Nutrapharmacology of Tocotrienols for Metabolic Syndrome

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Abstract: Metabolic syndrome is defined as a set of health risk factors that are associated with an increased chance of cardiovascular diseases and type 2 diabetes. These include abdominal obesity, hyperglycemia, impaired glucose tolerance, dyslipidemia, and hypertension. Interventions in metabolic syndrome include lifestyle interventions such as a healthy diet using functional foods together with increased physical activity to induce weight loss as the first aim of treatment. Nutraceuticals such as tocotrienols and tocopherols as members of the vitamin E family may be more targeted interventions. This review evaluates the effects of tocotrienols on the risk factors of metabolic syndrome using data from human, animal and in vitro studies. Tocotrienols improved lipid profiles and reduced atherosclerotic lesions, decreased blood glucose and glycated hemoglobin concentrations, normalized blood pressure, and inhibited adipogenesis. The differences in responses between tocopherols and tocotrienols in preventing obesity, diabetes, hypertension, atherosclerosis, ischemia, and inflammation suggest that different receptors or signaling mechanisms may be involved.

Keywords: Metabolic syndrome, tocotrienols, tocopherols, diabetes, hypertension, atherosclerosis, adipogenesis.

INTRODUCTION

Metabolic syndrome was recognized by the World Health Organization (1998) as the clustering of interrelated risk factors for cardiovascular disease and type 2 diabetes. Clinical definitions have different threshold values, but all emphasize hypertension, dyslipidemia, hyperglycemia, insulin resistance, and abdominal obesity as the main parameters of the syndrome [1-3].

The metabolic syndrome is common in adult populations throughout the world. Based on National Health and Nutrition Examination Survey (NHANES) 2003-06 data, 35.1% of US males and 32.6% of US females aged 20 and older met the criteria for metabolic syndrome [4]. Depending on the definition used, the prevalence of metabolic syndrome ranges between 13.4 and 30.7% in Australian adults [5]. In the Japanese population, 51% of male and 38% of female subjects met WHO criteria for metabolic syndrome [6]. In Chinese men, the prevalence of metabolic syndrome associated with hypertension was 32.9% and 53.1% by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III definition [7]. In an urban Indian population, the NCEP definition included 31.6% with metabolic syndrome, with higher prevalence in women (39.9%) than in men (22.9%) [8].

Metabolic syndrome is linked to increased plasma concentrations of reactive oxidant species, non-esterified fatty acids, oxidized low-density lipoprotein (LDL), and increased abdominal adipose tissue [3, 9]. Adipose tissue is much more than an energy storage depot as it is a complex and dynamic organ, including lipid-filled adipocytes, endothelial cells, pericytes, fibroblasts, preadipocytes, mast cells and immune cells such as resident macrophages and T-cells [10]. Adipocytes and immune cells within adipose tissue release many bioactive molecules, known as adipokines and pro-inflammatory cytokines, to initiate vascular dysfunction, atherosclerosis, and impaired glucose metabolism [9, 11]. Increased production of non-esterified fatty acids (NEFA) by adipocytes inhibits carbohydrate metabolism via substrate competition and impaired insulin signaling [12]. Adipose tissue growth is regulated by angiogenesis; the vasculature responds by increased blood vessel density to supply the growing adipose tissue with nutrients and oxygen [13]. Adipose tissue angiogenesis has been described as a cause of metabolic syndrome since modulators of angiogenesis can regulate the expansion and metabolism of fat mass [13, 14].

Inflammatory lipid mediators such as prostaglandins, thromboxanes, and leukotrienes are involved in many human diseases, including rheumatoid arthritis and chronic inflammatory diseases [15]. These mediators can act as ligands for important immune receptors such as class A G protein-coupled receptors and Toll-like receptors (TLRs), and this receptor activation can initiate and perpetuate an innate immune response [15]. It has been speculated that lipid mediators localized in the circulation and adipose tissue may bind to these immune receptors and induce low-grade tissue inflammation, leading to adipocyte and metabolic dysfunction [16]. In addition, excess accumulation of fat directly disturbs metabolism, altering synthesis and action of metabolic hormones, or gets deposited in liver, heart, muscle and pancreatic beta cells causing lipotoxicity, or initiates specialized extracellular and intra-cellular signaling through lipid-derived mediators leading to systemic inflammation and insulin resistance [17].

Inflammation may play a vital role in metabolic syndrome, causing both insulin resistance and vascular dysfunction [16, 17]. Tumor necrosis factor-α (TNFα) expression in adipose tissue is induced by obesity, hence contributing to systemic insulin resistance [18]. Adipocytes secrete proinflammatory cytokines which are detrimental to the vasculature, with endothelial dysfunction, plaque initiation, plaque progression, and plaque rupture worsening cardiovascular symptoms [19]. Production of proinflammatory cytokines is linked to nuclear factor-κB (NFκB) activation [20]. Activation of NFKB leads to TNFα-induced insulin resistance [21]. Peroxisome proliferator activated receptors (PPARs) may attenuate inflammatory signaling pathways and, as such, interfere with cardiac remodeling via inhibition of NFκB [22].

Dietary changes have been perceived as the first line intervention in metabolic syndrome, targeting insulin sensitivity and preventing or correcting the associated metabolic and cardiovascular abnormalities. Targeting selected components of foods as medications, defined as treatment with functional foods or nutrapharmacology, could provide protection against cardiovascular diseases and diabetes [23-25]. Functional or medicinal foods and phytonutrients are widely accepted for maintaining well-being, enhancing health, and modulating immune function to prevent specific diseases [26, 27]. Vitamin E is a family of closely-related phytochemicals, the tocopherols and tocotrienols, with potential health-
VITAMIN E: TOCOPHEROLS AND TOCOTRIENOLS

Tocopherols and tocotrienols share a common chromanol ring with the tocopherols having a saturated phytyl side chain, differing from the geranylgeranyl side chain with three double bonds in the tocotrienols (Fig. 1). Each group has α-, β-, γ - and δ-homologues.

Vitamin E has been an active research area for nearly a century since the first report as a micronutrient essential for reproduction in rats [28]. Many reviews on the physiological functions and metabolism of vitamin E have been generated [29-35]. The functions of vitamin E remain unclear, although several pathways and modes of action have been suggested [31, 32, 34, 36]. Early research on vitamin E focused on α-tocopherol since α-tocopherol was more abundant in plasma [37, 38] so it was assumed to be the most important vitamin E component in the body. α-Tocopherol functioned as a scavenger of lipid peroxyl radicals, with antioxidant, cell signaling and gene regulatory functions for the prevention of atherosclerosis and other chronic, oxidative stress-induced pathologies in human diseases [39]. The bioactive derivative of α-tocopherol could be α-tocopheryl phosphate, possibly functioning as a cofactor and active lipid mediator involved in signal transduction and gene expression [40]. Clinical studies have shown mixed results on the efficacy of α-tocopherol in the prevention and treatment of heart disease, cancer and Alzheimer’s disease [39]. While in vitro and in vivo studies demonstrated positive antioxidant and anti-atherogenic effects with tocopherols, clinical evidence was inconclusive [41]. Large clinical studies have not demonstrated benefits of tocopherols in the primary and secondary prevention of cardiovascular disease with supplementation possibly associated with increases in total mortality, heart failure, and hemorrhagic stroke [41].

As disappointment with the therapeutic value of the tocopherols grows, it is worthwhile investigating the efficacy of the tocotrienols, as new evidence has shown unique functions of these compounds [33, 34]. Tocotrienols possess neuroprotective, anticancer, and cholesterol-lowering properties that are often not exhibited by tocopherols [34, 42]. α-Tocotrienol, but not α-tocopherol, prevents neurodegeneration at nanomolar concentrations, relevant considering the low plasma concentrations [35]. This suggests that the molecular and therapeutic targets of the tocotrienols are distinct from those of the tocopherols [34].

VITAMIN E PHARMACOKINETICS

Most pharmacokinetic studies have used either α-tocopherol or α-tocopheryl acetate [31, 43–47], on the assumption that this is the most biologically relevant homologue. The absorption of α-tocopherol is often generalized and extrapolated to other homologues [48]. Dietary α-tocopherol is absorbed unesterified in the small intestine, incorporated into chylomicrons and secreted into the intestinal lymph. These chylomicrons are catabolized in the circulation by lipoprotein lipase to form chylomicron remnants [43, 44]. In the liver, chylomicron remnants and α-tocopherol are selectively transferred by α-tocopherol transfer protein (α-TTP) and repacked into nascent very low density lipoproteins (VLDL) by hepatocytes [31, 43]. Tocopherols are secreted with chylomicrons in the presence of microsomal triglyceride transfer protein, lipids and oleic acid. VLDL are then secreted into the blood stream, and undergo lipolysis by lipoprotein lipase becoming LDL and high density lipoprotein (HDL). Both HDL and LDL can readily transfer α-tocopherol to other lipoproteins and hence distribute α-tocopherol to all circulating lipoproteins. Any α-tocopherol that fails to bind to α-TTP will be subject to a series of α-oxidation followed by β-oxidation reactions, with final metabolism to carboxyethyl-6-hydroxychroman (CEHC) and excretion in the urine [48].

This process of absorption and distribution now seems more complex with two independent pathways [49, 50]. Enterocytes can also secrete tocopherol and tocotrienol with HDL, a pathway which is independent of lipids and microsomal triglyceride transfer protein availability [49]. Small HDL can deliver tocotrienols to peripheral tissues independent of α-TTP [48], the transporter which selectively transports α-tocopherol in the liver. Thus, in α-TTP-deficient rats, transport of tocotrienols occurs independently of α-TTP as indicated by tocotrienol accumulation in the brain, heart, adipose tissues, skin, spinal cord, and skeletal muscles [51]. Further, tocotrienol treatment leads to the restoration of reproductive capability in these rats [51]. Tocotrienols were present in all tissues except the brain of hamsters fed palm tocotrienol-rich fractions, with adipose tissues especially rich in tocotrienols [52]. Mouse skins are

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\begin{array}{c|c}
R_1 & R_2 \\
\hline
\text{CH}_3 & \text{CH}_3 & \alpha\text{-Tocopherol} \\
\text{CH}_3 & \text{H} & \beta\text{-Tocopherol} \\
\text{H} & \text{CH}_3 & \gamma\text{-Tocopherol} \\
\text{H} & \text{H} & \delta\text{-Tocopherol} \\
\end{array}
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\[
\begin{array}{c|c}
R_1 & R_2 \\
\hline
\text{CH}_3 & \text{CH}_3 & \alpha\text{-Tocotrienol} \\
\text{CH}_3 & \text{H} & \beta\text{-Tocotrienol} \\
\text{H} & \text{CH}_3 & \gamma\text{-Tocotrienol} \\
\text{H} & \text{H} & \delta\text{-Tocotrienol} \\
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Fig. (1). Structures of tocopherols and tocotrienols.
Tocotrienols and Metabolic Syndrome

The skin is able to accumulate appreciable amounts of tocotrienols, possibly primarily by the actions of lipoprotein lipase on chylomicrons to be deposited in the skin before selection by α-TTP in liver for subsequent distribution [34]. γ-Tocotrienol is accumulated and secreted at greater rates in Caco2 cells than α-tocopherol [50]. When administered 10mg orally to mice, γ-tocotrienol appeared faster in the plasma (peak at 2-4 hours) but at lower concentrations than α-tocopherol (peak at 6-8 hours) [50].

The biological responses of the tocopherols and tocotrienols are likely to be related to the concentrations in the relevant organs, rather than to plasma concentrations. Tocotrienols may produce larger responses than tocopherols [33-35, 42], even though the plasma concentrations of tocotrienols are much lower than tocopherols [37, 38], due to higher intracellular concentrations of tocotrienols relative to plasma concentrations [53]. A docking study of tocotrienols with several proteins involved in cellular transport, including tocopherol-associated protein, human serum albumin, and P-glycoprotein, showed higher binding affinity for tocotrienols compared to tocopherols [54]. In addition to their shared antioxidant activities, tocotrienols have anti-inflammatory [55, 56] and anti-angiogenic activities that are often not exhibited by tocopherols [57, 58]. These activities could play vital roles in attenuating metabolic syndrome; the next section will highlight these activities that have been summarised in Fig. 2.

NUTRAPHARMACOLOGY WITH TOCOTRIENOLS

The risk factors for metabolic syndrome are common and costly. The 2010 Australian Institute of Health and Welfare (AIHW) analysis reports the prevalence of overweight and obesity at 42.2% and 25.4% respectively for all adult Australian men and 31.1% and 23.7% respectively for all adult Australian women [59]. In 2008 alone, the overall cost of obesity to Australian society and governments was estimated to be $58.2 billion, with the total direct financial cost of obesity for the whole Australian community of approximately 22 million people estimated to be $8.3 billion, or around US$360 per person in 2008 [60]. Cardiovascular disease is the highest health-care cost in Australia, with direct costs of $5.94 billion in 2004-5. In 2004-2005, hypertension was reported in 11% of the whole population, with an increase in prevalence from 14% in the 45 to 54 age group to 41% of those aged 75 years and over [61]. The National Health Survey estimated 3.6% of Australians had diagnosed diabetes in 2004-05. Of those people reporting long-term diabetes, 13% had type 1 diabetes, 83% had type 2 diabetes and 4% had an unknown type of diabetes [62].

i. Tocotrienols in Atherosclerosis

Atherosclerosis is a chronic disease caused by the deposition of fatty material such as cholesterol in the innermost layer of endothelium of the large and medium-sized arteries, resulting in atherosclerotic plaque formation. The plaque often grows with time and thus progressively narrows the artery lumen. Narrowing the coronary arteries reduces blood supply to the myocardial tissues, especially at times of increased demand, and angina may ensue [63, 64]. Rupture of the plaque starts a rapid coagulation process leading to blockage of the vessel and myocardial infarction. Hypercholesterolemia is a major contributing factor for the risk of atherosclerosis [65]. Clinical trials have shown that lowering LDL cholesterol with 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors slows the progression of atherosclerosis, reducing morbidity and mortality from coronary artery disease [66].

The anti-inflammatory and antioxidant properties of α-tocopherol were suggested to be important in preventing atherosclerosis [40]. Decreased susceptibility of LDL to oxidation and protection against hepatic lipid peroxidation were shown in vitro and in vivo, preventing atherosclerotic plaque formation in mouse models. However, the majority of clinical studies have not demonstrated benefits of α-tocopherol in preventing atherosclerotic diseases [40].

Fig. (2). Some possible mechanisms for tocotrienols in the metabolic syndrome.
Responses to individual tocotrienols may also differ. Administration of tocotrienol-rich fraction containing all four tocotrienol homologues and α-tocopherol to patients reduced serum total cholesterol [67] and low density lipoprotein cholesterol [67, 68]; γ and δ-tocotrienols reduced triglycerides [69]. Combination of the AHA Step-I diet with either tocotrienol-rich fraction 50 mg or lovastatin 10 mg for 75 days lowered serum total cholesterol and LDL-cholesterol by about 15% in hypercholesterolemic subjects [67]. Additive effects with decreases of around 25% were observed when these doses of tocotrienol-rich fraction and lovastatin were combined [67]. Substitution of tocotrienol-rich fraction with α-tocopherol produced no further changes when given with lovastatin. Serum triglyceride concentrations were lowered by 28% after 8 weeks of treatment with 120 mg/day of γ- and δ-tocotrienols in a placebo-controlled trial in hypercholesterolemic humans [69].

In high-cholesterol and atherogenic diet-fed ApoE^{-/-} mice, 0.05% or 0.2% (w/w) tocotrienol-rich fraction attenuated the development of atherosclerosis by inducing the PPAR target gene, liver X receptor alpha, and its down-stream target genes, apolipoproteins and cholesterol transporters [70]. This effect was independent of antioxidant responses as a study using high triglyceride and cholesterol diet-fed ApoE^{-/-} mice showed insignificant atherosclerotic changes with 0.5% α-tocopherol yet 0.5% and 1.5% tocotrienol-rich fraction reduced lesion size by about 90% [71]. In vitro studies suggested that α-, γ- and δ-tocotrienols act post-transcriptionally in HepG2 cells in culture to lower the HMG-CoA reductase activity, which regulates cholesterol synthesis in liver [69, 72].

**ii. Tocotrienols in Ischemia**

Myocardial injury occurs during ischemia because of decreased oxygen supply to the muscle but also during reperfusion following ischemia (ischemia/reperfusion injury), probably due to an excessive production of reactive oxygen species [73]. Decreased vitamin E content of cardiac tissue could play a major role in the damage caused by myocardial ischemia/reperfusion. Previous studies using in vitro models had examined vitamin E deficiency and ischemia/reperfusion-induced myocardial damage with equivocal results but there was no difference in cardiac performance in ischemia-reperfused heart with or without α-tocopherol supplement [74]. However, α-tocopheryl phosphate ameliorated myocardial ischemic reperfusion injury by converting ischemia/reperfusion-mediated death signal into a survival signal via modulation of MAP kinase signalling, hence cardioprotection [75].

Supplementation of rats with α- or γ-tocotrienol (0.3 mg/kg/day), or tocotrienol-rich fraction (3.5% in food) for 4 weeks improved post-ischemic ventricular function and reduced myocardial infarct size [76]. Aortic flow, left ventricular developed pressure and contractility were markedly higher with tocotrienol treatment compared with control hearts after 30 minutes of global ischemia followed by 2 hours of reperfusion. γ-Tocotrienol increased these parameters more than α-tocotrienol and δ-tocotrienol [76]. In addition, γ-tocotrienol down-regulated phosphorylated c-Src and increased Akt phosphorylation [76]. Increases in phosphorylated PPARγ and c-Src are detrimental to the cell and are associated with pro-death signaling in myocytes while increases in Akt phosphorylation are generally associated with cardiomyocyte survival [76]. In a similar study, tocotrienol-rich fraction (7 g/kg diet) afforded greater protection against ischemia/reperfusion injury in isolated Langendorff hearts than 20g/kg α-tocopherol in the diet [77]. While the protective effects of individual tocotrienols and α-tocopherol were not directly compared, consumption of tocotrienol-rich fraction and α-tocopherol showed that protection by tocotrienol-rich fraction was more pronounced than that of α-tocopherol [77]. The likely mechanisms were complete suppression of lactate dehydrogenase leakage, decrease in ATP and creatine phosphate concentrations in the ischemic heart as well as inhibition of the formation of endogenous peroxide by tocotrienol-rich fraction [77].

**iii. Tocotrienols in Hypertension**

Chronic hypertension is a key risk factor for strokes, heart attacks, and heart failure [34]. The ability of tocopherol to lower blood pressure is inconsistent. While α-tocopherol prevented the increased blood pressure in high-salt diet (8% NaCl)-induced hypertension in Dahl salt-sensitive rats [78], neither α- nor γ-tocopherol ameliorated adrenocorticotropic hormone-induced hypertension in the rat [79].

Tocotrienols are consistently reported to decrease blood pressure. Tocotrienol-rich fraction or γ-tocotrienol depressed age-related increases in systolic blood pressure of Spontaneously Hypertensive Rats (SHR) [80, 81] by elevating total antioxidant status and superoxide dismutase activity with reduced lipid peroxidation [81] and improved endothelium-dependent arterial relaxation [82]. 12 weeks of treatment with γ-tocotrienol (15 mg/kg) normalized blood pressure in SHR [81]. Supplementation with α-tocopherol or tocotrienol-rich fraction (0.2% of diet for 3 weeks) in SHR reduced blood pressure and a similar trend was observed in another study, using α-lipoic acid as a regenerative antioxidant protocol [83]. Treatment with either α-tocopherol or tocotrienol-rich fraction (0.84 g/kg of food for 8 weeks) reversed or prevented the increases in blood pressure in rats with fructose-induced metabolic disorder [83]. No differences were observed between α-tocopherol or tocotrienol-rich fraction diet in this study.

The anti-hypertensive effect may be attributed to the antioxidant activity of these compounds. Acetylcholine-mediated vascular relaxation depends on an intact endothelium and the release of nitric oxide/endothelium-derived relaxing factor [84, 85]. Excess generation of reactive free radical species destroys the endothelium-derived relaxation factors [86], thus impairing vasodilation. This increases peripheral resistance and blood pressure [81]. Free radical scavenging activity as well as prevention of cellular damage by lipid peroxidation of α-tocopherol and tocotrienol-rich fraction may prevent hypertension. In addition, the anti-hypertensive responses to α-tocopherol and tocotrienol-rich fraction could be attributed to increased production of prostacyclin, also preventing thrombosis [80, 87].

**iv. Tocotrienols in Diabetes**

Diabetes involves chronic increased blood glucose concentrations, causing damage to the cardiovascular system. A recent review on α- and γ-tocopherol suggested the chemical similarities of α- and γ-tocopherol and the PPARγ agonists, the thiazolidinediones, possibly leading to upregulation of an endogenous ligand involved in activating PPARγ, hence increased adiponectin expression in 3T3-L1 [88]. Diabetes induces oxidative stress and the resulting damage may be mitigated by treatment with tocopherols [89-91]. Tocopherols appear to protect against macromolecule damage, especially lipid peroxidation, in experimental diabetes. There is also evidence to support a role of tocopherols in protection of the pancreas, kidney, eye, and nervous system against the development of diabetic complications in animals, inhibiting the increase in urinary protein, blood glucose, HbA1c and PAI-1 levels [90]. While relationships between low tocopherol status and increased risk of type 2 [92] and type 1 diabetes [93] were confirmed, other studies showed no association of tocopherol supplementation with type 2 diabetes development [94] or protection [95, 96].

A tocotrienol-rich diet (1g tocotrienol-rich fraction/kg diet for 12 weeks or 200mg/kg b.w. orally for 8 weeks) decreased blood glucose and glycated hemoglobin concentrations in streptozotocin-induced diabetic rats, a model of type 1 diabetes [97, 98]. Tocotrienols exerted these effects by increasing superoxide dismutase activity and vitamin C concentrations in plasma, decreasing malondialdehyde and 4-hydroxynonenal concentrations in plasma and aorta, and decreasing oxidative DNA damage [34]. Tocotrienol-rich fraction increased whole body glucose utilization and improved insulin sensitivity in diabetic db/db mice [99], attributed
to the activation of PPARs. Tocotrienols enhanced the interaction between the purified ligand-binding domain of PPARα and the receptor-interacting motif of coactivator PPARγ coactivator-1α [99].

Hyperglycemia induces an increased interstitial collagen deposition. This has been previously reported in a diet-induced model of type 2 diabetes, the high fructose diet-fed rat, where excess collagen deposition was observed in the perivascular and interstitial areas of the myocardium [100]. Increases in collagen deposition are linked to the excess production of advanced glycation end products (AGEs), as products of nonenzymatic glycosylation in hyperglycemic states [101]. Combined treatment of either α-tocopherol or tocotrienol-rich fraction (0.84g/kg food) with α-lipoic acid (1.6g/kg food) prevented and reversed the increase in ventricular collagen deposition and diastolic stiffness of rats with fructose-induced metabolic disorder [83].

v. Tocotrienols in Adiposity

Previous studies of associations between diet, obesity, and blood concentrations of α-tocopherol have been equivocal. Some previous studies reported negative associations between α-tocopherol concentrations and central adiposity, in both serum [102] and adipose tissue [103], while others observed a positive association between serum α-tocopherol concentration and central adiposity, and a positive association between BMI and serum α-tocopherol concentration in men [103].

Long-term use of glucocorticoid drugs such as dexamethasone will suppress adrenal function and lead to obesity as an adverse effect. An in vivo study showed that oral supplementation of 60 mg/kg γ-tocotrienol decreased body fat of adenectomized rats treated with 240 mg/kg dexamethasone [104]. In the same model, 60 mg/kg/day γ-tocotrienol for 8 weeks decreased fat mass by approximately 25 g/cm² more when compared with 60 mg/kg α-tocopherol [104]. The authors postulated that antioxidant effects were responsible [104]. However, investigations on 3T3-L1 cells in the presence of 1.8 μmol/L insulin suggested that α- and γ-tocotrienols reduced body fat by suppressing adipocyte differentiation and Akt phosphorylation [105]. Tocotrienols suppressed the insulin-induced mRNA expression of adipocyte-specific genes such as PPARγ, adipocyte fatty acid-binding protein, and CCAAT/enhancer binding protein-α (C/EBPα) that are required for adipocyte differentiation. The adipocyte inhibitory effect of γ-tocotrienol was greater (90 ± 2%) than other tocotrienol homologues at the same concentration (2.4 μmol/L) [105]. Tocotrienols have not been studied as anti-obesity agents in whole animal or human studies. It may be worthwhile to explore possible responses as in vivo studies show that tocotrienols accumulate in adipose tissue [51, 52, 106]. In addition, obesity is regulated by angiogenesis [13] and tocotrienols are anti-angiogenic [57, 107].

vi. Tocotrienols in Inflammation

Inflammation is a complex response that protects the body from various harmful agents such as microbes and toxins [108]. Nevertheless, unregulated inflammation is associated with many chronic diseases such as atherosclerosis, cancer, arthritis, and obesity. α-Tocopherol supplementation in human subjects and animal models showed both antioxidant and anti-inflammatory actions defined as decreasing plasma C-reactive protein (CRP) concentrations and release of proinflammatory cytokines, with chemokine IL-8 and PAI-1 concentrations especially at high doses [109]. In clinical studies of asthma, α-tocopherol supplementation of asthmatic patients was beneficial in Italy and Finland but α-tocopherol did not benefit asthmatic patients in the United States or the Netherlands [110]. Clinical reports caution that elevation of tocopherols can increase the incidence of high blood pressure, hemorrhagic stroke, all-cause mortality, or post-trial cerebral infarction [110]. These reports indicate contradictory outcomes for anti-inflammatory functions of α-tocopherol [110].

Tocotrienol-rich fraction and α-, δ-, and γ-tocotrienols inhibited the release of interleukin-6 and nitric oxide, pro-inflammatory cytokines involved in acute and chronic inflammation, in lipoplysaccharide-stimulated RAW264.7 macrophages; γ-tocotrienol was the most effective homologue in most assays [55]. Although this study did not directly examine the effects of tocotrienols on NFκB activity per se, the genes that were examined, such as TNFα, cyclooxygenase-2, and nitric oxide synthases (iNOS), are considered to be NFκB targets; therefore, the biological effects were probably mediated by this transcription factor [20].

The NFκB family of transcription factors is a central player that regulates genes critical for inflammation and immunity. Several independent lines of evidence suggest that tocotrienols block the activation of NFκB [20, 34, 56, 111]. Human myeloid KBM-5 cells incubated with 25 μM γ-tocotrienol for 24 hours did not activate NFκB, and TNFα-induced NFκB activation was almost maximally abolished at 12 h in contrast to the lack of response to α-tocopherol [56]. Suppression of the NFκB pathway will eventually lead to a lower expression of pro-inflammatory enzymes and cytokines [55]. In addition, combined treatment with subeffective doses of γ-tocotrienol (0.25 μM) and the cyclo-oxygenase 2 inhibitor, celecoxib (2.5 μM), resulted in a synergistic effect to downregulate NFκB, with reduced prostaglandin E2 (PGE2) synthesis, and decreased cyclo-oxygenase 2, phospho-Akt, and phospho-NFκB concentrations [111]. These mechanisms are summarised in Fig. 3.

vii. Tocotrienols in Angiogenesis

Development of obesity is associated with substantial modulation of adipose tissue structure, involving adipogenesis, angiogenesis, and extracellular matrix remodeling. Thus, adipose tissue development stimulates blood vessel formation, and in turn endothelial cells in adipose tissue promote pre-adipocyte differentiation [13]. Excessive angiogenesis may promote abdominal fat development [13, 14], thus decreased angiogenesis should impair adipose tissue development. TNP-470 is a synthetic analog of fumagillin, which selectively inhibits endothelial cell growth and angiogenesis [112]. Treatment with TNP-470 20 mg/kg for 16 weeks to mice fed with high fat diet reduced total body fat by 63% compared with controls [113].

Studies on the anti-angiogenic properties of tocotrienols have been undertaken in cancer [57, 107, 114]. δ-Tocotrienol suppressed the phosphorylation of phosphoinositide-dependent protein kinase (PDK) and Akt, and increased the phosphorylation of apoptosis signal-regulating kinase and p38 in fibroblast growth factor-treated human umbilical vein endothelial cells, indicating that the anti-angiogenic effects of tocotrienols are associated with changes in growth factor-dependent phosphatidylinositol-3 kinase/PDK/Akt signaling as well as induction of apoptosis in endothelial cells [58]. It was postulated that the anti-angiogenic effects of tocotrienols occur upstream of the phosphatidylinositol-3 kinase/PDK/Akt signaling pathway via the suppression of FGF receptor tyrosine phosphorylation by tocotrienols [58]. It may be worthwhile to explore the anti-angiogenic effects in adipose tissue as in vivo studies show tocotrienol accumulation [51, 106].

FUTURE DIRECTIONS OF TOCOTRIENOL RESEARCH

Tocopherols and tocotrienols share close structural similarity (Fig. 1) and both vitamin E families scavenged free radicals and increased superoxide dismutase activity [81]. This antioxidant activity could underlie the prevention of the increase in blood pressure [80, 83] and blood glucose concentrations [83] and the normalised cardiac stiffness [83] following administration of α-tocopherol and tocotrienol-rich fractions. However, tocotrienols may be more potent antioxidants in vivo than α-tocopherol, since α-tocotrienol appeared superior due to its better distribution in the fatty layers of...
Fig. (3). Mechanisms by which tocotrienols could suppress hyperglycemia and inflammation.

the cell membrane [115]. Thus, tocotrienols may exhibit a greater magnitude of antioxidant activity than tocopherols [116].

While tocopherols and tocotrienols are antioxidant vitamins, tocotrienols seem to have unique biological activities. Unlike tocopherols, tocotrienols reduced serum total cholesterol and LDL-cholesterol [67, 68] through regulation of fatty acid metabolic genes such as PPAR and HMG-CoA [69, 72]. Protection against ischemic heart disease was shown with both tocotrienol-rich fraction and α-tocopherol but it was more pronounced with tocotrienol-rich fractions [77]. This could be attributed to lactate dehydrogenase leakage suppression [77] and down-regulation of phosphorylated c-Src and increased phosphorylated Akt [76]. Tocotrienols activate PPAR, adipocyte fatty acid-binding protein, and C/EBPα [99]. This enables tocotrienols to inhibit adipogenesis [99] and decrease fat mass more than tocopherols [104]. Furthermore, the anti-angiogenic activity of tocotrienols [57, 107] may enhance the fat mass reduction. Tocotrienols exhibited anti-inflammatory activity by preventing the activation of NFκB but tocopherols did not [56]. These selective responses to tocotrienols by anti-inflammatory, anti-adiposity and anti-angiogenesis mechanisms suggest that tocotrienols activate different receptors or signaling pathways than α-tocopherol. This would justify further research on the tocotrienols, partly to redress the comment that only 1% of vitamin E research is on the tocotrienols [42].

A further justification for more research on the tocotrienols is the relative failure of the clinical trials with the tocopherols. Several large randomized controlled clinical trials on α-tocopherol acetate were unsuccessful in translating positive data in animal studies into improved human health. As an example, the Heart Outcomes Prevention Evaluation (HOPE-TOO) concluded that α-tocopherol acetate did not prevent cancer or major cardiovascular events [117]. In addition, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study showed an increased incidence of hemorrhagic stroke with α-tocopherol acetate and β-carotene co-supplementation and showed no benefits with α-tocopherol acetate supplementation on coronary artery disease [118]. These results have led to criticisms of the study design, subject recruitment health status, dose, route of administration, subject compliance, duration of treatment, toxicity, suppression effect of α-tocopherol on other homologues, and type of vitamin E homologues used [42, 63, 119]. A reductionist approach to these negative results in clinical trials would simply be that α-tocopherol acetate has minimal therapeutic effects in humans. However, this conclusion would only relate to α-tocopherol or α-tocopherol acetate as other homologues, especially the tocotrienols, have not been tested. Differences between vitamin E homologues and other signaling mechanisms [120] are plausible given the differences in biological actions outlined in previous sections. These possible differences between the tocopherols and tocotrienols also suggest that the term "vitamin E" is useful to describe the family of compounds but ambiguous and uninformative in describing the biological actions of the family members. Thus, all research studies on tocopherols and tocotrienols should emphasize the compound that has been tested and not generalize as actions of vitamin E.

There are several differences which may account for higher potency of tocotrienols than α-tocopherol. Structural differences may allow tocotrienols to be more uniformly distributed in the lipid bilayer. The unsaturated side chain of tocotrienols allows for more efficient penetration into tissues compared with all saturated side-chain of α-tocopherol [30, 115]. Apart from that, α-tocopherol has cellular uptake 70 times higher than that of α-tocopherol [121]. Therefore, further studies with tocotrienols are necessary despite the ineffectiveness of tocopherol supplements.

DISCLOSURES

The authors have no conflicts of interest.

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