

**Special Contribution**

## Alpha-Tocopherol Transfer Protein ( $\alpha$ -TTP): Insights from Alpha-Tocopherol Transfer Protein Knockout Mice\*

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### Abstract

Alpha-tocopherol transfer protein ( $\alpha$ -TTP) is a liver cytosolic transport protein that facilitates  $\alpha$ -tocopherol ( $\alpha$ -T) transfer into liver secreted plasma lipoproteins. Genetic defects in  $\alpha$ -TTP, like dietary vitamin E deficiency, are associated with infertility, muscular weakness and neurological disorders. Both human and  $\alpha$ -TTP deficient ( $\alpha$ -TTP<sup>-/-</sup>) mice exhibit severe plasma and tissue vitamin E deficiency that can be attenuated by sufficient dietary  $\alpha$ -T supplementations. In this review, we summarize the literature concerning studies utilizing the  $\alpha$ -TTP<sup>-/-</sup> mice. Levels of vitamin E in the  $\alpha$ -TTP<sup>-/-</sup> mice do not appear to be directly related to the amounts of dietary  $\alpha$ -T or to the levels of  $\alpha$ -TTP protein in tissues. The  $\alpha$ -TTP<sup>-/-</sup> mice appear to present a good model for investigating the specific role of  $\alpha$ -T in tissue vitamin E metabolism. Furthermore,  $\alpha$ -TTP<sup>-/-</sup> mice appear to be useful to elucidate functions of  $\alpha$ -TTP beyond its well recognized functions of transferring  $\alpha$ -T from liver to plasma lipoprotein fractions.

**Key Words:**  $\alpha$ -tocopherol,  $\alpha$ -tocopherol transfer protein, vitamin E

### Introduction

Vitamin E is a generic term which describes a group of lipophilic chain breaking antioxidants in biological membranes and includes 4 tocopherols ( $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -) and 4 tocotrienols ( $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -) (Traber, 1999). Vitamin E plays an important role in reproduction and in maintaining the structural integrity of the cells (Evans & Bishop, 1922; Packer, 1991).  $\alpha$ -tocopherol ( $\alpha$ -T) is the dominant isoform found in human and animals. Indeed, in the 2000 Dietary Reference Intakes, only  $\alpha$ -T was recommended as meeting human dietary vitamin E requirements (Food and Nutrition Board & Institute of Medicine, 2000).  $\alpha$ -T functions additionally beyond its well recognized antioxidant role of smooth muscle cells. For example,  $\alpha$ -T inhibits proliferation via a protein kinase C-dependent mechanism (Boscoboinik *et al.*, 1991a; Boscoboinik *et al.*, 1991b; Ricciarelli *et al.*, 1998). However,  $\alpha$ -T functions in most tissues remain to be fully clarified.

In humans,  $\alpha$ -T deficiency occurs as a result of genetic defects in the hepatic  $\alpha$ -tocopherol transfer protein ( $\alpha$ -TTP) and causes a rare neurological disease, ataxia with vitamin E deficiency (AVED) (Ouahchi *et al.*, 1995). Subsequently,  $\alpha$ -TTP knock out ( $\alpha$ -TTP<sup>-/-</sup>) mice were shown to exhibit many of the same phenotypes as humans with AVED (Yokota *et al.*, 2001). In the human disease the neurologic abnormalities are a result of the

$\alpha$ -T deficiency as neurologic manifestations can be ameliorated by a greatly augmented oral vitamin E intake. However,  $\alpha$ -TTP functions remain to be completely clarified, especially in the few non-hepatic tissues where it has been detected. Although  $\alpha$ -T enrichment of plasma is mediated by liver  $\alpha$ -TTP, the  $\alpha$ -TTP protein may also modulate tissue  $\alpha$ -T levels (Arita *et al.*, 1995; Traber & Arai, 1999). The molecular mechanisms of how  $\alpha$ -T levels are regulated in different tissues and the extent to which  $\alpha$ -TTP interrelate to these mechanisms, likewise remains incompletely understood. The following review will provide a summary of general characteristics and gene profiles of  $\alpha$ -TTP<sup>-/-</sup> mice, and regulation of plasma and tissue levels of  $\alpha$ -T manipulated by dietary  $\alpha$ -T in  $\alpha$ -TTP<sup>-/-</sup> mice.

#### *$\alpha$ -T transfer protein*

$\alpha$ -TTP is a 32 kDa cytosolic lipid binding protein known as a member of the *cis*-retinal binding motif sequence (CRAL-TRIO) protein family (Panagabko *et al.*, 2003).  $\alpha$ -TTP was first described in 1975 by Catignani *et al.* (1975) as a hepatic  $\alpha$ -T binding protein.  $\alpha$ -TTP is expressed primarily in liver, but has been detected in various tissues including lung, spleen, kidney, brain, adrenals, uterus (pregnant rodent) and placenta (Copp *et al.*, 1999; Gohil *et al.*, 2004; Hosomi *et al.*, 1998; Jishage *et al.*, 2001; Kaempf-Rotzoll *et al.*, 2003; Kaempf-Rotzoll *et al.*, 2002).

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All forms of dietary vitamin E are absorbed and transported via chylomicrons through lymph into the circulation (Arita *et al.*, 1995). Unlike non-specific absorption of vitamin E,  $\alpha$ -TTP mediates the selective transport of  $\alpha$ -T in its *RRR*- or *2R*- forms into nascent very low density lipoproteins (VLDL) and is responsible for secretion of  $\alpha$ -T from the liver into blood to maintain plasma  $\alpha$ -T concentrations (Arita *et al.*, 1995).

Liver  $\alpha$ -TTP has been isolated and its cDNA sequences reported from a variety of species. The human gene has been localized to the 8q13.1-13.3 region of chromosome 8 (Arita *et al.*, 1995; Doerflinger *et al.*, 1995).  $\alpha$ -TTP has been crystallized and the  $\alpha$ -T binding pocket identified (Meier *et al.*, 2003; Min *et al.*, 2003).  $\alpha$ -TTP has a hinge and a cover that closes entrapping  $\alpha$ -T in the binding pocket that causes  $\alpha$ -T to fold such that the 2-position is critical for its fit (Meier *et al.*, 2003; Min *et al.*, 2003).

$\alpha$ -TTP has ligand specificity and relative affinities towards different tocopherols ( $\alpha$ ->  $\beta$ ->  $\gamma$ ->  $\delta$ -T) *in vitro* (Hosomi *et al.*, 1997). Tocopherol binding affinity and transfer activity of  $\alpha$ -TTP and ligand selectivity discriminating physiological factor for  $\alpha$ -T retention were also demonstrated *in vitro* models (Morley *et al.*, 2004; Panagabko *et al.*, 2003; Panagabko *et al.*, 2002). However, binding affinity of  $\alpha$ -TTP to  $\alpha$ -T and roles of  $\alpha$ -TTP beyond incorporation of  $\alpha$ -T to very low density lipoproteins (VLDL) were not demonstrated in various tissues from *in vivo* models.

A recent study has shown that high concentration of hepatic  $\alpha$ -tocopherol caused by high  $\alpha$ -T injection increased hepatic  $\alpha$ -T concentrations but didn't induce either hepatic  $\alpha$ -TTP mRNA and protein levels in rats (Mustacich *et al.*, 2006). The result suggests that hepatic  $\alpha$ -T levels doesn't regulate  $\alpha$ -TTP gene expression.

To elucidate the importance of vitamin E *in vivo*,  $\alpha$ -TTP gene deletion leading to vitamin E deficiency was required due to difficulty of dietary depletion of vitamin E in humans and animals (Cuddihy *et al.*, 2004).  $\alpha$ -TTP<sup>-/-</sup> mice were generated by targeted disruption of  $\alpha$ -TTP (Jishage *et al.*, 2001; Terasawa *et al.*, 2000).

#### *Infertility in females with $\alpha$ -T deficiency*

Vitamin E was first recognized as a "reproductive factor" in 1922 by Evans and Bishop (Evans and Bishop, 1922). Impaired fertility, such as resorption of the fetus, was reported in female  $\alpha$ -TTP<sup>-/-</sup> mice (Jishage *et al.*, 2001; Terasawa *et al.*, 2000) while no signs of male infertility were detected (Terasawa *et al.*, 2000). In pregnant  $\alpha$ -TTP<sup>-/-</sup> mice, placental failure was effectively abrogated by dietary  $\alpha$ -T (567 mg/kg diet) or a synthetic antioxidant (BO-653) (Jishage *et al.*, 2001). Female rats with vitamin E deficiency demonstrated structural changes of ovary and uterus and abnormal estrus cycle (Das and Chowdhury, 1999).  $\alpha$ -T levels increased in the placental membrane as gestation progressed during fetoplacental development in normal humans and rodents (Jishage *et al.*, 2001; Qanungo *et al.*, 1999; Sen & Mukherjee, 1998). Moreover,  $\alpha$ -TTP was not expressed in the placenta but in the uterus to supply  $\alpha$ -T for placenta and

fetus during the development of placental labyrinthine trophoblasts in mice (Jishage *et al.*, 2001). These results suggest that  $\alpha$ -TTP is a critical fertility factor to provide enough  $\alpha$ -T for the maintenance of a regular estrous cycle and protection of placenta and fetus during pregnancy. It should be noted that mice and humans have very different placental structures and that  $\alpha$ -TTP has been detected in human placenta (Muller-Schmehl *et al.*, 2004).

#### *Neurological disorders in $\alpha$ -T deficiency*

Vitamin E deficiency is associated with neurological abnormalities in humans and animals suggesting  $\alpha$ -T's importance in the central nervous systems. The  $\alpha$ -TTP mRNA expression was demonstrated in cerebral cortex, especially Purkinje cells and in cerebellum (Hosomi *et al.*, 1998) possibly to protect the brain from oxidative stress by providing  $\alpha$ -T (Meier *et al.*, 2003). AVED patients with genetic defects of  $\alpha$ -TTP had very low or undetectable plasma  $\alpha$ -T levels and progressive spinocerebellar dysfunctions (Hoshino *et al.*, 1999; Hosomi *et al.*, 1998; Ouahchi *et al.*, 1995; Yokota *et al.*, 1997; Yokota *et al.*, 2000).

Although specific discrimination of  $\alpha$ -T by  $\alpha$ -TTP was shown in *in vitro* models (Hosomi *et al.*, 1997; Panagabko *et al.*, 2003; Panagabko *et al.*, 2002) some human  $\alpha$ -TTP mutants showed  $\alpha$ -T transfer activity similar to that of wild type protein suggesting that  $\alpha$ -TTP may have additional physiological functions *in vivo* (Morley *et al.*, 2004). Therefore, we should consider the importance of an *in vivo* animal model to investigate effects of  $\alpha$ -T in AVED and other possible genetic disorders in inbreeding mice as well.

#### *Gene profiles related to neurodegeneration*

$\alpha$ -TTP<sup>-/-</sup> mice showed many features of neurological diseases as seen in humans with AVED. Specifically, the mice with ataxia had very low plasma and brain  $\alpha$ -T levels (Yokota *et al.*, 2001). Gene profile studies in  $\alpha$ -TTP<sup>-/-</sup> cortex identified putative genes affected by genetic deletion of  $\alpha$ -TTP and may account for neurological disorders (Gohil *et al.*, 2004; Gohil *et al.*, 2003). These studies showed down-regulation in  $\alpha$ -TTP<sup>-/-</sup> cortex of genes related to neurogenesis and increased expression of genes related to neurodegeneration. The authors suggested that some of the affected genes may be regulated by retinoic acid receptor related orphan receptor  $\alpha$ . In female  $\alpha$ -TTP<sup>-/-</sup> cerebral cortex, selective genes, such as myelin associated oligodendrocyte specific protein and proteolipid protein, associated with synaptogenesis and myelination were repressed. Induction of neurodegenerative genes, such as synulein- $\alpha$ , repression of synulein- $\beta$  and induction of mitochondrial proapoptotic factor, cytochrome c, were also shown in  $\alpha$ -TTP<sup>-/-</sup> cerebral cortex (Gohil *et al.*, 2004; Gohil *et al.*, 2003). Although apparent neurological signs were not reported in 3-4 months old mice (Gohil *et al.*, 2003; Jishage *et al.*, 2001; Terasawa *et al.*, 2000; Yokota *et al.*, 2001),

**Table 1.** Relationship of plasma ( $\mu\text{mol/l}$ ) and tissue  $\alpha\text{-T}$  (nmol/g) and dietary  $\alpha\text{-T}$  in  $\alpha\text{-TTP}^{-/-}$  mice

Dietary $\alpha\text{-T}$ (mg/kg)	Feeding Period (wks)	Plasma ( $\mu\text{mol/l}$ )		Tissue $\alpha\text{-T}$ concentrations (nmol/g)										Reference.
				Liver		Lung		Heart		Cortex		Cerebellum		
		TTP+/+	TTP-/-	TTP+/+	TTP-/-	TTP+/+	TTP-/-	TTP+/+	TTP-/-	TTP+/+	TTP-/-	TTP+/+	TTP-/-	
99	27	~7	<0.1	~58	~20	~28	~2	~30	~3.5	~30**	<0.1**	-	-	(Terasawa <i>et al.</i> , 2000)
0	43	~0.9	<0.5	-	-	-	-	~0.9	<0.2	~1.2	<0.2	~1.6	<0.2	(Yokota <i>et al.</i> , 2001)
36		~4.4	-	-	-	-	-	~25.5	-	~26.7	-	~13.5	-	
600		-	~3.5	-	-	-	-	-	~20.9	-	~1.8	-	~2.6	
36	8	9.3	<0.1	-	-	-	-	-	-	-	-	-	-	(Jishage <i>et al.</i> , 2001)
600		16.3	4.7	-	-	-	-	-	-	-	-	-	-	
60*	12	21.3 $\pm$ 0.5	1.2 $\pm$ 0.3	122.4 $\pm$ 6.4	47.7 $\pm$ 17.7	62.9 $\pm$ 3.7	6.1 $\pm$ 2.1	74.1 $\pm$ 2.5	6.1 $\pm$ 1.5	48.3 $\pm$ 3.1**	0.9 $\pm$ 0.1**	-	-	(Leonard <i>et al.</i> , 2002)
35	11-15	4.5 $\pm$ 0.8	0.3 $\pm$ 0.0	13.0 $\pm$ 4.0	6.1 $\pm$ 2.2	-	-	-	-	24.0 $\pm$ 2.5	0.3 $\pm$ 0.1	-	-	(Gohil <i>et al.</i> , 2003)
66	~14	4.3 $\pm$ 0.6	0.3 $\pm$ 0.1	25.9 $\pm$ 3.3	8.8 $\pm$ 0.9	21.1 $\pm$ 2.6	2.3 $\pm$ 0.3	-	-	-	-	-	-	(Schock <i>et al.</i> , 2004)
0	5	0.5 $\pm$ 0.1	0.1 $\pm$ 0.1	1.0 $\pm$ 0.5	0.2 $\pm$ 0.2	2.0 $\pm$ 0.8	0.5 $\pm$ 0.1	1.3 $\pm$ 0.3	0.7 $\pm$ 0.1	3.4 $\pm$ 0.6	0.1 $\pm$ 0.4	3.7 $\pm$ 1.2	1.1 $\pm$ 0.1	(Schock <i>et al.</i> , 2004)
35		4.4 $\pm$ 0.8	0.4 $\pm$ 0.1	13.1 $\pm$ 3.2	6.0 $\pm$ 1.6	13.8 $\pm$ 3.3	1.4 $\pm$ 0.3	16.0 $\pm$ 4.5	1.9 $\pm$ 0.5	8.8 $\pm$ 2.1	0.7 $\pm$ 0.1	6.6 $\pm$ 0.8	3.4 $\pm$ 0.6	

\* 30mg d6-RRR- $\alpha$ -tocopherol and 30 mg d3 all rac- $\alpha$ -tocopherol per kg high polyunsaturated fat diet

\*\* whole brain

neurological behaviors such as balance, coordination, strength, exploratory behavior and anxiety were affected in  $\alpha\text{-TTP}^{-/-}$  mice fed a  $\alpha\text{-T}$  deficient or a normal diet up to at age of 6 months (Gohil *et al.*, 2003; Jishage *et al.*, 2001; Terasawa *et al.*, 2000; Yokota *et al.*, 2001). Learning and memory skills were significantly decreased in old female  $\alpha\text{-TTP}^{-/-}$  mice at age of 12 months old (Gohil *et al.*, 2004). However, by the age of 18 months  $\alpha\text{-TTP}^{-/-}$  mice fed normal  $\alpha\text{-T}$  diets developed abnormalities gradually while those fed deficient diets had obvious ataxia (Yokota *et al.*, 2001). These differences in response along with the slow progression of abnormalities might be due to different dietary  $\alpha\text{-T}$  concentrations and/or the duration of feeding, as well as different genetic backgrounds, ages and genders of mice (Table 1). The results suggest that the combination of genetic  $\alpha\text{-TTP}$  defect (or deficiency) and dietary  $\alpha\text{-T}$  deficiency would exacerbate neurodegeneration caused by prolonged  $\alpha\text{-T}$  deficiency. The neurological abnormalities were prevented or attenuated when high dietary  $\alpha\text{-T}$  was supplemented to  $\alpha\text{-TTP}^{-/-}$  mice, as well as to patients with AVED (Mariotti *et al.*, 2004; Schuelke *et al.*, 2000; Yokota *et al.*, 2001; Yokota *et al.*, 1997). Recent studies in phospholipid transfer protein (PLTP) deficient mice having brain  $\alpha\text{-T}$  concentrations at higher levels than in apparently normal  $\alpha\text{-TTP}^{-/-}$  mice demonstrated vitamin E deficiency-related anxiety but did not show any evidence of neurological disorders, including neuromotor coordination (Desrumaux *et al.*, 2005). It is likely that there will be tissue specific responses that are also dependent on  $\alpha\text{-T}$  concentrations in various brain region .

#### Gene profiles related to vasculogenesis, signal transduction pathways and cell proliferation

The gene profile studies also suggested that deletion of  $\alpha\text{-TTP}$

leading to  $\alpha\text{-T}$  deficiency causes tissue specific change of genes associated with vasculogenesis, signal transduction pathways and cell proliferation (Gohil *et al.*, 2003; Vasu *et al.*, 2007). In a  $\alpha\text{-TTP}^{-/-}$  liver, changes in genes associated with neovascularization including quaking, NMDA receptor regulated gene 1 and hyaluronidase I were found. In addition, cell proliferation pathways such as growth factor binding proteins, mitogen activated protein kinase 3 and apoptosis inhibitor 6 were activated in  $\alpha\text{-TTP}^{-/-}$  liver (Gohil *et al.*, 2003). Moreover, genes related to signal transduction such as casein kinase 1, doublecortin and calcium/calmodulin-dependent protein kinase-like 1 were downregulated in heart gene profile study of  $\alpha\text{-TTP}^{-/-}$  mice (Vasu *et al.*, 2007).

Taken together, the gene profile studies demonstrated that a  $\alpha\text{-TTP}^{-/-}$  mice are a useful model to find target genes related to neurological disorders or neurodegeneration which could be prevented or attenuated by dietary vitamin E treatment and vasculogenesis, signal transduction pathways and cell proliferation. Further gene profile studies identifying gender differences in different feeding regimen (time and dose) at different age and verifying reversibility of the neurological symptoms are recommended for future application of dietary  $\alpha\text{-T}$  in patients with AVED.

#### Oxidative stress, inflammatory and immune response related markers

Vitamin E is a widely accepted antioxidant and a potent anti-inflammatory agent.  $\alpha\text{-TTP}$  mRNA level was decreased in rat liver exposed to hypoxia (Ban *et al.*, 2002).  $\alpha\text{-TTP}$  deletion increased levels of lipid peroxidation measured by thiobarbituric acid reactive substances (TBARS) and 4-hydroxy-2-nonenal (HNE) in brain and by malondialdehydes in plasma (Terasawa

*et al.*, 2000; Yokota *et al.*, 2001).  $\alpha$ -TTP deletion with vitamin E deficiency increased another lipid peroxidation biomarkers demonstrated by 8-iso-prostaglandin F<sub>2a</sub> (8-iso-PGF<sub>2a</sub>) and hydroxyoctadecadienoic acid (tHODE) in retina and retinal pigment epithelium (RPE) fraction (Tanito *et al.*, 2007). Moreover, F<sub>2</sub>-isoprostanes levels in aortic lesions were increased in  $\alpha$ -TTP<sup>-/-</sup>ApoE<sup>-/-</sup> mice, which are atherosclerosis susceptible and vitamin E deficient, when compared to that of  $\alpha$ -TTP<sup>+/+</sup>ApoE<sup>-/-</sup> mice (Terasawa *et al.*, 2000).  $\alpha$ -TTP<sup>-/-</sup> mice challenged with lipopolysaccharide affected the inflammatory markers including heme oxygenase -1, inducible nitric oxide synthetase expression and nitric oxides (NOx) release in lung and liver (Schock *et al.*, 2004).

Additionally, protein oxidation (protein carbonyl) in lung lavage fluid, lung and liver and protein kinase C activity were increased in polymorphonuclear cells of  $\alpha$ -TTP<sup>-/-</sup> mice. However, levels of vitamin C and urate in lung tissues remained unchanged in  $\alpha$ -TTP<sup>-/-</sup> mice (unpublished data).

As a supportive evidence, genes regulated by antioxidant response elements such as metallothionein II and glutathione-S-transferase  $\pi$  were selectively induced in  $\alpha$ -TTP<sup>-/-</sup> liver. However, classical antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase were not induced in  $\alpha$ -TTP<sup>-/-</sup> liver, lung and heart (Gohil *et al.*, 2003; Oommen *et al.*, 2007; Vasu *et al.*, 2007). It was similar patterns shown in lung tissues of  $\alpha$ -TTP<sup>+/+</sup> mice fed an vitamin E deficient diet (Gohil *et al.*, 2004) as shown in the lung tissues of  $\alpha$ -TTP<sup>-/-</sup> mice (Gohil *et al.*, 2007). Interestingly, genes involved in detoxification of xenobiotics (cytochrome p450 1a2 and 2d9) were induced in  $\alpha$ -TTP<sup>-/-</sup> liver and heart (Gohil *et al.*, 2003; Vasu *et al.*, 2007).

Vit E has been known as a regulator of immune functions and vit E deficiency modulates immune responses (Beharka *et al.*, 1997). The immunoglobulin genes including immunoglobulin kappa chain variable 8, immunoglobulin joining chain, immunoglobulin heavy chain, complement factor D were down-regulated (Vasu *et al.*, 2007).

These results suggest that  $\alpha$ -TTP may play a role in modulation of inflammatory-immune responses and antioxidant systems. Moreover,  $\alpha$ -TTP<sup>-/-</sup> mice are mildly susceptible to oxidative stress and might use other indirect antioxidant defense systems instead of classical antioxidant defense mechanism. Therefore,  $\alpha$ -TTP<sup>-/-</sup> mice are a practical model to discriminate antioxidant and anti-inflammatory functions  $\alpha$ -T and to identify biomarkers of oxidative stress and inflammation in various disease states.

#### *The relationship between dietary $\alpha$ -T and plasma or tissue $\alpha$ -T*

$\alpha$ -TTP is the primary determinant of plasma  $\alpha$ -T levels (Gohil *et al.*, 2003; Jishage *et al.*, 2001; Leonard *et al.*, 2002; Schock *et al.*, 2004; Terasawa *et al.*, 2000; Yokota *et al.*, 2001). Plasma and tissue  $\alpha$ -T levels were very low in  $\alpha$ -TTP<sup>-/-</sup> mice fed  $\alpha$ -T deficient or normal diets but with vitamin E supplementation

concentrations were comparable to those of wild-type mice fed normal diets (Table 1). During chylomicron catabolism vitamin E is transported to other lipoproteins bypassing the sorting function of  $\alpha$ -TTP in the liver (Traber *et al.*, 1990). If vitamin E intakes are sufficiently high, then  $\alpha$ -TTP is not necessary to maintain plasma  $\alpha$ -T concentrations.

Liver  $\alpha$ -T levels in  $\alpha$ -TTP<sup>-/-</sup> mice were 34-47% of those of wild-type mice when fed normal diets (30-150 mg/kg) (Table 1). Brain  $\alpha$ -T concentrations in  $\alpha$ -TTP<sup>-/-</sup> mice were less than 10% of those of wild-type mice fed normal diets (Traber *et al.*, 2005). In long term (38 weeks) vitamin E deficient wild-type mice, the brain retained approximately 50% of its  $\alpha$ -T while liver was approximately 95% deficient (Cuddihy *et al.*, 2004; Traber *et al.*, 2005). Interestingly, liver  $\alpha$ -T in  $\alpha$ -TTP<sup>-/-</sup> mice on normal diets was relatively well-maintained compared to wild-type mice with dietary vitamin E deficiency. This might be due to non-specific transport of  $\alpha$ -T from gut to liver. Our group reported that  $\alpha$ -T modulates cytochrome p 450 system especially, cyp3a, which is responsible for xenobiotic metabolism and is regulated by pregnane X receptor (Goodwin *et al.*, 2002; Traber *et al.*, 2005). This regulatory role of  $\alpha$ -T in metabolizing toxic substances may be the reason that the liver maintains relatively high  $\alpha$ -T levels compared to other organs. The results also suggest that  $\alpha$ -TTP<sup>-/-</sup> mice are more susceptible to neurological disorders because brain  $\alpha$ -T levels are lower in  $\alpha$ -TTP<sup>-/-</sup> mice compared to dietary vitamin E deficient wild-type mice.

Lung, spleen, kidney and heart  $\alpha$ -T levels in  $\alpha$ -TTP<sup>-/-</sup> mice were very low (only 10-15%) compared with those of wild-type mice fed normal diets (Leonard *et al.*, 2002; Traber *et al.*, 2005). In chow (vitamin E, ~99 mg/kg)-fed mice for 27 weeks,  $\alpha$ -T levels of  $\alpha$ -TTP<sup>-/-</sup> liver, adipose tissue, adrenal gland and aorta were 15-35% of those of wild type mice (Terasawa *et al.*, 2000).  $\alpha$ -T levels were undetectable in the plasma (<0.46  $\mu$ mol/l) and tissues (<0.2 nmol/g) of  $\alpha$ -TTP<sup>-/-</sup> mice when fed  $\alpha$ -T deficient diet for ~43 weeks. After  $\alpha$ -T supplementation (600 mg/kg diet),  $\alpha$ -T levels in  $\alpha$ -TTP<sup>-/-</sup> mice increased to 70-90% of wild-type levels in the plasma (~3.5  $\mu$ mol/l) and heart (~21 nmol/g) but were slightly increased in the cerebrum cortex (6.8%), cerebellum (19.6%), and spinal cord (23.8%) as compared to those of wild type mice fed normal diets (Yokota *et al.*, 2001). Another study showed selective transport of RRR- or 2R-  $\alpha$ -T from 1:1 mixture of natural and synthetic  $\alpha$ -T by  $\alpha$ -TTP and relatively higher plasma and tissue  $\alpha$ -T levels compared to those of other studies in both  $\alpha$ -TTP<sup>-/-</sup> and wild-type mice; these latter diets were high in polyunsaturated fat (Leonard *et al.*, 2002).

Our group also demonstrated tissue specific gene expression profiles in 5 different tissues including liver, lung, brain, adrenal and spleen of  $\alpha$ -TTP<sup>-/-</sup> mice (Gohil *et al.*, 2004). The number of genes which are sensitive to  $\alpha$ -TTP in tissues was not correlated with levels of  $\alpha$ -T in the same tissues. Additionally, the levels of  $\alpha$ -TTP expressed in tissues are not directly correlated with the levels of  $\alpha$ -T (unpublished data). Each tissue might specifically preserve  $\alpha$ -T for its own functions i.e. spleen

and thymus for immune functions, plasma and liver for maintenance of homeostasis and lung and skin for protection from environmental insults such as ozone. In the plasma, the distribution of  $\alpha$ -T to tissues is related to plasma levels of low density lipoprotein (LDL) and high density lipoprotein (HDL) which deliver  $\alpha$ -T to tissues (Traber *et al.*, 1992). Thus,  $\alpha$ -T levels in plasma are dependent on plasma concentrations of these lipoproteins (Mardones *et al.*, 2002). Scavenger receptor class B1 type (SR-B1) binds HDL, then mediates selective lipid uptake from HDL (Trigatti *et al.*, 2000). SR-B1 knockout mice showed significant decreases in  $\alpha$ -T levels in various tissues including liver, spleen and kidney. SR-B1 may be responsible for tissue  $\alpha$ -T levels by regulating  $\alpha$ -T influx into cells (Mardones *et al.*, 2002). These results suggest that the levels of lipoproteins and SR-B1 may be responsible for regulation of plasma and tissue  $\alpha$ -T concentrations.

Variability of  $\alpha$ -T levels shown in the same tissues treated with normal diets may be due to the dietary form (natural or synthetic) of  $\alpha$ -T, the amount of dietary fat in diets and/or the duration of dietary treatments. With  $\alpha$ -T supplementation plasma and tissue levels of  $\alpha$ -T in  $\alpha$ -TTP<sup>-/-</sup> mice on the diets containing different levels of  $\alpha$ -T showed considerable variability suggesting that these differences are likely to be tissue specific.

Since there are still limitations to evaluate the relationship between dietary  $\alpha$ -T levels and tissue  $\alpha$ -T concentrations, and the roles of  $\alpha$ -TTP in different tissues further studies in this animal model are recommended.

## Conclusions

$\alpha$ -TTP<sup>-/-</sup> mice are characterized by abnormalities that can be reversed, prevented or attenuated by high  $\alpha$ -T supplementation. Additionally, specific genes sensitive to  $\alpha$ -TTP were discussed. Plasma and tissue  $\alpha$ -T levels in  $\alpha$ -TTP<sup>-/-</sup> mice reflected dietary  $\alpha$ -T levels in tissue specific manner.

These results emphasize the benefit of  $\alpha$ -T supplementation in vitamin E deficiency caused by genetic defects of  $\alpha$ -TTP in humans (AVED). However,  $\alpha$ -TTP expression in various tissues leads to questions such as tissue specific  $\alpha$ -T requirements and other  $\alpha$ -TTP functions beyond binding and transferring  $\alpha$ -T in cells.  $\alpha$ -TTP<sup>-/-</sup> mice combined with dietary vitamin E deficiency appear to present an ideal model for investigating the specific roles of  $\alpha$ -T and even other vitamin E analogues in different tissues. Furthermore,  $\alpha$ -TTP<sup>-/-</sup> mice would be a good genetic model to identify genes associated with  $\alpha$ -T in various diseases and for dissociating antioxidant functions of  $\alpha$ -T from its non-antioxidant functions.

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