observed.

Habitual L/Z dietary intake was not quantitively monitored over the full study duration in any of the studies. Therefore, it is unclear for the ten studies in this review whether habitual L/Z dietary intake influenced reported MPOD outcomes. The lack of habitual L/Z intake monitoring in these studies is a serious limitation and should be considered when interpreting MPOD outcomes in this review and in future research. To effectively monitor habitual dietary L/Z intake in future studies, standardisation of the dietary intake tools utilised is needed. Four of the ten studies in this review did assess habitual intake at one point throughout the study. [28,31,34,38] However, each study utilised different dietary intake tools, and none of these tools had been specifically validated to monitor dietary L/Z intake. To our knowledge, there are currently

no dietary intake tools specifically designed to quantitatively monitor habitual dietary L/Z intake. The development of such a tool is warranted.

Participant macular pigment optical density.

The variable MPOD response observed in the ten studies reviewed may have also been influenced by the protocol utilised to measure MPOD, HFP. HFP has been shown to have high test-retest reliability. However, HFP is a psychophysical measure as it relies on adequate participant input and understanding of the activity to complete the measure. As such, when using HFP, the effect of participant practice in measurement completion has been acknowledged as an important methodological consideration. [42] A minimum of two measurements of MPOD per session has been recommended to monitor the influence of intra-person variability and 'practice effect' associated with performing HFP. [39] Only four of the studies in this review clearly indicated that participants were familiarised and provided with education to understand the HFP procedure. [28,32-34] Five of the studies reported using the mean of three or more repeated MPOD measurements at a single timepoint, [28,30,31,35,36] and one study reported measuring twice at baseline but did not clearly indicate which value was utilised. [38] Four studies did not clearly indicate that repeat measures were conducted. [29,32-34] Thus, for these four studies whether the change in reported MPOD values is due to true change or due to the practice effect cannot be determined. In addition to the practice effect, MPOD values obtained were difficult to compare between studies due to multiple different HFP machines and protocols utilised. One study used a Maxwellian view system, [37] two studies used the QuantifEYE Macular Pigment Screener II [43], and seven studies used the Macular Densitometer [44]. These HFP machines and protocols differ in aspects such as degrees of eccentricity measured from the fovea in the macula, wavelengths of light used for measurement, accommodation of inter-individual differences in flicker thresholds, and whether an individual

is looking for a flicker to appear or disappear.[45] These differences between HFP methods may result in different MPOD values measured, and is described in detail in a review of MPOD techniques by Howells et al.[45]. Future research utilising HFP would be strengthened through completion of a minimum two MPOD measures at each time point as standard practice recommends, and reporting of the within-session variability, such as by coefficient of variation or similar reliability measures. Alternatively, utilisation of objective MPOD measures in future research, such as fundus autofluorescence, would remove the influence of the practice effect. [45]

Another factor that may influence MPOD response with increased L/Z intake is participant baseline MPOD. [24] Lower baseline MPOD has been associated with a greater MPOD response to L/Z supplementation. [24] In two of the ten studies in this review, the observed absence of MPOD response was proposed to be due to the high baseline participant MPOD. [29,31] However, this association of baseline MPOD influencing responsiveness to elevated L/Z/MZ intake does not appear as convincing in the studies within this review. Participants' mean baseline MPOD was above 0.38 ODU in three of six studies reporting statistically significant MPOD improvements from baseline, and was as high as 0.7 ODU (a study also reporting significant MPOD increase compared to the control group). [28,33,35] Any attempt to interpret the potential influence of baseline MPOD on responsiveness to elevated dietary L/Z intake is made more difficult by the inability to consider the influence of habitual dietary L/Z intake in this relationship. Without habitual dietary L/Z intake data, it cannot be determined whether baseline habitual intake is related to the baseline MPOD values and subsequent responses observed. Further research is needed to investigate the difference in MPOD response in participants with a baseline MPOD above or below 0.4 ODU when prescribed the same dietary L/Z intervention.

375 Other participant characteristics.

376 There was heterogeneity in the age, sex, AMD status, and body composition of participants
377 across the ten studies. Age and sex are not generally considered to be independent determinants
378 of MPOD status, [43,46] while AMD has been associated with lower MPOD status. [19,21-23] The
379 heterogeneity in AMD status of participant groups resulted in additional difficulty when
380 attempting to compare studies to interpret the trends in MPOD outcomes in relation to the
381 intervention food used, L/Z dose provided, and intervention duration.

382

383 Two of the ten studies suggested that the absence of any statistically significant increase in
384 MPOD may have occurred due to the higher body fat composition of the study population. [29,31]
385 This suggestion was based on the BMI being 25.0 kg/m$^2$ or greater in these participants. As
386 L/Z are fat soluble nutrients they can be deposited in adipose tissue, [47] although mechanisms
387 regulating carotenoid uptake or release from adipose tissue are not well understood. [48] Higher
388 percentage of body fat has been previously inversely associated with MPOD. [47] However, in
389 two of the ten studies, participants' BMI was 25.0 kg/m$^2$ or greater, and yet significant MPOD
390 improvement was observed [33,36]. Intervention group MPOD increased significantly compared
391 to the control group in one study, [33] and compared to baseline in the other study. [36] Clearly,
392 BMI is not an accurate measure of body fatness, and as such it is not possible to draw definitive
393 conclusions regarding the influence of body fat percentage on MPOD response. None of the
394 ten studies measured body fat percentage, thus future studies may benefit by including robust
395 measurement of body composition. An additional consideration is the current lack of
396 understanding surrounding mechanisms regulating carotenoid uptake or release from adipose
397 tissue. This consideration provides further reason to consistently monitor habitual dietary L/Z
398 intake and blood L/Z concentrations. These two measures are important as they may be used

to provide insight into fluctuations in L/Z bioavailability, and influential factors such as diet and adiposity.

*Lutein and zeaxanthin dietary intervention dosages*

It remains unclear how different prescribed L/Z intervention dosages influences MPOD response. The aforementioned meta-analysis of RCTs by Ma et al.[24] reported that MPOD increased by 0.004 ODU for each additional 1 mg / day in L/Z/MZ supplementation in healthy individuals. [24] However, this dose dependent relationship was not observed in the six studies investigating different dietary dosages of L/Z in this review. [29-32,34,36] In the study by Kelly et al.[32], the control group was prescribed no change to diet, and four groups were prescribed a range of different L/Z dosages (0.26–1.61 mg/day L/Z) from egg. [32] Despite a range of dosages from a single food source, no statistically significant within or between group differences were reported over the study duration. [32] Important to note is the difference in dosages between the dietary intervention trials and supplementation trials. In the meta-analysis of supplementation trials 15 of the 19 studies in healthy populations provided L/Z/MZ dosages above 10 mg per day. [24] These dosages are considerably higher than the doses provided by the dietary intervention studies included in this review (median dose was 0.98 mg/day, range 0.26–17.58 mg/day). Therefore, variation in habitual dietary L/Z intake is likely to exert a greater confounding influence on the effects observed after dietary modification providing lower additional doses of L/Z. Measurement habitual dietary intake must be considered in future investigations.

*Dietary intervention food source*

A statistically significant increase in MPOD from baseline was achieved after consumption of all of the intervention foods. However, only two prescribed interventions reported a significant

MPOD response compared to the control group, and both were in populations with early AMD (50 years of age and above). The difference in MPOD between the intervention and control groups was 8.33% after 52 weeks with a small L/Z dose (1.59 mg/day) consumed with a fat source, [33] and 15.8% after 12 weeks with a much larger L/Z dose (17.58 mg/day) consumed without fat respectively. [35] It has been demonstrated that bioavailability is improved with co-consumption with fat. [49] These two studies in individuals with early AMD demonstrate an MPOD response achieved through prescription of L/Z containing foods with or without fat. Further studies demonstrating this relationship are needed in healthy individuals.

*Dietary intervention duration*

The time course of MPOD response with dietary intervention prescription remains unclear. An intervention duration of 12 weeks was the minimum length in which a statistically significant MPOD response was observed. The durations of studies that did not observe a statistically significant MPOD increase compared to baseline or to the control group were 12 weeks, [29,32] eight weeks, [34] and five weeks. [31] The two studies in populations with AMD observed similar significant increases in MPOD compared to the control group over different intervention durations. In the study by Li et al.[35] the intervention group had a 16% greater increase over the 12 weeks compared to the control, whilst a 16% greater increase over 52 weeks compared to control was observed by Van Der Made et al.[33] MPOD was measured pre and post intervention in these two studies. With no interim measures it is not known when MPOD started to respond throughout the intervention.

The time course of MPOD response is also unknown in the studies in healthy populations in this review. Two studies that observed significant MPOD from baseline increases in the intervention group performed interim measures throughout the intervention. [28,30]

In the first study with interim measures by Wenzel et al.[30], a significant increase from a baseline mean MPOD of 0.18 ODU was observed by week four for Group 1 (provided 0.28 mg L daily from egg), and was not significantly different at week eight or 12 compared to week four. Meanwhile, for Group 2 (provided 0.83 mg L/Z daily from egg) a significant increase from a baseline mean MPOD of 0.37 ODU was observed at week four and eight, with a further significant increase compared to week four and eight observed by week 12. [30] Group 1 and 2 were not compared, and baseline MPOD of the groups were significantly different. An increase in MPOD was observed in as little as four weeks, however further MPOD increase by 12 weeks was only observed with the higher L/Z dosage.

The second study with interim measures provided a dose of just 0.5 mg of L daily from avocado for 26 weeks. [28] In this study, a significant 23% increase from a baseline mean MPOD of 0.39 ODU was observed at 12 weeks, with no further change between 12 and 26 weeks. [28] No further increase in MPOD despite three more months of daily L intake may be due to what has been termed as 'MPOD saturation'. MPOD saturation is the suggestion that MPOD may be saturable, and that the threshold of saturation may be different between individuals. [50] This has been demonstrated in a cohort of 172 adults with AMD, mean age 70 ± 10 years, that were randomized to 3 groups. [50] Sixty subjects were supplemented daily for 12 months with 10 mg L and 1 mg Z, 66 subjects with 20 mg L and 2 mg Z, and 46 subjects with a placebo. Significant increase in mean MPOD compared to baseline and placebo was observed in both treatment groups by one month, and continued to increase until six months. Between six months and 12 months mean MPOD remained elevated but did not significantly increase compared to the 6-month measure. The absence of continued MPOD increase was suggested to be due to MPOD saturation. [50] Within the studies of this review, a significant MPOD response from baseline was

been observed in as little as four weeks, and with a dietary intervention L/Z dosage less than that of the supplementation study [38]. Thus, the saturation theory may also have influenced the lack of MPOD response observed in four of the ten studies in this review. However, the potential influence of the saturation theory cannot be unpacked further as the studies in this review did not closely monitor habitual dietary L/Z intake. Measurement of habitual dietary L/Z intake is necessary to identify participants with regular consumption of L/Z rich foods that may influence MPOD saturability and the time course of MPOD.

## Conclusion

No clear relationship between dietary L/Z interventions and MPOD response could be determined in this review. Appraisal of the studies identified that factors limiting the determination of any relationship include the lack of quantitative monitoring of habitual dietary L/Z intake over the study duration, and heterogeneity in study design. Heterogeneity in study design included variety of food source, L/Z dosages administered, intervention duration, participant characteristics, and inclusion of a control group. Future studies investigating MPOD response to dietary L/Z interventions should consider the use of a validated dietary intake tool designed to quantitatively measure dietary L/Z intake over the study duration.

## Acknowledgements

Author Contributions: N.F., J.B., V.C., A.S., S.J., D.B., and S.C proposed and drafted the concept of the publication. N.F., V.C., J.B., contributed to writing the manuscript. N.F., J.B., V.C., A.S., S.J., D.B., and S.C contributed to writing and editing of manuscript, table, and figure. All authors have read and agreed on the final version.

Funding: Naomi Fitzpatrick is supported by an Australian Government Research Training Program (RTP) scholarship. The RTP support played no role in the conception and writing of the manuscript.

Declaration of Interests: none.

References

1. Mares J. Lutein and Zeaxanthin Isomers in Eye Health and Disease. *Annual review of nutrition.* 2016;36:571-602.
2. Chew EY, Clemons TE, SanGiovanni JP, et al. Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 report No. 3. *JAMA ophthalmology.* 2014;132(2):142-149.
3. Yao Y, Qiu Q-h, Wu X-W, Cai Z-y, Xu S, Liang X-q. Lutein supplementation improves visual performance in Chinese drivers: 1-year randomized, double-blind, placebo-controlled study. *Nutrition.* 2013;29(7-8):958-964.
4. Stringham JM, Garcia PV, Smith PA, McLin LN, Foutch BK. Macular pigment and visual performance in glare: benefits for photostress recovery, disability glare, and visual discomfort. *Investigative ophthalmology & visual science.* 2011;52(10):7406-7415.
5. Bovier ER, Renzi LM, Hammond BR. A double-blind, placebo-controlled study on the effects of lutein and zeaxanthin on neural processing speed and efficiency. *PLoS One.* 2014;9(9).
6. Liu T, Liu WH, Zhao JS, Meng FZ, Wang H. Lutein protects against beta-amyloid peptide-induced oxidative stress in cerebrovascular endothelial cells through modulation of Nrf-2 and NF-kappab. *Cell biology and toxicology.* 2017;33(1):57-67.

7. Frede K, Ebert F, Kipp AP, Schwerdtle T, Baldermann S. Lutein Activates the Transcription Factor Nrf2 in Human Retinal Pigment Epithelial Cells. *Journal of agricultural and food chemistry.* 2017;65(29):5944-5952.
8. Buscemi S, Corleo D, Di Pace F, Petroni ML, Satriano A, Marchesini G. The Effect of Lutein on Eye and Extra-Eye Health. *Nutrients.* 2018;10(9).
9. Jia YP, Sun L, Yu HS, et al. The Pharmacological Effects of Lutein and Zeaxanthin on Visual Disorders and Cognition Diseases. *Molecules.* 2017;22(4).
10. Kumari N, Cher J, Chua E, Hamzah H, Wong TY, Cheung CY. Association of serum lutein and zeaxanthin with quantitative measures of retinal vascular parameters. *PLoS One.* 2018;13(9):e0203868.
11. Wu J, Seregard S, Algvere PV. Photochemical damage of the retina. *Survey of ophthalmology.* 2006;51(5):461-481.
12. Widomska J, Subczynski WK. Why has Nature Chosen Lutein and Zeaxanthin to Protect the Retina? *J Clin Exp Ophthalmol.* 2014;5(1):326.
13. Bernstein PS, Li B, Vachali PP, et al. Lutein, zeaxanthin, and meso-zeaxanthin: The basic and clinical science underlying carotenoid-based nutritional interventions against ocular disease. *Progress in retinal and eye research.* 2016;50:34-66.
14. Shyam R, Gorusupudi A, Nelson K, Horvath MP, Bernstein PS. RPE65 has an additional function as the lutein to meso-zeaxanthin isomerase in the vertebrate eye. *Proceedings of the National Academy of Sciences of the United States of America.* 2017;114(41):10882-10887.
15. Ranard KM, Jeon S, Mohn ES, Griffiths JC, Johnson EJ, Erdman JW, Jr. Dietary guidance for lutein: consideration for intake recommendations is scientifically supported. *Eur J Nutr.* 2017;56(Suppl 3):37-42.
16. Federal Registrer. In. Vol 69: National Institues of Health; 2004:55821-55822.

17. Murphy SP, Yates AA, Atkinson SA, Barr SI, Dwyer J. History of nutrition: the long road leading to the Dietary Reference Intakes for the United States and Canada. *Advances in Nutrition.* 2016;7(1):157-168.
18. Lupton JR, Atkinson SA, Chang N, et al. Exploring the benefits and challenges of establishing a DRI-like process for bioactives. *Eur J Nutr.* 2014;53(1):1-9.
19. Beatty S, Murray IJ, Henson DB, Carden D, Koh H-H, Boulton ME. Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. *Investigative ophthalmology & visual science.* 2001;42(2):439-446.
20. Putnam CM. Clinical imaging of macular pigment optical density and spatial distribution. *Clinical and Experimental Optometry.* 2017;100(4):333-340.
21. Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE, Twaroska EE. Macular pigment in donor eyes with and without AMD: a case-control study. *Investigative ophthalmology & visual science.* 2001;42(1):235-240.
22. Trieschmann M, Spital G, Lommatzsch A, et al. Macular pigment: quantitative analysis on autofluorescence images. *Graefe's Archive for Clinical and Experimental Ophthalmology.* 2003;241(12):1006-1012.
23. Beatty S, Boulton M, Henson D, Koh H, Murray I. Macular pigment and age related macular degeneration. *British Journal of Ophthalmology.* 1999;83(7):867-877.
24. Ma L, Liu R, Du JH, Liu T, Wu SS, Liu XH. Lutein, Zeaxanthin and Meso-zeaxanthin Supplementation Associated with Macular Pigment Optical Density. *Nutrients.* 2016;8(7).
25. Ferrari R. Writing narrative style literature reviews. *Medical Writing.* 2015;24(4):230-235.

569 26. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for
570 systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.*
571 2009;6(7):e1000097.
572 27. *Evidence Analysis Manual: Steps in the Academy Evidence Analysis Process.*
573 Academy of Nutrition and Dietetics 120 South Riverside Plaza, Suite 2000, Chicago,
574 IL 60606-6995: American Dietetic Association;2016. 978-0-88091-429-1.
575 28. Scott TM, Rasmussen HM, Chen O, Johnson EJ. Avocado Consumption Increases
576 Macular Pigment Density in Older Adults: A Randomized, Controlled Trial.
577 *Nutrients.* 2017;9(9).
578 29. Edwards CG, Walk AM, Thompson SV, et al. Effects of 12-week avocado
579 consumption on cognitive function among adults with overweight and obesity. *Int J*
580 *Psychophysiol.* 2020;148:13-24.
581 30. Wenzel AJ, Gerweck C, Barbato D, Nicolosi RJ, Handelman GJ, Curran-Celentano J.
582 A 12-wk egg intervention increases serum zeaxanthin and macular pigment optical
583 density in women. *The Journal of nutrition.* 2006;136(10):2568-2573.
584 31. Vishwanathan R, Goodrow-Kotyla EF, Wooten BR, Wilson TA, Nicolosi RJ.
585 Consumption of 2 and 4 egg yolks/d for 5 wk increases macular pigment
586 concentrations in older adults with low macular pigment taking cholesterol-lowering
587 statins. *The American journal of clinical nutrition.* 2009;90(5):1272-1279.
588 32. Kelly ER, Plat J, Haenen GR, Kijlstra A, Berendschot TT. The effect of modified
589 eggs and an egg-yolk based beverage on serum lutein and zeaxanthin concentrations
590 and macular pigment optical density: results from a randomized trial. *PLoS One.*
591 2014;9(3):e92659.
592 33. Van der Made SM, Kelly ER, Kijlstra A, Plat J, Berendschot TT. Increased macular
593 pigment optical density and visual acuity following consumption of a buttermilk drink

containing lutein-enriched egg yolks: a randomized, double-blind, placebo-controlled trial. *Journal of ophthalmology.* 2016;2016.

34. Kelly D, Nolan JM, Howard AN, et al. Serum and macular response to carotenoid-enriched egg supplementation in human subjects: the Egg Xanthophyll Intervention clinical Trial (EXIT). *The British journal of nutrition.* 2017;117(1):108-123.

35. Li S, Liu N, Lin L, Sun ED, Li JD, Li PK. Macular pigment and serum zeaxanthin levels with Goji berry supplement in early age-related macular degeneration. *International journal of ophthalmology.* 2018;11(6):970-975.

36. Kopsell DA, Lefsrud MG, Kopsell DE, Wenzel AJ, Gerweck C, Curran-Celentano J. Spinach cultigen variation for tissue carotenoid concentrations influences human serum carotenoid levels and macular pigment optical density following a 12-week dietary intervention. *Journal of agricultural and food chemistry.* 2006;54(21):7998-8005.

37. Hammond BR, Jr., Johnson EJ, Russell RM, et al. Dietary modification of human macular pigment density. *Investigative ophthalmology & visual science.* 1997;38(9):1795-1801.

38. Hammond B, Johnson EJ, Russell RM, et al. Dietary modification of human macular pigment density. *Investigative ophthalmology & visual science.* 1997;38(9):1795-1801.

39. Howells O, Eperjesi F, Bartlett H. Improving the repeatability of heterochromatic flicker photometry for measurement of macular pigment optical density. *Graefe's Archive for Clinical and Experimental Ophthalmology.* 2013;251(3):871-880.

40. Ventura Marra M, Thuppal SV, Johnson EJ, Bailey RL. Validation of a Dietary Screening Tool in a Middle-Aged Appalachian Population. *Nutrients.* 2018;10(3).

41. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. A data-based approach to diet questionnaire design and testing. *American journal of epidemiology.* 1986;124(3):453-469.
42. Snodderly DM, Mares JA, Wooten BR, Oxton L, Gruber M, Ficek T. Macular pigment measurement by heterochromatic flicker photometry in older subjects: the carotenoids and age-related eye disease study. *Investigative ophthalmology & visual science.* 2004;45(2):531-538.
43. Van Der Veen RL, Berendschot TT, Hendrikse F, Carden D, Makridaki M, Murray IJ. A new desktop instrument for measuring macular pigment optical density based on a novel technique for setting flicker thresholds. *The journal of the college of optometrists.* 2012;29.
44. Wooten BR, Hammond BR, Land RI, Snodderly DM. A practical method for measuring macular pigment optical density. *Investigative ophthalmology & visual science.* 1999;40(11):2481-2489.
45. Howells O, Eperjesi F, Bartlett H. Measuring macular pigment optical density in vivo: a review of techniques. *Graefe's archive for clinical and experimental ophthalmology.* 2011;249(3):315-347.
46. Berendschot TT, van Norren D. On the age dependency of the macular pigment optical density. *Exp Eye Res.* 2005;81(5):602-609.
47. Nolan J, O'Donovan O, Kavanagh H, et al. Macular pigment and percentage of body fat. *Investigative ophthalmology & visual science.* 2004;45(11):3940-3950.
48. Bohn T, Desmarchelier C, Dragsted LO, et al. Host-related factors explaining interindividual variability of carotenoid bioavailability and tissue concentrations in humans. *Molecular nutrition & food research.* 2017;61(6).

642 49. Chung H-Y, Rasmussen HM, Johnson EJ. Lutein bioavailability is higher from lutein-
643 enriched eggs than from supplements and spinach in men. *The Journal of nutrition.*
644 2004;134(8):1887-1893.
645 50. Dawczynski J, Jentsch S, Schweitzer D, Hammer M, Lang GE, Strobel J. Long term
646 effects of lutein, zeaxanthin and omega-3-LCPUFAs supplementation on optical
647 density of macular pigment in AMD patients: the LUTEGA study. *Graefe's Archive*
648 *for Clinical and Experimental Ophthalmology.* 2013;251(12):2711-2723.
649
650 Figure Legend:
651 Figure 1. Flowchart of study selection adapted Preferred Reporting Items for Systematic
652 Reviews and Meta-Analyses [26]
653

654 Table 1. Study interventions and outcomes

| Author (date) [study quality] | Study design | Participant characteristics | Inclusion criteria | Intervention (mg L/Z/MZ per food serve) | Mean MPOD | | | Blood L/Z/MZ response | | Method to monitor habitual dietary intake. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | baseline (ODU ± SD) | Study end (ODU ± SD) | % change from baseline | L % change from baseline | Z % change from baseline | |
| Treatment food: avocado | | | | | | | | | | |
| Scott et al. (2017) [28] [+] | RCT, 26 weeks | n = 40 (52% female), ≥ 50 years | Healthy | G1: 135 g/day avocado (0.5 mg L) | G1: 0.39 ± 0.14 | G1: 0.49 ± 0.14 | G1: 26% **c** | G1: 26% **c** | G1: -10% | Baseline semi-quantitative, 132-item FFQ and monthly dietitian |
| | | | | G2: potato (0 mg L) | G2: 0.38 ± 0.17 | G2 0.42 ±0.15 | G2: 11% | G2: 15% **b** | G2: 20% **b** | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | administered interviews. |
| Edwards et al. (2020) [29] [+] | RCT, 12 weeks | n = 84 (63% female), 25-45 years | Healthy, BMI ≥ 25 kg/m$^2$ | G1: 1x 527-659 calorie meal/day with avocado (0.56-0.7 mg L/Z)<br>G2: 1x 529-662 calorie meal/day no avocado (0.16-0.21 mg L/Z) | G1: 0.47 ± 0.22<br>G2: 0.47 ± 0.19 | G1: 0.50 ±0.21<br>G2: 0.49 ± 0.20 | G1: 6%<br>G2: 5% | G1: 33% **b ***<br>G2: -7% | G1:NR<br>G2: NR | Not monitored. |
| Treatment food: egg | | | | | | | | | |
| | RCT, 12 weeks | n = 24 (100% female), 24-59 years | Healthy, BMI ≤ 30 kg/m$^2$ | G1: 6 eggs/week | G1: 0.18 ± 0.02 a | Values NR | G1: **c** | G1: 23% **b** | G1:NR **b** | Not monitored. |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | (0.20 mg L, 0.13 mg Z) | | | | | |
| Wenzel et al. (2006) [30] [Ø] | | | G2: 6 eggs/week (0.60 mg L, 0.37 mg Z) | G2: 0.37 ± 0.06 a | | G2: **b** | G2: 26% | G2: NR **b** |
| | | | G3: 1 x sugar pill/day (0 mg L/Z) | G3: 0.29 ± 0.04 a | | G3 | G3: 10% | G3: NR |
| Vishwanathan et al. (2009) [31] [+] | Cross-over trial, 4 week run in, 5 week | n = 52 (60% female), ≥ 60 years | Healthy | Phase 1: 2 egg yolks/day (0.44 mg L, 0.46 mg Z) | 0.49 ± 0.04 (at 0.5 ºE) | Phase 1: 0.52 ± 0.04 (at 0.5 ºE) | Phase 1: 6% (at 0.5 ºE) | Phase 1: 16% **b** | Phase 1: 36% **c** | 7-day diet diary once per study phase (4 total). |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | intervention, 4 week break, 5 week intervention | | | Phase 2, 4 egg yolks/day (0.96 L, 0.92 Z) | | Phase 2: 0.54 ± 0.03 (at 0.5 ºE) | Phase 2 (10%) (at 0.5 ºE) | Phase 2: 24% **c** | Phase 2: 82% **c** | |
| Kelly et al. (2014) [32] [+] | RCT, 12 weeks | n = 97 (59% female), ≥ 18 years | Healthy, BMI ≤ 30 kg/m$^2$ | G1: 1 non-enriched egg/day (0.17 mg L, 0.9 mg Z)<br>G2: 1 L enriched egg yolk in buttermilk | G1: 0.31 ± 0.14<br>G2: 0.38 ± 0.12 | G1: 0.35 ± 0.22<br>G2: 0.32 ± 0.16 | G1: 13%<br>G2: -16% | G1: 9%<br>G2: 78% **c *** | G1: 64%<br>G2: 93% | Not monitored. |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| drink (0.97 mg L, 0.34 mg Z) | | | | | | | |
| G3: 1 L enriched egg/day (0.92 mg L, 0.14 mg Z) | G3: 0.32 ± 0.12 | | G3: 0.36 ± 0.16 | | G3: 13% | G3: 60% a **c *** | G3: 92% |
| G4: 1 Z enriched egg/day (0.17 mg L, 0.49 mg Z) | G4: 0.35 ± 0.14 | | G4: 0.36 ± 0.21 | | G4: 2% | G4: 14% | G4: 337% **c *** |
| G5: nil change to diet | G5: 0.34 ± 0.15 | | G5: 0.35 ± 0.17 | | G5: 3% | G5: -2% | G5: 47% |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Van der Made et al. (2016) [33] [+] | Double-blind RCT, 52 weeks | n = 101 (67% female), ≥ 50 years | Early AMD, visual acuity >0.5 | G1: 1.5 L enriched egg yolk in buttermilk drink (1.38 mg L, 0.21 mg Z)<br>G2: buttermilk drink no egg yolks (0 mg L/Z) | G1: 0.45 ± 0.14<br>G2: 0.46 ± 0.16 | G1: 0.52<br>G2: 0.48 (SD NR) | G1: 16% **c** *****<br>G2: 4% | G1: 94% **c**<br>G2: NR | G1: NR b<br>G2: NR | Not monitored. |
| Kelly et al. (2017) [34] [Ø] | Placebo controlled trial, 8 weeks | n = 50 (38% female), 18-65 years | Healthy | G1: 1 L, Z, and MZ enriched egg/day (values NR) | G1: 0.45 ± 0.20 | G1: 0.41 ± 0.21 | G1: -9% | G1 126% **c *** | G1: 68% **c** | Dietary Screening Tool at baseline. |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | G2: 41% **b** MZ not detected at baseline for G1 or G2, and detected at 0.084 µmol/L for G1 only by week 8 **c *** |
| G2: 1 non-enriched egg/day (values NR) | G2: 0.41 ± 0.17 (at 0.5 ºE) | G2: 0.44 ± 0.20 (at 0.5 ºE) | G2: 7% (at 0.5 ºE) | G2: 31% **b** | |

Treatment food: goji berries

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Li et al. (2018) [35] [+] | RCT, 12 weeks | n = 114 (70% female), 51-92 years | Early AMD | G1: 25g/day goji berries (2.5 mg L, 15.08 mg Z)<br>G2: nil change to diet | G1: 0.73 ± 0.21<br>G2: 0.72 ± 0.19 | | G1: 0.88 ± 0.20<br>G2: 0.76 ± 0.19 | G1: 21% **c** *<br>G2: 6% | G1: 2%<br>G2: NR | G1: 248% **c** *<br>G2: 7% | Not monitored. |
| Treatment food: spinach | | | | | | | | | | | |
| Kopsell et al. (2006) [36] [+] | RCT, 12 weeks | n = 30 (70% female), 21-60 years | Healthy | G1: 50 g high L variety spinach 5 days/week (6.05 mg L) | G1: 0.34 ± 0.04 | | G1: 0.34 ± 0.04 | G1: 9% **b** | G1: 49% **b** | G1: 36% | Not monitored. |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | G2: 50 g lower L variety spinach 5 days/week (4.2 mg L) | G2: 0.35 ± 0.04 | | G2: 0.35 ± 0.04 | | G2: 0% | G2: 28% **b** | G2: -36% |
| | | | G3: nil change to diet | G3: 0.31 ± 0.04 | | G3: 0.31 ± 0.04 | | G3: 0% | G3: 5% | G3: -11% |
| Treatment food: spinach and corn | | | | | | | | | | |
| Hammond et al. (1997) [38] [Ø] | Open label intervention trial, 14 weeks | n = 10 (69% female), 30-65 years | Healthy | G1: 60 g spinach/day, 150 g corn/day (11.2 mg L, 0.6 mg Z) | Values NR | | | G1: b | G1: NR **b** | G1: NR | Healthy Habits and History Questionnaire at baseline |

655 Study quality assessed by ANDQCC for primary research: (+) relevant and valid study, low risk of bias; (∅), relevant study, moderate or unclear
656 validity and risk of bias [27]. [a] significant difference between groups at baseline $p < 0.05$, [b] significant MPOD increase from baseline $p < 0.05$, [C] $p \leq$
657 0.001, * significant MPOD change versus control group $p < 0.05$. Abbreviations: AMD, age-related macular degeneration; BMI, body mass index;
658 °E, degrees eccentricity from macular centre; G, group; L, lutein; MPOD, macular pigment optical density; n= number of participants; NR, not
659 reported; ODU, optical density units; %, percentage; SD, standard deviation; Z, zeaxanthin.
660