Characterization of the interindividual variability of lutein and zeaxanthin concentrations in the adipose tissue of healthy male adults and identification of combinations of genetic variants associated with it †

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Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/d4fo03087g

Characterization of the interindividual variability of lutein and zeaxanthin concentrations in the adipose tissue of healthy male adults and identification of combinations of genetic variants associated with it†

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Lutein (L) and zeaxanthin (Z) are involved in visual function and could prevent age-related macular degeneration and chronic diseases and improve cognitive performances. Adipose tissue is the main storage site for these xanthophylls (Xanth). The factors affecting their concentrations in this tissue remain poorly understood but in animal models, genetic variations in apolipoprotein E and β-carotene oxygenase 2 have been associated with adipose tissue L concentration. Therefore, the aims of this study were to better characterize the interindividual variability of adipose tissue Xanth concentration and to identify single nucleotide polymorphisms (SNPs) associated with it. Periumbilical subcutaneous adipose tissue samples were collected on 6 occasions in 42 healthy adult males and L and Z concentrations were measured by HPLC. Participants had their whole genome genotyped and the associations of 3589 SNPs in 49 candidate genes with the concentrations of L and Z were measured. Mean L and Z concentrations were 281 ± 27 and 150 ± 14 nmol g^{-1} proteins, respectively. There was no significant correlation between plasma and adipose tissue Xanth concentrations, although the correlation for L approached significance (Pearson's r = 0.276, p = 0.077). Following univariate filtering, 109 and 97 SNPs were then entered into a partial least squares regression analysis to identify the combination of SNPs that explained best adipose tissue concentration of L and Z, respectively. A combination of 7 SNPs in *ELOVL5*, *PPARG*, *ISX* and *ABCA1*, explained 58% of the variability in adipose tissue L concentration while 11 SNPs located in or near *PPARG*, *ABCA1*, *ELOVL5*, *CXCL8*, *IRS1*, *ISX*, *MC4R* explained 53% of the variance in adipose tissue Z concentration. This suggests that some genetic variations influence the concentrations of these Xanth in adipose tissue and could therefore indirectly influence the health effects of these compounds. Clinical Trial Registry: https://ClinicalTrials.gov registration number NCT02100774.

Received 28th June 2024,
Accepted 10th September 2024

DOI: 10.1039/d4fo03087g

rsc.li/food-function

Statement of significance

This is the first study to characterize the interindividual variability of lutein and zeaxanthin concentrations in adipose tissue in healthy humans and to show that genetic variants are associated with these concentrations. This could help explain why the health effects of these compounds vary between individuals.

Introduction

Lutein (L) and zeaxanthin (Z) are 2 carotenoids belonging to the xanthophyll (Xanth) subfamily, *i.e.* oxygenated carotenoids (Fig. 1). They are present in many fruits and vegetables.[1–4] Some like kale, parsley and broccoli being particularly rich in L and others like orange, pepper and corn being rich in Z. The interest in these phytochemicals comes in particular from the fact that they are the main constituents of the macular pigment.[5–7] Numerous studies suggest that they play a role in

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†Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d4fo03087g

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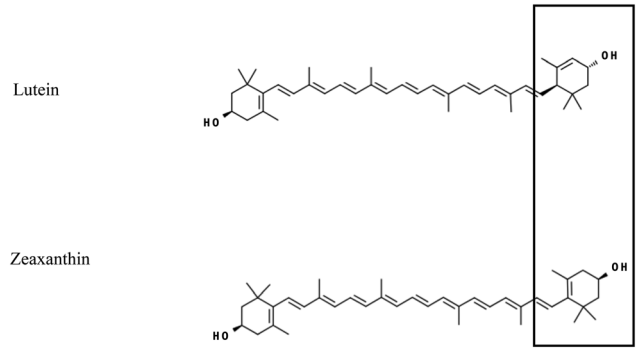

Fig. 1 Lutein and zeaxanthin chemical structures. The sole difference between lutein and zeaxanthin is the location of a single double bond in one of the end rings (enclosed in rectangle). (3*R*,3′*R*,6*R*)-4,5-Didehydro-5,6-dihydro-β,β-carotene-3,3′-diol, also called (all-*trans*-lutein), is the main naturally occurring stereoisomer of lutein. The configurations at zeaxanthin 2 chiral centers gives rise to 3 stereoisomeric forms: (3*R*,3′*R*), (3*R*,3′*S*), and (3*S*,3′*S*). The primary natural form of zeaxanthin is the (3*R*,3′*R*)-β,β-carotene-3,3′-diol, also called all-*trans*-zeaxanthin.

visual function[8] and that they can prevent the progression of late age-related macular degeneration,[9,10] which is the main cause of vision loss in the elderly. This is probably due to the ability of these Xanth to quench blue light[11] and their ability to neutralize light-induced free radicals. Interest in these compounds increased further when several studies, including randomized control trials, suggested that these compounds can improve our cognitive performance.[8,12,13] Finally, studies carried out in rodents have recently suggested that they could be involved in obesity[14,15] and adipocyte inflammation.[16] Mechanisms have been proposed, *e.g.* activation of β3-adrenergic receptor by Z or regulation of the NF-κB signaling pathway by L, as well as interactions with the nuclear hormone receptors RXR and PPARs, and the interested reader is referred to these publications.[17–22] It should be remembered, however, that the factors that modulate obesity and inflammation of adipocytes are very numerous and that the beneficial effect of a diet rich in fruits and vegetables on these parameters, for example fewer calories and saturated fats and an increased intake of many bioactive phytochemical compounds, is obviously not explained solely by the supposed effects on genetic modulation of these two xanthophylls.

Given the potential beneficial role of L and Z in the eye, the brain and the adipose tissue, several studies have been devoted to better understand their metabolism in humans. We thus have data on their fate in the lumen of the human digestive tract,[23,24] on their absorption mechanism[25] and on their transport in our body.[26] The liver and adipose tissue have been identified as their main storage sites[27,28] and using reported mean Xanth concentrations in adipose tissue[29] and liver[30] in healthy individuals, we estimated that the absolute proportions of Xanth in adipose tissue and liver are of approximately 99.8% (456.3 pmol mg^{-1}) and 0.2% (1.1 pmol mg^{-1}), respectively. Other works have later clarified the preferential localization of these compounds in the different deposition sites of adipose tissue[29,31] and they are preferentially located in the abdominal subcutaneous adipose tissue.[32]

Concentrations of Xanth in human adipose tissue apparently vary greatly between individuals.[27] This variability is assumed to be due to several factors, *e.g.* variability in their consumption, in their bioavailability in different food matrices, in their metabolism between individuals.[33] Since humans do not synthesize them, their dietary intake is regarded as the main factor and not consuming them would lead to not having them in our body as has been demonstrated on a primate model.[7] Furthermore, the bioavailability of these compounds also displays a high interindividual variability.[34] It has been shown to be modulated by several factors,[26,35] including genetic variations.[34] These genetic variations are located in genes involved in the intestinal absorption and postprandial plasma metabolism of these compounds. Knowing that the accumulation of these compounds in adipose tissue is not dependent on their physicochemical properties (*e.g.*, hydrophobicity, molar volume, surface tension, density, *etc.*),[36] knowing that it has been shown that proteins, and therefore genes, are involved in their metabolism in adipose tissue,[37,38] and finally knowing that genetic variants in *APOE*[39] and in *BCO2*[40] have been associated with the concentration of L in the adipose tissue of mice (with the ancestral *Apoe4* allele leading to lower L accumulation) and rabbits (where the *BCO2* del/del genotype results in higher L concentrations compared to the ins/ins and ins/del genotypes), respectively, it is reasonable to hypothesize that genetic variations can also modulate their concentration in human adipose tissue.

The aims were therefore firstly to characterize the interindividual variability of L and Z concentration in adipose tissue of healthy male adults, *i.e.* to precisely determine their concentrations (including interindividual variability), and secondly to determine whether genetic variations are associated with it. Such associations would indeed suggest that the associated genes can modulate these concentrations and would allow us to better understand the role of genetics on the concentrations of these compounds in their main storage site. The identification of genotypes that modulate these concentrations could also provide a better understanding of their effects on health.

Methods

Participants and study design

This study was a secondary analysis of a randomized crossover trial that was designed to identify genetic polymorphisms associated with the bioavailability of several fat-soluble vitamins and carotenoids.[34,41–43] Since no data linking genetic variations and L and Z concentration in adipose tissue has been published, it was not possible to carry out a power calculation to determine the number of participants required to demonstrate the effect of a genetic polymorphism on L or Z concentration in adipose tissue. Therefore, this study should be considered exploratory but its results will nonetheless provide a basis for future power calculations. The participants

were non-overweight, non-obese (BMI <25 kg m^{-2}) and non-smoking healthy male adults. Their baseline characteristics are shown in Table 1. Participants presented normal energy consumption, *i.e.* ≈2500 kcal d^{-1}, and drank ≤2% alcohol as total energy. Participants were provided with 3 different test meals taken in a random order and separated by a washout period of at least 3 weeks (see Fig. 2. Study design). The control test meal was composed of 70 g of semolina cooked in 200 mL of water, 40 g of white bread, 60 g of hard-boiled egg whites, 50 g of peanut oil and 330 mL of mineral water. The second test meal, which was intended to evaluate the bioavailability of lycopene,[43] was composed of the same ingredients as the control meal, to which 100 g of tomato puree was added. The third test meal, which was intended to evaluate the bioavailability of vitamin E,[41] was composed of the same ingredients as the control meal, to which a capsule providing 67 mg of α-tocopherol was added. Participants were provided a list of foods to avoid, *i.e.* those rich in the studied micronutrients and carotenoids, 48 h before each test meal. A day prior to each test meal, participants were asked to eat dinner between 7 and 8 p.m., without any alcohol intake. They were also asked to abstain from consuming any food or beverage other than water after the dinner and until they came to the Center for Clinical Investigation (la Conception Hospital, Marseille, France). On the day of the clinical trial, participants were instructed to consume the meal at a relatively uniform pace. Participants were advised to consume half of the meal within the initial 10 min and the remaining half in the next 10 min. After every meal consumption, they were asked to refrain from consuming any other food for the next 8 h (except the remaining water provided with the meal). The study was approved by the regional committee on human experimentation (no. 2008-A01354-51, Comité de Protection des Personnes Sud Méditerranée I, Marseille, France). Procedures followed were in accordance with the Declaration of Helsinki of 1975 as revised in 1983. Informed written consent was obtained from each participant.

Table 1 Baseline characteristics of the study participants (n = 42)

Characteristic	Mean (SEM)
Age, years	31.6 (1.9)
Weight, kg	73.2 (1.2)
BMI, kg m^{-2}	22.9 (0.3)
Fasting plasma concentration	
Total cholesterol, g L^{-1}	1.64 (0.06)
LDL-C, g L^{-1}	1.05 (0.05)
HDL-C, g L^{-1}	0.46 (0.02)
Triglycerides, g L^{-1}	0.80 (0.06)
Glucose, mmol L^{-1}	4.7 (0.1)
Hemoglobin, g dL^{-1}	15.0 (0.1)
Lutein, nmol L^{-1} [a]	307.1 (17.2)
Zeaxanthin, nmol L^{-1} [a]	45.0 (2.7)
Adipose tissue concentration	
Lutein, nmol g^{-1} proteins[b]	301.0 (35.1)
Zeaxanthin, nmol g^{-1} proteins[b]	164.1 (19.8)

[a] Mean of 3 samples collected at baseline for each test meal, at 3 weeks intervals. [b] Quantified in adipose tissue samples collected at fast and 8 h after test meal intake.

Plasma samples

To obtain a more precise measurement of fasting plasma L and Z concentrations, we measured L and Z concentrations in fasting plasma samples that were collected from each subject before each test meal. This allowed us to have 3 values for each subject and the concentrations of L and Z assigned

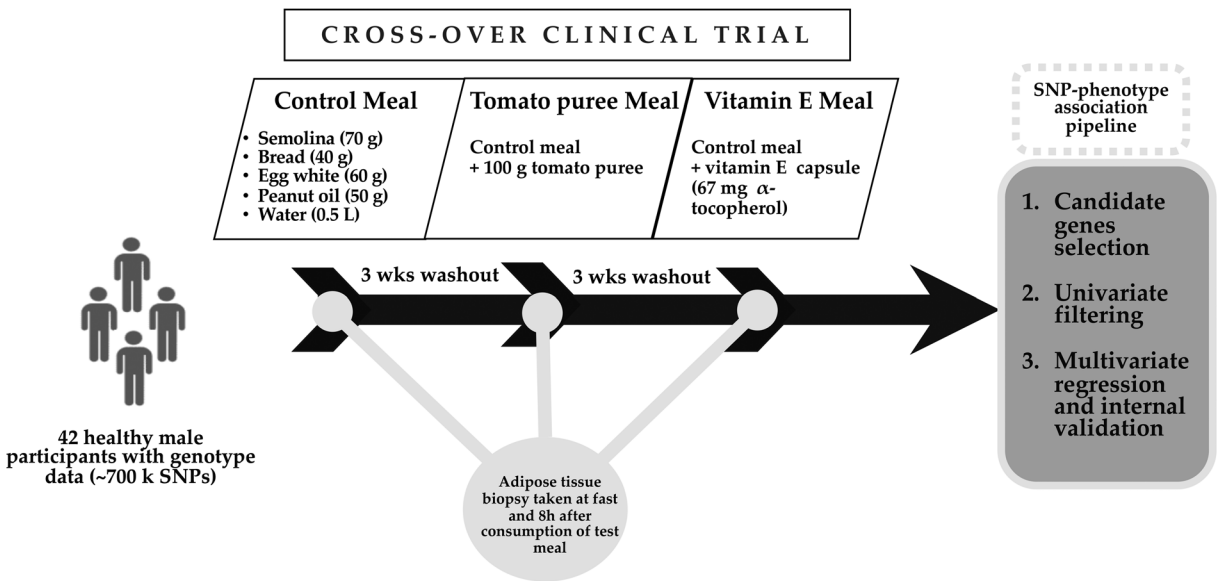

Fig. 2 Study design. Forty-two healthy male adults received, in a randomized crossover trial, 3 test meals consumed at least 3 weeks apart. Periumbilical adipose tissue samples were collected on 6 occasions, *i.e.* at fast and 8 h following the consumption of each test meal. L and Z concentrations were measured in the adipose tissue (following saponification). Participants were genotyped using whole-genome microarray.

to each subject were the averages of these different measurements.

Measurement of basic biochemical parameters

Baseline fasting plasma concentrations of total cholesterol, LDL-C, HDL-C triglyceride, glucose, and hemoglobin were measured using standard methods as previously described.[44] All analyte concentrations were measured on a Modular PP instrument (Roche Diagnostics, Meylan, France), except for hemoglobin which was measured on an ADVIA 120 instrument (Siemens Healthcare Diagnostics, Saint-Denis, France), at La Conception Hospital (Biochemistry Laboratory, Marseille).

Collection of adipose tissue samples

The location for sampling the white adipose tissue, *i.e.* subcutaneously in the periumbilical region, was chosen because it is the preferred storage location for L,[29,32] and probably for Z. Samples were taken at fast and 8 h after eating the test meals because we wanted to know if the concentration of some micronutrients in the white adipose tissue could significantly vary after the ingestion of a large quantity of these micronutrients. Each participant was therefore sampled 6 times (3 test meals × 2 sampling times), at different places around the navel. The collected adipose tissue samples were immediately placed into an ice-water bath and covered with aluminum foil. At arrival, a first adipose tissue biopsy was collected by a trained physician, according to the following protocol: after local anesthesia with 1 cm^3 non-adrenalized xylocaine (2%), approximately 100–200 mg of subcutaneous adipose tissue was aspirated from the periumbilical region. After 8 h, a second adipose tissue biopsy was taken from the periumbilical region, following the same procedure as that described above.

Adipose tissue Xanth were first extracted as follows: around 50 mg (fresh weight) adipose tissue was homogenized in 300 μL of phosphate buffered saline with 2 3 mm diameter stainless steel balls using an MM301 ball mill (Retsch, Eragny-sur-Oise, France). Then, a 50 μL aliquot was collected for protein quantification by the BiCinchoninic acid Assay kit (Pierce, Montluçon, France) after dilution to 1/5 in phosphate buffered saline buffer. Lipids, including Xanth, were extracted from the remaining 250 μL using 1.5 mL chloroform/methanol (0.5/1, v/v) and 0.2 mL phosphate buffered saline. All extractions were performed at room temperature under yellow light to minimize light-induced damage. The dried extract was incubated at 37 °C for 1 h 30 minutes with 100 μL of an ethanolic pyrogallol solution (12%, w/v) and 1 mL of an ethanolic potassium hydroxide solution (5.5%, w/v) to saponify triglycerides and potential xanth esters and to accurately quantify total L and Z. After incubation, the sample was cooled to room temperature and apo 8′-carotenal was added as an internal standard. The mixture was extracted twice by using 3 mL of hexane. The extract was evaporated to dryness under nitrogen and then resolubilized in 100 μL methanol/dichloromethane (65/35, v/v) for downstream HPLC analysis.

Xanth quantification in plasma and adipose tissue samples

Plasma Xanth were extracted as previously described.[45] About 90 μL of resolubilized samples of either plasma or adipose tissue were injected for HPLC analysis. Separation was achieved using a 10 mm × 4.0 mm Modulo-Cart guard column, with 2 μm particle size, (Interchim, Montluçon, France) followed by a 250 mm × 4.6 mm, 5 μm particle size YMC C30 column (Interchim) held at 35 °C. The mobile phase was composed of HPLC-grade methanol (A), methyl *tert*-butyl ether (B), and water (C) (Carlo Erba–SDS). A linear gradient from 96% A, 2% B, and 2% C at t = 0 to 18% A, 80% B, and 2% C at t = 27 min at a flow rate of 1 mL min^{-1} was used. The HPLC system consisted of a 2690 HPLC Pump associated with a photodiode array detector (Waters 2996) (Waters). L and Z were identified *via* UV spectra and retention time coincident with authentic standard (kindly provided by the DSM SA company, Lausanne, Switzerland) and quantitated at 445 nm and 452 nm, respectively. Peak integration and quantification were performed using Chromeleon CDS software (version 6.80, Dionex, Villebon sur Yvette, France), using external calibration curves and corrected on the basis of recovery of the internal standard.

Saliva DNA extraction and genotyping

Genomic DNA was extracted from saliva samples using the Oragene kit (DNA Genotek, Ottawa, Ontario, Canada). About 200 ng of DNA was hybridized overnight to HumanOmniExpress BeadChips (Illumina, San Diego, California, USA). Unhybridized and nonspecifically hybridized DNA were washed out. BeadChips, a type of microarray developed by Illumina, were then stained and scanned on an Illumina iScan scanner (Illumina, San Diego, California, USA) as previously described.[34] This process allowed for the genotyping of approximately 7.13 × 10^5 SNPs/DNA sample across the genome, with mean call frequency and reproducibility rate >99% and minimal log R deviation ranging from 0.11 to 0.30.

Candidate SNP selection

Following a literature search, a total of 49 candidate genes were selected (ESI Table S1 and ESI Fig. S1†). These were selected because their encoded proteins have been previously suggested to influence Xanth concentration in adipose tissue, whether directly, by being involved in adipocyte Xanth uptake and lipid droplet distribution, or indirectly, by influencing blood Xanth concentration. Of the corresponding 3589 SNPs on the DNA chips, we first excluded SNPs with a low, *i.e.* <95%, call rate and SNPs that presented a significant departure from the Hardy–Weinberg equilibrium (p < 0.05; chi-squared test) (536 SNPs excluded), thus leaving 3053 SNPs. Then, for each candidate gene, we excluded SNPs in linkage disequilibrium (LD, R^2 > 0.80) and kept the tag SNPs as identified by LD TAG SNP Selection tool from the SNPinfo Web Server (HapMap, European (CEU) population, accessible at **https://snpinfo.niehs.nih.gov**) (919 SNPs excluded). Further analysis was conducted for SNPs that were not present in the SNPinfo

Web Server database. When 2 SNPs were perfectly correlated (R^2 = 1.0), one was randomly kept for further analysis, resulting in the exclusion of 153 SNPs. The remaining 1981 SNPs were tested under both additive and dominant models. SNPs with fewer than 5 observations in each genotype group were excluded from downstream statistical analysis, leaving 537 and 1702 SNPs in the additive and dominant models, respectively.

Statistical analysis

Data were expressed as mean ± SEM. A linear mixed model (LMM) analysis was conducted to evaluate the effects of the 3 distinct test meals (fixed variables) on adipose tissue Xanth concentration, with time considered as a repeated measure and individual participant as random variables. These variables were subjected to 5 covariance structures: unstructured covariance, scaled identity, diagonal, compound symmetry, and autoregressive order one. The goodness of fit of the covariance structures was compared using Akaike's Information Criterion. Moreover, paired samples *t*-tests were performed to analyze the effect of individual test meal on adipose tissue L and Z concentration at 2 time points. Departure from normality was assessed by inspecting Q–Q plots of the standardized residuals while departure from homoscedasticity was assessed using scatterplots of residuals. Two-tailed Grubbs' test (accessible in **https://www.graphpad.com/quickcals/grubbs1**) was used to detect outliers among the technical replicates. The relationship between adipose tissue Xanth concentrations and other covariates (*i.e.* age, BMI, plasma lipid concentrations) was analyzed using Pearson's *r*, calculated with 95% confidence intervals (CI). Two-tailed *t*-test for independent samples was used to compare L and Z concentration ratio in adipose and fasting plasma. CV of plasma and adipose tissue Xanth concentrations were compared according to Forkman.[46] For all tests, the bilateral alpha risk was α = 0.05. Statistical analyses were performed using SPSS 28 (SPSS Inc., Chicago, IL, USA).

In order to determine which combination of SNPs best explained the variability in each Xanth, a 2-step approach was followed, combining (1) dimension reduction by univariate filtering followed by partial least squares (PLS) regression, as has been previously applied.[42,47,48] The step of univariate filtering consisted of selecting SNPs that showed a *p*-value <0.05 (Wald test asymptotic *p*-value) following analysis with PLINK (v1.07, **https://pngu.mgh.harvard.edu/purcell/plink/**). This univariate selection also considered other variables (*i.e.* fasting carotenoid concentration, fasting cholesterol, *etc.*) with a non-zero correlation value (Pearson's *r*) with respect to the Xanth concentration. A PLS regression model including all thus selected variables coded in units of variance was then built. Variables were ranked according to their variable importance in the projection (VIP) value, which estimates the contribution of each variable in the projection used in the PLS regression model. Several PLS regression models were then generated using different VIP threshold values as described in detail elsewhere.[44,49,50] The model that had the highest adjusted R^2 (eqn (1)) value with a significant *p*-value after cross-validation ANOVA,[51] was selected.

$$\text{Adjusted } R^2 = 1 - \frac{(1 - R^2)(n - 1)}{n - k - 1} \quad (1)$$

With n the sample size and k the number of variables in the model (excluding the constant). The selected models were internally validated through several methods. This included leave-*k*-out cross validation,[52] regression coefficient stability testing,[34] R^2 and adjusted R^2 after subjecting the Y variable, *i.e.* adipose tissue Xanth concentration, to 100 random permutations[53] (see ESI†). SIMCA® Multivariate Data Analytics Solution software (version 17.0.0.24543, Umetrics, Umeå, Sweden) was used for all multivariate data analyses, robustness and stability tests.

Retrospective multivariate power analysis calculations

A retrospective sample size power calculation was conducted using the online tool MetaboAnalyst 6.0 (accessible at **https://new.metaboanalyst.ca**). By utilizing the Power Analysis feature of MetaboAnalyst 6.0, we determined the minimum sample size required to achieve statistical significance for a dataset consisting of SNPs identified through PLS regression analysis (Table 3 – lutein; Table 4 – zeaxanthin). To facilitate this analysis, the sample was divided into 2 groups based on the median adipose tissue Xanth concentration. The evaluation indicated that a sample size of at least 15 participants per group was needed to achieve a statistical power of more than 80% at a false discovery rate (FDR) adjusted *p*-value of 0.001 (ESI Fig. S4A and B†). This suggests that the sample size used in this study was adequate.

Results

Characterization of adipose tissue Xanth concentrations

The concentrations of the 2 Xanth at fast were not significantly different when samples were taken on days of different test meals (L: p = 0.342; Z: p = 0.604). The mean concentrations were also not significantly different 8 h after the test meals *vs.* at fast before consumption of the test meals (L: p = 0.494; Z: p = 0.458) (also verified by paired *t*-tests; see ESI Table S3B†). Therefore, to decrease sampling variability and obtain a more accurate estimate of adipose tissue L and Z concentrations, we decided, in the following analyses, to use for each participant the mean of the 6 concentrations measured (Fig. 3A and B). Indeed, we assume that these means were in fact more representative of the real concentrations of the Xanth in adipose tissue than a single value which can be more affected by the variability of the measurement. Consequently, the mean concentration of L was 280.7 ± 26.9 nmol g^{-1} proteins, that of Z was 150.1 ± 14.3 nmol g^{-1} proteins. Interestingly, mean values of adipose tissue L and Z concentrations were correlated (Pearson's r = 0.896 [95% CI: 0.8115, 0.9415], $p = 4.76 \times 10^{-16}$).

Regarding interindividual variability, the coefficients of variation of L and Z were close, *i.e.* 62.2 and 61.6% for L and Z, respectively. These were significantly higher than both the interindividual variability values of fasting plasma L and Z, which had a CV of 34.3 and 33.1%, respectively (L: p = 0.004; Z: p = 0.002).

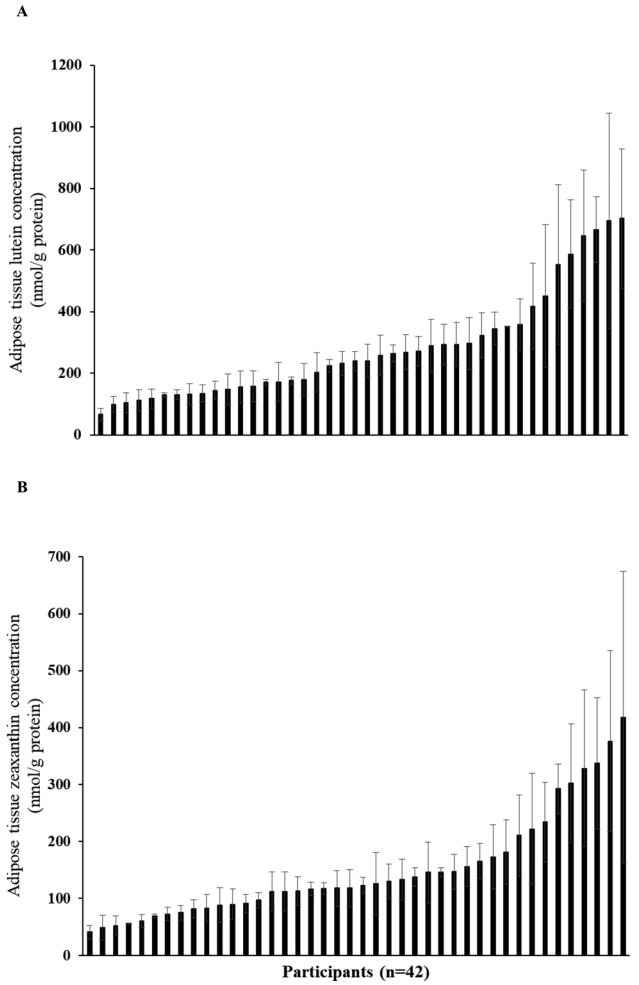

Fig. 3 Adipose tissue L and Z concentrations. Adipose tissue concentrations (nmol g^{-1} protein) of L (A) and Z (B) of each participant were the arithmetic mean of the concentrations measured in periumbilical adipose tissue samples taken at fast and 8 h after consumption of 3 test meals (usually 6 values for the participants who ingested the 3 test meals). Values are means with their SEM. Participants (n = 42) were sorted by increasing adipose tissue L and Z concentrations.

Correlations between adipose tissue Xanth concentrations and characteristics of the participants

Since these compounds are taken up by the adipose tissue through the blood circulation, we first calculated the correlation between their concentrations in the plasma and in the adipose tissue. We did not find a significant correlation even if that of L was close to significance (L: Pearson's r = 0.276 [95% CI: −0.0340, 0.5325], p = 0.077; Z: Pearson's r = 0.203 [95% CI: −0.1322, 0.4924], p = 0.228). Concerning other parameters measured in the participants at baseline, we found significant correlations with fasting total cholesterol (L: Pearson's r = 0.315 [95% CI: 0.0086, 0.5624], p = 0.042; Z: Pearson's r = 0.383 [95% CI: 0.0850, 0.6125], p = 0.012), and fasting LDL-C, only for L (Z: Pearson's r = 0.379 [95% CI: 0.0528, 0.6262], p = 0.022) (Table 2).

Genetic variants associated with the interindividual variability of adipose tissue Xanth concentrations

The list of associated SNPs which passed univariate filtering (p < 0.05) is presented in ESI Table S2† (in separate .xls file). With these SNPs, a PLS regression model was generated for each Xanth to find the combination of SNPs and covariates (among participant characteristics) that best explained the interindividual variability of their concentration in the adipose tissue.[34,41–44,54] As shown in ESI Table 3A,† the models including all selected variables (1st in the lists) could explain a high part of the variance in the phenotypes (indicated by the R^2) but this estimation was positively biased, as illustrated by low adjusted R^2 values. This is due to the high number of predictors included in the model. Therefore, to improve the model and find an association of SNPs more predictive of the adipose tissue Xanth concentrations, we filtered out SNPs and covariates that made no important contribution (*i.e.*, those that displayed the lowest VIP value). After the application of several thresholds of the VIP value, we showed that the best models obtained are highlighted in bold in ESI Table S4.† The PLS regression model for L included 7 SNPs in or near 4 genes and it explained 58% (adjusted R^2) of the variability in adipose tissue L concentration (Table 3). For Z, the best model included 13 SNPs, of which 11 were not in LD (ESI Table S5†). The 11 SNPs were located in or near 7 genes (Table 4) and

Table 2 Pearson's correlation coefficients between adipose tissue lutein (L) and zeaxanthin (Z) concentrations and selected anthropometric measurements and blood lipid concentrations

	Adipose tissue L Concentration			Adipose TISSUE Z concentration		
	Pearson's r	95% CI	p-Value	Pearson's r	95% CI	p-Value
Age, years	0.15	−0.16, 0.44	0.33	0.27	−0.04, 0.53	0.09
BMI, kg m^{-2}	0.10	−0.21, 0.39	0.54	0.09	−0.22, 0.38	0.56
Fasting lipid concentration, g L^{-1}						
Total cholesterol	0.32	0.01, 0.56	0.04	0.38	0.08, 0.61	0.01
LDL-C	0.32	−0.02, 0.58	0.06	0.38	0.05, 0.63	0.02
HDL-C	0.14	−0.20, 0.44	0.44	0.12	−0.21, 0.43	0.48
Triglycerides	0.03	−0.27, 0.33	0.83	0.17	−0.14, 0.45	0.29
Concentration in fasting plasma L and Z, μmol L^{-1} [a]	0.28	−0.03, 0.53	0.08	0.20	−0.13, 0.49	0.23

[a] Means of 3 samples collected in fasted state at 3 weeks intervals.

Table 3 Combination of SNPs associated with adipose tissue L concentration following partial least squares regression

Gene[a]	SNP	VIP value[b]	Regression coefficient[c]
ELOVL5	rs2817114	1.455	87.0
PPARG	rs709158	1.336	68.1
ISX	rs5755468	1.305	−68.4
PPARG	rs1152004	1.282	66.5
ABCA1	rs1561166	1.275	100.3
ABCA1	rs3904998	1.263	66.2
ISX	rs2413241	1.257	64.3

[a] Gene names can be found in ESI Table S1.† [b] Variables were ranked according to their variable importance in the projection (VIP) value, which estimates the contribution of each SNP in the projection used in the PLS regression model. [c] Regression coefficients are for untransformed variables and represent the mean change in adipose tissue L concentrations (nmol g^{-1} protein) for each additional copy of the minor allele under the additive model and in the presence of the minor allele under the dominant model. Abbr: ABCA1 – ATP binding cassette subfamily A member 1; ELOVL5 – ELOVL fatty acid elongase 5; ISX – Intestine specific homeobox; L – lutein; PPARG – Peroxisome proliferator activated receptor gamma; SNP – single nucleotide polymorphism.

Table 4 Combination of SNPs associated with adipose tissue Z concentration following partial least squares regression

Gene[a]	SNP	VIP value[b]	Regression coefficient[c]
PPARG	rs709158	1.364	19.4
ABCA1	rs3904998	1.283	27.3
ABCA1	rs4149275	1.282	29.3
PPARG	rs1152004	1.252	26.4
ELOVL5	rs2817114	1.241	30.1
ABCA1	rs1561166	1.182	37.7
CXCL8	rs4694627	1.172	−16.4
IRS1	rs16866816	1.130	33.4
ISX	rs2413241	1.125	23.4
ELOVL5	rs2493164	1.121	23.2
MC4R	rs489310	1.117	35.7

[a] Gene names can be found in ESI Table S1.† [b] Variables were ranked according to their variable importance in the projection (VIP) value, which estimates the contribution of each SNP in the projection used in the PLS regression model. [c] Regression coefficients are for untransformed variables and represent the mean change in adipose tissue Z concentrations (nmol g^{-1} protein) for each additional copy of the minor allele under the additive model and in the presence of the minor allele under the dominant model. Abbr: ABCA1 – ATP binding cassette subfamily A member 1; CXCL8 – Chemokine (C–X–C motif) ligand 8; ELOVL5 – ELOVL fatty acid elongase 5; IRS1 – Insulin receptor substrate 1; ISX – Intestine specific homeobox; MC4R – Melanocortin-4 receptor; PPARG – Peroxisome proliferator activated receptor gamma; SNP – single nucleotide polymorphism; Z – zeaxanthin.

explained 53% of the variance (adjusted R^2). The results of internal validation tests are presented in ESI Table S6 and ESI Fig. S2 and S3† (*i.e.* leave-*k*-out cross-validation, regression coefficient stability test, R^2 and adjusted R^2 after 100 random permutations).

Genetic score to explain adipose tissue Xanth concentrations

With the knowledge of a participant's genotype at the SNPs in the selected model, it was possible to calculate a participant's adipose tissue Xanth concentrations (in nmol g^{-1} proteins) using the following equations:

$$\text{Adipose tissue L concentration} = 175.1 + \sum_{i=1}^{7}(r_i) \times \text{number of minor allele SNP}_i \quad (2)$$

$$\text{Adipose tissue Z concentration} = 76.4 + \sum_{i=1}^{11}(r_i) \times \text{number of minor allele SNP}_i \quad (3)$$

with r_i the unstandardized regression coefficient of the i^{th} SNP in the PLS regression model (provided in Tables 3 and 4). When SNPs were entered under the dominant model, participants homozygous for the lesser frequent allele were grouped with heterozygous participants and the number of minor alleles for both these groups was considered to be 1.

Discussion

The first objective of this study was to characterize the concentration of these two Xanth in human adipose tissue. We chose to collect subcutaneous adipose tissue from the abdominal region because it is apparently the preferential site for Xanth storage.[32] In addition, to obtain a more precise estimate of their concentrations in this tissue, we calculated the average of the up to 6 concentrations of L and Z obtained in each participant, which is an improvement from previous studies in which only one measurement in the abdominal area was carried out in each participant.[29]

The data obtained first showed that the ratio of the concentrations of L to Z in this tissue was around 1.9 ± 0.07. This was approximately 4 times significantly lower than the one observed in plasma of this group of participants, *i.e.* 7.1 ± 0.41. This means that the proportion of Z in this tissue was significantly higher than that found in the plasma. Several hypotheses can be proposed, either Z is better captured by this tissue than L, or L is more metabolized in this tissue than Z, or L is more secreted by this tissue than Z. Whatever the mechanism(s), the fact that the ratio between these two Xanth is similar to the ratio found in retina,[5] *i.e.* around 4, suggests that either Z is preferentially bioaccumulated than L in eye and adipose tissue and/or L is preferentially metabolized in another compound than Z. This latter hypothesis comes from the observation that L is converted in meso-Z in the primate eye.[7] A second interesting observation is that, despite the differences in concentration of these two molecules in this tissue, their concentrations are closely correlated. This suggests that their bioavailability and transport to this tissue are governed by very similar mechanisms.

Regarding interindividual variability, we observe coefficients of variation of 62.2 and 61.6% for L and Z concentration in adipose tissue, respectively. When we compare these CV to the CV of their concentrations in the fasting plasma, *i.e.* 34.3 and 33.1%, respectively, we observe that they are approximately

1.8 times higher in adipose tissue. This suggests that factors additional to those which influence the concentration of these Xanth in plasma influence their concentration in adipose tissue. This is in support of our starting hypothesis, *i.e.* genetic variations in genes that encode proteins which are involved in the metabolism of these compounds in this tissue can modulate the activity or the expression of these proteins and in turn the concentrations of these compounds in this tissue.

Knowing that correlations between the concentration of L in the adipose tissue and macular pigment density have been previously described,[55,56] we investigated whether the concentrations of the two Xanth in the adipose tissue were correlated with other biochemical measurements or participants' demographics. There was no significant correlation with the fasting plasma concentrations of the Xanth, although this was close to significance for L. We also did not find any significant correlation with BMI, though likely because participants were all having normal weight. On the other hand, we found significant correlations with the fasting plasma concentrations of total and LDL-cholesterol.

The second objective of this study was to search for associations between interindividual variability of Xanth concentration in adipose tissue and genetic variations in candidate genes. To reach this goal, we used a 2-step procedure that we have previously used in several studies.[34,42,43,54,57] Firstly, we identified SNPs in candidate genes significantly associated with these concentrations. Then, these SNPs were entered into PLS regression analyses. This procedure allowed us to identify a combination of 7 SNPs in 4 genes (*ELOVL5*, *PPARG*, *ABCA1* and *ISX*) that explained best adipose tissue L concentration, and a combination of 11 SNPs in 7 genes (*ELOVL5*, *PPARG*, *ABCA1*, *CXCL8*, *ISX*, *IRS1* and *MC4R*) that explained best adipose tissue Z concentration. It is interesting to point out that 6 SNPs in 4 genes (*ELOVL5*, *PPARG*, *ISX* and *ABCA1*) were present in both models. This confirms what was assumed and supported by the high correlation between L and Z concentrations in adipose tissue, namely that the metabolism of these 2 Xanth is relatively similar. Regarding the 3 genes which were not found in both PLS regression models, either they are very specific to each Xanth, or they are less significantly associated with the second Xanth. For instance, the fact that Chemokine (C–X–C motif) ligand 8 (*CXCL8*) was classified in 8th (additive model) position in one of the non-selected PLS models of L supports its potential involvement in the concentration of L in adipose tissue.

Obviously, the associations we report do not prove causality. Nevertheless, based on the known functions of the 4 genes which were associated with the concentration of both Xanth in the adipose tissue, we can make hypotheses on their role in the adipose tissue metabolism, and therefore in the concentration, of these 2 Xanth. Regarding *ELOVL5*, it encodes an enzyme that catalyzes the elongation of eicosapentaenoic acid into docosapentaenoic acid, then into docosahexaenoic acid. A SNP in *ELOVL2*, which shares function with *ELOVL5* within the fatty acid elongation pathway, has been associated with L bioavailability,[34] probably due to the inhibitory effect of eicosapentaenoic acid on carotenoid intestinal absorption.[58] Knowing that chylomicrons are the lipoproteins responsible for the transport of newly absorbed Xanth from the intestine to the liver and that a significant proportion of fatty acids located on their surface are transferred to adipose tissue during their metabolism,[59,60] and knowing that Xanth are preferentially located on the surface of lipoproteins,[61] we can assume that a variation in their concentration in the surface of chylomicrons, due to a variation in their absorption induced by variants of *ELOVL5*, will modulate the quantity of Xanth which will be transferred to adipose tissue and, ultimately, their concentration in this tissue. Concerning *PPARG*, its association with the adipose tissue concentrations of both Xanth could be explained indirectly by its role in regulating the expression of genes involved in lipid metabolism in this tissue. In fact, this regulation has the effect of modulating the absorption of fatty acids by this tissue and their storage in the form of triglycerides. Knowing that Xanth are hydrophobic and solubilized in triglycerides,[62] we can assume that their concentration in this tissue is positively correlated with that of triglycerides and that it is therefore indirectly linked to the activity of PPARG. The third gene associated with the adipose tissue concentrations of both Xanth was *ABCA1*. This association could be due to the fact that this membrane transporter, which is well known to efflux cholesterol,[63,64] is also capable to efflux these 2 Xanth.[34,65] This would not be so surprising as it has been suggested that it is capable to efflux another lipophilic micronutrient, vitamin E, and perhaps also some carotenoids.[65] This association could also be due to the fact that this membrane transporter was associated with the concentration of L in chylomicrons in the postprandial period.[34] *ISX* SNPs were found in PLS regression models for L and for Z. Knowing that this gene is not expressed in this tissue (**https://www.proteinatlas.org**; accessed on June 25, 2024), we can explain its association by the effect of ISX on the bioavailability of L,[34,66] and therefore probably also on that of Z. ISX has been identified as a transcriptional repressor of SR-BI expression.[67] Given that SR-BI is crucial for L uptake,[68] we hypothesize that SNPs in *ISX* could influence its expression or its activity. This, in turn, may impact SR-BI expression and, consequently, the efficiency of Xanth uptake. Indeed, as previously suggested we can assume that the higher the concentration of these Xanth in chylomicrons, and the more these Xanth are transferred to this tissue during vascular metabolism of chylomicrons.

This study has of course limitations (see ref. 69 for a detailed discussion). The main one being that we did not estimate the Xanth intake by a dietary survey even though it is one of the main factors which affects the concentration of Xanth in our body. The results were obtained only in males, mainly Caucasians, and consequently we cannot extrapolate them to females and other population groups yet. We followed a candidate gene approach and it is likely that other genes are involved in these phenotypes. The SNPs analyzed were those on the DNA chips and other SNPs are probably involved. Finally, this is an association study and only functional genetic

studies could confirm the involvement of these SNPs in these phenotypes. On the other hand, this is the first time that the interindividual variability of the concentration of these 2 Xanth in human adipose tissue is characterized, and that genetic variations potentially involved in this variability are identified. The results described herein are very interesting from a pathophysiological point of view. Indeed, the fact that we are not all equal in terms of our capacity to store Xanth in adipose tissue, a major storage site for Xanth, suggests that we do not benefit the same way from their health effect.[14,15] This also suggests that our genetic characteristics could affect our ability to benefit from the effects of these compounds on visual function,[8] cardiometabolic health,[70] cognitive performance[8,12,13] as well as on AMD and cancer prevention.[9,10,21] We therefore hope that these preliminary results will encourage other researchers to investigate the metabolism of these compounds in adipose tissue and to identify the factors influencing their concentration.

Abbreviations

ABCA1	ATP binding cassette subfamily A member 1
AMD	Age-related macular degeneration
CXCL8	Chemokine (C–X–C motif) ligand 8
ELOVL5	ELOVL fatty acid elongase 5
IRS1	Insulin receptor substrate 1
ISX	Intestine specific homeobox
L	Lutein
MC4R	Melanocortin-4 receptor
PLS	Partial least squares
PPARG	Peroxisome proliferator activated receptor gamma
Xanth	Xanthophyll(s)
Z	Zeaxanthin

Author contributions

The authors' responsibilities were as follows. M. P. Z.: choice of candidate genes, statistical analysis, visualization, review & editing. C. D.: methodology, choice of candidate genes, statistical analysis, validation, supervision, writing – review & editing. B. G.: quantification of L and Z in the adipose tissue. M. N.: organization of the clinical study, preparation of test meals, collection and storage of samples. D. O. A.: collection of adipose tissue samples. P. B.: conceptualization, methodology, funding acquisition, resources, project administration, supervision, choice of candidate genes, validation, writing – original draft, review & editing. All authors have read and approved the final manuscript.

Data availability

Data described in the manuscript will not be made available because this was not stated in the ethics application.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This study was supported by the European Community's Sixth Framework Program. The funding was attributed to the Lycocard project (no. 016213), which was an Integrated Project within the framework of the "Food Quality and Safety" program. We would like to thank the Department of Science and Technology-Science Education Institute, Philippines (DOST-SEI) and the Embassy of France to the Philippines and Micronesia for the PhilFrance-DOST Fellowship of M. P. Z.

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