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Essay in Biomedical Sciences:

THE CONTROVERSY AROUND VITAMIN E SUPPLEMENTATION: A POTENTIAL TREATMENT FOR TYPE 2 DIABETES-RELATED OXIDATIVE STRESS?

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SUMMARY:

Vitamin E, a collective group of eight lipids, is an essential dietary antioxidant and its protective function against oxidative stress plays a highly important role in human health and disease. Despite a physiological low level, elevated oxidative stress is associated with several pathologies, including diabetes mellitus type 2. A low circulating vitamin E status was positively correlated with diabetes type 2 incidence, hence, raising the questions whether vitamin E plays a role in diabetes-related oxidative stress and its potential as treatment to prevent the development or to ameliorate the morbidity of the disease. Accumulating evidence of *in vitro*, preclinical and observational studies positively associate vitamin E with antioxidative, protective properties in diabetes type 2, in particular, 2'R-4'R-6'R-α-tocopherol as predominant isoform in the human body. Several interventional trials report beneficial effects of vitamin E on intermediate, diabetes type 2-related phenotypes, suggesting a high potential of vitamin E as treatment. However, large hard-endpoint randomized controlled trials remain inconclusive, revealing adverse outcomes in some cases which has raised a highly controversial debate around this topic.

This essay discusses several studies from *in vitro* to interventional level regarding the role of vitamin E in diabetes-related oxidative stress and points out possible arguments why large interventional trials have remained unsuccessful. Briefly, this concerns the supplemented isoform and amount, the status of co-antioxidants to prevent pro-oxidative effects, assessment methods of status and intake and lastly, screening for subpopulations which might be particularly prone to vitamin E deficiency due to the Hp 2-2 genotype and HbA1c baseline levels.

Overall, although the connection between vitamin E and oxidative stress in diabetes type 2 is apparent, the exact role and potential as treatment application require further research. In light of the failure of large interventional trials to successfully implement vitamin E as treatment, more attention should be drawn to subpopulations with individual demands regarding the amount and duration of treatment and potential co-deficiencies driving the function as pro-oxidant.

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1 INTRODUCTION

Oxidative Stress in Diabetes Mellitus Type 2

Diabetes mellitus type 2 is one of the ten most important non-communicable diseases faced by the global population according to the World Health Organization [1] with a prevalence of one out of five people aged 60 years or older and approximately 8 % of the general population [2]. This chronic disease is hallmarked by insulin resistance and pancreatic beta cell dysfunction, leading to diminished insulin synthesis or secretion. It is further characterized by systemic generation of oxidative stress, particularly in vascular and myocardial cells [3]. Oxidative stress is hallmarked by an aberrant, relative decrease of protective cellular compounds such as antioxidants in favour of factors producing reactive oxygen species (ROS), disturbing the highly sensitive balance [2]. ROS is an umbrella term for several radical and non-radical oxidizing, oxygen-containing molecules including superoxide anion ($O_2 \cdot^-$), hydroxyl radical ($OH\cdot$) and hydrogen peroxide (H_2O_2) which damage cellular macromolecules [4]. The major factors leading to diabetes-related oxidative stress are obesity, hyperglycaemia and elevated levels of free fatty acids, which in turn causes insulin resistance [2]. Consequently, a complex antioxidative defence system including the superoxide dismutase (SOD), glutathione (GSH) and the vitamins C and E has evolved and proved critical for detoxification and cellular survival [4]. It is proposed that hyperglycaemia promotes ROS generation mainly in microvascular cells whereas, autoxidation of free fatty acids is suspected to play a major role in macro- and cardiovascular ROS production and tissue damage [3]. Beyond negative effects during hyperglycaemia, pathogenic consequences were observed even after normalization of glucose levels, a phenomenon referred to as “hyperglycaemic memory”, defined as chronic inflammation which is persistent even under post-hyperglycaemic conditions [3].
In short, oxidative stress strongly contributes to the aetiology of diabetes mellitus type 2 and is caused primarily by high levels of glucose and lipids as main drivers for insulin resistance. This suggests that using antioxidative agents to ameliorate oxidative stress could decrease the development, burden and mortality of diabetes type 2, in particular by targeting ROS-induced insulin resistance. However, the potential of implementing such antioxidants as treatment is subject to current research and challenged by the complexity of the human antioxidant system.

Vitamin E as Potential Diabetes Type 2 Treatment

The apparent connection between oxidative stress and diabetes type 2 drives the research for antioxidants which are effective in reducing glucose- or lipid induced oxidative stress.
Attention was drawn to vitamin E which in particular is known inhibit lipid peroxidation efficiently due to the hydrophobic nature of both compounds [5]. Polyunsaturated fatty acids (PUFA) are prone to oxidation, generating a peroxyl radical which induces an autoxidative chain reaction and results in further lipid radicals [6], promoting the pathology of diabetes type 2. Vitamin E effectively inhibits this chain propagation by reduction of lipid radicals, leading to oxidation into a relatively stable vitamin E radical which reacts with other radicals to non-radical products [6]. Hence, it constitutes a highly interesting candidate as potential treatment to reduce oxidative stress and by that, prevent the development and decrease the morbidity and mortality of diabetes type 2.
Numerous studies on different levels have investigated the question whether the antioxidative capacity of vitamin E might be beneficial in diabetes type 2, ranging from *in vitro* to interventional studies but inconclusive results have raised a scientific debate about the potential of vitamin E as treatment for diabetes type 2. For clarity, a short background about vitamin E regarding its isoforms, bioavailability and bioactivity as well as assessment methods will be given in the following section.

Isomerism of vitamin E

Due to the lacking ability of humans to endogenously synthesize vitamin E, it is one of the most important dietary antioxidants. It comprises a collective group of eight naturally occurring lipids which are divided into two subgroups: α-δ tocopherol (TOH) and α-δ tocotrienol [6], as shown in figure 1. Despite their structural similarity, the isomers are not interconvertible in humans [7] and furthermore, considerable differences in the *in vitro* antioxidative properties were observed, with the α-isoform having the highest activity [6]. Several stereoisomers exist for each isoform depending of the S- or R-conformation of the chiral centres of each compound which are not interconvertible through rotation [8]. Naturally, TOHs and tocotrienols appear in the 2'R,4'R,8'R and 2'R configuration, respectively [9]. In a synthetic mixture of α-TOH, all stereoisomers are generated, referred to as all racemic α-TOH (all-rac-α-TOH) [10]. In short, vitamin E comprises several isoforms and enantiomers with specific antioxidative properties *in vitro* and *in vivo* and thus, the specific bioavailability and -activity of the compounds must be respected when searching for a treatment candidate.

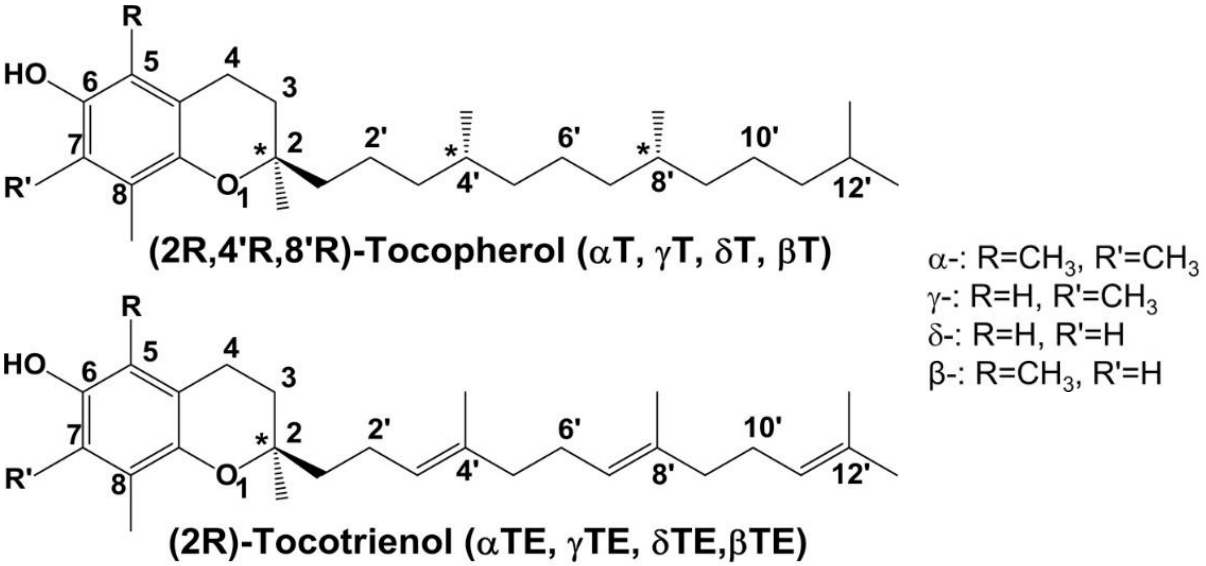

Figure 1: Natural isoforms of vitamin E Naturally occurring in eight different isoforms, vitamin E is divided into α-δ tocohopherol (TOH) and α-δ tocotrienol, which contain either a methyl group or a hydrogen at the 3' and 7' carbon residues in the chromanol ring. Moreover, stereoisomers are determined by the S or R conformation of the three chiral centres in TOHs and one chiral centre of tocotrienols. Naturally, all vitamin E forms exhibit the R conformations. *: chiral centre; figure adapted from REF[9]

RRR-α-TOH is the predominant isoform in humans

The different vitamin E compounds do not occur in the same concentrations in the human circulation, which is influenced by the food availability and metabolism and very important to consider when selecting a potential vitamer as treatment approach.

Vitamin E is exclusively found in plant food sources which are mostly rich in RRR-α-TOH and γ-TOH and hence, the two isomers are mainly consumed [9]. Although the intestinal absorption does not discriminate between the different isoforms [11], RRR-α-TOH is primarily retained as opposed to non-α-TOH forms which are predominantly metabolized and excreted [12]. This takes place in the liver, coordinating distribution metabolism and excretion of vitamin E [10, 12]. Due to its hydrophobic nature, circulating vitamin E needs to be bound by lipophilic carriers such as very low density lipoproteins (VLDL), low density lipoproteins (LDL) or high density lipoproteins (HDL), which are rich in triacylglycerides (TAG) and cholesterol [13]. This allows the release of vitamin E into the circulation and transport to extra-hepatic tissue. The high bioavailability of α-TOH is determined in hepatocytes

due to its high affinity to the α-tocopherol transfer protein (α-TTP), which is firstly, important for integration into lipid carriers and secondly, for protection from hepatic degradation of vitamin E. Contrarily, non-α-TOHs are not bound by the α-TTP and consequently, primarily subjected to degradation and excretion [10, 12].
In short, due to the natural occurrence in food sources and preferable retention in the human metabolism, RRR-α-TOH has the highest bioavailability and -activity as opposed to non-α-TOH compounds. This renders RRR-α-TOH a highly interesting candidate for research on vitamin E as treatment in diabetes type 2.

Biomarkers of vitamin E

Research on vitamin E and diabetes type 2 associations requires the consideration of vitamin E consumption and status in subjects, hence, reference values, assessment methods and biomarkers to evaluate optimal or insufficient intake and deficiencies are crucial.
The Recommended Dietary Allowance (RDA) of vitamin E is 15 mg (35 µmol) per day and the recommended adequate status requires a plasma concentration above 12 mmol/L [7]. Both refer solely to α-TOH due to the above-mentioned predominance. Although the RDA is seldom achieved, the occurrence of deficiencies and associated symptoms is rare in the general population and mainly associated with co-morbidities [11]. Thus, correlations between vitamin E and diabetes type 2 warrant accurate assessment of consumption, plasma level and functionality by using suitable intake, status and functional biomarkers.
Status biomarkers assess the presence of the different circulating vitamin E compounds. As vitamin E is bound to lipophile carriers, the concentration must be adjusted for a lipid reference base [14]. As this implies correction for cholesterol as well as TAG levels this harbours the risk of overcorrection, hampering an accurate estimation. Considering this, a recent study suggested erythrocytic vitamin E as lipid-independent, representative alternative to estimate the vitamin E status and associate it more reliably with health outcomes [15]. Status markers constitute a widely implemented, relatively accurate method to evaluate an optimal, insufficient or deficient state, however, the actual antioxidative capacity remains unconsidered.
The biological activity beyond the presence of vitamin E is assessed by functional biomarkers. Generally, two different types of functional markers for vitamin E have been established, namely, the induction of haemolysis upon hydrogen peroxide [14] and markers for lipid peroxidation [16] as vitamin E is a protective factor for both phenomena. As markers for haemolysis and lipid peroxidation are often not specific for deficient vitamin E levels [7] functional markers as less accurate for vitamin E and hence, should be evaluated in combination with status markers.
Because of the highly regulated homeostasis, plasma and intracellular levels of vitamin E, *i.e.* status and functional markers, do not represent dietary intake. A common way to investigate vitamin E intake, primarily in epidemiological studies, are conventional dietary assessment methods such as 24-hour recall [17], Food Frequency Questionnaires (FFQ) [18] and food records [19]. All three methods have the advantage that intake of other macro- and micronutrients is recorded as well, which might reveal as important factors to consider, however, also harbour a relatively high risk of inaccurately assessing certain micronutrients such as vitamins. Hence, measurement of biomarkers in 24-urine samples has been suggested as more accurate alternative, not underlying possible confounders such as memory, awareness, estimation of portions size or nutrient content [20]. The collection of 24-hour urine is critical because spot samples normalized to creatine clearance are less reliable due to variations in urine concentration and time-dependent excretion [21]. Moreover, this allows the discrimination of the different isoforms as they are independently catabolized and excreted [5]. Although intermediate catabolic products are excreted via bile into the faeces, the end product of vitamin E metabolism, CEHC (2,5,7,8-tetramethyl-2(2'-carboxyethyl)-6-hydroxychroman), is released into the urine and measured in 24-hour urine samples [11]. As predominant form of vitamin E, α-CEHC levels were validated as

intake biomarker for α-TOH [22]. In short, metabolic urinary products of vitamin E constitute an accurate method to assess dietary consumption.
In summary, a combination of several markers is necessary to assess vitamin E status accurately and furthermore, the choice of marker depends on the research questions as for dietary consumption, intake markers in 24-hour urine samples are to be analysed whereas status markers are more suitable for assessment of sufficient, insufficient or deficient vitamin E levels.

Thesis of This Essay

Due to the apparent clinical, social and economic relevance of diabetes type 2, treatment to reliably prevent or cure the disease are highly important. Attention was drawn to oxidative stress as part of diabetes type 2 aetiology and the potential of implementing naturally occurring antioxidative agents to tackle the disease. This idea was followed by observations correlating diabetes type 2 incidence with vitamin E deficiencies and led researchers to focus on vitamin E as potential treatment approach for diabetic patients, reducing oxidative stress and its associated pathogenic consequences.
Promising results of *in vitro* and preclinical studies associated vitamin E with decreased oxidative markers and insulin resistance which could be confirmed in intermediate endpoint randomized clinical trials (RCT)s. However, hard endpoint RCTs remain yet inconclusive and even report adverse effects of vitamin E supplementation.
This essay discusses the current state of research on vitamin E supplementation in diabetes type 2 in the light of *in vitro* to interventional studies. The discrepancy of beneficial findings and the controversial large RCTs will be critically viewed by elaborating possible arguments why vitamin E supplementation has been unsuccessful. This follows my hypothesis that vitamin E is a promising factor in decreasing oxidative stress-induced insulin resistance which can either prevent the development of diabetes type 2 or ameliorate the progression and thus the disease-related morbidity and mortality and that in my opinion, RRR-α-TOH as predominant isoform in humans has the highest potential as potential treatment approach.

2 PRO'S AND CON'S ON VITAMIN E IN DIABETES TYPE 2

Research on vitamin E as potential treatment for diabetes type 2 requires several stages from *in vitro* studies and preclinical trials to minimize safety concerns and elucidate mechanistic explanations to observational and interventional human studies, validating certain hypotheses in humans. Beneficial, insignificant to adverse effects led to the controversial debate around this topic. Selected studies investigating the antioxidative and protective potential of vitamin E isoforms related to diabetes type 2 will be discussed in the following chapter, an overview is provided in table 1.

Table 1: Overview about the discussed studies regarding design, used isoform (if applicable, dosage), endpoint and outcome. RCT: Randomized controlled trial, ↗: beneficial effect, →: no effect, ↘: adverse effect

Study design	Isoform	Endpoint	Outcome	Ref.
In vitro	α-TOH	intermediate	↗	[23]
	Not specified	intermediate	↗	[24]
Preclinical	α-TOH	intermediate	↗	[25]
	Not specified	intermediate	↗	[26]
	Not specified	intermediate	↗	[27]
Observational	α-TOH	intermediate	↗	[19]
	Not specified	Hard	↗	[28]
	α-TOH	Hard	↗	[29]
Interventional	α-TOH (1200 IU)	intermediate	↗	[30]
	Not specified (900 IU)	intermediate	↗	[31]
	Not specified (800 IU, 1200 IU)	intermediate	↗	[32]
	α-TOH (400 IU)	Hard	↗	[33]
	α-TOH (400 IU)	Hard	→	[34]
	α-TOH (900 IU)	Hard	→	[35]
RCT meta-analysis	Not specified	intermediate	↗	[36]
	Not specified	intermediate	↗	[37]
	Not specified (<400 IU)	Hard	↘	[38]
	Not specified (900 IU)	Hard	→	[39]
	Not specified (900 IU)	Hard	→	[40]

2.1 *IN VITRO* AND PRECLINICAL STUDIES

In vitro evidence

Cell culture studies on vitamin E and diabetes type 2 analyse the influence of vitamin E on oxidative stress marker expression, often mimicking diabetic cells using hyperglycaemic conditions. In doing so, several studies directly associated the presence of vitamin E with relieved oxidative stress.

For example, α-TOH reduced protein kinase C (PKC) expression and thus, ROS production in a diabetic monocyte model [23]. In human erythrocytes, vitamin E ameliorated lipid peroxidation and dysbalanced osmosis following hyperglycaemia [24]. Both studies demonstrate the function of vitamin E, in particular α-TOH, as gene expression modulator and free radical scavenger *in vitro* and indicate a direct causal relation between high glucose-induced oxidative stress and a protective antioxidative effect of vitamin E. Hence, this study type is highly valuable to determine isolated mechanistic explanations between the vitamers and cellular damage through glucose-mediated oxidative stress. However, the two most important limitations are firstly, that these *in vitro* studies are restricted to acute effects and secondly, do not take into account the absorption, transport and

metabolism of the vitamers which could alter the antioxidative capacity *in vivo*. Moreover, some studies do not specify the used isoform which is a critical information as the vitamers differ in their *in vitro* activity and bioavailability and -activity [11, 41].
Overall, interpretation of cell culture studies is restricted to acute effects as no long-term influence can be observed. Nevertheless, accumulating *in vitro* evidence on antioxidative properties of vitamin E isoforms, particularly α-TOH, constitutes an important foundation for preclinical and clinical trials and furthermore, provides causal and mechanistic explanations for observed relations between vitamin E and oxidative stress reduction.

Preclinical evidence
Next to cell culture studies, preclinical trials were performed largely to examine vitamin E supplementation *in vivo* using diabetic animal models of which most studies use streptozotocin-induced diabetic rats.
Supplementation of vitamin E revealed striking antioxidative effects counteracting the glucose-induced oxidative damage in diabetic rat models, improving oxidative stress markers and renal function [27] as well as reduction of aberrantly activated mitogen-activated protein kinases and apoptotic pathways [26]. In particular, α-TOH exhibited strong beneficial effects by suppression of lipid peroxidation and ROS production in addition to promoting the detoxifying radical scavengers GSH and SOD [25]. The most important strength of preclinical trials is the strong causal relationship between vitamin E supplementation and alleviated oxidative stress trough placebo-controlled trials which is highly valuable for mechanistic explanations in human diabetes type 2 cases, although the extrapolation is limited. Secondly, vitamin E is administered to a living organism, allowing the consideration of as digestion, absorption, metabolism and excretion. As this is a large source for known and unknown confounding factors in human trials it is important to conduct these studies first. Nonetheless, the most significant limitation is that animal models cannot fully represent humans and thus, evidence cannot be included in dietary recommendations. Equally important is that the studies discussed in this section assessed oxidative stress markers as primary outcome which constitutes an intermediate endpoint of diabetes and does not inevitably cause diabetes and the short duration does not allow extrapolation to possible long-term outcomes. Next to these major limitations, it is also to be noted that diabetes was induced through streptozotocin in the rats whereas human studies are conducted in actual diabetes cases and that the specific stereoisomer was often not specified.

In conclusion, accumulating evidence in *in vitro* and preclinical studies report positive correlations between vitamin E supplementation, primarily α-TOH, and alleviated oxidative stress related to hyperglycaemia and diabetes, providing valuable causal and mechanistic insights. However, these study designs are limited and cannot simply be extrapolated to humans and thus, rather form a foundation for human trials but cannot be included in evidence for treatment recommendations.

2.2 HUMAN OBSERVATIONAL STUDIES

Observational human studies are important to associate dietary intake and status of vitamin E with intermediate and hard outcomes of which the strongest type are prospective cohort studies which are be discussed here. By definition, observational studies do not include an intervention.

A positive correlation between vitamin E and insulin sensitivity was found primarily for circulating α-TOH, but not ß- or γ-TOH levels and not for dietary intake in pre-diabetic men with chronic kidney disease [19]. Thus, the most important conclusion is that α-TOH deficiency may be related with decreased insulin resistance which is a known risk factor for development of diabetes type 2, emphasizing the potential of targeting plasma α-TOH levels for treatment approaches. The main

limitation however is the restricted external validation to chronic kidney diseases and the cross-sectional design. Insulin resistance is one of the most important causal factors for diabetes and thus, strongly indicating the risk for the disease, however, it does not inevitable cause diabetes type 2. For that, a long-term observation considering the incidence is needed. Less critical but considerable uncertainties are the group size (n = 273) and the dietary assessment via food records which, despite considering the complete dietary pattern, harbour a high risk for inaccurate results. This might have caused the weak association between vitamin E consumption and insulin sensitivity. Briefly, despite several limitations, this study clearly demonstrates a positive association between the α-TOH plasma status and insulin sensitivity.

For estimation of clinical relevance, cohort studies need to consider hard endpoints such as disease incidence and mortality. Two studies associated reduced plasma vitamin E with increased risk for diabetes type 2 development, clinically diagnosed after WHO criteria. While Salonen *et al.* [28] did not discriminate between the circulating isoforms, the Insulin Resistance and Atherosclerosis Study (IRAS) [29] observed a strong association between total lipid-adjusted plasma levels of α-TOH and development of type 2 diabetes. This further emphasizes the relevance of α-TOH over non-α-TOH compounds as previously demonstrated [19]. The most considerable strength of both studies is the longitudinal design with four and five years follow-up, respectively, allowing the deduction of long-term effects and hard endpoints, *i.e.* disease incidence. Secondly, the large cohort sizes of 944 [28] and 895 [29] subjects, respectively, render the studies valuable. Dietary habits were only considered in the IRAS via FFQ, which might have resulted in inaccurate results, hampering a potential association between dietary intake and diabetes type 2. However, supplement and non-supplement users of vitamin E were analysed separately and the association between plasma α-TOH and diabetes type 2 was rendered insignificant when vitamin E was supplemented. This points out the important of a α-TOH deficiency over dietary supplementation. Overall, the studies provide strong but plausible evidence indicating that a vitamin E deficiency might increase the risk for developing type 2 diabetes.

In summary, several human prospective observational studies revealed strong associations between plasma vitamin E with insulin resistance and diabetes type 2 incidence, in particular for α-TOH as opposed to other isoforms. Hence, this emphasizes the importance of targeting vitamin E deficiencies which, due to the lacking significant correlation, cannot be simply connected to dietary intake. The assessment of dietary patterns is important to elucidate whether a generally poor diet is associated with vitamin E deficiency and thus, promoting diabetes type 2, however, more accurate measurements of vitamin E consumption are warranted. The strong but plausible evidence of deficient, circulating α-TOH levels promoting diabetes type 2 development requires interventional trials to analyse if this deficiency can be ameliorated and to generate a potential causal relationship regarding the disease incidence, progression and mortality.

2.3 Human Interventional Trials

Several RCTs supplementing vitamin E in a diabetes and other disease-related context have been conducted and vary largely in the amount supplemented, study population, group size, duration and endpoint among other factors. The amount often is defined in international units (IU) with 1 IU corresponding to 0.67 mg of the natural RRR-α-TOH or 0.91 mg of synthetic all racemic α-TOH [7]. The compiled evidence controversially points towards beneficial as well as adverse correlations between vitamin E and health outcomes.

Supporting evidence

Successful clinical trials on vitamin E supplementation in type 2 diabetes are mainly limited to the effects on oxidative stress, insulin resistance and inflammation, all of which are important but intermediate endpoints of the disease.

One study daily administered 1200 IU of synthetic all-rac-α-TOH to 25 type 2 diabetic patients and a control group for three months, focussing on monocytic activity [30]. The treatment significantly lowered LDL oxidation, monocytic adhesion and monocytic release of oxidative and inflammatory markers in both groups. The study clearly demonstrates a protective benefit of high α-TOH supplementation on known pathogenic characteristics of diabetes type 2 and hence, the potential to decrease disease burden and progression as they are known to promoting atherosclerosis [30]. However, a placebo-controlled study design would have allowed to evaluate the benefit of α-TOH compared to non-supplemented but diabetic patients. Another RCT in 43 type 2 diabetic subjects supplemented 900 IU of vitamin E for three months [31] which significantly reduced blood pressure and circulating glucose levels. Furthermore, vitamin E promoted the antioxidative capacity through elevating SOD and glutathione peroxidase (GPx) activities and reducing plasma levels of glycosylated haemoglobin (HbA1c). The study additionally administered a combined supplementation of vitamin E and C which constitutes a promising approach as the two vitamins function as co-antioxidants, however, the authors do not state which treatment was more effective. The findings on antioxidative properties and health benefits of vitamin E agree with another RCT, supplementing vitamin E or a placebo to two groups of 40 overweight adults for six months [32]. After three months, the administration was increased to from 800 IU 1,200 IU. Following the treatment, the authors report a persistent improvement in oxidative stress and liver function but only a transient protection against insulin resistance after three months. The diminished effect after six months might be due to the increased administration as vitamin E in high concentrations was previously observed to act pro-oxidatively, particularly upon exhausted co-antioxidant levels such as vitamin C [42].

In spite of the direct causal effect as strength of these trials, a critical limitation is the short duration lacking a follow-up. In this respect, disease incidence, complication or mortality have not been considered but the outcomes were restricted to intermediate endpoints such as oxidative stress which limits the extrapolation of these studies. However, focussing on this causal factor is also highly important as this might prevent the disease development in the first place. This is particularly interesting in the third study which focussed on overweight adults as high-risk group for diabetes type 2 [32]. As further uncertainty it is to note that the discrimination of the stereochemistry and isomerism is seldomly respected, but critical due to the primary retention of RRR-α-TOH [43]. The high supplemented amount might have generated adversities of possible pro-oxidative properties of vitamin E. In this regard, the role of other factors such as co-antioxidants was not considered despite previous findings demonstrating the importance of co-antioxidative agents for the α-TOH recycling system [42]. Moreover, the diet as potential confounder was not considered, hence, a crossover interventional study design may have been more representative and also feasible due to the only short-term study design. These arguments will be discussed in a later chapter of this essay in more detail. Lastly, the small group sizes hamper identification of significant and reliable effects.

Contrary to the limitations mentioned above, a large RCT allocated 400 IU natural RRR-α-TOH or placebo to 1434 middle-aged diabetes type 2 patients for 18 months [33]. Strikingly, the treatment reduced cardiovascular events (Figure 2) with the primary outcomes being myocardial infarction, stroke and cardiovascular death. Importantly, only patients with the Haptoglobin (Hp) 2-2 genotype of the antioxidative Hp protein were included by genotyping prior selection of the study cohort. Hence, this trial demonstrated a successful implementation of RRR-α-TOH as treatment for hard endpoints in a Hp 2-2, diabetic subpopulation and is one of the very few RCTs reporting beneficial effects of vitamin E in diabetes type 2 patients.

In summary, several clinical trials have investigated the role of vitamin E in diabetes-related risk factors and reported beneficial health effects of vitamin E supplementation, in particular for the Hp 2-2 subpopulation. Compiling these arguments, the present studies suggesting α-TOH or vitamin E in general as prevention or treatment for diabetes provide promising results which need further validation by hard endpoint clinical trials.

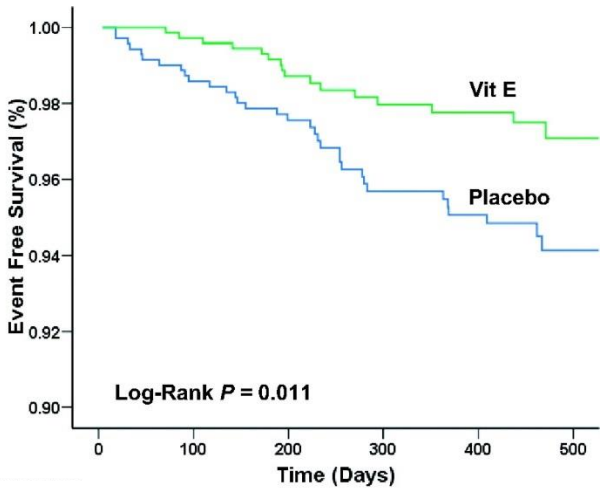

Figure 2: Supplementation of RRR-α-TOH in Hp 2-2 diabetes type 2 patients Natural RRR-α-TOH (Vit E) administered to 1434 type 2 diabetes patients harbouring the Hp 2-2 allele, significantly reduced cardiovascular events compared to a placebo control after 18 months [33]. Myocardial infarction, stroke and cardiovascular death were defined as primary outcome. The relative event free survival is plotted against the days of treatment.

Inconclusive or contradictive evidence

The numerous studies demonstrating positive influences of vitamin E on diabetes-related oxidative stress are challenged by several clinical trials reporting non-significant or significantly adverse health effects following vitamin E supplementation.

The Heart Outcomes Prevention Evaluation (HOPE) [34] study is a large RCT assessing the effects of vitamin E supplementation in elderly with diabetes type 2 or another cardiovascular disease. The study included 3,654 diabetic participants, randomly divided for daily administration of 400 IU RRR-α-TOH acetate or placebo for 4,5 years. Although plasma levels of vitamin E in the treatment group increased, the treatment did not affect the incidences of myocardial infarction stroke or cardiovascular death which were defined as primary outcome (Figure 3A). Neither was any improvement in secondary outcomes such as total mortality found. Furthermore, no influence of RRR-α-TOH on the incidence of diabetes type 2 in non-diabetic participants (n = 5,887) was revealed compared to the placebo group. In the Heart Protection Study [35], a similar large RCT including 20,536 high-risk subjects, supplementing 600 mg (approximately 900 IU) synthetic all-rac-α-TOH did not improve the outcomes of mortality, vascular diseases or cancer after a 5-year follow up (Figure 3B).
Both studies did not obtain beneficial nor harmful evidence of α-TOH supplementation, regardless if RRR-α-TOH or synthetic-all-rac-α-TOH was administered. The large group sizes and robust study design through placebo-controlled, randomized administration account for the high quality of the trials. Furthermore, the primary outcomes were hard endpoints after long follow up periods, emphasizing the clinical relevance of these results. Overall, these findings agree with other large clinical trials, reporting no benefits of vitamin E supplementation in a cardiovascular disease-related context [44-47]. Critique points however are most importantly, that the baseline vitamin E status and intake remained unconsidered raising the question whether the treatment might be more effective in a subpopulation

with insufficient consumption or deficient plasma levels of vitamin E. Secondly, the daily dosage was mainly either very high (400 to >800 IU), which might induce pro-oxidative properties of vitamin E and concomitantly increase the demand of co-antioxidative agents [48], or too low (50 mg or 55 IU) for a detectable effect. Lastly, despite stating the usage of α-TOH, the stereochemistry is often not respected.
Overall, these RCTs are of high quality and provide strong evidence challenging the use of vitamin E as antioxidative treatment but the large group sizes might have rendered potential benefits in certain subpopulations insignificant or undetectable.

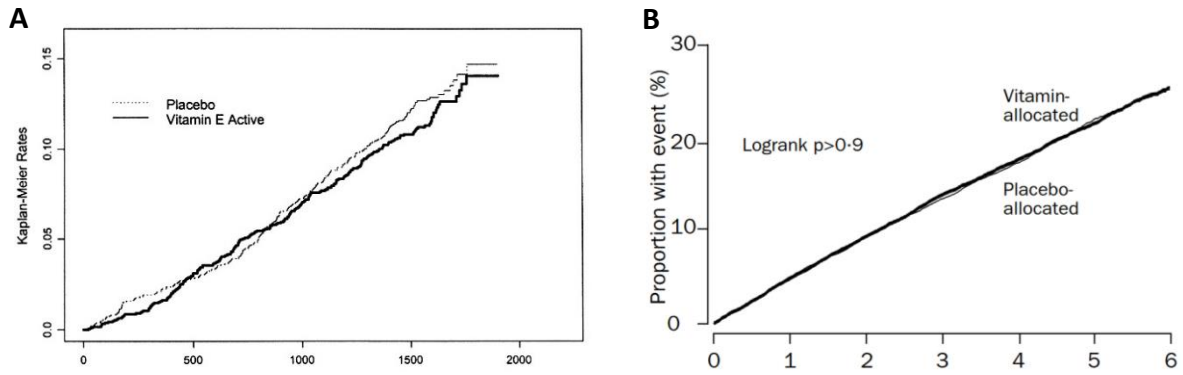

Figure 3: No difference in all-cause mortality between vitamin E compared to placebo supplementation Large RCTs investigated the influence of vitamin E supplementation on all-cause mortality after follow-up of several years in subjects with diabetes or other cardiovascular diseases. **A** The HOPE study [34] included 3,654 patients and administered 400 IU RRR-α-TOH daily in the intervention group. The Kaplan-Meier survival is plotted against the days of follow-up. **B** The Heart Protection Study [35] assessed all-cause mortality in 20,536 subjects, supplementing a placebo or 900 IU all-rac-α-TOH per day. The y-axis indicates the percentage of deaths and the x-axis indicates the duration of follow-up in years.

Following individual RCTs on vitamin E supplementation, several meta-analyses resulted in conflicting and overall inconclusive results. A recent meta-analysis compiling nine RCTs in diabetic patients reported no overall improvement of glycaemic control, assessed via plasma HbA1c measurement, following vitamin E supplementation [36]. However, subgroup analysis revealed a beneficial effect in patients with deficient baseline vitamin E status and aberrant baseline glycaemic control. Thus, the authors suggest a targeted rather than generic treatment approach as vitamin E supplementation might be beneficial in certain subgroups, but also point out that further research on long-term outcomes is warranted. These findings agree with a similar meta-analysis of RCTs assessing glycaemic control, revealing no generic benefit of vitamin E supplementation but beneficial reduction in HbA1c and fasting insulin following vitamin E administration in subjects with initially low vitamin E levels [37]. The supplied compounds in the RCTs varied between RRR-α-TOH, all-rac-TOH, tocotrienols and non-specified vitamin E. Additionally, the amount and duration of supplementation differed largely which together with other factors such as ethnic group, baseline glucose metabolism etc. might have acted confounding. Overall, this further emphasizes the importance of subgroup analysis to identify subpopulations in which α-TOH supplementation is particularly effective.
Long-term safety issues of vitamin E supplementation are marked by a highly controversial debate centring around a meta-analysis by Miller *et al.* in 2005 [38]. This meta-analysis on 135,967 subjects with a chronic disease-related background in 19 trials revealed an increased risk for all-cause mortality following high-dosage dietary supplementation of vitamin E (≥400 IU per day). The authors conclude a clinically highly relevant, dose-dependent harmful effect of vitamin E. However, these findings were challenged recently by another meta-analysis using the same data but a different statistical method, reporting no harmful effects of vitamin E, independent of the dose [39]. A third meta-analysis included 57 trials and 246,371 subjects [40] and, contrarily to the first two analyses, did not exclude RCTs

reporting less than ten deaths, neither found a relationship between vitamin E supplementation and all-cause mortality, even when high dosages were administered. As recent meta-analyses could not prove the suggested adversity of vitamin E supplementation, this issue remains inconclusive.

In summary, accumulating evidence supports the effectiveness of vitamin E supplementation, in particular α-TOH, to reduce oxidative stress and promote beneficial health outcomes in diabetes type 2 and various other diseases. This however is mainly limited to *in vitro*, preclinical and observational studies as well as short-term clinical trials assessing intermediate endpoints as primary outcome. Large hard endpoint clinical trials are further needed to validate and reliably interpret the findings, especially the role of subpopulations determined by the Hp 2-2 genotype and baseline HbA1c, but many remain inconclusive or even contradictive so far. This discrepancy marks the yet unresolved controversy around vitamin E supplementation in diabetes- and other disease-related oxidative stress.

3 WHY HAS VITAMIN E SUPPLEMENTATION NOT BEEN SUCCESSFUL?

The inconsistency in clinical trials supplementing vitamin E is opposed to the positive outcomes in *in vitro*, preclinical and cohort studies. The reasons for this discrepancy and more importantly, the resolve remain unknown yet. Extrapolation from *in vitro*, preclinical and observational studies to RCTs is hampered by several confounding factors, often causing insignificant or even adverse outcomes. Estimation of and adjustment for these factors reduces their influence, although uncertainties, especially in human studies, cannot be abolished completely. This chapter will elaborate on possible confounders in trials supplementing vitamin E in a diabetes-related context such as isoforms, dosage (with regard to potential pro-oxidative properties), accuracy of vitamin E assessment and the role of subpopulations due to the Hp 2.2 genotype and baseline glycaemic control.

3.1 ISOMERISM OF VITAMIN E

When associating dietary intake of natural or supplemented vitamin E with potentially beneficial health effects, consideration of the specific isoform and stereochemistry is critical.

Despite the rising attention drawn to the individual isoforms, studies often do not specify which isoform or if a mix of TOHs and tocotrienols was used. In the light of current findings emphasizing the relevance of discrimination between the isoforms [49], by limiting the specification to “vitamin E” the study reliability, quality and identification of isoforms-specific effects is hampered. For example, a very recent meta-analysis on vitamin E supplementation and inflammatory biomarkers in highly insulin resistant patients reported significant anti-inflammatory benefits following the α-TOH administration as opposed to other isoforms [49]. Moreover, novel findings discriminated the regulatory functions of α-TOH and γ-TOH in oxidative stress, inflammation and ageing after renal transplantation, associating improvements with high α-TOH levels, whereas γ-TOH was correlated with adverse effects [15]. Circulating γ-TOH levels were inversely correlated with total vitamin E intake and circulating α-TOH in a cross-sectional, observational study [50] which might dilute, if not even disturb the health promoting effect associated with high plasma α-TOH. Several non-antioxidative functions have been described specifically for α-TOH [51] which might further contribute to its benefit.

Beyond discrimination of α-δ isoforms, the stereochemistry might be another important, but often neglected contributor to inconsistency. The four 2’R-α-TOH stereoisomers are biologically more active than the 2’S isoforms [52], presumably, this confirmation is important for the binding to the hepatic α-TTP [9]. Pure, natural RRR-α-TOH has a three times higher bioavailability and -activity higher than all-rac-α-TOH [52]. Vitamers other than RRR-α-TOH, together with excess RRR-α-TOH, are predominantly excreted and treated as xenobiotics by conjugation of the CEHC metabolites with sulfate or glucuronide [53]. This unequal distribution of the vitamers is due to the affinity to the α-TTP, leading to the most efficient incorporation of RRR-α-TOH into circulating lipids [54]. Moreover, it cannot be excluded that other isoforms, which are primarily metabolized and excreted, not only dilute but even deteriorate RRR-α-TOH-mediated positive effects. For example, the demonstrated α-TOH-induced reduction of PKC activity cannot be achieved by ß-TOH but in fact, ß-TOH disturbs the protective function of α-TOH [55]. This is coherent with the study discussed in 2.1 (Ref [23] in table 1) demonstrating a reduction in PKC activity upon α-TOH addition. As all vitamers exhibit antioxidative activity *in vitro* the difference *in vivo* is presumably caused by unwanted side-reactions or non-antioxidative functions [55]. Hence, concomitant supplementation of different vitamers might cause negative reactions, constituting an additional burden to the subject. As RRR-α-TOH is preferably retained and biologically most active, using only on RRR-α-TOH instead of all-rac-TOH constitutes a promising approach to observe clearer effects. The most reliable implementation of RRR-αTOH would imply a purity measurement to exclude contaminations. This is apparent in studies discussed above, for example in 2.2 an observational study correlated plasma α-TOH but no other isoforms to improved

insulin sensitivity (Ref [19] in table 1). Several studies in the chapters 2.2 and 2.3 (Refs [28, 31, 32] in table 1) and all RCT meta-analyses did not specify the investigated vitamer which, based on this knowledge, might have resulted in more significant positive effects.
Overall, the importance to discriminate between the isomers of vitamin E becomes apparent and might shed light on controversial results on vitamin E supplementation in clinical trials, creating a new perspective on previous conclusions and for integration in further studies.

3.2 Dose-Dependent Antioxidant Paradox

The pro-oxidative nature of vitamin E induced by high concentrations is a widely discussed observation described as antioxidant paradox [56].
Aggressive α-TOH supplementation can promote pro-oxidative properties of vitamin E, particularly when co-antioxidants such as vitamin E are deficient [48]. Adverse health outcomes of high-dosage vitamin E administration are often related to co-morbidities or -deficiencies, indicating that a healthy homeostasis is able to deal with high concentrations whereas diseased are more vulnerable to oxidative damage [12]. Further *in vitro* research demonstrated α-TOH-induced LDL peroxidation in the presence of high α-TOH concentrations or absence of co-antioxidants such as vitamin C [57]. These are critical as the antioxidant activity of α-TOH produces an oxidized α-tocopheroxyl radical (TO·), requiring regeneration by other redox-active agents [42]. The interaction between vitamin E and C in the detoxification of oxidative radicals is *e.g.* important for the oxidation-prone membrane-located PUFA. Oxidation of PUFA results in the formation of a peroxyl radical which initiates an autoxidizing chain reaction, promoting further PUFA oxidation. Embedded in the membrane lipid layer, α-TOH interferes with and stops this chain reaction through reduction of the peroxyl radicals to PUFA. The generated less oxidizing TO· radical exhibits a lower cellular toxicity. However, TO· recycling, *i.e.* the reduction back to α-TOH, is critical for its function as protective antioxidant. It is believed that ascorbate (vitamin C) mediates the reduction of TO·, generating an ascorbate radical with even lower oxidizing properties. Subsequently, ascorbate is recycled by thiol compounds such as GSH and NAD(P)H using enzyme systems [10, 58] (Figure 4). Hence, vitamin C and E constitute an efficient and protective network against oxidative damage in proteins, membranes and lipids [42]. The synergistic benefit combining vitamin E and C was demonstrated in diabetic rats [59].
Next to vitamin C, several micronutrients are essential for the antioxidative network around vitamin E. An important factor is selenium which is integrated into enzymes, referred to as selenoproteins, involved in the recycling of antioxidants such as GPx in the thiol cycle of the vitamin E recycling network [60] (Figure 4). A recent meta-analysis including 20 observational studies associated a deficient selenium status with increased diabetes incidence [61].
Considering this, the adverse outcomes of several large clinicals might be caused by high dosage of vitamin E together with insufficient co-antioxidative agents. The daily RDA for vitamin E of 15 mg (approximately 16 IU) is often highly exceeded by RCTs using 800 IU or more. This is coherent with the dose-dependent increase in mortality observed by Miller *et al.* [38], demonstrating a U-shaped vitamin E dependence, as very low and very high intake entails adverse health consequences which is discussed in chapter 2.3 (Ref [38] in table 1). The RCT in overweight adults (Ref [32] in table 1) observed a transiently improved insulin sensitivity upon supplementation of 800 IU vitamin E which was diminished after increasing the dosage to 1,200 IU. A possible reason for this might be that the adversity dominated the benefit in the higher dose. In a similar manner, the lacking effect in the α-TOH supplementing RTCs (Ref [34, 35] in table 1) might be caused by an unconsidered co-deficiency which hampered the full antioxidative capacity of α-TOH.
In conclusion, integration of this knowledge into RCTs should raise awareness for the dosage and the role of other antioxidative compounds. Baseline screening for potentially low levels or concomitant supplementation might reduce the risk for vitamin E-mediated pro-oxidative damage.

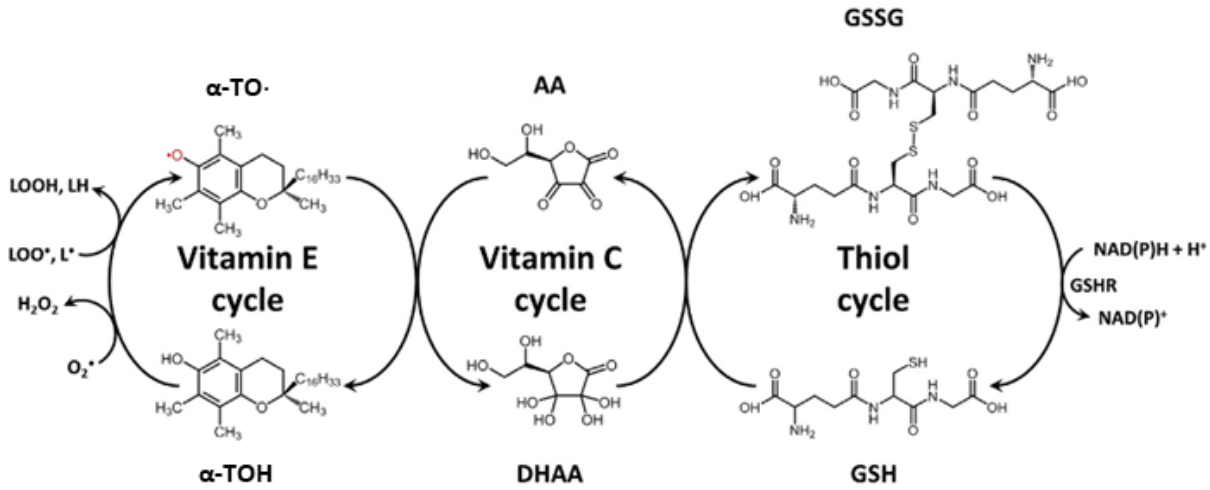

Figure 4: The antioxidative network and recycling system Detoxification of radicals such as superoxide anion (O_2·), peroxyl radicals (LOO·) and alkyl radicals (L·) to hydrogen peroxide (H_2O_2), lipid hydroperoxides (LOOH) and alkanes (LH) by α-TOH generates an α-tocopheroxyl radical (α-TO·). It is recycled, *i.e.* reduced to α-TOH, by ascorbic acid (DHAA) oxidation to dehydroascorbic acid (AA) which is then converted back to DHAA by a glutathione (GSH) and NAPDH or NADPH using enzyme system. Figure adapted from REF[10].

3.3 Assessment of Vitamin E Status and Intake

A second potential confounder is the vitamin E status and intake assessment.

Vitamin E intake is an important variable in observational studies and RCTs commonly assessed by dietary questionnaires such as the retrospective FFQs or 24-hour recall or the prospective food record, taking the potential influences of other nutrients into account. For example, a γ-TOH-rich diet often entails high n-6 PUFA intake whereas α-TOH-rich products are typically high in MUFA [9]. Although n-6 PUFA were previously reported to decrease low-density cholesterol levels, excess n-6 PUFA was associated with increased risk for cardiovascular diseases [62], presumably as they are particularly prone to peroxidation [58]. Moreover, the food matrix is important as total fatty acid intake is critical for uptake and bioavailability of vitamin E [63]. While natural vitamin E consumption often entails a higher lipid intake, this is not necessarily true for vitamin E supplementation [10]. Hence, a sufficient fatty acid intake must be ensured, but excess lipid consumption might be problematic, especially in high-risk groups for diabetes. Despite the importance of considering dietary patterns and nutrient intake, highly accurate assessment of vitamin E intake with respect to the isomerism is critical. Awareness or recall bias, poor estimation of portion size, etc. render the questionnaires less reliable than intake biomarkers such as CEHC in 24-hour urine samples and thus, should constitute an additional rather than primary method. However, no study discussed in chapter 2 implemented CEHC measurement as intake marker. Instead they relied on dietary questionnaires such as the observational IRAS in chapter 2.2 (Ref [29] in table 1) which associated α-TOH status but not intake with type 2 diabetes. The dietary assessment performed via FFQ combined with 24-hour urine CHECs might have allowed a more accurate estimation and revealed a stronger correlation to the vitamin E consumption. Other studies did not consider dietary intake in the first place such as Salonen *et al.* (Ref [28] in table 1). In my opinion, this accounts for the lacking understanding of the discrepancy between vitamin E intake, status and disease-related outcomes. Overall, accurate measurement of dietary vitamin E consumption is limited and intake markers provide high-quality data. Studies solely relying on dietary questionnaires might incur a high risk of uncertainty in the quantities and isomers consumed.

Beyond intake, studies evaluate optimal, insufficient and deficient vitamin E levels by measuring status and functional markers. The ratio between plasma vitamin E and total lipid levels is the most common

method. A strength of this assessment is the discrimination between the isoforms, however, a recent study pointed out the uncertainty by overcorrection as vitamin E adjusted to circulating cholesterol as well as TAG concentration [15]. The authors propose measuring vitamin E in erythrocyte membranes as lipid-independent and hence, more reliable method. Beyond the presence of vitamin E, considering its functionality as antioxidative agent via functional biomarkers is essential to allow the identification of true deficiencies, *i.e.* reduced antioxidative capacity. Lipid peroxidation or induction of hydrogen peroxide-induced haemolysis in erythrocytes serve as functional markers as both are inhibited by vitamin E [64]. Lipid peroxidation is less specific for vitamin E antioxidative activity, rendering the latter method more reliable [7].

The two intermediate endpoint RCT meta-analyses (Refs [36, 37] in table 1) point out the importance of accurate vitamin E status assessments which significantly influenced the outcome. As most trials assess the vitamin E to lipid ratio, a potential overcorrection might render this effect undetectable in the individual RCTs. Hence, lipid-independent measurements could reveal a stronger correlation between baseline vitamin E status and supplementation. Notably, many studies do not consider the functionality, including all studies in chapter 2 but solely analyse vitamin E presence. However, the discrimination between deficient plasma levels, according to reference values, and truly exhausted antioxidative capacities would help to identify subgroups with a higher vitamin E demand in particular in large RTCs such as the HOPE and Heart Protection Study (Refs [34, 35] in table 1).

In summary, variations in human studies might be promoted by uncertainties in assessment methods for vitamin E intake as well as status. Integration of recent findings suggesting alternative methods including CEHC as intake and lipid-independent vitamin E as status marker might allow the identification of true deficiencies and thus, subgroups with an increased demand for α-TOH supplementation which in turn could reduce the confounding influence.

3.4 Identification of Subpopulations

The individual demand of daily vitamin E is influenced by predeterminant genetic variables. Recently, attention was drawn to the haptoglobin (Hp) genotype.

The circulating, haemoglobin-scavenging antioxidative Hp protein prevents iron-mediated oxidative damage [12]. It is marked by a polymorphism as either the Hp-1 or Hp-2 allele can be expressed. Studies suggest a higher antioxidative capacity by the homozygous Hp 1-1 genotype whereas the Hp 2-2 genotype was correlated with increased susceptibility to cardiovascular diseases ,indicating that Hp 2-2 subgroups might have an increased demand for vitamin E [12]. In line with this, a meta-analysis [65] and a systematic review [66] concluded a more effective vitamin E supplementation in type 2 diabetes patients harbouring the Hp 2-2 allele, reducing the risk for myocardial infarction, stroke and cardiovascular mortality. Hence, this further supports the suggested relation between the Hp 2-2 genotype and vitamin E demand. This is highly apparent in the hard endpoint RCT successfully reducing cardiovascular events in the diabetic type 2, Hp 2-2 population following 400 IU RRR-α-TOH supplementation (Ref [33] in table 1), demonstrating subgroups as another essential factor to consider. Interestingly, no other discussed study screened for the Hp 2-2 polymorphism, which could have allowed significantly positive results for this subgroup such as in the HOPE study [34].

Another potentially important confounder may be the genetic predisposition of HbA1c levels. Diagnosis as well as treatment of type 2 diabetes were suggested to centre around the HbA1c levels as increased plasma concentrations are associated with a disturbed glucose metabolism [67]. As vitamin E was demonstrated to effectively lower circulating HbA1c, screening for genetic factors driving the vulnerability to high Hb1Ac levels might help to identify individuals with impaired glycaemic control as target group for vitamin E supplementation. This is coherent with two RCT meta-analyses in chapter 2.3 (Refs [36, 37] in table 1) reporting that subgroups with initial vitamin E deficiencies and

impaired glycaemic control (*i.e.* high Hb1Ac) are more prone to oxidative stress-induced diabetes type 2 and supplementation is more efficient whereas a more generic analysis was insignificant.
Overall, factors such as genetic diversity have a large influence on the outcome of vitamin E supplementation which might have caused inconclusive results in trials not considering this confounder. This shows the benefit of subgroup analysis, particularly the Hp 2-2 genotype and baseline HbA1c levels.

4 CONCLUSION AND OUTLOOK

4.1 CONCLUSION

Drawing a final conclusion, accumulating evidence from *in vitro* studies to interventional RCTs revealed a strong and relevant association between the vitamin E status, oxidative stress and type 2 diabetes. However, the exact involvement cannot be explained mechanistically yet and despite accumulating evidence demonstrating improved intermediate endpoints following vitamin E treatment, the long-term benefits of implementing vitamin E as prevention or treatment approach remain inconclusive, raising a highly controversial debate in this scientific field.

For the time being, recommendations are confined to vitamin E as potential biomarker for vulnerability to oxidative stress and inflammation, potentially causing diabetes type 2. Interventional trials are necessary to prove a causal relationship and convincing evidence, however, successful results are limited to the influence of vitamin E on diabetes-associated phenotypes such as markers of oxidative stress and inflammation, insulin resistance of HbA1c levels. In spite of these phenotypes contributing to the development and morbidity of diabetes type 2, they cannot be considered as definite cause for disease onset and mortality. Contrarily, many long-term RCTs considering diabetes type 2 incidence and mortality show non-significant or even slightly adverse effects, although the potential toxicity of vitamin E was challenged in following large trials. The lacking availability of sufficient large interventional studies on clinically relevant, hard endpoints does not allow the recommendation of vitamin E as treatment for diabetes type 2 development, progression or mortality so far.

This discrepancy may be explained by several potential confounders. Most importantly is the genetic diversity underlying the diabetic or high-risk population regarding the Hp polymorphism. In contrast to generic large RCTs, a restriction to Hp 2-2 genotypic patients was the only successful trial reducing cardiovascular mortality, rendering subgroup analysing an essential tool to identify significant effects in certain subpopulations following vitamin E supplementation. This further concerns baseline glycaemic control by screening for high HbA1c levels. Hence, trials not considering these confounders might have obtained false negative results. The second main reason is the vitamin E isomerism with respect to the stereochemistry as the bioavailability and activity varies largely between the isoforms. The predominant RRR-α-TOH has been demonstrated to be associated with and significantly impact oxidative stress and diabetes type 2. However, studies often do not specify the exact isoform used which might lead to a dilution or deterioration of potential benefits. Thirdly, the observed duality of vitamin E supplementation could be caused high-dosage induced oxidative properties. This dose-, but also time- and duration-, dependent adversity is accelerated by a concomitant exhaustion of important co-antioxidative agents which is often not respected in clinical trials. Lastly, evaluating the role of dietary vitamin E consumption, from food sources but also supplements, is hampered by uncertainties regarding the accuracy of measuring vitamin intake and status. Most commonly, intake is assessed via dietary questionnaires and status via the circulating α-TOH to total lipids ratio which neglects recent findings suggesting the implementation of more reliable biomarkers such as 24-hour urinal CEHC as intake and erythrocyte membrane α-TOH as status marker.

Overall, strong associations and promising results of the relationship between vitamin E, oxidative stress and diabetes type 2 face the inconclusiveness of several large, high-quality clinical trials reporting no hard endpoint effect, leading to the current, controversial debate about this discrepancy. Certainly, more research is required aiming to identify and resolve confounders to develop a clear picture of the potential of vitamin E in oxidative stress and diabetes type 2 and allow recommendations for the implementation as treatment.

4.2 OUTLOOK

From my point of view, the potential of vitamin E, especially RRR-α-TOH, as treatment for oxidative stress-related diseases such as diabetes type 2 is promising as has been demonstrated on several levels. The discrepancy to successful hard endpoint RCTs needs to be overcome for which the above discussed, potentially confounding factors constitute a promising foundation for future research.

In my opinion, the most critical factor is the screening for and identification of subpopulations which are more vulnerable to oxidative stress and diabetes type 2 and consequently more receptive to vitamin E treatment. I am convinced that a focus on target subgroups, the Hp 2-2 genotype in particular but also impaired glycaemic control, harbours a considerable potential for large interventional trials to obtain significant outcomes. Although approaches in the general diabetic population allow even larger group sizes, this presumably renders significant effects of subpopulations undetectable. Hence, this points towards the suitability of personalized medicine as opposed to generic treatment approaches.

In addition to that, the specific stereoisomers need to be assessed separately to discriminate the biological effects of each compound. For clinical trials it would be advisable to limit the intervention to one stereoisomer and measure the purity of the administered supplement. This allows the deduction of clear and direct isoform-effect relationships. Furthermore, the dosage, duration and time of the intervention should be considered together with the status of co-antioxidants to enable the recycling of α-TOH and prevent the emergence of the pro-oxidative nature and related tissue damage. Lastly, reliable assessment of vitamin E is important to identify deficiencies which correspond to the demand, but also the consumption of vitamin E and related dietary habits. For this, current knowledge on suitability of dietary assessment methods and biomarkers should be implemented in future cohort and interventional studies to reduce the influence of uncertain measurements.

In short, I consider all of these factors highly important in future clinical trials but as the most promising RCT was restricted to Hp 2-2 diabetic patients, a special emphasis should be given on screening for a genetically predetermined vulnerability for oxidative stress-induced diabetes type 2 to specifically target these subgroups.

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