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NON-DIETARY CORRELATES AND DETERMINANTS OF PLASMA LUTEIN AND ZEAXANTHIN CONCENTRATIONS IN THE IRISH POPULATION

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Abstract: *Objective:* To investigate non-dietary correlates and determinants of plasma lutein (L) and zeaxanthin (Z) concentrations in The Irish Longitudinal Study on Ageing (TILDA) sample. *Design:* Cross-sectional study. *Setting:* Community dwelling adults in the Republic of Ireland (ROI). *Participants:* 3,681 participants aged 50 years and older. *Measurements:* TILDA is a nationally representative prospective cohort study of community dwelling adults aged 50 years and over in the ROI. Demographic and health variables were collected during a face-to-face interview carried out in the home (n=8175), and a substantial proportion of these (n=5035; 62%) also attended a study visit in a health assessment centre. Blood samples collected at baseline (wave 1, the subject of the current study), were analysed for plasma concentrations of L and Z by reversed-phase high performance liquid chromatography, and macular pigment (MP) optical density was also measured (using customized heterochromatic flicker photometry). *Results:* After excluding participants with eye disease, data from 3,681 participants were available for analysis. For this group of participants, plasma L and Z were inversely and significantly associated with body mass index (BMI), and were positively and significantly associated with MP, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) ($p<0.001$, for all). Plasma L and Z were significantly lower in males, current smokers, participants reporting less physical exercise, and participants reporting lower levels of education ($p<0.05$, for all). Plasma L was significantly higher in participants reporting a family history of age-related macular degeneration (AMD) ($p=0.001$), and in the group of ≥75 years old ($p<0.05$). For each of these variables, the significant associations remained after controlling for other potential confounding variables. *Conclusion:* The findings of this large study indicate that plasma concentrations of L and Z were lower in association with indicators of a poor lifestyle (high BMI, tobacco use, and less physical exercise) and in association with lower education, indicating that modifying lifestyle in a positive way is likely to be reflected in higher concentrations of plasma carotenoids, with consequential and putative health benefits.

Key words: Lutein, zeaxanthin, ageing, nutrition, lifestyle.

Introduction

Three carotenoids, lutein (L), zeaxanthin (Z) and meso-zeaxanthin (MZ), accumulate in the macula, where they are collectively referred to as macular pigment (MP) (1, 2). The macula, a specialized area of the retina, is responsible for central and colour vision. The blue light-filtering (3) and the antioxidant properties (4) of MP render this pigment important for optimising visual function in humans. Indeed, MP has been shown to enhance visual function in diseased (5, 6) and non-diseased eyes (7) and reduces the risk of visual loss in, and progression of, age-related macular degeneration (AMD) (8), the leading cause of blindness in adults over the age of 65 (9-12). Also, several epidemiological studies have shown that participants with a high dietary intake of carotenoids (including L and Z) have a lower prevalence of AMD, when compared to participants with a poor dietary intake of carotenoids (13-15).

Recent studies have demonstrated that L and Z are found in the monkey and human brain (16-18), and work by Johnson et al. has shown that brain concentrations of these carotenoids correlate positively with MP levels (19). Also, various groups have reported that MP and serum concentrations of L and Z correlate positively to measures of global cognitive function (20-22), and work from our group has shown that MP and serum concentrations of L and Z are significantly lower in patients with Alzheimer's disease, when compared with age-matched controls (23). Accordingly, there is a need to understand factors that influence the circulating concentrations of these nutrients deemed important for eye and brain health.

Carotenoids are entirely of dietary origin and, as a result, the plasma concentrations of these compounds are dependent on an individual's dietary intake of food containing these nutrients (e.g. leafy greens, vegetables, fruits and eggs) (24). We know that the concentration of L and Z in human plasma varies dramatically between individuals (25). We also know that supplementation with the macular carotenoids (L, Z, and MZ) increases serum concentrations of each respective carotenoid (26, 27) and MP, in different subject populations (e.g. participants free of retinal disease (7), participants with AMD (6), and participants with Alzheimer's disease (28)). However, it has been shown that many other variables (both modifiable and unmodifiable) are likely to influence the concentrations

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of L and Z in human plasma and host tissues (e.g. retina and adipose tissue (29)). Previous studies suggest that variables such as sex, age, BMI, alcohol consumption, smoking status, physical exercise, serum lipids, genetic background, and ethnicity are associated with plasma concentrations of L and Z (30-38). However, not all reports are in agreement, probably due to differences in methodologies and populations studied.

In the current report, we present findings from The Irish Longitudinal Study on Ageing (TILDA). Baseline data on the social, economic, and health status of 8,175 participants aged 50 years and older was collected between 2009 and 2011 from a random sample in the Republic of Ireland (ROI) (39). Given the putative and proven health and functional benefits of L and Z for eye and brain, we hypothesised that it was important to study correlates and determinants of these nutrients in an ageing population. Of note, TILDA allowed us to address this research question, using its large, uniquely homogeneous and randomly selected study sample.

Methods

Study design and sampling

Full details of the design, sampling and methodology of TILDA have been previously reported (39). In summary, a nationally representative sample of community dwelling adults was drawn from the Irish Geodirectory, a current and comprehensive record of all residential addresses in the ROI. Addresses were selected by means of RANSAM (a random sampling design for Ireland) using a three stage process where all household residents aged 50 years or older were eligible to participate (40). Wave 1 (baseline) recruitment had an overall response rate of 62% (n=8175). It is planned that these participants will be interviewed every 2 years and the health assessment repeated every 4 years over a ten year period. As TILDA began in 2009, it has now completed wave 1 and 2, and wave 3 is underway. The focus of the current study was baseline (wave 1) only. Participants were required to provide written informed consent prior to participation in the study. This study was approved by the Faculty of Health Sciences Research Ethics Committee of Trinity College Dublin and the local Ethics committee at the Waterford Institute of Technology. All experimental procedures adhered to the tenets of the Declaration of Helsinki.

Interview/questionnaire

As part of wave 1, participants completed a computer-aided personal interview (CAPI) carried out by a trained social interviewer in the participants' home. The questionnaire collected detailed information on many aspects of the participants' lives (demographics, lifestyle and behaviours), socio-economic status, self-reported health (physical and medical history) and medication use. A list of medications taken on a daily basis (coded using Anatomical Therapeutic Chemical (41)), including food supplements (defined according to the Directive 2002/46/EC of the European Parliament and the Council of the European Union, 10 June 2002) was recorded for each participant. The interview was followed by a self-completion questionnaire, which the participant could complete and return via post. Of note, participants were asked whether a doctor had diagnosed them with the following medical conditions: high blood pressure, AMD (including family history), diabetic maculopathy, diabetic retinopathy, cataracts or glaucoma. It is important to note that self-reported data is susceptible to bias, and this limitation must be taken into account when interpreting results here.

Physical health assessment

Participants were invited to attend a comprehensive health assessment carried out by a team of trained nurses in one of two dedicated health centres in Dublin and Cork or have a modified assessment carried out in their own home (39). Height and weight were measured with Seca™ (Seca Ltd., Birmingham, UK) using a standardized protocol, and body mass index (BMI) was calculated as weight (kg)/height (m^2). According to their BMI, participants were categorized into normal (<25 kg/m^2), overweight (25.1-29.9 kg/m^2) or obese (≥30 kg/m^2). Assessment of MP optical density and retinal photographs for AMD grading were only conducted on the 5,035 participants who attended the health centre (i.e. excluding participants who had home health assessments).

Assessment of Macular Pigment Optical Density

Customized heterochromatic flicker photometry (cHFP), a fast and non-invasive procedure, using Macular Densitometer™ (Macular Metrics Corp., Providence, RI, USA) was used to measure MP at the fovea (0.5° eccentricity) with a reference set at 7° eccentricity (parafoveal reference locus). The eye with the best visual acuity was chosen for MP assessment. A full description of the protocol used for the TILDA study is published elsewhere (42, 43). In summary, the subject is required to achieve isoluminance between a blue light wavelength (absorbed by MP), and a green reference wavelength light (not absorbed by MP). This psychophysical technique is customized for each subject, by optimizing the method, taking into account the subject's age and critical flicker frequency (to allow participants reach their end point in testing with minimal variance) (44).

Retinal photography and AMD grading

TILDA nurses were trained and certified by experts (from the Ocular Epidemiology Reading Centre at the University of Wisconsin, Madison, USA) to take retinal photographs using the NIDEK AFCE-210 non-mydriatic auto-fundus camera, through a non-dilated pupil. Pupils were not dilated as this could influence the results of other health measurements such as gait assessment, which was carried out after retinal photography. Retinal photographs were graded by a trained and certified grader using a modified version of the International

Classification and Grading System for AMD under the supervision of Moorfields Eye Hospital Reading Centre, London, UK (9).

Blood collection and processing

Separate written and verbal consent was required to obtain blood samples from participants. Non fasting venous blood samples were collected into one 5 ml Lithium Heparin tube (BD, Becton, Dickinson Limited, Oxford, UK) for immediate analysis and two 10 ml EDTA (BD, Becton, Dickinson Limited, Oxford, UK) tubes for long term storage. Samples were immediately analysed for lipid profile by a commercial laboratory, which includes total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride. One of the EDTA tubes was immediately protected from direct light. This blood sample was centrifuged and 1 ml of EDTA plasma was dedicated to carotenoid assessment and stored at -80°C until time of analysis.

Plasma L and Z assessment

Each participant had 1 ml of frozen EDTA plasma wrapped in tinfoil transported to the Macular Pigment Research Group, Vision Research Centre, Waterford, Ireland (www.mprg.ie). In 2013, L and total Z was analysed using a reversed phase high performance liquid chromatography (HPLC) method. Details of extraction procedures and HPLC analysis are previously described by our research group (23). Method validation was carried out using 968e Fat-Soluble Vitamins, Carotenoids, and Cholesterol in Human Serum Reference Standard from National Institute of Standards and Technology (NIST) and quality checks were frequently evaluated using control plasma samples. Average coefficients of variation were 4% and 6.8% interassay for L and Z, respectively. The limits of quantification were determined to be 0.0021 μmol/L and 0.0027 μmol/L for L and Z, respectively.

Statistical analysis

The statistical package IBM SPSS Statistics for Windows Version 22.0 was used for analysis. General linear models, with plasma L and Z as dependent variables, were the principal method of analysis. We included a core set of explanatory variables in all linear models, consisting of variables which had been identified in previous studies as being associated with plasma L and Z: age, sex, BMI, highest level of education (primary/none, secondary, and third level), smoking status (never, past or current smoker), and family history of AMD (yes, no and don't know). Controlling for these core variables, we also included the following explanatory variables, one at a time, in the general linear models: geographic location (Dublin city/county, another city or town, and rural), plasma cholesterol (TC, HDL, LDL, and triglyceride), alcohol consumption (number of drinks per week), physical exercise derived from the International Physical Activity Questionnaire-short form (inactive [low], minimally active [medium] and health enhancing physical exercise [high]), food supplement use (number of food supplements taken on a regular basis), and self-reported high blood pressure (yes/no). The 5% level of significance was used throughout, without adjustment for multiple testing.

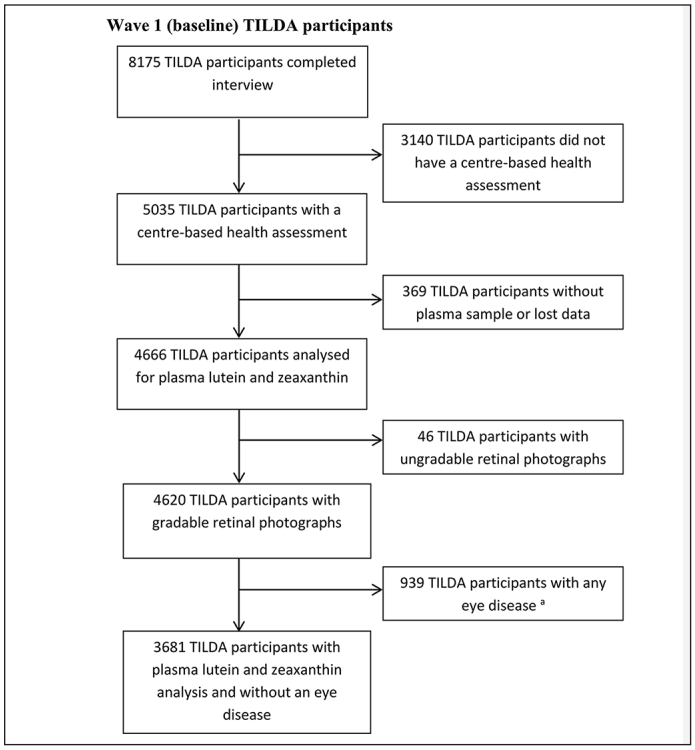
Results

After excluding participants with AMD or self-reported eye disease (diabetic maculopathy, diabetic retinopathy, cataracts or glaucoma), data from 3,681 participants (99% of whom were white, mainly native Irish) were available for analysis (Figure 1). Demographic characteristics for these 3,681 participants studied are reported in Table 1. Of note, the 50-64 age group (70% of our sample) and third level educated respondents (32% of our sample) are over-represented, relative to the population from which the sample was drawn.

Health and lifestyle variables are also presented in Table 1. Mean plasma L and Z concentrations were 0.2047 ± 0.115 μmol/L and 0.0567 ± 0.047 μmol/L, respectively. Plasma concentrations of L and Z were highly correlated with each other; Pearson correlation: $r = 0.647$, $p<0.001$. There was also a positive and significant relationship between plasma concentrations of both L and Z, and MP optical density; Pearson correlation: $r = 0.242$, $p<0.001$ and $r = 0.213$ $p<0.001$, respectively.

Figure 1

The Irish Longitudinal Study on Ageing (TILDA) participants included in this investigation and the time frame for collection and analysis of data; [a] any eye disease includes age-related macular degeneration (AMD) and self-reported: diabetic maculopathy, diabetic retinopathy, cataracts or glaucoma



Relationship of plasma L and Z to core study variables

In the general linear model with putative core explanatory variables (age, sex, MP, education level, BMI, smoking status and family history of AMD), these variables were significantly related to plasma L after controlling for the other variables, with highly significant relationships ($p<0.001$) for sex, BMI, education level, family history of AMD, MP, and smoking status. Most of these core variables were also significantly related to plasma Z ($p<0.001$), the exceptions being age and family history of AMD. Tables 2 and 3 summarise these findings. After the inclusion of food supplement use as a potential explanatory variable, these significant relationships persisted.

Table 1
Demographic, health and lifestyle characteristics of TILDA participants in this investigation

Variable (n=3681)	Mean ± SD or %
Age (years)	60.68 ± 7.70
50-64	70%
65-74	24.4%
75+	5.6%
Sex (Female)	53.1%
Education level	
Primary/none	20.2%
Secondary	42.8%
Third level	37.0%
BMI (kg/m^2)	27.45 ± 4.90
Total Cholesterol, mmol/L	5.185 ± 1.050
HDL, mmol/L	1.556 ± 0.436
LDL, mmol/L	2.960 ± 0.940
Smoking status*	
Never/past smoker	84.5%
Current smoker	15.2%
MP Optical Density (0.5°)	0.208 ± 0.159
Plasma L, μmol/L	0.2047 ± 0.115
Plasma Z, μmol/L	0.0567 ± 0.047
Exercise (per week)*	
Low	26.9%
Moderate	35.7%
High	37.4%
Family History of AMD*	5.0%

Data displayed are mean ± standard deviation (SD) for interval data and percentages for categorical data: Variables, variables analysed in the study; n, number of participants; Education level, highest level of education (primary/none, secondary, and third level); BMI, body mass index; Cholesterol, total cholesterol, high density lipoprotein (HDL), and low density lipoprotein (LDL); Smoking status, never-smokers (non-smoker), past smoker and current smoker; MP Optical Density, measured by customized heterochromatic flicker photometry at 0.5°; Plasma L and Z, concentrations measured by high performance liquid chromatography, Exercise, % exercise per week (inactive [low], minimally active [medium] and health enhancing physical exercise [high]); Family history of AMD, % of participants self-reporting family history of age-related macular degeneration; * Self-reported.

As seen in Table 2, females and third level educated participants have significantly higher plasma L and Z concentrations, on average, compared to males and lower educated participants; current smokers have significantly lower average L and Z concentrations compared to past smokers and non-smokers. As seen in Table 3, plasma L and Z concentrations were significantly higher in the low and medium BMI tertile groups when compared to the high BMI tertile group, and were also significantly higher in the medium and high MP tertile groups when compared to the low MP tertile group.

Relationship of plasma L and Z to other study variables

The following potentially confounding variables were then added to the model (which continued to include the core variables), one at a time, with the following statistically significant and positive results: total cholesterol ($p<0.001$, for both plasma L and Z models), HDL ($p<0.001$, for both plasma L and Z models), LDL ($p<0.001$, for both plasma L and Z models). Physical exercise was also significantly related to both plasma concentrations of L and Z, reflected in significantly lower plasma concentrations of these carotenoids amongst participants in the lowest exercise group when compared to the highest exercise group ($p<0.005$). These results are summarised in Tables 2 and 3.

After controlling for the core variables, alcohol consumption was not significantly related to plasma concentrations of L or Z ($p>0.05$, for both), whereas plasma triglycerides were significantly and positively related to plasma concentrations of Z ($p<0.001$) but not to plasma concentrations of L ($p=0.057$), and self-reported hypertension was positively and significantly related to plasma concentrations of Z ($p=0.042$) but not to plasma concentrations of L ($p=0.058$). Use of food supplements was positively related to plasma concentrations of L ($p=0.008$) but not to plasma concentrations of Z ($p=0.059$); of note, after controlling for food supplement use, the significant positive association between plasma L concentration and age remained. Finally, plasma concentrations of L were significantly lower amongst urban dwellers (another city/town, other than Dublin) versus dwellers of rural areas ($p=0.001$).

Discussion

To our knowledge, this is the largest study of its kind to report on the relationships between plasma concentrations of L and Z and non-dietary correlates and determinants of these carotenoids in an older Irish population. The main finding from our study is that plasma concentrations of both L and Z are associated with the following variables: tobacco use, sex, BMI, education, physical exercise, cholesterol status, age (partially), family history of AMD and MP levels. Importantly, the modifiable variables reported here are associated with lifestyle and behavioural habits, and we discuss these findings, and their implications, below.

Table 2
Demographic and lifestyle variables as determinants of plasma lutein and zeaxanthin concentrations

	Mean ± SD[a]					Mean ± SD[a]					Mean ± SD[a]			
	Lutein	**Sig.**	**Zeaxanthin**	**Sig.**		**Lutein**	**Sig.**	**Zeaxanthin**	**Sig.**		**Lutein**	**Sig.**	**Zeaxanthin**	**Sig.**
Sex					**Smoking Status**					**Education level**				
Male	0.1869 ± 0.098	<0.001	0.0515 ± 0.038	<0.001	Never	0.2153 ± 0.126	<0.001	0.0607 ± 0.052	<0.001	Primary/none	0.1837 ± 0.106	<0.001	0.0450 ± 0.034	<0.001
Female	0.2203 ± 0.127	-	0.0614 ± 0.053	-	Past	0.2059 ± 0.111	<0.001	0.0563 ± 0.045	<0.001	Secondary	0.1974 ± 0.113	<0.001	0.0553 ± 0.047	<0.001
					Current	0.1690 ± 0.086	-	0.0461 ± 0.031	-	Third/higher	0.2245 ± 0.121	-	0.0649 ± 0.051	-
Age					**Exercise**					**Family history of AMD**				
50-64	0.2051 ± 0.112	0.055	0.0594 ± 0.049	0.299	Low	0.1913 ± 0.105	0.002	0.0517 ± 0.045	0.004	Yes	0.252 ± 0.163	-	0.066 ± 0.004	-
65-74	0.1195 ± 0.110	0.018	0.0495 ± 0.037	0.197	Medium	0.2105 ± 0.120	0.197	0.0579 ± 0.048	0.183	No	0.203 ± 0.113	0.001	0.057 ± 0.0008	0.495
≥ 75	0.2215 ± 0.167	-	0.0554 ± 0.051	-	High	0.2092 ± 0.119	-	0.0594 ± 0.048	-	Don't know	0.194 ± 0.102	<0.001	0.052 ± 0.003	0.074

Data displayed are mean ± standard deviation (SD); a. data expressed as µmol/L, plasma L and Z, concentrations measured by high performance liquid chromatography; Sig, significance difference between groups (dashes indicate reference group e.g. p values for education for comparison of primary and secondary educated subjects with third level educated subjects); smoking status, never-smokers (non-smoker), past smoker and current smoker; exercise, % exercise per week(inactive [low], minimally active [medium] and health enhancing physical exercise [high]); education level, highest level of education (primary/none, secondary, and third level); family history of AMD, % of participants self-reporting family history of age-related macular degeneration (AMD).

Table 3
Health variables as determinants of plasma lutein and zeaxanthin concentrations

	Plasma lutein concentration (Mean ± SD)[a]				Plasma zeaxanthin concentration (Mean ± SD)[a]			
	1st tertile group	**2nd tertile group**	**3rd tertile group**	**Sig.**	**1st tertile group**	**2nd tertile group**	**3rd tertile group**	**Sig.**
BMI	0.2344 ± 0.139	0.2038 ± 0.105	0.1755 ± 0.091	<0.001	0.0648 ± 0.055	0.0574 ± 0.047	0.0480 ± 0.035	<0.001
MPOD	0.1770 ± 0.098	0.1970 ± 0.106	0.2379 ± 0.130	<0.001	0.0478 ± 0.035	0.0539 ± 0.044	0.0688 ± 0.056	<0.001
TC	0.1751 ± 0.095	0.2036 ± 0.116	0.2381 ± 0.127	<0.001	0.0453 ± 0.039	0.0554 ± 0.041	0.0706 ± 0.056	<0.001
HDL	0.1690 ± 0.091	0.1993 ± 0.101	0.2478 ± 0.137	<0.001	0.0469 ± 0.043	0.0549 ± 0.043	0.0691 ± 0.051	<0.001
LDL	0.1869 ± 0.112	0.2054 ± 0.113	0.2228 ± 0.118	<0.001	0.0494 ± 0.044	0.0559 ± 0.042	0.0653 ± 0.053	<0.001

Data displayed are mean ± standard deviation (SD); a. data expressed as µmol/L, plasma L and Z, concentrations measured by high performance liquid chromatography; Sig, significance difference between groups; BMI, body mass index (kg/m^2); MPOD, macular pigment optical density 5°; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein.

Firstly, TILDA is a unique longitudinal study, in that it contains data on plasma concentrations of L and Z and MP collected from a large, random, racially homogenous sample of older Irish adults. Secondly, we report here baseline (cross-sectional) data from TILDA, comparing our findings to the findings of other studies including: the Third National Health and Nutrition Examination Survey (NHANES III) (33), the Carotenoids in Age-Related Eye Disease Study (CAREDS) (32), the European Prospective Investigation into Cancer and Nutrition (EPIC) (31), the European Eye study (EUREYE) (30), and some other observational studies (34-37). These studies were conducted between 1988 and 2004, and TILDA (data for this report captured between 2009 and 2011) is much more recent. This is an important point, because some of the factors known to influence circulating concentrations of plasma L and Z have changed in recent years (45, 46), such as trends in tobacco use, lifestyle and dietary habits, and use of dietary supplements.

The mean L and Z plasma concentrations reported in our study were comparable with the EUREYE study (30), but were lower when compared to the EPIC study (31). Population and lifestyle differences are likely explanations of these disparities (30, 31).

Consistent with previous reports, we found that tobacco use is associated with significantly lower circulating plasma concentrations of L and Z (33, 34). Current cigarette smokers exhibited 24% and 27% lower plasma concentrations of L and Z, respectively, when compared with non-cigarette smokers. This finding is unsurprising, because cigarette smokers have been shown to have diets lacking in fruits and vegetables (the source of L and Z) (47, 48), but also because it has been shown that cigarette smokers have an increased overall oxidant load, thus reducing circulating plasma carotenoid concentrations (49).

We also found a statistically significant inverse relationship between BMI (a measure of obesity) and plasma concentrations of L and Z, which is also consistent with previous reports (30, 31, 34, 37, 50, 51). Explanations in the literature include an association between BMI and oxidative stress (52), between BMI and diet (53), and competition between adipose tissue and the retina for uptake of the carotenoids from serum (29, 54). Our findings are consistent with the CAREDS study (32), and Kimmons et al. (NHANES III) (55).

Another finding from our study is that plasma L and Z concentrations were positively related to both HDL and LDL. This finding is not surprising, because carotenoids are transported in plasma by these lipoproteins, in a way that relates to the degree of hydrophobicity of those particles (56). However, Clevidence et al. reported that HDL is the primary

carrier of L and Z (57), but our data shows that both HDL and LDL are comparable correlates (and possibly determinants) of plasma concentrations of L and Z, suggesting that L and Z are transported on both lipoproteins. Our findings are consistent with some studies (37, 58, 59) but others report a relationship only with HDL (60-62).

Consistent with the findings reported previously by our group (36, 61), we found in the current TILDA sample that participants reporting a family history of AMD (n=185) had significantly higher plasma L (but not Z) concentrations when compared to participants with no known family history of AMD (n=3496). Importantly, and similar to Nolan et al. (36) and Loane et al. (61), our study compares plasma concentrations of L and Z (separately) for participants with and without a confirmed family history of AMD. In contrast, the CAREDS study reported that a family history of AMD was not significantly related to serum concentrations of L and Z (combined) in elderly women (32). We believe reporting serum concentrations of L and Z (combined) could confound any potential association between serum concentrations of either of these carotenoids (in isolation) and potential correlates and determinants. Our finding that participants with a reported family history of AMD exhibited significantly higher concentrations of L is important, and may reflect carotenoid supplement use in those with a family history of this condition (63). Of note, the ROI is a small country with a population of only 4.6 million (64) with the prevalence of AMD in the population 50 years and older estimated at 7.2% (9). These findings may reflect food supplement use, which is typically more common in the older population, and given that food supplements typically contain high amounts of L (and little or no Z) in their formulations (65). Of note, following extensive media coverage, awareness of AMD in ROI is high, leading to possibly increased supplement use (and consequent higher plasma L) among these 185 participants with family history of AMD. The same explanation may apply to the two older groups (i.e. participants ≥75 years of age having higher plasma L concentrations than those aged 65-74); however, when we controlled for food supplement use in this study, older participants still had significantly higher plasma L concentrations. Our findings are consistent with those of Olmedilla-Alonoso et al., who reported higher serum L concentrations in older adults compared to younger adults, and no association between plasma Z and increasing age (66). Interestingly, O'Connell et al. found that increasing age was associated with reduced dietary intake of Z, but not with reduced dietary intake of L (67).

Also, and consistent with other studies, we found that plasma concentrations of L and Z were higher in female participants when compared to male participants (31, 34, 68), and higher in participants reporting more physical activities (32), which may be explained, at least in part, by better dietary habits in female participants (35, 69) and those performing physical exercise (34, 70). Other correlates of plasma L and Z concentrations in our study include MP and education levels, each of which was significantly and positively related to plasma concentrations of these carotenoids, findings that are consistent with previous reports (32, 71-73). For example, it has been previously shown that plasma L and Z concentrations are important determinants of MP, which is unsurprising given that retinal capture of circulating carotenoids is required to accumulate these nutrients at the macula. Our finding that plasma concentrations of L and Z are related to education levels are consistent with those of Rock et al. and the EUREYE study, which reported that higher serum concentrations of L and Z were associated with third level education (30, 34). Indeed, our findings that education level is a determinant of plasma concentrations of L and Z is also consistent with our previous report from this sample (see Nolan et al. (42)), which found that the level of education was positively and significantly related to MP. Lack of education is associated with many negative correlates (and possibly determinants) of plasma concentration of L and Z including obesity, low physical exercise, tobacco use, and poor diet (74, 75).

The main strengths of this study can be summarised as follows: it is a large (n=3681) randomly selected, racially homogeneous sample (99% white and of Irish birth). Furthermore, standardized methods (including blood processing and storage) and use of a single laboratory to carry out the carotenoid analysis minimized variability. The main limitations of the TILDA study were an underrepresentation of the age group of 75 years and older and underrepresentation of participants who attended primary level education compared with the overall population of ROI (76). Also, dietary data and additional information on food supplement use (ingredients for multivitamins, dose and dose regimen) would have allowed for more detailed analysis with respect to correlates (and putative determinants) of plasma L and Z concentrations in the Irish population.

Conclusion

We report on the demographic, lifestyle and health status of 3,681 mainly white Irish adults over the age of 50 years, in order to investigate correlates and identify potential determinants of plasma concentrations of L and Z. The findings of this large study indicate that plasma concentrations of L and Z are lower in association with indicators of a poor lifestyle (high BMI, tobacco use, and less physical exercise) and lower education, indicating that modifying lifestyle in a positive way is likely to be reflected in higher concentrations of plasma carotenoids with consequential health benefits.

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Conflict of interests: S. Beatty and J.M. Nolan are Directors of Nutrasight Consultancy Ltd, where they do consultancy work for companies with an interest in supplements for eye care. All other authors report no potential conflict of interest.

Ethical Standards: The authors declare that the study procedures comply with the

current ethical standards for investigation involving human participants in the Republic of Ireland. This study was approved by the Faculty of Health Sciences Research Ethics Committee of Trinity College Dublin and the local Institute committee at the Waterford Institute of Technology.

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