Can Genetic Variability in α-Tocopherol Bioavailability Explain the Heterogeneous Response to α-Tocopherol Supplements?

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Can genetic variability in α-tocopherol bioavailability explain the heterogeneous response to α-tocopherol supplements?

Abbreviated title: Variability in α-tocopherol bioavailability

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ABSTRACT

AIM: Both vitamin E (VE) consumption and blood VE status have been negatively associated with the incidence of degenerative diseases and some cancers. However, the response to VE supplementation is very variable among individuals. This could be due to interindividual variability in VE bioavailability, due, at least partly, to genetic variations in genes involved in VE metabolism. Thus, the main objective was to identify single nucleotide polymorphisms (SNPs) that may be involved in the interindividual variability in α-tocopherol (TOL) bioavailability.

RESULTS: The postprandial chylomicron TOL response (area under the curve of the postprandial chylomicron TOL concentration) to a TOL rich meal was highly variable (CV=81%; n=38). This response was positively correlated with the fasting plasma TOL concentration (r = 0.5, P = 0.004). A significant (P=1.8.10^{-8}) partial least squares regression model, which included 28 SNPs in 11 genes, explained 82% of this response.

INNOVATION: First evidence that the interindividual variability in TOL bioavailability is, at least partly, modulated by a combination of SNPs.

CONCLUSIONS: TOL bioavailability is, at least partly, modulated by genetic variations that can affect long-term TOL status. This allows us to propose a new hypothesis that links the biological response to VE supplementation with one's individual genetic characteristics.

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INTRODUCTION

Vitamin E (VE), which consists of eight isomers known as α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols, is considered to be the primary fat-soluble dietary antioxidant consumed by humans and has also been suggested to have biological activities independent of its antioxidant effects (6). For example tocotrienols could up-regulate genes related to osteoblastogenesis and might prevent colorectal cancer better than tocopherols. It has also been suggested that α-tocotrienol and γ-tocopherol are more effective than the other VE isomers for reducing cerebral infarcts.

VE has been hypothesized to be involved in the prevention of cardiovascular diseases and cancers following the negative associations observed in epidemiological studies between its dietary intake or fasting blood concentration and the incidence of these diseases. Nevertheless, most prospective randomized controlled trials (RCTs) designed to provide definite evidence of the beneficial effects of TOL supplementation have failed to support these associations. Indeed, no effects and even adverse effects (2) of TOL supplementation have been reported. Several hypothesis have been proposed to explain this discrepancy. A first one suggests that, epidemiological studies being inherently biased, the absence of beneficial effect reported by most RCTs demonstrates that TOL is unrelated to these diseases. A second hypothesis suggests that TOL supplementation in RCTs was not effective because the TOL status among most participants was already close to optimal, thus providing no opportunity to observe any significant benefit of TOL supplementation. A third hypothesis might reconcile these two opposing views by suggesting that the benefit of TOL supplementation depends on the haptoglobin (Hp) genotype of the subjects (1). Finally it has been suggested that any potential health benefits of TOL supplementation have been offset by diminution in the bioavailability of other VE isomers.

We suggest that the variability in the response to TOL supplementation observed in RCTs is in part due to interindividual differences in absorption and metabolism of TOL. These differences can be due to several factors, among them genetic variations that have been proposed as a determining factor in antioxidant status. Indeed, several genetic polymorphisms have been shown to influence the fasting blood TOL concentration (5). Nevertheless, this parameter reflects the complex interactions between TOL intake, TOL absorption efficiency, blood clearance (e.g. liver secretion and tissue uptake), utilisation, and catabolism (7) and its variability is hence highly dependent on environmental factors. Conversely, the chylomicron (CM) VE response to a VE rich test meal, which is acknowledged as a good estimate of VE absorption efficiency, also called bioavailability, is less affected by environmental factors and is hypothesized to be mostly dependent on genetic factors when similar VE rich test meals are given to different individuals. TOL bioavailability has systematically been shown to display an elevated interindividual variability in clinical studies of TOL supplementation, hence supporting the possible involvement of genetic factors. Yet, no study has attempted to decipher the mechanisms underlying this phenomenon.

Thus, the objectives of this study were to i) better characterize the interindividual variability of TOL bioavailability in healthy subjects, ii) assess whether this variability might affect the fasting TOL status, and iii) identify single nucleotide polymorphisms (SNPs) that are involved in this phenomenon.

RESULTS

Interindividual variability in the chylomicron TOL response to the meal

The postprandial **chylomicron (**CM) TOL response after consumption of the meal containing the TOL supplement is shown in **Figure 1**. The coefficient of variation (CV) of the CM TOL response was 81%, as illustrated in **Figure 2**.

Correlation between the CM TOL response to the TOL-rich meal and the fasting plasma TOL concentration

To assess whether the fasting plasma TOL concentration, which is known to be a marker of TOL status, was related to the ability to respond to dietary TOL, we calculated the correlation between the CM TOL response of the subjects (subjects' characteristics shown in **Table 1**) and their fasting plasma TOL concentration. Results showed that there was a significant positive correlation between the CM TOL response to the meal containing the TOL supplement and the fasting plasma TOL concentration (Pearson's $r = 0.46$, $P = 0.004$; Spearman's rank correlation coefficient = 0.38, $P = 0.022$).

Genetic variants associated with the TOL response

The PLS regression model that included all 3769 candidate SNPs (used as qualitative X variables) described the group variance with good accuracy ($R^2 = 0.81$) but was not predictive of this variance ($Q^2 = -0.10$), as shown in **Table 2.** Therefore, to improve the model and find an association of SNPs more predictive of the TOL response, we filtered out those that displayed the lowest variable importance in the projection (VIP) value (i.e. those that made no important contribution). After the application of increasing thresholds of VIP value (**Table 2**), the model which presented the highest Spearman's Rho between the measured and

the predicted postprandial CM TOL response was selected. The Spearman's Rho value of this model was 85.2 % ($P < 0.001$) and it included 36 SNPs (28 not in linkage disequilibrium).. The 28 SNPs were located in or near 11 genes (*ABCA1*, *ABCG1*, *APOB*, *BET1*, *IRS1*, *LIPC*, *NAT2*, *PNLIP*, *SLC10A2*, *SREBF2*, *ZNF664*) (**Table 3)** and described 82 % of the group variance, with a predicted variance (Q^2) of 74 % (**Table 2**). The robustness and the stability of the model were validated by 4 independent procedures (see **Notes section**).

The association of these 28 SNPs with the CM TOL response was further evaluated using univariate statistics by comparing for each SNP the CM TOL response of the subjects who bore different genotypes (**Table 3**). For all the SNPs, subjects with different genotypes exhibited a significantly different CM TOL response (*p* after Benjamini-Hochberg correction < 0.05).

Genetic score to predict the CM TOL response of a genotyped subject

With the knowledge of a subject's genotype at the 28 aforementioned loci, it was possible to predict the subject's ability to respond to TOL according to the following equation:

$$\mathrm{RP} = a + \sum_{1}^{28} ri \ \mathrm{genotype.(SNPi)}$$

With RP as the responder phenotype (i.e. the CM TOL response to a TOL rich meal), *a* as a constant (9.93), r_i as the regression coefficient of the i^{th} SNP included in the PLS regression model, and "genotype.(SNP_i)" as a Boolean variable indicating the subject's genotype at the i^{th} SNP. S*ee* **Table 4** for the list of regression coefficients calculated by the SIMCA-P12 software.

DISCUSSION

The first aim of this study was to accurately determine the interindividual variability in TOL bioavailability. Thus, we chose to measure the postprandial CM TOL response to a TOL-rich meal, a widely used method for clinical assessment of TOL bioavailability. Note that the choice of TOL as the dietary source of VE limits our conclusions to TOL, which is the main form of VE used in VE supplementation trials and which is the main form of VE found in human tissues, and cannot be extrapolated to tocotrienols. Indeed it is not known whether absorption mechanisms of TOL and tocotrienols are similar in humans. The first observation from this study was the large range of interindividual variability in TOL bioavailability, estimated at 81% (CV), which is similar to previous observations (4). The second observation from this study was that a significant part of the interindividual variability in TOL bioavailability was explained by 28 SNPs in or near 11 genes. It was first striking to observe that 7 of these genes (**Table 3)** were shown to be involved in the postprandial CM triacylglycerol response in the same group of subjects (3). This observation was not surprising, as most newly absorbed VE is carried from the intestine to peripheral organs and the liver via CM (8). Thus, SNPs in genes that modulate CM metabolism likely have an indirect effect on the CM VE response (3). Interestingly, SNPs in the 4 remaining genes were not associated with the CM triacylglycerol response and were thus specifically associated with the CM TOL response. These genes were: solute carrier family 10 (sodium/bile acid cotransporter), member 2 (*SLC10A2*), pancreatic lipase (*PNLIP*), sterol regulatory element binding transcription factor 2 (*SREBF2*), and ATP-binding cassette, sub-family G (WHITE), member 1 (*ABCG1*). *SLC10A2* encodes an apical sodium-dependent bile acid transporter (ASBT). It has been shown that mutations in this gene cause primary bile acid malabsorption and SNPs have been suggested to impair bile acid transport. The association of this gene with the CM TOL response was therefore not surprising, as genetic defects in bile acid metabolism

have been shown to cause fat-soluble vitamin deficiency. *PNLIP* encodes for pancreatic lipase, which is responsible for the intestinal hydrolysis of triacylglycerols (i.e. the primary form of dietary fat). Since VE is fat-soluble, it must be released from lipid droplets in the digestive tract to be incorporated into mixed micelles for efficient absorption. Thus, we hypothesize that SNPs that affect PNLIP activity or expression may affect fat hydrolysis and thus VE absorption. *SREBF2* encodes for a transcription factor, SREBP-2, that controls cholesterol homeostasis. Interestingly, SREBP-2 modulates the expression of *NPC1L1*, which is involved in TOL absorption/transport. Thus the association between SNPs in *SREBF2* could be explained by an indirect effect on *NPC1L1* expression. ABCG1 is an ATP-binding cassette transporter involved in macrophage and liver cholesterol and phospholipid transport. There is no published study on its involvement in TOL transport. Nevertheless, unpublished *in vitro* and *in vivo* studies from our laboratory suggest that it is involved in the cellular efflux of TOL. Finally note that it was surprising to did not find some TOL transporters, i.e. *α-TTP*, *CD36*, *SCARB1*, *SEC14L*, associated with the TOL response. We have two hypothesis to explain this finding, the first one is that SNPs in these genes are indeed not associated to this phenotype, or their association is weaker than that of SNPs we have found associated. The second hypothesis is that SNPs in these 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 (for not respecting the Hardy-Weinberg equilibrium or because their genetic call rate was <95%). We acknowledge this limitation but it does not change the important finding of this study: TOL bioavailability is associated with a combination of genetic variants and we have found some SNPs that likely explain a significant part of the variability in TOL bioavailability.

The third noteworthy observation of this study was that the plasma TOL concentration was positively correlated with the CM TOL response to the TOL-rich meal. Since the fasting

plasma TOL concentration is the result of interactions between several factors, e.g. dietary TOL intake, TOL absorption efficiency, TOL catabolism rate, etc., this association suggests that the individual ability to respond to dietary TOL is a key factor that governs blood TOL concentration. This likely affects tissue concentrations as well. Obviously, the size of the studied population and the candidate gene approach used in this study suggest that the list of SNPs involved in this phenotype will be corrected and/or complemented in future studies. It is also possible that additional SNPs will be involved in the CM response of other VE forms, e.g. tocotrienols, whether some different proteins are involved in absorption and metabolism of TOL and tocotrienols. However, the current results strongly support the conclusion that a significant percentage of the interindividual variability in TOL, and likely other VE isomers, bioavailability is explained by a combination of genetic variants.

We believe these results can be extrapolated to help to understand the interindividual differences observed when TOL supplements are given to healthy subjects. Indeed, we suggest that the interindividual variability in TOL bioavailability can partially explain the variability in the response to TOL supplementation, following the model proposed in **Figure 3**. In summary, the TOL responder characteristics of the subjects could affect both the initial TOL status and the amplitude of the blood and tissue response to TOL supplementation.

In conclusion, there is a high interindividual variability in TOL bioavailability, which is partly due to genetic variations. As suggested recently, future clinical studies designed to elucidate the impact of VE supplementation on various diseases should take into account the genetic characteristics of the subjects for a better interpretation of the results (6,9).

INNOVATION

Vitamin E (VE) supplementation has been hypothesized to reduce the incidence of oxidative stress-related diseases. However, results of RCTs do not support this hypothesis and show a very heterogeneous response to α-tocopherol (TOL) supplementation. We here show that TOL bioavailability is associated with the long term blood TOL status and that its variability is modulated, at least partly, by a combination of SNPs in 11 genes. These findings allow us to propose a model where the biological effects of TOL supplementation depend on one's individual genetic characteristics and could have significant implications in the design of future VE supplementation trials.

TABLES

Table 1. Characteristics of subjects included in the statistical analysis of the results[*]

Parameter	Mean ± SEM
Age (y)	33.3 ± 2.1
Weight (kg)	73.7 ± 1.3
BMI (kg/m²)	22.9 ± 0.4
Glucose (mmol/L) †	4.7 ± 0.1
Triacylglycerol (g/L) †	0.7 ± 0.1
Total cholesterol (g/L) †	1.7 ± 0.1
Hemoglobin (g/dL) †	15.1 ± 0.1
α-tocopherol (μmol/L) †	27.8 ± 1.0

[*] n = 38.

† Fasting plasma variables.

Table 2. Performances of different Partial Least Square regression models to explain the postprandial chylomicron α-tocopherol response [*]

VIP threshold	R^2	Q^2	SNPs no.	*P* (CV-ANOVA)	Average R^2 of 100 permuted models	Spearman's Rho
No selection	0.81	-0.10	3759	1	0.90	0.926
>0.5	0.99	0.75	1077	0.16	0.98	0.986
>1.0	0.90	0.79	342	6.24×10^{-6}	0.70	0.893
>1.5	0.97	0.83	142	1.04×10^{-4}	0.86	0.981
>1.6	0.91	0.78	123	5.71×10^{-6}	0.69	0.914
>1.7	0.90	0.79	102	1.54×10^{-6}	0.66	0.912
>1.8	0.85	0.78	78	3.09×10^{-8}	0.45	0.825
>1.9	0.84	0.77	72	2.51×10^{-8}	0.42	0.826
>2.0	0.83	0.75	61	1.84×10^{-8}	0.40	0.828
>2.1	0.83	0.76	54	2.12×10^{-8}	0.38	0.845
>2.2	0.82	0.74	44	2.09×10^{-8}	0.33	0.838
>2.3	0.82	0.74	41	1.93×10^{-8}	0.33	0.841
>2.35	0.82	0.74	36	1.81×10^{-8}	0.30	0.852
>2.4	0.79	0.70	32	8.61×10^{-8}	0.27	0.842
>2.5	0.77	0.68	30	1.36×10^{-7}	0.25	0.827
>2.6	0.73	0.65	21	1.04×10^{-6}	0.20	0.788

* Different partial least squares regression (PLS) models were built by using increasing VIP threshold values. We first tested the validity of the models using the permutation technique (see the **Notes section** for more details): we considered a PLS regression model as validated when the average of the R^2 values of 100 permuted models was less than half the R^2 value of the original model, indicating that more than half of the explained variability was not due to chance. Then, the validated model presenting the highest Spearman's Rho between the measured and the predicted area under the curve of the postprandial plasma chylomicron α-tocopherol concentration was selected. This is the model with VIP >2.35, shown in the table. Validation criteria and procedures of the PLS regression models are described **in the Notes section.** CV-ANOVA, ANOVA after cross-validation; Q^2, predicted variance; R^2, explained variance; SNP, single nucleotide polymorphism; VIP, variable importance in the projection

Table 3. Genes and single nucleotide polymorphisms associated with the postprandial chylomicron α-tocopherol response*

Gene and SNP rs no.	VIP value	SNP minor allele frequency	*q*†
APOB-rs4643493	3.66	0.103	0.006
LIPC-rs4238329	3.28	0.139	0.003
ABCA1-rs4149314	3.18	0.077	0.006
SLC10A2-rs1571513	3.09	0.242	0.006
LIPC-rs8041525	3.08	0.097	0.006
ZNF664-rs7296124	3.04	0.096	0.006
ABCA1-rs11789603	3.04	0.088	0.007
PNLIP-rs2915775	3.04	0.250	0.007
ABCA1-rs2274873	3.03	0.086	0.006
APOB-rs1042031	2.94	0.153	0.013
ZNF664-rs1048497	2.88	0.081	0.007
BET1-rs10464587	2.86	0.293	0.013
LIPC-rs7164909	2.83	0.144	0.006
LIPC-rs8035357	2.77	0.131	0.010
LIPC-rs12591216	2.71	0.096	0.011
PNLIP-rs3010494	2.67	0.282	0.018
ABCA1-rs4149297	2.67	0.088	0.026
SREBF2-rs2839715	2.59	0.148	0.017
APOB-rs1713222	2.58	0.152	0.019
SREBF2-rs4822062	2.57	0.132	0.015
SLC10A2-rs9558203	2.54	0.203	0.006
LIPC-rs12593880	2.51	0.075	0.011
SLC10A2-rs16961116	2.50	0.166	0.006
SLC10A2-rs12874168	2.49	0.209	0.027
SLC10A2-rs2065550	2.47	0.149	0.024
IRS1-rs1316328	2.38	0.142	0.006

NAT2-rs4921920	2.37	0.101	0.023
ABCG1-rs468320	2.35	0.218	0.026

* SNPs present in the selected partial least squares regression model shown in **Table 2**. SNPs are ranked by decreasing variable importance in the projection (VIP) value. Note that 8 of the 36 SNPs present in the selected model were in linkage disequilibrium. Because these SNPs provided redundant information in the model, we randomly kept one of each SNP (those presented in this table), in the final selected partial least squares regression model. S*ee* **the Notes section** for a complete list of gene names and symbols. rs, reference single nucleotide polymorphism; SNP, single nucleotide polymorphism; VIP, variable importance in the projection.

† Student's *t* test with the Benjamini-Hochberg correction was carried out to test differences between the postprandial chylomicron α-tocopherol response according to genotype groups for each SNP.

Table 4. Regression coefficients of the genetic score equation that aims to predict the α-tocopherol response of a genotyped subject to a α-tocopherol rich meal

Genes and SNP rs number	Base substitution	Homozygous 1*	Heterozygous	Homozygous 2
ABCA1-rs11789603	[C/T]	0.00	0.56	-0.42
ABCA1-rs2274873	[A/G]	0.00	1.11	-0.83
ABCA1-rs4149297	[C/T]	-0.34	0.49	0.00
ABCA1-rs4149314	[A/G]	-0.43	0.58	0.00
ABCG1-rs468320	[C/T]	-0.31	0.46	0.00
APOB-rs1042031	[A/G]	0.00	-0.43	0.58
APOB-rs1713222	[C/T]	0.00	-0.81	1.09
APOB-rs4643493	[C/T]	0.00	-0.57	0.71
BET1-rs10464587	[A/G]	0.00	-0.45	0.59
IRS1-rs1316328	[A/G]	-0.58	0.86	0.00
LIPC-rs12591216	[C/T]	1.67	-1.24	0.00
LIPC-rs12593880	[A/C]	0.00	-0.40	0.54
LIPC-rs4238329	[A/C]	-0.44	0.58	0.00
LIPC-rs7164909	[C/T]	0.00	0.51	-0.37
LIPC-rs8035357	[C/T]	-0.74	1.02	0.00
LIPC-rs8041525	[A/G]	0.62	-0.48	0.00
NAT2-rs4921920	[C/T]	-0.31	0.45	0.00
PNLIP-rs2915775	[A/G]	0.00	0.56	-0.42
PNLIP-rs3010494	[G/T]	-0.35	0.49	0.00
SLC10A2-rs12874168	[A/G]	0.00	0.49	-0.34
SLC10A2-rs1571513	[C/T]	-0.42	0.56	0.00
SLC10A2-rs16961116	[A/G]	0.00	0.90	-0.62
SLC10A2-rs2065550	[C/T]	0.00	0.48	-0.34
SLC10A2-rs9558203	[C/T]	0.00	0.47	-0.33
SREBF2-rs2839715	[C/T]	0.00	-0.78	1.07
SREBF2-rs4822062	[A/G]	0.00	-0.39	0.54
ZNF664-rs1048497	[A/G]	0.00	0.54	-0.40
ZNF664-rs7296124	[C/T]	-0.43	0.57	0.00

* For a given SNP, homozygous 1 refers to subjects homozygous for the first allele. The responder phenotype (RP), *i.e.* the incremental area under the curve of the postprandial plasma chylomicron α-tocopherol concentration, can be predicted as follows: $\widehat{RP} = 9.93 + \sum_1^{28} ri * genotype.(SNPi)$, where r_i is the regression coefficient of the i^{th} SNP included in the PLS regression model and *genotype.(SNP$_i$)* is a Boolean variable indicating the subject's genotype at the i^{th} SNP. A positive regression coefficient indicates that a genotype is associated with an increase in the postprandial chylomicron α-tocopherol response (*e.g.*

ABCA1- rs11789603 (CT)) whereas a negative regression coefficient indicates that a genotype is associated with a decrease in this response (*e.g. ABCA1*- rs11789603 (TT)). A regression coefficient of 0 for a given SNP (*e.g. ABCA1*- rs11789603 (CC)) indicates that a genotype was not carried by enough subjects to reach statistical significance in the selected PLS regression model.

FIGURES

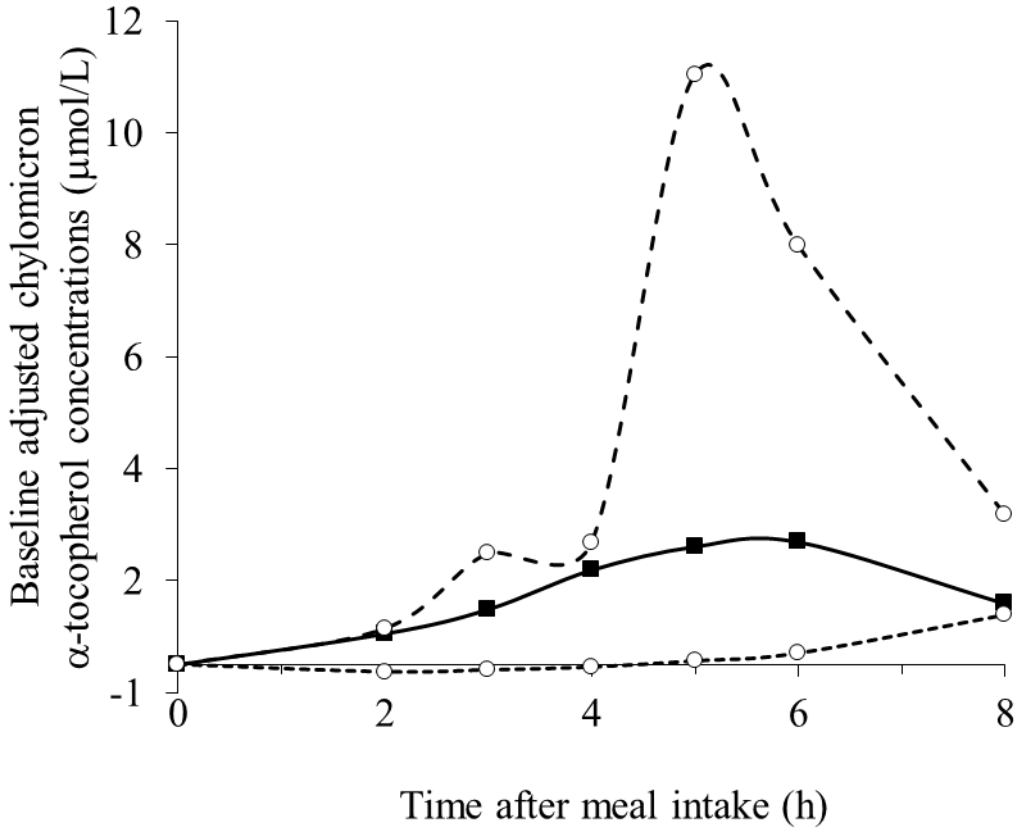

Figure 1: Baseline-adjusted chylomicron α-tocopherol concentration over 8 hours after consumption of the vitamin E-rich meal.

For each subject, postprandial chylomicron α-tocopherol (CM TOL) concentrations were baseline adjusted by using the fasting CM TOL concentration. The bold curve shows mean ± SEM of 38 subjects combined. The smaller dashed curve shows the concentration of CM TOL measured in the lowest single responder. The larger dashed curve shows the concentration of CM TOL measured in the highest single responder.

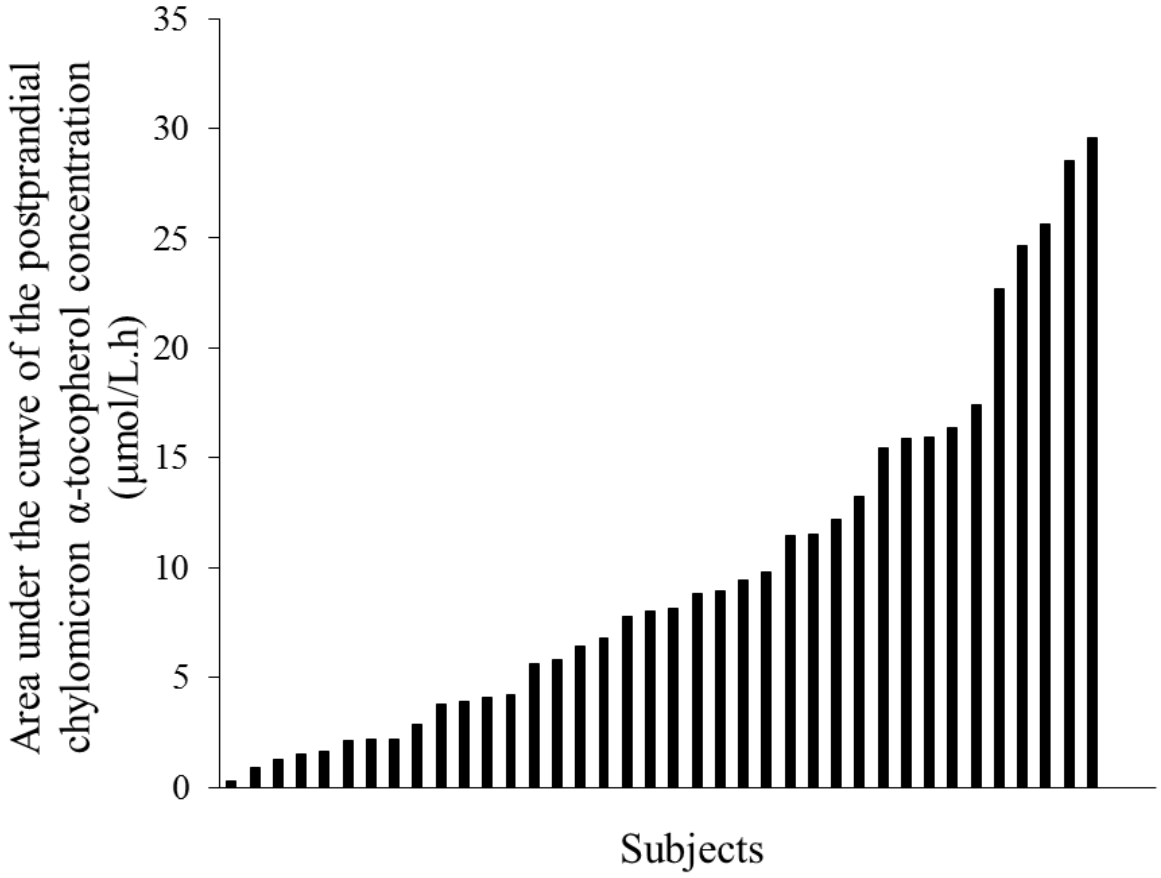

Figure 2: Individual AUCs of the postprandial chylomicron α-tocopherol response after consumption of the α-tocopherol rich meal.

Subjects were sorted by increasing postprandial chylomicron α-tocopherol response (i.e. 0-8 h AUC).

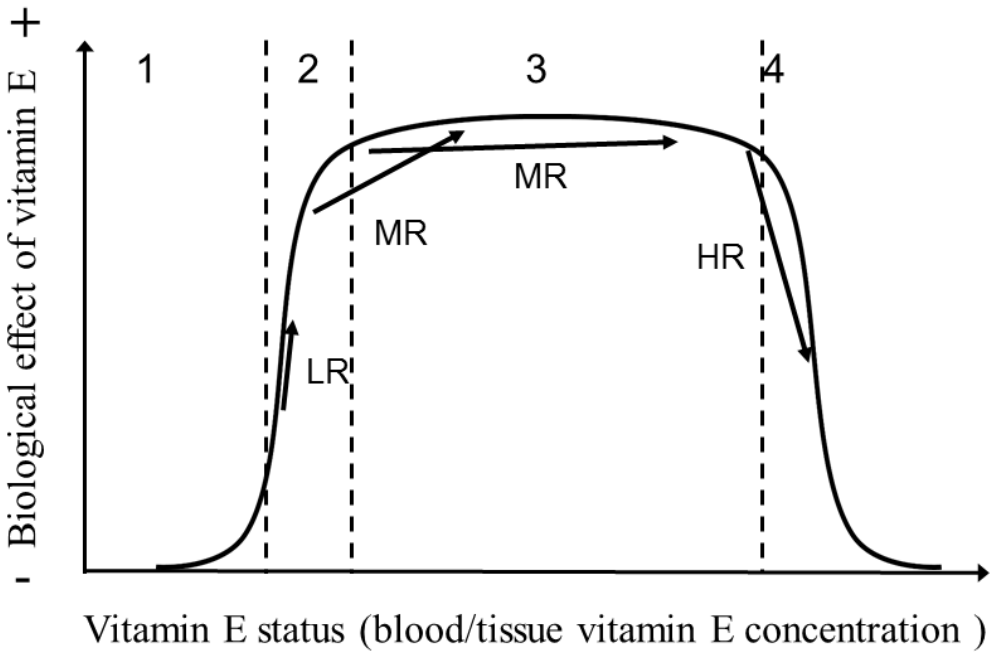

Figure 3: Proposed model of the effect of the interindividual differences in vitamin E bioavailability on the biological effects of vitamin E supplementation.

The curve shows the theoretical biological effects of vitamin E (VE) supplementation as a function of vitamin E status (i.e. blood and tissue concentration). We hypothesize that this model can be applied to the different forms of VE, e.g. tocopherols and tocotrienols. When VE concentration is below optimal levels (i.e. when the concentration falls in intervals 1 or 2), then the subject either displays symptoms of deficiency (interval 1) or does not fully experience the beneficial biological effects of VE (interval 2). When the VE concentration is optimal (interval 3), the maximum beneficial biological effects of VE are obtained. Finally, when VE concentration is above optimal (interval 4), adverse biological effects of VE can be observed. In studies with VE supplementation, several possibilities can take place depending on the VE responder characteristic of the subjects. Some examples are shown on the figure. The first example is a subject who is medium responder (MR) and who has a barely

suboptimal status (interval 2), in case of VE supplementation we hypothesize that he will benefit from the VE supplementation as his blood/tissue VE concentration can be expected to reach interval 3. A second example is a subject who is low responders (LR), in case of VE supplementation we hypothesize that he will not optimally benefit from the the supplementation because his VE status will not increase enough to reach interval 3. A third example with a subject who is high responder (HR) and who has an initial VE status close to interval 4. In that case there is a possibility that he might undergo adverse effect of VE supplementation because his blood/tissue VE concentration will reach the interval 4.

LIST OF ABBREVIATIONS

ABCG1 (ATP-binding cassette, sub-family G (WHITE), member 1); AUC (area under the curve); CM (chylomicron); CV (coefficient of variation); HPLC (high performance liquid chromatography); PNLIP (pancreatic lipase); PLS (partial least squares); SLC10A2 (solute carrier family 10 (sodium/bile acid cotransporter), member 2); SNPs (single nucleotide polymorphisms); SREBF2 (sterol regulatory element binding transcription factor 2); TOL (α-tocopherol); VE (vitamin E); VIP (variable importance in the projection).

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NOTES

Subject number and characteristics

Forty healthy, non-obese, non-smoking men were recruited for the study. This group of subjects included the individuals who were initially recruited to determine if a combination of SNPs significantly explained the postprandial CM triacylglycerol response to dietary fat. Subjects reported normal energy consumption (i.e. 2500 kcal/d) with <2% alcohol as total energy intake on a kcalorie basis. Subjects had no history of chronic disease, hyperlipidemia, or hyperglycemia and were not taking any medication known to affect VE absorption or lipid metabolism, e.g. tetrahydrolipstatin, ezetimibe, phytosterols, cholestyramine, fibrates…, during the month before the study or during the study period. Because of the relatively large volume of blood that was drawn during the study, blood hemoglobin concentration >1.3 g/L was an inclusion criteria. 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. Objectives and requirements of the study were fully explained to all participants before beginning the study, and written informed consent was obtained from each subject. Two subjects left the study for personal reasons before they participated in the postprandial experiment, which left 38 subjects whose baseline characteristics are reported in **Table 1.**

DNA preparation and genotyping methods

An average of 25 µg of DNA was isolated from a saliva sample from each subject using the Oragene kit (DNA Genotek Inc., Kanata, ON, Canada). DNA concentration and purity were checked by spectrophotometry (Nanodrop ND1000, Thermo Scientific, Villebon

sur Yvette, France) at 260 nm and 280 nm. All genotyping procedures were carried out by the Integragen company (Evry, France). The whole-genome was genotyped as follows: 200 ng of DNA was hybridized overnight to HumanOmniExpress BeadChips (Illumina, San Diego, CA, USA), which allowed for the analysis of approximately ~ 7.33 x 10^5 SNPs/DNA sample. Unhybridized and non-specifically hybridized DNA was then washed away. Afterward, the BeadChips were stained and scanned on an Illumina iScan scanner (Illumina). Detailed methods are provided in the Infinium HD Assay Ultra Protocol Guide (Illumina). Eleven additional SNPs were genotyped as previously described (refer to "Choice of candidate genes" below).

Postprandial experiment

Subjects were asked to refrain from the consumption of vitamin supplements and VE-rich foods (an exclusion list was provided by a dietitian) 48 h before the postprandial visit. In addition, the subjects were asked to eat dinner between 1900 and 2000 the day before the postprandial visit and to abstain from alcohol and any food or beverage other than water until they arrived at the clinic. After the overnight fast, subjects arrived at the local Center for Clinical Investigation (la Conception Hospital, Marseille, France) and consumed a meal including a VE supplement. The meal consisted of semolina (70 g) cooked in 200 mL of hot water, white bread (40 g), cooked egg whites (60 g), peanut oil (50 g), and mineral water (330 mL). The VE supplement was provided as a capsule containing 67 mg α-tocopherol (TOL) equivalent (100 IU) of d-α-tocopheryl acetate (Holland & Barrett, Nuneaton, Warwickshire, England). The pill also contained gelatin, glycerin and soy bean oil. Subjects were asked to consume the meal at a steady pace, with one-half of the meal consumed in 5 min and the remainder of the meal consumed within 10 min (to diminish the variability due to different rates of intake and, thus, gastric emptying). No other food was permitted over the next 8 h.

Subjects were only allowed to consume any remaining bottled water from the meal. A baseline blood sample was drawn before administration of the meal (i.e. in the fasted state) as well as at 2, 3, 4, 5, 6 and 8 h after meal consumption. Blood was taken up into evacuated tubes containing K-EDTA. Tubes were immediately placed into an ice-water bath and covered with aluminum foil to avoid light exposure. Plasma was isolated by centrifugation (10 min at 4 °C and 878 *g*) < 2 h after collection.

CM preparation

Plasma (6 mL) was overlaid with 0.9% NaCl solution (4.5 mL) and centrifuged for 28 min at 130,000 *g* at 10 °C using a SW41Ti rotor (Beckman Coulter, Villepinte, France) in a Thermo Sorvall WX100 ultracentrifuge (Thermo Scientific, Saint Herblain, France). The upper phase, containing primarily chylomicrons (CM) and large CM remnants, was collected. Immediately after recovery, CM were stored at -80 °C before TOL analysis.

Plasma and CM TOL extraction and analysis

All extractions were performed at room temperature under yellow light to minimize light-induced damage. Samples (0.5 mL for plasma or < 2 mL for CM) were deproteinated by adding one volume of ethanol containing tocopheryl nicotinate as an internal standard. After the addition of 2 volumes of hexane, the mixture was vortexed for 5 min and centrifuged at 1200*g* for 10 min at 4 °C. The upper phase (containing TOL) was collected, and the sample was extracted a second time with hexane following the same procedure. The hexane phases were pooled and evaporated to dryness using nitrogen gas. The dried extract was redissolved in 200 µL of methanol. A volume of 90 µL was injected into the HPLC system, which consisted of a separation module (P680 HPLC Pump and ASI-100 Automated Sample Injector, Dionex SA, Villebon sur Yvette, France) and a UVD340U photodiode array detector

(Dionex SA). Separation was achieved using a 10 mm x 4.0 mm Modulo-Cart guard column, with 2 µm particle size, (Interchim, Montluçon, France) followed by a 250 mm x 4.6 mm , 5-µm particle size C_{18} Zorbax Uptisphere column (Interchim). The isocratic mobile phase was composed of 100 % HPLC-grade methanol (Carlo Erba – SDS, Peypin, France) maintained at 35° C with a flow rate of 1.5 mL/min. TOL was detected at 290 nm and identified via spectra and a retention time coincident with authentic standard (Sigma-Aldrich, Saint Quentin Fallavier, France). Peaks were integrated using Chromeleon software (version 6.80, Dionex SA). Quantitation was performed using external calibration curves normalized to internal standard.

Calculations

Trapezoidal approximation was used to calculate the AUC of the postprandial plasma CM TOL concentration over 8 hours.

Choice of candidate genes

Candidate genes included those for which the encoded protein has been shown *in vitro* to be involved in TOL uptake by the enterocyte [i.e. scavenger receptor class B, member 1 and Niemann-Pick disease, type C1, gene-like 1]. They also included genes that have been suggested to be involved, directly or indirectly, in enterocyte TOL metabolism, e.g. liver-fatty acid binding protein, intestinal-fatty acid binding protein, and microsomal triglyceride transfer protein. Finally, they included genes that have been associated with circulating TOL concentration in genome-wide association studies or candidate gene association studies. This choice resulted in the selection of 59 genes (see **Notes Table 1 below**), representing 4474 SNPs. In addition, we added 29 SNPs in 15 genes that were shown to be associated with the postprandial CM triacylglycerol response in the same group of subjects (3). Indeed, CMs are

the main carrier of newly absorbed VE in the blood, and we hypothesized that genetic variants that affect the secretion and clearance of CMs in the postprandial period can also likely affect the postprandial blood response of VE. We also added 11 SNPs that have been previously associated with lipid metabolism and which were not genotyped on the BeadChips (see **Notes Table 2 below**). After the genotyping of the subjects (*see* DNA preparation and genotyping methods), SNPs for which the genotype call rate was <95%, or SNPs that presented a significant departure from the Hardy-Weinberg equilibrium ($P < 0.05$; Chi-squared test), were excluded from all subsequent analysis [745 SNPs excluded, leaving 3769 SNPs for the PLS regression analysis].

Notes Table 1. Candidate genes selected

Gene name	Gene symbol
Genes that are assumed to play a role in α-tocopherol metabolism or that have been associated with blood α-tocopherol concentration[a]	
ATP-binding cassette, sub-family A (ABC1), member 1	*ABCA1**
Apolipoprotein A-I	*APOA1**
Apolipoprotein A-IV	*APOA4*
Apolipoprotein A-V	*APOA5**
Apolipoprotein B	*APOB**
Apolipoprotein C-I	*APOC1*
Apolipoprotein C-II	*APOC2*
Apolipoprotein C-III	*APOC3*
Apolipoprotein C-IV	*APOC4*
Apolipoprotein E	*APOE*
ATP-binding cassette, sub-family B (MDR/TAP), member 1 (PGP, MDR1)	*ABCB1*
ATP-binding cassette, sub-family G (WHITE), member 1	*ABCG1*
ATP-binding cassette, sub-family G (WHITE), member 2	*ABCG2*
ATP-binding cassette, sub-family G (WHITE), member 5	*ABCG5*
ATP-binding cassette, sub-family G (WHITE), member 8	*ABCG8*
Bile acid CoA: amino acid N-acyltransferase (glycine N-choloyltransferase)	*BAAT*
BUD13 homolog (S. cerevisiae)	*BUD13*
Carboxyl ester lipase	*CEL*
CD36 molecule (thrombospondin receptor)	*CD36**
Colipase, pancreatic	*CLPS*
Cytochrome P450, family 3, subfamily A, polypeptide 4	*CYP3A4*

Cytochrome P450, family 3, subfamily A, polypeptide 5	*CYP3A5*
Cytochrome P450, family 4, subfamily F, polypeptide 2	*CYP4F2*
Fatty acid binding protein 1, liver	*FABP1*
Fatty acid binding protein 2, intestinal	*FABP2*
Fatty acid binding protein 6, ileal (I-BABP)	*FABP6*
Intestine Specific Homebox	*ISX*
Lipase, gastric	*LIPF*
Lipase, hepatic	*LIPC**
Lipoprotein lipase	*LPL**
Microsomal Triglyceride Transfer Protein	*MTTP*
Na+/K+ transporting ATPase interacting 3	*NKAIN3*
Niemann-Pick disease, type C1	*NPC1*
Niemann-Pick disease, type C2	*NPC2*
NPC1-like 1	*NPC1L1*
Nuclear receptor subfamily 1, group H, member 2 (LXRβ)	*NR1H2*
Nuclear receptor subfamily 1, group H, member 3 (LXRα)	*NR1H3*
Nuclear receptor subfamily 1, group H, member 4 (FXR)	*NR1H4*
Nuclear receptor subfamily 1, group I, member 2 (PXR)	*NR1I2*
Pancreatic lipase	*PNLIP*
Pancreatic lipase-related protein 2	*PNLIPRP2*
Peroxisome proliferator-activated receptor alpha	*PPARA*
SAR1 homolog B (S. cerevisiae)	*SAR1B*
Scavenger receptor class B, member 1	*SCARB1*
SEC14-like 2 (S. cerevisiae)	*SEC14L2*
SEC14-like 3 (S. cerevisiae)	*SEC14L3*
SEC14-like 4 (S. cerevisiae)	*SEC14L4*
Solute carrier family 10 (sodium/bile acid cotransporter), member 2 (ASBT, IBAT)	*SLC10A2*
Solute carrier family 27 (fatty acid transporter), member 5	*SLC27A5**
Solute carrier family 51, alpha subunit	*SLC51A*
Solute carrier family 51, beta subunit	*SLC51B*
Solute carrier organic anion transporter family, member 1A2 (OATP)(Na^+-independent)	*SLCO1A2*
Sterol carrier protein 2	*SCP2*
Sterol regulatory element binding transcription factor 2	*SREBF2*
Tocopherol (alpha) transfer protein	*TTPA*
Transmembrane 6 superfamily member 2	*TM6SF2*
UDP glucuronosyltransferase 1 family, polypeptide A1	*UGT1A1*
UDP glucuronosyltransferase 1 family, polypeptide A10	*UGT1A10*
Zinc finger protein 259	*ZNF259*

[a] The candidate genes included those whose encoded proteins have been shown by *in vitro* methods to be involved in α-tocopherol uptake by the enterocyte, genes that are suspected to be involved, directly or indirectly, in α-tocopherol metabolism, e.g. genes involved in bile salt

metabolism or transcription factors involved in the expression of proteins involved in α-tocopherol metabolism, and genes that have been associated in genome-wide association studies or candidate gene association studies with blood α-tocopherol concentration.* genes that have been involved in postprandial chylomicron triacylglycerol response in the same group of subjects (3).

Note Table 2: Additional candidate SNPs selected[1].

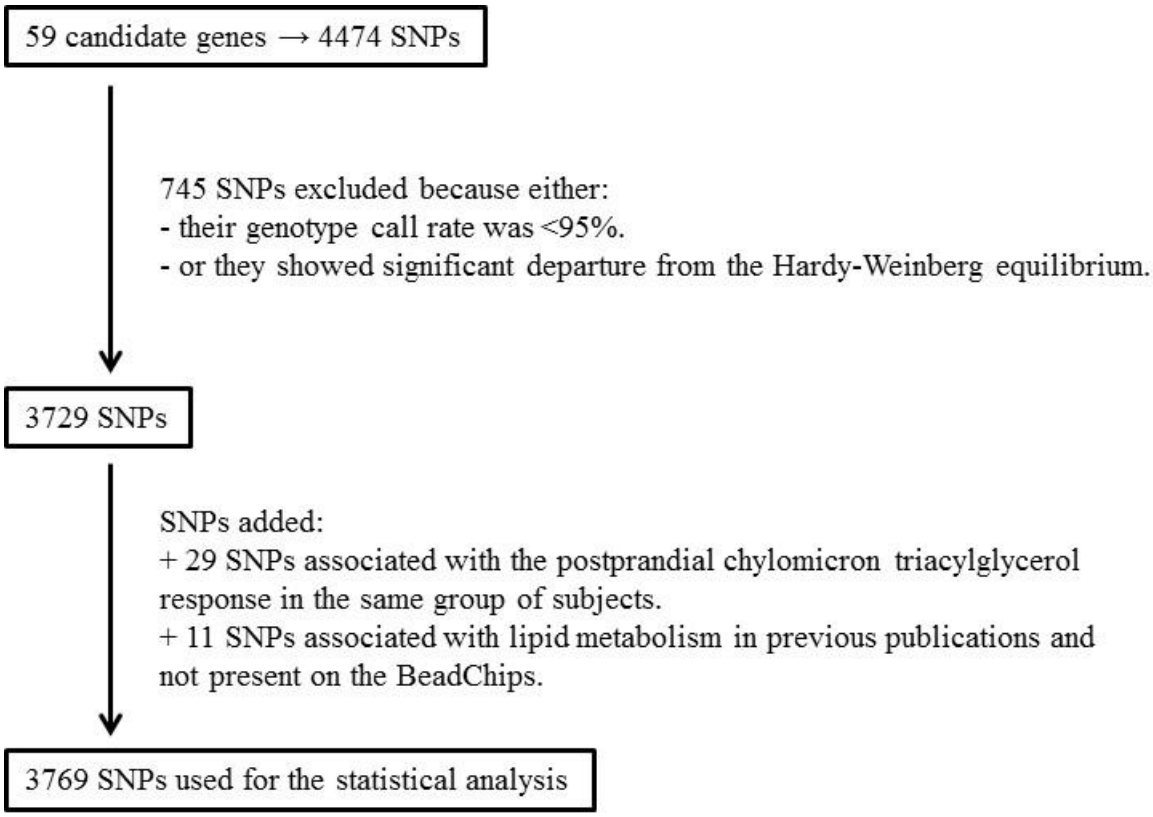
Gene name	Gene symbol	SNP
ATP-binding cassette, sub-family A (ABC1), member 1	*ABCA1*	rs2066718*
		rs2230805*
		rs2230806*
		rs2230808*
		rs2472458*
		rs4149313*
		rs7862756*
ATP-binding cassette, sub-family G, member 2	*ABCG2*	rs2231142
		rs4148152*
ATP-binding cassette, sub-family G (WHITE), member 5	*ABCG5*	rs17031672*
		rs6720173*
ATP-binding cassette, sub-family G (WHITE), member 8	*ABCG8*	rs11887534*
		rs147194762*
		rs4148211*
		rs41448217*
		rs6544718*
CD36 molecule (thrombospondin receptor)	*CD36*	rs13230419*
		rs1527479
		rs1527483
		rs1761667
		rs1984112
		rs3211805
Fatty acid binding protein 1, liver	*FABP1*	rs1545223*
		rs1545224*
		rs2197076
		rs224188*
		rs2919871
Microsomal Triglyceride Transfer Protein	*MTTP*	rs1800591
		rs2255119*
		rs745075*
		rs881980*

NPC1 (Niemann-Pick disease, type C1, gene)-like 1	*NPC1L1*	rs17655652* rs217428 rs217434*
Scavenger receptor class B, member 1	*SCARB1*	rs4238001* rs5888* rs61932577

[1]These SNPs have been selected because previous publications have shown that these SNPs, or SNPs in linkage disequilibrium with some of them, have a phenotypic effect on lipid metabolism. * SNPs excluded from all statistical analysis because either their genotype call rate was <95%, or they presented a significant departure from Hardy-Weinberg equilibrium (P<0.05 following the Chi-squared test), or only one genotype for the SNP was present in the studied population, or they were already genotyped on the HumanOmniExpress BeadChips.

Selection of the SNPs used in the statistical analysis

The following scheme explains how we arrived to the 3769 SNPs used in the PLS regression.



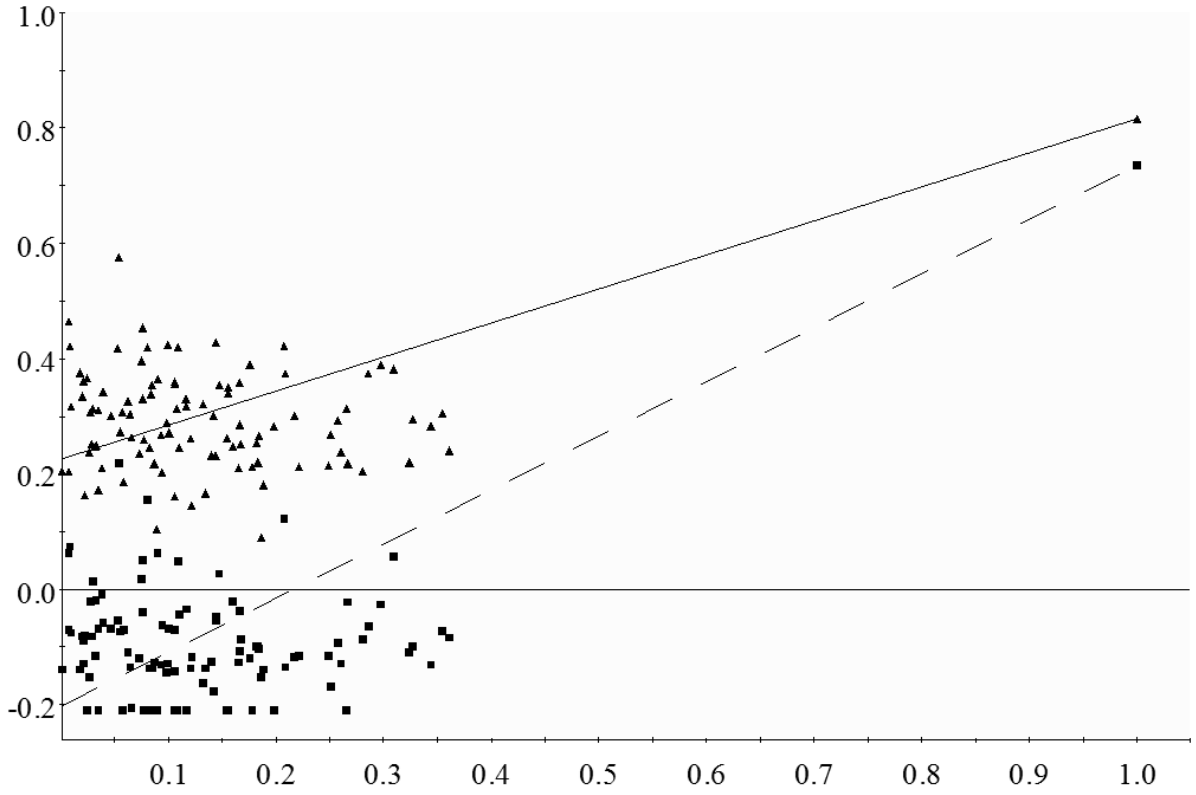
Statistics: PLS regression

To identify SNPs associated with the variability observed in the TOL response, we used PLS regression using previously published rationale and model assumptions. SIMCA-P12 software (Umetrics, Umeå, Sweden) was used for all multivariate data analyses and modeling. PLS regression was chosen to identify SNPs (of the 3769 candidate SNPs) that were predictive of the postprandial CM TOL response according to their VIP value. A general genetic model was assumed (i.e. the 3 genotypes of each SNP were treated as separate categories with no assumption made about the effect conferred by the variant allele for homozygotes or heterozygotes on the postprandial CM TOL response). Different PLS regression models were built using increasing VIP threshold values. The validated model presenting the highest Spearman's Rho between the measured and the predicted postprandial CM TOL response was selected. The model explained 72.2% of the genotypic variable variation (R^2X), and 81.6% of

the phenotypic variation (R^2Y) (prediction value Q^2= 73.6% after cross-validation). Four procedures of validation were applied to validate the model :

1. *Permutation technique*

This procedure 1) assesses the risk that the PLS regression model is spurious, i.e. the model fits the current data set well but does not predict Y well for new observations, and 2) tests for overfitting. For this, the accuracy of fit (R^2 and Q^2) of the original model was compared with the accuracy of fit of 100 models based on data where the order of the Y matrix for the subjects (postprandial CM TOL response) was randomly permuted, while the X matrix (the genotype for the selected SNPs of each subjects) was kept intact. Thus, a robust model (where the fit between X and Y is high) should be unable to predict the permuted Y variables with the intact X variables. We considered a PLS regression model as validated when the average of the R^2 values of the 100 permuted models was less than half the R^2 value of the original model, indicating that more than half of the explained variability was not due to chance. **Note Figure 1** (below) shows the results of these permutations for the selected PLS regression model.



Note Figure 1. The horizontal axis represents the correlation between the permuted Y's and the original Y's. The vertical axis represents the R^2 (solid line and triangles) and Q^2 (dashed line and squares) values obtained in the permuted models. Values of the original model are on the far right (at correlation = 1), values of the 100 Y-permuted models are further to the left. Note that all the R^2 and Q^2 values of the permuted models are lower than the R^2 and Q^2 values of the original non permuted model. This strongly supports the conclusion that the ability of the original, non-permuted model to predict the phenotype was not due to chance.

2. Single cross-validation

In this second validation procedure, 4-5 subjects were kept out of model development, their postprandial CM TOL response was then predicted by the model and subsequently compared with their measured postprandial CM TOL response. In this test, the predicted response values (Q^2, fraction of the total variation of the response Y that can be predicted) should be

close to the measured values (R^2), with predicted values Q^2>0.5 (50%). This validation was repeated until all subjects were left out once and only once. *P*-values after cross-validation ANOVA were calculated.

3. Leave-k-out

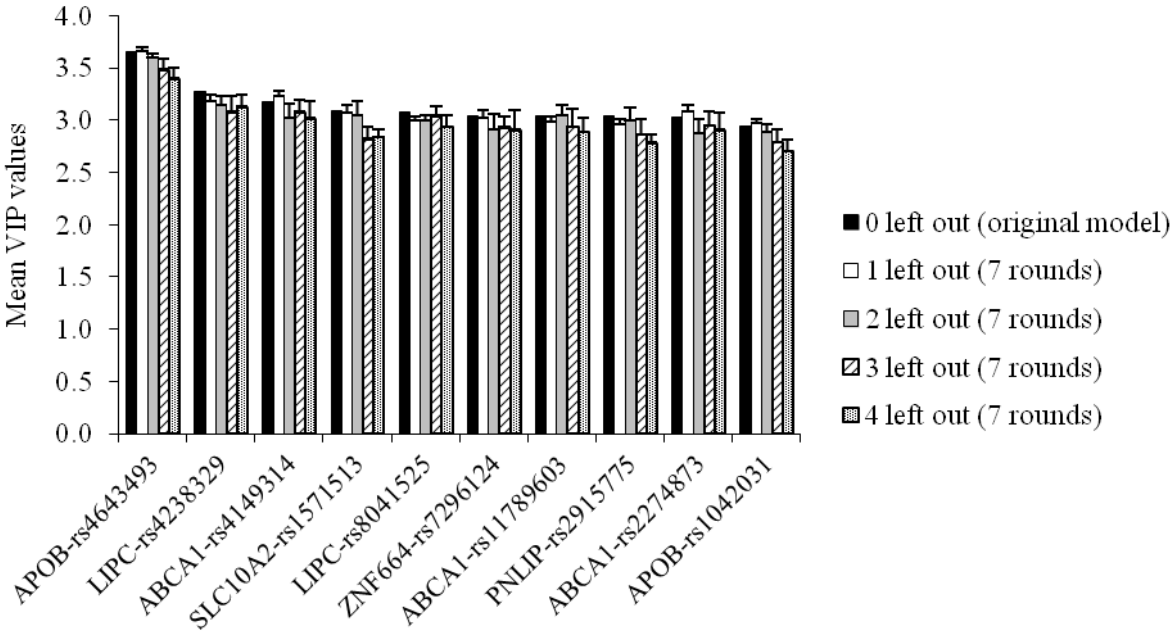
In this third validation procedure, we challenged our PLS regression model by randomly taking out k subjects (k = {1, 2, 3, 4}) from the original dataset, thus leaving a training dataset. The k subjects taken out were then reintroduced into this training set to assess whether the training models built without these k subjects were able to predict their postprandial CM TOL response accurately. This test was performed thirty eight times for k=1 and eight times for k = {2, 3, 4}. The Spearman's Rho between the measured and the predicted AUC of the postprandial plasma CM TOL concentration of the left out subjects is shown in **Notes Table 2**. The correlation coefficient was >80%, even when up to four subjects were left out of the model.

Notes Table 3. Relative prediction error following the leave-k-out procedure

	Number of subjects left out				
	0	1	2	3	4
Relative prediction error (%)	85.2	81.0	86.2	84.4	84.0

4. VIP stability following the leave-k-out procedure

In this last procedure, we checked that the top 10 SNPs (top 10 VIP values in **Table 3**) remained unchanged following the leave-k-out procedure described above. **Notes Figure 2** (below) shows good stability of the selected model with this validation (no variables missing across the validation rounds, similar 'weight' in the response).



Statistics: univariate analyses

In a second approach, we performed univariate analyses to compare the postprandial CM TOL response between subgroups of subjects who bore different genotypes for SNPs selected from the PLS regression model. Differences obtained in the different genotype subgroups were analyzed using a Student's *t* test with the Benjamini-Hochberg correction with QVALUE software (version 1.0, designed by researcher) and R software (version 3.0.2, R foundation of statistical computing). For all tests, a false discovery rate $q < 0.05$ was considered significant. Results are shown in **Table 3**.

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AUTHORS' CONTRIBUTIONS TO MANUSCRIPT:

PB (UMR INRA 1260 NORT) designed the research; MN (UMR INRA 1260 NORT) conducted the clinical research; RB (UMR INRA 1260 NORT) and FT (UMR INRA 1260 NORT) analysed α-tocopherol by HPLC; CD (UMR INRA 1260 NORT) and PB analyzed data; CD performed statistical analyses; PB and CD wrote the paper and had primary responsibility for final content of the manuscript.