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The Interindividual Variability of Phytofluene Bioavailability is Associated with a Combination of Single Nucleotide Polymorphisms

*Mark Pretzel Zumaraga, Patrick Borel, Romain Bott, Marion Nowicki, Denis Lairon, and Charles Desmarchelier**

Scope: Phytofluene is a colorless carotenoid with potential health benefits that displays a higher bioavailability compared to carotenoids such as lutein, β-carotene or lycopene. Several studies suggest its bioavailability displays an elevated interindividual variability. The aim of this work is to investigate whether a combination of SNPs is associated with this variability.
Methods and Results: Thirty-seven healthy adult males consume a test meal that provides phytofluene from a tomato puree. Phytofluene concentrations are measured at fast and in chylomicrons at regular time intervals after meal intake. Identification of the combination of SNPs that best explained the interindividual variability of the phytofluene response is assessed by partial least squares regression. There is a large interindividual variability in the phytofluene response, with CV = 88%. Phytofluene bioavailability is positively correlated with fasting plasma phytofluene concentration ($r = 0.57$; $p = 2 \times 10^{-4}$). A robust partial least squares regression model comprising 14 SNPs near or within 11 genes (*ABCA1*-rs2487059, rs2515629, and rs4149316, *APOC1*-rs445925, *CD36*-rs3211881, *ELOVL5*-rs6941533, *FABP1*-rs10185660, *FADS3*-rs1000778, *ISX*-rs130461, and rs17748559, *LIPC*-rs17240713, *LPL*-rs7005359, *LYPLAL1*-rs1351472, *SETD7*-rs11936429) explains 51% (adjusted R^2) of the interindividual variability in phytofluene bioavailability.
Conclusions: This study reports a combination of SNPs that is associated with a significant part of the interindividual variability of phytofluene bioavailability in a healthy male adult population.

1. Introduction

Phytofluene (7,8,11,12,7′,8′-hexahydro-ψ,ψ-carotene, PTF) is an outlier within the carotenoid family as, like phytoene (PT), it lacks one of its distinctive characteristics, the visible color. This linear C_{40} carotenoid is formed by the action of PT desaturases, which insert one double bond in the polyene chain, extending the system of conjugated double bonds by two. PTF is known to be a universal precursor in the plant biosynthesis of the colored carotenoids, for example, lycopene, β-carotene and lutein (**Figure 1**).[1–3]

PTF is naturally present in commonly consumed fruits and vegetables such as carrots, tomatoes and tomato-based products, apricots, and orange juice.[1,4,5] PTF is generally not included in food carotenoid databases but its daily per capita intake was estimated between 0.5 and 0.7 mg, depending on the population and dietary habits.[6–8] In a Spanish population, its average daily intake (0.5 mg) was lower than that of lycopene (3.1 mg), PT (1.9 mg), β-carotene

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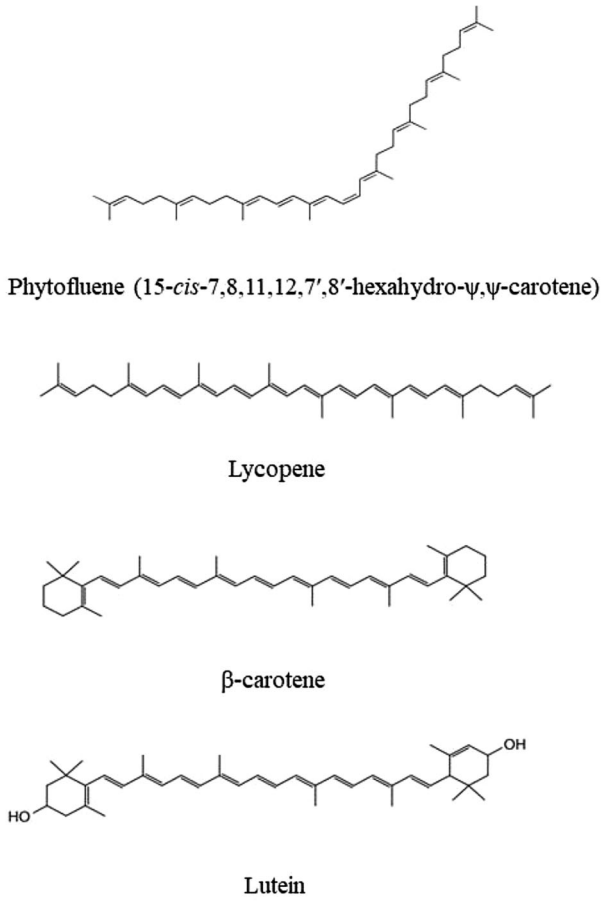

Figure 1. Molecular structure of commonly consumed carotenoids.

(1.5 mg), and lutein (1.2 mg), but higher than that of β-cryptoxanthin (0.3 mg) and α-carotene (0.3 mg).[7] Nonetheless, PTF is found among the predominant carotenoids in human plasma and tissues.[1,4,5,9] This is partly due to its relatively high bioavailability. Indeed, it has been shown in mice to display a higher bioavailability compared to PT, lycopene, and β-carotene.[10] To date, no study in humans has compared the bioavailability of PTF to that of other major carotenoids. PTF elevated bioavailability could participate in its attributed biological actions and health benefits.[3] From a mechanistic point of view, PTF could participate in antioxidant and anti-inflammatory actions[1,11,12] and decrease DNA damage in lymphocytes.[11] Moreover, due to its high UVA absorption activity, PTF might be considered as a promising UVA photoprotective for medical purposes.[1,13]

Similarly to what has been observed for other carotenoids (see refs.[14, 15] for review), PTF bioavailability displays an elevated interindividual variability (**Table 1**). Several proteins are involved, or are suspected of being involved, at different stages in carotenoid bioavailability, as reviewed in.[15,16] This suggests that variations in the genes encoding for these proteins could modulate their expression or activity and could in turn affect carotenoid bioavailability. Following a candidate gene approach, we have previously reported combinations of SNPs associated with the interindividual variability of the bioavailability of lutein,[17] lycopene[18] and β-carotene[19] in a group of healthy male participants. Among the carotenoid-containing meals consumed was a meal containing tomato puree, which also contains PTF. Hence, we here aimed to characterize the interindividual variability of PTF bioavailability in the same group of participants and assess whether SNPs are involved, as is the case for other carotenoids.

2. Experimental Section

2.1. Study Participants

Thirty-seven apparently healthy, non-overweight, non-obese (BMI <25 kg m^{-2}) and non-smoking males were recruited for the study. Participants had no history of chronic disease, hyperlipidemia, or hyperglycemia. They were not taking any medication or dietary supplement that might affect PTF or lipid metabolism (e.g., tetrahydrolipstatin, ezetimibe, cholestyramine, fibrates, statins) at least a month prior to the study or during the study period. Because of the relatively large volume of blood that was drawn during the study, participants were required to have a blood hemoglobin concentration >130 g L^{-1} as additional inclusion criterion. Participants presented normal energy consumption, that is, $\approx$ 2500 kcal per days, and drank $\leq$ 2% alcohol as total energy. Their baseline characteristics are shown in **Table 2**. The study was approved by the regional committee on human experimentation (N°2008-A01354-51, Comité de Protection des Personnes Sud Méditerranée I, 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.

2.2. Postprandial Experiment

Participants were asked to refrain from consuming carotenoid-rich foods for 48 h before the postprandial experiment. A day prior to the visit, they 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 the clinic visit. On the day of the intervention, participants arrived at the local Center for Clinical Investigation (la Conception Hospital, Marseille, France) and consumed a test meal including 100 g of tomato puree purchased from a local supermarket, providing around 1 mg PTF.[20,21] The remainder of the test meal consisted of semolina (70 g) cooked in 200 mL hot water, white bread (40 g), egg whites (60 g), peanut oil (50 g), and mineral water (330 mL). The participants were asked to consume the meal at a steady pace, with half of the meal consumed in the first 10 min, and the remainder of the meal consumed in the next 10 min, in order to reduce any variability in gastric emptying due to variation in rates of intake. No other food was permitted over the following 8 h, but participants were permitted to finish the remainder of the 330 mL water they had not drunk during the meal. A fasting baseline blood sample was drawn before meal consumption as well as at 2, 3, 4, 5, 6 and 8 h after meal intake. Blood was taken up into evacuated tubes containing K-EDTA. The tubes were immediately placed into an ice-water bath and covered with aluminum foil to avoid light exposure. Plasma was isolated

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Table 1. Interindividual variability of phytofluene bioavailability.[a)]

PTF source	PTF dose	Duration	Sample used	Bioavailability estimation	*n*	Mean	SD	% CV	PMID
Tomato nutrient complex soft gel capsules	0.16 mg per day[b)] 0.83 mg per day 2.5 mg per day	2, 3 and 4 w[c)]	Fasting plasma	Change from baseline	25	0.088 µM 0.176 µM 0.380 µM	0.283[d)] 0.293 0.297	321.8 167.7 78.0	[12]
Lyc-o-Guard drink Lyc-o-Mato soft gel capsule	2 × 1.6 mg per day 2 × 0.4 mg per day	12 w	Fasting serum	Change from baseline	12	0.600 µM 0.500 µM	0.422 0.550	70.3 110.1	[13]
Carotenoid rich meal with carrot and tomato juice	1.01 mg PTF	10 h	Postprandial plasma triglyceride-rich lipoprotein	0–10 h incremental AUC of triglyceride-rich lipoprotein PTF concentration	13	0.423 µmol L^{-1} × h	0.322	76.3	[20]
Carotenoid-rich tomato extract capsule	4 mg of combined PT and PTF	3 w	Fasting plasma	Change from w2 to w3	75	0.250 µM	0.177	70.9	[56]
Carotenoid-rich tomato nutrient complex soft gel capsules	2 × 2.9 mg per day of combined PT and PTF	12 w	Fasting plasma	Change from baseline	75	Median PTF increased from 0.051 µM (IQR: 0.018–0.024) to 0.156 µM (IQR: 0.066–0.390) after supplementation			[57]

[a)] Methodology for the literature review can be found in Supplementary methods; [b)] Since only the lycopene dose given (2, 5, and 15 mg) and the PTF/lycopene ratio (1/6) were reported, the PTF dose was calculated; [c)] Results are presented only after 4 weeks; [d)] SD of the change in concentration was calculated using error propagation formula on the summary data as follows: let C = B-A, then $\sigma_c = \sqrt{(\sigma_A^2 + \sigma_B^2)}$. Abbreviations: AUC, area under the curve; IQR, interquartile range; PT, phytoene; PTF, phytofluene.

Table 2. Baseline characteristics of the study participants ($n = 37$).[a)]

Characteristic	Mean (SEM)
Age, years	32.8 (2.2)
Weight, kg	73.3 (1.3)
BMI, kg m^{-2}	23.0 (0.4)
Total cholesterol[b)], mmol L^{-1}	1.6 (0.1)
Triglycerides[b)], mmol L^{-1}	0.7 (0.1)
Glucose[b)], mmol L^{-1}	4.7 (0.1)
Hemoglobin[b)], g dL^{-1}	15.1 (0.1)
Phytofluene[b)], nmol L^{-1}	83.5 (8.0)

[a)] All participants were Caucasian males; [b)] These analytes were quantified in fasting plasma samples.

by centrifugation (10 min at 4°C and 878 *g*) within 2 h following collection.

2.3. Biochemical Measurements

Fasting plasma total cholesterol, triglyceride, glucose, and hemoglobin concentrations were measured at baseline using standard methods as previously described[22]: 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). All analyses were performed according to manufacturers' instructions.

Fasting plasma PTF concentration was measured by HPLC as described thereafter.

2.4. Chylomicron PTF Extraction and Analysis

Total chylomicron (CM) PTF concentration was determined at baseline and at the above-mentioned time points during the postprandial period. Preparation and extraction of CM from a starting volume of 6 mL of plasma were previously explained in detail.[18] All extractions were performed at room temperature under yellow light to minimize light-induced damage, using echinenone as an internal standard. The dried extract was then resolubilized in 200 µL of a dichloromethane/methanol mixture (65:35, v/v). A volume of 90 µL was injected for HPLC analysis. Separation was achieved using a 10 × 4.0 mm, 2 µm particle size Modulo-Cart QS guard column (Interchim, Montluçon, France) followed by a 250 × 4.6 mm, 5 µm particle size YMC C30 column (Interchim, Montluçon, France) held at 35°C. The mobile phase was composed of HPLC grade methanol (A), methyl tert-butyl ether (B) and water (C). A linear gradient of 96% A, 2% B, 2% C at t = 0 to 18% A, 80% B, 2% C at t = 27 min at a flow rate of 1 mL min^{-1} was used. The HPLC system consisted of a pump (Waters 2690) connected in-line with a photodiode-array detector (Waters 2996) (Waters, Saint Quentin en Yvelines, France). PTF was identified via UV–Vis spectra and retention time coincident with authentic standard (CaroteNature, Münsingen, Switzerland) and

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quantified at 348 nm.[23] Peak integration was performed using Chromeleon software (version 6.80, Dionex, Villebon sur Yvette, France).

The initial objective of this work was to characterize the interindividual variability of the bioavailability of both colorless carotenoids, that is, PT and PTF, and to identify SNPs associated with it. Indeed, both are present in tomatoes and tomato-based products, with PT usually found in greater quantities compared to PTF.[6,7,24] The chromatographic conditions used, which, as far as we knew, were the best at separating most carotenoids from fruits and vegetables, did not allow to quantify PT correctly. Indeed, the PT peak was contaminated with at least two other compounds. The first gave a peak that did not return to baseline until the PT peak started, the second gave a peak that started before the PT peak returned to baseline (Figure S1, Supporting Information). This carotenoid was therefore not quantified accurately, and so data on PT are not presented here.

2.5. DNA Isolation and Genotyping Analysis

An average of 25 µg of DNA was isolated from saliva samples using the Oragene kit (DNA Genotek, Ottawa, Ontario, Canada). DNA preparation and genotyping methods were performed as previously described.[18] The whole genome was genotyped using HumanOmniExpress BeadChips (Illumina, California, USA), which allow for the analysis of $\approx 7.33 \times 10^5$ SNPs/DNA sample.

2.6. Candidate SNP Selection

Literature search in PubMed for genes whose pathways are involved in carotenoid absorption or metabolism resulted in the selection of 57 candidate genes (Table S1, Supporting Information). Of the 2369 corresponding SNPs on the chips, only the SNPs deemed relevant following the procedure described in Figure S2 (Supporting Information) were selected for subsequent analyses. More precisely, SNPs for which the genotype call rate was <95% and SNPs that presented a significant departure from the Hardy–Weinberg equilibrium ($p < 0.05$; Chi-squared test) were first excluded (369 SNPs excluded), leaving 2000 SNPs. Then, SNPs in high linkage disequilibrium (LD, $R^2 > 0.80$) were removed, leaving 1396 SNPs (604 SNPs excluded). LD information was retrieved using the LD TAG SNP Selection tool from the SNPinfo Web Server (https://snpinfo.niehs.nih.gov), considering genotype data from dbSNP in the European population. Finally, SNPs were tested under both additive and dominant models. The additive model assumes that there is a uniform, linear increase, or decrease in the quantitative trait for each copy of the minor allele. For SNPs under the dominant model, participants homozygous for the lesser frequent allele were grouped with heterozygous participants and were compared with participants homozygous for the more frequent allele. SNPs with fewer than five observations in the minor group were excluded from further analysis, leaving 294 and 1115 SNPs in the additive and dominant model, respectively.

2.7. SNP Function Prediction

PolyPhen-2 within the Ensembl variant effect predictor (VEP) (http://www.ensembl.org/Tools/VEP) was used to predict the effect of the exonic variants. PolyPhen-2 predicts the functional significance of an allele replacement from its individual features by Naive Bayes classifier trained using supervised machine-learning algorithm. PolyPhen-2 classifies a mutation qualitatively as benign, possibly damaging, or probably damaging by estimating the false positive rate (i.e., the chance that the mutation is classified as damaging when it is in fact non-damaging) and the true positive rate (i.e., the chance that the mutation is classified as damaging when it is indeed damaging).[25] Functional effects of other forms of genetic variants (intronic and intergenic variants) were predicted using RegulomeDB (https://regulomedb.org/regulome-search/). RegulomeDB is a database that annotates SNPs with known and predicted regulatory elements in the intergenic regions such as DNase hypersensitivity, binding sites of transcription factors, and promoter regions that had been biochemically characterized to regulate transcription. The RegulomeDB probability score is ranging from 0 to 1, with a value of 1 indicating the variant is most likely a regulatory one.[26]

2.8. Calculations and Statistics

To estimate PTF bioavailability, incremental, i.e., baseline-corrected, area under the curves (AUC) of the 0 to 8 h postprandial CM PTF concentrations were calculated, hereafter named PTF responses. The trapezoidal approximation rule was used to calculate the PTF responses.

Partial least squares (PLS) regression,[27] a multivariate regression extension of principal component analysis,[28] was used to identify the combination of SNPs explaining best the variance in PTF response. A 2-step approach was followed, combining dimension reduction by univariate filtering followed by PLS regression, as has been previously applied with SNPs.[22,29,30] 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, http://pngu.mgh.harvard.edu/purcell/plink/), which is an open-source online toolset designed to perform tests that determine level of association between genotypic and phenotypic data. 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 SNP in the projection used in the PLS regression model, and several PLS regression models were then built using increasing VIP threshold values as described in detail elsewhere.[31] The model maximizing the adjusted R^2 (Equation 1) and validated following cross-validation ANOVA was selected.

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

With n the sample size and k the number of predictors in the model (excluding the constant). Robustness and stability of the selected model were estimated by leave-k-out cross-validation,[32] regression coefficient stability testing,[17] cross-

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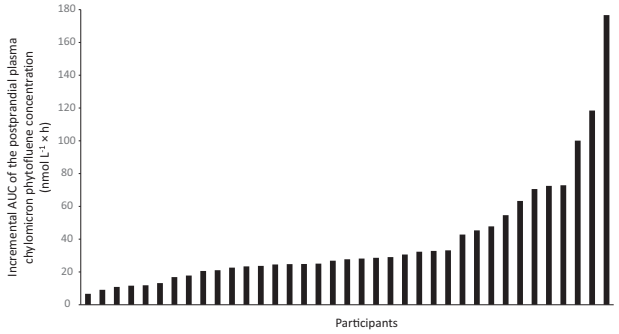

Figure 2. Phytofluene bioavailability in the study participants. Phytofluene bioavailability was assessed by measuring the 0–8 h incremental area under the curve of the postprandial plasma chylomicron phytofluene concentrations after consumption of a meal that provided phytofluene from a tomato puree. Participants ($n = 37$) were sorted by increasing phytofluene response.

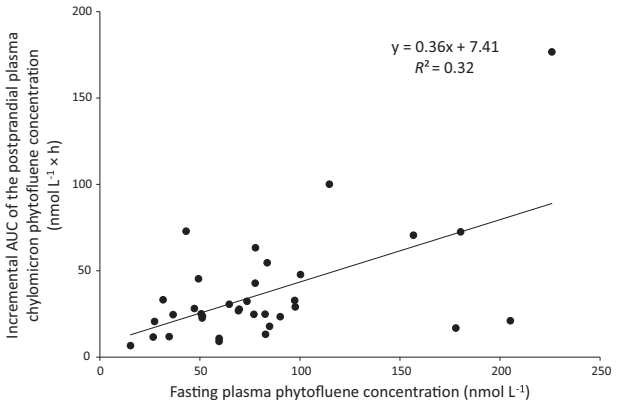

Figure 3. Linear correlation between phytofluene status and bioavailability. Phytofluene status was assessed by measuring the fasting plasma phytofluene concentration while phytofluene bioavailability was assessed by measuring the 0–8 h incremental area under the curve of the postprandial plasma chylomicron phytofluene concentrations after consumption of a meal that provided phytofluene from a tomato puree. There was a significant and positive correlation between the two variables (Pearson's $r = 0.57$; $p = 2 \times 10^{-4}$, $n = 37$).

validation ANOVA[33] and response permutation testing (*see* additional validations of the PLS regression model in the Supplements). For response permutation testing, the explained variance after cross-validation of the original model was compared with the explained variance after cross-validation of 100 models based on data where the order of the Y matrix for the participants (PTF response) was randomly permuted, while the X matrix (SNPs and covariates) was kept intact. SIMCA® Multivariate Data Analytics Solution software (Version 17.0.0.24543, Umetrics, Umeå, Sweden) was used for all multivariate data analyses and modeling.

3. Results

3.1. PTF Bioavailability

Figure 2 shows the PTF response of the 37 participants after consumption of the tomato puree meal. The PTF response displayed a high interindividual variability (CV = 88%), with the highest responder exhibiting a 26 times greater PTF response compared to the lowest responder (AUC of 176.6 and 6.7 nmol $L^{-1} \times h$, respectively). The PTF response was significantly and positively correlated with the fasting plasma PTF concentration (Pearson's $r = 0.57$, $p = 2 \times 10^{-4}$; $R^2 = 0.32$) (**Figure 3**).

3.2. SNPs Associated with the Interindividual Variability of the PTF Response

We first measured the association between SNPs, analyzed individually, and the interindividual variability of the PTF response (**Table 3**). Five SNPs (within 4 genes) exhibited a significant *p*-value under the additive model while 45 (within 18 genes) exhibited a significant *p*-value under the dominant model. Unstandardized regression coefficients (*B* coefficients), which represent the mean change in the PTF response for each additional copy of the minor allele under the additive model and in the presence of the minor allele under the dominant model, are also provided in Table 3. Two exonic SNPs in *PKD1L2*, namely rs16954717 and rs4889244 (not in linkage disequilibrium, R^2 <0.8), were predicted as "probably damaging" by PolyPhen-2. An intergenic SNP, rs7005359, near the *LPL* gene received a probability score of 1.0 by RegulomeDB, indicating that this SNP might contribute to the variability of the PTF response by acting via a gene regulatory element. The majority of the lead PTF-associated SNPs had high RegulomeDB scores, signaling a high probability that they are indeed located within a gene regulatory region and hence, may influence gene function (Table 3). Their proposed roles in PTF uptake, enterocyte metabolism and postprandial CM transport are illustrated in **Figure 4**.

3.3. Combinations of SNPs Associated with the Interindividual Variability of the PTF Response

We used PLS regression to find whether a combination of the SNPs exhibiting *p*-values <0.05 and covariates (age and BMI) could explain a significant part of the interindividual variability of the PTF response. As shown in Supplemental Table S2, the model including all thus selected variables (51 SNPs, age and BMI) could explain a high part of the variance in PTF response ($R^2 = 0.78$) but this estimation was positively biased, as illustrated by the high R^2 value after permutations, due to the high number of predictors. Therefore, to improve the model and find a combination of SNPs more predictive of the PTF response, we sequentially filtered out SNPs that made a less important contribution, that is, those that displayed the lowest VIP values. With the application of several VIP value thresholds, the best model obtained had an adjusted R^2 of 0.51 (Supplemental Table S2). It included 14 SNPs (not in linkage disequilibrium) in or near 11 genes (**Table 4**). The model was first validated by cross-validation ANOVA ($p = 3.8 \times 10^{-7}$) (Supplemental Table S2). Then, its robustness and its stability were validated by three additional methods (Table S3, Figure S3 and S4, Supporting Information).

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Table 3. SNPs significantly associated with the phytofluene response following univariate analysis.

SNP	Gene	Alleles	Alternate allele frequency (European population)	Coding status[a)]	Unstandardized regression coefficient[b)]	*p*-value[c)]	Variant effect prediction score[d)]
Additive model							
rs5755607	*ISX*	A>G	G = 0.73		−16.7 ± 7.1	0.025	0.135
rs380609	*SOD2*	C>A	A = 0.41		19.6 ± 8.4	0.025	0.609
rs10086580	*LPL*	A>G	G = 0.48		18.1 ± 7.8	0.027	0.135
rs10948750	*ELOVL5*	C>T	T = 0.54		−15.1 ± 7.2	0.042	0.184
rs2758317	*SOD2*	C>A	A = 0.54		−17.2 ± 8.2	0.042	0.954
Dominant Model[e)]							
rs17748559	*ISX*	C>T	T = 0.12		26.0 ± 6.4	2.0×10^{-4}	0.609
rs2515629	*ABCA1*	A>G	G = 0.17	Intron	16.7 ± 5.2	0.003	0.617
rs130461	*ISX*	T>C	C = 0.86		20.1 ± 6.4	0.004	0.184
rs3211881	*CD36*	A>G	G = 0.07	Intron	17.2 ± 5.7	0.005	0.843
rs6941533	*ELOVL5*	A>G	G = 0.10		20.4 ± 6.9	0.006	0.135
rs445925	*APOC1*	G>A	A = 0.10	Upstream	20.0 ± 7.0	0.007	0.305
rs10185660	*FABP1*	T>C	C = 0.15		17.7 ± 6.2	0.008	0.644
rs2487059	*ABCA1*	T>G	G = 0.26	Intron	−14.3 ± 5.2	0.009	0.696
rs7005359	*LPL*	A>G	G = 0.20		−14.6 ± 5.3	0.010	1.000
rs1000778	*FADS3*	A>G	G = 0.74	Intron	14.7 ± 5.4	0.010	0.135
rs1351472	*LYPLAL1*	C>T	T = 0.05		20.2 ± 7.6	0.012	0.184
rs17240713	*LIPC*	C>T	T = 0.07		19.6 ± 7.6	0.015	0.609
rs4149316	*ABCA1*	C>T	T = 0.08	Intron	19.5 ± 7.7	0.016	0.654
rs3904998	*ABCA1*	T>C	C = 0.21	Intron	13.8 ± 5.5	0.017	0.135
rs7165301	*LIPC*	T>C	C = 0.18		−13.8 ± 5.5	0.017	0.810
rs11789603	*ABCA1*	C>T	T = 0.10	Intron	15.9 ± 6.4	0.018	0.135
rs10518968	*LIPC*	T>C	C = 0.15		14.0 ± 5.8	0.020	0.099
rs11936429	*SETD7*	C>T	T = 0.03		13.9 ± 5.8	0.021	0.135
rs6532059	*ABCG2*	C>T	T = 0.10		15.4 ± 6.4	0.022	0.609
rs9395867	*ELOVL5*	C>T	T = 0.28		−12.7 ± 5.3	0.023	0.135
rs443757	*ABCA1*	T>C	C = 0.72		−12.6 ± 5.3	0.023	0.184
rs391045	*SOD2*	T>C	C = 0.56		14.0 ± 6.0	0.025	0.609
rs9787661	*PNLIPRP2*	C>T	T = 0.20	Intron	13.0 ± 5.5	0.025	0.609
rs651821	*APOA5*	C>T	T = 0.93	5' UTR	18.1 ± 7.8	0.025	0.554
rs1527470	*CD36*	A>G	G = 0.44		13.4 ± 5.8	0.028	0.244
rs16842	*LPL*	T>C	C = 0.28		12.2 ± 5.3	0.028	0.135
rs4921920	*NAT2*	T>C	C = 0.13		13.3 ± 5.8	0.028	0.417
rs16954717	*PKD1L2*	C>T	T = 0.15	Missense	13.3 ± 5.8	0.029	0.882
rs2178704	*IRS1*	A>G	G = 0.13		13.6 ± 6.0	0.029	0.169
rs2899632	*LIPC*	A>G	G = 0.82	Intron	−12.7 ± 5.7	0.032	0.135
rs2602248	*SETD7*	G>A	A = 0.66		−12.2 ± 5.5	0.033	0.867
rs192138	*SOD2*	G>A	A = 0.87		13.2 ± 6.0	0.035	0.212
rs446303	*ABCA1*	A>G	G = 0.63		−12.5 ± 5.7	0.035	0.388
rs2970892	*FABP1*	A>G	G = 0.66		−11.6 ± 5.4	0.037	1.000
rs4149314	*ABCA1*	T>C	C = 0.10	Intron	13.5 ± 6.2	0.038	0.609
rs17092018	*LPL*	G>A	A = 0.10		13.9 ± 6.5	0.040	0.184
rs625284	*SOD2*	G>T	T = 0.83		13.3 ± 6.2	0.041	0.533
rs35427222	*ABCG2*	T>C	C = 0.29	Intron	−11.7 ± 5.5	0.041	0.183
rs1890582	*SOD2*	G>A	A = 0.18		11.5 ± 5.5	0.043	0.000
rs1027472	*SETD7*	C>T	T = 0.53		11.6 ± 5.5	0.043	0.609

(*Continued*)

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Table 3. (Continued).

SNP	Gene	Alleles	Alternate allele frequency (European population)	Coding status[a)]	Unstandardized regression coefficient[b)]	*p*-value[c)]	Variant effect prediction score[d)]
rs4149292	*ABCA1*	G>A	A = 0.11	Intron	14.4 ± 6.9	0.044	0.135
rs4889244	*PKD1L2*	C>T	T = 0.69	Missense	11.7 ± 5.6	0.045	0.986
rs2275542	*ABCA1*	C>T	T = 0.32	Intron	−11.2 ± 5.4	0.045	0.851
rs1955164	*PNLIPRP2*	C>T	T = 0.50		−12.4 ± 6.1	0.048	0.135
rs17827934	*ELOVL5*	C>T	T = 0.13		−12.9 ± 6.3	0.048	0.763

[a)] Alternate allele frequencies were retrieved from dbSNP (https://www.ncbi.nlm.nih.gov/snp/) using the Allele Frequency Aggregator (ALFA) dataset (pooled allele frequency data from dbSNP and the dbGaP) in the European population (19.05.2022). Coding status: intron, missense, upstream or unstranslated region (UTR). The 5′ UTR is a regulatory DNA region situated at the 5′ end of all protein-coding genes that is transcribed into mRNA but not translated into protein. All SNPs were otherwise intergenic; [b)] Unstandardized regression coefficients represent the mean change in the phytofluene response for each additional copy of the minor allele under the additive model and in the presence of the minor allele under the dominant model; [c)] SNPs are ranked by increasing *p*-values; [d)] Variant Effect Prediction Score was estimated using https://www.ensembl.org/Homo_sapiens/Tools/VEP/ for exonic/missense and RegulomeDB for intron, upstream, UTR or intergenic SNPs (accessed on 25.03.2022); [e)] For SNPs under the dominant model, participants homozygous for the lesser frequent allele were grouped with heterozygous participants and were compared with participants homozygous for the more frequent allele. Abbreviations: TFBS, transcription factor binding site; miRNA, micro RNA; Gene names can be found in Table S1, Supporting Information.

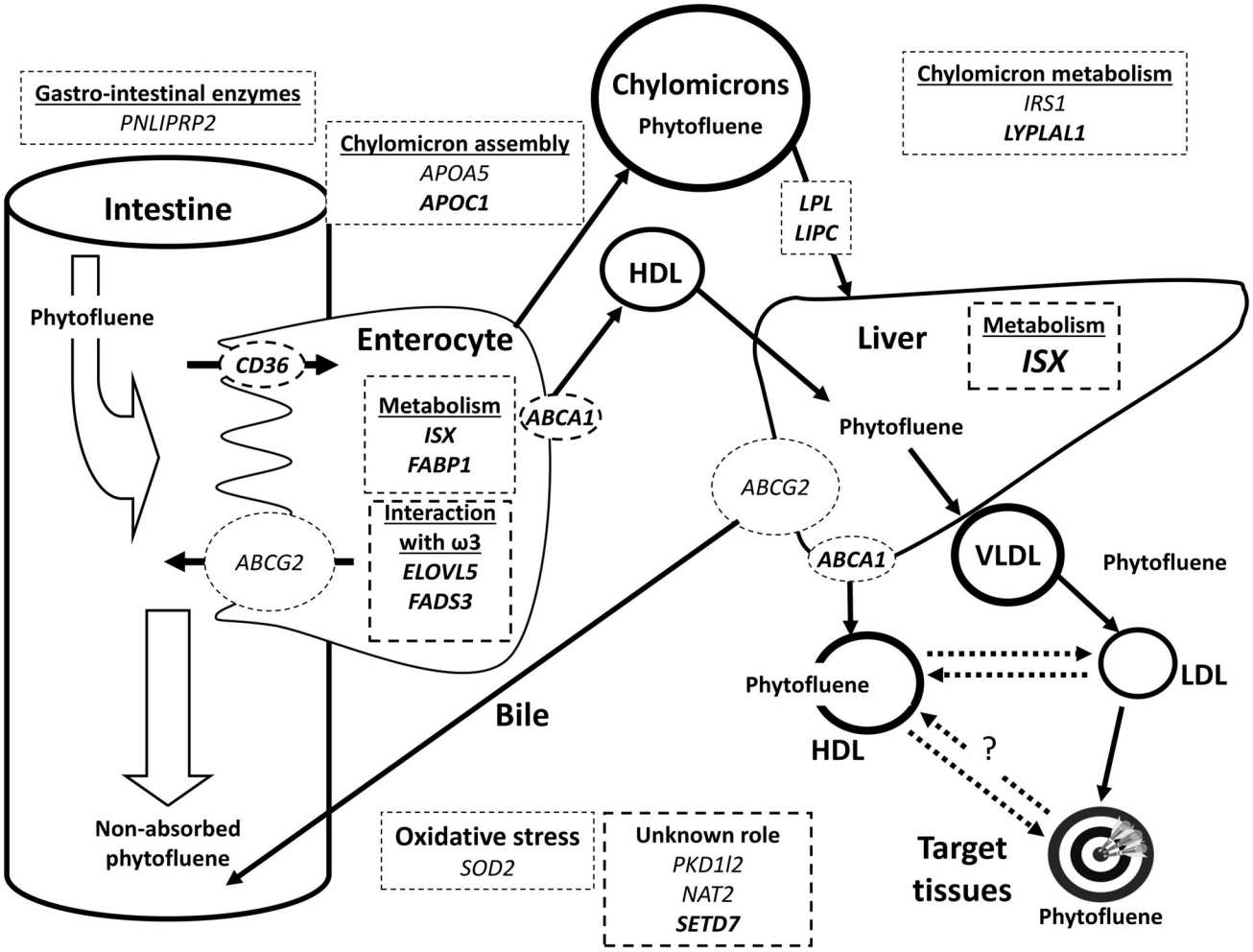

Figure 4. Genes whose SNPs are involved in the interindividual variability of phytofluene bioavailability. Phytofluene bioavailability was assessed by measuring the 0–8 h incremental area under the curve of the postprandial plasma chylomicron phytofluene concentrations after consumption of a meal that provided phytofluene from a tomato puree. Genes displayed are those for which SNPs were associated with the interindividual variability of phytofluene response, following univariate analysis (5 SNPs within 4 genes under the additive model, 45 SNPs within 18 genes under the dominant model). Genes displayed in bold are those for which SNPs were associated with the interindividual variability of phytofluene response, following partial least squares regression (14 SNPs in 11 genes). *See* Section 4 for gene names and a description of their associations.

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Table 4. Combination of SNPs associated with the phytofluene response.

Gene[a)]	SNP	VIP value[b)]	Regression coefficient[c)]
ISX	rs17748559	1.53	13.3
ABCA1	rs2515629	1.29	8.5
ISX	rs130461	1.25	10.3
CD36	rs3211881	1.22	8.8
ELOVL5	rs6941533	1.20	10.4
APOC1	rs445925	1.17	10.2
FABP1	rs10185660	1.16	9.1
ABCA1	rs2487059	1.14	7.3
LPL	rs7005359	1.13	-7.5
FADS3	rs1000778	1.11	7.3
LYPLAL1	rs1351472	1.10	10.3
SETD7	rs11936429	1.10	8.1
LIPC	rs17240713	1.07	10.1
ABCA1	rs4149316	1.06	10.0

[a)] Gene names can be found in Table S1, Supporting Information; [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 coefficient are for untransformed variables and represent the mean change in the PTF response in the presence of the minor allele under the dominant model.

With the knowledge of a participant's genotype at the 14 SNPs in the selected model, it was possible to calculate a participant's PTF response using the following equation:

$$PTF = \sum_{i=1}^{14} (ri) \times number\ of\ minor\ allele\ SNPi \quad (2)$$

with PTF the PTF response and r_i the unstandardized regression coefficient of the i^{th} SNP in the PLS regression model (provided in Table 4). Note that for the SNPs 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 1.

4. Discussion

We here report a combination of 14 SNPs in 11 genes involved in carotenoid metabolism and transport that is associated with a significant part of the interindividual variability of PTF bioavailability in 37 healthy adult males following consumption of a PTF-rich meal. Our first noteworthy observation was the high interindividual variability in PTF bioavailability (CV = 88%) in this group of apparently healthy participants. Only 5 studies reported data that allowed us to calculate the interindividual variability of PTF bioavailability (Table 1). However, 4 of these studies estimated PTF bioavailability by measuring the change (from 24 h to a few weeks) in its fasting blood concentration following a dietary intervention. On the other hand, Iddir et al. estimated PTF bioavailability by measuring its postprandial concentration in triglyceride-rich lipoproteins,[20] which reflects more accurately PTF bioavailability. The CV of PTF bioavailability in the 13 male participants was 76% (following our own calculations), therefore supporting the finding of the present study. In addition, the CV we report for PTF was in the range of those reported for lycopene, that is, 70%,[18] lutein, that is, 75%,[17] and beta-carotene,that is, 105%,[19] in the same sample population. Another noteworthy observation is that PTF status, assessed by measuring its fasting plasma concentration, and PTF bioavailability were significantly and positively correlated. This suggests that the ability of an individual to absorb PTF has a significant impact on his long term PTF status. Nevertheless, the correlation was of intermediate effect size (Pearson's $r = 0.57$), highlighting the fact that factors other than PTF bioavailability also explain PTF status. Indeed, the PTF status results from several determinants, that is, PTF consumption, absorption, distribution, metabolism and excretion, while our estimation of PTF bioavailability mostly reflects PTF absorption and distribution.

Several host-related factors can affect the interindividual variability of carotenoid bioavailability, including diseases, lifestyle habits, gender, age, the gut microbiota as well as genetic variations (reviewed in ref.[14]). To the best of our knowledge, this is the first study that explores the putative contributions of genetic factors in explaining interindividual differences in PTF bioavailability. Using a 2-step statistical approach, univariate filtering followed by PLS regression, we found a combination of 14 SNPs that could explain 51% (adjusted R^2) of the interindividual variability of the PTF response. The validity of the selected PLS model was verified internally by several tests, including cross-validation and permutations (*see* Supplemental information). Our approach was similar to our previous published works that identified, in the same sample population, combinations of SNPs associated with the interindividual variability of the bioavailability of fat-soluble vitamins[34–36] and carotenoids, that is, lycopene,[18] lutein,[17] and beta-carotene.[19]

Of the 14 SNPs associated with PTF bioavailability, 2, namely rs7005359 near or within *LPL* and rs1351472 near or within *LYPLAL1*, were found to be also involved in the postprandial CM triglyceride response in the same group of participants.[37] This was not surprising as most newly absorbed PTF is carried from the intestine to the circulation in CM. LPL is involved in CM triglyceride hydrolysis and CM clearance and can thus also affect PTF bioavailability, with most likely a higher effect on PTF distribution than on PTF absorption. Interestingly, RegulomeDB annotated the intergenic rs7005359 SNP with a probability score of 1.0, indicating that it is highly likely to act as a regulatory variant, which might explain its association with PTF bioavailability.

It was striking to observe that among the top 5 genetic variants, based on VIP values, associated with PTF bioavailability, there were 2 SNPs (rs17748559 and rs130461) located near or within *ISX*. *ISX* encodes for an intestine specific homeodomain transcription factor that has been shown to act as a transcriptional repressor of *BCO1* and *SCARB1* expression.[38] *SCARB1* encodes for SR-B1, an apical membrane protein, which we have previously shown to be involved in the intestinal uptake of dietary PTF.[39] We hypothesize that SNPs in *ISX* may affect *SCARB1* expression and/or SR-B1 activity which would in turn affect PTF intestinal uptake efficiency.[40]

Three SNPs in *ABCA1* (rs2515629, rs2487059, rs4149316) were also associated with PTF bioavailability. ABCA1 is a basolateral membrane protein that is involved in the efflux of lipid molecules, including carotenoids,[41,42] to APOA1/HDL in the

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lymph.[41] This has been confirmed in vitro and in a hamster model for lutein and zeaxanthin where up- and down-regulation of *ABCA1* expression, via activation of the liver X receptor and statin treatment respectively, led to increased and decreased HDL-lutein and -zeaxanthin concentrations.[42] Moreover, our studies in the same sample population showed that several SNPs in *ABCA1* were associated with the interindividual variability of lycopene,[18] β-carotene,[19] and lutein.[17] Thus, this study adds to the evidence of the involvement of ABCA1 in carotenoid transport.

CD36 encodes for a protein involved in the intestinal uptake of fat-soluble vitamins and carotenoids.[16] A study from our group in 2018 suggested that CD36 is not a major player in PTF absorption based on the fact it was less expressed in the distal intestine where PTF accumulation was more important.[10] However, this study was performed in mice after a gavage with standardized emulsions and it is not known whether the same pattern of CD36 expression exists in humans. Furthermore, since CD36 promotes the assembly and secretion of CM,[43] it is possible that *CD36* rs3211881 had an effect on the PTF response through its effect on CM.

The observed association of *FABP1* rs10185660 with PTF bioavailability suggests that fatty acid binding protein 1 could be involved in the intracellular transport of this carotenoid.[44] Indeed, PTF has to cross the aqueous environment of the intestinal cell to reach its basolateral side and this protein, which has a broad substrate specificity, could be involved in that function. Functional studies employing cell cultures or transgenic mice should be performed to confirm this involvement.

Our study highlights the putative contribution of rs445925 in *APOC1* to PTF bioavailability. Previous genome-wide linkage and association studies have shown high LD between *APOC1* rs445925 and *APOE* rs7412.[45] Although rs445925, which is a 2 kb upstream regulatory variant, has not been extensively studied, functional in vitro and in vivo assays have shown *APOC1* inhibitory activity against LPL.[46]

A SNP in *LIPC*, rs17240713, was also associated with PTF bioavailability. *LIPC* encodes for hepatic lipase, which converts large, triglyceride-rich HDL2 into small, dense HDL3.[47] Hepatic lipase is also involved in the removal of remnant particles, including CM remnants by the liver,[48,49] which provides a potential link between a SNP in its encoding gene and PTF bioavailability, since CM carry newly absorbed PTF. Similarly to *LPL, LIPC* effect is most likely higher on PTF distribution than on PTF absorption. Additionally, SNPs in *LIPC* have previously been associated with the bioavailability of other carotenoids, that is, β-carotene,[19] lycopene,[18] lutein,[17] in the same sample population.

A SNP in *ELOVL5*, which encodes for an enzyme that catalyzes the elongation of eicosapentaenoic acid to docosapentaenoic acid (DPA), and subsequently to docosahexaenoic acid (DHA), was also associated with the interindividual variability of PTF bioavailability. This is possibly due to the inhibitory effect of eicosapentaenoic acid on carotenoid absorption, as has been shown with β-carotene.[50] Moreover, omega-3 fatty acids have also been shown to accelerate CM triglyceride clearance.[51] Furthermore, another SNP in a gene involved in the synthesis of long-chain polyunsaturated fatty acids, namely rs1000778 in *FADS3*, was identified as one of the key contributory SNPs, based on VIP value, to the PTF response. Murine hepatic ELOVL5 expression levels were increased significantly in the absence of FADS3, which may explain the significant increase of hepatic DPA concentrations and implicate FADS3 as a factor influencing elongase activity.[52] All the above observations suggest that DHA biosynthesis and/or regulation may modify PTF bioavailability.

A SNP in *SETD7*, rs11936429, was also associated with the PTF response. *SETD7* was selected as a candidate gene in our study following its association with lycopene concentrations carried out in a mixed-gender, Caucasian population.[53] *SETD7* expression has been shown to activate the nuclear factor erythroid 2–related factor 2 (NRF2)/antioxidant response element signaling pathway to prevent the formation of reactive oxygen species.[54] It may be speculated that PTF may play an important role in the action of *SETD7* to activate NRF2 signaling pathway against oxidative stress and cancer progression.

This study has several strengths. We used a very commonly consumed food source of PTF and we measured its bioavailability postprandially in the lipoproteins that carry most newly absorbed lipids, which constitutes the best method, apart from the use of stable isotopes of PTF. Moreover, due to the acute intervention design used, this sample population is well-controlled, which has allowed us to characterize the bioavailability of several other lipid moieties.[17–19,22,37,55] We acknowledge some limitations of the study. PTF bioavailability is a complex process that involves numerous genes. Thus, a thorough investigation of the association between candidate genetic variants and this phenotype should ideally include all SNPs in genes that are assumed to be involved in this phenotype. Yet, several SNPs in candidate genes were not entered in the PLS regression analysis because either they were not expressed on the BeadChips or they were excluded from the analysis (as explained in the Methods). Furthermore, it should be reminded that SNPs and other genetic variations represent only a portion of the host-related factors that affect the interindividual variability of the bioavailability of most dietary compounds, albeit they are stable over the lifespan. Other factors, such as the pathophysiological status or the gut microbiota can also affect bioavailability of numerous molecules. Finally, since only Caucasian males participated in this study, the associations observed need to be validated in females as well as in other ethnic groups in order to be generalized.

Taken together, our results show that, in this sample of healthy adult males, there is a relatively high interindividual variability of PTF bioavailability. This variability is significantly associated with a combination of 14 SNPs, including SNPs in genes that encode transcriptional regulators of key genes involved in carotenoid transport and metabolism (*ISX*), cellular transporters (*ABCA1* and *CD36*), intracellular transport proteins *(FABP1*), as well as genes encoding proteins indirectly linked to carotenoid metabolism (i.e., *ELOVL5, FADS3, SETD7*). Furthermore, PTF bioavailability and PTF status are significantly correlated, thus stressing the relevance of determining factors, including genetic ones, influencing PTF bioavailability. Future studies may gear towards identification of other genes/SNPs involved in PTF bioavailability that may contribute to improvement of the model and hence predict more precisely the bioavailability of this phytochemical. Although a large part of this variability remains unexplained, we believe this approach could constitute an important step in providing nutritionists an evidence-based, accurate and validated genetic tool to predict PTF bioavailability for future

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supplementation studies and for personalized dietary recommendations.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

C.D. is a member of the Scientific Advisory Board of Nutritional Fundamentals for Health Inc., Canada.

Author Contributions

M.P.Z. and P.B. contributed equally to this work and should be considered co-first authors. The authors' responsibilities were as follows: M.P.Z.: Methodology, Software, Validation, Visualization, Writing-original draft; P.B.: Conceptualization, Funding acquisition, Project Administration, Resources, Supervision, Writing – review & editing. R.B. and M.N.: Investigation, Methodology; D.L.: Conceptualization, Writing – review & editing; C.D.: Conceptualization, Methodology, Software, Validation, Visualization, Supervision, Writing – review & editing. All authors have read and approved the final manuscript.

Data Availability Statement

Data described in the manuscript shall be made available upon request to the corresponding author.

Keywords

absorption, carotenoids, chylomicrons, genetic polymorphisms, genetic variants, postprandial

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