Aus dem Institut für Epidemiologie
der Christian-Albrechts-Universität zu Kiel

Vitamin E (α- and γ-tocopherol) - determinants and associated outcomes

Dissertation
zur Erlangung des Doktorgrades
der Agrar- und Ernährungswissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel

vorgelegt von
M.Sc. Sabina Waniek
aus Sohrau

Kiel, 2018

Dekan: Prof. Dr. Dr. Christian Henning
1. Berichterstatter: Prof. Dr. Wolfgang Lieb
2. Berichterstatter: Prof. Dr. Gerald Rimbach
Tag der mündlichen Prüfung: 04. Juli 2018

Gedruckt mit Genehmigung der Agrar- und Ernährungswissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel

Danksagung

An dieser Stelle möchte ich mich bei allen Personen bedanken, die mich bei der Anfertigung dieser Doktorarbeit unterstützt haben.

An erster Stelle gilt mein Dank meinem Doktorvater Prof. Dr. Wolfgang Lieb für die Bereitstellung und Betreuung des Themas. Für die gemeinsamen Gespräche, die hilfreichen Anregungen und die konstruktive Kritik bei der Erstellung dieser Arbeit möchte ich mich herzlich bedanken.

Ein weiterer Dank gilt Prof. Dr. Gerald Rimbach und Dr. habil. Tuba Esatbeyoglu für die Durchführung der Vitamin E-Messungen.

Bei Prof. Dr. Marcus Both, Priv.-Doz. Dr. Jan Borggrefe, Prof. Dr. Ute Nöthlings, Prof. Dr. Jan Kassubek, Priv.-Doz. Dr. Hans-Peter Müller und Dr. Gunnar Jacobs bedanke ich mich ganz herzlich für ihre Kooperationsbereitschaft.

Ich bedanke mich außerdem bei allen Koautoren, die mich mit ihren wissenschaftlichen Ideen und Anregungen unterstützt haben.

Mein Dank gilt allen Kollegen des Instituts für Epidemiologie für die freundschaftliche Arbeitsatmosphäre und Hilfsbereitschaft. Ilka Ratjen, Dr. Janna Enderle und Dr. Romina di Giuseppe bin ich für viele gemeinsame Diskussionen und Ratschläge sehr dankbar. Ein besonderer Dank gilt meiner Mitdoktorandin Ilka Ratjen. Ich danke ihr für ihre freundschaftliche Unterstützung und für die schöne Zeit während unserer Promotion.

Ein großer Dank gilt allen Studienteilnehmern der PopGen-Kontrollkohorte und den Mitarbeitern der Biobank PopGen, ohne deren Teilnahme und Engagement wäre diese Arbeit nicht möglich gewesen.

Von ganzem Herzen möchte ich mich bei meiner Familie und meinen Freunden bedanken, die mir während der Promotionszeit immer unterstützend und liebevoll zur Seite standen.

Herzlichen Dank.

Table of Contents

List of publications and presentations

Articles incorporated into the thesis

Waniek S, di Giuseppe R, Plachta-Danielzik S, Ratjen I, Jacobs G, Koch M, Borggrefe J, Both M, Müller HP, Kassubek J, Nöthlings U, Esatbeyoglu T, Schlesinger S, Rimbach G, Lieb W. Association of Vitamin E Levels with Metabolic Syndrome, and MRI-derived Body Fat Volumes and Liver Fat Content. Nutrients 2017 Oct 18;9(10)

Waniek S, di Giuseppe R, Ratjen I, Jacobs G, Koch M, J, Nöthlings U, Esatbeyoglu T, Schlesinger S, Rimbach G, Lieb W. Vitamin E (α- and γ-tocopherol) levels in the community: Distribution, clinical and biochemical correlates, and association with dietary patterns. Nutrients 2017 Dec 21;10(1)

Waniek S, di Giuseppe R, Ratjen I, Jacobs G, Koch M, J, Nöthlings U, Esatbeyoglu T, Schlesinger S, Rimbach G, Lieb W. Association of Vitamin E Levels with Gallstone Disease. Nutrients 2018 Jan 27;10(2)

Articles not incorporated into the thesis

Ratjen I, Schafmayer C, di Giuseppe R, **Waniek S**, Plachta-Danielzik S, Koch M, Burmeister G, Nöthlings U, Hampe J, Schlesinger S, Lieb W. Postdiagnostic physical activity, sleep duration, and TV watching and all-cause mortality among long-term colorectal cancer survivors: a prospective cohort study. BMC Cancer 2017 Oct 25;17(1)

Ratjen I, Schafmayer C, di Giuseppe R, **Waniek S**, Plachta-Danielzik S, Koch M, Nöthlings U, Hampe J, Schlesinger S, Lieb W. Postdiagnostic Mediterranean and Healthy Nordic Dietary Patterns Are Inversely Associated with All-Cause Mortality in Long-Term Colorectal Cancer Survivors. J Nutr 2017 Apr;147(4)

Presentations at scientific conferences

Plasma Vitamin E (α- und γ-Tocopherol)-Status in der Bevölkerung: Einflussfaktoren und Assoziation mit Ernährungsmustern. Abstract presented in the Congress of the German Society of Nutrition (DGE), 01.-03. March 2017, Kiel (Germany)

Assoziation von Vitamin E (α- und γ-Tocopherol)-Plasmaspiegeln mit dem Metabolischen Syndrom und mittels MRT-quantifiziertem Körperfett und Leberfettgehalt. Abstract presented in the Congress of German Association for the Study of Obesity (DAG), 28.-30. September 2017, Berlin (Germany)

Index of abbreviations

BfR	Federal Institute for risk assessment
BMI	Body mass index
BIA	Bioelectrical impedance analysis
CAT	Catalase
CEHC	Carboxyethyl hydroxychroman
DGE	German Society of Nutrition
DXA	Dual energy X-ray absorptiometry
FFQ	Food frequency questionnaire
FLD	Fatty liver disease
GWAS	Genome-wide association studies
HDL	High density lipoprotein
HPLC	High performance liquid chromatography
IL	Interleukin
LDL	Low density lipoprotein
LPO	Lipid peroxides
LSI	Liver signal intensity
MET	Metabolic equivalent value
MetS	Metabolic syndrome
MRI	Magnetic resonance imaging
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NHANES	National Health and Nutrition Examination Survey

NO	Nitric oxides
NVS	National Nutrition Survey
SAT	Subcutaneous adipose tissue
SNP	Single nucleotide polymorphism
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
TTP	Tocopherol transfer protein
US	United States
VAT	Visceral adipose tissue
VLDL	Very low density lipoprotein

1 General introduction

The term vitamin E refers to a group of naturally occurring compounds that include tocopherols and tocotrienols [1]. α- and γ-tocopherol are the main forms of vitamin E found in human diet [2]. α-tocopherol represents over 90% of the total vitamin E in plasma and is, thus, the most abundant and biologically active form of vitamin E [3, 4]. The concentration of α-tocopherol in plasma is, therefore, the most commonly used biomarker of vitamin E status in humans [3, 4]. Vitamin E is known to have important anti-oxidative and potentially anti-inflammatory functions that may reduce the risk for many chronic disease conditions, including atherosclerosis, diabetes, kidney disease, and cancer [1, 5, 6].

1.1 Properties and relevance of vitamin E

1.1.1 Chemistry and function

The vitamin E family encompasses eight lipophilic molecules, including four tocopherols (α, β, γ, δ) and four tocotrienols (α, β, γ, δ) [1]. All vitamin E forms possess a similar chemical structure consisting of a chromanol ring with a side chain with 16 carbon atoms (C16). Specifically, α-tocopherol has three methyl (CH_3) groups at the 5-, 7-, and 8-positions of the chromanol ring, whereas γ-tocopherol is dimethylated at the 7-, and 8-positions of the chromanol ring [1, 6].

The anti-oxidative properties of vitamin E are well established [1]. Specifically, vitamin E is able to react with free radicals in cell membranes, thereby preventing polyunsaturated fatty acids from being damaged by lipid peroxidation [1, 7, 8]. Compared to α-tocopherol, γ-tocopherol is slightly less efficient as a scavenger of free radicals, but it is superior to trap reactive nitrogen oxide species and also possesses anti-inflammatory activities [6, 9]. Furthermore, vitamin E also exhibits non-anti-oxidant activities, including modulation of gene expression and inhibition of cell proliferation [2].

1.1.2 Physiology of vitamin E

This paragraph summarizes intestinal absorption, vascular transport, hepatic metabolism, and the excretion of vitamin E [10].

After the intake of dietary vitamin E, various tocopherol forms are absorbed in the small intestine. In the intestinal lumen, dietary vitamin E is solubilized into mixed micells by bile salts and other lipids. Absorption of dietary vitamin E is highly variable, ranging from 20% to 80% [10, 11]. Unabsorbed vitamin E is excreted in faeces, while absorbed and metabolized vitamin E is excreted via the bile or urine (as α- and γ-carboxyethyl hydroxychroman (CEHC)) [11, 12].

For the absorption, vitamin E requires the presence of fat and is involved in biochemical and molecular processes which are related to lipid and lipoprotein metabolism [13]. After the intestinal absorption through a mechanism that is not entirely clarified, vitamin E is packed into chylomicrons and enters the systemic circulation via the lymphatic system [10, 14].

After entering the systemic circulation, vitamin E has several possible metabolic pathways:

A small amount of vitamin E is transferred from chylomicrons to high density lipoproteins (HDL), from where vitamin E can be distributed to circulating lipoproteins [14].

Another pathway comprises the degradation of chylomicrons by the action of lipoprotein lipase, whereas a small proportion of vitamin E is transported to extrahepatic tissues such as lungs, kidney, adipose tissue, and skeletal muscle [15].

The major pathway includes the transport of vitamin E via chylomicron remnants to the liver [10, 14]. To this point, there is no discrimination between different vitamin E forms [16]. In the liver, the hepatic α-tocopherol transfer protein (TTP) sorts out α-tocopherol for incorporating into very low density lipoproteins (VLDL) [10]. Of note, a-TTP preferentially transfers α-tocopherol due to its specific biochemical structure [12, 17]. The mechanism by which VLDLs are enriched with α-tocopherol is poorly understood [2, 12]. In the blood circulation, α-tocopherol is transported back to the liver and to extrahepatic tissues by circulating lipoproteins, including, e.g., low density lipoproteins (LDL). [10, 16]. α-TTP is considered to be a major regulator for

maintaining normal α-tocopherol concentrations in plasma [18]. Indeed, the relative affinity of α-TTP to other tocopherols is substantially lower (β-tocopherol=38%, γ-tocopherol=9%, and δ-tocopherol=2%) than to α-tocopherol (100%) [19].

1.1.3 Sources of vitamin E and recommendations for dietary vitamin E intake and plasma vitamin E levels

Sources of vitamin E

Vitamin E is an essential nutrient for the human body and, thus, it must be provided by foods [20]. Almost 90% of the German population consume vitamin E in the form of α- and γ-tocopherol [21]. Foods containing high concentrations of both, α- and γ-tocopherol, are mainly vegetable oils [1]. Besides, the most commonly consumed oil in Germany is rapeseed oil [22], which is rich in α- and γ-tocopherol and is, therefore, the most important source of vitamin E [23]. In the European diet, α-tocopherol is the predominant vitamin E form, while γ-tocopherol is the major dietary form of vitamin E in the United States (US) [1, 9].

Recommendations of vitamin E - dietary intake and plasma levels

The recommended daily intake for vitamin E is based on the reported effects of vitamin E on the prevention of lipid peroxidation [10]. The German Society of Nutrition (DGE, Deutsche Gesellschaft für Ernährung) recommends a vitamin E (α-tocopherol equivalent) intake of 12 mg/day for women and 13-15 mg/day for men [24]. For infants, children, and the elderly, a different daily vitamin E intake is recommended. So far, no adverse effects associated with vitamin E consumption from naturally occurring vitamin E in foods have been described [14]. With respect to vitamin E recommendations for the European population, the European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies set an Adequate Intake of 11 mg/day for women and 13 mg/day for men for α-tocopherol [25].

With respect to circulating vitamin E levels, the DGE recommends plasma vitamin E levels between 12 and 46 μmol/L [24]. Plasma concentrations <12 μmol/L may result in functional deficiencies in the human body [26], while plasma α-tocopherol

concentrations of about 30 µmol/L have been recommended to prevent nutrition-related diseases and to reduce the risk for common disease conditions, including cardiovascular diseases and different types of cancer [8]. In contrast to α-tocopherol, γ-tocopherol has received less scientific attention, so far, because its bioavailability and bioactivity are lower than for α-tocopherol [6]. Furthermore, γ-tocopherol is not included in the current dietary intake recommendations [24]. However, increasing evidence suggests that γ-tocopherol may have beneficial biological properties such as anti-inflammatory activities [27].

1.2 Assessment of vitamin E in epidemiological studies

In epidemiological studies, dietary intake of vitamin E can be estimated using established questionnaires [28]. Furthermore, vitamin E concentrations can be measured in blood using high performance liquid chromatography (HPLC) [29].

1.2.1 Questionnaire-based assessment of vitamin E intake

Established instruments to retrospectively assess self-reported dietary vitamin E intake include, for example, the 24-hour dietary recall and the food frequency questionnaire (FFQ) [28].

The FFQ inquiries the dietary intake of the past 12 months and is, thus, an instrument to assess rather long-term eating habits, whereas the 24-hour dietary recall focusses on short-term intake [28, 30].

Specifically, the FFQ records the frequency, and in some cases portion size information, by which an individual consumes foods (food groups, single foods) and beverages [28]. For the papers included in the present thesis, the dietary vitamin E intake from the consumed food recorded in the FFQ was determined from the Department of Epidemiology of the German Institute of Human Nutrition Potsdam Rehbrücke by using the German Food Code and Nutrient Data Base (version II.3) [31]. In our Northern German sample, we used a FFQ that was specifically adjusted for the German population [32]. The FFQ tends to underestimate the dietary intake

of vitamin E because the FFQ does not capture foods (e.g., olives), which are contributing to vitamin E intake [33, 34].

In a 24-hour dietary recall, participants are asked to describe in detail and in an open-ended manner the foods and beverages consumed in the last 24 hours [35]. Detailed data regarding the time of the consumed food, food preparation methods, recipe ingredients, and the brand name of products are necessary [28]. To estimate the average dietary intake of given individuals, several 24-hour dietary recalls are needed [28].

1.2.2 Measuring vitamin E levels in plasma

Several methods have been used for the assessment of the vitamin E status, including measurements of α-tocopherol concentrations in plasma, blood components (e.g., red blood cells, platelets), and body stores (e.g., adipose tissue) [36]. In fact, plasma α- and γ-tocopherol levels are the most commonly used biomarker of the current vitamin E status in epidemiological studies and are technically simple to measure [37]. Circulating vitamin E levels either in plasma or in serum can be determined by HPLC, which is the method of choice [38]. This method is able to measure simultaneously various tocopherols, particularly α- and γ-tocopherol [29, 39].

With regard to other measurements of the vitamin E status, for example, the measurement of α-tocopherol concentrations in red blood cells and platelets can provide a valid measurement of the vitamin E status [38]. However, both approaches are technically difficult and are therefore not commonly used to determine the vitamin E status [38]. Furthermore, the α-tocopherol concentration in red blood cells is only a fraction (about 20%) of the α-tocopherol concentration in plasma [36]. Moreover, vitamin E can also be determined in adipose tissue. Vitamin E concentrations in adipose tissue reflect more the long-term vitamin E status than the current status [40]. However, adipose tissue biopsies are invasive and, thus, not applicable in large scale epidemiological studies [26].

1.3 Correlates of circulating vitamin E levels: Dietary intake

Dietary intake is an important determinant of circulating vitamin E concentrations and, as mentioned above, vegetable oils are important sources of vitamin E [1]. However, several prior studies reported relatively poor or no correlations between dietary vitamin E intake (assessed, e.g., by FFQ) and circulating vitamin E concentrations [41-43].

In order to look beyond the health-related effects of individual nutrients or foods, dietary pattern analysis has emerged as a more comprehensive assessment of diet, which reflects more complex combinations of nutrients that may interact in their biological effects [44]. Thus far, only two prior US studies have related circulating plasma vitamin E levels to a posteriori derived dietary patterns [42, 45]. Gao et al. observed [42] that participants (n=602) in a “sweet” dietary pattern had the lowest α-tocopherol levels as compared to participants in a “fruit and breakfast cereal” pattern, and a “milk and milk products” pattern. Further, in African-Americans (n=373), those in a “juice” cluster (characterized by high intakes of fruit juice) had higher serum α-tocopherol levels relative to those in a “fast food” cluster (characterized by high intakes of fast food, salty snacks, non-diet soft drinks, and meat) [45].

In Northern European populations, the association of vitamin E levels with dietary patterns is not well explored.

1.4 Vitamin E and metabolic conditions and gallstone disease

In the present thesis, circulating vitamin E levels will be related to a broad spectrum of cardiometabolic traits. These traits and some prior evidence regarding their associations with vitamin E levels are described below.

1.4.1 Association of circulating vitamin E levels with the metabolic syndrome

The metabolic syndrome (MetS) is a clustering of metabolic risk factors and conditions and considered present when at least 3 of the 5 following conditions are present: Abdominal obesity, hyperglycemia, elevated blood pressure, reduced concentration of HDL-cholesterol, and elevated triglyceride concentrations [46]. Individuals with the MetS are at high risk for developing type 2 diabetes mellitus and cardiovascular diseases [47, 48].

Some prior studies have related circulating vitamin E levels to the MetS in clinical and epidemiological settings, but the results were partially conflicting [49-52]. Godala et al. [51] reported lower levels of vitamin E in 182 individuals with the MetS compared to 91 healthy adults, whereas Li et al. [52] found no differences in serum vitamin E levels between individuals with the MetS (n=221) and healthy adults (n=329). Likewise, Beydoun et al. [49] observed no association of vitamin E levels with the MetS (n=3008). With respect to individual components of the MetS, vitamin E adjusted for lipids was positively related to high triglyceride levels but not to low HDL-cholesterol levels in samples from the National Health and Nutrition Examination Survey (NHANES; n=4322 and n=8465, respectively) [49, 50].

1.4.2 Association of circulating vitamin E levels with body fat volumes

Obesity is generally defined by an excessive body fat accumulation and represents a major risk factor for a number of chronic diseases, including cardiovascular diseases, type 2 diabetes mellitus, and cancer [53].

Abdominal adipose tissue can be divided in different compartments, including subcutaneous (SAT) and visceral adipose tissue (VAT) with SAT representing almost 90% of the adipose tissue [54]. Compared to SAT, VAT adipocytes are more metabolically active, more insulin-resistant, and produce a higher number of inflammatory cells and hormones [54]. Both types of adipose tissues are positively related to metabolic risk factors but VAT tends to be more strongly associated with greater cardiometabolic risk than SAT [55].

Many prior studies used body mass index (BMI) or waist circumference as surrogate measures for adiposity and some of these studies explored the associations of BMI

or waist circumference with vitamin E levels [56-60]. Two studies related vitamin E levels to body fat volumes, as assessed by dual energy X-ray absorptiometry (DXA) [59] and bioelectrical impedance analysis (BIA) [58]. So far, there were no studies published assessing the association of vitamin E levels with body fat volumes derived by magnetic resonance imaging (MRI).

1.4.3 Association of circulating vitamin E levels with liver fat content

Fatty liver disease (FLD) is defined as an excess accumulation of liver fat and comprises a wide spectrum of liver diseases ranging from simple fatty liver disease to non-alcoholic steatohepatitis (NASH), which may progress to fibrosis and to cirrhosis [61]. FLD has been shown to be associated with metabolic disturbances such as dyslipidemia, hypertension, and type 2 diabetes mellitus [62-64].

In some prior studies, the association of vitamin E levels with NASH has been assessed in rather small samples [65-67]. For example, Machado et al. [65] observed higher serum vitamin E levels in patients with NASH (n=43, histologically proven) compared to healthy controls (n=33). However, Erhardt et al. [66] observed lower vitamin E levels in 50 patients with NASH (biopsy-proven) compared to 40 healthy controls. Likewise, Bahcecioglu et al. [67] reported lower vitamin E levels in patients with biopsy-proven NASH (n=29) than in healthy controls (n=10).

1.4.4 Association of circulating vitamin E levels with gallstone disease

Gallstone disease refers to the presence of stones in the gallbladder or common bile duct system [68]. Gallstones are composed mainly of cholesterol, bilirubin, and calcium salts [69]. In most cases, gallstones are clinically asymptomatic (80%), but they can lead to complications such as acute cholecystitis or gallstone pancreatitis [70]. A few well-established risk factors have been identified, including age, female gender, obesity, and dyslipidemia [70-72].

Associations of vitamin E levels with gallstone disease have been observed in some prior reports in rather small studies [73-75]. For example, in a study from Worthington et al. [73], lower levels of the α-tocopherol/cholesterol ratio were

observed in patients with gallstone disease (n=18) compared to healthy controls (n=47). Likewise, Rocchi et al. [74] reported lower levels of the α-tocopherol/cholesterol ratio in 16 patients with gallstone disease compared to 20 healthy controls. Besides, Shukla et al. [75] observed lower levels of α-tocopherol in individuals with gallstone disease (n=30) compared to healthy controls (n=30).

1.5 Aims

The aims of this doctoral thesis were:

1. To assess the distribution of vitamin E levels in a general population sample (Chapter 2).
2. To investigate clinical and biochemical correlates of vitamin E, and its associations with a priori and a posteriori derived dietary patterns (Chapter 2).
3. To study the associations of vitamin E levels with MetS, with MRI-derived body fat volumes and with liver fat content in a general population sample (Chapter 3).
4. To evaluate the association between vitamin E levels and gallstone disease (Chapter 4).

These 4 scientific questions were addressed in 3 separate articles and published in peer-reviewed journals.

For this present thesis, data from the first follow-up examination of the PopGen control cohort from Northern Germany (Kiel) were used, as described in detail in Chapter 2, 3, and 4.

1.6 References

1. Jiang, Q., *Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy.* Free Radic Biol Med, 2014. **72**: p. 76-90.
2. Borel, P., D. Preveraud, and C. Desmarchelier, *Bioavailability of vitamin E in humans: an update.* Nutr Rev, 2013. **71**(6): p. 319-31.
3. Kim, Y.N. and Y.O. Cho, *Vitamin E status of 20- to 59-year-old adults living in the Seoul metropolitan area of South Korea.* Nutr Res Pract, 2015. **9**(2): p. 192-8.
4. Albahrani, A.A. and R.F. Greaves, *Fat-Soluble Vitamins: Clinical Indications and Current Challenges for Chromatographic Measurement.* Clin Biochem Rev, 2016. **37**(1): p. 27-47.
5. Roberts, C.K. and K.K. Sindhu, *Oxidative stress and metabolic syndrome.* Life Sci, 2009. **84**(21-22): p. 705-12.
6. Jiang, Q., et al., *gamma-tocopherol, the major form of vitamin E in the US diet, deserves more attention.* Am J Clin Nutr, 2001. **74**(6): p. 714-22.
7. Traber, M.G. and J.F. Stevens, *Vitamins C and E: beneficial effects from a mechanistic perspective.* Free Radic Biol Med, 2011. **51**(5): p. 1000-13.
8. Raederstorff, D., et al., *Vitamin E function and requirements in relation to PUFA.* Br J Nutr, 2015. **114**(8): p. 1113-22.
9. Wagner, K.H., A. Kamal-Eldin, and I. Elmadfa, *Gamma-tocopherol--an underestimated vitamin?* Ann Nutr Metab, 2004. **48**(3): p. 169-88.
10. Schmolz, L., et al., *Complexity of vitamin E metabolism.* World J Biol Chem, 2016. **7**(1): p. 14-43.
11. Traber, M.G., *Mechanisms for the prevention of vitamin E excess.* J Lipid Res, 2013. **54**(9): p. 2295-306.
12. Brigelius-Flohe, R., et al., *The European perspective on vitamin E: current knowledge and future research.* Am J Clin Nutr, 2002. **76**(4): p. 703-16.
13. Rigotti, A., *Absorption, transport, and tissue delivery of vitamin E.* Mol Aspects Med, 2007. **28**(5-6): p. 423-36.
14. Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds, *Vitamin E*, in *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. 2000: Washington (DC). p. 186-283.
15. Herrera, E. and C. Barbas, *Vitamin E: action, metabolism and perspectives.* J Physiol Biochem, 2001. **57**(2): p. 43-56.
16. Debier, C. and Y. Larondelle, *Vitamins A and E: metabolism, roles and transfer to offspring.* Br J Nutr, 2005. **93**(2): p. 153-74.

17. Brigelius-Flohe, R. and M.G. Traber, *Vitamin E: function and metabolism.* FASEB J, 1999. **13**(10): p. 1145-55.

18. Traber, M.G., G.W. Burton, and R.L. Hamilton, *Vitamin E trafficking.* Ann N Y Acad Sci, 2004. **1031**: p. 1-12.

19. Hosomi, A., et al., *Affinity for alpha-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs.* FEBS Lett, 1997. **409**(1): p. 105-8.

20. Azzi, A. and A. Stocker, *Vitamin E: non-antioxidant roles.* Prog Lipid Res, 2000. **39**(3): p. 231-55.

21. Yamashita, K., *Dietary tocotrienol and UVB-induced skin damage*, in *Handbook of Diet, Nutrition and the Skin*, V.R. Preedy, Editor. 2012, Wageningen Academic Publishers: Wageningen. p. 165-179.

22. Verband der Ölsaatenverarbeitenden Industrie in Deutschland. *Verbrauch Pflanzenöle Deutschland*. 2015 [cited April 2018]; Available from: http://www.ovid-verband.de/unsere-branche/daten-und-grafiken/pflanzenoel/.

23. Shahidi, F. and A.C. de Camargo, *Tocopherols and Tocotrienols in Common and Emerging Dietary Sources: Occurrence, Applications, and Health Benefits.* Int J Mol Sci, 2016. **17**(10).

24. Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, and Schweizerische Gesellschaft für Ernährung, *Vitamin E (Tocopherole)*, in *Referenzwerte für die Nährstoffzufuhr*. 2015, DGE: Bonn. p. 1-5.

25. European Food Safety Authority, *Scientific opinion on Dietary Reference Values for vitamin E as alpha-tocopherol.* EFSA, 2015. **13**(7): p. 1-72.

26. Traber, M.G., *Vitamin E inadequacy in humans: causes and consequences.* Adv Nutr, 2014. **5**(5): p. 503-14.

27. Hensley, K., et al., *New perspectives on vitamin E: gamma-tocopherol and carboxyelthylhydroxychroman metabolites in biology and medicine.* Free Radic Biol Med, 2004. **36**(1): p. 1-15.

28. Shim, J.S., K. Oh, and H.C. Kim, *Dietary assessment methods in epidemiologic studies.* Epidemiol Health, 2014. **36**: p. e2014009.

29. Mayne, S.T., M.E. Wright, and B. Cartmel, *Assessment of antioxidant nutrient intake and status for epidemiologic research.* J Nutr, 2004. **134**(11): p. 3199S-3200S.

30. Shahar, D., et al., *Development of a semi-quantitative Food Frequency Questionnaire (FFQ) to assess dietary intake of multiethnic populations.* Eur J Epidemiol, 2003. **18**(9): p. 855-61.

31. Dehne, L.I., et al., *The German Food Code and Nutrient Data Base (BLS II.2).* Eur J Epidemiol, 1999. **15**(4): p. 355-9.

32. Nothlings, U., et al., *Fitting portion sizes in a self-administered food frequency questionnaire.* J Nutr, 2007. **137**(12): p. 2781-6.

33. Grilo, E.C., et al., *Alpha-tocopherol and gamma-tocopherol concentration in vegetable oils.* Food Science and Technology (Campinas), 2014. **34**: p. 379-385.

34. El-Sohemy, A., et al., *Population-based study of alpha- and gamma-tocopherol in plasma and adipose tissue as biomarkers of intake in Costa Rican adults.* Am J Clin Nutr, 2001. **74**(3): p. 356-63.

35. Naska, A., A. Lagiou, and P. Lagiou, *Dietary assessment methods in epidemiological research: current state of the art and future prospects.* F1000Res, 2017. **6**: p. 926.

36. Gibson, R.S., *Assessment of the status of vitamins A, D, and E*, in *Principles of Nutritional Assessment* 2005, Oxford University Press: Oxford. p. 508-528.

37. Jansen, E. and T. Ruskovska, *Serum Biomarkers of (Anti)Oxidant Status for Epidemiological Studies.* Int J Mol Sci, 2015. **16**(11): p. 27378-90.

38. Sauberlich, H.E., *Vitamin E (Tocopherols)*, in *Laboratory Tests for the Assessment of Nutritional Status*. 1999, CRC Press: Boca Raton. p. 249-259.

39. Augustin, K., et al., *Dietary green tea polyphenols do not affect vitamin E status, antioxidant capacity and meat quality of growing pigs.* J Anim Physiol Anim Nutr (Berl), 2008. **92**(6): p. 705-11.

40. Kayden, H.J., L.J. Hatam, and M.G. Traber, *The measurement of nanograms of tocopherol from needle aspiration biopsies of adipose tissue: normal and abetalipoproteinemic subjects.* J Lipid Res, 1983. **24**(5): p. 652-6.

41. Boeing, H., et al., *The relative validity of vitamin intakes derived from a food frequency questionnaire compared to 24-hour recalls and biological measurements: results from the EPIC pilot study in Germany. European Prospective Investigation into Cancer and Nutrition.* Int J Epidemiol, 1997. **26 Suppl 1**: p. S82-90.

42. Gao, X., et al., *alpha-Tocopherol intake and plasma concentration of Hispanic and non-Hispanic white elders is associated with dietary intake pattern.* J Nutr, 2006. **136**(10): p. 2574-9.

43. Kardinaal, A.F., et al., *Relations between antioxidant vitamins in adipose tissue, plasma, and diet.* Am J Epidemiol, 1995. **141**(5): p. 440-50.

44. Hu, F.B., *Dietary pattern analysis: a new direction in nutritional epidemiology.* Curr Opin Lipidol, 2002. **13**(1): p. 3-9.

45. Talegawkar, S.A., et al., *Serum carotenoid and tocopherol concentrations vary by dietary pattern among African Americans.* J Am Diet Assoc, 2008. **108**(12): p. 2013-20.

46. Alberti, K.G., et al., *Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity.* Circulation, 2009. **120**(16): p. 1640-5.

47. Ballantyne, C.M., et al., *Metabolic syndrome risk for cardiovascular disease and diabetes in the ARIC study.* Int J Obes (Lond), 2008. **32 Suppl 2**: p. S21-4.

48. Klein, B.E., R. Klein, and K.E. Lee, *Components of the metabolic syndrome and risk of cardiovascular disease and diabetes in Beaver Dam.* Diabetes Care, 2002. **25**(10): p. 1790-4.

49. Beydoun, M.A., et al., *Serum antioxidant status is associated with metabolic syndrome among U.S. adults in recent national surveys.* J Nutr, 2011. **141**(5): p. 903-13.

50. Ford, E.S., et al., *The metabolic syndrome and antioxidant concentrations: findings from the Third National Health and Nutrition Examination Survey.* Diabetes, 2003. **52**(9): p. 2346-52.

51. Godala, M.M., et al., *Lower Plasma Levels of Antioxidant Vitamins in Patients with Metabolic Syndrome: A Case Control Study.* Adv Clin Exp Med, 2016. **25**(4): p. 689-700.

52. Li, Y., et al., *Serum and dietary antioxidant status is associated with lower prevalence of the metabolic syndrome in a study in Shanghai, China.* Asia Pac J Clin Nutr, 2013. **22**(1): p. 60-8.

53. Karastergiou, K. and S.K. Fried, *Multiple adipose depots increase cardiovascular risk via local and systemic effects.* Curr Atheroscler Rep, 2013. **15**(10): p. 361.

54. Ibrahim, M.M., *Subcutaneous and visceral adipose tissue: structural and functional differences.* Obes Rev, 2010. **11**(1): p. 11-8.

55. Liu, J., et al., *Impact of abdominal visceral and subcutaneous adipose tissue on cardiometabolic risk factors: the Jackson Heart Study.* J Clin Endocrinol Metab, 2010. **95**(12): p. 5419-26.

56. Kabat, G.C., et al., *Longitudinal association of measures of adiposity with serum antioxidant concentrations in postmenopausal women.* Eur J Clin Nutr, 2016. **70**(1): p. 47-53.

57. Chai, W., et al., *Associations between obesity and serum lipid-soluble micronutrients among premenopausal women.* Nutr Res, 2010. **30**(4): p. 227-32.

58. Wallstrom, P., et al., *Serum concentrations of beta-carotene and alpha-tocopherol are associated with diet, smoking, and general and central adiposity.* Am J Clin Nutr, 2001. **73**(4): p. 777-85.

59. Garcia, O.P., et al., *Zinc, vitamin A, and vitamin C status are associated with leptin concentrations and obesity in Mexican women: results from a cross-sectional study.* Nutr Metab (Lond), 2012. **9**(1): p. 59.

60. Galan, P., et al., *Serum concentrations of beta-carotene, vitamins C and E, zinc and selenium are influenced by sex, age, diet, smoking status, alcohol consumption and corpulence in a general French adult population.* Eur J Clin Nutr, 2005. **59**(10): p. 1181-90.

61. Paschos, P. and K. Paletas, *Non alcoholic fatty liver disease and metabolic syndrome.* Hippokratia, 2009. **13**(1): p. 9-19.

62. Anstee, Q.M., G. Targher, and C.P. Day, *Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis.* Nat Rev Gastroenterol Hepatol, 2013. **10**(6): p. 330-44.

63. Tarantino, G., et al., *Non-alcoholic fatty liver disease: further expression of the metabolic syndrome.* J Gastroenterol Hepatol, 2007. **22**(3): p. 293-303.

64. Chatrath, H., R. Vuppalanchi, and N. Chalasani, *Dyslipidemia in patients with nonalcoholic fatty liver disease.* Semin Liver Dis, 2012. **32**(1): p. 22-9.

65. Machado, M.V., et al., *Blood oxidative stress markers in non-alcoholic steatohepatitis and how it correlates with diet.* Scand J Gastroenterol, 2008. **43**(1): p. 95-102.

66. Erhardt, A., et al., *Plasma levels of vitamin E and carotenoids are decreased in patients with Nonalcoholic Steatohepatitis (NASH).* Eur J Med Res, 2011. **16**(2): p. 76-8.

67. Bahcecioglu, I.H., et al., *Levels of serum vitamin A, alpha-tocopherol and malondialdehyde in patients with non-alcoholic steatohepatitis: relationship with histopathologic severity.* Int J Clin Pract, 2005. **59**(3): p. 318-23.

68. Lammert, F., et al., *Gallstones.* Nat Rev Dis Primers, 2016. **2**: p. 16024.

69. Njeze, G.E., *Gallstones.* Niger J Surg, 2013. **19**(2): p. 49-55.

70. Stinton, L.M. and E.A. Shaffer, *Epidemiology of gallbladder disease: cholelithiasis and cancer.* Gut Liver, 2012. **6**(2): p. 172-87.

71. Shaffer, E.A., *Gallstone disease: Epidemiology of gallbladder stone disease.* Best Pract Res Clin Gastroenterol, 2006. **20**(6): p. 981-96.

72. Marschall, H.U., et al., *The genetic background of gallstone formation: an update.* Biochem Biophys Res Commun, 2010. **396**(1): p. 58-62.

73. Worthington, H.V., et al., *Dietary antioxidant lack, impaired hepatic glutathione reserve, and cholesterol gallstones.* Clin Chim Acta, 2004. **349**(1-2): p. 157-65.

74. Rocchi, E., et al., *Antioxidant liposoluble vitamins and carotenoids in chronic hepatitis.* Eur J Intern Med, 2001. **12**(2): p. 116-121.

75. Shukla, V.K., et al., *Micronutrients, antioxidants, and carcinoma of the gallbladder.* J Surg Oncol, 2003. **84**(1): p. 31-5.

2 Vitamin E (α- and γ-tocopherol) levels in the community: Distribution, clinical and biochemical correlates, and association with dietary patterns

Sabina Waniek[1], Romina di Giuseppe[1], Tuba Esatbeyoglu[2], Sandra Plachta-Danielzik[1], Ilka Ratjen[1], Gunnar Jacobs[1,3], Ute Nöthlings[4], Manja Koch[1,5], Sabrina Schlesinger[1,6], Gerald Rimbach[2], Wolfgang Lieb[1,3]

[1]Institute of Epidemiology, Christian-Albrechts University Kiel, 24105 Kiel, Germany

[2]Institute of Human Nutrition and Food Science, Christian-Albrechts University of Kiel, 24118 Kiel, Germany

[3]Biobank PopGen, University Hospital Schleswig-Holstein, Campus Kiel, 24105 Kiel, Germany

[4]Department of Nutrition and Food Sciences, University of Bonn, 53113 Bonn, Germany

[5]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA

[6]Institute for Biometry and Epidemiology, Leibniz Institute for Diabetes Research, Heinrich Heine University Düsseldorf, 40225 Düsseldorf, Germany

Published in: Nutrients 2017 Dec 21;10(1)

nutrients

MDPI

Article

Vitamin E (α- and γ-Tocopherol) Levels in the Community: Distribution, Clinical and Biochemical Correlates, and Association with Dietary Patterns

Sabina Waniek [1], Romina di Giuseppe [1,*], Tuba Esatbeyoglu [2], Sandra Plachta-Danielzik [1], Ilka Ratjen [1], Gunnar Jacobs [1,3], Ute Nöthlings [4], Manja Koch [1,5], Sabrina Schlesinger [1,6], Gerald Rimbach [2] and Wolfgang Lieb [1,3]

1 Institute of Epidemiology, University of Kiel, 24105 Kiel, Germany; sabina.waniek@epi.uni-kiel.de (S.W.); sandra.plachta-danielzik@epi.uni-kiel.de (S.P.-D.); ilka.ratjen@epi.uni-kiel.de (I.R.); jacobs@popgen.de (G.J.); mkoch@hsph.harvard.edu (M.K.); sabrina.schlesinger@DDZ.uni-duesseldorf.de (S.S.); wolfgang.lieb@epi.uni-kiel.de (W.L.)
2 Institute of Human Nutrition and Food Science, University of Kiel, 24118 Kiel, Germany; tuba.esatbeyoglu@mri.bund.de (T.E.); rimbach@foodsci.uni-kiel.de (G.R.)
3 Biobank PopGen, University Hospital Schleswig-Holstein, Campus Kiel, 24105 Kiel, Germany
4 Department of Nutrition and Food Sciences, University of Bonn, 53113 Bonn, Germany; noethlings@uni-bonn.de
5 Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA
6 Institute for Biometry and Epidemiology, Leibniz Institute for Diabetes Research, Heinrich Heine University Duesseldorf, 40225 Duesseldorf, Germany
* Correspondence: romina.digiuseppe@epi.uni-kiel.de; Tel.: +49-431-500-30220; Fax: +49-431-500-30204

Received: 18 October 2017; Accepted: 18 December 2017; Published: 21 December 2017

Abstract: Little is known about the distribution and determinants of circulating vitamin E levels in a German population. In this cross-sectional study we assessed the distribution of both α- and γ-tocopherol levels, identified their clinical and biochemical correlates, and assessed their relationships with a priori and *a posteriori* derived dietary patterns. Plasma α- and γ-tocopherol concentrations were measured using high performance liquid chromatography (HPLC) with fluorescence detection in 641 individuals (mean-age: 61 years; 40.6% women). Correlates of both markers were determined using linear regression with backward selection. Using a validated food-frequency questionnaire (FFQ), an a priori defined vitamin E-rich dietary pattern was constructed, and three *a posteriori* derived dietary patterns were identified by principal component analysis. Each pattern was related to α- and γ-tocopherol levels using linear regression. Median concentrations of α- and γ-tocopherol were 31.54 μmol/L and 1.35 μmol/L, respectively. 57.6% of participants had α-tocopherol levels >30 μmol/L. Triglycerides, high density lipoprotein (HDL)- and low density lipoprotein (LDL)-cholesterol, and vitamin E supplementation were identified as correlates of vitamin E levels. After excluding supplement users, a dietary pattern rich in meat, bread, fats, potatoes, and sugar/confectionery was inversely related to α-tocopherol levels (β, −0.032, SE = 0.016; $p = 0.047$). Prospective studies are warranted to evaluate the actual impact of the reported findings in terms of nutrition and health outcomes.

Keywords: vitamin E; α-tocopherol; γ-tocopherol; dietary patterns

1. Introduction

Vitamin E encompasses 4 tocopherols (α-, β-, γ-, and δ-tocopherol) and 4 tocotrienols (α-, β-, γ-, and δ-tocotrienol), with α-tocopherol representing over 90% of total tocopherol [1,2].

Vitamin E acts mainly as an antioxidant preventing polyunsaturated fatty acids from being damaged by lipid peroxidation [2,3]. Oxidative damage has been linked to numerous chronic diseases conditions, including cardiovascular diseases and cancer [4], and dietary vitamin E intake and lower circulating vitamin E concentrations have been linked to cardiovascular disease and cancer in observational studies [5–11]. However, despite the potential relevance of vitamin E for health and disease, little is known about the distribution of vitamin E levels in the general German population and about clinical and biochemical correlates of circulating α- and γ-tocopherol concentrations. To prevent nutrition-related diseases, plasma α-tocopherol concentrations of about 30 μmol/L have been recommended [12], and recommendations for the daily dietary vitamin E intake have been issued in Germany stratified by age and gender [13], but it is not well known how well these recommendations are met in the general population. In fact, a recent report from Germany indicates that about half of the analyzed population (n = 7532) does not meet the recommended intakes [14].

One determinant of circulating vitamin E concentrations is its dietary intake and vegetable oils, nuts, wheat germ, olives, green leafy vegetables, and fruits, which are important sources of vitamin E [2,15,16]. However, the association of vitamin E levels with dietary patterns, reflecting complex combinations of nutrients and foods that may interact in their biological effects [17], is not well explored, particularly not in Northern European populations. Only two prior US studies assessed the association between circulating plasma vitamin E and *a posteriori* derived dietary patterns [18,19].

Therefore, in the present cross-sectional study from an elderly community-based sample of Northern Germany, we aimed to assess the distribution of both α- and γ-tocopherol levels in the community, to investigate their clinical and biochemical correlates and to study the association of circulating α- and γ-tocopherol levels with both a priori- and *a posteriori* derived dietary patterns.

2. Methods

2.1. Study Sample and Design

The study sample is a subsample of the PopGen control cohort, originally encompassing 1316 participants (747 individuals from a random community based-sample and 569 blood donors) [20]. Between 2010 and 2012, a first follow-up examination was attended by 952 participants. Participants received a physical examination at the study center and provided blood samples, obtained by trained nurses. Furthermore, participants filled-in standardized questionnaires on demographics, education, smoking status, diet [21] (detailed below), physical activity (detailed below), and various health-related characteristics [20]. For the present cross-sectional analysis, we used data from this first follow-up examination.

Blood samples were taken from participants in a sitting position after overnight fasting. lithium-heparin (LH)-plasma (Sarstedt, Germany) tubes were used for triglyceride, cholesterol, and high density lipoprotein (HDL)- and low density lipoprotein (LDL)-cholesterol, plasma C-reactive protein (CRP) and glucose measurements. HbA1c was measured from potassium-ethylenediaminetetraacetic acid (EDTA) blood tubes.

For immediate laboratory analyses, unfrozen blood samples were analyzed under standard clinical conditions on the same day in the Institute of Clinical Chemistry at the University Hospital Schleswig-Holstein, Campus Kiel, and the following measurements were obtained: plasma glucose, HbA1c, total cholesterol, high HDL- and LDL-cholesterol, triglycerides, and CRP (more detailed information is provided in Supplementary Materials Table S1. Vitamin E (α- and γ-tocopherol) was measured in frozen plasma, generated from LH-blood after centrifugation (3000× g for 15 min, room temperature), aliquotation, and storage at −80 °C.

Plasma vitamin E concentrations were measured in a subsample of 641 study participants.

The study protocol was approved by the Ethics Committee of the Medical Faculty of the University of Kiel (Project identification code A 156/03). All participants provided written informed consent.

2.2. Clinical Examination and Definitions

Weight (in kg, nearest to 0.1 kg) and height (in cm, measured at one decimal) were measured with the participants wearing light clothing, using the same digital scale with height rod, between 08:00 and 12:00 in the morning. From the measured weight, 2.0 kg were subtracted to correct for the remaining clothes. Body mass index (BMI) was calculated as body weight (kg)/height (m^2). Waist circumference (in cm, measured at one decimal) was measured at the midpoint between the lower ribs and iliac crest, and the hip circumference (in cm, measured at one decimal) was measured at the level of the trochanter major. After the participants had rested 5 min in a sitting position, blood pressure was measured twice (2 min interval) using a sphygmomanometer [22]. Average systolic and diastolic blood pressures were computed as arithmetic mean of the two measurements. Prevalent hypertension was defined as systolic blood pressure $\geq$140 mmHg, or diastolic blood pressure $\geq$90 mmHg, or use of antihypertensive medication, or self-reported hypertension history. Type 2 diabetes was defined as use of anti-diabetic medication, glycated hemoglobin (HbA1c) $\geq$6.5% (48 mmol/mol), or fasting serum glucose $\geq$126 mg/dL, or self-report physician diagnosis.

Participants also responded to validated questions [23] related to physical activity during the past 12 months including participation in several activities (walking, cycling, "do-it-yourself" activities, gardening, sports, and household chores), separately averaged for summer and winter, and the average number of stairs climbed per day. These activities were then multiplied by the corresponding metabolic equivalent of task (MET)-values and summed over all activities [24,25]. In order to assess whether participants meet the recommended dietary allowance of vitamin E, the individual dietary vitamin E intake of each person was compared to the recommended dietary vitamin E intake (stratified by age and sex), as issued by the German Nutrition Society [13].

2.3. Assessment of Dietary Variables

Dietary intake was assessed using a validated, self-administered, semi-quantitative 112-item food-frequency questionnaire (FFQ) designed especially for the German population [21]. Participants were asked to report the frequency of consumption of 112 food and beverage items during the previous 12 months. Nutrients and energy intakes were determined using the German Food Code and Nutrient Data Base (version II.3) and were provided by the Department of Epidemiology of the German Institute of Human Nutrition Potsdam Rehbrücke [26]. The FFQ included questions related to the use of vitamin E supplements. To reduce the arbitrariness of food-item grouping for exploratory dietary patterns analysis, food and beverage items were grouped into 39 food groups according to Kröger et al. [27].

2.4. Laboratory Analyses

Vitamin E (α- and γ-tocopherol) levels were determined at the Institute of Human Nutrition and Food Science from the University of Kiel in Germany using a high performance liquid chromatography (HPLC) method with fluorescence detection. Regarding the HPLC conditions, vitamin E concentrations were quantified by an external standard curve using a Jasco HPLC system (Jasco GmbH Deutschland, Gross-Umstadt, Germany; equipped with an autosampler (Jasco AS-2057; temperature 4 °C), pump (PU-2080), ternary gradient unit (LG-2080-02), 3 line degasser (DG-2080-53), and fluorescence detector (FP2020 Plus)) with a Waters Spherisorb ODS-2,3 µm column (100 × 4.6 mm) protected with a guard column. The chromatographic separation was done by isocratic elution with methanol:water (98:2, *v*/*v*) as mobile phase. The flow rate of the mobile phase was set at 1.2 mL/min and oven temperature at room temperature. The fluorescence detector operated an excitation wavelength of 290 nm and emission wavelength of 325 nm. The analytic run time was 7 min. The injection volume was set at

40 µL, and duplicate measurements of technical replications were performed. Preparing samples for analyses, plasma (50 µL) was homogenized in 2 mL 1% ascorbic acid (in ethanol), 700 µL deionised water, 50 µL 0.1% butylated hydroxytoluol (in ethanol), and 2 mL n-hexane. The samples were centrifuged (1000× *g* for 5 min at 4 °C). After separating the phases, 1000 µL of the upper phase was dried under vacuum in a RC-1010 centrifugal evaporator (Jouan, Saint-Herblain, France), and the samples were re-suspended in 200 µL mobile phase (methanol:water, 98:2, *v*/*v*) [28]. The coefficients of variation for α- and γ-tocopherol were 1.05% and 1.29%, respectively. Intra- and inter-day variations of α- and γ-tocopherol levels are provided in Supplementary Materials Table S2.

2.5. Statistical Analyses

Few missing values (n = 12) of categorical variables were replaced by the most commonly observed category of that respective variable. Missing values of normally distributed continuous variables (n = 10) were substituted by the respective mean, and skewed continuous variables were substituted by the sex-specific median (n = 2). Of the 641 CRP values, 247 were below the detection limit (0.9 mg/dL). For these values, participants were assigned a value equal to the half of the detection limit. γ-tocopherol values below the detection limit (n = 14) were replaced by the lowest γ-tocopherol value measured in our sample. Because vitamin E is bound to lipids in the circulation [2], the α- and γ-tocopherol/cholesterol ratios (µmol/mmol) were calculated by dividing α-tocopherol (µmol/L) and γ-tocopherol (µmol/L) concentrations by total cholesterol (mmol/L), as reported elsewhere [29].

Participants were categorized into tertiles based on the distribution of their α- and γ-tocopherol/cholesterol ratios. Mean values and the prevalence of baseline characteristics across α- and γ-tocopherol/cholesterol ratio tertiles were assessed using a general linear model, adjusting for age and sex.

2.6. Correlates of Circulating Vitamin E Biomarkers

Clinical, anthropometric, and biochemical correlates of plasma α- and γ-tocopherol levels and of the α-tocopherol/cholesterol ratio and the γ-tocopherol/cholesterol ratio (4 biomarkers, each considered separately) were determined using linear regression models with backward selection (variables with $p > 0.10$ were eliminated). Eligible covariates for these models were age; sex; the residual of waist circumference regressed on BMI; physical activity; systolic and diastolic blood pressure; triglycerides; CRP, HDL-, and LDL-cholesterol; HbA1c; fasting serum glucose; vitamin E supplement use; smoking status (never, current, former); education level (≤9, 10, or ≥11 years); and total energy intake. Age and sex were forced in the model, and categorical variables with more than two categories were included as indicator variables.

Correlations of vitamin E concentrations measured in plasma with the estimated intake of food groups rich in vitamin E and with estimated dietary vitamin E intake were determined using Spearman partial correlation coefficients adjusted for age, sex, and total energy intake.

2.7. Dietary Pattern Analyses

Two approaches were used to derive dietary patterns. First, Principal Component Analysis (PCA) was performed on 39 food groups. Briefly, PCA selects factors that explain as much predictor variation as possible [30]. The PCA works only with one set of variables, called predictors (food groups in g/day), and the number of factors that can be extracted is equal to the number of predictor variables. To identify the number of factors to retain, the Kaiser criterion (eigenvalue > 1.0) and the visual inspection of the screen plot were applied. The orthogonal varimax rotation method was used to enhance the difference between loadings, which allowed for easier interpretability.

For each participant, the extracted dietary pattern score was calculated as a sum of the products of the intake of 39 food groups with the corresponding factor loadings. Factor loadings represent the correlations of each food group with the dietary pattern score. The criterion of factor loadings was determined as greater than 0.2.

Second, an a priori dietary pattern rich in vitamin E reported by Gonzalez [31] was constructed to analyse the associations between a vitamin E-rich dietary pattern with 3 outcome variables (each outcome considered separately: (i) dietary α-tocopherol intake; (ii) circulating α-tocopherol levels (plasma); and (iii) circulating γ-tocopherol levels (plasma). Gonzalez [31] chose the top ten food groups that explained the most variance in energy-adjusted vitamin E intake to construct the food pattern rich in vitamin E.

To determine the association of *a posteriori* and a priori derived dietary patterns (exposure) with plasma α- and γ-tocopherol levels (outcome, each biomarker considered separately), a linear regression model was built. Correlations between dietary patterns (exposure) and dietary α-tocopherol intake (outcome) were tested with Spearman partial correlation coefficients adjusted for age, sex, and other covariates (see below). In addition, a sensitivity analysis was conducted by excluding vitamin E supplement users (n = 48). In a further sensitivity analysis, we excluded individuals with missing γ-tocopherol values (n = 14).

All statistical models were adjusted for total energy intake and for significant correlates of the respective biomarkers that were identified in the backward selection linear regression, as described above ("Correlates of circulating vitamin E biomarkers"). Additionally, interactions between dietary patterns and age, sex, and vitamin E supplementation were tested by including respective interaction terms into the regression models.

All analyses were conducted using SAS software version 9.4 (SAS Institute, Inc., Cary, NC, USA). All p-values were two-sided, and $p < 0.05$ was considered statistically significant.

3. Results

General characteristics of the study sample are displayed in Table 1. The median intakes of dietary α-tocopherol were 11.6 mg/day (men: 11.9 mg/day, women: 11.3 mg/day). 36.3% of men and 41.2% of women met the recommended dietary allowance for vitamin E from food as defined by the German Nutrition Society. The median levels of α-tocopherol and for the α-tocopherol/cholesterol ratio were 31.54 µmol/L and 5.53 µmol/mmol, respectively. A total of 57.6% of the study sample had adequate circulating α-tocopherol levels (above 30 µmol/L). No vitamin E deficiency (defined as <12 µmol/L) was observed in the study sample. For γ-tocopherol and the γ-tocopherol/cholesterol ratio, the median values were 1.35 µmol/L and 0.24 µmol/mmol, respectively. Overall, 7.5% of the study sample were taking vitamin E supplements.

Table 1. General characteristics of the study sample (n = 641).

Characteristics	
Men, %	59.4
Age, years	61.2 (11.6)
Body mass index, kg/m^2	27.2 (4.5)
Weight, kg	80.5 (15.7)
Hip circumference, cm	102.3 (8.8)
Waist circumference, cm	96.2 (13.0)
Systolic blood pressure, mmHg	139.6 (18.2)
Diastolic blood pressure, mmHg	85.0 (8.9)
Prevalent hypertension, %	69.0

Table 1. *Cont.*

Characteristics	
Current smokers, %	10.9
High education (≥11 years), %	35.9
Prevalent diabetes, %	10.8
Vitamin E supplementation, %	7.5
Physical activity, MET-hour/week	90.0 (58.3, 131.6)
Alcohol consumption, g/day	9.6 (3.7, 18.7)
Dietary α-tocopherol intake (FFQ), mg/day	11.6 (9.7, 13.9)
Biochemical features	
α-tocopherol, μmol/L	31.5 (27.27, 37.03)
α-tocopherol > 30 μmol/L, % *	57.6
α-tocopherol/cholesterol ratio, μmol/mmol	5.53 (4.88, 6.33)
γ-tocopherol, μmol/L	1.35 (0.99, 1.79)
γ-tocopherol/cholesterol ratio, μmol/mmol	0.24 (0.18, 0.31)
HbA1c, %	5.60 (5.40, 5.90)
C-reactive protein, mg/dL	1.20 (0.45, 2.50)
HDL-cholesterol, mg/dL	65.81 (18.61)
LDL-cholesterol, mg/dL	131.36 (34.07)
Total cholesterol, mg/dL	223.42 (41.38)
Triglycerides, mg/dL	106.0 (76.0, 139.0)

MET: Metabolic equivalent; FFQ: Food frequency questionnaire; IQR: interquartile range; HDL: High density lipoprotein; LDL: Low density lipoprotein. Values are presented as mean (standard deviation), median (IQR: Q_1, Q_3) or percentages (%). * Participants with α-tocopherol above the criterion of α-tocopherol adequacy.

3.1. Correlates of Vitamin E Biomarkers

In exploratory analyses, age- and sex-adjusted characteristics of the study sample stratified by tertiles for α- and γ-tocopherol/cholesterol ratios are shown in Supplementary Materials Tables S3 and S4. All lipid traits were strongly associated with the α-tocopherol/cholesterol ratio. Furthermore, HbA1c tended to be slightly higher, with a higher α-tocopherol/cholesterol ratio (Supplementary Materials Table S3). Similarly, all lipid traits were associated with the γ-tocopherol/cholesterol ratio. In addition, prevalent diabetes was slightly more common, whereas vitamin E supplementation was slightly less common in the top tertile of the γ-tocopherol/cholesterol ratio. Finally HbA1c, CRP, BMI, waist circumference, and triglyceride levels were slightly higher in the top compared to the bottom tertile of γ-tocopherol/cholesterol ratio levels (Supplementary Materials Table S4).

After backward elimination with age and sex forced into the models, only triglycerides, HDL-, and LDL-cholesterol and vitamin E supplementation were statistically significant correlates of α-tocopherol levels and the α-tocopherol/cholesterol ratio. The identified set of correlates explained 35.9% and 19.7% of total variation in α-tocopherol and the α-tocopherol/cholesterol ratio (Table 2). Similarly, γ-tocopherol and the γ-tocopherol/cholesterol ratio were correlated with lipid traits and vitamin E supplementation after backward elimination with age and sex forced into the models. These correlates explained 12.7% and 10.6% of total variation of γ-tocopherol and the γ-tocopherol/cholesterol ratio (Table 2).

Table 2. Correlates for (a) α-tocopherol (b) α-tocopherol/cholesterol ratio, (c) γ-tocopherol, and (d) γ-tocopherol/cholesterol ratio.

(a) α-Tocopherol *	β Estimate	SE	*p* Value
Age	−0.0006	0.0007	0.358
Sex	−0.0108	0.0184	0.556
Triglycerides	0.0015	0.0001	<0.0001
HDL-cholesterol	0.0033	0.0005	<0.0001
LDL-cholesterol	0.0027	0.0002	<0.0001
Vitamin E supplementation	0.0863	0.0307	0.005
$R^2 = 0.359$			
(b) α-Tocopherol/Cholesterol Ratio *			
Age	−0.0014	0.0007	0.040
Sex	−0.0096	0.0187	0.606
Triglycerides	0.0009	0.0001	<0.0001
HDL-cholesterol	−0.0013	0.0005	0.015
LDL-cholesterol	−0.0021	0.0002	<0.0001
Vitamin E supplementation	0.0777	0.0311	0.013
$R^2 = 0.197$			
(c) γ-Tocopherol *			
Age	−0.0011	0.1562	0.474
Sex	−0.0681	0.0016	0.108
Triglycerides	0.0019	0.0423	<0.0001
HDL-cholesterol	0.0031	0.0003	0.011
LDL-cholesterol	0.0018	0.0012	0.001
Vitamin E supplementation	−0.3170	0.0006	<0.0001
$R^2 = 0.127$			
(d) γ-Tocopherol/Cholesterol Ratio *			
Age	−0.0020	0.0016	0.201
Sex	−0.0454	0.0381	0.235
Triglycerides	0.0015	0.0003	<0.0001
LDL-cholesterol	−0.0030	0.0006	<0.0001
Vitamin E supplementation	−0.3225	0.0707	<0.0001
$R^2 = 0.106$			

SE: Standard Error; HDL: High density lipoprotein; LDL: Low density lipoprotein. * Log transformed values. Variables with $p > 0.10$ were eliminated; age and sex were forced in the model.

No correlations were observed between plasma vitamin E levels and the intakes of vitamin E rich foods and dietary vitamin E intake from food. The correlation coefficient observed between plasma α- and γ-tocopherol levels and dietary α-tocopherol intake did not change in magnitude after exclusion of vitamin E supplement users (Table 3).

Table 3. Correlations of vitamin E rich food groups and estimated vitamin E intake from food frequency questionnaire (FFQ) with plasma vitamin E adjusted for age, sex, and total energy intake.

	α-Tocopherol		γ-Tocopherol	
	rho	95% CI	rho	95% CI
Leafy vegetables	0.03	−0.05, 0.10	0.01	−0.07, 0.08
Fruiting and root vegetables	0.01	−0.06, 0.09	0.04	−0.04, 0.12
Cabbages	−0.04	−0.12, 0.04	0.05	−0.02, 0.13
Other vegetables	−0.04	−0.12, 0.04	0.03	−0.04, 0.11
Legumes	0.02	−0.06, 0.10	0.05	−0.03, 0.12
Nuts	0.08	−0.002, 0.15	0.07	−0.01, 0.15

Table 3. *Cont.*

	α-Tocopherol		γ-Tocopherol	
	rho	**95% CI**	**rho**	**95% CI**
Other fruits	0.11	0.04, 0.20	0.12	0.04, 0.19
Breakfast cereals	0.06	−0.001, 0.14	0.03	−0.05, 0.11
Other cereals	0.03	−0.04, 0.11	0.01	−0.07, 0.09
Margarine	0.01	−0.07, 0.08	0.02	−0.06, 0.10
Vegetables oils	0.06	−0.07, 0.14	0.02	−0.06, 0.10
Dietary α-tocopherol intake (FFQ)	0.01	−0.07, 0.09	0.005	−0.07, 0.08
Dietary α-tocopherol intake (FFQ) *	0.01	−0.07, 0.09	0.01	−0.07, 0.09

Values are presented as Spearman correlation coefficient with 95% Confidence Interval (CI). Other vegetables: grain and pod vegetables, onion, garlic, stalk vegetables, mushrooms, sprouts, mixed salad, and mixed vegetables; other fruits: mixed fruits, and olives; other cereals: flour, flakes, starches, semolina, dough and pastry, salty biscuits, and crackers. * Non vitamin E supplement users (n = 593).

3.2. Dietary Pattern Analyses

Three major patterns were identified through PCA: dietary pattern 1 was characterized by high intakes of vegetables oils, fruiting and root vegetables, condiments and yeast, leafy vegetables, cabbages, and other vegetables. The second dietary pattern included high intakes of processed meat, red meat and game, bread, other fats, potatoes, sugar and confectionery, and butter. Dietary pattern 3 was characterized by high intakes of breakfast cereals, other cereals, nuts, fish and milk, and dairy products (Figure 1).

These patterns are explained in our sample 4.4%, 3.5%, and 2.3% variation in food intake, respectively. A full list of factor loadings from PCA-derived patterns is shown online in Supplementary Materials Table S5. In multivariable-adjusted models, both the dietary pattern 1 and the dietary pattern 3 showed a positive correlation with dietary α-tocopherol intake, which was stronger for the dietary pattern 1, whereas dietary pattern 2 showed an inverse, though weak, correlation. However, in multivariable-adjusted regression analyses, none of the PCA-derived patterns was significantly related to circulating plasma vitamin E levels (Table 4). After excluding supplement users, a borderline significant inverse association was observed between the dietary pattern 2 and plasma α-tocopherol levels (β, SE = −0.032, 0.016; p = 0.047). No interaction was observed between the dietary patterns and sex, age, and vitamin E supplementation in relation to plasma vitamin E levels (data not shown).

The a priori defined dietary pattern was associated with dietary α-tocopherol intake ($p < 0.0001$). However, this pattern was not related to circulating vitamin E levels in our sample (p = 0.475, p = 0.431, respectively for α- and γ-tocopherol) (Table 5).

In a sensitivity analysis, after excluding individuals with missing γ-tocopherol values (n = 14), the results were essentially unchanged (data not shown).

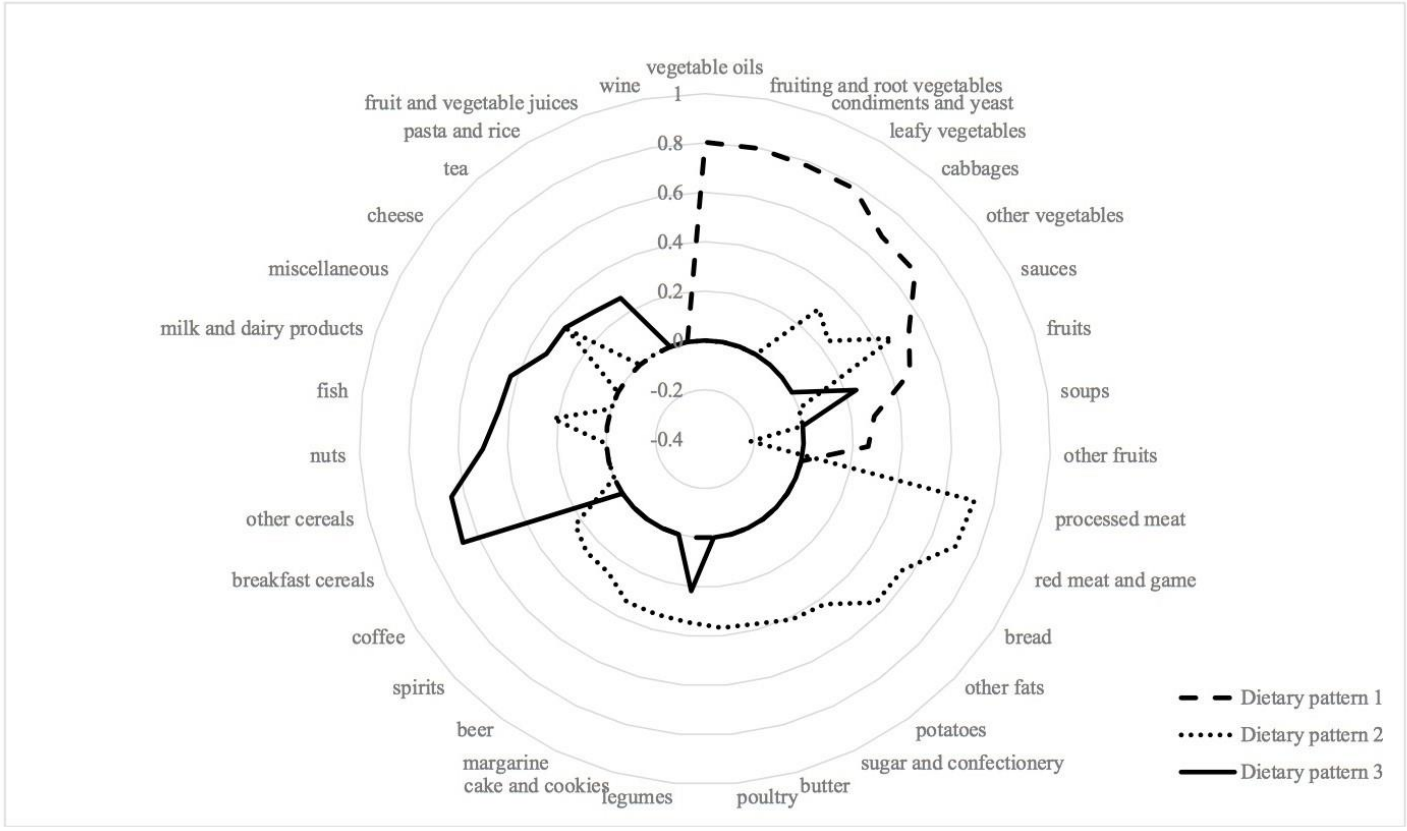

Figure 1. Spider web diagram from the Principal Component Analysis (factor loadings of food groups |> 0.20|).

Table 4. Multivariable-adjusted linear regression models for the association between a Principal Component Analysis (PCA)-derived dietary patterns with plasma α- and γ-tocopherol levels and dietary α-tocopherol intake.

	α-Tocopherol								
Overall (*n* = 641)	**Dietary Pattern 1**			**Dietary Pattern 2**			**Dietary Pattern 3**		
	***β* Estimate**	**SE**	***p* Value**	***β* Estimate**	**SE**	***p* Value**	***β* Estimate**	**SE**	***p* Value**
α-tocopherol, μmol/L [*,†]	−0.007	0.008	0.415	−0.026	0.015	0.089	0.015	0.008	0.077
α-tocopherol/cholesterol ratio, μmol/mmol [*,†]	−0.006	0.009	0.508	−0.020	0.016	0.207	0.015	0.009	0.087
Dietary α-tocopherol intake (FFQ), mg/day [†]	0.72 [‡]	0.68, 0.75	<0.0001	−0.18 [‡]	−0.25, −0.10	0.002	0.32 [‡]	0.24, 0.39	<0.0001
Non vitamin E supplement users (*n* = 593)									
α-tocopherol, μmol/L [*,§]	−0.003	0.009	0.712	−0.032	0.016	0.047	0.015	0.009	0.097
α-tocopherol/cholesterol ratio, μmol/mmol [*,§]	−0.002	0.009	0.790	−0.025	0.016	0.135	0.014	0.009	0.112
Dietary α-tocopherol intake (FFQ), mg/day [§]	0.71 [‡]	0.67, 0.75	<0.0001	−0.16 [‡]	−0.24, −0.08	0.0001	0.34 [‡]	0.27, 0.41	<0.0001
	γ-Tocopherol								
Overall (*n* = 641)									
γ-tocopherol, μmol/L [*,‡]	0.006	0.019	0.759	−0.013	0.036	0.715	0.027	0.019	0.165
γ-tocopherol/cholesterol ratio, μmol/mmol [*,‖]	0.007	0.019	0.715	−0.004	0.036	0.920	0.024	0.019	0.216
Non vitamin E supplement users (*n* = 593)									
γ-tocopherol, μmol/L [*,§]	0.004	0.020	0.844	0.004	0.036	0.906	0.022	0.019	0.262
γ-tocopherol/cholesterol ratio, μmol/mmol [*,¶]	0.005	0.020	0.790	0.014	0.036	0.695	0.018	0.019	0.352

SE: Standard Error; FFQ: Food frequency questionnaire. * Log transformed values. [†] Adjustment for: sex, age, triglycerides, HDL-cholesterol, LDL-cholesterol, vitamin E supplementation, total energy intake. [‡] Spearman correlation coefficient with 95% Confidence Interval. [§] Adjustment for all the covariates in model [†] excluding vitamin E supplementation. [‖] Adjustment for: sex, age, triglycerides, LDL-cholesterol, vitamin E supplementation, total energy intake. [¶] Adjustment for all the covariates in model [‖] excluding vitamin E supplementation.

Table 5. Multivariable-adjusted linear regression models for the association between an a prioriderived, vitamin E rich dietary pattern with plasma α- and γ-tocopherol levels and dietary α-tocopherol intake.

α-Tocopherol				
Overall (n = 641)	**β Estimate**	**SE**	**p Value**	**R^2**
α-tocopherol, μmol/L *,†	−0.002	0.003	0.475	0.359
α-tocopherol/cholesterol ratio, μmol/mmol *,†	−0.001	0.004	0.683	0.198
Dietary α-tocopherol intake (FFQ), mg/day †	0.51 ‡	0.45, 0.56	<0.0001	
Non vitamin E supplement users (n = 593)				
α-tocopherol, μmol/L *,§	−0.001	0.004	0.749	0.355
α-tocopherol/cholesterol ratio, μmol/mmol *,§	−0.0002	0.004	0.947	0.179
Dietary α-tocopherol intake (FFQ), mg/day §	0.49 ‡	0.42, 0.55	<0.0001	
γ-Tocopherol				
Overall (n = 641)				
γ-tocopherol, μmol/L *,†	0.006	0.008	0.431	0.100
γ-tocopherol/cholesterol ratio, μmol/mmol *,‖	0.008	0.008	0.346	0.080
Non vitamin E supplement users (n = 593)				
γ-tocopherol, μmol/L *,§	0.005	0.008	0.549	0.107
γ-tocopherol/cholesterol ratio, μmol/mmol *,¶	0.007	0.008	0.412	0.084

SE: Standard Error; FFQ: Food frequency questionnaire. * Log transformed value. † Adjustment for: sex, age, triglycerides, HDL-cholesterol, LDL-cholesterol, vitamin E supplementation, total energy intake. ‡ Spearman correlation coefficient with 95% Confidence Interval. § Adjustment for all the covariates in model † excluding vitamin E supplementation. ‖ Adjustment for: sex, age, triglycerides, LDL-cholesterol, vitamin E supplementation, total energy intake. ¶ Adjustment for all the covariates in model ‖ excluding vitamin E supplementation.

4. Discussion

4.1. Principal Findings

Our main observations were as follows: first, nearly 40% of the participants in our sample met the recommended dietary allowance for α-tocopherol from foods, as recommended by the German Nutrition Society. Close to 60% of our participants (57.6%) had adequate circulating α-tocopherol levels above 30 μmol/L. As expected, triglycerides, HDL- and LDL-cholesterol, and vitamin E supplementation were important correlates of both plasma α- and γ-tocopherol and of the ratio of each biomarker to total cholesterol. Third, we confirmed the association of a previously reported dietary pattern with dietary α-tocopherol intake. However, this pattern was not related to circulating plasma α- and γ-tocopherol levels in our sample. Similarly, three dietary patterns derived by PCA were not associated with circulating plasma α- and γ-tocopherol levels in the overall sample. One of these patterns, however, pattern 2 was inversely related to plasma α-tocopherol concentrations when supplement users were excluded.

4.2. In the Context of the Published Literature

Dietary Vitamin E Intake and Distribution of Circulating Vitamin E Levels in the Population

The dietary α-tocopherol intake in our sample (men: 11.9 mg/day, women: 11.3 mg/day) was slightly lower than in two German subgroups within the European Prospective Investigation into Cancer and Nutrition study [32]. Consistently, the proportion of individuals who met the requirement for dietary vitamin E intake (38.8%) was slightly lower than that observed in a prior study from Germany (n = 15,371, 52%, and 51% of men and women, respectively) [14]. In a report from the US, based on data from the National Health and Nutrition Examination Survey (NHANES), only 4.9% of men and 4.5% of women met the Recommended Daily Allowance (15 mg/day) for dietary vitamin E [33]. It has to be kept in mind that the vitamin E equivalent of the German Nutrient Database

is mainly based on α-tocopherol without consideration of other vitamin E compounds. Therefore, the calculated values represent an underestimation of the actual vitamin E intake [34].

The median circulating levels of plasma α- (31.54 μmol/L) and γ-tocopherol (1.35 μmol/L) were in agreement with prior studies conducted in Germany [35,36]. Compared to data from NHANES [37], values for α-tocopherol and the α-tocopherol/cholesterol ratio in the present sample were slightly higher. Using a criterion of adequacy of 30 μmol/L [12], about 60% of individuals in our sample had adequate α-tocopherol concentrations, a proportion that is higher than in other studies in Europe (39%) and in the US (13%) [38].

Also, the levels of circulating γ-tocopherol were comparable to the results conducted in six European countries (total sample size, $n = 2118$) [39]. By contrast, most studies conducted in the US reported higher average plasma γ-tocopherol levels as compared to studies conducted in Europe [16]. This is likely explained by the fact that γ-tocopherol is the major form (≈70%) of vitamin E in the US diet [40]. The prevalence of vitamin E supplement users in our sample is slightly lower (7.5%) than in the second German National Nutritional Survey (11.4%) [14] and in a report from the US (11.4%) [41].

4.3. Correlates of Vitamin E Biomarkers

We identified lipid traits and the use of vitamin E supplements as key correlates of circulating α- and γ-tocopherol in our sample. The association of vitamin E levels with circulating lipid concentrations is well established and explained by the fact that vitamin E is transported in plasma in lipoproteins [2]. Also, the association of circulating vitamin E levels with the use of vitamin E supplementation is plausible. As in prior studies, α-tocopherol was positively related, and γ-tocopherol was inversely related to intake of vitamin E from dietary supplements [11,42,43].

Vitamin E consumed from supplements is predominantly in the form of α-tocopherol and may also explain increased α-tocopherol levels and decreased γ-tocopherol levels [42]. It is well documented that supplementation with α-tocopherol reduces γ-tocopherol levels in humans [44] and rodents [45]. This observation is partly explained by the function of the hepatic α-tocopherol transfer protein (α-TTP) [46], which has a higher affinity for α-tocopherol compared to other vitamin E forms (α-tocopherol = 100%, β-tocopherol = 38%, γ-tocopherol = 9%, δ-tocopherol = 2%) [47]. Once vitamin E is absorbed and taken up by the liver, the hepatic α-TTP preferentially transfers α-tocopherol into circulating lipoproteins and accounts for the higher concentration of α-tocopherol in plasma, whereas non-α-tocopherol forms are largely degraded in the liver and excreted [46,48]. An additional explanation of why γ-tocopherol levels are decreased after α-tocopherol supplementation may be found in a degradation of desmethyl vitamers via an induction of cytochrome P450 enzymes, which regulate vitamin E metabolism [45,49].

Nevertheless, in the present study the inclusion of supplement users (7.5% of the sample) in the analyses did not change the magnitude of the correlation between plasma and dietary α-tocopherol intake.

4.4. Lack of Association between Estimated Dietary α-Tocopherol Intake and Circulating Vitamin E Levels

One rather small study conducted in Germany [50] ($n = 92$) reported very weak correlations between plasma α-tocopherol and dietary α-tocopherol intake ($r = 0.14$), whereas others have reported higher correlations between plasma α-tocopherol levels and dietary α-tocopherol when supplement intake was taken into account [51–53].

In our study, however, we observed no evidence for a correlation between dietary α-tocopherol intake and circulating α-tocopherol levels, which is in line with several other studies [51,54,55]. Yet, these controversial observations may suggest that variation in circulating vitamin E levels is not only determined by dietary vitamin E intake but also by many other factors, including age, gender, lifestyle factors (e.g., smoking and alcohol consumption) [56,57], circulating lipid levels [29,46], genetic variation and variation in the absorption, metabolism, and excretion of vitamin E, as reviewed in detail elsewhere [56,57]. For example, substantial inter-individual variation has been reported regarding the intestinal absorption of vitamin E (20–80%) [56], in part explained by other dietary factors such as the

intake of competing nutrients, the food matrix, or the amount of fat [15,56]. Additionally, the renal excretion of vitamin E [56], as well as its metabolism, might vary with different metabolic conditions such as obesity or the metabolic syndrome [58,59].

Further, the FFQ, the most commonly used tool to assess dietary intake in large cohort studies, does not capture all foods rich in vitamin E (e.g., olives) and reflects a very different time interval than circulating vitamin E levels. While the FFQ inquires about the food intake in the past 12 months, the half-life of α-tocopherol in plasma is 48 h, and the ingested α-tocopherol appears in plasma within 2–4 h and peaks in 5–14 h [60]. All these factors might have contributed to the poor correlation between dietary α-tocopherol intake and plasma α-tocopherol levels observed in our sample and in other studies [51,54,55], as well as to the partially conflicting results between studies [51–53]. Yet, we cannot completely rule out whether an unknown bias such as the unreported consumption of special or local foods might have affected the lack of correlation between vitamin E intake and plasma levels. Indeed, it will be interesting to re-evaluate the present findings once the FFQs have been recalculated when new data from the Federal Institute for Risk Assessment (BfR) Meal Study, which will cover at least 90% of the foods consumed in Germany, has been included in the newly generated FFQ database representative of the typical German eating habits.

4.5. Association of Dietary Patterns with Dietary Vitamin E Intake and Circulating Vitamin E Levels

We assessed the association between three dietary patterns derived by PCA and one previously reported as a vitamin E-rich pattern with dietary vitamin E intake in our sample, as well as with circulating α- and γ-tocopherol levels. The previously reported, vitamin E-rich dietary pattern, as well as dietary patterns 1–3 derived by PCA, were not associated with circulating plasma vitamin E levels in the overall sample. However, the vitamin E-rich dietary pattern and dietary pattern 1 and dietary pattern 3 displayed moderate to good positive correlations with dietary α-tocopherol intake. Given the lack of association between dietary vitamin E intake and circulating α- and γ-tocopherol concentrations (discussed above), this discrepancy in the association of the dietary patterns with dietary vitamin E intake and circulating vitamin E concentrations is not surprising.

When supplement users were excluded, circulating plasma α-tocopherol levels were only weakly inversely related to the dietary pattern (dietary pattern 2) characterized by high intakes of meat (processed meat, red meat, and game), bread, other fats, potatoes, sugar, and confectionery and butter. Only two prior US studies assessed the association between circulating plasma vitamin E and dietary patterns [18,19]. In 602 participants a "sweet" dietary pattern, which was similar to our second dietary pattern, had the lowest α-tocopherol levels relative to a "fruit and breakfast cereal" pattern and a "milk and milk products" pattern [18]. In 373 African Americans, a "juice" cluster (characterized by high intakes of fruit juice) showed higher serum α-tocopherol concentrations as compared to a "fast food" cluster [19]. In the present study, we did not identify a "juice" pattern, but fruit was part of our dietary patterns 1 and 3. Additionally, our dietary pattern 1 was characterized by high intakes of vegetable oils, the most important source of dietary vitamin E.

4.6. Strength and Limitations

Strengths of the present study include the population-based design, the availability of standardized information regarding dietary intake of vitamin E, and the detailed assessment of potential correlates, obtained in a standardized fashion by trained personnel. Some limitations merit consideration. Vitamin E status was defined based on α- and γ-tocopherol measurements in a single blood sample. Since dietary intake was assessed using a self-administered FFQ only, misreporting of vitamin E may have occurred. The relatively modest sample size is a further limitation.

5. Conclusions

In conclusion, most of the study participants in this Northern German sample had adequate plasma α-tocopherol levels, suggesting an overall adequate supply of this vitamin from the diet.

However, the inverse trend observed between a "western" dietary pattern and lower plasma α-tocopherol concentrations should raise awareness that unhealthy eating patterns could negatively affect the vitamin E status. Because of the many physiological actions of vitamin E and the intense interest in possible health effects, prospective studies are warranted to confirm our results and to evaluate the actual impact of the reported findings in terms of nutrition and health outcomes.

Supplementary Materials: The following are available online at www.mdpi.com/2072-6643/10/1/1/s1, Table S1: details about the measurements of glucose, cholesterol, high density lipoprotein (HDL)-, and low density lipoprotein (LDL)-cholesterol, triglyceride, C-reactive protein (CRP), and HbA1c; Table S2: intra- and inter-day variations of plasma α- and γ-tocopherol levels. Table S3: age- and sex-adjusted characteristics of the study sample according to tertiles (T) of α-tocopherol/cholesterol ratio; Table S4: age- and sex-adjusted characteristics of the study sample according to tertiles (T) of γ-tocopherol/cholesterol ratio; Table S5: factor loadings for food groups that highly |>0.2| loaded in principal component analysis (PCA).

Acknowledgments: Manja Koch is recipient of a Postdoctoral Research Fellowship from the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG, KO 5187/1-1). RdG is supported by the Deutsche Forschungsgemeinschaft Excellence Cluster "Inflammation Interfaces" (grants EXC306 and EXC306/2). The PopGen 2.0 Network is supported by the German Federal Ministry of Education and Research (grant 01GR0468 and 01EY1103). The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Author Contributions: W.L. and G.R. formulated the research question; W.L., S.S. and U.N. designed the study; T.E. performed the vitamin E measurements, S.W. and R.d.G. performed the statistical analyses, S.W., W.L., R.d.G., S.P.-D., M.K. and S.S. contributed to the interpretation of the data; S.W. and W.L. drafted the manuscript. All authors (S.W., R.d.G., S.P.-D., T.E., M.K., S.S., I.R., G.J., U.N., G.R. and W.L.) critically reviewed and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sauberlich, H.E. Vitamin E (Tocopherols). In *Laboratory Tests for the Assessment of Nutritional Status*, 2nd ed.; CRC Press: Boca Raton, FL, USA, 1999; pp. 249–266.
2. Jiang, Q. Natural forms of vitamin E: Metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. *Free Radic. Biol. Med.* **2014**, *72*, 76–90. [CrossRef] [PubMed]
3. Institute of Medicine (US). Panel on Dietary Antioxidants and Related Compounds. In *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*; Hardcopy Version at National Academies Press: Washington, DC, USA, 2000; pp. 186–283.
4. Pham-Huy, L.A.; He, H.; Pham-Huy, C. Free radicals, antioxidants in disease and health. *Int. J. Biomed. Sci.* **2008**, *4*, 89–96. [PubMed]
5. Ohrvall, M.; Sundlof, G.; Vessby, B. Gamma, but not alpha, tocopherol levels in serum are reduced in coronary heart disease patients. *J. Intern. Med.* **1996**, *239*, 111–117. [CrossRef] [PubMed]
6. Wright, M.E.; Lawson, K.A.; Weinstein, S.J.; Pietinen, P.; Taylor, P.R.; Virtamo, J.; Albanes, D. Higher baseline serum concentrations of vitamin E are associated with lower total and cause-specific mortality in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *Am. J. Clin. Nutr.* **2006**, *84*, 1200–1207. [PubMed]
7. Stampfer, M.J.; Hennekens, C.H.; Manson, J.E.; Colditz, G.A.; Rosner, B.; Willett, W.C. Vitamin E consumption and the risk of coronary disease in women. *N. Engl. J. Med.* **1993**, *328*, 1444–1449. [CrossRef] [PubMed]
8. Rimm, E.B.; Stampfer, M.J.; Ascherio, A.; Giovannucci, E.; Colditz, G.A.; Willett, W.C. Vitamin E consumption and the risk of coronary heart disease in men. *N. Engl. J. Med.* **1993**, *328*, 1450–1456. [CrossRef] [PubMed]
9. Riemersma, R.A.; Wood, D.A.; Macintyre, C.C.; Elton, R.A.; Gey, K.F.; Oliver, M.F. Risk of angina pectoris and plasma concentrations of vitamins A, C, and E and carotene. *Lancet* **1991**, *337*, 1–5. [CrossRef]
10. Dong, Y.; Liu, Y.; Shu, Y.; Chen, X.; Hu, J.; Zheng, R.; Ma, D.; Yang, C.; Guan, X. Link between risk of colorectal cancer and serum vitamin E levels: A meta-analysis of case-control studies. *Medicine* **2017**, *96*, e7470. [CrossRef] [PubMed]
11. Huang, H.Y.; Appel, L.J. Supplementation of diets with alpha-tocopherol reduces serum concentrations of gamma- and delta-tocopherol in humans. *J. Nutr.* **2003**, *133*, 3137–3140. [PubMed]
12. Raederstorff, D.; Wyss, A.; Calder, P.C.; Weber, P.; Eggersdorfer, M. Vitamin E function and requirements in relation to PUFA. *Br. J. Nutr.* **2015**, *114*, 1113–1122. [CrossRef] [PubMed]

13. German Nutrition Society (2017). Vitamin E (Tocopherole). Available online: https://www.dge.de/wissenschaft/referenzwerte/vitamin-e/ (accessed on 18 June 2017).
14. Max Rubner-Institut. *Nationale Verzehrsstudie II. Ergebnisbericht Teil 2*; MRI: Karlsruhe, Germany, 2008.
15. Borel, P.; Preveraud, D.; Desmarchelier, C. Bioavailability of vitamin E in humans: An update. *Nutr. Rev.* **2013**, *71*, 319–331. [CrossRef] [PubMed]
16. Wagner, K.H.; Kamal-Eldin, A.; Elmadfa, I. Gamma-tocopherol—An underestimated vitamin? *Ann. Nutr. Metab.* **2004**, *48*, 169–188. [CrossRef] [PubMed]
17. Hu, F.B. Dietary pattern analysis: A new direction in nutritional epidemiology. *Curr. Opin. Lipidol.* **2002**, *13*, 3–9. [CrossRef] [PubMed]
18. Gao, X.; Martin, A.; Lin, H.; Bermudez, O.I.; Tucker, K.L. alpha-Tocopherol intake and plasma concentration of Hispanic and non-Hispanic white elders is associated with dietary intake pattern. *J. Nutr.* **2006**, *136*, 2574–2579. [PubMed]
19. Talegawkar, S.A.; Johnson, E.J.; Carithers, T.C.; Taylor, H.A., Jr.; Bogle, M.L.; Tucker, K.L. Serum carotenoid and tocopherol concentrations vary by dietary pattern among African Americans. *J. Am. Diet. Assoc.* **2008**, *108*, 2013–2020. [CrossRef] [PubMed]
20. Nothlings, U.; Krawczak, M. PopGen. A population-based biobank with prospective follow-up of a control group. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* **2012**, *55*, 831–835. [PubMed]
21. Nothlings, U.; Hoffmann, K.; Bergmann, M.M.; Boeing, H. Fitting portion sizes in a self-administered food frequency questionnaire. *J. Nutr.* **2007**, *137*, 2781–2786. [PubMed]
22. Barbaresko, J.; Siegert, S.; Koch, M.; Aits, I.; Lieb, W.; Nikolaus, S.; Laudes, M.; Jacobs, G.; Nothlings, U. Comparison of two exploratory dietary patterns in association with the metabolic syndrome in a Northern German population. *Br. J. Nutr.* **2014**, *112*, 1364–1372. [CrossRef] [PubMed]
23. Haftenberger, M.; Schuit, A.J.; Tormo, M.J.; Boeing, H.; Wareham, N.; Bueno-de-Mesquita, H.B.; Kumle, M.; Hjartaker, A.; Chirlaque, M.D.; Ardanaz, E.; et al. Physical activity of subjects aged 50–64 years involved in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Public Health Nutr.* **2002**, *5*, 1163–1176. [CrossRef] [PubMed]
24. Friedenreich, C.; Norat, T.; Steindorf, K.; Boutron-Ruault, M.C.; Pischon, T.; Mazuir, M.; Clavel-Chapelon, F.; Linseisen, J.; Boeing, H.; Bergman, M.; et al. Physical activity and risk of colon and rectal cancers: The European prospective investigation into cancer and nutrition. *Cancer Epidemiol. Biomarkers Prev.* **2006**, *15*, 2398–2407. [CrossRef] [PubMed]
25. Ainsworth, B.E.; Haskell, W.L.; Herrmann, S.D.; Meckes, N.; Bassett, D.R., Jr.; Tudor-Locke, C.; Greer, J.L.; Vezina, J.; Whitt-Glover, M.C.; Leon, A.S. 2011 Compendium of Physical Activities: A second update of codes and MET values. *Med. Sci. Sports Exerc.* **2011**, *43*, 1575–1581. [CrossRef] [PubMed]
26. Dehne, L.I.; Klemm, C.; Henseler, G.; Hermann-Kunz, E. The German Food Code and Nutrient Data Base (BLS II.2). *Eur. J. Epidemiol.* **1999**, *15*, 355–359. [CrossRef] [PubMed]
27. Kroger, J.; Ferrari, P.; Jenab, M.; Bamia, C.; Touvier, M.; Bueno-de-Mesquita, H.B.; Fahey, M.T.; Benetou, V.; Schulz, M.; Wirfalt, E.; et al. Specific food group combinations explaining the variation in intakes of nutrients and other important food components in the European Prospective Investigation into Cancer and Nutrition: An application of the reduced rank regression method. *Eur. J. Clin. Nutr.* **2009**, *63* (Suppl. S4), S263–S274. [CrossRef] [PubMed]
28. Augustin, K.; Blank, R.; Boesch-Saadatmandi, C.; Frank, J.; Wolffram, S.; Rimbach, G. Dietary green tea polyphenols do not affect vitamin E status, antioxidant capacity and meat quality of growing pigs. *J. Anim. Physiol. Anim. Nutr.* **2008**, *92*, 705–711. [CrossRef] [PubMed]
29. Thurnham, D.I.; Davies, J.A.; Crump, B.J.; Situnayake, R.D.; Davis, M. The use of different lipids to express serum tocopherol: Lipid ratios for the measurement of vitamin E status. *Ann. Clin. Biochem.* **1986**, *23 Pt 5*, 514–520. [CrossRef] [PubMed]
30. Hoffmann, K.; Schulze, M.B.; Schienkiewitz, A.; Nothlings, U.; Boeing, H. Application of a new statistical method to derive dietary patterns in nutritional epidemiology. *Am. J. Epidemiol.* **2004**, *159*, 935–944. [CrossRef] [PubMed]
31. Gonzalez, Z.C. Vitamin E Intake and Cardiovascular Diseases in the EPIC-Potsdam Study. Ph.D. Thesis, Technical University of Berlin, Berlin, Germany, 2011.

32. Jenab, M.; Salvini, S.; van Gils, C.H.; Brustad, M.; Shakya-Shrestha, S.; Buijsse, B.; Verhagen, H.; Touvier, M.; Biessy, C.; Wallstrom, P.; et al. Dietary intakes of retinol, beta-carotene, vitamin D and vitamin E in the European Prospective Investigation into Cancer and Nutrition cohort. *Eur. J. Clin. Nutr.* **2009**, *63* (Suppl. S4), S150–S178. [CrossRef] [PubMed]
33. Gao, X.; Wilde, P.E.; Lichtenstein, A.H.; Bermudez, O.I.; Tucker, K.L. The maximal amount of dietary alpha-tocopherol intake in U.S. adults (NHANES 2001–2002). *J. Nutr.* **2006**, *136*, 1021–1026. [PubMed]
34. Max Rubner-Institut. *Nationale Verzehrsstudie II. Lebensmittelverzehr und Nährstoffzufuhr auf Basis von 24h-Recalls*; MRI: Karlsruhe, Germany, 2013.
35. Wolters, M.; Hermann, S.; Golf, S.; Katz, N.; Hahn, A. Selenium and antioxidant vitamin status of elderly German women. *Eur. J. Clin. Nutr.* **2006**, *60*, 85–91. [CrossRef] [PubMed]
36. Kompauer, I.; Heinrich, J.; Wolfram, G.; Linseisen, J. Association of carotenoids, tocopherols and vitamin C in plasma with allergic rhinitis and allergic sensitisation in adults. *Public Health Nutr.* **2006**, *9*, 472–479. [CrossRef] [PubMed]
37. Ford, E.S.; Schleicher, R.L.; Mokdad, A.H.; Ajani, U.A.; Liu, S. Distribution of serum concentrations of alpha-tocopherol and gamma-tocopherol in the US population. *Am. J. Clin. Nutr.* **2006**, *84*, 375–383. [PubMed]
38. Péter, S.; Friedel, A.; Roos, F.F.; Wyss, A.; Eggersdorfer, M.; Hoffmann, K.; Weber, P. A Systematic Review of Global Alpha-Tocopherol Status as Assessed by Nutritional Intake Levels and Blood Serum Concentrations. *Int. J. Vitam. Nutr. Res.* **2016**, 1–21. [CrossRef] [PubMed]
39. Stuetz, W.; Weber, D.; Dolle, M.E.; Jansen, E.; Grubeck-Loebenstein, B.; Fiegl, S.; Toussaint, O.; Bernhardt, J.; Gonos, E.S.; Franceschi, C.; et al. Plasma Carotenoids, Tocopherols, and Retinol in the Age-Stratified (35–74 Years) General Population: A Cross-Sectional Study in Six European Countries. *Nutrients* **2016**, *8*, 614. [CrossRef] [PubMed]
40. Jiang, Q.; Christen, S.; Shigenaga, M.K.; Ames, B.N. gamma-tocopherol, the major form of vitamin E in the US diet, deserves more attention. *Am. J. Clin. Nutr.* **2001**, *74*, 714–722. [PubMed]
41. Millen, A.E.; Dodd, K.W.; Subar, A.F. Use of vitamin, mineral, nonvitamin, and nonmineral supplements in the United States: The 1987, 1992, and 2000 National Health Interview Survey results. *J. Am. Diet. Assoc.* **2004**, *104*, 942–950. [CrossRef] [PubMed]
42. White, E.; Kristal, A.R.; Shikany, J.M.; Wilson, A.C.; Chen, C.; Mares-Perlman, J.A.; Masaki, K.H.; Caan, B.J. Correlates of serum alpha- and gamma-tocopherol in the Women's Health Initiative. *Ann. Epidemiol.* **2001**, *11*, 136–144. [CrossRef]
43. McBurney, M.I.; Yu, E.A.; Ciappio, E.D.; Bird, J.K.; Eggersdorfer, M.; Mehta, S. Suboptimal Serum alpha-Tocopherol Concentrations Observed among Younger Adults and Those Depending Exclusively upon Food Sources, NHANES 2003-20061-3. *PLoS ONE* **2015**, *10*, e0135510. [CrossRef] [PubMed]
44. Handelman, G.J.; Machlin, L.J.; Fitch, K.; Weiter, J.J.; Dratz, E.A. Oral alpha-tocopherol supplements decrease plasma gamma-tocopherol levels in humans. *J. Nutr.* **1985**, *115*, 807–813. [PubMed]
45. Gaedicke, S.; Zhang, X.; Huebbe, P.; Boesch-Saadatmandi, C.; Lou, Y.; Wiswedel, I.; Gardemann, A.; Frank, J.; Rimbach, G. Dietary vitamin E, brain redox status and expression of Alzheimer's disease-relevant genes in rats. *Br. J. Nutr.* **2009**, *102*, 398–406. [CrossRef] [PubMed]
46. Traber, M.G. Mechanisms for the prevention of vitamin E excess. *J. Lipid Res.* **2013**, *54*, 2295–2306. [CrossRef] [PubMed]
47. Hosomi, A.; Arita, M.; Sato, Y.; Kiyose, C.; Ueda, T.; Igarashi, O.; Arai, H.; Inoue, K. Affinity for alpha-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. *FEBS Lett.* **1997**, *409*, 105–108. [CrossRef]
48. Traber, M.G. Vitamin E regulatory mechanisms. *Annu. Rev. Nutr.* **2007**, *27*, 347–362. [CrossRef] [PubMed]
49. Sontag, T.J.; Parker, R.S. Cytochrome P450 omega-hydroxylase pathway of tocopherol catabolism. Novel mechanism of regulation of vitamin E status. *J. Biol. Chem.* **2002**, *277*, 25290–25296. [CrossRef] [PubMed]
50. Boeing, H.; Bohlscheid-Thomas, S.; Voss, S.; Schneeweiss, S.; Wahrendorf, J. The relative validity of vitamin intakes derived from a food frequency questionnaire compared to 24-h recalls and biological measurements: Results from the EPIC pilot study in Germany. European Prospective Investigation into Cancer and Nutrition. *Int. J. Epidemiol.* **1997**, *26* (Suppl. S1), S82–S90. [CrossRef] [PubMed]

51. McNaughton, S.A.; Marks, G.C.; Gaffney, P.; Williams, G.; Green, A. Validation of a food-frequency questionnaire assessment of carotenoid and vitamin E intake using weighed food records and plasma biomarkers: The method of triads model. *Eur. J. Clin. Nutr.* **2005**, *59*, 211–218. [CrossRef] [PubMed]
52. Ascherio, A.; Stampfer, M.J.; Colditz, G.A.; Rimm, E.B.; Litin, L.; Willett, W.C. Correlations of vitamin A and E intakes with the plasma concentrations of carotenoids and tocopherols among American men and women. *J. Nutr.* **1992**, *122*, 1792–1801. [PubMed]
53. Stryker, W.S.; Kaplan, L.A.; Stein, E.A.; Stampfer, M.J.; Sober, A.; Willett, W.C. The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels. *Am. J. Epidemiol.* **1988**, *127*, 283–296. [CrossRef] [PubMed]
54. Kardinaal, A.F.; van 't Veer, P.; Brants, H.A.; van den Berg, H.; van Schoonhoven, J.; Hermus, R.J. Relations between antioxidant vitamins in adipose tissue, plasma, and diet. *Am. J. Epidemiol.* **1995**, *141*, 440–450. [CrossRef] [PubMed]
55. Andersen, L.F.; Solvoll, K.; Johansson, L.R.; Salminen, I.; Aro, A.; Drevon, C.A. Evaluation of a food frequency questionnaire with weighed records, fatty acids, and alpha-tocopherol in adipose tissue and serum. *Am. J. Epidemiol.* **1999**, *150*, 75–87. [CrossRef] [PubMed]
56. Schmolz, L.; Birringer, M.; Lorkowski, S.; Wallert, M. Complexity of vitamin E metabolism. *World J. Biol. Chem.* **2016**, *7*, 14–43. [CrossRef] [PubMed]
57. Galli, F.; Azzi, A.; Birringer, M.; Cook-Mills, J.M.; Eggersdorfer, M.; Frank, J.; Cruciani, G.; Lorkowski, S.; Ozer, N.K. Vitamin E: Emerging aspects and new directions. *Free Radic. Biol. Med.* **2017**, *102*, 16–36. [CrossRef] [PubMed]
58. Mah, E.; Sapper, T.N.; Chitchumroonchokchai, C.; Failla, M.L.; Schill, K.E.; Clinton, S.K.; Bobe, G.; Traber, M.G.; Bruno, R.S. alpha-Tocopherol bioavailability is lower in adults with metabolic syndrome regardless of dairy fat co-ingestion: A randomized, double-blind, crossover trial. *Am. J. Clin. Nutr.* **2015**, *102*, 1070–1080. [CrossRef] [PubMed]
59. Traber, M.G.; Mah, E.; Leonard, S.W.; Bobe, G.; Bruno, R.S. Metabolic syndrome increases dietary alpha-tocopherol requirements as assessed using urinary and plasma vitamin E catabolites: A double-blind, crossover clinical trial. *Am. J. Clin. Nutr.* **2017**, *105*, 571–579. [CrossRef] [PubMed]
60. Chuang, J.C.; Matel, H.D.; Nambiar, K.P.; Kim, S.H.; Fadel, J.G.; Holstege, D.M.; Clifford, A.J. Quantitation of [5-14CH3]-(2R, 4′R, 8′R)-alpha-tocopherol in humans. *J. Nutr.* **2011**, *141*, 1482–1488. [CrossRef] [PubMed]

Table S1: Details about the measurements of glucose, cholesterol, high density lipoprotein (HDL) - and low density lipoprotein (LDL) -cholesterol, triglyceride, C-reactive protein (CRP) and HbA1c

Glucose	
TestName	Glucose HK Gen.3 (2200 Tests), Art. Nr. 05168791 190
Instrument	Roche Cobas 8000/ c702
Controls	PreciControl Clin. Chem. Multi 1; Roche, PreciControl Clin.Chem. Multi 2; Roche
Intra-assay variation	CV=max. 0.8 %
Inter-assay variation	CV=max. 1.3 %
Cholesterol	
TestName	Cholesterol Gen.2 (2100 Tests), Art. Nr. 05168538 190
Instrument	Roche Cobas 8000/ c702
Controls	PreciControl Clin. Chem. Multi 1; Roche, PreciControl Clin. Chem. Multi 2; Roche
Intra-assay variation	CV=max. 0.8 %
Inter-assay variation	CV=max. 1.6 %
HDL-cholesterol	
TestName	HDL-cholesterol plus 3rd generation (450 Tests), Art. Nr. 05168805 190
Instrument	Roche Cobas 8000/ c702
Controls	PreciControl Clin. Chem. Multi 1; Roche, PreciControl Clin. Chem. Multi 2; Roche
Intra-assay variation	CV=max. 0.8 %
Inter-assay variation	CV=max. 1.5 %
LDL-cholesterol	
TestName	LDL-cholesterol plus 2nd generation (500 Tests), Art.Nr. 05171369 190
Instrument	Roche Cobas 8000/ c702
Controls	PreciControl Clin. Chem. Multi 1; Roche, PreciControl Clin. Chem. Multi 2; Roche
Intra-assay variation	CV=max. 0.9 %
Inter-assay variation	CV=max. 2.7 %
Triglyceride	
TestName	Triglycerides (800 Tests), Art.Nr. 05171407 190
Instrument	Roche Cobas 8000/ c702
Controls	PreciControl Clin. Chem. Multi 1; Roche, PreciControl Clin. Chem. Multi 2; Roche
Intra-assay variation	CV=max. 0.9 %
Inter-assay variation	CV=max. 2.0 %
CRP	
TestName	C-Reactive Protein Gen.3 (500 Tests), Art.Nr. 05172373 190
Instrument	Roche Cobas 8000/ c702
Controls	PreciControl Clin. Chem. Multi 1; Roche, PreciControl Clin. Chem. Multi 2; Roche
Intra-assay variation	CV=max. 3.7 %
Inter-assay variation	CV=max. 4.0 %
HbA1c	
TestName	VARIANT™ II Turbo HbA1c Kit - 2.0, Art.Nr. 270-2455EX
Instrument	VARIANT™ II Turbo HPLC-Analyser Hemoglobin HbA1c Program, BioRad
Controls	Lyphocheck™ Diabetes Control, Bilevel, BioRad
Intra-assay variation	CV=0.78 %
Inter-assay variation	CV=0.53 %

CV: Coefficient of variation.

Table S2: Intra- and inter-day variation of plasma α- and γ-tocopherol levels.

	Intra-day variation			Inter-day variation		
	mean	**SD**	**CV (%)**	**mean**	**SD**	**CV (%)**
α-tocopherol (µmol/L)	34.8	0.264	0.757	32.4	2.44	7.53
γ-tocopherol (µmol/L)	1.44	0.056	3.90	1.33	0.173	13.0

SD: Standard deviation; CV: Coefficient of variation.

Table S3: Age- and sex-adjusted characteristics of the study sample (n=641) according to tertiles (T) of α-tocopherol/cholesterol ratio.

	Tertiles α-tocopherol/cholesterol ratio			
Characteristics	**T1**	**T2**	**T3**	**P trend**
n	213	214	214	
Median α-tocopherol/cholesterol ratio (IQR), µmol/mmol	4.63 (4.25,4.88)	5.53 (5.36,5.72)	6.74 (6.33,7.59)	
Men, %	54.5	60.7	61.3	0.181
Age, years	62.3 (60.7,63.9)	60.6 (59.0,62.2)	60.6 (59.0,62.1)	0.118
Body mass index, kg/m^2	26.5 (25.7,27.2)	26.7 (25.9,27.4)	26·9 (26.2,27.7)	0.314
Waist circumference, cm				
Men	98.12 (95.6,100.6)	98.4 (96.1,100.8)	99.1 (96.7,101.5)	0.509
Women	86.9 (83.5,90.4)	89.2(85.7,92.6)	90.0 (86.8,93.2)	0.193
Hip circumference, cm	101.3 (99.8,102.9)	102.7 (101.2,104.2)	102.3 (100.8,103.8)	0.269
Prevalent hypertension, %	56.4	60.3	58.0	0.713
Current smokers, %	31.1	38.3	34.4	0.494
Physical activity, MET-hour/week *	79.2 (71.0,88.3)	76.7 (68.9,85.3)	85.4 (77.0,94.8)	0.237
High education (≥11 years), %	38.8	47.6	43.0	0.375
Alcohol consumption, g/day *	6.5 (5.3,8.9)	7.3 (5.9,8.9)	8.2 (6.7,10.0)	0.048
Vitamin E supplementation, %	4.6	5.9	9.1	0.097
Prevalent diabetes, %	5.2	5.6	10.8	0.078
HbA1c, % *	5.50 (5.41,5.58)	5.51 (5.44,5.60)	5.62 (5.54,5.70)	0.014
C-reactive protein, mg/dL *	1.15 (0.96,1.38)	1.17 (0.98,1.40)	1.27 (1.07,1.51)	0.365
HDL-cholesterol, mg/dL	70.93 (68.02,73.83)	68.18 (65.33,71.03)	62.81 (60.04,65.59)	<.0001
LDL-cholesterol, mg/dL	137.71 (132.03,143.39)	126.83 (121.25,132.40)	118.24 (112.81,123.66)	<.0001
Triglycerides, mg/dL *	88.34 (81.29,96.00)	95.21 (87.75,103.30)	117.47 (108.50,127.18)	<.0001
γ-tocopherol, µmol/L *	1.11 (1.02,1.20)	1.23 (1.13,1.34)	1.39 (1.28,1.51)	<.0001

* Log-transformed variables were reported as geometric means and 95% Confidence Interval (CI). IQR: Interquartile range; MET: Metabolic equivalent; HDL: High density lipoprotein; LDL: Low density lipoprotein.

Table S4: Age- and sex-adjusted characteristics of the study sample according (n=641) to tertiles (T) of γ-tocopherol/cholesterol ratio.

	Tertiles γ-tocopherol/cholesterol ratio			
Characteristics	**T1**	**T2**	**T3**	**P trend**
n	213	214	214	
Median γ-tocopherol/cholesterol ratio (IQR), μmol/mmol	0.16 (0.13,0.18)	0.24 (0.22,0.26)	0.35 (0.31,0.41)	
Men, %	59.7	59.6	58.0	0.722
Age, years	61.9 (60.2,63.4)	60.7 (59.2,62.3)	60.8 (59.2,62.4)	0.389
Body mass index, kg/m^2	25.9 (25.2,26.7)	26.9 (26.2,27.7)	27.2 (26.4,28.0)	0.004
Waist circumference, cm				
Men	96.9 (94.6,99.2)	99.0 (96.6,101.5)	99.9 (97.5,102.2)	0.040
Women	84.9 (81.5,88.2)	89.2 (85.9,92.5)	91.9 (88.8,95.1)	0.002
Hip circumference, cm	100.6 (99.1,102.1-9	102.8 (101.3,104.4)	102.9 (101.4,104.4)	0.011
Prevalent hypertension, %	57.1	62.0	55.7	0.750
Current smokers, %	35.5	34.6	33.9	0.726
Physical activity, MET-hour/week *	78.4 (70.5,87.1)	82.2 (73.9,91.5)	81.3 (73.1,90.3)	0.561
High education (≥11 years), %	47.9	39.5	42.2	0.216
Alcohol consumption, g/d*	6.7 (5.5,8.2)	7.8 (6.4,9.5)	7.5 (6.2,9.2)	0.354
Vitamin E supplementation, %	14.1	1.6	4.4	0.0002
Prevalent diabetes, %	4.2	6.0	11.9	0.013
HbA1c, %*	5.50 (5.42,5.59)	5.52 (5.44,5.61)	5.62 (5.54,5.71)	0.014
C-reactive protein, mg/dL *	1.02 (0.85,1.21)	1.28 (1.07,1.53)	1.32 (1.11,1.57)	0.012
HDL-cholesterol, mg/dL	69.56 (66.71,72.41)	67.18 (64.29,70.06)	64.40 (61.55,67.25)	0.002
LDL-cholesterol, mg/dL	132.49 (126.87,138.11)	127.26 (121.58,132.93)	121.21 (115.61,126.83)	0.0007
Triglycerides, mg/dL *	93.68 (86.29,101.71)	96.79 (89.08,105.16)	111.95 (103.13,121.52)	0.0002
α-tocopherol, μmol/L*	30.09 (28.86,31.37)	31.14 (29.86,32.48)	34.10 (32.71,35.54)	<.0001

* Log-transformed variables are reported as geometric means and 95% Confidence Interval (CI). IQR: Interquartile range; MET: Metabolic equivalent; HDL: High density lipoprotein; LDL: Low density lipoprotein.

Table S5: Factor loadings for food groups that highly |>0.2| loaded in principal component analysis (PCA).

	Dietary pattern 1	Dietary pattern 2	Dietary pattern 3
Vegetable oils	0.80	-	-
Fruiting and root vegetables	0.80	-	-
Condiments and yeast	0.78	-	-
Leafy vegetables	0.78	-	-
Cabbages	0.69	0.30	-
Other vegetables	0.69	0.24	-
Sauces	0.54	0.46	-
Fruits	0.47	-	0.24
Soups	0.29	-	-
Other fruits	0.26	-0.21	-
Water	-	-	-
Processed meat	-	0.72	-
Red meat and game	-	0.70	-
Bread	-	0.56	-
Other fats	-	0.56	-
Potatoes	-	0.43	-
Sugar and confectionery	-	0.41	-
Butter	-	0.38	-
Poultry	-	0.37	-
Legumes	-	0.35	0.22
Cake and cookies	-	0.34	-
Margarine	-	0.33	-
Beer	-	0.27	-
Spirits	-	0.26	-
Coffee	-	0.23	-
Eggs	-	-	-
Other alcoholic beverages	-	-	-
Soft drinks	-	-	-
Breakfast cereals	-	-	0.67
Other cereals	-	-	0.65
Nuts	-	-	0.50
Fish	-	0.22	0.44
Milk and dairy products	-	-	0.43
Miscellaneous	-	-	0.33
Cheese	-	0.31	0.32
Tea	-	-	0.28
Pasta and rice	-	-	0.27
Fruit and vegetable juices	-	-	-
Wine	-	-	-

Only food groups with factor loadings |>0.20| are displayed and listed in order for simplicity and interpretation.

3 Association of vitamin E levels with metabolic syndrome, and MRI-derived body fat volumes and liver fat content

Sabina Waniek[1], Romina di Giuseppe[1], Sandra Plachta-Danielzik[1], Ilka Ratjen[1], Gunnar Jacobs[1,2], Manja Koch[1,3], Jan Borggrefe[4], Marcus Both[5], Hans-Peter Müller[6], Jan Kassubek[6], Ute Nöthlings[7], Tuba Esatbeyoglu[8], Sabrina Schlesinger[9], Gerald Rimbach[8], Wolfgang Lieb[1,2]

[1]Institute of Epidemiology, Christian-Albrechts University of Kiel, 24105 Kiel, Germany

[2]Biobank PopGen, University Hospital Schleswig-Holstein, Campus Kiel, 24105 Kiel, Germany

[3]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA

[4]Institute of Diagnostic and Interventional Radiology, University Hospital Cologne, 50937 Cologne, Germany

[5]Department of Radiology and Neuroradiology, University Hospital Schleswig-Holstein, Campus Kiel, 24105 Kiel, Germany

[6]Department of Neurology, University of Ulm, 89081 Ulm, Germany

[7]Department of Nutrition and Food Science, University of Bonn, 53113 Bonn, Germany

[8]Institute of Human Nutrition and Food Science, Christian-Albrechts University of Kiel, 24118 Kiel, Germany

[9]Institute for Biometrics and Epidemiology, German Diabetes Center (DDZ) at Heinrich Heine University Düsseldorf, 40225 Düsseldorf, Germany

Published in: Nutrients 2017 Oct 18;9(10)

nutrients

MDPI

Article

Association of Vitamin E Levels with Metabolic Syndrome, and MRI-Derived Body Fat Volumes and Liver Fat Content

Sabina Waniek [1], Romina di Giuseppe [1], Sandra Plachta-Danielzik [1], Ilka Ratjen [1], Gunnar Jacobs [1,2], Manja Koch [1,3], Jan Borggrefe [4], Marcus Both [5], Hans-Peter Müller [6], Jan Kassubek [6], Ute Nöthlings [7], Tuba Esatbeyoglu [8], Sabrina Schlesinger [9], Gerald Rimbach [8] and Wolfgang Lieb [1,2,*]

1 Institute of Epidemiology, Christian-Albrechts University of Kiel, 24105 Kiel, Germany; sabina.waniek@epi.uni-kiel.de (S.W.); romina.digiuseppe@epi.uni-kiel.de (R.d.G.); sandra.plachta-danielzik@epi.uni-kiel.de (S.P.-D.); ilka.ratjen@epi.uni-kiel.de (I.R.); jacobs@popgen.de (G.J.); mkoch@hsph.harvard.edu (M.K.)
2 Biobank PopGen, University Hospital Schleswig-Holstein, Campus Kiel, 24105 Kiel, Germany
3 Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA
4 Institute of Diagnostic and Interventional Radiology, University Hospital Cologne, 50937 Cologne, Germany; jan.borggrefe@uk-koeln.de
5 Department of Radiology and Neuroradiology, University Hospital Schleswig-Holstein, Campus Kiel, 24105 Kiel, Germany; Marcus.both@uksh.de
6 Department of Neurology, University of Ulm, 89081 Ulm, Germany; hans-peter.mueller@uni-ulm.de (H.-P.M.); jan.kassubek@uni-ulm.de (J.K.)
7 Department of Nutrition and Food Science, University of Bonn, 53113 Bonn, Germany; noethlings@uni-bonn.de
8 Institute of Human Nutrition and Food Science, Christian-Albrechts University of Kiel, 24118 Kiel, Germany; tuba.esatbeyoglu@mri.bund.de (T.E.); rimbach@foodsci.uni-kiel.de (G.R.)
9 Institute for Biometrics and Epidemiology, German Diabetes Center (DDZ) at Heinrich Heine University Düsseldorf, 40225 Düsseldorf, Germany; sabrina.schlesinger@DDZ.uni-duesseldorf.de
* Correspondence: wolfgang.lieb@epi.uni-kiel.de; Tel.: +49-431-500-30200; Fax: +49-431-500-30204

Received: 21 July 2017; Accepted: 11 October 2017; Published: 18 October 2017

Abstract: We aimed to relate circulating α- and γ-tocopherol levels to a broad spectrum of adiposity-related traits in a cross-sectional Northern German study. Anthropometric measures were obtained, and adipose tissue volumes and liver fat were quantified by magnetic resonance imaging in 641 individuals (mean age 61 years; 40.6% women). Concentrations of α- and γ-tocopherol were measured using high performance liquid chromatography. Multivariable-adjusted linear and logistic regression were used to assess associations of circulating α- and γ-tocopherol/cholesterol ratio levels with visceral (VAT) and subcutaneous adipose tissue (SAT), liver signal intensity (LSI), fatty liver disease (FLD), metabolic syndrome (MetS), and its individual components. The α-tocopherol/cholesterol ratio was positively associated with VAT (β scaled by interquartile range (IQR): 0.036; 95%Confidence Interval (CI): 0.0003; 0.071) and MetS (Odds Ratio (OR): 1.83; 95% CI: 1.21–2.76 for 3rd vs. 1st tertile), and the γ-tocopherol/cholesterol ratio was positively associated with VAT (β scaled by IQR: 0.066; 95% CI: 0.027; 0.104), SAT (β scaled by IQR: 0.048; 95% CI: 0.010; 0.087) and MetS (OR: 1.87; 95% CI: 1.23–2.84 for 3rd vs. 1st tertile). α- and γ-tocopherol levels were positively associated with high triglycerides and low high density lipoprotein cholesterol levels (all $P_{trend} < 0.05$). No association of α- and γ-tocopherol/cholesterol ratio with LSI/FLD was observed. Circulating vitamin E levels displayed strong associations with VAT and MetS. These observations lay the ground for further investigation in longitudinal studies.

Keywords: vitamin E; α- and γ-tocopherol; metabolic syndrome; body fat volumes; liver fat content

1. Introduction

Metabolic conditions like metabolic syndrome (MetS), fatty liver disease (FLD), and obesity have been linked to increased inflammation and oxidative stress [1–3]. Vitamin E is a lipid-soluble vitamin, encompassing different tocopherols (α-, β-, γ-, and δ-tocopherol) with important anti-oxidative and potentially anti-inflammatory functions [4,5]. In a recent randomized trial [6], vitamin E administration over 96 weeks performed better than pioglitazone and better than placebo in patients with non-alcoholic steatohepatitis (NASH). It is, therefore, conceivable that vitamin E levels are altered in patients with MetS or FLD and that vitamin E levels are correlated with other adiposity-related traits.

Previous clinical and epidemiologic studies on the association of circulating vitamin E levels with different anthropometric adiposity measures (e.g., waist circumference, body mass index (BMI)) [7–11], the MetS [12–16], and NASH [17–19] produced partially conflicting results. Among the different fat depots, subcutaneous (SAT) and, particularly, visceral adipose tissue (VAT) are considered relevant for metabolic conditions, such as MetS. Whether vitamin E levels are associated with MetS, FLD and other adiposity measures, including SAT and VAT, as determined by magnetic resonance imaging (MRI), is unknown.

Therefore, we aimed to relate circulating levels of α- and γ-tocopherol to a broad spectrum of adiposity-related traits in a community-based sample from Northern Germany. Specifically, we assessed the associations of plasma α- and γ-tocopherol levels with MetS and its individual components, MRI-determined VAT, SAT, and liver signal intensity (LSI), as well as with the presence or absence of FLD. We hypothesize that vitamin E levels are altered in individuals with MetS and that vitamin E levels are associated with VAT, SAT, and liver fat, as determined by MRI.

2. Materials and Methods

2.1. Study Sample

Between 2005 and 2007, a total of 1316 individuals from Northern Germany were recruited by the PopGen biobank [20]. Specifically, the sample consisted of 747 individuals who were identified through official population registries and from 569 blood donors. The first follow-up examination, conducted between 2010 and 2012, was attended by 952 individuals, who received a physical examination conducted by trained personnel and provided blood and urine samples. Furthermore, all participants filled-in a standardized questionnaire on demographic and health-related characteristics (including dietary intake, education, smoking status, and physical activity) and medical history [20,21]. A subsample of participants (n = 641) agreed to undergo whole-body MRI. From these participants concentrations of circulating α- and γ-tocopherol levels were measured. Thus, the association between plasma vitamin E concentrations and MetS was investigated in 641 individuals. A total of 91 individuals had to be excluded from the analyses related to MRI phenotypes because of insufficient imaging quality (n = 35), non-adherence to the MRI breathing protocol (n = 40), and missing information on quality of MRI assessment (n = 16). Further, individuals with self-reported liver disease (hepatitis A, B, C, or D virus infection, hemochromatosis, autoimmune liver disease, or liver cirrhosis (n = 29)) were excluded. Thus, the association between circulating vitamin E biomarkers and VAT and SAT was assessed in 591 individuals and the association with liver fat and FLD was evaluated in 571 individuals.

The study has been approved by the Ethics Committee of the Medical Faculty of the Christian-Albrechts University Kiel. Written informed consent was obtained from all study participants.

2.2. Physical Examination and Standardized Questionnaires

Weight and height were measured with the participant wearing light clothing and no shoes, and 2.0 kg were subtracted to correct for the remaining clothes. BMI was calculated as body weight (kg)/height (m^2). Waist circumference was measured at the midpoint between the lower ribs and iliac crest on the anterior axillary line in a resting expiratory position. After the participants had rested 5 min in a sitting position, blood pressure was measured twice (2 min interval) using a

sphygmomanometer [22]. Dietary intake, including information on alcohol consumption during the last 12 months, was assessed by a self-administered semi-quantitative 112-item food-frequency questionnaire (FFQ) designed and validated especially for the German population [23]. The German Food Code and Nutrient Data Base (version II.3) was used to determine energy intake and was provided by the Department of Epidemiology of the German Institute of Human Nutrition Potsdam Rehbrücke [24]. Participants were asked to report their use of vitamin E supplements in the FFQ.

2.3. Assessment of SAT, VAT, and Liver Fat Using MRI

Liver fat and adipose tissue (AT) volumes (defined as VAT and SAT) were measured by MRI using a 1.5-T scanner (Magnetom Avanto; Siemens Medical solution, Erlangen, Germany), as described in detail elsewhere [25–27]. VAT was determined as the sum of VAT voxels from the top of the liver to the femoral heads inside the abdominal muscular wall as anatomical border and SAT was determined as the sum of AT voxels underneath the skin layer surrounding the abdomen from the top of the liver to the femur heads. To obtain the volumes (in dm^3) of VAT and SAT the voxel size ($3.9 \times 2 \times 8$ mm^3) was multiplied by the number of voxels [26].

Liver fat was quantified as relative LSI difference of the liver on out-of-phase compared with in-phase images in arbitrary units. Both in- and out- of phase images were acquired during a breath hold by using axial T1-weighted gradient echo sequences. Signal intensities were obtained by measuring the average of three circular regions of interest in the liver parenchyma [27].

2.4. Definitions

Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg, or self-reported hypertension history or use of antihypertensive medication.

MetS was defined according to the harmonized criteria [28] and was considered present when at least three of the following five criteria were met: (1) elevated triglyceride concentration (≥150 mg/dL); (2) reduced high density lipoprotein (HDL)-concentration (<40 mg/dL in men or <50 mg/dL in women); (3) elevated blood pressure (systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg or anti-hypertensive treatment); (4) dysglycaemia, defined as elevated plasma fasting glucose (≥100 mg/dL) or anti-diabetic treatment; and (5) abdominal obesity (waist circumference ≥94 cm for men and ≥80 cm for women). In the present definition, information about triglyceride-lowering and HDL-increasing medications were not included because this information was not available in detail in our sample. Type 2 diabetes was defined as glycated hemoglobin (HbA1c) ≥6.5% (48 mmol/mol) or fasting glucose ≥126 mg/dL, or use of anti-diabetic medication or self-report physician diagnosis. FLD was defined as log liver signal intensity ≥3.0 according to a cut-off, which corresponds to the maximum Youden Index and was derived using spectroscopically determined FLD (liver fat ≥ 5.56%) as the reference method [27,29].

Total physical activity was defined as the reported frequency (hour/week) of different activities (leisure and working-time) [30], multiplied by the corresponding metabolic equivalent (MET)-value, and summed up for all activities [22,31]. Participants were classified into 3 categories to determine smoking status: no-smokers if they had never smoked; former smokers if they had smoked in the past and quit smoking more than 1 year ago; and current smokers if they were currently smoking 1 or more cigarettes per day. Participants were categorized according to the level of education into three categories: low (≤9 years), middle (10 years), or high (≥11 years).

2.5. Laboratory Analyses

Fasting blood (EDTA whole-blood and lithium heparin) samples were obtained from participants in a sitting position. All blood samples were centrifuged, aliquoted, and stored at −80 °C. In fresh blood samples, concentrations of C-reactive protein (CRP), triglycerides, HDL-cholesterol, and total cholesterol were analyzed by enzymatic colorimetry (Roche Diagnostic, Mannheim, Germany); the concentration of glucose was determined by using enzymatic ultraviolet tests (Roche Diagnostic,

Mannheim, Germany), and HbA1c concentrations were determined by using high performance liquid chromatography (HPLC) and photometric detection (Bio-Rad Laboratories, Munich, Germany) in EDTA plasma.

Laboratory blood analyses were performed in the laboratory for clinical chemistry of the University Hospital Schleswig-Holstein, Campus Kiel in Germany.

The Institute of Human Nutrition and Food Science at the Christian-Albrechts-University of Kiel in Germany measured plasma vitamin E (α- and γ-tocopherol) levels using a HPLC with fluorescence detection. An external standard curve was used to quantify vitamin E concentrations using a Jasco HPLC system (Jasco GmbH Deutschland, Gross-Umstadt, Germany; equipped with an autosampler (Jasco AS-2057), pump (PU-2080), ternary gradient unit (LG-2080-02), 3 line degasser (DG-2080-53), and fluorescence detector (FP2020 Plus)) with a Waters Spherisorb ODS-2,3 µm column (100 × 4.6 mm) using methanol:water (98:2, *v/v*) as mobile phase. The fluorescence detector operated an excitation wavelength of 290 nm and emission wavelength of 325 nm. The flow rate of the mobile phase was set at 1.2 mL/min. Duplicate measurements were performed and the injection volume was set at 40 µL. Plasma (50 µL) was homogenized in 2 mL 1% ascorbic acid (in ethanol), 700 µL deionised water, 50 µL 0.1% butylated hydroxytoluol (in ethanol), and 2 mL n-hexane were prepared for analysing the samples. The samples were centrifuged (1000× *g* for 5 min at 4 °C). After separating the phases, 1000 µL of the upper phase was dried under vacuum in a RC-1010 centrifugal evaporator (Jouan, Saint-Herblain, France). The samples were re-suspended in 200 µL mobile phase (methanol:water, 98:2, *v/v*) [32]. The coefficients of variation for α- and γ-tocopherol were 1.05% and 1.29%, respectively.

2.6. Statistical Analyses

Some few missing values of covariates were replaced by a simple imputation, as follows: When values of categorical variables were missing, they were replaced by the most commonly observed category of that respective variable (n = 10). Normally distributed continuous missing variables were imputed by the respective mean and skewed variables by the sex-specific median (n = 2). Detailed information of missing covariates are provided in Supplementary Materials Table S1.

CRP values below 0.9 mg/dL (detection limit) were assigned a value equal to the half of the detection limit (n = 247). Values of γ-tocopherol (n = 14, respectively) were imputed by the lowest γ-tocopherol concentration measured in our sample. Detailed information of missing covariates are provided in Supplementary Materials Table S1.

Because vitamin E is bound to lipoproteins in the blood stream [33], cholesterol-adjusted α- and γ-tocopherol levels (µmol/mmol) were calculated by dividing α- and γ-tocopherol concentrations (µmol/L) by total cholesterol (mmol/L) [34].

We performed the following analyses: For descriptive purposes, anthropometric, lifestyle, and clinical factors were compared across tertiles of the α- and γ-tocopherol/cholesterol ratios. Differences in median of continuous variables were tested by using Wilcoxoń s rank-sum test, and differences in categorical variables were assessed by using a chi-square test.

Restricted cubic splines analyses displayed linear associations between vitamin E biomarkers and continuous (VAT, SAT, LSI) and binary (MetS, and FLD) outcomes. Third, linear and logistic regression models were used to relate circulating vitamin E (α- and γ-tocopherol/cholesterol ratio, each biomarker considered separately) levels to continuous outcomes (VAT, SAT, LSI) and binary outcomes (MetS, individual components of MetS, FLD), respectively. In linear regression models, both α- and γ-tocopherol levels were scaled to their interquartile range (IQR) and β coefficients interpreted as comparing VAT, SAT, and LSI values of a person with a typical "high" α- or γ-tocopherol value to a person with a typical "low" value.

Adjusted means of VAT, SAT, and LSI were calculated by general linear models, respectively. We ran age- and sex-adjusted, as well as multivariable-adjusted, models which included, based on literature research [12,13], age (continuous in years) and sex, education (low, medium, high), physical activity (continuous in MET-hour/week), smoking status (never, current, former), vitamin E

supplementation (yes, no), alcohol intake (continuous in g/day), and total energy intake (continuous in kJ/day) as potential confounders. Furthermore, the models with continuous VAT and SAT as outcome variables were additionally adjusted for BMI (continuous in kg/m^2) to assess whether VAT and SAT were associated with circulating vitamin E levels independent of BMI. Individual components of MetS were adjusted for each of the other four criteria for the MetS.

Potential interactions of age, sex, and vitamin E supplementation with each metabolic outcome (VAT, SAT, LSI, MetS, individual components of MetS, FLD) were tested by including multiplicative interaction terms into the regression models. In a sensitivity analysis, we excluded vitamin E supplement users and we related α- and γ-tocopherol/cholesterol ratio levels (each biomarker considered separately) to each selected metabolic outcome (Supplementary Materials Tables S4–S6). Furthermore, we excluded individuals who reported a consumption of alcohol more than 20 g/day (n = 134) when examining the association of α- and γ-tocopherol/cholesterol ratio with FLD.

Categorical variables with more than two categories were included as indicator variables. P_{trend} was calculated across tertiles using median values of α- and γ-tocopherol/cholesterol ratio within each tertile and we used these values as continuous variables.

All statistical tests were two-sided and considered to be significant when p values < 0.05. All analyses were performed with SAS 9.4 (SAS Institute, Cary, NC, USA).

3. Results

3.1. General Characteristics

General characteristics of the study sample according to tertiles of the α- and γ-tocopherol/cholesterol ratio are depicted in Tables 1 and 2, respectively. Triglycerides levels were higher in the 3rd tertile compared to the 1st tertile of the α-tocopherol/cholesterol ratio. Furthermore, the proportion of individuals with MetS was higher in the upper tertiles of the α-tocopherol/cholesterol ratio. Similarly, triglycerides levels were higher in the 3rd tertile compared to the 1st tertile of the γ-tocopherol/cholesterol ratio. Furthermore, BMI and waist circumference, CRP levels, as well as VAT and SAT were higher in the 3rd tertile compared to the 1st tertile of the γ-tocopherol/cholesterol ratio. In addition, the prevalence of the MetS and of diabetes rose with tertiles of the γ-tocopherol/cholesterol ratio. The proportion of vitamin E supplement users was highest in the bottom tertile of γ-tocopherol/cholesterol ratio.

Table 1. General characteristics of the PopGen control study population (n = 641) according to tertiles (T) of α-tocopherol/cholesterol ratio.

Characteristics	Tertiles α-Tocopherol/Cholesterol Ratio						p
	T1 (n = 213)		T2 (n = 214)		T3 (n = 214)		
Median α-tocopherol/cholesterol ratio (IQR), µmol/mmol	4.63	(4.25–4.88)	5.53	(5.36–5.72)	6.74	(6.33–7.59)	
Men, %	55.9		61.7		60.8		0.422
Age, years	63.0	(56.0–70.0)	61.5	(54.0–71.0)	62.0	(51.0–71.0)	0.411
Body mass index, kg/m^2	26.6	(23.3–29.8)	26.7	(24.8–29.4)	26.7	(24.6–29.2)	0.633
Waist circumference, cm							
Men	100.0	(92.8–107.4)	100.2	(92.7–105.9)	99.4	(93.5–106.8)	0.956
Women	87.1	(78.5–96.4)	88.5	(83.2–97.4)	92.4	(80.2–99.6)	0.199

Table 1. *Cont.*

Characteristics	Tertiles α-Tocopherol/Cholesterol Ratio						*p*
	T1 (*n* = 213)		T2 (*n* = 214)		T3 (*n* = 214)		
Systolic blood pressure, mm/Hg	139.0	(127.5–150.0)	140.0	(125.0–150.0)	138.3	(125.0–150.0)	0.856
Diastolic blood pressure, mm/Hg	85.0	(80.0–90.0)	85.0	(80.0–90.0)	82.3	(80.0–90.0)	0.341
Prevalent hypertension, %	68.1		71.0		67.8		0.723
Current smokers, %	10.1		9.8		12.2		0.640
Physical activity, MET-hour/week	98.3	(61.5–141.6)	84.2	(54.8–120.1))	90.0	(59.3–131.7)	0.074
High education (≥11 years), %	29.1		40.7		37.9		0.143
Alcohol consumption, g/day	8.67	(2.76–17.0)	8.58	(4.09–17.95)	10.96	(4.15–20.05)	0.114
Vitamin E supplementation, %	5.6		6.5		10.3		0.154
Prevalent diabetes, %	8.9		8.9		14.5		0.099
Metabolic syndrome, %	36.6		36.0		48.1		0.016
C-reactive protein, mg/dL	1.10	(0.45–2.60)	1.20	(0.45–2.40)	1.40	(0.45–2.20)	0.531
HDL-cholesterol, mg/dL	67.0	(56.0–82.0)	63.5	(54.0–76.0)	57.5	(49.0–72.0)	<0.0001
Triglycerides, mg/dL	96.0	(71.0–123.0)	104.0	(78.0–132.0)	123.0	(84.0–169.0)	<0.0001
Diabetes medication, % *	3.6		7.1		14.6		0.015
Lipid-lowering medication, % *	13.6		29.3		45.8		<0.0001
Fatty liver disease, % †	38.9		38.5		40.3		0.928
Liver signal intensity †	18.6	(14.9–23.4)	18.2	(15.0–22.1)	18.0	(14.5–24.7)	0.925
Visceral adipose tissue, dm^3 ‡	3.70	(2.18–5.02)	3.90	(2.41–5.25)	3.94	(2.54–5.37)	0.478
Subcutaneous adipose tissue, dm^3 ‡	5.91	(4.45–8.23)	6.45	(4.75–8.53)	6.10	(4.88–8.24)	0.546

Data are reported as percentages (%) or median and interquartile range (IQR). * *n* = 305, † *n* = 571, ‡ *n* = 591; MET: Metabolic equivalent; HDL: High density lipoprotein

Table 2. General characteristics of the PopGen control study population (*n* = 641) according to tertiles of γ-tocopherol/cholesterol ratio.

Characteristics	Tertiles (T) γ-Tocopherol/Cholesterol Ratio						*p*
	T1 (*n* = 213)		T2 (*n* = 214)		T3 (*n* = 214)		
Median γ-tocopherol/cholesterol ratio (IQR), μmol/mmol	0.16	(0.13–0.18)	0.24	(0.22–0.26)	0.35	(0.31–0.42)	
Men, %	60.06		57.9		59.8		0.851
Age, years	63.0	(55.0–71.0)	61.5	(55.0–71.0)	62.0	(54.0–69.0)	0.709
Body mass index, kg/m2	26.1	(23.4–28.9)	27.3	(24.8–29.6)	26.8	(24.9–30.7)	0.005
Waist circumference, cm							
Men	98.9	(91.5–105.3)	100.8	(93.5–108.3)	100.7	(94.6–106.9)	0.271
Women	85.3	(77.4–93.6)	89.0	(82.4–98.0)	91.8	(80.2–103.5)	0.002
Systolic blood pressure, mm/Hg	139.0	(126.5–150.0)	140.0	(125.0–150.0)	139.0	(127.5–150.0)	0.858
Diastolic blood pressure, mm/Hg	85.0	(80.0–90.0)	85.0	(80.0–90.0)	85.0	(80.0–90.0)	0.853
Prevalent hypertension, %	67.1		71.5		68.2		0.598
Current smokers, %	8.0		14.5		10.3		0.278
Physical activity, MET-hour/week	86.3	(58.8–130.0)	89.5	(59.8–138.2)	90.8	(56.8–125.4)	0.932
High education (≥11 years), %	40.9		31.3		35.5		0.168
Alcohol consumption, g/d	8.87	(3.20–16.79)	10.18	(3.82–20.3)	9.74	(4.0–20.13)	0.504
Vitamin E supplementation, %	14.6		2.8		5.1		<0.0001
Prevalent diabetes, %	8.0		8.9		15.4		0.026
Metabolic syndrome, %	32.9		41.6		46.3		0.017
C-reactive protein, mg/dL	1.0	(0.45–1.90)	1.30	(0.45–2.80)	1.40	(0.45–2.60)	0.009
HDL-cholesterol, mg/dL	66.0	(54.0–79.0)	62.0	(53.0–79.0)	60.0	(51.0–74.0)	0.023
Triglycerides, mg/dL	100.0	(76.0–131.0)	103.0	(72.0–133.0)	115.5	(80.0–158.0)	0.004
Diabetes medication, % *	4.0		3.9		17.0		0.0005
Lipid-lowering medication, % *	22.8		26.9		37.0		0.073
Fatty liver disease, % †	37.4		36.7		43.7		0.303
Liver signal intensity †	18.5	(14.7–22.4)	17.9	(14.5–24.1)	18.8	(14.8–24.2)	0.599
Visceral adipose tissue, dm^3 ‡	3.55	(2.26–4.95)	3.82	(2.46–5.16)	4.15	(2.71–5.77)	0.013
Subcutaneous adipose tissue, dm^3 ‡	5.85	(4.33–7.70)	6.33	(4.81–8.46)	6.30	(4.89–9.09)	0.018

Data are reported as percentages (%) or median and interquartile range (IQR). * *n* = 305, † *n* = 571, ‡ *n* = 591; MET: Metabolic equivalent; HDL: High density lipoprotein.

3.2. Association of α-Tocopherol/Cholesterol Ratio with Metabolic Traits

In multivariable-adjusted linear regression models, plasma α-tocopherol/cholesterol ratio displayed a statistically significant association with VAT (β scaled by IQR: 0.036; 95% Confidence Interval (CI): 0.0003; 0.071), including a model additionally adjusted for BMI (β scaled by IQR: 0.026; 95% CI: 0.002; 0.050) (Table 3). Furthermore, consistent associations of plasma α-tocopherol/cholesterol ratio with the MetS were observed (Odds Ratio (OR): 1.83; 95% CI: 1.21–2.76 for 3rd vs. 1st tertile; P_{trend} = 0.003) (Table 5), driven by positive associations with high triglycerides (OR: 3.02; 95% CI: 1.80–5.06 for 3rd vs. 1st tertile; P_{trend} < 0.0001) and low HDL-cholesterol levels (OR: 2.52; 95% CI: 0.97–6.56 for 3rd vs. 1st tertile; P_{trend} = 0.033) (Supplementary Materials Table S2). The α-tocopherol/cholesterol ratio was neither associated with SAT, nor with LSI, modeled as a continuous or binary trait (FLD) (Table 3 and Table 5).

Table 3. Multivariable-adjusted means and 95% CI of VAT, SAT, and LSI according to tertiles of α-tocopherol/cholesterol ratio, and scaled by IQR.

Outcome	Tertiles (T) α-Tocopherol/Cholesterol Ratio			P_{trend}	β Scaled by IQR and 95% CI
	T1	T2	T3		
N	196	199	196		
Median α-tocopherol/cholesterol ratio (IQR), µmol/mmol	4.49 (4.41–4.57)	5.53 (5.44–5.63)	7.18 (7.05–7.30)		
VAT, dm^3 (n = 591)					
Model 1	2.99 (2.74–3.26)	3.20 (2.94–3.48)	3.29 (3.04–3.57)	0.056	0.035 (−0.002; 0.071)
Model 2	2.92 (2.63–3.26)	3.13 (2.82–3.47)	3.23 (2.93–3.57)	0.043	0.036 (0.0003; 0.071)
Model 3	3.09 (2.87–3.32)	3.31 (3.09–3.32)	3.34 (3.13–3.57)	0.016	0.026 (0.002; 0.050)
SAT, dm^3 (n = 591)					
Model 1	6.07 (5.78–6.61)	6.38 (5.88–6.93)	6.32 (5.83–6.84)	0.437	0.025 (−0.011; 0.062)
Model 2	5.98 (5.38–6.66)	6.23 (5.62–6.91)	6.22 (5.64–6.87)	0.433	0.026 (−0.009; 0.062)
Model 3	6.36 (6.00–6.74)	6.64 (6.28–7.02)	6.46 (6.12–6.81)	0.572	0.015 (−0.004; 0.034)
N	190	191	190		
Median α-tocopherol/cholesterol ratio (IQR), µmol/mmol	4.50 (4.42–4.58)	5.54 (5.44–5.64)	7.19 (7.05–7.32)		
LSI (n = 571)					
Model 1	16.86 (15.57–18.24)	16.67 (15.43–18.01)	17.70 (16.41–19.10)	0.491	0.014 (−0.019; 0.047)
Model 2	17.10 (15.47–18.90)	16.91 (15.43–18.01)	17.70 (16.41–19.10)	0.486	0.011 (−0.023; 0.045)

VAT: Visceral adipose tissue; SAT: Subcutaneous adipose tissue; LSI: Liver signal intensity; BMI: Body mass index; IQR: Interquartile range; CI: Confidence Interval. Model 1: Adjusted for age and sex. Model 2 is model 1 but additionally adjusted for education, physical activity, smoking status, vitamin E supplementation, alcohol intake, and total energy intake. Model 3 is model 2 but additionally adjusted for BMI.

3.3. Association of γ-Tocopherol/Cholesterol Ratio with Metabolic Traits

In multivariable-adjusted linear and logistic regression models, plasma γ-tocopherol/cholesterol ratio showed statistically significant associations with VAT (β scaled by IQR: 0.066; 95% CI: 0.027; 0.104), SAT (β scaled by IQR: 0.048; 95% CI: 0.010; 0.087), and the MetS (OR: 1.87; 95% CI: 1.23–2.84 for $3r^d$ vs. 1st tertile; P_{trend} = 0.004) (Tables 4 and 5). The association with VAT persisted upon additional adjustment for BMI (β scaled by IQR: 0.037; 95% CI: 0.011; 0.063), whereas adding BMI to the model rendered the association of γ-tocopherol/cholesterol ratio with SAT statistically non-significant (β scaled by IQR: 0.015; 95% CI: −0.006; 0.037) (Table 4). Regarding the individual components of the MetS, the γ-tocopherol/cholesterol ratio was positively related to hypertriglyceridemia (OR: 1.81; 95% CI: 1.08–3.06 for 3rd vs. 1st tertile; P_{trend} = 0.014) and low HDL-cholesterol levels (OR: 4.67; 95% CI: 1.42–15.41 for 3rd vs. 1st tertile; P_{trend} = 0.018) in multivariable-adjusted models (Supplementary Materials Table S3).

Table 4. Multivariable-adjusted means and 95% CI of VAT, SAT, and LSI according to tertiles of γ-tocopherol/cholesterol ratio, and scaled by IQR.

Outcome	Tertiles (T) γ-Tocopherol/Cholesterol Ratio			P_{trend}	β Scaled by IQR and 95% CI
	T1	T2	T3		
N	196	199	196		
Median γ -tocopherol/cholesterol ratio (IQR), µmol/mmol	0.14 (0.13–0.14)	0.24 (0.23–0.25)	0.37 (0.36–0.39)		
VAT, dm^3 (n = 591)					
Model 1	2.90 (2.68–3.15)	3.17 (2.92–3.44)	3.48 (3.21–3.78)	0.0002	0.073 (0.034; 0.111)
Model 2	2.92 (2.65–3.21)	3.21 (2.88–3.57)	3.45 (3.11–3.83)	0.0006	0.066 (0.027; 0.104)
Model 3	3.16 (2.97–3.37)	3.25 (3.02–3.49)	3.48 (3.24–3.73)	0.0034	0.037 (0.011; 0.063)
SAT, dm^3 (n = 591)					
Model 1	5.80 (5.35–6.29)	6.40 (5.90–6.95)	6.65 (6.13–7.21)	0.006	0.059 (0.020; 0.099)
Model 2	5.81 (5.28–6.39)	6.51 (5.84–7.25)	6.58 (5.92–7.30)	0.011	0.048 (0.010; 0.087)
Model 3	6.36 (6.04–6.70)	6.61 (6.23–7.01)	6.64 (6.27–7.03)	0.103	0.015 (−0.006; 0.037)
N	190	191	190		
Median γ-tocopherol/cholesterol ratio (IQR), µmol/mmol	0.14 (0.14–0.15)	0.24 (0.23–0.25)	0.38 (0.36–0.39)		
LSI (n = 571)					
Model 1	16.32 (15.00–17.61)	17.41 (16.11–18.82)	17.60 (16.30–19.00)	0.193	0.017 (−0.020; 0.055)
Model 2	16.80 (15.35–18.40)	18.05 (16.30–20.00)	17.96 (16.28–19.82)	0.304	0.012 (−0.026; 0.051)

VAT: Visceral adipose tissue; SAT: Subcutaneous adipose tissue; LSI: Liver signal intensity; BMI: Body mass index; IQR: Interquartile range; CI: Confidence interval. Model 1: Adjusted for age and sex. Model 2 is model 1 but additionally adjusted for education, physical activity, smoking status, vitamin E supplementation, alcohol intake, and total energy intake. Model 3 is model 2 but additionally adjusted for BMI.

Table 5. Odds Ratio and 95% Confidence Interval for the association of α- and γ-tocopherol/cholesterol ratio with metabolic syndrome (MetS) and fatty liver disease (FLD).

Outcome	Tertiles (T) of α-Tocopherol/Cholesterol Ratio			P_{trend}
	T1	T2	T3	
Median α-tocopherol/cholesterol ratio (IQR), µmol/mmol	4.63 (4.25–4.88)	5.53 (5.36–5.72)	6.74 (6.33–7.59)	
MetS (yes/no) (258/383)	(78/135)	(77/137)	(103/111)	
Model 1	Reference	1.01 (0.67–1.51)	1.72 (1.15–2.58)	0.006
Model 2	Reference	1.09 (0.72–1.65)	1.83 (1.21–2.76)	0.003
Median α-tocopherol/cholesterol ratio (IQR), µmol/mmol	4.61 (4.25–4.87)	5.52 (5.35–5.73)	6.75 (6.29–7.57)	
FLD (yes/no) (224/347)	(72/113)	(75/120)	(77/114)	
Model 1	Reference	1.03 (0.67–1.58)	1.11 (0.72–1.70)	0.631
Model 2	Reference	1.01 (0.65–1.55)	1.09 (0.70–1.68)	0.694
	Tertiles (T) of γ-Tocopherol/Cholesterol Ratio			**P_{trend}**
	T1	**T2**	**T3**	
Median γ-tocopherol/cholesterol ratio (IQR), µmol/mmol	0.16 (0.13–0.18)	0.24 (0.22–0.26)	0.35 (0.31–0.41)	
MetS (yes/no) (258/383)	(70/143)	(89/125)	(99/115)	
Model 1	Reference	1.58 (1.05–2.39)	1.92 (1.28–2.89)	0.002
Model 2	Reference	1.50 (0.98–2.29)	1.87 (1.23–2.84)	0.004
Median γ-tocopherol/cholesterol ratio (IQR), µmol/mmol	0.16 (0.13–0.18)	0.24 (0.22–0.26)	0.34 (0.31–0.42)	
FLD (yes/no) (224/347)	(72/113)	(75/120)	(77/114)	
Model 1	Reference	0.99 (0.65–1.52)	1.38 (0.90–2.10)	0.124
Model 2	Reference	0.97 (0.62–1.51)	1.31 (0.85–2.02)	0.204

IQR: Interquartile range; MetS: Metabolic syndrome; FLD: Fatty liver disease model 1: adjusted for age and sex. Model 2 is model 1 but additionally adjusted for education, physical activity, smoking status, vitamin E supplementation, alcohol intake, and total energy intake.

No association of γ-tocopherol/cholesterol ratio with LSI (continuous trait) or FLD (binary trait) was observed (Tables 4 and 5).

3.4. Assessment of Interactions and Sensitivity Analyses

No significant interactions between circulating α- and γ-tocopherol/cholesterol concentrations and age, sex, or vitamin E supplementation in relation to each selected outcome were observed ($p > 0.05$ for all). In a sensitivity analysis, excluding vitamin E supplement users, the magnitude and the direction of the associations were essentially unchanged (Supplementary Materials Tables S4–S6).

With respect of the association of vitamin E levels with FLD, the results were essentially unchanged after excluding individuals with an alcohol consumption of more than 20 g/day. In multivariable-adjusted models, the α- and γ-tocopherol/cholesterol ratio were not statistically significantly related to the probability of having FLD (OR: 1.09; 95% CI: 0.66–1.80 for 3rd vs. 1st tertile; $P_{trend} = 0.268$ and OR: 1.51; 95% CI: 0.94–2.55 for 3rd vs. 1st tertile; $P_{trend} = 0.239$, respectively).

4. Discussion

4.1. Principal Observations

In a community-based sample from Northern Germany, the α-tocopherol/cholesterol ratio and the γ-tocopherol/cholesterol ratio were positively associated with VAT, SAT, MetS, and its components, high triglycerides and low HDL-cholesterol levels. No significant associations were observed when α- and γ-tocopherol/cholesterol ratios were studied in relation to LSI or FLD.

4.2. In the Context of the Published Literature

4.2.1. Vitamin E Levels and Measures of Adiposity and Adipose Tissue Volumes

We observed a consistent association of α- and γ tocopherol/cholesterol ratio with VAT; the γ-tocopherol/cholesterol ratio was also associated with SAT. This is in line with several prior studies that reported positive associations of circulating vitamin E levels with adiposity measures (e.g., BMI, waist circumference, waist-to-hip ratio, and waist-to-height ratio) [7–9]. For example, in a subsample of participants in the Women's Health Initiative ($n = 2672$), circulating γ-tocopherol levels were positively and strongly associated with BMI, waist circumference, and waist-to-height ratio, while α-tocopherol levels were only positively associated with waist-to-hip ratio [7]. Likewise, Chai et al. [8] reported in 180 premenopausal women that γ-tocopherol levels were significantly higher in obese individuals, whereas α-tocopherol levels did not differ among BMI subgroups.

With respect to α-tocopherol, Wallström et al. [9] reported that serum levels of α-tocopherol were positively related to central adiposity (defined as waist circumference and waist-to-hip ratio), but BMI was only associated with α-tocopherol in men. Body fat percentage (determined by bioelectrical impedance analysis), however, was not significantly associated with vitamin E [9]. Interestingly, in a subsample of healthy postmenopausal women ($n = 48$), α-tocopherol was identified as predictor of MRI-determined total fat [35]. By contrast, in a sample of 580 women, no association of vitamin E levels with measures of adiposity (BMI, waist circumference, waist-to-height ratio, visceral adiposity, and total body fat) determined by dual-energy x-ray absorptiometry was observed [10].

4.2.2. Vitamin E Levels and the Metabolic Syndrome

We observed consistent positive associations of α- and γ-tocopherol/cholesterol ratio levels with MetS in different multivariable-adjusted statistical models. These associations were driven by a positive association with low HDL-cholesterol levels and high triglycerides levels. The association of vitamin E levels with lipid traits is biologically plausible because the lipid-soluble vitamin E is transported in the blood by lipoproteins [33].

In contrast to our observations, in one study, lower levels of plasma α-tocopherol levels were reported in individuals with MetS ($n = 182$) compared to healthy adults ($n = 91$) [14]. In a much larger sample from the 2001–2006 National Health Examination Survey (NHANES; $n = 3008$), no association of vitamin E levels with MetS was reported. However, in further analyses, the authors observed that

vitamin E concentrations were significantly positively related to the number of MetS criteria [12], which lends some support to our results.

Regarding individual components of MetS, vitamin E controlled for lipids showed a positive association with hypertriglyceridemia but not with low HDL-cholesterol levels in NHANES samples [12,13]. A possible explanation for the discrepancies might be that we considered each biomarker separately (α- and γ-tocopherol/cholesterol ratio, respectively), whereas in the other studies vitamin E was defined as the sum of α- and γ-tocopherol. Additionally, we only had a low number of individuals (n = 37) with low HDL-cholesterol levels, whereas both of the other studies [12,13] included more participants (n = 4322 and n = 8465, respectively).

4.2.3. Vitamin E Levels, Fatty Liver Disease, and Liver Fat Content

The association of vitamin E levels with NASH has been assessed in some prior clinical settings with rather small samples sizes [17–19]. One study [17] reported, on average, higher serum vitamin E levels in 43 patients with histologically proven NASH as compared to 33 healthy controls. In two other studies [18,19], however, vitamin E levels were lower in biopsy-proven NASH patients (n = 50 and n = 29, respectively) than in controls (n = 40 and n = 10, respectively).

Regarding liver fat content in postmenopausal healthy women, α-tocopherol was identified as a predictor of MRI-determined liver fat, along with other biomarkers [35]. However, this study was based on a rather small sample (n = 48) of postmenopausal women, with lack of generalizability to other women and to men [35]. We expand these analyses by assessing in a much larger sample (n = 571) from the general population, including men and women, the associations of circulating α- and γ-tocopherol/cholesterol ratio levels with MRI-determined LSI, a proxy for liver fat, modeled on a continuous scale and as a binary trait (FLD).

Yet, albeit FLD is commonly subdivided into non-alcoholic FLD and alcoholic FLD [36], others questioned such a distinction, e.g., because of, in part, similar pathological findings in alcoholic and non-alcoholic FLD, in part overlapping pathophysiological features, sharing of alcohol and other risk factors for FLD in a substantial fraction of the population, and a lack of a consensus regarding harmless alcohol consumption [37]. Furthermore, both non-alcoholic FLD and alcoholic FLD have been associated with premature atherosclerosis, and these findings support the paradigm that steatosis might be a precursor of an increased cardiovascular risk [38]. Therefore, considering FLD as a complex, multifactorial condition [37], we did not distinguish between alcoholic and non-alcoholic FLD but focused on MRI-derived LSI as a proxy for liver fat content.

Interestingly, vitamin E therapy (800 UI per day) for 96 weeks performed better than pioglitazone and placebo in a randomized trial in patients with NASH [6]. The primary endpoint of the study was a histological improvement of NASH features [6].

However, in contrast to the studies mentioned above, we observed no association between vitamin E levels and LSI or FLD in our sample. One potential explanation is that vitamin E levels are altered preferentially in patients with advanced liver disease [39], but not in relatively healthy men and women from the general population with rather modest alterations in liver fat, a premise that merits further investigations.

4.3. Potential Mechanisms for the Observed Associations

Our data suggest that circulating vitamin E (α- and γ-tocopherol/cholesterol ratio) levels are positively associated with MetS and MRI-determined body fat volumes (particularly VAT).

Circulating vitamin E levels are affected by several factors: Dietary vitamin E is absorbed in the gastro-intestinal system (the efficiency of vitamin E absorption is widely variable, ranging from 20–80%) and transported via chylomicrons to the liver [40]. Taken up by the liver, α-tocopherol has several possible metabolic pathways. The hepatic α-tocopherol transfer protein (α-TTP) is the major regulator for maintaining normal plasma α-tocopherol concentrations [41]. γ-tocopherol has much less affinity (α-tocopherol = 100%, γ-tocopherol = 9% [42]) for α-TTP and is largely metabolized in the

liver and secreted in the bile [43]. Experimental evidence indicates that α-TTP activity is modulated by oxidative stress potentially influencing vitamin E status [44–47].

α-TTP can facilitate α-tocopherol transfer to very low density lipoproteins (VLDL), whereas the underlying mechanism is still not understood, and facilitate its return to the liver [40]. It is suggested that VLDL is enriched with α-tocopherol to a lesser extent in MetS compared to healthy adults and therefore transported in VLDL to a lesser degree to extrahepatic tissues in individuals with MetS because of a slower α-tocopherol catabolism in MetS compared to healthy adults [16,48]. Therefore, it might be that the disappearance of α-tocopherol from plasma is slower in individuals with MetS [16,48], which would explain the positive association of plasma α-tocopherol levels with MetS as observed in our analyses.

Looking at the excretion, α-tocopherol can be secreted in bile for fecal excretion, but it is not known if this pathway is altered in individuals with MetS [48]. Interestingly, data from a recent study [48] indicate that MetS may inhibit the hepatic metabolism of α-tocopherol to the α-tocopherol metabolite α-carboxyethyl hydroxychromanol (CEHC) (secreted in bile for elimination via feces or excreted via urine [43]). In a recent clinical trial, Traber et al. [48] observed that individuals with MetS (n = 10) excrete less vitamin E (lower amounts of α- and γ-CEHC were detected in the urine) as compared to healthy adults (n = 10). The authors speculated that individuals with MetS might need more vitamin E because of increased oxidative and inflammatory stressors, thereby suggesting they had increased requirements for α-tocopherol and therefore retained more vitamin E compared to healthy adults [48]. We observed that α- and γ-tocopherol/cholesterol ratios were more strongly and positively associated with VAT than with SAT. Indeed, it is known that adipose tissue, as an endocrine organ, contains a large number of pro-inflammatory cytokines including tumor necrosis factor-α, interleukin (IL)-1β, and IL-6-promoting inflammatory response and oxidative stress [49,50]. Of note, VAT has been shown to release more inflammatory markers (e.g., two to three times more IL-6) than SAT [50]; a rise in concentration of inflammatory markers could be responsible for increased oxidative stress leading to higher vitamin E levels as a compensatory mechanism. Furthermore, in our study, adjustment for BMI rendered the association of γ-tocopherol/cholesterol with SAT statistically non-significant, but not the association with VAT. This might be explained by the fact that BMI is more strongly correlated with SAT than with VAT [51].

4.4. Strengths and Limitation

Strengths of the present study include the assessment of VAT, SAT and liver fat by MRI in a moderate-sized sample from the general population, the measurement of vitamin E in plasma, and the detailed assessment of covariates. The following limitation merits consideration. The cross-sectional study design precludes causal inferences, because exposure and outcome were assessed at the same time. Furthermore, we cannot completely rule out reverse causality. Besides, the cross-sectional study design and the small regression coefficients observed for the associations of VAT and SAT, with both α- and γ-tocopherol values, limit our ability to quantify and translate the observed associations into clinical meaningful findings. Moreover, we had no information about why individuals were taking vitamin E supplements and about the use of statins. However, we did have self-reported information regarding the use of lipid-lowering medications for a subsample (n =305).

In summary, we observed significant associations of circulating vitamin E concentrations with MetS and MRI-determined body fat volumes (particularly VAT). Further investigations of longitudinal relationships between α- and γ-tocopherol levels and metabolic conditions and liver fat are warranted.

Supplementary Materials: The following are available online at www.mdpi.com/2072-6643/9/10/1143/s1, Table S1: Missing covariates information; Table S2: Odds ratio and 95% Confidence Interval for the association of α-tocopherol/cholesterol ratio with individual components of MetS; Table S3: Odds ratio and 95% Confidence Interval for the association of γ-tocopherol/cholesterol ratio with individual components of MetS; Table S4: Sensitivity analysis: multivariable-adjusted means and 95% CI of VAT, SAT, and LSI according to tertiles of α-tocopherol/cholesterol ratio, and scaled by IQR after excluding vitamin E supplement users; Table S5: Sensitivity analysis: multivariable-adjusted means and 95% CI of VAT, SAT, and LSI according to tertiles of γ-tocopherol/cholesterol ratio, and scaled by IQR after excluding vitamin E supplement users; Table S6: Sensitivity analysis: odds Ratio and 95% Confidence Interval for the association of α- and γ-tocopherol/cholesterol ratio with MetS and FLD after excluding vitamin E supplement users.

Acknowledgments: Manja Koch is recipient of a Postdoctoral Research Fellowship from the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG, KO 5187/1-1). Romina di Giuseppe is supported by the Deutsche Forschungsgemeinschaft Excellence Cluster "Inflammation at Interfaces" (grants EXC306 and EXC306/2). The PopGen 2.0 Network is supported by the German Federal Ministry of Education and Research (grant 01GR0468 and 01EY1103). The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Author Contributions: W.L. and G.R. formulated the research question; W.L., S.S. and U.N. designed the study; T.E. performed the vitamin E measurements, S.W. and R.d.G. performed the statistical analyses, S.W., W.L., R.D.G., S.P.D., M.K., I.R. and S.S. contributed to the interpretation of the data; S.W. and W.L. drafted the manuscript. All authors (S.W., R.d.G., S.P.D., T.E., M.K., S.S., I.R., M.B., J.B., J.K., H.-P. M., G.J., U.N., G.R. and W.L.) critically reviewed and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Roberts, C.K.; Sindhu, K.K. Oxidative stress and metabolic syndrome. *Life Sci.* **2009**, *84*, 705–712. [CrossRef] [PubMed]
2. Bonomini, F.; Rodella, L.F.; Rezzani, R. Metabolic syndrome, aging and involvement of oxidative stress. *Aging Dis.* **2015**, *6*, 109–120. [CrossRef] [PubMed]
3. Polimeni, L.; Del Ben, M.; Baratta, F.; Perri, L.; Albanese, F.; Pastori, D.; Violi, F.; Angelico, F. Oxidative stress: New insights on the association of non-alcoholic fatty liver disease and atherosclerosis. *World J. Hepatol.* **2015**, *7*, 1325–1336. [CrossRef] [PubMed]
4. Jiang, Q. Natural forms of vitamin E: Metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. *Free Radic. Biol. Med.* **2014**, *72*, 76–90. [CrossRef] [PubMed]
5. Borel, P.; Preveraud, D.; Desmarchelier, C. Bioavailability of vitamin E in humans: An update. *Nutr. Rev.* **2013**, *71*, 319–331. [CrossRef] [PubMed]
6. Sanyal, A.J.; Chalasani, N.; Kowdley, K.V.; McCullough, A.; Diehl, A.M.; Bass, N.M.; Neuschwander-Tetri, B.A.; Lavine, J.E.; Tonascia, J.; Unalp, A.; et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N. Engl. J. Med.* **2010**, *362*, 1675–1685. [CrossRef] [PubMed]
7. Kabat, G.C.; Heo, M.; Ochs-Balcom, H.M.; LeBoff, M.S.; Mossavar-Rahmani, Y.; Adams-Campbell, L.L.; Nassir, R.; Ard, J.; Zaslavsky, O.; Rohan, T.E. Longitudinal association of measures of adiposity with serum antioxidant concentrations in postmenopausal women. *Eur. J. Clin. Nutr.* **2016**, *70*, 47–53. [CrossRef] [PubMed]
8. Chai, W.; Conroy, S.M.; Maskarinec, G.; Franke, A.A.; Pagano, I.S.; Cooney, R.V. Associations between obesity and serum lipid-soluble micronutrients among premenopausal women. *Nutr. Res.* **2010**, *30*, 227–232. [CrossRef] [PubMed]
9. Wallstrom, P.; Wirfalt, E.; Lahmann, P.H.; Gullberg, B.; Janzon, L.; Berglund, G. Serum concentrations of beta-carotene and alpha-tocopherol are associated with diet, smoking, and general and central adiposity. *Am. J. Clin. Nutr.* **2001**, *73*, 777–785. [PubMed]
10. Garcia, O.P.; Ronquillo, D.; Caamano Mdel, C.; Camacho, M.; Long, K.Z.; Rosado, J.L. Zinc, vitamin A, and vitamin C status are associated with leptin concentrations and obesity in Mexican women: Results from a cross-sectional study. *Nutr. Metab.* **2012**, *9*, 59. [CrossRef] [PubMed]
11. Galan, P.; Viteri, F.E.; Bertrais, S.; Czernichow, S.; Faure, H.; Arnaud, J.; Ruffieux, D.; Chenal, S.; Arnault, N.; Favier, A.; et al. Serum concentrations of beta-carotene, vitamins C and E, zinc and selenium are influenced by sex, age, diet, smoking status, alcohol consumption and corpulence in a general French adult population. *Eur. J. Clin. Nutr.* **2005**, *59*, 1181–1190. [CrossRef] [PubMed]

12. Beydoun, M.A.; Shroff, M.R.; Chen, X.; Beydoun, H.A.; Wang, Y.; Zonderman, A.B. Serum antioxidant status is associated with metabolic syndrome among U.S. Adults in recent national surveys. *J. Nutr.* **2011**, *141*, 903–913. [CrossRef] [PubMed]
13. Ford, E.S.; Mokdad, A.H.; Giles, W.H.; Brown, D.W. The metabolic syndrome and antioxidant concentrations: Findings from the third national health and nutrition examination survey. *Diabetes* **2003**, *52*, 2346–2352. [CrossRef] [PubMed]
14. Godala, M.M.; Materek-Kusmierkiewicz, I.; Moczulski, D.; Rutkowski, M.; Szatko, F.; Gaszynska, E.; Tokarski, S.; Kowalski, J. Lower plasma levels of antioxidant vitamins in patients with metabolic syndrome: A case control study. *Adv. Clin. Exp. Med.* **2016**, *25*, 689–700. [CrossRef] [PubMed]
15. Li, Y.; Guo, H.; Wu, M.; Liu, M. Serum and dietary antioxidant status is associated with lower prevalence of the metabolic syndrome in a study in Shanghai, China. *Asia Pac. J. Clin. Nutr.* **2013**, *22*, 60–68. [PubMed]
16. Mah, E.; Sapper, T.N.; Chitchumroonchokchai, C.; Failla, M.L.; Schill, K.E.; Clinton, S.K.; Bobe, G.; Traber, M.G.; Bruno, R.S. Alpha-tocopherol bioavailability is lower in adults with metabolic syndrome regardless of dairy fat co-ingestion: A randomized, double-blind, crossover trial. *Am. J. Clin. Nutr.* **2015**, *102*, 1070–1080. [CrossRef] [PubMed]
17. Machado, M.V.; Ravasco, P.; Jesus, L.; Marques-Vidal, P.; Oliveira, C.R.; Proenca, T.; Baldeiras, I.; Camilo, M.E.; Cortez-Pinto, H. Blood oxidative stress markers in non-alcoholic steatohepatitis and how it correlates with diet. *Scand. J. Gastroenterol.* **2008**, *43*, 95–102. [CrossRef] [PubMed]
18. Erhardt, A.; Stahl, W.; Sies, H.; Lirussi, F.; Donner, A.; Haussinger, D. Plasma levels of vitamin E and carotenoids are decreased in patients with nonalcoholic steatohepatitis (NASH). *Eur. J. Med. Res.* **2011**, *16*, 76–78. [CrossRef] [PubMed]
19. Bahcecioglu, I.H.; Yalniz, M.; Ilhan, N.; Ataseven, H.; Ozercan, I.H. Levels of serum vitamin A, alpha-tocopherol and malondialdehyde in patients with non-alcoholic steatohepatitis: Relationship with histopathologic severity. *Int. J. Clin. Pract.* **2005**, *59*, 318–323. [CrossRef] [PubMed]
20. Nothlings, U.; Krawczak, M. PopGen. A population-based biobank with prospective follow-up of a control group. *Bundesgesundhbl. Gesundheitsforsch. Gesundheitsschutz* **2012**, *55*, 831–835.
21. Krawczak, M.; Nikolaus, S.; Von Eberstein, H.; Croucher, P.J.; El Mokhtari, N.E.; Schreiber, S. Popgen: Population-based recruitment of patients and controls for the analysis of complex genotype-phenotype relationships. *Community Genet.* **2006**, *9*, 55–61. [CrossRef] [PubMed]
22. Barbaresko, J.; Siegert, S.; Koch, M.; Aits, I.; Lieb, W.; Nikolaus, S.; Laudes, M.; Jacobs, G.; Nothlings, U. Comparison of two exploratory dietary patterns in association with the metabolic syndrome in a northern German population. *Br. J. Nutr.* **2014**, *112*, 1364–1372. [CrossRef] [PubMed]
23. Nothlings, U.; Hoffmann, K.; Bergmann, M.M.; Boeing, H. Fitting portion sizes in a self-administered food frequency questionnaire. *J. Nutr.* **2007**, *137*, 2781–2786. [PubMed]
24. Dehne, L.I.; Klemm, C.; Henseler, G.; Hermann-Kunz, E. The German food code and nutrient data base (BLS II.2). *Eur. J. Epidemiol.* **1999**, *15*, 355–359. [CrossRef] [PubMed]
25. Muller, H.P.; Raudies, F.; Unrath, A.; Neumann, H.; Ludolph, A.C.; Kassubek, J. Quantification of human body fat tissue percentage by MRI. *NMR Biomed.* **2011**, *24*, 17–24. [CrossRef] [PubMed]
26. Fischer, K.; Moewes, D.; Koch, M.; Muller, H.P.; Jacobs, G.; Kassubek, J.; Lieb, W.; Nothlings, U. Mri-determined total volumes of visceral and subcutaneous abdominal and trunk adipose tissue are differentially and sex-dependently associated with patterns of estimated usual nutrient intake in a northern German population. *Am. J. Clin. Nutr.* **2015**, *101*, 794–807. [CrossRef] [PubMed]
27. Koch, M.; Borggrefe, J.; Barbaresko, J.; Groth, G.; Jacobs, G.; Siegert, S.; Lieb, W.; Muller, M.J.; Bosy-Westphal, A.; Heller, M.; et al. Dietary patterns associated with magnetic resonance imaging-determined liver fat content in a general population study. *Am. J. Clin. Nutr.* **2014**, *99*, 369–377. [CrossRef] [PubMed]
28. Alberti, K.G.; Eckel, R.H.; Grundy, S.M.; Zimmet, P.Z.; Cleeman, J.I.; Donato, K.A.; Fruchart, J.C.; James, W.P.; Loria, C.M.; Smith, S.C., Jr.; et al. Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; national heart, lung, and blood institute; American heart association; World heart federation; International atherosclerosis society; and International association for the study of obesity. *Circulation* **2009**, *120*, 1640–1645. [PubMed]

29. Szczepaniak, L.S.; Nurenberg, P.; Leonard, D.; Browning, J.D.; Reingold, J.S.; Grundy, S.; Hobbs, H.H.; Dobbins, R.L. Magnetic resonance spectroscopy to measure hepatic triglyceride content: Prevalence of hepatic steatosis in the general population. *Am. J. Physiol. Endocrinol. Metab.* **2005**, *288*, E462–E468. [CrossRef] [PubMed]
30. Haftenberger, M.; Schuit, A.J.; Tormo, M.J.; Boeing, H.; Wareham, N.; Bueno-de-Mesquita, H.B.; Kumle, M.; Hjartaker, A.; Chirlaque, M.D.; Ardanaz, E.; et al. Physical activity of subjects aged 50–64 years involved in the European prospective investigation into cancer and nutrition (EPIC). *Public Health Nutr.* **2002**, *5*, 1163–1176. [CrossRef] [PubMed]
31. Ainsworth, B.E.; Haskell, W.L.; Herrmann, S.D.; Meckes, N.; Bassett, D.R., Jr.; Tudor-Locke, C.; Greer, J.L.; Vezina, J.; Whitt-Glover, M.C.; Leon, A.S. 2011 compendium of physical activities: A second update of codes and MET values. *Med. Sci. Sports Exerc.* **2011**, *43*, 1575–1581. [CrossRef] [PubMed]
32. Augustin, K.; Blank, R.; Boesch-Saadatmandi, C.; Frank, J.; Wolffram, S.; Rimbach, G. Dietary green tea polyphenols do not affect vitamin E status, antioxidant capacity and meat quality of growing pigs. *J. Anim. Physiol. Anim. Nutr.* **2008**, *92*, 705–711. [CrossRef] [PubMed]
33. Kayden, H.J.; Traber, M.G. Absorption, lipoprotein transport, and regulation of plasma concentrations of vitamin E in humans. *J. Lipid Res.* **1993**, *34*, 343–358. [PubMed]
34. Thurnham, D.I.; Davies, J.A.; Crump, B.J.; Situnayake, R.D.; Davis, M. The use of different lipids to express serum tocopherol: Lipid ratios for the measurement of vitamin E status. *Ann. Clin. Biochem.* **1986**, *23*, 514–520. [CrossRef] [PubMed]
35. Lim, U.; Turner, S.D.; Franke, A.A.; Cooney, R.V.; Wilkens, L.R.; Ernst, T.; Albright, C.L.; Novotny, R.; Chang, L.; Kolonel, L.N.; et al. Predicting total, abdominal, visceral and hepatic adiposity with circulating biomarkers in Caucasian and Japanese American women. *PLoS ONE* **2012**, *7*, e43502. [CrossRef] [PubMed]
36. Italian Association for the Study of the Liver. AISF position paper on nonalcoholic fatty liver disease (NAFLD): Updates and future directions. *Dig. Liver Dis.* **2017**, *49*, 471–483.
37. Volzke, H. Multicausality in fatty liver disease: Is there a rationale to distinguish between alcoholic and non-alcoholic origin? *World J. Gastroenterol.* **2012**, *18*, 3492–3501. [CrossRef] [PubMed]
38. Loria, P.; Marchesini, G.; Nascimbeni, F.; Ballestri, S.; Maurantonio, M.; Carubbi, F.; Ratziu, V.; Lonardo, A. Cardiovascular risk, lipidemic phenotype and steatosis. A comparative analysis of cirrhotic and non-cirrhotic liver disease due to varying etiology. *Atherosclerosis* **2014**, *232*, 99–109. [CrossRef] [PubMed]
39. Pacana, T.; Sanyal, A.J. Vitamin E and nonalcoholic fatty liver disease. *Curr. Opin. Clin. Nutr. Metab. Care* **2012**, *15*, 641–648. [CrossRef] [PubMed]
40. Schmolz, L.; Birringer, M.; Lorkowski, S.; Wallert, M. Complexity of vitamin E metabolism. *World J. Biol. Chem.* **2016**, *7*, 14–43. [CrossRef] [PubMed]
41. Traber, M.G. Vitamin E regulatory mechanisms. *Annu. Rev. Nutr.* **2007**, *27*, 347–362. [CrossRef] [PubMed]
42. Hosomi, A.; Arita, M.; Sato, Y.; Kiyose, C.; Ueda, T.; Igarashi, O.; Arai, H.; Inoue, K. Affinity for alpha-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. *FEBS Lett.* **1997**, *409*, 105–108. [CrossRef]
43. Traber, M.G. Mechanisms for the prevention of vitamin E excess. *J. Lipid Res.* **2013**, *54*, 2295–2306. [CrossRef] [PubMed]
44. Etzl, R.P.; Vrekoussis, T.; Kuhn, C.; Schulze, S.; Poschl, J.M.; Makrigiannakis, A.; Jeschke, U.; Rotzoll, D.E. Oxidative stress stimulates alpha-tocopherol transfer protein in human trophoblast tumor cells bewo. *J. Perinat. Med.* **2012**, *40*, 373–378. [CrossRef] [PubMed]
45. Ulatowski, L.; Dreussi, C.; Noy, N.; Barnholtz-Sloan, J.; Klein, E.; Manor, D. Expression of the alpha-tocopherol transfer protein gene is regulated by oxidative stress and common single-nucleotide polymorphisms. *Free Radic. Biol. Med.* **2012**, *53*, 2318–2326. [CrossRef] [PubMed]
46. Usenko, C.Y.; Harper, S.L.; Tanguay, R.L. Fullerene C60 exposure elicits an oxidative stress response in embryonic zebrafish. *Toxicol. Appl. Pharmacol.* **2008**, *229*, 44–55. [CrossRef] [PubMed]
47. Otulakowski, G.; Engelberts, D.; Arima, H.; Hirate, H.; Bayir, H.; Post, M.; Kavanagh, B.P. Alpha-tocopherol transfer protein mediates protective hypercapnia in murine ventilator-induced lung injury. *Thorax* **2017**, *72*, 538–549. [CrossRef] [PubMed]
48. Traber, M.G.; Mah, E.; Leonard, S.W.; Bobe, G.; Bruno, R.S. Metabolic syndrome increases dietary alpha-tocopherol requirements as assessed using urinary and plasma vitamin E catabolites: A double-blind, crossover clinical trial. *Am. J. Clin. Nutr.* **2017**, *105*, 571–579. [CrossRef] [PubMed]

49. Marseglia, L.; Manti, S.; D'Angelo, G.; Nicotera, A.; Parisi, E.; Di Rosa, G.; Gitto, E.; Arrigo, T. Oxidative stress in obesity: A critical component in human diseases. *Int. J. Mol. Sci.* **2014**, *16*, 378–400. [CrossRef] [PubMed]
50. Fonseca-Alaniz, M.H.; Takada, J.; Alonso-Vale, M.I.; Lima, F.B. Adipose tissue as an endocrine organ: From theory to practice. *J. Pediatr.* **2007**, *83*, S192–S203. [CrossRef]
51. Camhi, S.M.; Bray, G.A.; Bouchard, C.; Greenway, F.L.; Johnson, W.D.; Newton, R.L.; Ravussin, E.; Ryan, D.H.; Smith, S.R.; Katzmarzyk, P.T. The relationship of waist circumference and BMI to visceral, subcutaneous, and total body fat: Sex and race differences. *Obesity* **2011**, *19*, 402–408. [CrossRef] [PubMed]

Table S1. Missing covariates information.

Covariate	Number of Missing Values
Cholesterol	1
Physical activity	1
Education (years)	1
Smoking status	8
Vitamin E supplementation	1

Table S2. Odds ratio and 95% Confidence Interval for the association of α-tocopherol/cholesterol ratio with individual components of MetS.

Outcome	Tertiles (T) of α-Tocopherol/Cholesterol Ratio			P_{trend}
	T1	T2	T3	
Median α-tocopherol/cholesterol ratio (IQR), μmol/mmol	4.63 (4.25–4.88)	5.53 (5.36–5.72)	6.74 (6.33–7.59)	
Hypertension (yes/no) (135/506)	(170/43)	(171/43)	165/49)	
Model 1	Reference	0.97 (0.59–1.60)	0.70 (0.42–1.16)	0.135
Model 2	Reference	0.99 (0.60–1.65)	0.71 (0.43–1.18)	0.145
Low HDL-cholesterol (yes/no) (37/604)	(7/206)	(9/205)	(21/193)	
Model 1	Reference	1.16 (0.41–3.25)	2.38 (0.95–5.99)	0.034
Model 2	Reference	1.25 (0.43–3.62)	2.52 (0.97–6.56)	0.033
Hypertriglyceridemia (yes/no) (279/362)	(29/184)	(29/185)	(96/118)	
Model 1	Reference	0.89 (0.50–1.58)	3.02 (1.81–5.04)	<0.0001
Model 2	Reference	0.91 (0.51–1.62)	3.02 (1.80–5.06)	<0.0001
Hyperglycemia (yes/no) (129/512)	(92/121)	(91/123)	(71/143)	
Model 1	Reference	0.99 (0.66–1.48)	0.97 (0.64–1.48)	0.888
Model 2	Reference	1.10 (0.72–1.66)	1.02 (0.67–1.57)	0.994
Abdominal obesity (yes/no) (468/173)	(148/65)	(161/53)	(159/55)	
Model 1	Reference	1.54 (0.96–2.42)	1.14 (0.72–1.82)	0.842
Model 2	Reference	1.42 (0.89–2.27)	1.13 (0.70–1.83)	0.834

IQR: Interquartile range; MetS: Metabolic syndrome. Model 1: Adjusted for age and sex. Model 2 is model 1 but additionally adjusted for education, physical activity, smoking status, vitamin E supplementation, alcohol intake, and total energy intake.

Table S3. Odds ratio and 95% Confidence Interval for the association of γ-tocopherol/cholesterol ratio with individual components of MetS.

Outcome	Tertiles (T) of γ-Tocopherol/Cholesterol Ratio			P_{trend}
	T1	T2	T3	
Median γ-tocopherol/cholesterol ratio (IQR), μmol/mmol	0.16 (0.13–0.18)	0.24 (0.22–0.26)	0.35 (0.31–0.41)	
Hypertension (yes/no) (135/506)	(166/47)	(167/47)	(173/421)	
Model 1	Reference	0.97 (0.59–1.57)	1.01 (0.61–1.68)	0.948
Model 2	Reference	0.92 (0.56–1.53)	0.97 (0.58–1.61)	0.907
Low HDL-cholesterol (yes/no) (37/604)	(4/209)	(14/200)	(19/195)	
Model 1	Reference	3.58 (1.13–11.33)	3.63 (1.18–11.15)	0.045
Model 2	Reference	3.95 (1.14–13.65)	4.67 (1.42–15.41)	0.018
Hypertriglyceridemia (yes/no) (279/362)	(33/180)	(37/177)	(59/155)	
Model 1	Reference	0.98 (0.58–1.68)	1.65 (0.99–2.73)	0.042
Model 2	Reference	1.06 (0.61–1.87)	1.81 (1.08–3.06)	0.014
Hyperglycemia (yes/no) (129/512)	(89/124)	(86/128)	(104/110)	
Model 1	Reference	0.92 (0.61–1.39)	1.25 (0.83–1.89)	0.249
Model 2	Reference	0.78 (0.51–1.21)	1.14 (0.74–1.75)	0.437
Abdominal obesity (yes/no) (468/173)	(141/72)	(159/55)	(168/46)	
Model 1	Reference	1.56 (1.00–2.45)	1.69 (1.07–2.69)	0.029
Model 2	Reference	1.57 (0.98–2.52)	1.59 (0.98–2.58)	0.065

IQR: Interquartile range; MetS: Metabolic syndrome. Model 1: Adjusted for age and sex. Model 2 is model 1 but additionally adjusted for education, physical activity, smoking status, vitamin E supplementation, alcohol intake, and total energy intake.

Table S4. Sensitivity analysis: Multivariable-adjusted means and 95% CI of VAT, SAT and LSI according to tertiles of α-tocopherol/cholesterol ratio, and scaled by IQR after excluding vitamin E supplement users.

Outcome	Tertiles (T) α-Tocopherol/Cholesterol Ratio			P_{trend}	β Scaled by IQR and 95% CI
	T1	T2	T3		
N	184	185	177		
Median α-tocopherol/cholesterol ratio (IQR), µmol/mmol	4.59 (4.24–4.83)	5.52 (5.35–5.71)	6.73 (6.35–7.57)		
VAT, dm³ (*n* = 546)					
Model 1	3.15 (2.95–3.38)	3.51 (2.28–3.76)	3.51 (3.27–3.76)	0.037	0.036 (−0.001; 0.074)
Model 2	3.06 (2.86–3.78)	3.42 (3.20–3.66)	3.42 (3.19–3.66)	0.026	0.037 (0.0004; 0.074)
Model 3	3.18 (3.03–3.33)	3.44 (3.28–3.60)	3.49 (3.33–3.65)	0.005	0.028 (0.003; 0.053)
SAT, dm³ (*n* = 546)					
Model 1	6.07 (5.67–6.50)	6.68 (6.24–7.16)	6.35 (5.92–6.80)	0.387	0.024 (−0.013; 0.062)
Model 2	5.95 (5.55–6.38)	6.49 (6.07–6.94)	6.20 (5.79–6.64)	0.423	0.024 (−0.012; 0.061)
Model 3	6.21 (5.97–6.46)	6.53 (6.29–6.78)	6.34 (6.10–6.59)	0.421	0.014 (−0.006; 0.035)
N	175	183	170		
Median α-tocopherol/cholesterol ratio (IQR), µmol/mmol	4.59 (4.24–4.86)	5.53 (5.35–5.73)	6.72 (6.29–7.48)		
LSI (*n* = 528)					
Model 1	18.64 (17.42–19.92)	18.79 (17.59–20.07)	18.83 (17.59–20.17)	0.825	0.003 (−0.023; 0.049)
Model 2	18.44 (17.24–19.84)	18.45 (17.25–19.73)	18.52 (17.26–19.87)	0.976	0.010 (−0.026; 0.046)

VAT: Visceral adipose tissue; SAT: Subcutaneous adipose tissue; LSI: Liver signal intensity; BMI: Body mass index; IQR: Interquartile range; CI: Confidence Interval. Model 1: Adjusted for age and sex. Model 2 is model 1 but additionally adjusted for education, physical activity, smoking status, alcohol intake, and total energy intake. Model 3 is model 2 but additionally adjusted for BMI.

Table S5. Sensitivity analysis: Multivariable-adjusted means and 95% CI of VAT, SAT and LSI according to tertiles of γ-tocopherol/cholesterol ratio, and scaled by IQR after excluding vitamin E supplement users.

Outcome	Tertiles (T) γ-Tocopherol/Cholesterol Ratio			P_{trend}	β Scaled by IQR and 95% CI
	T1	T2	T3		
N	167	193	186		
Median γ -tocopherol/cholesterol ratio (IQR), µmol/mmol	0.16 (0.13–0.18)	0.24 (0.22–0.26)	0.34 (0.31–0.41)		
VAT, dm³ (*n* = 546)					
Model 1	3.07 (2.87–3.29)	3.40 (3.18–3.64)	3.71 (3.47–3.98)	0.0001	0.079 (0.038; 0.120)
Model 2	3.03 (2.83–3.24)	3.32 (3.10–3.55)	3.59 (3.36–3.85)	0.0004	0.072 (0.032; 0.112)
Model 3	3.25 (3.11–3.41)	3.31 (3.16–3.47)	3.55 (3.39–3.72)	0.0072	0.0395 (0.012; 0.066)
SAT, dm³ (*n* = 546)					
Model 1	5.75 (5.37–6.15)	6.26 (5.85–6.70)	6.58 (6.15–7.04)	0.004	0.065 (0.024; 0.106)
Model 2	5.82 (5.44–6.22)	6.35 (5.93–6.80)	6.53 (6.10–7.00)	0.011	0.055 (0.015; 0.095)
Model 3	6.31 (6.08–6.56)	6.34 (6.10–6.58)	6.45 (6.20–7.00)	0.290	0.017 (−0.006; 0.040)
N	164	185	179		
Median γ-tocopherol/cholesterol ratio (IQR), µmol/mmol	0.16 (0.13–0.18)	0.24 (0.22–0.26)	0.34 (0.31–0.42)		
LSI (*n* = 528)					
Model 1	17.73 (16.55–19.00)	18.99 (17.79–20.26)	19.49 (18.24–20.83)	0.053	0.023 (−0.018; 0.064)
Model 2	17.72 (16.52–19.00)	18.86 (17.64–20.17)	19.20 (17.93–20.57)	0.096	0.018 (−0.022; 0.059)

VAT: Visceral adipose tissue; SAT: Subcutaneous adipose tissue; LSI: Liver signal intensity; BMI: Body mass index; IQR: Interquartile range; CI: Confidence Interval. Model 1: Adjusted for age and sex. Model 2 is model 1 but additionally adjusted for education, physical activity, smoking status, alcohol intake, and total energy intake. Model 3 is model 2 but additionally adjusted for BMI.

Table S6. Sensitivity analysis: Odds ratio and 95% Confidence Interval for the association of α- and γ-tocopherol/cholesterol ratio with MetS and FLD after excluding vitamin E supplement users.

Outcome	Tertiles (T) of α-Tocopherol/Cholesterol Ratio			P_{trend}
	T1	T2	T3	
Median α-tocopherol/cholesterol ratio (IQR), µmol/mmol	4.61 (4.24–4.87)	5.53 (5.36–5.72)	6.72 (6.34–7.55)	
MetS (yes/no) (242/351)	(74/127)	(74/126)	(94/98)	
Model 1	Reference	1.07 (0.70–1.63)	1.78 (1.17–2.71)	0.006
Model 2	Reference	1.15 (0.75–1.76)	1.84 (1.20–2.82)	0.004
Median α-tocopherol/cholesterol ratio (IQR), µmol/mmol	4.59 (4.24–4.86)	5.53 (5.35–5.73)	6.72 (6.29–7.48)	
FLD (yes/no) (206/322)	(67/108)	(71/112)	(68/102)	
Model 1	Reference	1.10 (0.71–1.71)	1.13 (0.72–1.77)	0.597
Model 2	Reference	1.09 (0.70–1.71)	1.12 (0.71–1.76)	0.628
	Tertiles (T) of γ–Tocopherol/Cholesterol Ratio			
	T1	T2	T3	P_{trend}
Median γ-tocopherol/cholesterol ratio (IQR), µmol/mmol	0.16 (0.13–0.18)	0.24 (0.22–0.26)	0.35 (0.31–0.41)	
MetS (yes/no) (242/351)	(59/123)	(87/121)	(107/96)	
Model 1	Reference	1.60 (1.04–2.47)	1.99 (1.29–3.06)	0.002
Model 2	Reference	1.55 (1.00–2.41)	1.94 (1.23–3.00)	0.004
Median γ-tocopherol/cholesterol ratio (IQR), µmol/mmol	0.16 (0.13–0.18)	0.24 (0.22–0.26)	0.34 (0.31–0.42)	
FLD (yes/no) (206/322)	(61/103)	(67/118)	(78/101)	
Model 1	Reference	0.97 (0.62–1.51)	1.35 (0.87–2.11)	0.155
Model 2	Reference	0.93 (0.59–1.47)	1.32 (0.84–2.07)	0.196

IQR: Interquartile range; MetS: Metabolic syndrome; FLD: Fatty liver disease. Model 1: Adjusted for age and sex. Model 2 is model 1 but additionally adjusted for education, physical activity, smoking status, alcohol intake, and total energy intake.

4 Association of circulating vitamin E (α- and γ-tocopherol) levels with gallstone disease

Sabina Waniek[1], Romina di Giuseppe[1], Tuba Esatbeyoglu[2], Ilka Ratjen[1], Janna Enderle[1], Gunnar Jacobs[1,3], Ute Nöthlings[4], Manja Koch[1,5], Sabrina Schlesinger[6], Gerald Rimbach[2], Wolfgang Lieb[1,3]

[1]Institute of Epidemiology, University of Kiel, 24105 Kiel, Germany

[2]Institute of Human Nutrition and Food Science, University of Kiel, 24118 Kiel, Germany

[3]Biobank PopGen, University Hospital Schleswig-Holstein, Campus Kiel, 24105 Kiel, Germany

[4]Department of Nutrition and Food Science, University of Bonn, 53113 Bonn, Germany

[5]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA

[6]Institute for Biometrics and Epidemiology, German Diabetes Center (DDZ) at Heinrich Heine University Düsseldorf, 40225 Düsseldorf, Germany

Published in: Nutrients 2018 Jan 27;10(2)

nutrients

MDPI

Article

Association of Circulating Vitamin E (α- and γ-Tocopherol) Levels with Gallstone Disease

Sabina Waniek [1], Romina di Giuseppe [1], Tuba Esatbeyoglu [2], Ilka Ratjen [1], Janna Enderle [1], Gunnar Jacobs [1,3], Ute Nöthlings [4], Manja Koch [1,5], Sabrina Schlesinger [6], Gerald Rimbach [2] and Wolfgang Lieb [1,3,*]

1 Institute of Epidemiology, University of Kiel, 24105 Kiel, Germany; sabina.waniek@epi.uni-kiel.de (S.W.); romina.digiuseppe@epi.uni-kiel.de (R.d.G.); ilka.ratjen@epi.uni-kiel.de (I.R.); janna.enderle@epi.uni-kiel.de (J.E.); jacobs@popgen.de (G.J.); mkoch@hsph.harvard.edu (M.K.)
2 Institute of Human Nutrition and Food Science, University of Kiel, 24118 Kiel, Germany; tuba.esatbeyoglu@mri.bund.de (T.E.); rimbach@foodsci.uni-kiel.de (G.R.)
3 Biobank PopGen, University Hospital Schleswig-Holstein, Campus Kiel, 24105 Kiel, Germany
4 Department of Nutrition and Food Science, University of Bonn, 53113 Bonn, Germany; noethlings@uni-bonn.de
5 Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA
6 Institute for Biometrics and Epidemiology, German Diabetes Center (DDZ) at Heinrich Heine University Duesseldorf, 40225 Duesseldorf, Germany; sabrina.schlesinger@DDZ.uni-duesseldorf.de
* Correspondence: wolfgang.lieb@epi.uni-kiel.de; Tel.: +49-500-30200

Received: 19 December 2017; Accepted: 23 January 2018; Published: 27 January 2018

Abstract: In addition to well-established risk factors like older age, female gender, and adiposity, oxidative stress may play a role in the pathophysiology of gallstone disease. Since vitamin E exerts important anti-oxidative functions, we hypothesized that circulating vitamin E levels might be inversely associated with prevalence of gallstone disease. In a cross-sectional study, we measured plasma levels of α- and γ-tocopherol using high performance liquid chromatography in a community-based sample (582 individuals; median age 62 years; 38.5% women). Gallstone disease status was assessed by ultrasound. Multivariable-adjusted logistic regression models were used to estimate the association of circulating α- and γ-tocopherol/cholesterol ratio levels with prevalent gallstone disease. Lower probabilities of having gallstone disease were observed in the top (compared to the bottom) tertile of the plasma α-tocopherol/cholesterol ratio in multivariable-adjusted models (OR (Odds Ratio): 0.31; 95% CI (Confidence Interval): 0.13–0.76). A lower probability of having gallstone disease was also observed for the γ-tocopherol/cholesterol ratio, though the association did not reach statistical significance (OR: 0.77; 95% CI: 0.35–1.69 for 3rd vs 1st tertile). In conclusion, our observations are consistent with the concept that higher vitamin E levels might protect from gallstone disease, a premise that needs to be further addressed in longitudinal studies.

Keywords: vitamin E; α- and γ-tocopherol; gallstone disease

1. Introduction

In addition to well-established risk factors such as age, female gender, obesity, and dyslipidemia [1–3], oxidative stress is thought to play a role in the pathophysiology of gallstone disease [4–6]. Vitamin E is a lipid-soluble vitamin encompassing different tocopherols (α-, β-, γ-, and δ-tocopherol) and tocotrienols (α-, β-, γ-, and δ-tocotrienol) with important anti-oxidative functions [7], and could, therefore, potentially affect the risk to develop gallstones. Indeed, in prior, rather small studies, α-tocopherol levels [8] and the α-tocopherol/cholesterol ratio [9,10] were found to be lower in individuals with gallstone disease (n = 16–30) compared to healthy controls (n = 20–47) [8–10]. While these results are

intriguing, population-based analyses regarding the association of vitamin E levels (including both α- and γ-tocopherol) with gallstone disease in larger samples are currently lacking.

Considering that α- and γ-tocopherol are the two major vitamers in the human body [11], and that more than 90% of the German population consume vitamin E in the form of α- and γ-tocopherol [12], we aimed to investigate cross-sectional associations of both α- and γ-tocopherol plasma levels with gallstone disease in a community-based sample from Northern Germany.

2. Subjects and Methods

2.1. Study Design and Sample

The study sample comprised 1316 individuals from Northern Germany, recruited by the PopGen biobank in Kiel, Germany. Of them, 747 participants were derived from a random sample of the general population of Kiel, and 569 individuals were blood donors recruited at the University Hospital Schleswig-Holstein in Kiel (Germany), between 2005 and 2007 [13]. A total of 952 individuals agreed to participate in the first follow-up examination (between 2010 and 2012) where blood sampling and a medical examination was performed. Furthermore, participants filled-out standardized questionnaires on demographics and various health-related characteristics, including smoking status, medical history, dietary intake, and physical activity (please see below for details) [13–15]. Ultrasound examinations of the upper abdomen were performed in a subsample of 846 participants (please see below for details). Participants with missing information on ultrasound examination (n = 4) or diagnosis of gallbladder sludge (n = 6) were excluded, leaving 836 participants with ultrasound information regarding gallstone disease. In 582 of these participants, circulating vitamin E (α- and γ-tocopherol) concentrations were available. The study was approved by the Ethics Committee of the Medical Faculty of the Christian-Albrechts University of Kiel (Project identification code A 156/03). All participants provided written informed consent.

2.2. Ultrasound Examination

Gallstone disease was defined as gallbladder stones visualized at the ultrasound examination. To this end, an ultrasound examination of the upper abdomen was conducted under standard conditions using a Logiq 400 cl real-time ultrasound system (General Electric Healthcare, Bedford, UK) with a convex array probe (3-Mhz). Ultrasound examinations were carried out in supine position, the right arm raised over the head, and was performed under deep inspiration. The gallbladder was examined in 3 planes (longitudinal, cross-sectional, and diagonal) to provide the examiner with a 3-dimensional impression of the organ. In cases in which gallstones were present, the mobility of the stone(s) was assessed to differentiate gallstones from gallbladder polyps. Biliary sludge was identified as low-amplitude echoes without acoustic shadowing.

2.3. Physical Examinations and Standardized Questionnaires

Weight and height were measured in light clothing without shoes. Two kilograms was subtracted from weight measurements to account for clothing. Body mass index (BMI) was calculated as body weight (kg) divided by height squared (m^2). Waist circumference was measured at the midpoint between the lower ribs and iliac crest. After the participants had rested at least 5 min in a sitting position, blood pressure was measured two times at the right arm using a sphygmomanometer [16], and the arithmetic mean of both measurements was used for our analyses. Information on dietary intake, including vitamin E supplementation, and alcohol consumption during the past 12 months, were assessed using a self-administered semi-quantitative 112-item food-frequency questionnaire (FFQ), designed and validated for the German population [14]. Total energy intakes were determined using the German Food Code and Nutrient Data Base (version II.3), and were provided by the Department of Epidemiology of the German Institute of Human Nutrition Potsdam Rehbrücke [17].

2.4. Definition of Covariates Assessment

Prevalent hypertension was defined as systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg, or use of antihypertensive medication, or self-reported hypertension history. Type 2 diabetes was defined as use of anti-diabetic medication or glycated hemoglobin (HbA1c) ≥6.5% (48 mmol/mol) or fasting serum glucose ≥126 mg/dL, or self-reported physician diagnosis. Liver disease was defined as self-reported hepatitis A, B, C, or D virus infection, hemochromatosis, autoimmune liver disease, or liver cirrhosis diagnosis.

In order to quantify the amount of physical activity, participants were asked to report their time spent walking, cycling, "do-it-yourself" activities, gardening, sports, and household chores during the past 12 months, and the average number of stairs climbed per day [18]. The duration of each physical activity was then multiplied by the corresponding metabolic equivalent task (MET)-values and summed over all activities [19,20]. To determine smoking status, participants were classified into three categories: no-smokers (never smoked), former smokers (smoked in the past and quit smoking more than 1 year ago), and current smokers (currently smoking 1 or more cigarettes per day). According to their level of education, participants were categorized into three categories: low (≤9 years), middle (10 years), or high (≥11 years).

2.5. Biochemical Measurements

Blood samples were obtained from participants in a sitting position after overnight fasting. HbA1c, glucose, triglycerides, HDL-cholesterol, and low density lipoprotein (LDL)-cholesterol and total cholesterol were determined in fresh blood samples under standard conditions on the same day in the Institute of Clinical Chemistry at the University Hospital Schleswig-Holstein, Campus Kiel. The Institute of Human Nutrition and Food Science from the University of Kiel (Germany) measured plasma vitamin E (α- and γ-tocopherol) levels using high performance liquid chromatography (HPLC) with fluorescence detection. The methods have been described in detail elsewhere [21], with the coefficients of variation for α- and γ-tocopherol being 1.09% and 1.29%, respectively.

2.6. Statistical Analyses

Missing covariate values (n = 13) were imputed as follows: missing categorical variables were imputed by the most commonly observed category of that respective variable (n = 9), and missing values of normally distributed continuous variables were replaced by the respective mean, whereas skewed variables were imputed by the sex-specific median (n = 4). When values of γ-tocopherol were below the detection limit (n = 14), they were imputed by the lowest γ-tocopherol concentration measured in our study sample. Because vitamin E circulates in the blood bound to lipoproteins [22,23], circulating vitamin E (α- and γ-tocopherol) levels (μmol/L) were divided by circulating total cholesterol levels (mmol/L), and used as α-tocopherol/total cholesterol ratio (μmol/mmol) and γ-tocopherol/total cholesterol ratio (μmol/mmol) as primary exposure variables in the statistical analyses.

The following analyses were performed: first, for descriptive purposes, differences in the characteristics of the participants with and without gallstone disease were tested for statistical significance using the chi-squared test or the Fisher's exact test, as appropriate, for categorical variables, and the Wilcoxon's rank-sum test for continuous variables. Second, logistic regression models were conducted to estimate the associations of the α- and γ-tocopherol/cholesterol ratio (each ratio considered separately and modeled in tertiles) with gallstone disease. Age- and sex-adjusted, as well as multivariable-adjusted logistic regression models, were performed. The multivariable-adjusted model controlled for age (continuous in years), sex (female, male), education (low, medium, high), physical activity (continuous in MET-hour/week), smoking status (never, current, former), vitamin E supplementation (yes, no), alcohol intake (continuous in g/day), total energy intake (continuous

in kJ/day), and BMI (continuous in kg/m^2). Categorical variables with more than 2 categories were included as indicator variables.

Third, we performed several sensitivity analyses: we repeated the above mentioned logistic regression models, after (a) excluding vitamin E supplement users (n = 44, prevalent gallstone cases = 3); (b) excluding individuals with self-reported liver disease (hepatitis A, B, C, or D virus infection, hemochromatosis, autoimmune liver disease, or liver cirrhosis) (n = 30, prevalent gallstone cases = 1) from our analyses; and (c) excluding individuals with type 2 diabetes (n = 61, prevalent gallstone cases = 7). In further sensitivity analyses, we used α- and γ-tocopherol (without adjustment for cholesterol) as the exposure variables, and assessed the associations of these biomarkers with gallstone disease. Finally, we excluded all individuals with any missing values, and performed a complete case analysis (n = 558, prevalent gallstone cases = 45).

Analyses were performed with SAS 9.4 (SAS Institute, Cary, NC, USA). Statistical tests were 2-sided, and p values <0.05 were considered statistically significant.

3. Results

3.1. General Characteristics

Overall, 46 individuals (7.9%) of the study sample had gallstones detected by ultrasound. Participants with gallstones were older and had lower circulating α-tocopherol and α-tocopherol/cholesterol ratio levels compared to participants without gallstones. A total of 44 participants (7.6%) of the study sample were taking vitamin E supplements (Table 1).

Table 1. General characteristics of the PopGen control study population by gallstone disease status (n = 582).

Clinical Features	All (n = 582)	Gallstones, Yes (n = 46)	Gallstones, No (n = 536)	p
Men, %	61.5	54.4	62.1	0.273
Age, years	62.0 (55.0, 70.0)	67.0 (60.0, 73.0)	62.0 (54.0, 70.0)	0.019
Body mass index, kg/m^2	26.6 (24.2, 29.3)	27.4 (25.2, 29.8)	26.5 (24.1, 29.3)	0.292
Waist circumference, cm	96.0 (87.9, 104.2)	96.9 (83.7, 105.8)	95.9 (88.3, 104.2)	0.659
Systolic blood pressure, mmHg	138.5 (125.0, 150.0)	135.0 (122.5, 145.0)	138.8 (125.0, 150.0)	0.277
Diastolic blood pressure, mmHg	85.0 (80.0, 90.0)	83.8 (80.0, 90.0)	85.0 (80.0, 90.0)	0.736
Prevalent hypertension, %	68.7	73.9	68.3	0.421
Current smokers, %	11.3	6.52	11.8	0.464
High education (≥11 years), %	37.3	39.1	37.1	0.874
Prevalent diabetes, %	10.5	15.2	10.1	0.285
Vitamin E supplementation, %	7.6	5.5	7.7	0.999
Physical Activity, MET-hour/week	89.8 (58.0, 130.5)	95.5 (64.8, 130.0)	88.3 (57.8, 131.1)	0.288
Alcohol consumption, g/day	9.91 (4.09, 19.2)	7.41 (3.14, 15.0)	10.2 (4.2, 19.8)	0.576
Biochemical features				
α-Tocopherol, μmol/L	31.5 (27.0, 37.1)	28.1 (24.0, 34.7)	31.8 (27.4, 37.4)	0.003
α-Tocopherol/cholesterol ratio, μmol/mmol	5.53 (4.88, 6.39)	5.24 (4.63, 5.73)	5.58 (4.91, 6.44)	0.015
γ-Tocopherol, μmol/L	1.34 (0.98, 1.79)	1.25 (0.88, 1.76)	1.35 (0.98, 1.79)	0.507
γ-Tocopherol/cholesterol ratio, μmol/mmol	0.24 (0.18, 0.31)	0.22 (0.19, 0.32)	0.24 (0.18, 0.31)	0.810
HbA1c, %	5.60 (5.40, 5.90)	5.80 (5.50, 5.90)	5.60 (5.40, 5.90)	0.088
HDL-cholesterol, mg/dL	62.5 (53.0, 77.0)	65.0 (54.0, 75.0)	62.0 (52.0, 78.0)	0.605
LDL-cholesterol, mg/dL	130.0 (108.0, 152.0)	125.0 (97.0, 152.0)	131.0 (108.0, 152.0)	0.237
Total cholesterol, mg/dL	220.0 (196.0, 249.0)	200.0 (185.0, 251.0)	221.0 (198.0, 248.0)	0.121
Triglycerides, mg/dL	105.0 (75.0, 138.0)	108.0 (72.0, 131.0)	104.5 (76.0, 138.5)	0.656

Values are presented as median and interquartile range or percentages (%). HDL: high density lipoproteins; LDL: low density lipoproteins; MET: metabolic equivalent. Differences in the characteristics of the participants with and without gallstone disease were tested for statistical significance using the chi-squared test or the Fisher's exact test (when one of the expected values in one of the cells is less than 5) for categorical variables and the Wilcoxon's rank-sum test for continuous variables.

3.2. Association of α- and γ-Tocopherol/Cholesterol Ratio with Gallstone Disease

Lower probabilities of having gallstone disease were observed in the top tertile compared to the bottom tertile of the plasma α-tocopherol/cholesterol ratio in both age- and sex-adjusted (OR: 0.32; 95% CI: 0.13–0.78), as well as in multivariable-adjusted models (OR: 0.31; 95% CI: 0.13–0.76). Likewise, a lower probability of having gallstones for the γ-tocopherol/cholesterol ratio was observed in the top as compared to the bottom tertile, but this association did not reach statistical significance (Table 2).

Table 2. Odds ratio (OR) and 95% confidence interval (CI) for the association of α- and γ-tocopherol/cholesterol ratio with gallstone disease.

Outcome	Tertiles of α-Tocopherol/Cholesterol Ratio		
	1	2	3
Median α-tocopherol/cholesterol ratio (IQR), μmol/mmol	4.61 (4.24, 4.87)	5.52 (5.36, 5.72)	6.79 (6.38, 7.70)
Gallstones (yes/no) (46/536)	(21/173)	(18/176)	(7/187)
Age- and sex-adjusted OR (95% CI)	1.00 (reference)	0.89 (0.45–1.73)	0.32 (0.13–0.78)
Multivariable-adjusted OR (95% CI) *	1.00 (reference)	0.82 (0.42–1.63)	0.31 (0.13–0.76)
	Tertiles of γ-Tocopherol/Cholesterol Ratio		
	1	2	3
Median γ-tocopherol/cholesterol ratio (IQR), μmol/mmol	0.16 (0.13–0.18)	0.24 (0.22–0.26)	0.34 (0.31–0.41)
Gallstones (yes/no) (46/536)	(16/178)	(17/177)	(13/181)
Age- and sex-adjusted OR (95% CI)	1.00 (reference)	1.12 (0.54–2.29)	0.81 (0.38–1.74)
Multivariable-adjusted OR (95% CI) *	1.00 (reference)	1.08 (0.51–2.29)	0.77 (0.35–1.69)

IQR: Interquartile range; * Adjusted for age, sex, education, physical activity, smoking status, vitamin E supplementation, BMI, alcohol intake, and total energy intake.

3.3. Sensitivity Analyses

The associations between α- and γ-tocopherol/cholesterol ratio and gallstone disease did not substantially change in the sensitivity analyses after (a) excluding vitamin E supplement users ($n = 44$); (b) excluding individuals with self-reported liver disease ($n = 30$); and (c) excluding individuals with type 2 diabetes ($n = 61$). Likewise, the results were similar when we modeled both α- and γ-tocopherol as exposure variables, without dividing them by cholesterol, and when we performed a complete case analysis ($n = 558$) (Supplementary Table S1).

4. Discussion

4.1. Principle Observations

We examined the cross-sectional association of circulating vitamin E (α- and γ-tocopherol) levels with ultrasound-detected gallstone disease in a population-based sample from Northern Germany. Our main observations were as follows: first, the prevalence of gallstone disease in our sample was 7.9%. Second, participants with gallstone disease had lower circulating α-tocopherol and α-tocopherol/cholesterol ratio levels compared to participants without gallstone disease, and this inverse association of the α-tocopherol/cholesterol ratio with gallstone disease remained statistically significant in multivariable-adjusted models.

4.2. In the Context of the Current Literature

4.2.1. Prevalence of Gallstone Disease

The prevalence of gallstone disease in our sample (7.9%) is slightly higher compared to other German studies, likewise detecting gallstones by ultrasound (3.9%, $n = 1116$ [24] and 4.1%, $n = 2147$ [25], respectively). However, the prevalence of gallstone disease in our study sample is lower than in the Study of Health in Pomerania (SHIP), a community-based sample from North-East Germany ($n = 4202$), where a slightly higher prevalence (10.1%) of gallstone disease was reported [26]. Yet, the region where

the SHIP study is conducted is known for its strong clustering of metabolic risk factors [27]. Similar to our observations, data from the National Health and Nutrition Examination Survey III (n = 14,238), collecting nationally representative data from the population of the United States, documented a prevalence of gallstone disease of about 8.1% [28].

4.2.2. Vitamin E (α- and γ-Tocopherol) Levels and Gallstone Disease

Our data suggest that higher circulating vitamin E levels are associated with a lower probability of having gallstone disease. This is in line with some prior observations, obtained in clinical settings on rather smaller samples [8–10].

Rocchi et al. [10] observed lower levels of the plasma α-tocopherol/cholesterol ratio in patients with gallstone disease (n = 16) compared to healthy controls (n = 20), and Shukla et al. [8] reported lower levels of serum α-tocopherol in 30 individuals with gallstone disease compared to 30 healthy controls. Consistently, Worthington et al. [9] reported lower levels of the serum α-tocopherol/cholesterol ratio in 18 patients with (ultrasound detected) gallstone disease, compared to 47 healthy controls. We extend these prior observations by demonstrating an inverse association of higher α-tocopherol/cholesterol ratio levels with gallstone disease in a larger, community-based sample, including 46 participants with ultrasound evidence of gallstone disease and 536 individuals where gallstones had been ruled out by ultrasound.

4.3. Potential Mechanisms for the Observed Association

Free radicals and oxidative stress may play a role in the pathophysiology of gallstone disease [6]. In agreement with this concept, higher levels of oxidative stress markers have been found locally in the gallbladder mucosa as well as in the circulation of patients with gallbladder disease as compared to individuals free of gallbladder disease [4,5].

Thus, one potential explanation for the observed inverse association between circulating plasma α-tocopherol levels and gallstone disease would be that individuals with higher vitamin E levels are better protected from oxidative stress and, therefore, have a lower probability to develop gallstones. However, due to the cross-sectional design of our analyses, we cannot rule out reverse causality, which would mean that the lower vitamin E levels observed in individuals with gallstone disease could be secondary to increased oxidative stress in those with gallstone disease.

Furthermore, bile acids are synthesized in the liver and secreted into the intestine, where they emulsify dietary lipids, including fat-soluble vitamins [29]. Some reports indicated impaired vitamin E absorption under cholestatic conditions [30,31]. It has been shown that disturbed bile secretion due to cholestatic diseases is associated with lipid-soluble vitamin deficiencies [32].

4.4. Strength and Limitations

Strengths of the present study include the moderate-sized population-based sample, the measurement of plasma vitamin E (α- and γ-tocopherol) levels, and the detailed and standardized assessment of covariates. The following limitations merit consideration: the cross-sectional design precludes causal inferences, because exposure and outcome were assessed at the same time point. Besides, the present cohort is not representative of the general population, due to the inclusion of a subgroup of blood donors. However, considering our research question, this limitation could play a limited role with respect to the overall research findings. Lastly, we cannot completely rule out model misspecification, though we identified important covariates affecting both exposures and outcome. Therefore, we built parsimonious models incrementally adjusted for covariates to provide the reader with a comprehensive picture.

5. Conclusions

In conclusion, we observed an inverse association between circulating vitamin E (α- and γ-tocopherol) levels and the prevalence of gallstone disease in a community-based sample. This observation supports the concept that higher levels of the antioxidant vitamer α-tocopherol may protect against gallstone disease, a premise that needs to be addressed in a prospective setting. Furthermore, it needs to be established if, and to what, extent dietary vitamin E supplementation may prevent gallstone formation.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Sensitivity analyses: multivariable-adjusted odds ratio (OR) and confidence interval (CI) for the association of α- and γ-tocopherol/cholesterol ratio with gallstone disease after (a) excluding vitamin E supplement users, (b) excluding individuals with self-reported disease, (c) excluding individuals with type 2 diabetes, (d) modeling α- and γ-tocopherol without dividing them by cholesterol, and (e) performing a complete case analysis.

Acknowledgments: Romina di Giuseppe is supported by the Deutsche Forschungsgemeinschaft Excellence Cluster "Inflammation at Interfaces" (grants EXC306 and EXC306/2). The PopGen 2.0 Network was supported by the German Federal Ministry of Education and Research (01EY1103). The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Author Contributions: Wolfgang Lieb, Gerald Rimbach and Romina di Giuseppe formulated the research question; Wolfgang Lieb, Manja Koch and Ute Nöthlings designed the study; Tuba Esatbeyoglu performed the vitamin E measurements, Sabina Waniek and Romina di Giuseppe performed the statistical analyses, Sabina Waniek, Wolfgang Lieb, and Romina di Giuseppe, contributed to the interpretation of the data; Sabina Waniek and Wolfgang Lieb drafted the manuscript. All authors (Sabina Waniek, Romina di Giuseppe, Tuba Esatbeyoglu, Manja Koch, Sabrina Schlesinger, Ilka Ratjen, Janna Enderle, Gunnar Jacobs, Ute Nöthlings, Gerald Rimbach and Wolfgang Lieb) critically reviewed and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Shaffer, E.A. Gallstone disease: Epidemiology of gallbladder stone disease. *Best Pract. Res. Clin. Gastroenterol.* **2006**, *20*, 981–996. [CrossRef] [PubMed]
2. Stinton, L.M.; Shaffer, E.A. Epidemiology of gallbladder disease: Cholelithiasis and cancer. *Gut Liver* **2012**, *6*, 172–187. [CrossRef] [PubMed]
3. Marschall, H.U.; Katsika, D.; Rudling, M.; Einarsson, C. The genetic background of gallstone formation: An update. *Biochem. Biophys. Res. Commun.* **2010**, *396*, 58–62. [CrossRef] [PubMed]
4. Zhou, J.F.; Chen, P.; Yang, J.L.; Zhu, Y.G.; Peng, C.H.; Wu, Y.L. Oxidative stress before and after operation in patients with chronic cholecystitis containing gallstone. *Biomed. Environ. Sci.* **2000**, *13*, 254–262. [PubMed]
5. Geetha, A. Evidence for oxidative stress in the gall bladder mucosa of gall stone patients. *J. Biochem. Mol. Biol. Biophys.* **2002**, *6*, 427–432. [CrossRef] [PubMed]
6. Sipos, P.; Gamal, E.M.; Blazovics, A.; Metzger, P.; Miko, I.; Furka, I. Free radical reactions in the gallbladder. *Acta Chir. Hung.* **1997**, *36*, 329–330. [PubMed]
7. Jiang, Q. Natural forms of vitamin E: Metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. *Free Radic. Biol. Med.* **2014**, *72*, 76–90. [CrossRef] [PubMed]
8. Shukla, V.K.; Adukia, T.K.; Singh, S.P.; Mishra, C.P.; Mishra, R.N. Micronutrients, antioxidants, and carcinoma of the gallbladder. *J. Surg. Oncol.* **2003**, *84*, 31–35. [CrossRef] [PubMed]
9. Worthington, H.V.; Hunt, L.P.; McCloy, R.F.; Ubbink, J.B.; Braganza, J.M. Dietary antioxidant lack, impaired hepatic glutathione reserve, and cholesterol gallstones. *Clin. Chim. Acta* **2004**, *349*, 157–165. [CrossRef] [PubMed]
10. Rocchi, E.; Casalgrandi, G.; Ronzoni, A.; Rosa, M.C.; Cioni, G.; Marazzi, A.; Manenti, A.; Marchini, S.; Ventura, E. Antioxidant liposoluble vitamins and carotenoids in chronic hepatitis. *Eur. J. Intern. Med.* **2001**, *12*, 116–121. [CrossRef]
11. Frank, J.; Chin, X.W.; Schrader, C.; Eckert, G.P.; Rimbach, G. Do tocotrienols have potential as neuroprotective dietary factors? *Ageing Res. Rev.* **2012**, *11*, 163–180. [CrossRef] [PubMed]

12. Preedy, V.R. *Handbook of Diet, Nutrition and the Skin*; Wageningen Academic Publishers: Wageningen, The Netherlands, 2012; Volume 12.
13. Nothlings, U.; Krawczak, M. PopGen. A population-based biobank with prospective follow-up of a control group. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* **2012**, *55*, 831–835. [PubMed]
14. Nothlings, U.; Hoffmann, K.; Bergmann, M.M.; Boeing, H. Fitting portion sizes in a self-administered food frequency questionnaire. *J. Nutr.* **2007**, *137*, 2781–2786. [CrossRef] [PubMed]
15. Koch, M.; Borggrefe, J.; Barbaresko, J.; Groth, G.; Jacobs, G.; Siegert, S.; Lieb, W.; Muller, M.J.; Bosy-Westphal, A.; Heller, M.; et al. Dietary patterns associated with magnetic resonance imaging-determined liver fat content in a general population study. *Am. J. Clin. Nutr.* **2014**, *99*, 369–377. [CrossRef] [PubMed]
16. Barbaresko, J.; Siegert, S.; Koch, M.; Aits, I.; Lieb, W.; Nikolaus, S.; Laudes, M.; Jacobs, G.; Nothlings, U. Comparison of two exploratory dietary patterns in association with the metabolic syndrome in a northern German population. *Br. J. Nutr.* **2014**, *112*, 1364–1372. [CrossRef] [PubMed]
17. Dehne, L.I.; Klemm, C.; Henseler, G.; Hermann-Kunz, E. The german food code and nutrient data base (bls ii.2). *Eur. J. Epidemiol.* **1999**, *15*, 355–359. [CrossRef] [PubMed]
18. Haftenberger, M.; Lahmann, P.H.; Panico, S.; Gonzalez, C.; Seidell, J.; Boeing, H. Prevalence of overweight, general and central obesity in 50- to 64-year-olds involved in the epic cohort. *IARC Sci. Publ.* **2002**, *156*, 249–252. [PubMed]
19. Friedenreich, C.; Norat, T.; Steindorf, K.; Boutron-Ruault, M.C.; Pischon, T.; Mazuir, M.; Clavel-Chapelon, F.; Linseisen, J.; Boeing, H.; Bergman, M.; et al. Physical activity and risk of colon and rectal cancers: The european prospective investigation into cancer and nutrition. *Cancer Epidemiol. Biomark. Prev.* **2006**, *15*, 2398–2407. [CrossRef] [PubMed]
20. Ainsworth, B.E.; Haskell, W.L.; Herrmann, S.D.; Meckes, N.; Bassett, D.R., Jr.; Tudor-Locke, C.; Greer, J.L.; Vezina, J.; Whitt-Glover, M.C.; Leon, A.S. 2011 compendium of physical activities: A second update of codes and met values. *Med. Sci. Sports Exerc.* **2011**, *43*, 1575–1581. [CrossRef] [PubMed]
21. Waniek, S.; di Giuseppe, R.; Plachta-Danielzik, S.; Ratjen, I.; Jacobs, G.; Koch, M.; Borggrefe, J.; Both, M.; Muller, H.P.; Kassubek, J.; et al. Association of vitamin e levels with metabolic syndrome, and mri-derived body fat volumes and liver fat content. *Nutrients* **2017**, *9*, 1143. [CrossRef] [PubMed]
22. Thurnham, D.I.; Davies, J.A.; Crump, B.J.; Situnayake, R.D.; Davis, M. The use of different lipids to express serum tocopherol: Lipid ratios for the measurement of vitamin E status. *Ann. Clin. Biochem.* **1986**, *23*, 514–520. [CrossRef] [PubMed]
23. Traber, M.G.; Jialal, I. Measurement of lipid-soluble vitamins—Further adjustment needed? *Lancet* **2000**, *355*, 2013–2014. [CrossRef]
24. Kratzer, W.; Kachele, V.; Mason, R.A.; Hill, V.; Hay, B.; Haug, C.; Adler, G.; Beckh, K.; Muche, R. Gallstone prevalence in Germany: The ulm gallbladder stone study. *Dig. Dis. Sci.* **1998**, *43*, 1285–1291. [CrossRef] [PubMed]
25. Walcher, T.; Haenle, M.M.; Kron, M.; Hay, B.; Mason, R.A.; von Schmiesing, A.F.; Imhof, A.; Koenig, W.; Kern, P.; Boehm, B.O.; et al. Pregnancy is not a risk factor for gallstone disease: Results of a randomly selected population sample. *World J. Gastroenterol.* **2005**, *11*, 6800–6806. [CrossRef] [PubMed]
26. Volzke, H.; Baumeister, S.E.; Alte, D.; Hoffmann, W.; Schwahn, C.; Simon, P.; John, U.; Lerch, M.M. Independent risk factors for gallstone formation in a region with high cholelithiasis prevalence. *Digestion* **2005**, *71*, 97–105. [CrossRef] [PubMed]
27. Volzke, H.; Ittermann, T.; Schmidt, C.O.; Baumeister, S.E.; Schipf, S.; Alte, D.; Biffar, R.; John, U.; Hoffmann, W. Prevalence trends in lifestyle-related risk factors. *Dtsch. Arztebl. Int.* **2015**, *112*, 185–192. [PubMed]
28. Everhart, J.E.; Khare, M.; Hill, M.; Maurer, K.R. Prevalence and ethnic differences in gallbladder disease in the United States. *Gastroenterology* **1999**, *117*, 632–639. [CrossRef]
29. Hofmann, A.F. Bile acids: The good, the bad, and the ugly. *News Physiol. Sci.* **1999**, *14*, 24–29. [CrossRef] [PubMed]
30. Phillips, J.R.; Angulo, P.; Petterson, T.; Lindor, K.D. Fat-soluble vitamin levels in patients with primary biliary cirrhosis. *Am. J. Gastroenterol.* **2001**, *96*, 2745–2750. [CrossRef] [PubMed]

31. Kowdley, K.V.; Emond, M.J.; Sadowski, J.A.; Kaplan, M.M. Plasma vitamin k1 level is decreased in primary biliary cirrhosis. *Am. J. Gastroenterol.* **1997**, *92*, 2059–2061. [PubMed]
32. Werner, A.; Kuipers, F.; Verkade, H.J. Fat absorption and lipid metabolism in cholestasis. In *Molecular Pathogenesis of Cholestasis*; Springer: New York, NY, USA, 2000.

Table S1: Sensitivity analyses: Multivariable-adjusted Odds Ratio (OR) and Confidence Interval (CI) for the association of α- and γ-tocopherol/cholesterol ratio with gallstone disease after a) excluding vitamin E supplement users, b) excluding individuals with self-reported disease, c) excluding individuals with type 2 diabetes, d) modeling α- and γ-tocopherol without dividing them by cholesterol, and e) performing a complete-case analysis.

	Tertiles of α-Tocopherol/Cholesterol Ratio		
	1	**2**	**3**
a) excluding vitamin E supplement users ($n = 44$) *	1.00 (Reference)	0.71 (0.35–1.46)	0.27 (0.10–0.69)
b) excluding individuals with self-reported disease ($n = 30$) †	1.00 (Reference)	0.86 (0.43–1.71)	0.32 (0.13–0.80)
c) excluding individuals with type 2 diabetes ($n = 61$) †	1.00 (Reference)	0.81 (0.38–1.71)	0.33 (0.13–0.87)
d) modeling α-tocopherol ††	1.00 (Reference)	0.43 (0.12–0.89)	0.43 (0.18–1.04)
e) performing a complete-case analysis ($n = 558$) †	1.00 (Reference)	0.69 (0.34–1.38)	0.29 (0.12–0.72)
	Tertiles of γ-Tocopherol/Cholesterol Ratio		
	1	**2**	**3**
a) excluding vitamin E supplement users ($n = 44$) *	1.00 (Reference)	1.14 (0.53–2.47)	0.73 (0.32–1.67)
b) excluding individuals with self-reported disease ($n = 30$) †	1.00 (Reference)	1.04 (0.49–2.20)	0.71 (0.31–1.60)
c) excluding individuals with type 2 diabetes ($n = 61$) †	1.00 (Reference)	1.13 (0.50–2.58)	0.87 (0.37–2.05)
d) modeling γ-tocopherol ††	1.00 (Reference)	1.06 (0.49–2.38)	0.90 (0.40–2.02)
e) performing a complete-case analysis ($n = 558$) †	1.00 (Reference)	1.14 (0.54–2.41)	0.64 (0.28–1.46)

* Adjusted for age, sex, education, physical activity, smoking status, BMI, alcohol intake, and total energy intake; † Adjusted for age, sex, education, physical activity, smoking status, BMI, alcohol intake, total energy intake, and vitamin E supplementation; †† Adjusted for age, sex, education, physical activity, smoking status, BMI, alcohol intake, total energy intake, vitamin E supplementation, and cholesterol.

5 General discussion

The main results of the presented papers were as follows:

1. Almost 40% of the participants of our sample met the national recommendations for the dietary vitamin E intake. Close to 60% of our participants had adequate circulating α-tocopherol levels above 30 µmol/L (Chapter 2).
2. Triglycerides, HDL- and LDL-cholesterol, and vitamin E supplementation were statistically significant correlates of vitamin E levels. Both, a priori and a posteriori derived dietary patterns were not associated with circulating vitamin E levels (Chapter 2).
3. The α- and γ-tocopherol/cholesterol ratios were positively associated with different metabolic traits, including VAT, SAT, the MetS, and two of its components, high triglyceride levels and low HDL-cholesterol levels. No significant associations were observed when α- and γ-tocopherol/cholesterol ratios were studied in relation to LSI or FLD (Chapter 3).
4. A lower probability of having gallstone disease was observed with increasing α-tocopherol/cholesterol ratio. An inverse association of the γ-tocopherol/cholesterol with gallstone disease was observed, even though this association did not reach statistical significance (Chapter 4).

5.1 Dietary vitamin E intake and distribution of vitamin E levels

In our sample from Northern Germany, 38.8% of the participants met the national guidelines for dietary vitamin E intake [1]. This proportion was slightly lower than in a report from Germany (n=15.371), where 52% of men and 51% of women met the respective guidelines [2]. In a study from the US (n=4351), only 4.9% of men and 4.5% of women met the current recommendation (15 mg/day) for dietary vitamin E [3]. It has to be kept in mind that the current dietary intake recommendations of the DGE are based on α-tocopherol and do not consider other vitamin E forms [1]. Thus, the calculated vitamin E intakes are lower than the actual intakes of dietary vitamin E [4].

With respect to circulating vitamin E levels, the observed median of plasma α- (31.54 µmol/L) and γ-tocopherol (1.35 µmol/L) concentrations in our sample were in agreement with prior studies conducted in Germany, reporting, e.g., mean serum concentrations of α-tocopherol of 34.4 µmol/L [5] and 30.4 µmol/L [6]. In the present sample, circulating levels of α-tocopherol and the α-tocopherol/cholesterol ratio (median: 5.53 µmol/mmol) were slightly higher than in the NHANES sample (median of α-tocopherol: 26.9 µmol/L, median of α-tocopherol/cholesterol ratio: 4.93 µmol/mmol) [7].

About 60% of individuals in our sample had circulating α-tocopherol levels above 30 µmol/L. This proportion is slightly higher than in other studies on Europeans (39%) and higher than in samples from the US (13%) [8]. There are only few studies on the distribution of γ-tocopherol in Europe, but most of them reported similar levels as observed in our sample [9, 10]. However, most studies conducted in the US reported higher average plasma γ-tocopherol levels as compared to Europeans [10, 11], because γ-tocopherol is the major form (≈70%) of vitamin E consumed in the US [12].

Regarding vitamin E supplementation, the prevalence of vitamin E supplement users in our sample was relatively low (7.5%). The proportion of vitamin E supplement users in the German National Nutrition Survey (NVS) II (11.4%) [2] and in the National Health Interview Survey from the US is slightly higher (11.4%) [13] than in our sample. Data from the NVS II reported a median intake of supplemented vitamin E of about 10 mg/day, assessed by diet history interviews [2].

In our sample, participants provided information about their use of vitamin E supplements within the FFQ, which only included a single question related to vitamin E supplementation (intake of vitamin E supplements, ‘yes’ or ‘no’). More information about vitamin E supplementation, e.g., about the frequency, the amount, and the concentration of vitamin E supplements were unfortunately not part of the FFQ and, therefore, not available for our analyses.

5.2 Lack of association between estimated dietary α-tocopherol intake and circulating vitamin E levels

Of note, we observed no evidence for a correlation between dietary α-tocopherol intake and circulating α-tocopherol levels in our sample (Chapter 2). Even though this observation is surprising, a review of the literature revealed that several prior studies also reported poor or no correlations of dietary vitamin E intake and circulating α-tocopherol concentrations [14-16]. Other studies, however, reported positive correlations, which were likely due to supplemental intake [17-19]. For example, in a study from Stryker et al. [18] (n=330), a poor correlation of plasma vitamin E with dietary vitamin E intake (r=0.12) was observed, while a strong correlation with dietary vitamin E intake including the use of supplements was reported (r=0.51). Likewise, Ascherio et al. [19] observed in a sample of 307 men and women a strong positive association between plasma α-tocopherol concentrations (r=0.51 in men, r=0.41 in women) and dietary vitamin E intake due to supplemental intake.

Several factors could contribute to the observed lack of correlation between dietary vitamin E intake as assessed by FFQ and circulating vitamin E levels.

First, the FFQ does not capture all foods rich in vitamin E (e.g., olives) and while the vitamin E content of oils differs by type (e.g., refined oils have lower vitamin E content as compared to unrefined oils) and brand [20, 21], the FFQ is not able to differentiate between different oils. Furthermore, vitamin E content in foods is influenced by light, temperature, and oxygen availability promoting rancidity in vegetable oils. Therefore, the oxidative stability of vitamin E in edible oils is limited and vegetable oils might contribute less to vitamin E intake [22].

Second, individuals might underreport the intake of vitamin E, because it might be difficult to assess the amount of fats and oils added during food preparations [22, 23].

Third, circulating vitamin E levels and the FFQ capture very different time intervals. The FFQ assesses a more long-term intake (the past 12 months), while α-tocopherol appears in plasma within 2-4 hours, peaks in 5-14 hours and disappears from plasma with a half-life of 48 hours [24, 25].

Fourth, substantial inter-individual differences with respect to the efficiency of vitamin E absorption (between 20% and 80%) have been reported [26, 27]. The parallel intake of additional food ingredients (e.g., retinoic acid, plant sterols, and dietary fiber) can decrease the absorption of vitamin E [27, 28].

Fifth, in addition to dietary intake, circulating vitamin E concentrations are also determined by other factors such as genetic variability, and physiological (gender, age) and lifestyle (e.g., smoking, obesity, and alcohol consumption) factors [27-29].

All these factors might have contributed to the poor correlation between dietary α-tocopherol intake and plasma α-tocopherol levels observed in this thesis.

5.3 Association of circulating vitamin E levels with metabolic traits

Vitamin E has been found to reduce the risk for several chronic disease conditions due to its anti-oxidant and its anti-inflammatory properties [30].

5.3.1 Metabolic syndrome, body fat volumes, and liver fat content

We observed in our sample from the general population that circulating vitamin E levels were positively associated with the prevalence of the MetS and with MRI-determined body fat volumes (particularly VAT). By contrast, we did not find evidence for a statistically significant association of vitamin E levels with MRI-measures of liver fat content and FLD.

Previous studies that have examined the association of circulating vitamin E concentrations with MetS revealed inconsistent results [31-34], as described in detail in the introduction. With respect to adiposity-related traits, our observations are in line with several prior studies that displayed a positive association of circulating vitamin E levels with adiposity measures (e.g., BMI, waist circumference) [35-37]. However, no association of circulating vitamin E levels with body fat determined by DXA and BIA was reported [35, 38].

Potential mechanisms for the positive associations of vitamin E levels with MetS and VAT include the following:

First, decreased catabolism of α-tocopherol from plasma has been reported in individuals with the MetS as compared to healthy adults [34], possibly leading to higher plasma α-tocopherol levels in individuals with the MetS as compared to individuals without the MetS.

Second, with respect to α-TTP, several experimental studies suggest that oxidative stress could modulate the activity of α-TTP and, thus, potentially influences the vitamin E status and leading to higher circulating α-tocopherol levels [39-42].

Third, regarding the excretion of vitamin E, α-tocopherol can be secreted in bile, but it is still unknown if excretion via bile is altered in individuals with the MetS [34]. Traber et al. [34] suggest that the hepatic metabolism of α-tocopherol to the α-tocopherol metabolite α-CEHC may be inhibited in individuals with the MetS. They observed that individuals with the MetS excrete lower amounts of urinary α- and γ-CEHC as compared to individuals without the MetS [34], suggesting that individuals with the MetS need more vitamin E, because of increased oxidative stress. Thus, individuals with the MetS retain more vitamin E than individuals without the MetS [34], possibly leading to higher plasma α-tocopherol levels.

Fourth, the adipose tissue produces several pro-inflammatory cytokines such as tumor necrosis factor-α and IL (interleukin)-6, which promote inflammation and oxidative stress [43, 44]. Of note, VAT releases 2 to 3 times more IL-6 than SAT [44]. High concentrations of these inflammatory biomarkers could be responsible for increased oxidative stress and, thus, leading to higher circulating vitamin E levels. In this thesis, stronger associations of γ-tocopherol/cholesterol ratio as compared to α-tocopherol/cholesterol ratio with VAT and SAT were observed, which might be explained by the unique anti-inflammatory properties of γ-tocopherol [45].

The lack of association of circulating vitamin E levels with parameters of liver fat in our sample is in contrast with some clinical studies, which have reported lower vitamin E levels in patients with NASH than in healthy controls [46, 47]. It is conceivable that circulating vitamin E levels are primarily altered in individuals with an advanced liver disease [48], but not in relatively healthy individuals from the general population with modest alterations in liver fat content, as in our sample. With

respect to vitamin E as an anti-oxidant, in clinical studies, elevated systemic markers of oxidative stress and lipid peroxidation have been found in patients with non-alcoholic fatty liver disease (NAFLD) and NASH [49-52]. For example, Yesilova et al. [49] observed higher levels of oxidative stress markers (malondialdehyde) and lower levels of anti-oxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT) in patients with NAFLD (n=30) compared to healthy adults (n=30). Furthermore, higher levels of oxidized LDLs and thiobarbituric acid reactive substances (TBARS) were reported in 21 NASH patients compared to 19 healthy controls [50].

5.3.2 Gallstone disease

In our sample, we found an inverse association of α-tocopherol with gallstone disease. Thus, higher levels of α-tocopherol were associated with a lower probability of having gallstone disease. As detailed in the introduction, this is in line with prior studies [53-55].

Some studies reported higher levels of oxidative stress markers in patients with gallbladder disease as compared to healthy controls [56, 57]. Specifically, higher levels of conjugated dienes, lipid hydroperoxides, and TBARS and lower levels of anti-oxidative enzymes including CAT and SOD as well as glutathione related enzymes have been found in the gallbladder mucosa of 30 patients with cholecystectomy due to gallstone disease compared to 15 individuals without gallstones [56]. Furthermore, higher levels of lipid peroxides (LPO) and nitric oxides (NO) were reported in 107 patients with chronic cholecystitis containing gallstones as compared to 100 healthy controls [57]. Moreover, it has been shown that levels of oxidative stress markers (LPO and NO) decrease after gallbladder surgery compared to the preoperative state [57]. Thus, individuals with higher circulating vitamin E levels could be better protected from oxidative stress and, therefore, develop less gallstones.

Further, previous studies observed impaired vitamin E absorption in patients with cholestatic diseases [58, 59]. Due to cholestatic diseases inadequate amounts of bile salts, essential for absorption of lipid-soluble vitamins [60], are delivered to the

intestinal lumen and, consequently, result in a lipid-soluble vitamin malabsorption [61].

5.4 Future research

Based on the results from the present thesis, there are many research questions related to vitamin E that could be addressed in the future. The role of vitamin E as an anti-oxidant and its relation to the MetS, body fat volumes, liver fat content, and gallstone disease is not fully understood and has been addressed in cross-sectional analyses in the present thesis. Further investigations in a prospective setting within the general population, relating vitamin E to the incidence of the above mentioned metabolic traits are warranted.

As indicated above, the FFQ does not capture all vitamin E rich foods and is not able to differentiate between different oil types and brands [20, 21]. Therefore, an update of the vitamin E content in foods, food groups, or meals is of major interest. A representative data basis for all substances (e.g, nutrients, environmental contaminants, food additives) in foods will be established in the first total diet study, called the Federal Institute for risk assessement (BfR, Bundesinstitut für Risikobewertung) Meal study. In the BfR Meal Study, foods are being analyzed in ready-to-eat condition (foods are prepared as consumed). The BfR Meal Study provides a newly generated FFQ database reflecting typically eating habits in Germany. In this way, the BfR Meal Study makes it possible to get new detailed data about the content of vitamin E in foods, food groups, and meals [62]. Thus, it would be very interesting to re-evaluate the findings in this thesis.

Another important area of research in the context of vitamin E would be genetic analyses related to different vitamin E biomarkers. Two prior genome-wide association studies (GWAS) for vitamin E levels have been reported so far [63, 64]. The first GWAS, conducted in a moderately-sized Italian sample (n=1190), reported a single nucleotide polymorphism (SNP) near the apolipoprotein A5 gene to be associated with α-tocopherol levels [63]. While a p-value of 7.8×10^{-10} was reached in a meta-analysis including the discovery and the replication samples, this association was substantially attenuated upon adjustment for triglyceride levels

(p=0.002). US-based researchers conducted a GWAS for α- and γ-tocopherol [64] in a much larger sample (n=5006) and reported 3 new SNPs that reached genome-wide significance in their association with α-tocopherol, explaining together 1.7% of the inter-individual variation in α-tocopherol. Furthermore, a previously reported SNP, associated with α-tocopherol, could be replicated. However, no genetic variant convincingly associated with circulating γ-tocopherol levels could be identified. Further genetic analyses, also including rare and low-frequency variants, could provide a better understanding of the genetic underpinning of inter-individual variation in vitamin E levels.

5.5 Conclusion

The present thesis explored in detail the distribution of circulating plasma vitamin E levels (α- and γ-tocopherol) in a general population sample of moderate size, assessed clinical and nutritional correlates of vitamin E; and related circulating vitamin E biomarkers (α- and γ-tocopherol) to cardiometabolic traits, including the MetS, body fat volumes, liver fat content, and gallstone disease.

We observed that nearly 40% of the participants in our sample met the recommendations for dietary α-tocopherol intake from foods (issued by the DGE). An even higher proportion (57.6%) had adequate α-tocopherol levels in plasma above 30 µmol/L.

Because of the increasing interest in possible health effects of vitamin E and the anti-oxidative functions that vitamin E participates in, prospective studies between α- and γ-tocopherol levels and metabolic conditions, liver fat content, and gallstone disease in general populations studies are warranted.

5.6 References

1. Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, and Schweizerische Gesellschaft für Ernährung, *Vitamin E (Tocopherole)*, in *Referenzwerte für die Nährstoffzufuhr*. 2015, DGE: Bonn. p. 1-5.
2. Max Rubner-Institut, *Vitamin E*, in *Nationale Verzehrsstudie II. Ergebnisbericht Teil 2*. 2008, MRI: Karlsruhe. p. 111-113.
3. Gao, X., et al., *The maximal amount of dietary alpha-tocopherol intake in U.S. adults (NHANES 2001-2002).* J Nutr, 2006. **136**(4): p. 1021-6.
4. Max Rubner-Institut, *Nationale Verzehrsstudie II. Lebensmittelverzehr und Nährstoffzufuhr auf Basis von 24h-Recalls*. 2013, MRI: Karlsruhe. p. 1-12.
5. Wolters, M., et al., *Selenium and antioxidant vitamin status of elderly German women.* Eur J Clin Nutr, 2006. **60**(1): p. 85-91.
6. Kompauer, I., et al., *Association of carotenoids, tocopherols and vitamin C in plasma with allergic rhinitis and allergic sensitisation in adults.* Public Health Nutr, 2006. **9**(4): p. 472-9.
7. Ford, E.S., et al., *Distribution of serum concentrations of alpha-tocopherol and gamma-tocopherol in the US population.* Am J Clin Nutr, 2006. **84**(2): p. 375-83.
8. Szabolcs, P., et al., *A Systematic Review of Global Alpha-Tocopherol Status as Assessed by Nutritional Intake Levels and Blood Serum Concentrations.* Int J Vitam Nutr Res, 2016: p. 1-21.
9. Stuetz, W., et al., *Plasma Carotenoids, Tocopherols, and Retinol in the Age-Stratified (35-74 Years) General Population: A Cross-Sectional Study in Six European Countries.* Nutrients, 2016. **8**(10).
10. Midttun, O., et al., *Circulating concentrations of biomarkers and metabolites related to vitamin status, one-carbon and the kynurenine pathways in US, Nordic, Asian, and Australian populations.* Am J Clin Nutr, 2017.
11. Wagner, K.H., A. Kamal-Eldin, and I. Elmadfa, *Gamma-tocopherol--an underestimated vitamin?* Ann Nutr Metab, 2004. **48**(3): p. 169-88.
12. Jiang, Q., et al., *gamma-tocopherol, the major form of vitamin E in the US diet, deserves more attention.* Am J Clin Nutr, 2001. **74**(6): p. 714-22.
13. Millen, A.E., K.W. Dodd, and A.F. Subar, *Use of vitamin, mineral, nonvitamin, and nonmineral supplements in the United States: The 1987, 1992, and 2000 National Health Interview Survey results.* J Am Diet Assoc, 2004. **104**(6): p. 942-50.
14. Boeing, H., et al., *The relative validity of vitamin intakes derived from a food frequency questionnaire compared to 24-hour recalls and biological*

measurements: results from the EPIC pilot study in Germany. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol, 1997. **26 Suppl 1**: p. S82-90.

15. Gao, X., et al., *alpha-Tocopherol intake and plasma concentration of Hispanic and non-Hispanic white elders is associated with dietary intake pattern.* J Nutr, 2006. **136**(10): p. 2574-9.
16. Kardinaal, A.F., et al., *Relations between antioxidant vitamins in adipose tissue, plasma, and diet.* Am J Epidemiol, 1995. **141**(5): p. 440-50.
17. McNaughton, S.A., et al., *Validation of a food-frequency questionnaire assessment of carotenoid and vitamin E intake using weighed food records and plasma biomarkers: the method of triads model.* Eur J Clin Nutr, 2005. **59**(2): p. 211-8.
18. Stryker, W.S., et al., *The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels.* Am J Epidemiol, 1988. **127**(2): p. 283-96.
19. Ascherio, A., et al., *Correlations of vitamin A and E intakes with the plasma concentrations of carotenoids and tocopherols among American men and women.* J Nutr, 1992. **122**(9): p. 1792-801.
20. Grilo, E.C., et al., *Alpha-tocopherol and gamma-tocopherol concentration in vegetable oils.* Food Science and Technology (Campinas), 2014. **34**: p. 379-385.
21. El-Sohemy, A., et al., *Population-based study of alpha- and gamma-tocopherol in plasma and adipose tissue as biomarkers of intake in Costa Rican adults.* Am J Clin Nutr, 2001. **74**(3): p. 356-63.
22. Péter, S., et al., *A Systematic Review of Global Alpha-Tocopherol Status as Assessed by Nutritional Intake Levels and Blood Serum Concentrations.* Int J Vitam Nutr Res, 2016: p. 1-21.
23. Institute of Medicine (US) Panel on Dietary Antioxidants and Related Compounds, *Vitamin E*, in *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. 2000: Washington (DC). p. 186-283.
24. Chuang, J.C., et al., *Quantitation of [5-14CH3]-(2R, 4'R, 8'R)-alpha-tocopherol in humans.* J Nutr, 2011. **141**(8): p. 1482-8.
25. Shim, J.S., K. Oh, and H.C. Kim, *Dietary assessment methods in epidemiologic studies.* Epidemiol Health, 2014. **36**: p. e2014009.
26. Traber, M.G., *Mechanisms for the prevention of vitamin E excess.* J Lipid Res, 2013. **54**(9): p. 2295-306.
27. Schmolz, L., et al., *Complexity of vitamin E metabolism.* World J Biol Chem, 2016. **7**(1): p. 14-43.

28. Borel, P., D. Preveraud, and C. Desmarchelier, *Bioavailability of vitamin E in humans: an update.* Nutr Rev, 2013. **71**(6): p. 319-31.

29. Jenab, M., et al., *Biomarkers in nutritional epidemiology: applications, needs and new horizons.* Hum Genet, 2009. **125**(5-6): p. 507-25.

30. Brigelius-Flohe, R., et al., *The European perspective on vitamin E: current knowledge and future research.* Am J Clin Nutr, 2002. **76**(4): p. 703-16.

31. Beydoun, M.A., et al., *Serum antioxidant status is associated with metabolic syndrome among U.S. adults in recent national surveys.* J Nutr, 2011. **141**(5): p. 903-13.

32. Ford, E.S., et al., *The metabolic syndrome and antioxidant concentrations: findings from the Third National Health and Nutrition Examination Survey.* Diabetes, 2003. **52**(9): p. 2346-52.

33. Godala, M.M., et al., *Lower Plasma Levels of Antioxidant Vitamins in Patients with Metabolic Syndrome: A Case Control Study.* Adv Clin Exp Med, 2016. **25**(4): p. 689-700.

34. Traber, M.G., et al., *Metabolic syndrome increases dietary alpha-tocopherol requirements as assessed using urinary and plasma vitamin E catabolites: a double-blind, crossover clinical trial.* Am J Clin Nutr, 2017. **105**(3): p. 571-579.

35. Wallstrom, P., et al., *Serum concentrations of beta-carotene and alpha-tocopherol are associated with diet, smoking, and general and central adiposity.* Am J Clin Nutr, 2001. **73**(4): p. 777-85.

36. Kabat, G.C., et al., *Longitudinal association of measures of adiposity with serum antioxidant concentrations in postmenopausal women.* Eur J Clin Nutr, 2016. **70**(1): p. 47-53.

37. Chai, W., et al., *Associations between obesity and serum lipid-soluble micronutrients among premenopausal women.* Nutr Res, 2010. **30**(4): p. 227-32.

38. Garcia, O.P., et al., *Zinc, vitamin A, and vitamin C status are associated with leptin concentrations and obesity in Mexican women: results from a cross-sectional study.* Nutr Metab (Lond), 2012. **9**(1): p. 59.

39. Etzl, R.P., et al., *Oxidative stress stimulates alpha-tocopherol transfer protein in human trophoblast tumor cells BeWo.* J Perinat Med, 2012. **40**(4): p. 373-8.

40. Ulatowski, L., et al., *Expression of the alpha-tocopherol transfer protein gene is regulated by oxidative stress and common single-nucleotide polymorphisms.* Free Radic Biol Med, 2012. **53**(12): p. 2318-26.

41. Usenko, C.Y., S.L. Harper, and R.L. Tanguay, *Fullerene C60 exposure elicits an oxidative stress response in embryonic zebrafish.* Toxicol Appl Pharmacol, 2008. **229**(1): p. 44-55.

42. Otulakowski, G., et al., *alpha-Tocopherol transfer protein mediates protective hypercapnia in murine ventilator-induced lung injury.* Thorax, 2017. **72**(6): p. 538-549.

43. Marseglia, L., et al., *Oxidative stress in obesity: a critical component in human diseases.* Int J Mol Sci, 2014. **16**(1): p. 378-400.

44. Fonseca-Alaniz, M.H., et al., *Adipose tissue as an endocrine organ: from theory to practice.* J Pediatr (Rio J), 2007. **83**(5 Suppl): p. S192-203.

45. Reiter, E., Q. Jiang, and S. Christen, *Anti-inflammatory properties of alpha- and gamma-tocopherol.* Mol Aspects Med, 2007. **28**(5-6): p. 668-91.

46. Erhardt, A., et al., *Plasma levels of vitamin E and carotenoids are decreased in patients with Nonalcoholic Steatohepatitis (NASH).* Eur J Med Res, 2011. **16**(2): p. 76-8.

47. Bahcecioglu, I.H., et al., *Levels of serum vitamin A, alpha-tocopherol and malondialdehyde in patients with non-alcoholic steatohepatitis: relationship with histopathologic severity.* Int J Clin Pract, 2005. **59**(3): p. 318-23.

48. Pacana, T. and A.J. Sanyal, *Vitamin E and nonalcoholic fatty liver disease.* Curr Opin Clin Nutr Metab Care, 2012. **15**(6): p. 641-8.

49. Yesilova, Z., et al., *Systemic markers of lipid peroxidation and antioxidants in patients with nonalcoholic Fatty liver disease.* Am J Gastroenterol, 2005. **100**(4): p. 850-5.

50. Chalasani, N., M.A. Deeg, and D.W. Crabb, *Systemic levels of lipid peroxidation and its metabolic and dietary correlates in patients with nonalcoholic steatohepatitis.* Am J Gastroenterol, 2004. **99**(8): p. 1497-502.

51. Narasimhan, S., et al., *Oxidative stress is independently associated with non-alcoholic fatty liver disease (NAFLD) in subjects with and without type 2 diabetes.* Clin Biochem, 2010. **43**(10-11): p. 815-21.

52. Irie, M., et al., *Levels of the oxidative stress marker gamma-glutamyltranspeptidase at different stages of nonalcoholic fatty liver disease.* J Int Med Res, 2012. **40**(3): p. 924-33.

53. Worthington, H.V., et al., *Dietary antioxidant lack, impaired hepatic glutathione reserve, and cholesterol gallstones.* Clin Chim Acta, 2004. **349**(1-2): p. 157-65.

54. Rocchi, E., et al., *Antioxidant liposoluble vitamins and carotenoids in chronic hepatitis.* Eur J Intern Med, 2001. **12**(2): p. 116-121.

55. Shukla, V.K., et al., *Micronutrients, antioxidants, and carcinoma of the gallbladder.* J Surg Oncol, 2003. **84**(1): p. 31-5.

56. Geetha, A., *Evidence for oxidative stress in the gall bladder mucosa of gall stone patients.* J Biochem Mol Biol Biophys, 2002. **6**(6): p. 427-32.

57. Zhou, J.F., et al., *Oxidative stress before and after operation in patients with chronic cholecystitis containing gallstone.* Biomed Environ Sci, 2000. **13**(4): p. 254-62.

58. Phillips, J.R., et al., *Fat-soluble vitamin levels in patients with primary biliary cirrhosis.* Am J Gastroenterol, 2001. **96**(9): p. 2745-50.

59. Kowdley, K.V., et al., *Plasma vitamin K1 level is decreased in primary biliary cirrhosis.* Am J Gastroenterol, 1997. **92**(11): p. 2059-61.

60. Hofmann, A.F., *Bile Acids: The Good, the Bad, and the Ugly.* News Physiol Sci, 1999. **14**: p. 24-29.

61. Werner, A., F. Kuipers, and H.J. Verkade, *Fat absorption and lipid metabolism in cholestasis*. 2000.

62. Sarvan, I., et al., *[Dietary exposure assessment of substances in foods: The BfR MEAL study - the first German total diet study].* Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz, 2017. **60**(7): p. 689-696.

63. Ferrucci, L., et al., *Common variation in the beta-carotene 15,15'-monooxygenase 1 gene affects circulating levels of carotenoids: a genome-wide association study.* Am J Hum Genet, 2009. **84**(2): p. 123-33.

64. Major, J.M., et al., *Genome-wide association study identifies common variants associated with circulating vitamin E levels.* Hum Mol Genet, 2011. **20**(19): p. 3876-83.

6 Summary

Vitamin E is a lipid-soluble vitamin encompassing different vitamin E forms (α-, β-, γ-, δ-tocopherols, and tocotrienols) with important anti-oxidative functions. There is limited knowledge regarding the distribution of plasma vitamin E concentrations in the general Northern German population, the relation of vitamin E to dietary patterns, and its association with cardiometabolic traits. Therefore, in this thesis, the distribution of both α- and γ-tocopherol levels was assessed in a sample from the general population; and biochemical and dietary correlates of vitamin E were explored. We observed, that nearly 60% of the Northern German population had adequate plasma α-tocopherol levels (>30 µmol/L), and that lipid traits and the intake of vitamin E supplementation were statistically significantly associated with circulating vitamin E levels. However, neither a priori nor a posteriori derived dietary patterns displayed an association with circulating α- and γ-tocopherol levels. Furthermore, we explored associations of plasma vitamin E levels with the following cardiometabolic traits: The metabolic syndrome (MetS), visceral (VAT) and subcutaneous (SAT) adipose tissue, liver signal intensity (LSI), fatty liver disease (FLD), and gallstone disease. The α-tocopherol/cholesterol ratio was positively associated with MetS (Odds Ratio (OR): 1.83; 95% Confidence Interval (CI): 1.21-2.76 for 3rd vs. 1st tertile) and VAT (β scaled by interquartile range (IQR): 0.036; 95% CI: 0.0003; 0.071); and the γ-tocopherol/cholesterol ratio was positively associated with MetS (OR: 1.87; 95% CI: 1.23-2.84 for 3rd vs. 1st tertile), VAT (β scaled by IQR: 0.066; 95% CI: 0.027; 0.104), and SAT (β scaled by IQR: 0.048; 95% CI: 0.010; 0.087). However, no association of the α-tocopherol/cholesterol ratio or of the γ-tocopherol/cholesterol ratio with LSI and FLD was observed. Lower odds of having gallstone disease were observed in the top (compared to the bottom) tertile of the plasma α-tocopherol/cholesterol ratio (OR: 0.31; 95% CI: 0.13-0.76). A similar trend was observed for the γ-tocopherol/cholesterol ratio, but the association did not reach statistical significance. All analyses were conducted using data from the first follow-up examination (2010-2012) of the PopGen control cohort, a population-based sample from Northern Germany. Populations based, prospective studies are warranted to confirm our results and to further explore the potential impact of the reported findings on other nutritional and health outcomes.

7 Zusammenfassung

Vitamin E ist ein fettlösliches Vitamin und umfasst verschiedene Vitamin E-Formen (α-, β-, γ-, δ-Tocopherole und Tocotrienole) mit anti-oxidativen Funktionen. Bislang ist wenig über die Verteilung von Vitamin E-Plasmaspiegeln in der Allgemeinbevölkerung in Norddeutschland bekannt. Weiterhin ist unklar, welche Ernährungsmuster und Einflussfaktoren mit Vitamin E-Plasmaspiegeln assoziiert sind. Im Rahmen der vorliegenden Arbeit wurden der Vitamin E (α- und γ-Tocopherol)-Status in einer Stichprobe der Allgemeinbevölkerung bestimmt und biochemische und ernährungsbezogene Einflussfaktoren identifiziert, die mit Vitamin E-Plasmaspiegeln korrelieren. Ca. 60% unserer Stichprobe wiesen adäquate Vitamin E-Plasmaspiegel (>30 µmol/L) auf. Allerdings zeigten a priori und a posteriori Ernährungsmuster keinen statistisch signifikanten Zusammenhang mit Vitamin E-Plasmaspiegeln. Weiterhin wurde die Assoziation von Vitamin E-Plasmaspiegeln mit dem Metabolischen Syndrom (MetS), dem viszeralen (VAT) und subkutanen (SAT) Körperfett, der Lebersignalintensität (LSI), der Fettlebererkrankung (FLD) und mit Gallensteinen untersucht. Das α-Tocopherol/Cholesterol Verhältnis war mit dem MetS (Chancenverhältnis (OR): 1,83; 95% Konfidenzintervall (CI): 1,21-2,76; 3.Tertil vs. 1.Tertil) und mit VAT (β skaliert durch den IQR (Interquartilsabstand): 0,036; 95% CI: 0,003; 0,017) positiv assoziiert. Das γ-Tocopherol/Choesterol Verhältnis war positiv mit dem MetS (OR:1,87; 95% CI: 1,23-2,84; 3.Tertil vs. 1.Tertil), VAT (β skaliert durch den IQR: 0,066; 95% CI: 0,027; 0,104) und SAT (β skaliert durch den IQR: 0,048; 95% CI: 0,010; 0,087) assoziiert. Allerdings fand sich kein Zusammenhang zwischen dem Vitamin E/Cholesterol Verhältnis und der LSI und der FLD. Studienteilnehmer/innen mit höheren Werten für das α-Tocopherol/Cholesterol Verhältnis (3. Tertil) wiesen im Vergleich zu Personen mit niedrigeren Werten (1. Tertil) eine geringere Wahrscheinlichkeit auf, Gallensteine zu haben (OR: 0,31; 95% CI: 0,13-0,76). Ein ähnlicher Trend konnte auch für das γ-Tocopherol/Choesterol Verhältnis beobachtet werden, jedoch war dieser Zusammenhang nicht statistisch signifikant. Für die vorliegenden Analysen wurden Daten der 1. Nachuntersuchung (2010-2012) der PopGen-Kontrollkohorte verwendet, die eine populationsbasierte Stichprobe aus Norddeutschland darstellt. Populationsbasierte, prospektive Studien

sind nötig, um die vorliegenden Ergebnisse zu bestätigen und deren Relevanz auf andere ernährungswissenschaftliche und gesundheitliche Aspekte zu untersuchen.

8 Appendix

Curriculum vitae

Persönliche Daten

Name	Sabina Waniek
Geburtsdatum	13.09.1986
Geburtsort	Sohrau (Polen)
Staatsangehörigkeit	deutsch

Ausbildung und Studium

Seit 10/2016 **Jade Hochschule Wilhelmshaven/Oldenburg/Elsfleth**

Studium Public Health, berufsbegleitend
Abschluss: Master, voraussichtlich 04/2019

10/2013 - 10/2015 **Christian-Albrechts-Universität zu Kiel**

Studium der Ernährungs- und Lebensmittelwissenschaften
Abschluss: Master

10/2010 - 10/2013 **Christian-Albrechts-Universität zu Kiel**

Studium der Ökotrophologie
Schwerpunkt: Ernährungswissenschaften
Abschluss: Bachelor

10/2007 - 09/2010 **MTA-Schule Lübeck**

Ausbildung zur Medizinisch-technischen-Radiologie-Assistentin
Abschluss: Examen

08/1999 - 06/2006 **Gabriele-von-Bülow-Oberschule (Gymnasium) Berlin**

Abschluss: Abitur

Berufliche Tätigkeit

10/2015 - 07/2018 **Institut für Epidemiologie**
Universitätsklinikum Schleswig-Holstein, Campus Kiel

Wissenschaftliche Mitarbeiterin (Doktorandin)

Seit 05/2018 **Institut für Epidemiologie**
Universitätsklinikum Schleswig-Holstein, Campus Kiel

Wissenschaftliche Mitarbeiterin
Leiterin des Studienzentrums Kiel der NAKO Gesundheitsstudie