BMJ Nutrition, Prevention & Health

Haptoglobin polymorphism, vitamin E and mortality: the Ludwigshafen Risk and Cardiovascular Health Study

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To cite: Delgado GE, Kleber ME, Moissl AP, *et al*. Haptoglobin polymorphism, vitamin E and mortality: the Ludwigshafen Risk and Cardiovascular Health Study. *BMJ Nutrition, Prevention & Health* 2024;**0**:e001061. doi:10.1136/bmjnph-2024-001061

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/bmjnph-2024-001061).

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Received 14 September 2024
Accepted 30 September 2024

ABSTRACT

Objective In humans, haptoglobin (Hp) exists in two allelic forms, Hp1 and Hp2, that differ significantly in their ability to protect the organism from oxidative stress. It has been proposed that in patients with diabetes mellitus carriers of the Hp2-2 genotype may benefit from vitamin E supplementation. Aim of our study was to investigate if there is evidence regarding a potential interaction between the Hp polymorphism and vitamin E with regard to mortality in individuals at medium-to-high cardiovascular risk with and without diabetes mellitus.

Research design and methods Data from 3176 participants of the Ludwigshafen Risk and Cardiovascular Health study, a monocentric hospital-based study of patients referred for coronary angiography, were analysed using Cox proportional hazard regression.

Results Participants with the Hp2-2 genotype demonstrated significantly lower Hp levels, while carriers of at least one Hp-2 allele displayed elevated levels of the inflammatory markers high-sensitive C reactive protein and serum amyloid A. No notable differences in comorbidities were observed among the various HP genotype groups. While the HP genotype showed no direct association with mortality, a borderline significant correlation between α-tocopherol plasma concentration and overall mortality was noted. An interaction between vitamin E status and the HP genotype regarding mortality risk was evident, particularly among patients with diabetes mellitus, with a p value of 0.021 for the interaction term. In restricted cubic splice analysis, patients with diabetes mellitus who are carriers of the Hp2-2 genotype seem to benefit from higher γ-tocopherol concentrations whereas for the other genotype groups there was a direct association with mortality risk.

Conclusion Particularly in patients with diabetes mellitus we could show a significant interaction of γ-tocopherol plasma concentration and HP genotype. Carriers of the Hp2-2 genotype seemed to benefit from higher plasma concentrations of γ-tocopherol. Further research is warranted to elucidate the underlying mechanisms and potential therapeutic implications in cardiovascular disease management.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The role of the HP genotype in patients with diabetes mellitus is unclear. It has been proposed that in patients with diabetes mellitus carriers of the Hp2-2 genotype may benefit from vitamin E supplementation.

WHAT THIS STUDY ADDS

⇒ An interaction between vitamin E status and the HP genotype regarding mortality risk was observed in a cohort of patients with medium-to-high cardiovascular risk, especially in patients with diabetes mellitus and the Hp2-2 genotype, who seemed to benefit from higher plasma concentrations of γ-tocopherol.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE AND POLICY

⇒ There may be a beneficial effect of vitamin E supplementation for patients with diabetes mellitus who are carriers of the Hp2-2 genotype. We recommend testing the Hp genotype prior to supplementation.

INTRODUCTION

Haptoglobin (Hp) is a positive acute-phase protein and its main task is the binding of free haemoglobin (Hb) in order to prevent iron loss, oxidative damage and especially kidney damage during haemolysis. The Hp-Hb complex is rapidly cleared by macrophages via the CD163 receptor. This leads to anti-inflammatory signalling, offering protection against inflammation and oxidative stress and thereby preventing low-density lipoprotein (LDL) and high-density lipoprotein (HDL) oxidation, endothelial dysfunction and atherosclerosis.[1]

In humans, Hp exists in two allelic forms, Hp1 and Hp2 resulting in three different genotypes: Hp1-1, Hp2-1 and Hp2-2. These genotypes arise as products of two closely related genes called HP1 and HP2.[2] The allele frequency varies considerably among different ethnicities with 7% for the Hp1 allele in Calcutta to ~40% in Europe, ~25% in Asia and >70% in parts of West Africa and South America.[3] The product of the HP1

gene is monovalent and forms dimers in the blood, while the product of HP2 is bivalent, and forms polymers of 2–10 covalently linked monomers.[4] In Hp2-2 individuals, this leads to the formation of cyclic polymers, in Hp2-1 individuals to the formation of linear chains.[4]

The three HP isoforms are associated with different capacities to bind Hb as well as different concentrations of Hp.[5] Total Hp concentrations are lower in individuals carrying the Hp2-2 genotype than in individuals with the Hp1-1 or the 2-1 genotype[6–8] and Hp2-2 also exhibits the weakest affinity for Hb.

The HP phenotypes also differ significantly in their ability to protect the organism from oxidative stress and resulting vascular damage. This may be most relevant to conditions like diabetes mellitus, which is characterised by a marked increase in reactive oxygen species in the hyperglycaemic state. In the ACCORD trial, intensive glucose-lowering was effective for preventing incident coronary heart disease and cardiovascular events in participants with the Hp2-2 phenotype, but not in Hp1 carriers, who had increased mortality risk during intensive therapy.[9] A recent study demonstrated that a high glycaemic variability, assessed by using a continuous glucose monitoring system for 3 consecutive days, was associated with a higher prevalence of diabetic macroangiopathy in Hp1 carriers, but not in those with Hp2-2 genotype.[10]

Supplementation with antioxidants has widely been investigated as an approach to provide protection from cardiovascular events, especially also in patients suffering from diabetes mellitus. The preferred antioxidant has been vitamin E because (a) it is recycled into its reduced form by other antioxidants,[11] (b) it is well known that vitamin E protects LDL from oxidation[11] and (c) high dietary vitamin E intake is inversely associated with cardiovascular risk.[12]

A number of clinical trials however failed to provide evidence for a cardiovascular benefit of vitamin E supplementation,[13] a notable exception being the SPACE trial that showed that haemodialysis patients with preexisting cardiovascular disease and an exceedingly high level of oxidative stress benefited from 800 IU of vitamin E per day.[14]

A meta-analysis of two large vitamin E supplementation trials, HOPE and ICARE, demonstrated that vitamin E could significantly reduce the composite endpoint of CV death, MI and stroke in Hp2-2 carriers with diabetes mellitus (OR 0.58, CI 0.4 to 0.86), while having no effect in diabetic patients with Hp1-1 or Hp2-1 genotype.[15]

The aim of this study was to examine the association of the Hp polymorphism with mortality in a population of medium-to-high cardiovascular risk and further investigate a possible interaction of the polymorphism with vitamin E with regard to mortality risk, particularly in patients with diabetes mellitus.

Research design and methods

Subjects

The Ludwigshafen Risk and Cardiovascular Health (LURIC) study enrolled 3316 individuals between 1997 and 2000 at the Ludwigshafen Heart Center in South-West Germany.[16] All participants were of European ancestry and were referred to elective coronary angiography for the evaluation of established or suspected coronary artery disease (CAD). For 3177 study participants, data on the HP polymorphism was available. One participant was excluded due to an implausibly high Hp concentration of >1000 mg/dL leaving a total of 3176 participants for analyses.

Follow-up on mortality

Information on vital status was obtained from local registries. Death certificates, medical records of local hospitals and autopsy data were reviewed independently by two experienced clinicians who were blinded to patient characteristics and who classified the causes of death. During a median follow-up of 9.9 years (8.5–10.7) 958 (30.2%) LURIC participants died, 605 from cardiovascular causes. Death from cardiovascular causes included sudden cardiac death, fatal myocardial infarction, death due to heart failure, death after intervention to treat CAD, stroke and other deaths due to heart diseases. In cases of disagreement or uncertainty concerning the cause of death, the decision was made by a principal investigator (WM).

Laboratory

Fasting blood samples were obtained by venipuncture at the study entry. A summary of analytic methods has been reported previously.[16] Cholesterol and triglycerides were measured with enzymatic reagents from WAKO (Neuss, Germany) on an Olympus AU640 analyser; apolipoproteins A1 and B by turbidimetry with reagents from Greiner (Flacht, Germany). Lipoproteins were separated with a combined ultracentrifugation-precipitation method (β-quantification).

Serum creatinine was determined using the Jaffé method (Roche, Mannheim, Germany) on a Hitachi 717 Analyzer. Cystatin C (N LATEX Cystatin C), highsensitive C reactive protein (hsCRP (N High Sensitivity CRP) and serum amyloid A (N LATEX SAA) were assessed using nephelometric assays on a nephelometer II (Dade Behring GmbH, Marburg, Germany). ELISAs (R&D Systems, Minneapolis, MN) were used to measure plasma levels of interleukin (IL)-6. Fibrinogen was determined using the Clauss method (STA fibrinogen, Roche, Mannheim, Germany). Gamma-glutamyl transferase, aspartate aminotransferase, alanine aminotransferase and glucose were measured using enzymatic reagents (Roche Diagnostics, Mannheim, Germany). Plasma concentrations of α-tocopherol and γ-tocopherol concentrations were determined by reversed-phase high-performance liquid chromatography with fluorescence detection.[17 18]

Within-day reproducibility (CV) was 2.3% for α-tocopherol and 2.4% for γ-tocopherol.

Cholesterol efflux capacity was quantified in blood samples as previously described.[19] Hp was measured using N antisera to human transferrin, Hp, ceruloplasmin and haemopexin (Dade Behring GmbH) on a Behring nephelometer analyser. Hp genotyping was done using an exonuclease (TaqMan) assay as previously described.[20]

Definition of clinical variables and endpoints

The presence of a visible luminal narrowing (>20% stenosis) in at least one of 15 coronary segments was used to define CAD according to the classification of the American Heart Association.[16] Diabetes mellitus was defined according to 2010 guidelines of the American Diabetes Association as increased fasting (≥126 mg/dL) and/or post-challenge (2 hours after the 75 g glucose load >200 mg/dL) glucose and/or elevated glycated Hb (>6.5%) and/or history of diabetes mellitus. Hypertension was defined as a systolic and/or diastolic blood pressure ≥140 and/or ≥90 mm Hg or a history of hypertension. The glomerular filtration rate was estimated by using the 2012 CKD-EPI eGFRcreat-cys equation.[21]

Statistical analyses

The cohort was stratified for Hp genotype. Continuous data are presented as the means and SD when normally distributed or as the medians and 25th and 75th percentiles for non-normally distributed variables. Categorical data are presented as percentages. Statistical differences of continuous variables between groups were determined using analysis of variance. Non-normally distributed variables were log-transformed before entering analysis. The χ^2 test was used for categorical variables. Missing values for covariates were imputed using the R-package 'Hmisc' (V.5.1-2). The association between Hp and coronary artery disease was analysed by t-test using the R-package 'ggstatsplot' (V.0.12.3).

Kaplan-Meier curves were drawn using the R-package 'survminer' (V.0.4.9). Cox proportional hazard models were built to assess the effect of vitamin E concentration or the Hp polymorphism on all-cause mortality and cardiovascular mortality. The proportional hazard assumption was checked by examination of scaled Schoenfeld residuals. Besides crude analyses a model adjust for age and sex was calculated. In order to test for interaction effects, a Cox regression model including an interaction term for a-/g-tocopherol and HP genotype was calculated. For the generation of HR plots, vitamin E concentration was modelled as restricted cubic spline. All tests were two-sided and a p value <0.05 was considered statistically significant. All analyses were carried out using R V.4.4.1 (http://www.r-project.org[22]) and SPSS (V.27.0.0, IBM). HR plots were drawn using the R-package 'rms' (V.6.80).

Data and resource availability

Due to the statutes of the LURIC Study GmbH, which must recognise the Federal Data Protection Act and the consent of the study participants, the data cannot be disclosed to the public.

The use of the (LURIC) study database is governed by the statutes of the non-profit LURIC Study GmbH, which is registered under the number HRB 7668 in the commercial register of Freiburg im Breisgau, Germany.

According to the organisation's statutes, data may be made available to researchers on request and approval; such requests may not be unreasonably denied. This procedure means the data cannot be publicly released without formal consent. It ensures that the rules of good scientific practice are followed and that the people responsible for the study's design and organisation are named.

Interested researchers are invited to address their queries or suggestions to Kai Grunwald (kai.grunwald@weitnauer.net) or the principal investigator of the LURIC study, WM (winfried.maerz@luric-online.de). Finally, the authors confirm that they have accessed these data with permission from LURIC and that all other researchers can access the data the same way as the authors.

RESULTS

Characteristics of the study participants, stratified for Hp genotype are shown in table 1. As expected, participants carrying the Hp2-2 genotype had significantly lower levels of Hp. The distributions of Hp in the three genotype groups are also shown in online supplemental figure S1. Carriers of at least one Hp-2 allele (Hp2-1 and Hp2-2) showed higher levels of the inflammatory markers hsCRP and SAA as compared with participants homozygous for the Hp1 allele. For IL-6 and fibrinogen, a similar trend was observed, however it did not reach statistical significance. For leucocytes and all the other biomarkers examined no significant differences were observed. Regarding comorbidities, no difference was observed between the three genotype groups. Whereas there was no association of the HP genotype with coronary artery disease, measured Hp levels were significantly increased in patients with coronary artery disease (online supplemental figure S2).

Association of HP genotype and vitamin E with mortality

During a median follow-up of 9.9 (8.5–10.7) years, 958 (30.2%) study participants died, 605 (19.2%) from cardiovascular causes. Mortality rates were 30.2%, 31.3% and 28.7% for Hp1-1, Hp1-2 and Hp2-2, respectively. In Kaplan-Meier analyses, the HP genotype was not significantly associated with all-cause or cardiovascular mortality in our cohort, also not in the subgroup with diabetes mellitus (online supplemental figures 3–5). Regarding vitamin E, we observed a borderline significant association of α-tocopherol concentration with all-cause mortality with the first tertile showing the highest risk (online supplemental figure 6). For γ-tocopherol concentration, no significant association with mortality was observed (online supplemental figure 7).

Table 1 Study demographics according to haptoglobin genotype (mean and SD or median (25th to 75th percentile)

Variable	Hp1-1 (N=473)	Hp2-1 (N=1506)	Hp2-2 (N=1197)	P value*
Age (years)	62.5 (10.8)	62.8 (10.6)	62.8 (10.7)	0.851
Female sex (%)	29.6	30.1	30.4	0.947
BMI (kg/m^2)	27.5 (4.14)	27.4 (3.97)	27.6 (4.2)	0.630
Total-C (mg/dL)	193 (40.8)	191 (38.1)	193 (39.9)	0.522
LDL-C (mg/dL)	118 (36.4)	116 (33.3)	116 (34.7)	0.502
HDL-C (mg/dL)	38.6 (11)	38.3 (10.3)	39.2 (11.3)	0.078
Apo-A1 (mg/dL)	130 (25.4)	129 (24.1)	130 (26)	0.214
Apo-A2 (mg/dL)	41.6 (9.21)	41.2 (9.17)	42.0 (9.89)	0.135
Cholesterol efflux (%)	86.3 (27)	89.4 (27.5)	86.3 (26.6)	0.006
FC/CE	0.371 (0.351–0.397)	0.374 (0.352–0.399)	0.374 (0.352–0.4)	0.531
CETP (µg/mL)	1.07 (0.82–1.43)	1.07 (0.82–1.43)	1.11 (0.861–1.46)	0.055
TG (mg/dL)	149 (112–201)	148 (109–203)	145 (108–196)	0.864
FGLUC (mg/dL)	105 (95.4–119)	102 (94–118)	101 (92.7–118)	0.162
HbA1c (%)	6.31 (1.31)	6.31 (1.23)	6.31 (1.25)	0.998
Systolic BP (mm Hg)	142 (24.1)	141 (23.9)	141 (23.1)	0.903
Diastolic BP (mm Hg)	81.2 (11.8)	80.9 (11.4)	81.1 (11.3)	0.787
eGFR (mL/min/1.73m^2)	80.8 (21.4)	81.5 (19.8)	82.2 (20.4)	0.413
hsCRP (mg/L)	2.71 (1.21–7)	3.49 (1.35–8.83)	3.54 (1.28–8.59)	0.032
IL-6 (mg/L)	2.86 (1.68–5.39)	3.24 (1.8–6.47)	3.29 (1.91–5.93)	0.058
SAA (mg/L)	4.4 (2.5–8.5)	5.3 (2.82–13.4)	5.2 (3–12.2)	0.002
Fibrinogen (mg/dL)	369 (316-438)	374 (316-449)	380 (325-453)	0.065
Leucocytes (/nL)	6.7 (5.7–8.1)	6.75 (5.6–8.1)	6.72 (5.7–8.2)	0.638
NT-proBNP (pg/mL)	323 (109–940)	288 (109–870)	307 (104–894)	0.914
Haptoglobin (mg/dL)	167 (141–198)	159 (121–206)	126 (89–174)	<0.001
GGT (U/L)	17 (10–29)	17 (10–29)	17 (11–29)	0.909
GPT (U/L)	12 (9–19)	13 (9–19)	13 (9–19)	0.764
α-tocopherol (µmol/L)	31.7 (26.8–36.9)	31.2 (26.7–37.6)	31.6 (27.1–37.9)	0.875
α-Tocopherol/cholesterol	5.9 (5.18–6.74)	5.93 (5.22–6.88)	5.95 (5.24–6.85)	0.808
γ-tocopherol (µmol/L)	1.49 (1.12–2.04)	1.47 (1.1–2.02)	1.45 (1.08–2.02)	0.535
γ-tocopherol/cholesterol	0.278 (0.211–0.382)	0.278 (0.214–0.372)	0.276 (0.209–0.376)	0.564
Coronary artery disease (%)	74.6	78.9	78.1	0.148
Hypertension (%)	70.8	73	72.6	0.641
Active smoking (%)	21.6	23.6	23.4	0.653
Diabetes mellitus (%)	38.3	40.3	40.5	0.679

*Analysis of variance for continuous variables (non-normally distributed variables were log-transformed before entering analysis) and χ^2 test for categorical variables.
BMI, body mass index; eGFR, estimated glomerular filtration rate; FGLUC, fasting glucose; GGT, gamma-glutamyl transferase; GPT, glutamic-pyruvate transaminase; HDL, high-density lipoprotein; hsCRP, high-sensitive C reactive protein; IL, interleukin; LDL, low-density lipoprotein; NT-proBNP, N-terminal B-type natriuretic peptide; SAA, serum amyloid A; TG, triglycerides; Total-C, total cholesterol.

Interaction between vitamin E and diabetes mellitus on mortality risk

To further study a possible interaction of vitamin E and HP genotype regarding mortality risk we stratified the analysis for both factors. The HR plots shown in figure 1 revealed an increasing risk for all-cause mortality with increasing concentrations of γ-tocopherol for carriers of Hp1-1 of Hp2-1 genotypes regardless of diabetes mellitus status.

For carriers of the Hp2-2 genotype there is a striking difference in patients with diabetes mellitus who seem to benefit from higher γ-tocopherol concentrations. Indeed, adding the HP genotype and an interaction term between HP genotype and γ-tocopherol concentration to

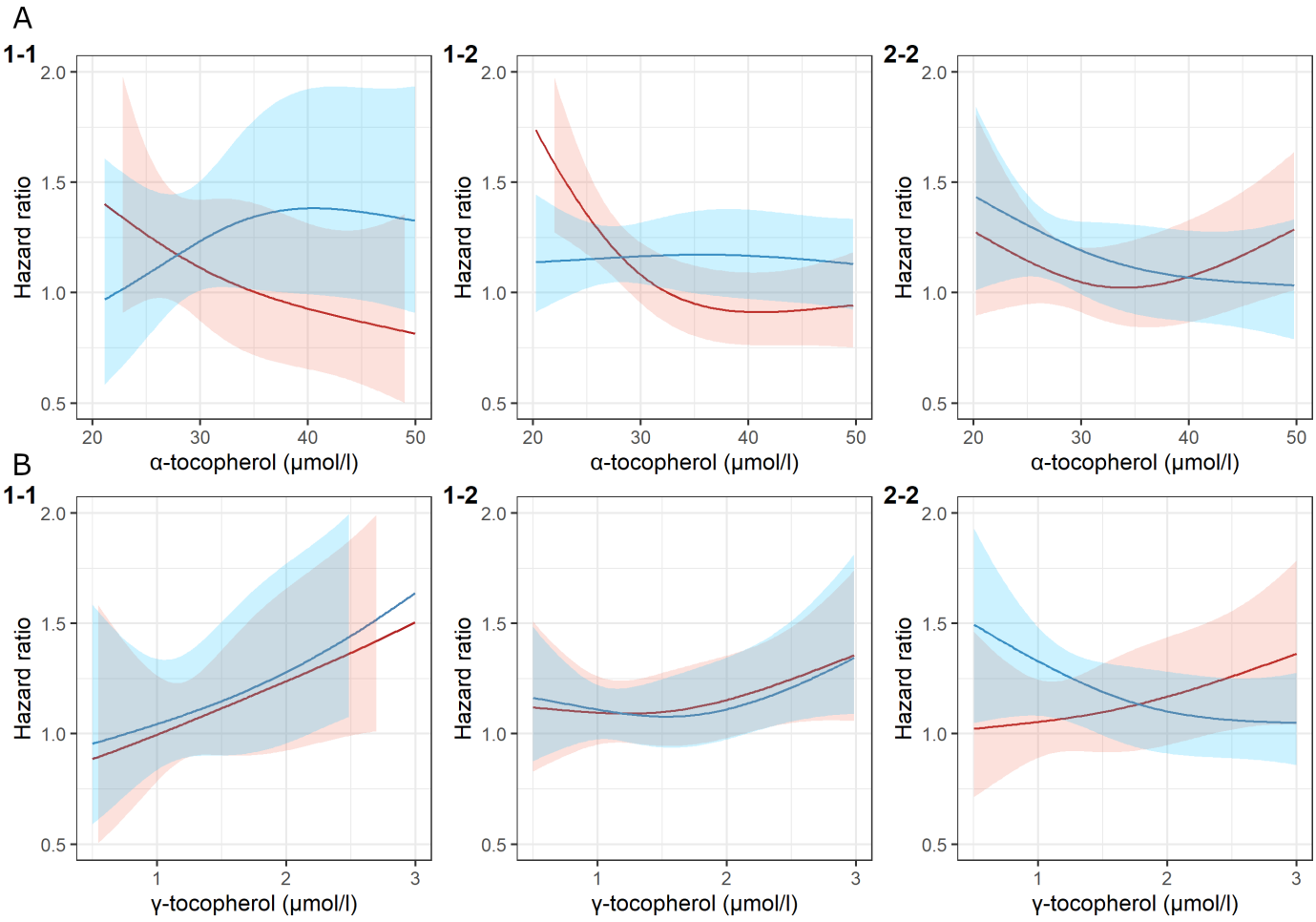

Figure 1 Interaction between HP polymorphism, diabetes mellitus and α-tocopherol (A) or γ-tocopherol (B) regarding all-cause mortality. Hazards curves for patients without diabetes mellitus are shown in red and curves for patients with diabetes mellitus are shown in blue. α-/γ-tocopherol modelled as restricted cubic spline with three knots, analyses adjusted for age and sex.

a model containing age, sex and γ-tocopherol concentration showed a statistically significant interaction in the subgroup of patients with diabetes mellitus with an HR (95% CI) for the main effect of 1.30 (1.07 to 1.59; p=0.009) and a p value for the interaction term of 0.021 (table 2). In the subgroup of patients without diabetes mellitus we found no evidence for an interaction of γ-tocopherol and HP genotype regarding mortality risk ($P_{interaction}$=0.688). For α-tocopherol, we found evidence for a potential interaction with the HP genotype in patients

Table 2 Interaction between HP genotype and vitamin E with regard to all-cause mortality

	γ-tocopherol			α-tocopherol		
	HR (95% CI)*	P value	$P_{interaction}$	HR (95% CI)*	P value	$P_{interaction}$
All						
Model 1	1.12 (1.05 to 1.20)	0.001		0.997 (0.991 to 1.00)	0.381	
Model 2	1.26 (1.11 to 1.43)	<0.001	0.049	0.993 (0.981 to 1.01)	0.279	0.481
Only patients with diabetes mellitus						
Model 1	1.05 (0.96 to 1.16)	0.283		0.997 (0.990 to 1.00)	0.411	
Model 2	1.30 (1.07 to 1.59)	0.009	0.021	1.00 (0.988 to 1.02)	0.797	0.443
Patients without diabetes mellitus						
Model 1	1.15 (1.03 to 1.27)	0.011		0.995 (0.986 to 1.00)	0.297	
Model 2	1.18 (0.98 to 1.42)	0.075	0.688	0.977 (0.957 to 0.997)	0.024	0.035

*Per 1-unit increase; model 1: adjusted for age and sex, model 2: additionally adjusted for HP and HP-α-tocopherol or HP-γ-tocopherol interaction.

Table 3 Association of α-tocopherol and γ-tocopherol with all-cause mortality stratified for diabetes mellitus status and HP genotype

	Diabetes mellitus			No diabetes mellitus		
HP genotype	N	HR (95% CI)*	P value	N	HR (95% CI)*	P value
α-tocopherol						
1-1	181	1.03 (0.816 to 1.30)	0.805	292	0.839 (0.626 to 1.12)	0.238
1-2	607	0.967 (0.856 to 1.09)	0.595	899	0.856 (0.727 to 1.01)	0.062
2-2	485	0.929 (0.796 to 1.08)	0.349	712	1.10 (0.944 to 1.29)	0.213
γ-tocopherol						
1-1	181	1.23 (0.985 to 1.55)	0.067	292	1.19 (0.99 to 1.44)	0.064
1-2	607	1.09 (0.963 to 1.24)	0.169	899	1.09 (0.942 to 1.25)	0.256
2-2	485	0.91 (0.77 to 1.08)	0.270	712	1.13 (0.958 to 1.32)	0.150

*Per 1-SD increase in α/ γ–tocopherol, adjusted for age and sex.

free from diabetes mellitus (HR (95% CI) for the main effect: 0.977 (0.957 to 0.997); p=0.024; $P_{interaction}$=0.03).

HRs for α- and γ-tocopherol from an analysis stratified for diabetes mellitus are shown in table 3. For α-tocopherol there was a trend towards an association with reduced mortality risk with an HR (95% CI) of 0.856 (0.727 to 1.01; p=0.062) per 1-SD increase for carriers of the Hp2-1 genotype without diabetes mellitus. For all other groups, there was no evidence for an association with mortality.

DISCUSSION

The main findings of our study in patients referred for coronary angiography are: first, there was no significant association of γ-tocopherol with all-cause or cardiovascular mortality in our cohort and only a borderline significant association for α-tocopherol. Second, there was no significant association of the Hp genotype with mortality. Third, stratifying the analyses for the presence of diabetes mellitus revealed a striking difference regarding the association of γ-tocopherol with mortality with a direct association in patients without diabetes mellitus and an inverse association in patients with diabetes mellitus. Introducing an interaction term for Hp genotype and diabetes mellitus showed a significant interaction of these two markers with mortality risk.

Vitamin E, as a major fat-soluble vitamin, is an essential nutrient and crucial for human health. The umbrella term describes a number of different molecular species (α-, β-, γ-, δ-tocopherols and α-, β-, γ-, δ-tocotrienols), of which α-tocopherol has for a long time been thought most relevant to human health, because it is preferentially retained after vitamin E intake by binding to the α-tocopherol transfer protein, that only poorly binds the other forms of vitamin E. Furthermore, it is this form that reverses human deficiency symptoms.[23] However, also for γ-tocopherol beneficial health effects have been proposed in the past,[24] partly mediated by specific effects of this species on inflammation.[25] Recently it has been suggested that its supplementation may ameliorate hepatic damage by attenuating inflammation and oxidative stress, at least in a mouse model of diabetes mellitus.[26]

As an antioxidant, vitamin E scavenges peroxyl radicals and can thereby protect lipids, especially polyunsaturated fatty acids, in membranes from oxidation and further plays a role in the regulation of the production of reactive oxygen and nitrogen species.[23]

Numerous studies have investigated the preventive role of vitamin E intake or circulating α-tocopherol levels for a number of different diseases, most notably cardiovascular disease and cancer. A meta-analysis of observational studies showed that vitamin E supplementation was associated with a reduction in mortality from cardiovascular disease.[27] However, a recent umbrella review of meta-analyses came to the conclusion that only an inverse association between circulating α-tocopherol and wheeze or asthma in children was consistently robust, with suggestive evidence for an effect on endothelial function, serum C reactive protein (CRP) concentrations and several types of cancer.[28] The US Preventive Services Task Force also does not recommend vitamin E supplementation for the prevention of CVD or cancer in their recently updated statement on this topic.[29] Similarly, the European Society of Cardiology and the American College of Cardiology report that supplementation of vitamin E is not recommended for CVD prevention or treatment and state that intake through the diet is more effective and safer.[30 31] Some reports even reported an increased mortality risk associated with vitamin E supplementation[32] and excessive supplementation may lead to vitamin E toxicity, most notably manifested by an increased risk of bleeding.[33]

Nevertheless, there have been reports that vitamin E supplementation may be beneficial in certain groups of patients, for example, patients with diabetes mellitus who carry the Hp2-2 genotype.[34 35] A mechanism potentially explaining this effect is that the ability of diabetic human and mice HDLs to promote cholesterol efflux is impaired in carriers of Hp2-2 as lecithin cholesterol acyl transferase, critical for the esterification and maturation of HDL

cholesterol, is markedly reduced in Hp2 carriers.[36–38] However, using the ratio of free cholesterol to cholesterol ester as a proxy for LCAT activity we observed no difference between the Hp genotype groups. For CETP there was an increase in carriers of the Hp2-2 genotype as compared with the other groups but it failed to reach statistical significance. Cholesterol efflux was significantly higher in carriers of the Hp2-1 genotype as compared with homozygous patients.

We observed a trend towards an inverse association with mortality in carriers of the Hp2-2 genotype only in patients with diabetes mellitus, but not in those free from diabetes mellitus. Interaction terms between HP genotype and α-/γ-tocopherol concentrations were significant for γ-tocopherol only in diabetic patients and for α-tocopherol only for patients without diabetes mellitus. While there was a trend towards slightly higher HDL-C in carriers of the Hp2-2 genotype, there were no differences in ApoA1 and ApoA2. Markers of inflammation, hsCRP, SAA, IL-6, were increased in carriers of a Hp2 allele (Hp2-1 and Hp2-2) compared with homozygous carriers of the Hp1 allele which may point towards more systemic inflammation and/or dysfunctional HDL particles.[39] Hp is an acute phase protein and its concentration is therefore positively correlated with the concentration of other acute phase proteins like SAA or CRP (Spearman's rho of 0.44 and 0.53 in LURIC for SAA and CRP, respectively). The distribution of hsCRP, SAA and IL-6 stratified for CAD status and HP genotype is shown in online supplemental figure 8. An association of the Hp2-2 genotype with increased inflammation has also been shown in patients with hepatitis B and concurrent hepatic steatosis.[40]

Strengths and limitations

A limitation of this study is the fact that data on vitamin E supplementation of the study participants has not been systematically obtained at study entry. Furthermore, participants were of European origin and had an indication for coronary angiography, so the results may not apply to other ethnicities or the general population. On the other hand, LURIC has several strengths: a precise clinical and metabolic characterisation of the study participants including detailed information on glucose metabolism and diabetes mellitus status, the availability of coronary angiograms, and a complete 10-year follow-up on mortality.

In conclusion, while there was no significant association of the Hp genotype and vitamin E plasma concentration with mortality in the LURIC study we observed a trend towards a possible beneficial effect of higher vitamin E, especially γ-tocopherol concentration, in diabetes mellitus patients who are carriers of the Hp2-2. The stronger association for γ-tocopherol might be explained by specific antioxidant and anti-inflammatory effects. On basis of the current findings, there may be a beneficial effect of vitamin E supplementation for patients with diabetes mellitus who are carriers of the Hp2-2 genotype. We recommend testing the Hp genotype prior to supplementation. Given that vitamin E supplementation, which in most cases provides exclusively α-tocopherol, increases not only α-tocopherol but simultaneously decreases γ-tocopherol concentrations,[41] further intervention studies in this patient group are warranted.

Twitter summary

In our study, participants with diabetes mellitus and the Hp2-2 genotype seemed to benefit from higher plasma concentrations of γ-tocopherol whereas participants without diabetes mellitus did not.

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Acknowledgements The authors extend appreciation to the participants of the LURIC study, without their collaboration this article would not have been written.

Contributors GED and MEK were involved in the conception of the study, the analysis of the data and interpretation of the results and wrote the first draft of the manuscript. APM was involved in the analysis of data. WR, TL, BMW-R were involved in the acquisition of data and the interpretation of the results. BKK, TBD, FPA and WM were involved in the conception of the study and the interpretation of the results. All authors edited, reviewed and approved the final version of the manuscript. MEK is the guarantor of this work and, as such, has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests GED, BKK, WR, TL, BMW-R and APM do not report any conflict of interest. TBD is employed with Immundiagnostik AG, Bensheim, Germany. FPA, is CEO of Immundiagnostik AG, Bensheim, Germany. MEK and WM are employed with SYNLAB Holding Deutschland GmbH, Germany.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and the ethics committee of the 'Landesärztekammer Rheinland-Pfalz' (LURIC, #837.255.97(1394)) approved the study and it was conducted in accordance with the Declaration of Helsinki. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; internally peer reviewed.

Data availability statement Data are available upon request.

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