REVIEW

Molecular mechanisms of membrane transport of vitamin E

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Vitamin E is an essential fat-soluble micronutrient for higher mammals and functions as an antioxidant for lipids and also as a regulator of gene expression and a modulator of cell signaling and proliferation. To exert its physiological functions, vitamin E must achieve an appropriate disposition throughout the body *via* several processes, such as intestinal absorption, uptake and efflux in peripheral tissues and biliary secretion. In this review, we mainly discuss membrane proteins involved in these transport processes (ATP-binding cassette transporter A1, scavenger receptor class B type I, Niemann-Pick C1-like 1 and multidrug resistance 3) and vitamin E-mediated regulation of their expression.

Keywords:
Biliary secretion / Ezetimibe / Intestinal absorption / Transporter / Vitamin E

1 Introduction

Vitamin E is a group of lipophilic micronutrients composed of four tocopherols and four tocotrienols (Fig. 1A). Besides its well-known function as an antioxidant for preventing peroxidative damage to lipids in cell membranes and on lipoprotein surfaces, several other important biological functions have been demonstrated, such as the regulation of gene expression and modulation of cell signaling and proliferation [1].

To carry out its physiological roles, the distribution of vitamin E in the whole body, *i. e.* dietary intake, transfer between blood and tissues, metabolism (ω-hydroxylation, β-oxidation and conjugation) and excretion from the body, must be adequately regulated. Besides the well-characterized importance of α-tocopherol transfer protein (α-TTP) in cytosol and of plasma phospholipid transfer protein, both of which are responsible for the homeostasis of vitamin E levels in the body [2, 3], recent research has revealed that several membrane proteins play crucial roles in these transport processes. The main focus of this review is on proteins involved in the membrane transport of vitamin E and their vitamin E-mediated regulation.

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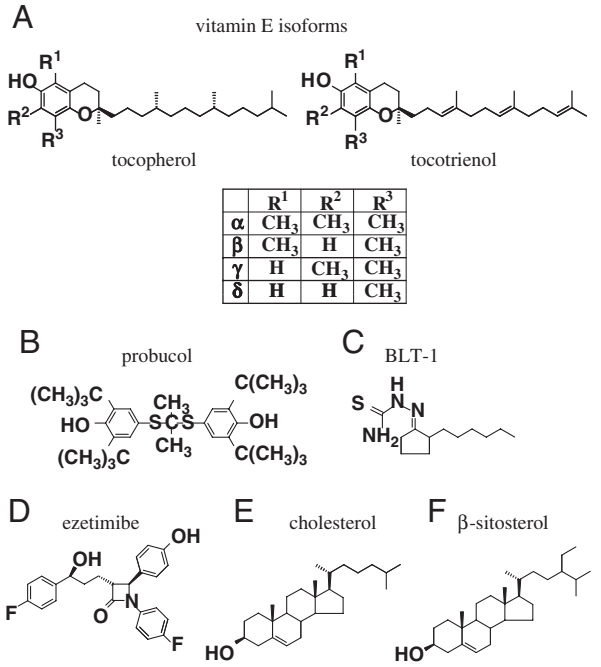
Abbreviations: ABCA1, ATP-binding cassette transporter A1; **apoA-I,** apolipoprotein A-I; **BHK cells,** baby hamster kidney cells; **MDR3,** multidrug resistance 3; **NPC1L1,** Niemann-Pick C1-like 1; **PXR,** pregnane X receptor; **SR-BI,** scavenger receptor class B type I; **α-TTP,** α-tocopherol transfer protein

2 ATP-binding cassette transporter A1

ATP-binding cassette transporter A1 (ABCA1) is known to be a crucial factor for the biogenesis of HDL and loss of its function results in Tangier disease, which is associated with a high risk of arteriosclerosis secondary to a deficiency of plasma HDL [4–6]. ABCA1 functions as an ATP-dependent efflux transporter for cholesterol and phospholipids on the basolateral membranes of various tissues and requires an acceptor protein, apolipoprotein A-I (apoA-I), to form premature HDL particles [7, 8].

In Abca1-deficient mice [9], in addition to a severely diminished plasma HDL-cholesterol level, the plasma concentration of vitamin E also falls to an undetectable level, which suggests that ABCA1 is important for vitamin E homeostasis. However, it was unclear how (directly transported or secondary to promotion of HDL formation)



	R^1	R^2	R^3
α	CH_3	CH_3	CH_3
β	CH_3	H	CH_3
γ	H	CH_3	CH_3
δ	H	H	CH_3

Figure 1. Chemical structures of vitamin E isoforms (A), probucol (B), BLT-1 (C), ezetimibe (D), cholesterol (E) and β-sitosterol (F).

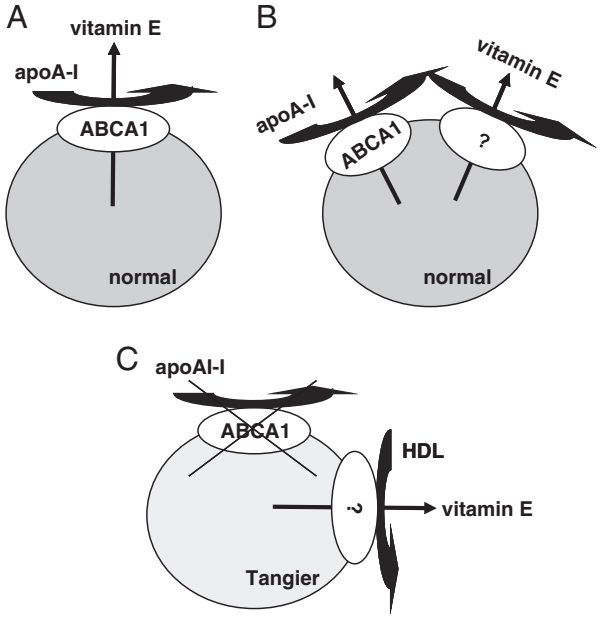


Figure 2. Hypothetical scheme of ABCA1-mediated transport of vitamin E. To determine whether ABCA1 mediates the transport of vitamin E directly (A) or ABCA1 produces apoA-I-originated acceptors of vitamin E for an unknown ABCA1-independent process (B), pre-incubated medium was transferred from ABCA1-transfected cells to untransfected control cells [10]. As the transferred medium did not stimulate the efflux of α-tocopherol from the control cells, ABCA1 is suggested to transport vitamin E to apoA-I directly. Fibroblasts derived from Tangier disease patients lack the apoA-I-driven vitamin E transport *via* ABCA1, whereas HDL promotes α-tocopherol efflux even from Tangier disease fibroblasts (C) [10], suggesting that an HDL-driven transport pathway is present in Tangier disease patients. These results show that ABCA1 contributes to the transport of vitamin E *via* initiation of HDL formation as well as *via* direct transport.

ABCA1 contributes to the transport of vitamin E and the site/organ at which ABCA1 predominantly affects the *in vivo* disposition of vitamin E.

In 2001, Oram *et al.* [10] provided direct evidence of ABCA1-mediated transport of α-tocopherol (Fig. 2). They showed that apoA-I-mediated efflux of α-tocopherol was absent in fibroblasts derived from Tangier disease patients and that overexpression of ABCA1 in baby hamster kidney (BHK) cells clearly increased the efflux of α-tocopherol to apoA-I. As the transfer of medium from ABCA1-transfected BHK cells to untransfected BHK cells did not promote α-tocopherol efflux, it is likely that ABCA1 participates directly in the transport of α-tocopherol from cells to apoA-I, not *via* generating acceptors of α-tocopherol for an unknown ABCA1-independent process. However, it was also demonstrated that the addition of HDL to the medium stimulated the cellular efflux of α-tocopherol even from Tangier disease fibroblasts, suggesting that the contribution of ABCA1 to α-tocopherol transport takes place also as secondary to HDL formation. As HDL particles have the ability to extract cellular α-tocopherol in the absence of ABCA1, the presence of other transporter(s) capable of transporting α-tocopherol to HDL is also indicated.

It has been suggested that hepatic efflux of vitamin E involves α-TTP, a cytosolic protein that is highly expressed in hepatocytes and maintains vitamin E homeostasis *via* cellular storage and trafficking of vitamin E [2]. Although it was reported that α-TTP overexpression in McARH7777 cells resulted in the facilitation of the cellular efflux of α-tocopherol [11], Shichiri *et al.* [12] recently demonstrated that ABCA1 is involved in this α-TTP-mediated process. The efflux of α-tocopherol from the α-TTP-overexpressing cells was stimulated by the addition of extracellular apoA-I in a concentration-dependent manner. In addition, this efflux was inhibited by probucol (Fig. 1B), which inactivates ABCA1 by preventing its binding to apoA-I, and also inhibited by the introduction of siRNA against ABCA1 mRNA. Furthermore, the plasma concentration of α-tocopherol in mice was reduced to approximately 10% by administration of probucol for several weeks. These results suggest that ABCA1 is essential for the hepatic efflux of α-tocopherol in conjunction with α-TTP. Intestinal absorption of γ-tocopherol in Abca1-deficient mice was one-fourth that of control mice, suggesting that ABCA1 plays a role also in the small intestine [13].

In addition to the role of ABCA1 in the transport of vitamin E, it has been suggested that vitamin E is involved in the transcriptional regulation of ABCA1. Transcriptional regulation of the ABCA1 gene has been extensively studied [14] because of its potential as a pharmacological target for

dyslipidemia. In the presence of pregnane X receptor (PXR) agonists, ABCA1 expression (mRNA and protein) and the transcriptional activity of the ABCA1 promoter were significantly decreased in HepG2 cells [15]. Because most vitamin E isoforms can function as PXR ligands [16], ABCA1 may be transcriptionally down-regulated by vitamin E *via* a PXR-mediated pathway, which has yet to be directly demonstrated. It was also shown in *in vitro* experiments that treatment with α-tocopherol reduced the transcriptional activity of liver X receptor α, an essential nuclear receptor for cholesterol homeostasis, and consequently reduced the expression level of ABCA1 [17]. However, the physiological significance of the PXR- and liver X receptor α-mediated down-regulation of ABCA1 expression and the relationship between these regulations remain unknown.

3 Scavenger receptor class B type I

Scavenger receptor class B type I (SR-BI) is also involved in vitamin E transport. SR-BI was initially identified as an oxidized LDL receptor [18] but is now predominantly known as a high-affinity HDL receptor on the basolateral membrane [19]. However, because of its wide tissue distribution and expression on the apical membrane under certain conditions [20, 21], SR-BI is believed to have many physiological functions in the body, including vitamin E membrane transport.

SR-BI-deficient mice were reported to have an abnormal disposition of α-tocopherol [22]. Although the plasma concentration of α-tocopherol was increased by more than twofold in SR-BI-deficient mice, α-tocopherol concentrations in the ovary, testis, lung and brain were reduced by more than 50%. This evidence, together with the finding that forced expression of SR-BI induced cellular uptake of HDL α-tocopherol [22], indicates that SR-BI functions as an acceptor of α-tocopherol from plasma HDL to tissues. On the other hand, despite an unaltered α-tocopherol level in the liver of SR-BI-deficient mice, the α-tocopherol concentration in bile was reduced by approximately 80%, suggesting that SR-BI is involved in the biliary secretion of α-tocopherol.

It has also been suggested that SR-BI is involved in intestinal absorption of vitamin E [23]. The bioavailability of γ-tocopherol was significantly (2.7-fold) higher in intestine-specific SR-BI transgenic mice than in wild-type mice. This observation is consistent with the fact that vitamin E uptake into Caco-2 TC7 cells was sensitive to SR-BI antibody and BLT-1 (Fig. 1C), a chemical inhibitor of lipid transport that acts *via* SR-BI [23]. SR-BI was also shown to contribute to apical secretion of α-tocopherol in Caco-2 TC7 cells [23], which implies the bidirectional transport of vitamin E *via* the SR-BI-mediated pathway. Taking the occasional expression of SR-BI on the bile canalicular membrane [20] into consideration, it is possible that SR-BI mediates the biliary secretion of vitamin E. Collectively, these results indicate that SR-BI hastens both intestinal absorption and biliary secretion of vitamin E, which may accelerate its enterohepatic circulation.

Vitamin E-mediated regulation of SR-BI has also been suggested. Treatment of HepG2 cells with agonists of PXR, which recognize most vitamin E isoforms as ligands [16], reduced the promoter activity and expression of SR-BI [15], which is similar to the regulation of the ABCA1 gene. Moreover, it was also demonstrated that α-tocopherol regulates the expression of SR-BI protein post-transcriptionally [24]. When Wistar rats were fed α-tocopherol-depleted chow over 38–40 days, an 11-fold increase in the expression of SR-BI protein was observed in the liver, which was partly reversed by feeding them α-tocopherol-enriched chow for 2 days. Hepatic SR-BI mRNA level remained constant, indicating that α-tocopherol exerted its effect at a post-transcriptional level. In addition, similar results were obtained using HepG2 cells, and an association between protein kinase C signaling and the α-tocopherol-mediated post-transcriptional regulation of SR-BI was reported [24]. Assuming that SR-BI functions as an importer of vitamin E [22], the existence of α-tocopherol-mediated negative feedback regulation of SR-BI expression seems reasonable to control cellular vitamin E level, although the exporter function of SR-BI [23] complicates the discussion. Intracellular localization of SR-BI and/or direction of SR-BI-mediated transport of vitamin E may be regulated by intracellular levels of vitamin E, as cholesterol loading induces movement of basolateral SR-BI to the apical bile canaliculus [21].

4 Niemann-Pick C1-like 1

Niemann-Pick C1-like 1 (NPC1L1) is essential for the intestinal absorption of cholesterol [25] and is recognized as a pharmacological target of ezetimibe (Fig. 1D) [26], a cholesterol absorption inhibitor clinically used for the treatment of dyslipidemia. In addition to cholesterol (Fig. 1E), phytosterols have been shown to be transported *via* the NPC1L1-mediated pathway [27, 28] and ezetimibe has been approved for the treatment of sitosterolemia, a hereditary disorder in which high plasma concentrations of phytosterols such as β-sitosterol (Fig. 1F) are present.

As ABCA1 transports both cholesterol and vitamin E [10], we assumed the NPC1L1-mediated transport of vitamin E and it was clearly demonstrated that NPC1L1 mediates the cellular uptake of vitamin E [29]. Based on the hypothesis that some lipophilic molecules may be taken up in the intestinal lumen *via* a pathway shared with cholesterol, some fat-soluble vitamins and drugs were tested using NPC1L1-overexpressing Caco-2 cells. Although the uptake of retinol (vitamin A) and cyclosporin A was not sensitive to ezetimibe, α-tocopherol transport was increased by the overexpression of NPC1L1 and inhibited by ezetimibe in a concentration-dependent manner. In addition, nonsynonymous variants of NPC1L1 found from cholesterol low

absorbers (A395V, G402S, R417W and G434R) showed similarly reduced transport activities for both cholesterol and α-tocopherol [30], suggesting that the recognition and transport of α-tocopherol did not differ from that of cholesterol among the four variants and wild type of NPC1L1. Moreover, the intestinal absorption of α-tocopherol was significantly inhibited by ezetimibe in short-term experiments with Wistar rats, suggesting that α-tocopherol is absorbed, at least partly, *via* the NPC1L1-dependent pathway *in vivo* [29].

The inhibitory effect of ezetimibe on the uptake of other isoforms of vitamin E in NPC1L1-overexpressing cells was also examined *in vitro* (Fig. 3). The uptake of all of the vitamin E isoforms tested was higher in NPC1L1 cells and was significantly reduced by ezetimibe. This result is consistent with the well-known fact that the different behavior among isoforms of vitamin E is not caused by differences in intestinal absorption [31] but by the higher affinity binding of α-TTP for α-tocopherol compared with that for the other isoforms [32]. The results that serum concentrations of vitamin E were not significantly altered by 12-wk administration of ezetimibe in a clinical study [33] may be explained also by the fact that the maintenance of vitamin E homeostasis is generally mediated *via* α-TTP-mediated storage and transfer [2], not *via* intestinal absorption.

Since the expression of NPC1L1 in the liver of mice is lower than in humans, transgenic mice were generated for the liver-specific expression of human NPC1L1 [34]. In these mice, human NPC1L1 was expressed on the canalicular membrane of hepatocytes and biliary secretion of cholesterol was drastically reduced, which suggest that NPC1L1-dependent reabsorption of cholesterol from bile is present in humans. Considering the substrate specificity of NPC1L1, some of vitamin E secreted into bile may be reabsorbed by NPC1L1 to prevent excessive loss.

Regulation of NPC1L1 expression by a membrane protein CD36 has also been reported. CD36 is involved in the uptake of substrates including fatty acids and oxidized lipoproteins [35], and increased expression of NPC1L1 protein was observed in the small intestine of Cd36-null mice [36]. As α-tocopherol-induced down-regulation of CD36 is supposed to be one of the important beneficial effects of α-tocopherol for the prevention of atherosclerosis [37, 38], the proposed regulation of NPC1L1 *via* CD36-related signaling may play an important role in maintaining the

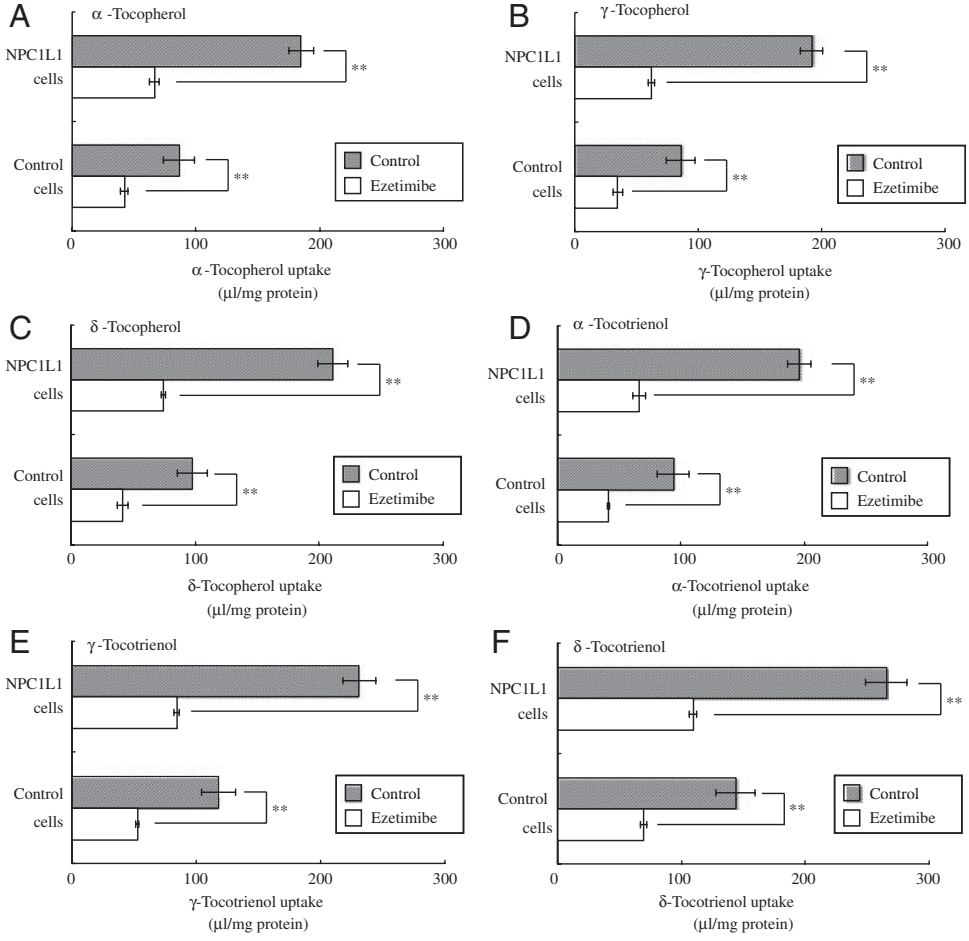


Figure 3. NPC1L1-mediated and ezetimibe-sensitive transport of vitamin E isoforms. The uptake of α-tocopherol (A), γ-tocopherol (B), δ-tocopherol (C), α-tocotrienol (D), γ-tocotrienol (E) and δ-tocotrienol (F) by control and human NPC1L1-overexpressing Caco-2 cells (NPC1L1 cells) was examined for 120 min in a medium containing 2 mM taurocholate, 50 μM phosphatidylcholine, 1 μM cholesterol and 10 μM vitamin E isoforms in the presence and absence of 40 μM ezetimibe as described previously [28, 29]. For quantification of vitamin E isoforms, UV detection was performed at 292 nm for α-tocopherol and α-tocotrienol, and at 298 nm for γ-tocopherol, δ-tocopherol, γ-tocotrienol and δ-tocotrienol after separation by ultra-performance liquid chromatography (Waters Corporation, Milford, MA, USA). Each column and horizontal bar represents the mean ± SD ($n = 3$). *Significantly different by Student's *t*-test ($p < 0.05$). **Significantly different by Student's *t*-test ($p < 0.01$).

homeostasis of both lipids and vitamin E. In addition, the expression of SR-BI mRNA was dramatically reduced in NPC1L1-knockdown Caco-2 cells [39], suggesting the mutual complicated regulations of vitamin E-associated membrane proteins.

5 Multidrug resistance 3

Multidrug resistance 3 (MDR3/ABCB4) plays an important role in the secretion of phosphatidylcholine from the canalicular membrane of hepatocytes into bile to form mixed biliary micelles of adequate composition. A deficiency of MDR3 results in a severe hereditary disorder, progressive familial intrahepatic cholestasis type 3, the symptoms of which are caused by toxic effects of bile salts on hepatocytes and cells lining the biliary tract [40, 41]. Mutations in the MDR3 gene also increase the risk of other liver diseases, such as adult biliary cirrhosis, low phospholipids-associated cholelithiasis syndrome, transient neonatal cholestasis and intrahepatic cholestasis of pregnancy [42].

Besides phosphatidylcholine, biliary secretion of α-tocopherol was also reduced in mdr2 (the counterpart of human MDR3) knockout mice [43]; the bile concentration of α-tocopherol in mdr2 knockout mice was approximately 25% of that in wild-type mice. Although the significance of biliary secretion of vitamin E in the homeostasis of this vitamin has not been clarified, it was proposed in the report [43] that α-tocopherol in bile may prevent peroxidation of phospholipids, which may be involved in the formation of cholesterol gallstones [44]. As α-tocopherol has also been detected in human bile [45], biliary secretion of α-tocopherol may also depend on an MDR3-mediated process in humans. However, it remains to be clarified whether mdr2/MDR3 mediates vitamin E secretion directly or indirectly *via* the formation of biliary micelles.

6 Concluding remarks

In this review, transmembrane proteins mediating membrane transport of vitamin E were discussed (Fig. 4). In contrast to the clear difference in the affinity of α-TTP for vitamin E isoforms [32], the recognition properties of the membrane proteins seem similar to each isoform of vitamin E. It should also be noted that vitamin E and cholesterol have common transport processes despite their dissimilar structures (Figs. 1A and E). Considering its role as an antioxidant for lipids such as cholesterol, an association between vitamin E and cholesterol transport seems reasonable. These findings have potential implications also for the development of drugs for controlling plasma cholesterol levels. Nowadays, several types of anti-dyslipidemic drugs have been developed or are under development, the molecular targets of which are cholesterol transporters [46–48]. We have to be aware that the treatment with these drugs may affect the behavior of vitamin E.

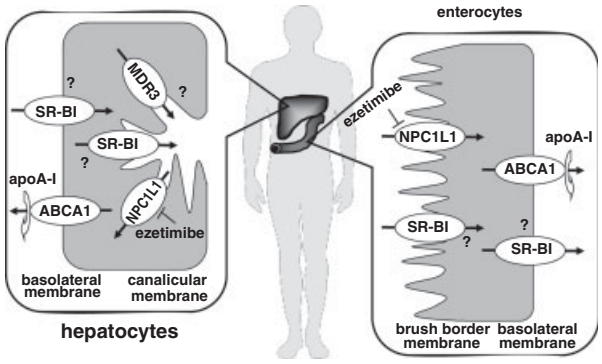


Figure 4. Membrane proteins mediating the transport processes of vitamin E in hepatocytes and enterocytes. Recent research has revealed that several transmembrane proteins play crucial roles in the appropriate disposition of vitamin E, such as ABCA1, SR-BI, NPC1L1 and MDR3/ABCB4.

The involvement of membrane proteins has been demonstrated in several processes, such as intestinal absorption (ABCA1, SR-BI and NPC1L1), uptake (SR-BI) and efflux (ABCA1 and SR-BI) in peripheral tissues and biliary secretion (SR-BI and MDR3), and these transport processes should have important roles to exhibit the various functions of vitamin E. However, the physiological and pathophysiological contribution of each protein to these processes and the precise kinetics of vitamin E in the body are still largely unknown, especially in humans. Detailed and matching analyses of each transport process using knockout or transgenic animals of these genes, preferably with tissue-specific genetic modifications, and clinical research into the effects of their genetic variations on the kinetics and efficacy of vitamin E in humans should allow great progress in this field. By also focusing on the transport characteristics of vitamin E derivatives, such as α-tocopheryl phosphate [49, 50] and 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman (γ-CEHC, γ-tocopherol and γ-tocotrienol metabolite with natriuretic activity) [51], elucidation of these subjects will contribute to a better understanding of the physiology of vitamin E.

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