REVIEW

Dietary α-tocopherol and neuromuscular health: Search for optimal dose and molecular mechanisms continues!

Kishorchandra Gohil, Vihas T. Vasu and Carroll E. Cross

Department of Internal Medicine, Genome and Biomedical Sciences Facility, University of California, Davis, CA 95616, USA

Received: November 27, 2009
Revised: January 14, 2010
Accepted: January 15, 2010

Rodents fed α-tocopherol (αT)-depleted diets develop neuromuscular deficits. Unequivocal role of αT in the prevention of these deficits is confounded by possible neurotoxic oxidant products generated, *ex vivo* in αT-depleted diets. The discovery that large doses of αT could ameliorate neuromuscular deficits, attributed to very low serum αT caused by mutations in either the microsomal triglyceride transfer protein or the αT-transfer protein (αTTP), underscores the necessity of αT for neuromuscular health in humans. The discovery of human αTTP provided physiological relevance to biochemical data from rodents documenting αT–binding transfer protein, expressed exclusively in liver. The cloning of αTTP gene and the creation of αTTP-knockout mice allowed to achieve severe systemic αT deficiency in brain and muscles, possibly at birth, eliminating the possible confounding effects of *ex vivo*-generated oxidant products in vitamin E-stripped diets. αTTP-knockout mice have proven useful models to discover αT-regulated phenotypes and molecular actions of αT *in vivo*. The results suggest that anti-oxidant and non-antioxidant actions of αT *in vivo* may not be mutually exclusive. These studies also suggest that low levels of dietary αT can achieve in excess of nanomolar αT levels in tissues and maintain normal neuromuscular functions. This is consistent with biochemical and crystallographic data of α-TTP and of other αT-binding proteins that have dissociation constants in nanomolar range. Molecular mechanisms that cause a long delay for the development of deficiency symptoms remain enigmatic. It is likely that αT is metabolically stable in post-mitotic neurons and myocytes and, if it undergoes redox-cycling *in vivo*, a large repertoire of αT-regenerating systems maintains its biological activity before it is totally depleted.

Keywords:
Ataxia / Antioxidant / Sarcolipin / TTP-KO / Vitamin E

1 Introduction

α-Tocopherol (αT) has been touted as an essential micro-nutrient for a broad spectrum of physio-pathological processes that include fertility, aging, cardiovascular and pulmonary health, cancers and, cognitive and neuromuscular functions. Of these, the predominant non-controversial vitamin function is the prevention of female sterility [1] attributed to failure in the development of placenta [2]. This action is primarily assigned to αT, the most abundant isoform of the eight-member vitamin E family in mammals [3].

Similar to the requirement of αT for rodent fertility, experimental evidence for physiological functions of αT in the prevention of neuromuscular dysfunction is unequivocal. This review surveys some of the initial research in rodents describing the development of neuromuscular dysfunction caused by feeding rodents αT-depleted diets during their pre-weaning and post-weaning periods. Interpretation of the contribution of vitamin E to the health of the neuromuscular system in these experiments has been complicated by the methods used to deplete vitamin E from

Correspondence: Professor Kishorchandra Gohil, Department of Internal Medicine, Genome and Biomedical Sciences Facility, University of California, Suite 6510, Davis, CA 95616, USA
E-mail: kgohil@ucdavis.edu
Fax: +1 530 752 8632

Abbreviations: αT, α-tocopherol; **αTTP,** αT-transfer protein; **αTTP-KO,** αTTP-knockout; **AVED,** ataxia with vitamin E deficiency; **CNS,** central nervous system; **ESR,** electron spin resonance; **GSH,** glutathione; **GSSG,** oxidized glutathione; **HGF,** hepatocyte growth factor; **MTP,** microsomal triglyceride transfer protein; **SEPs,** somatosensory-evoked potentials

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

the diet. For example, lipid oxidation products generated *ex vivo* in rodent αT-depleted diets have not been fully characterized [4] and they exhibit toxic actions *in vivo* to the neuromuscular system [5]. It is possible that the uncharacterized presence of lipid and protein oxidation products in vitamin E-depleted diets could potentially contribute to many of the observations documented in rodents fed vitamin E-depleted diets.

This review describes research aimed to identify the nutritional and genetic basis of vitamin E deficiencies associated with ataxias in humans. The observations that mutations in the gene encoding microsomal triglyceride transfer protein (MTP) can cause neuromuscular deficits [6–8] that can be ameliorated by large doses of αT in the diet was a significant discovery relevant to the importance of αT for human health. Later, the discovery of very low αT in ataxic patients with normal MTP led to the postulation of a hepatocyte αT-binding protein [9] and to the discovery of the α-TTP gene [10]. The description of human neuromuscular syndromes that could be attributed to mutations in a hepatic gene that caused systemic αT deficiency, in spite of normal dietary αT intake [10], is a significant landmark in vitamin E research.

The identification of mutations in the human α-TTP gene [10] and cloning of the rat αT-transfer protein (αTTP) gene [11] led to the development of a mouse model of αT deficiency [12, 13] by homologous recombination technology [14]. This represents another significant milestone in vitamin E research because severe systemic αT deficiency can be obtained in extra-hepatic tissues, particularly in brain and muscles, without the need for chemically ''stripping'' vitamin E from oils used as source of fats in rodent diets, thereby avoiding any spontaneous and undesirable productions of lipid peroxidation products. The current review concludes with a survey of research that has utilized αTTP-knockout (αTTP-KO) mice to test possible antioxidant actions of αT in mouse models of Parkinson's Disease [15], Alzheimer's Disease [16, 17] and ataxia [13]. A key outcome of these studies in mice suggests that dietary αT is required in very small and possibly catalytic amounts (< 5 mg/Kg diet). Similar suggestions were stated in 1943 [18] and in 1987 [19], before the discoveries of αT-binding proteins. This is consistent with some of the more recently described kinetic properties of α-TTP and other members of vitamin E-binding proteins that have dissociation constants in the nanomolar range and these αT-binding proteins are expected to be saturated with αT in tissues obtained from rodents fed ''basal'' diets containing up to 90 mg αT/Kg diet (Fig. 1).

Members of αT-binding proteins belong to the CRAL-TRIO protein family [20]. One exception is afamin, which belongs to the albumin gene family [21]. Mutations in three genes, αT transfer protein, phospholipid transfer protein and caytaxin, appear to display age-related ataxia in mice [22] and humans [23, 24]. This suggests that actions of these diverse biomolecules converge on molecular pathways that affect tissue-specific genome expressions and cause very similar neuromuscular phenotypes. Experiments that were designed to identify αT-sensitive, genome-wide changes in mRNA expression of muscles [25–27] and cerebral cortex [28–30] show distinct and phenotype specific changes in gene expression profiles. It is remarkable that these tissues do not express αTTP, the best characterized αT-binding protein [20, 31], suggesting that other αT-binding molecules transduce changes in αT levels. The identity of tissue-specific, αT-sensitive molecular networks that orchestrate gene expression and change cellular composition and functions remain to be characterized. This goal is feasible with the availability of αTTP-KO mice, ''omic'' technologies and bioinformatics tools.

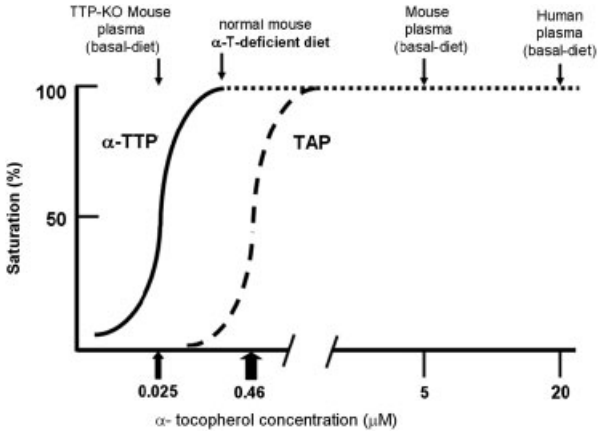

Figure 1. αTTP and tocopherol associated proteins (TAPs) are expected to be saturated with αT in normal rodents and humans with minimal supplementations (1–30 mg αT/Kg diet). Figure constructed from data obtained from several reports [29, 80, 158, 163, 166].

2 Nutritional myopathy and ataxia in animal models of dietary vitamin E deficiency

Experimental evidence in support for the role of substances designated by the term vitamin E in muscle function was assembled in a 1943 review [18]. Extensive necrosis of voluntary muscles from suckling rats, in the absence or presence of central nervous system (CNS) lesions, was described in rats fed vitamin E-depleted diets. Similar lesions of skeletal muscles were described in rabbits, guinea pigs, sheep, goats, the tree kangaroo, hamsters and ducks. However, some caution should be exercised in assigning deficiency in vitamin E alone as to the only contributory factor in the causation of these skeletal muscle lesions. The diets used for these experiments were likely to contain oxidative products, due to the lack of anti-rancidity actions of vitamin E, thus depleting other micronutrients such as ascorbic acid, biotin, vitamin A and unsaturated fatty acids. This is particularly likely in investigations where vitamin

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

E depletion was obtained by treating the diet with ethereal ferric chloride [18]. The amelioration of the deficiency symptoms by addition of wheat germ extract to vitamin E-depleted diets does not necessarily prove an *in vivo* role for vitamin E in neuromuscular health. A strong support for the ''toxicity'' of these early diets to neuromuscular system is provided by observations that describe very early onset of the disease phenotype. The descriptions of early onsets of αT deficiency phenotypes have not been reproduced in later experiments in mice or rats. The possible toxicities of vitamin E-depleted diets caused by uncontrolled oxidation of other diet constituents were recognized by early investigators, but not completely addressed in their investigative strategies. A very recent study related to the toxicities of trace metals provides experimental evidence of the need for thorough chemical characterization of diets in nutritional studies [32].

The 1943 review also suggests a lack of agreement on the presence of CNS lesions when there were severe muscular lesions in rats on a vitamin E-deficient diet for 1 year. In spite of these limitations, the early studies are noteworthy for the amount of dl-αT acetate required to correct the deficiency symptoms. The deficiency symptoms in rabbits could be prevented by the addition of 0.32–1.06 mg of dl-αT acetate/Kg of diet, a quantity much lower than that (60–90 mg/Kg) currently recommended for rodents [33].

The observation that long period of feeding vitamin E-depleted diet to develop neuromuscular disturbances in rats was noted in later studies [34]. These studies were remarkable for the detailed clinical and histological description of the CNS and muscles of rats subjected to chronic vitamin E deficiency. The investigators may be the first group to publish the description of ''prominent ataxia'' in addition to muscle dysfunctions as one of the many clinical features of vitamin E deficiency in rats. Particularly noteworthy was the observation that: ''Its onset is insidious after many months on the experimental diet, and it proceeds very slowly; from its first appearance it takes six months to 15 months to develop completely. The neuron muscular disturbances mostly begin caudally and progress towards the head, affecting the front and hind legs and hind quarters in a remarkably symmetrical manner.'' [34]. Remarkably, some of these clinical features are replicated in mice that have almost undetectable amounts of αT in CNS and muscle, in spite of feeding an αT-sufficient diet [13, 27, 28] in which *ex vivo* generation of neurotoxic dietary factors is less concerning.

2.1 Electrophysiological studies

Development of ataxia together with the data of electrophysiological recordings were described in rats after 10 months of feeding either a vitamin E-depleted or a normal diet (100 mg αT acetate/Kg diet) in 1988 [35]. This study is significant because it appears to be a first report of careful electrophysiogical recordings in anaesthetized, temperature-controlled, vitamin E-deficient rats. Feeding vitamin E-depleted diets resulted in ataxia and abnormal myographic recordings in all the rats. In addition, small and selective CNS-effects of vitamin E deficiency were also recorded in experiments that assayed somatosensory-evoked potentials (SEPs). In contrast, another study that simultaneously assayed electromyographic, spinal and SEPs, motor nerve conduction velocities, histopathology and tissue αT concentrations in rats fed either a normal diet or an αT-depleted diet for up to 15 months found normal electrophysiology but abnormal histopathology of type 1 (slow-twitch, oxidative) muscle fibers in αT-depleted rats [36]. Ataxia was not described, but the authors observed muscle degeneration before that of peripheral nerves, which were severely depleted in αT (<1% of controls). The investigators raised the issue of the possible difference in the selenium content of the αT-deficient diets in the two studies to account for the discrepancies between the two studies [36]. The data from this longitudinal study were also in contrast with those of a later study that detected abnormal electrophysiology of nerve and muscles [37]. This study described the minimal dose of dietary RRR α-T acetate needed to ''marginally protect nerves from vitamin E deficiency'' to be 1.0 mg/Kg diet [37]. Eight months were required to develop significant differences in conduction velocities and amplitudes of SEPs between rats fed αT-depleted and rats fed a diet containing 1 mg αT/Kg diet. Neither myographic recordings nor ataxia were reported in these studies.

Collectively, these studies in rats show that at least 8 months of severe dietary αT deficiency are required to display symptoms of neuromuscular defects. Equally noteworthy is that 1 mg of αT/Kg of diet is sufficient to prevent the deficiency symptoms. It seems that larger amounts of dietary αT supplements, 36 mg/Kg diet, are necessary to reverse the symptoms caused by dietary αT deficiency [38]. The effects of the differences in micronutrient composition on ''stability'' of other labile dietary constituents, such as vitamin A, essential aminoacids and unsaturated fatty acids, remain uncharacterized. These factors appear to confound the assignment of specific effects of αT on the chronology, severity and selectivity (nerve or muscle) of the proposed αT-deficiency syndromes in rodent models.

2.2 Does αT affect exercise endurance capacity?

Functional correlates of neuromuscular αT deficiency have also been evaluated by whole body exercise performance. Two or five months of αT deficiency imposed in male rats, post weaning, caused a 40% decrease in endurance (run time to exhaustion on rodent treadmill) compared with those on a normal diet [39, 40]. A diet containing <1 IU/Kg of vitamin E caused blood αT deficiency after 1 month, assayed by erythrocyte hemolysis [41]. Neither study reported the development of ataxia, once again suggesting that

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

more than 8 months of severe αT deficiency are required to develop ataxia.

Since an important determinant of exercise endurance capacity is muscle mitochondria [42] the activities of the latter were assayed [39, 40]. Maximal oxygen uptake, assayed in whole homogenates, was not different between the groups of rats fed normal or vitamin E-depleted diets for 5 months [39]. This was in agreement with lack of significant difference between the two groups of rats in whole body maximal oxygen uptake (VO2max) [39]. Interestingly, lack of effects of chronic vitamin E deficiency on whole body oxygen uptake of guinea pigs was previously reported [18]. Also, the maximal activity of muscle mitochondrial electron transport chain was not affected by two months of αT deficiency [40]. αT deficiency did not affect exercise training or post-training endurance capacity of female rats [43, 44]. Hence, decrease in endurance capacity could not be attributed to loss of maximal substrate oxidation capacity of muscle mitochondria, suggesting the possible sensitivity of other cellular targets, such as sarcoplasmic reticulum or myofibrils, to muscle αT. The lack of effects of short-term but severe αT deficiency on maximal mitochondrial activities is in contrast to decreased muscle mitochondrial activities of rats on αT-deficient diets for 12 months [45].

Vitamin E supplementations did not improve human physical performance [46, 47]. Contrasting effects of αT status on rodent and human physical performance may be attributed to the severity of αT depletion. Unlike rodents, human subjects were not depleted of αT but received αT supplements to the basal diet. This would imply that 13–20 μM plasma αT maintained with unsupplemented diets is sufficient to maintain normal muscle function.

2.3 Does αT scavenge free radicals generated by active muscles *in vivo* and *in vitro*?

Molecular mechanisms that decrease endurance capacity after short-term αT deficiency remain to be identified. Increased free radical activity, assayed by electron spin resonance (ESR) technique, was detected in muscle and liver homogenates from rats run to exhaustion [39]. Interestingly, muscles and livers from αT-deficient rats also showed increased free radical activity in the absence of endurance stress and the ESR signal was not augmented by exhaustive exercise of αT-deficient rats. Because the mitochondrial respiratory index was lower in αT-deficient muscles, and in those of rats on normal αT diet run to exhaustion, it was hypothesized that increased free radical activity ''damaged'' mitochondria and muscle membranes, resulting in decrease in muscle function and endurance capacity [39]. Experimental evidence for contraction dependent free radical generation in isolated muscle preparations [48] gives some support for this hypothesis.

Mechanism by which exercise augments muscle-free radicals and affects muscle function remain to be fully clarified [49, 50]. Furthermore, the link between muscle αT, free radicals and muscle function is unclear. Metabolically generated free radicals affect redox cycling of cellular thiols, including those of proteins that determine cell fate [51–55]. Free radicals from active muscle also affect thiol status [56]. Hence, depletion of free thiols in αT-depleted tissues was attributed to the free radical scavenging potential of αT [39]. Inactivation of enzymes such as citrate synthase and malate dehydrogenase by exercise, possibly by oxidation of free thiols, and deregulation of intramitochondrial redox state, was proposed [57]. A similar conclusion, oxidation of protein thiols and glutathione (GSH), was made in a study that subjected extensor digitorum longus (EDL) muscle to lengthening contractions, *in situ*, in rats [58]; total (GSH+ oxidized glutathione (GSSG)) was unchanged but GSSG in muscle increased after 3 days of stimulations.

Two studies assayed GSH status in whole blood during rest, sub-maximal and maximal exercise, and during recovery from exercise in moderately trained human subjects [59, 60]. GSH was oxidized (GSSG) during submaximal exercise. GSH recovered during the recovery phase and exceeded baseline levels. Total GSH levels showed depletion during exercise and they increased during recovery. Since most of blood GSH is in erythrocytes, the authors associated GSG-GSSG changes during exercise-recovery cycle with those of oxyhemoglobin-methemoglobin cycle [59]. Auto-oxidation of hemoglobin generates superoxide radicals [61] and hydrogen peroxide, which is reduced by erythrocyte glutathione peroxidase and GSH [62]. If myoglobin undergoes similar redox-cycling during exercise-recovery phase, then it would provide a molecular link between exercise, muscle oxygenation and intramuscular GSH-GSSG cycles. In addition, myoglobin has nitrite reductase activity [63–65], which generates nitric oxide required to prevent cardiac ischemia-reperfusion injury by inhibiting mitochondrial respiration [66, 67]. Similar mechanisms may operate during exercise-recovery cycles of skeletal muscle and mitochondrial respiration, contributing to exercise-induced muscle fatigue.

Muscle mitochondria may also be a source of activity-dependant free radicals. Previous studies, using ESR techniques and isolated cardiac mitochondria or submitochondrial particles, have established increased free radical generation by mitochondrial electron transport chain during substrate oxidations [68, 69]. Therefore, it was reasonable to hypothesize that increased demand for ATP by active muscles would augment free radical generation from mitochondria to meet the energy demands during exercise. There is substantial experimental evidence to support this hypothesis [39, 48, 58, 70–73]. However, mitochondria may not be the only source of exercise induced free radicals [74]. Increased superoxide generation has been implicated in the causation of fatigue in diaphragm [72]. Dysregulated calcium homeostasis through free radical-mediated oxidation of thiols has been implicated in muscle fatigue [50]. Oxidation of vicinal (adjacent) cysteines of sarcoplasmic reticulum Ca(2+)-ATPase (SERCA1) under conditions that

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

simulate titanic stimulation induced muscle fatigue has also been suggested [75].

Irrespective of the precise identity of the electron transport systems that generate free radicals, the co-localization of αT and free radical generators increase the probability of interactions between the two systems. Acute augmentation of αT and spin trappers have resulted in increased endurance of swimming stress in mice [76], possibly suggestive of antioxidant actions of αT. Other antioxidants, such as DMSO, purines and allopurinol, were suggested to scavenge free radicals generated by stimulation of isolated mouse soleus or canine gastrocnemius, and decrease fatigue [77].

Hence, one or more of these mechanisms may contribute to the decreased endurance capacity described in αT-deficient rats [39, 40]. Experiments with cells, skin fibroblasts [78] or myocytes [79] suggest interactions between αT and GSH-GSSG cycle. However, in heart and brain regions of αTTP-KO mice that are severely depleted in αT, total GSH levels were not significantly different from those of the congenic wild-type mice [80]. Therefore, the link between intracellular GSH-GSSG cycle and αT status, if any, remains equivocal.

2.4 Does increased muscle activity increase catabolism and redox-cycling of αT? Is αT consumed by exercised-induced free radical formation?

To the best of our knowledge, these questions remain unanswered, possibly due to technical limitations in quantifying organ, cell, and in the case of muscle, myofibril-specific αT metabolism. Endurance training decreases αT concentrations of rat quadriceps muscle groups, when assayed immediately after exercise [81]. The mechanism(s) that cause acute, exercise-induced depletion of muscle αT remain to be rigorously clarified. αT can be oxidatively catabolized by microsomal cytochrome P450 and, peroxisomal and mitochondrial β-oxidation pathways [82–84]. These pathways are robust in liver and are hypothesized to regulate systemic concentrations of αT and of other members of the vitamin E family.

The kinetic properties of β-oxidation pathway, relevant to αT catabolism, in skeletal muscle remain uncharacterized. β-oxidation pathway is induced by endurance training [42, 85]. However, the expression of mRNAs encoding the subset of cytochrome P450 members implicated in ω-hydoxylations were undetectable in gastrocnemius of mice [86]. Therefore, if ω-oxidation is an obligatory initial step in αT degradation, then αT is less likely to be catabolized in muscle. The contribution of mitochondrial β-oxidation pathway to the catabolism of αT, in the absence of cytochrome P450 activity, remains to be determined. However, it is possible that there is ''trans-organ'' catabolism of αT when it is delivered in high doses. For example, liver with active ω-oxidation pathways generates αT oxidant products that are released into systemic circulation and catabolized by β-oxidation pathways in cardiac and skeletal muscles. Perhaps these mechanisms may contribute to increased mortality in humans taking high levels of αT supplementations in combination with other antioxidant supplements, such as β-carotene, vitamin A, vitamin C (ascorbic acid) and selenium [87, 88]. One of these studies analyzed data from 232 606 healthy participants in 68 randomized trials and concluded that β-carotene, vitamin A, and vitamin E may increase mortality [88]. The second study by the same group of investigators included participants with various diseases (gastrointestinal, cardiovascular, neurological, ocular, dermatological, rheumatoid, renal, endocrinological or unspecified) [87]. The cause of mortality was not identified.

It is interesting to note that 3 months of dietary αT deficiency decreased the ratio of αT/ubiquione 9 in mitochondria from endurance trained rats [44]. In rats that were fed a αT-depleted diet, the concentration of muscle αT decreased from an estimated 25 to 2 nM after 3 months. Endurance training of the rats fed the αT-depleted diet did not affect either the rate of αT depletion from muscle or the biogenesis of mitochondria [44], suggesting that adaptive responses to endurance training remain robust, at least during the training period. It would be of interest to discover if endurance training protocols [42, 85] will accelerate the onset of ataxia, which occurs after 12 months in rodents fed αT-depleted diets.

Another challenging question that remains to be addressed is the possible association between redox-cycling of αT and its catabolic removal. It has been suggested that ascorbic acid can regenerate αT from tocopheroxy radical formed by endogenous free radicals in human erythrocytes [89, 90]. A stable tocopheroxy radical was detected, by ESR technique, in microsomal and mitochondrial membranes isolated from livers of rats fed αT-supplemented diet [91]. The ESR signal for tocopheroxy radical in these membrane preparations decayed to undetectable levels within 2 min during the assay. This decay was associated with a 50% loss of αT, assayed by HPLC, suggesting ''activity'' dependant loss of αT. Interestingly, it was reported, much earlier, that, ''…in lipid systems subject to autooxidation in vitro,… peroxidation did not occur without concomitant destruction of tocopherol.'' [92]. Chemical mechanism(s) that may selectively catabolize tocopheroxy radical remains unclear. Similarly, the physiological significance of activity-dependent catabolism of αT, if it occurs *in vivo*, is also unclear because vitamin E supplementations do not improve human physical performance [46, 47]. It is likely that as long as there is sufficient αT regenerating capacity, such as the presence of vitamin C and possibly ubiquinone, very small, catalytic amounts (nM) of αT that is sequestered in mitochondrial and sarcoplasmic membranes is sufficient to maintain normal muscle biochemistry and function.

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

3 αT-sensitive ataxias in humans consuming normal diets

3.1 Mutations in MTP cause very low levels of systemic αT and, myopathy and neuropathy with aging

Although neuromuscular deficits in rodents fed αT-depleted diets are well established, a role for αT in neuromuscular health of humans remained to be proven, decades after the discovery of vitamin E. The identification of human ataxias that could be ameliorated by dietary αT supplementations must be considered compelling proof for the requirement of αT to maintain neuromuscular health [93–96]. These studies also suggest that prolonged deficiency of αT (>10 years) is required for the development of CNS deficits [97–100]. Particularly remarkable, from these studies, is that αT supplementations can ameliorate the effects of genetic mutations that are primarily expressed in gut and liver but cause deficits in CNS and muscles.

αT is undetectable in abetaliproteinemia patients due to mutations in the gene encoding MTP [6–8]. These patients have defects in the biogenesis of lipoproteins (chylomicroms and VLDL). Hence the two lipoproteins were hypothesized to play vital role in the absorption, secretion and transport of αT and other lipid soluble vitamins [6, 101–103]. Levels of αT in muscles of these patients have not been reported. However, αT in adipose tissue [104, 105] and nerves [105] are very low or undetectable. Its noteworthy that tissue αT deficiency precedes long before the histological evidence of nerve degeneration [105], suggesting a time lag between the onset of the biochemical deficiency and detectable histologic/neuropathic-myopathic defects described in rodent models of αT deficiency.

3.2 Mutations in the gene encoding αTTP cause low levels of plasma and extra-hepatic tissue αT and ataxia with aging

Development of ataxia over the first two decades of life has been described in siblings with very low levels of αT in the absence of fat malabsorption [9]. These investigators postulated, ''…that an inherited defect in hepatocyte secretion of vitamin E into lipoproteins may account for this disorder…'' [9]. Further studies in this group of patients, designated patients with familial isolated vitamin E deficiency, led to the suggestions that, ''…these patients are lacking or have a defective liver ''tocopherol binding protein'' that incorporates alpha-tocopherol into nascent VLDL.''[102, 106].

Decades earlier, a study in rats showed that orally administered α-[^{3}H] tocopherol was associated with VLDL fraction of serum [107]. Although, more recent investigations in mice excluded the role of VLDL in αT transport [108] the importance of the liver and its cellular and intracellular compartments for αT homeostasis was appreciated by early investigators. Specifically, in an *in vivo* study, ~25% of liver α-[^{3}H] tocopherol, from rats gavaged with α-[^{3}H] tocopherol, was associated with the cytosolic fraction, an equal fraction was associated with microsomes, and the rest, ~50% with mitochondria [109]. In addition, and particularly relevant to the putative function of the cytosolic αT-binding protein, the authors also showed a preferential transfer of αT from cytosol into mitochondria. In another study, a ~30 kDa αT-binding protein was isolated from the cytosolic fraction of rat liver homogenates [110, 111]; the protein was detected only in the liver. A 34 kDa cytosolic protein that could transfer αT to membranes was detected in liver but not in heart or lung [112]. Rabbit anti αT-binding protein showed that the protein was expressed in lysates of fractionated hepatocytes but not in those of endothelial or Kupffer cells. Neither was the protein detected in lungs, heart, spleen or testes [113].

A more detailed molecular description of the αT-binding protein with αT transfer activity, αTTP, was obtained when a cDNA clone that encoded a 31.845 kDa protein was isolated from rat liver and was shown to be expressed exclusively in the liver [11]. Thus, there appeared to be unequivocal experimental evidence for a gene that encoded a protein that specifically bound to αT and was able to transfer it to mitochondria, other membranes and lipoproteins secreted by hepatocytes. Equally important is the evidence that the gene was expressed primarily in the liver; its expression was undetectable in brain, lung, heart, intestine, kidney and adrenal glands [11]. However, the authors stated that, ''The physiological role of αTTP remains unknown.'' [11].

Physiological importance of liver αTTP for neuromuscular health became clear when it was discovered that patients displaying Friedreich ataxia-like phenotype have very low serum αT levels. These patients, designated ataxia with vitamin E deficiency (AVED) patients were discovered to have mutations in αTTP gene [10], which could explain the postulated deficiency in systemic αT [11, 102, 106]. After this discovery, several mutations in the αTTP gene have been described and appear to determine the severity of the phenotype and its sensitivity to amelioration by augmenting dietary αT [114–117]. The heterogeneity in the resultant severity of neuromuscular phenotypes suggest that different regions of the αTTP molecule may interact and cooperate with other molecules in the binding, trafficking and transfer of αT. Elegant biochemical studies with recombinant αTTP harboring three different AVED mutations expressed in HepG2 cells ''found that these mutations impair the ability of αTTP to facilitate the secretion of vitamin E from cells. Furthermore, the degree of impairment corresponded to the severity of the AVED pathology associated with each mutation.'' [118]. Since αTTP must interact with other ligands, proteins or lipids, to orchestrate exchange of αT, it is likely that at least some of the mutations affect interactions between αTTP and other ligands in the cytosol and membranes. The identities of these putative αTTP-binding

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

ligands, including other proteins [119], remain to be discovered . The availability transgenic mice with the deletion of αTTP gene [13, 120, 121] may expedite the identification of molecular partners of αTTP.

3.3 Creation of αTTP-deficient mice that recapitulate some of the phenotypes of familial isolated vitamin E deficiency and AVED patients

The cloning of the rat αTTP gene [11] and the discovery of αTTP mutation in AVED patients laid the foundations to create transgenic mice by homologous recombination technology combined with manipulation of embryonic stem cells [14, 122–125]. Two research teams independently engineered the deletion of mice αTTP gene and created αT-deficient mice [13, 120, 121]. Homozygous αTTP-deficient (αTTP-KO) mice were infertile and had very low or undetectable serum αT levels (<0.2 nM) in spite of being fed a basal diet (36 mg αT-/Kg) [121]. The infertility was abolished by supplementing the diets of αTTP-KO breeding pairs with 567 mg αT/Kg of diet, reinforcing the need for dietary αT for fertility. A more recent study has suggested that tocotrienol may prevent sterility in mice rendered αT deficient by the deletion of αT transfer protein gene [126]. This is a surprising discovery that needs confirmation because tocotrienol is hypothesized to be preferentially excluded from incorporation into lipoproteins, the primary carriers of tocopherol, and is targeted for catabolism by liver [127].

Histological examination of pregnant uteri suggested that αT deficiency causes dysfunction in the first epithelial cells, labyrinth trophoblasts [121], generated by fertilized eggs. αT may also be essential for placentation [2], suggesting a vital role for αT in the development of placenta and embryo. Molecular and cellular networks that αT must modulate during these very initial stages of embryogenesis *in utero* remain to be clarified. It is remarkable that histological descriptions and chronology of labyrinth trophoblasts malfunctions in αT-deficient mice [2, 121] are similar to those described in mice with the deletion of hepatocyte growth factor (HGF) expression [128]. These observations suggest αT:αT receptor (putative) and HGF:HGF receptor signaling pathways may share common molecular mechanisms that determine mesenchyme-trophoblastic cellular networks and early stages of mouse embryonic stem cell differentiation. Antioxidant properties of αT have been invoked as a mechanism for normal fertility [121]. However, mechanism(s) of action of αT *in vivo* are actively debated and unresolved today [129–132] as they were more than 40 years ago [18, 92, 133, 134]. Physicochemical properties of αT dictate its localization and distribution to biological membranes [135]; hence, it is most likely to modulate activities of membranes and intrinsic membrane proteins, including those of nuclear membranes. Many of these intrinsic membrane proteins serve structural and signaling functions [136, 137].

Mild ataxia, abnormal motor performance and abnormal electrophysiological recordings of retina and SEPs were detected in αTTP-KO mice after they were fed the basal diet for 1 year [13], reproducing the observations of a long delay in the development of neuromuscular phenotype after the onset of tissue αT deficiency. Interestingly, wild-type mice fed an αT-depleted diet in the same set of experiments did not develop ataxia [13], suggesting the possibility that prenatal depletion of αT, such as that predicted to occur in αTTP-KO mice, accelerates the onset of the αT deficiency phenotype. Another group of investigators have used αTTP-KO mice originating from a separate colony of transgenic mice and have reproduced the neuromuscular phenotype and have noted additional behavioral defects [28, 29]. A possible involvement of brain αT in modulation of anxiety was also suggested in a later study in rats fed a αT-depleted diet [138].

The αT levels of five brain regions and of four peripheral organs from 5-month-old αTTP-KO mice were below the limits of detection (<0.2 nM) when compared with those of the congenic wild-type littermates [80]. These 5-month-old mice did not display ataxia, once again underscoring the dissociation of the onset of biochemical deficiency from phenotype expression. In contrast, the onset of neuromuscular phenotype precedes the detection of histological lesions that were undetectable in spinal cord and muscle of 12-month-old ataxic mice but were detected at 20 months of age [13]. In another study, muscle lesions in the absence of any lesions in the CNS were apparent in 12-month-old ataxic, αTTP-KO mice but not in the age-matched congenic wild-type littermates [86], possibly suggesting that muscle histological lesions precede those in the CNS and probably suggests that hind limb skeletal muscle dysfunction precedes motor nerve degeneration. Similar observations were reported in rats after 16 months of feeding an αT-depleted diet [36].

Surprisingly, the onset of the ataxic phenotype was not accelerated by feeding the αTTP-KO mice a αT-deficient diet [13, 86]. These observations are in contrast to those that suggest acceleration of motor deficits [139] or retinal degeneration [140] or Alzheimer disease phenotype [17] in α-TTP-KO mice fed a diet depleted in αT. These experiments suggest that very low levels of αT in plasma and tissues of αTTP-KO mice are sufficient to maintain normal retinal, motor (rotarod performance) and memory functions. This is also suggested by lack of CNS or muscular deficits in 1-year-old mice whose brain αT levels are low due to the deletions of scavenger receptor B1 [141, 142].

Attempts to further deplete brains and CNS of αTTP-KO mice by feeding αT-depleted diets raises the possibility of feeding diet derived oxidant products with potential neurotoxic actions. Increased biomarkers of oxidative stress detected in these studies [139, 143] may not be, unequivocally, assigned to *in vivo* actions of αT. If αT has antioxidant actions then the effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, an inducer of oxidative stress

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

[144, 145], should be potentiated in αTTP-KO mice. However, the loss of dopaminergic neurons was not increased by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in αTTP-KO mice as compared with those of wild-type mice [15]. Another study showed decreased indices of oxidative stress in cerebral cortex caused by prolonged αT deficiency in αTTP-KO mice that were fed a chow diet (dietary αT levels were not described) [146]. The αTTP-KO mice for this study were obtained from Jackson laboratory and there was no mention of the development of neuromuscular phenotypes [146]. The pathophysiological relevance of the detailed biochemical data that assayed mitochondrial functions and superoxide production from cerebral cortex in this study remains to be addressed. Others have detected small (~20%) but significant increase ($p < 0.03$, $n = 3$) in isoprostanes (assayed by gas chromatography/mass spectrometry [147]) but not in histologically assayed 8-hydroxy guanosine, heme oxygenase 1, 4-hydroxynonenal, isoprostanes or free iron in cerebral cortices of 2-year-old, ataxic mice that showed ataxia-specific gene expression signature [27], compared with those of age- and sex-matched congenic wild-type littermates [86].

3.4 Search for molecular mechanisms of neuromuscular deficits caused by severe and chronic αT deficiency

Lack of large and statistically significant difference in biomarkers of oxidative stress in tissues severely depleted in αT for very long periods suggests that the concentrations of these metabolites are tightly controlled during normal and pathophysiology. If so, the proponents of antioxidant actions of αT [13, 127, 139] face a challenge similar to that proposed by A.V. Hill to muscle biochemists to prove that ATP is the direct source of energy for muscle contraction [148]. A direct, causative relationship between the chronology of changes in concentrations αT, its metabolites, biomarkers of oxidant stress and development of phenotypes remain to be established.

Elegant biochemical [20, 31, 118, 149] and crystallographic [150–152] studies of the best characterized, physiologically relevant αT-binding protein, αTTP have been interpreted to suggest that, ''… only α-TTP is likely to serve as the physiological mediator of αT's biological activity.'' [20]. However, in a normal mouse the expression of αTTP in the CNS and muscles, the anatomical targets in ataxia, is below the limits of detection [11, 27, 80], suggesting that other molecular entities may be transducing the changes in αT levels in these tissues. A very weak signal for immunoreactive αTTP was detected in tissue extracts of mouse cerebral cortex, cerebellum and spinal cord, compared with that in the liver [13]. It has been suggested that these, very low levels of αTTP do not account for the ''bulk'' tissue levels of αT [80] but they may have a role in affecting gene expression in a few cells. Immunohistochemical assays have detected αTTP in cerebellar cells, possibly in Bergmann glial cells, but not in those of cerebral cortex, in rats [153] or in cerebellar Purkinje cells in humans [154]. The physiological significance of αTTP in these cells remains to be clarified. If the primary function αTTP is to preferentially select αT from the other seven members of vitamin E family in the diet [127, 155], then αTTP in these cells may offer a second ''molecular filter'' to exclude other members of vitamin E, suggesting exquisite preference for αT for the function and survival of these cells. It cannot be excluded that αTTP in these few cells may serve other functions [119]. A third possibility is that the immunoreactive signals are technical artifacts. All these possibilities can be tested in αTTP-KO mice.

Global mRNA (exonomic) expression analysis show αT-sensitive changes in mRNA signatures that are specific for cerebral cortex and muscles, which that have undetectable expression of αTTP [26–27, 80, 156–158], suggesting other sensors and transducers of αT concentrations must exist. Some of the changes in exonomes of cerebral cortex [29] and skeletal muscles [25, 27] offer transcriptional correlates of ataxic and histological phenotypes attributed to chronic tissue deficiency of αT obtained either by dietary depletion of αT or genetic deletion of αTTP. For example, ~14 months of dietary αT deficiency in rats identified activation of four cathepsin genes [25], two of which are associated with muscle lysosomes and muscle histopathology [159]. The other gene of interest that showed robust activation by the chronic αT deficiency was that encoding cardiac ankyrin repeat protein [25], which regulates transcription and is implicated in cardiac disease [160]. In another study, aimed to identify exonomic signature of ataxia in αTTP-KO mice, a cluster of nine genes, designated ''the sarcolipin cluster'', was overexpressed in ataxic muscles compared with that in the muscles of non-ataxic, αT-deficient or congenic wild-type mice [27]. The functional significance of this cluster of genes is that it regulates muscle contraction–relaxation cycles by regulating calcium cycling between sarcoplasm and sarcoplasmic reticulum [27]. The authors suggested that overexpression of this cluster of genes in, ataxic, αT-deficient muscle of αTTP-KO mice, compared with that of congenic wild-type litter mates, would prevent relaxation of the muscle and contribute to the shorter-stride lengths, an indicator of ataxia in mice [27]. It is also remarkable that two independent studies of αT-deficient skeletal muscles [25, 27] detected overexpression of genes that are associated with cardiac muscle, suggesting that αT suppresses cardiac-specific genes in skeletal muscle.

A distinct set of genes that could contribute to development of neurological deficits, such as anxiety, in young mice and ataxia in older mice were detected in cerebral cortex of 3-month-old αTTP-KO mice compared with those of congenic wild-type mice [29]. A cluster of ten genes that encode synaptic proteins, particularly those associated with the synaptic vesicles, were coordinately repressed by αT deficiency caused by deletion of the αTTP gene. Genes that encode synaptic proteins were also repressed in the cerebral cortex of rats fed an αT-depleted diet for 14 months [30]. The

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

possible role of αT in regulating the expression of vesicle assembly and transport was further underscored in another study that focused on rat liver [161]. A unifying theme that emerges from these gene profiling studies is that αT affects calcium mediated excitation-secretion or excitation-contraction coupling. Hence, although the genes modulated in cerebral cortex were distinct from those in skeletal muscles, their encoded proteins such as hippocalcin, visinin-like 1 and protein kinase Cγ, are related to the sarcolipin cluster in muscle because they participate in calcium-regulated functions. These data and those described by others, such as in [162, 163], provide strong support for αT actions on gene expression.

The mechanisms by which αT affects gene expression remains to be characterized and promise to be a major challenge in αT research. In addition to αTTP, other αT-binding proteins have been discovered [21, 164–166], which may contribute to biological and gene expression activities of this vitamin. Orally delivered, ^{3}[H]-αT was recovered from liver nuclear membranes [167] and was associated with sub-nuclear, non-histone proteins of cardiac nuclei and mitochondria of perfused rabbit hearts [168, 169]. In the rabbit cardiac muscle, increased nuclear ^{3}[H]-αT-binding was associated with increased RNA synthesis [169]. A more recent study using COS (CV-1 (simian) in Origin, and carrying the SV40 genetic material) cells has suggested that the tocopherol-associated protein, which has significant sequence similarity to αTTP, specifically binds αT and translocates to the nucleus and increases transcription [170]. Similar αT-binding proteins in nuclei of brain and muscle cells may transduce changes in cellular αT levels to account for changes in gene expression profiles reported in [25, 27–30, 86].

4 Future directions

4.1 Utility of αTTP-KO mice for characterizing molecular mechanisms of age-related neuropathologies

Although αT deficiency caused by mutations in MTP and αTTP genes is rare, rigorous characterization of chronology of cellular and molecular mechanisms that cause the well-defined and preventable age-related neuromuscular phenotypes, such ataxia, may have relevance to more common age-related health issues, such as sarcopenia [171–173], macular degeneration [174, 175] and dementia [176, 177]. αTTP-KO mice that have constitutive deletion of αTTP gene recapitulate some of the age-related phenotypes of AVED patients [13, 27–29]. These age-dependant αT deficiency phenotypes are ameliorated by pharmacological, oral doses of αT. Hence, αTTP-KO mice offer *in vivo* models to address mechanisms by which severely αT-depleted CNS and muscular systems are sustained for up to 9 months of age and then begin to fail. The αTTP-KO mice may also offer important models for preclinical evaluation of physical exercise, dietary and pharmacological intervention strategies for the treatment and prevention of age-related decline in neuromuscular health.

4.2 Identification of molecular partners of αTTP in the presence or absence of αT

Although it is established that αTTP specifically binds αT, which is buried deep within the αTTP molecule, it is unclear how αT is ejected from its binding pocket and transferred to lipoproteins in liver [150, 151]. It is likely that specific proteins, yet to be identified, participate in the release and transfer of αT from αTTP and contribute to systemic αT levels. It has also been suggested that the severity of AVED phenotype cannot be completely accounted for by the αT-transfer activity of αTTP [119]. Thus, the mutations in αTTP may affect its interactions with other proteins or lipids that may contribute to the development of AVED phenotypes. Alternatively, multiple genetic defects may be present in AVED patients [178]. The availability of αTTP-KO mice in which a single gene is deleted or creation of mice with specific mutations [179] in the αTTP-gene offers unique opportunities to test these hypotheses, identify biochemical and molecular mechanisms, and evaluate their biological and clinical relevance.

4.3 Is αTTP expressed in the CNS? Generation of tissue-specific αTTP-KO mice

The currently available αTTP-KO mice have constitutive deletion of αTTP gene resulting in abrogation of αTTP expression in all the tissues during embryogenesis [13, 120, 121]. Although there is strong experimental evidence for the lack of expression of αTTP in the CNS and muscles tissues, some studies suggest cell-specific expression of αTTP in the CNS [153, 154]. Genetic engineering techniques that target a specific gene in a specific cell-type [180, 181] such as αTTP in cerebellar Purkinje cells may rigorously test the proposed contribution of αTTP expression in these cells to the development of neuromuscular phenotypes. Alternatively, genetic engineering techniques may be used to knock in a specific gene [182], such as the αTTP gene in the CNS, skeletal and cardiac tissues to evaluate the functions of αTTP in αT-dependant modulation of age-related neuromuscular functions and pathologies.

4.4 Adipose tissue atrophy/metabolic effects of severe and chronic αT/αTTP deficiency

In addition to the development of age-related ataxia, the αTTP-KO mice failed to gain body weight after 6–8 months on a diet that contained 35 mg αT/Kg (Fig. 2, [86]). Interestingly, a previous study showed a similar, age-related

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

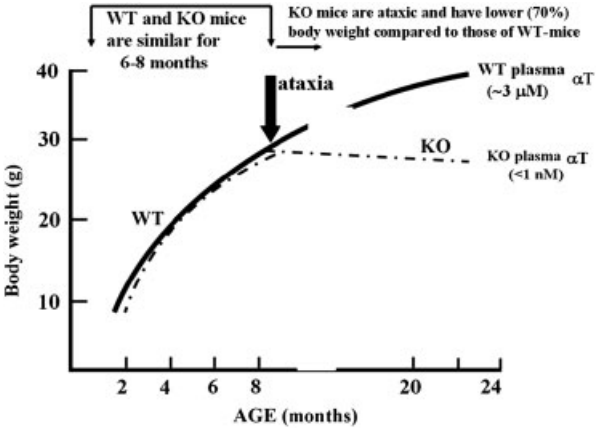

Figure 2. Figure constructed from data obtained from congenic wildtype (WT) and αTTP-KO mice (authors' laboratory, unpublished). Mice, (~20/group, males and females) were fed a 35 mg αT/Kg diet. The KO mice were indistinguishable from WT mice for the initial 6–8 months post-weaning. After 8 months, KO mice failed to maintain body weight and developed ataxia. After 12 months, body weight of KO mice was ~70% of WT mice. At autopsy, the KO mice had less white adipose tissue. Plasma leptin was ~50% ($p<0.03$) lower in KO mice compared with those of WT mice.

effect on body weights of rats fed an αT-depleted diet [38]. Autopsy of the old αTTP-KO mice was remarkable for decreased amount of abdominal white adipose tissue. In addition, cytokines and chemokines (granulocyte-monocyte-colony stimulating factor, interferon-γ, interlukin-6, glucagon, grehelin, insulin, leptin, plasminogen activator inhibitor type 1 and resistin) were assayed in plasma from 2-year-old αTTP-KO mice (that displayed ataxia) and their congenic wild-type littermates. Plasma leptin of αTTP-KO mice was lower (50%, $p<0.05$) than that of the wild-type mice. All other cytokines and chemokines were not significantly different between the two groups of mice. These preliminary data suggest a role for αT/αTTP in age-related deregulation of adipose tissue homeostasis. The observations are particularly remarkable because they suggest another tissue, in addition to the CNS and muscles, that does not express αTTP, is terminally differentiated, whose functions are affected by αT. The specific molecular pathways that may contribute to age-related decline in function are most likely to be uncovered by integrated analysis of the transcriptomes [183, 184], proteomes [185], epigenomes [186] and metabolomes [187] of the CNS, muscle tissues and the adipose tissues.

4.5 Antioxidant *versus* non-antioxidant actions of αT

Experimental evidence for free radical scavenging actions of αT *in vivo* remain unresolved [92, 129–131]. It is likely that αT acts *via* non-antioxidant and antioxidant actions in the CNS and muscle tissues. Gene expression profiling studies of the CNS [28–30, 86], cardiac [26] and skeletal muscles [25, 27] do not show induction of oxidative stress genes such as those driven by free radical sensitive transcription factors Keap1-Nrf2 [188–190]. However, some of the genes induced by severe αT deficiency in cerebral cortex [29] appeared to be regulated by transcription factors that may be regulated by oxidative stress, suggesting that free radical-mediated processes may contribute to mRNA expressions. Protein thiols are frequently invoked as targets of free radical actions [191, 192] including those of muscle proteins [75, 193]. However, it has also been proposed that redox cycling of thiols can also occur without free radicals [54]. Hence, it is technically difficult to assign a specific mechanism that αT may affect to generate changes in mRNA expression. Advances in techniques that assay specific free radicals *in vivo* are necessary to evaluate antioxidant actions of αT. Since skeletal muscles generate free radicals when rested and increase free radical generation when activated [50, 70], muscles of αTTP-KO mice and their congenic littermates may offer valuable *in vivo* systems to test the proposed antioxidant functions of αT.

5 Summary and concluding remarks

This review is focused on *in vivo* effects of αT on the neuromuscular system in rodents and humans. Amelioration of neuromuscular deficiencies by pharmacological doses of αT in patients harboring mutations in MTP and αTTP genes underscore the dietary need of αT for neuromuscular health. The discovery of human αTTP gene gave a functional significance for biochemical data from rodents that showed expression of αTTP in the liver. The creation of αTTP-KO mice confirmed the role of this liver-specific protein in the selection of αT from other eight members of vitamin E family, the regulation of systemic αT and its biological action as a fertility vitamin whose chronic deficiency causes neuromuscular deficits. The CNS of αTTP-KO mice has almost undetectable levels of αT. This is not achieved by feeding αT-depleted diets that may contain neurotoxic oxidant products, generated *ex vivo* in the absence of αT. Supplementation of 1–30 mg of αT/Kg of diet is sufficient to prevent the neuromuscular deficits in normal rodents. αTTP-KO mice develop well-defined neuromuscular deficits after 9 months in spite of feeding a diet containing 35 mg αT/Kg diet. The molecular and cellular mechanisms that maintain the CNS and muscle functions for 9 months and then cause neuromuscular deficits remain to be characterized.

This review is dedicated to Professor Lester Packer on his 80th Birthday.

We apologize to all the researchers whose work was not cited because of our primary focus on neuromuscular system; their comments are invited so that they can be addressed in future

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

publications. We thank the two anonymous reviewers who made constructive criticisms to improve this review. Expert technical assistance from undergraduate students, Brad Hobson, Sean Ott and Vania Rashidi is sincerely appreciated. The research from authors' laboratories was supported by several sources that included the following: NIH Grant ♯ES011985, USDA grant ♯ 35200-13456, University of California Clinical Nutrition Research Unit and University of California, Davis, Center for Human Nutrition Research Pilot Research Grant. Data from our unpublished experiments cited in this review will be made available upon request. The assays for plasma cytokines and chemokines were made possible by generous collaboration with Drs. Paul Davis, (Nutrition Department) and Imran Khan (Center for Comparative Medicine). Isoprostane quantizations in cerebral cortices were performed by Professor Jason D. Morrow's laboratory (Vanderbilt University Medical Center). Histological assays for markers of oxidative stress were done in the laboratory of Professor Mark A. Smith (Case Western Reserve University, Cleveland). Muscle tissue (gastroscnemius and diaphragm) and CNS histology was done by Professor Robert Higgins (Pathology, Microbiology and Immunology Department, Veterinary Medicine, University of California, Davis).

The authors have declared no conflict of interest.

6 References

[1] Evans, H. M., Bishop, K. S., On the existence of a hitherto unrecognized dietary factor essential for reproduction. *Science* 1922, *56*, 650–651.

[2] Jishage, K., Tachibe, T., Ito, T., Shibata, N. *et al.*, Vitamin E is essential for mouse placentation but not for embryonic development itself. *Biol. Reprod.* 2005, *73*, 983–987.

[3] Lee, B. L., New, A. L., Ong, C. N., Simultaneous determination of tocotrienols, tocopherols, retinol, and major carotenoids in human plasma. *Clin. Chem.* 2003, *49*, 2056–2066.

[4] Kubow, S., Lipid oxidation products in food and atherogenesis. *Nutr. Rev.* 1993, *51*, 33–40.

[5] Shivaprasad, H. L., Crespo, R., Puschner, B., Lynch, S., Wright, L., Myopathy in brown pelicans (*Pelicanus occidentalis*) associated with rancid feed. *Vet. Rec.* 2002, *150*, 307–311.

[6] Berriot-Varoqueaux, N., Aggerbeck, L. P., Samson-Bouma, M., Wetterau, J. R., The role of the microsomal triglygeride transfer protein in abetalipoproteinemia. *Ann. Rev. Nutr.* 2000, *20*, 663–697.

[7] Shoulders, C. C., Brett, D. J., Bayliss, J. D., Narcisi, T. M. *et al.*, Abetalipoproteinemia is caused by defects of the gene encoding the 97 kDa subunit of a microsomal triglyceride transfer protein. *Hum. Mol. Genet.* 1993, *2*, 2109–2116.

[8] Wetterau, J. R., Aggerbeck, L. P., Bouma, M. E., Eisenberg, C. *et al.*, Absence of microsomal triglyceride transfer protein in individuals with abetalipoproteinemia. *Science* 1992, *258*, 999–1001.

[9] Sokol, R. J., Kayden, H. J., Bettis, D. B., Traber, M. G. *et al.*, Isolated vitamin E deficiency in the absence of fat malabsorption--familial and sporadic cases: characterization and investigation of causes. *J. Lab. Clin. Med.* 1988, *111*, 548–559.

[10] Ouahchi, K., Arita, M., Kayden, H., Hentati, F. *et al.*, Ataxia with isolated vitamin E deficiency is caused by mutations in the alpha-tocopherol transfer protein. *Nat. Genet.* 1995, *9*, 141–145.

[11] Sato, Y., Arai, H., Miyata, A., Tokita, S. *et al.*, Primary structure of alpha-tocopherol transfer protein from rat liver. Homology with cellular retinaldehyde-binding protein. *J. Biol. Chem.* 1993, *268*, 17705–17710.

[12] Terasawa, Y., Cases, S. J., Wong, J. S., Jamil, H. *et al.*, Apolipoprotein B-related gene expression and ultrastructural characteristics of lipoprotein secretion in mouse yolk sac during embryonic development. *J. Lipid Res.* 1999, *40*, 1967–1977.

[13] Yokota, T., Igarashi, K., Uchihara, T., Jishage, K. *et al.*, Delayed-onset ataxia in mice lacking alpha-tocopherol transfer protein: model for neuronal degeneration caused by chronic oxidative stress. *Proc. Natl. Acad. Sci. USA* 2001, *98*, 15185–15190.

[14] Capecchi, M. R., The new mouse genetics: altering the genome by gene targeting. *Trends Genet.* 1989, *5*, 70–76.

[15] Ren, Y. R., Nishida, Y., Yoshimi, K., Yasuda, T. *et al.*, Genetic vitamin E deficiency does not affect MPTP susceptibility in the mouse brain. *J. Neurochem.* 2006, *98*, 1810–1816.

[16] Nishida, Y., Ito, S., Ohtsuki, S., Yamamoto, N. *et al.*, Depletion of vitamin E increases Abeta accumulation by decreasing its clearances from brain and blood in a mouse model of Alzheimer disease. *J. Biol. Chem.* 2009, *284*, 33400–33408.

[17] Nishida, Y., Yokota, T., Takahashi, T., Uchihara, T. *et al.*, Deletion of vitamin E enhances phenotype of Alzheimer disease model mouse. *Biochem. Biophys. Res. Commun.* 2006, *350*, 530–536.

[18] Pappenheimer, A. M., Muscular disorders associated with deficiency of vitamin E. *Physiol. Rev.* 1943, *23*, 37–50.

[19] Bell, E. F., History of vitamin E in infant nutrition. *Am. J. Clin. Nutr.* 1987, *46*, 183–186.

[20] Panagabko, C., Morley, S., Hernandez, M., Cassolato, P. *et al.*, Ligand specificity in the CRAL-TRIO protein family. *Biochemistry* 2003, *42*, 6467–6474.

[21] Jerkovic, L., Voegele, A. F., Chwatal, S., Kronenberg, F. *et al.*, Afamin is a novel human vitamin E-binding glycoprotein characterization and in vitro expression. *J. Proteome Res.* 2005, *4*, 889–899.

[22] Drouineaud, V., Lagrost, L., Klein, A., Desrumaux, C. *et al.*, Phospholipid transfer protein deficiency reduces sperm motility and impairs fertility of mouse males. *FASEB J.* 2006, *20*, 794–796.

[23] Kono, S., Otsuji, A., Hattori, H., Shirakawa, K. *et al.*, Ataxia with vitamin E deficiency with a mutation in a phospholipid transfer protein gene. *J. Neurol.* 2009, *256*, 1180–1181.

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

[24] Bomar, J. M., Benke, P. J., Slattery, E. L., Puttagunta, R. *et al.*, Mutations in a novel gene encoding a CRAL-TRIO domain cause human Cayman ataxia and ataxia/dystonia in the jittery mouse. *Nat. Genet.* 2003, *35*, 264–269.

[25] Nier, B., Weinberg, P. D., Rimbach, G., Stocklin, E., Barella, L., Differential gene expression in skeletal muscle of rats with vitamin E deficiency. *IUBMB Life* 2006, *58*, 540–548.

[26] Vasu, V. T., Hobson, B., Gohil, K., Cross, C. E., Genome-wide screening of alpha-tocopherol sensitive genes in heart tissue from alpha-tocopherol transfer protein null mice (ATTP(−/−)). *FEBS Lett.* 2007, *581*, 1572–1578.

[27] Vasu, V. T., Ott, S., Hobson, B., Rashidi, V. *et al.*, Sarcolipin and ubiquitin carboxy-terminal hydrolase 1 mRNAs are over-expressed in skeletal muscles of alpha-tocopherol deficient mice. *Free Radic. Res.* 2009, *43*, 106–116.

[28] Gohil, K., Godzdanker, R., O'Roark, E., Schock, B. C. *et al.*, Alpha-tocopherol transfer protein deficiency in mice causes multi-organ deregulation of gene networks and behavioral deficits with age. *Ann. N. Y. Acad. Sci.* 2004, *1031*, 109–126.

[29] Gohil, K., Schock, B. C., Chakraborty, A. A., Terasawa, Y. *et al.*, Gene expression profile of oxidant stress and neurodegeneration in transgenic mice deficient in alpha-tocopherol transfer protein. *Free Radic. Biol. Med.* 2003, *35*, 1343–1354.

[30] Hyland, S., Muller, D., Hayton, S., Stoecklin, E., Barella, L., Cortical gene expression in the vitamin E-deficient rat: possible mechanisms for the electrophysiological abnormalities of visual and neural function. *Ann. Nutr. Metab.* 2006, *50*, 433–441.

[31] Manor, D., Morley, S., The alpha-tocopherol transfer protein. *Vitam. Horm.* 2007, *76*, 45–65.

[32] Kozul, C. D., Nomikos, A. P., Hampton, T. H., Warnke, L. A. *et al.*, Laboratory diet profoundly alters gene expression and confounds genomic analysis in mouse liver and lung. *Chem. Biol. Interact.* 2008, *173*, 129–140.

[33] Reeves, P. G., Nielsen, F. H., Fahey, G. C., Jr., AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* 1993, *123*, 1939–1951.

[34] Einarson, L., Deposits of fluorescent acid-fast products in the nervous system and skeletal muscles of adult rats with chronic vitamin-E deficiency. *J. Neurol. Neurosurg. Psychiatr.* 1953, *16*, 98–109.

[35] Goss-Sampson, M. A., Kriss, A., Muddle, J. R., Thomas, P. K., Muller, D. P., Lumbar and cortical somatosensory evoked potentials in rats with vitamin E deficiency. *J. Neurol. Neurosurg. Psychiatr.* 1988, *51*, 432–435.

[36] Pillai, S. R., Traber, M. G., Kayden, H. J., Cox, N. R. *et al.*, Concomitant brainstem axonal dystrophy and necrotizing myopathy in vitamin E-deficient rats. *J. Neurol. Sci.* 1994, *123*, 64–73.

[37] Hayton, S. M., Muller, D. P., Vitamin E in neural and visual function. *Ann. N. Y. Acad. Sci.* 2004, *1031*, 263–270.

[38] Hayton, S. M., Kriss, T., Wade, A., Muller, D. P., Effects on neural function of repleting vitamin E-deficient rats with alpha-tocopherol. *J. Neurophysiol.* 2006, *95*, 2553–2559.

[39] Davies, K. J., Quintanilha, A. T., Brooks, G. A., Packer, L., Free radicals and tissue damage produced by exercise. *Biochem. Biophys. Res. Commun.* 1982, *107*, 1198–1205.

[40] Gohil, K., Packer, L., de Lumen, B., Brooks, G. A., Terblanche, S. E., Vitamin E deficiency and vitamin C supplements: exercise and mitochondrial oxidation. *J. Appl. Physiol.* 1986, *60*, 1986–1991.

[41] Draper, H. H., Csallany, A. S., A simplified hemolysis test for vitamin E deficiency. *J. Nutr.* 1969, *98*, 390–394.

[42] Holloszy, J. O., Adaptation of skeletal muscle to endurance exercise. *Med. Sci. Sports* 1975, *7*, 155–164.

[43] Tiidus, P. M., Houston, M. E., Vitamin E status does not affect the responses to exercise training and acute exercise in female rats. *J. Nutr.* 1993, *123*, 834–840.

[44] Packer, L., Almada, A. L., Rothfuss, L. M., Wilson, D. S., Modulation of tissue vitamin E levels by physical exercise. *Ann. N. Y. Acad. Sci.* 1989, *570*, 311–321.

[45] Thomas, P. K., Cooper, J. M., King, R. H., Workman, J. M. *et al.*, Myopathy in vitamin E deficient rats: muscle fibre necrosis associated with disturbances of mitochondrial function. *J. Anat.* 1993, *183*, 451–461.

[46] Gerster, H., Function of vitamin E in physical exercise: a review. *Z. Ernahrungswiss.* 1991, *30*, 89–97.

[47] Takanami, Y., Iwane, H., Kawai, Y., Shimomitsu, T., Vitamin E supplementation and endurance exercise: are there benefits? *Sports Med.* 2000, *29*, 73–83.

[48] Koren, A., Sauber, C., Sentjurc, M., Schara, M., Free radicals in tetanic activity of isolated skeletal muscle. *Comp. Biochem. Physiol.* 1983, *74*, 633–635.

[49] Vasilaki, A., Mansouri, A., Remmen, H., van der Meulen, J. H. *et al.*, Free radical generation by skeletal muscle of adult and old mice: effect of contractile activity. *Aging Cell* 2006, *5*, 109–117.

[50] Reid, M. B., Free radicals and muscle fatigue: Of ROS, canaries, and the IOC. *Free Radic. Biol. Med.* 2008, *44*, 169–179.

[51] Meister, A., Selective modification of glutathione metabolism. *Science* 1983, *220*, 472–477.

[52] Le Moan, N., Tacnet, F., Toledano, M. B., Protein-thiol oxidation, from single proteins to proteome-wide analyses. *Methods Mol. Biol.* 2009, *476*, 175–192.

[53] Winterbourn, C. C., Hampton, M. B., Thiol chemistry and specificity in redox signaling. *Free Radic. Biol. Med.* 2008, *45*, 549–561.

[54] Jones, D. P., Radical-free biology of oxidative stress. *Am. J. Physiol. Cell Physiol.* 2008, *295*, C849–C868.

[55] Moriarty-Craige, S. E., Jones, D. P., Extracellular thiols and thiol/disulfide redox in metabolism. *Ann. Rev. Nutr.* 2004, *24*, 481–509.

[56] Ferreira, L. F., Reid, M. B., Muscle-derived ROS and thiol regulation in muscle fatigue. *J. Appl. Physiol.* 2008, *104*, 853–860.

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

[57] Ji, L. L., Stratman, F. W., Lardy, H. A., Enzymatic down regulation with exercise in rat skeletal muscle. *Arch. Biochem. Biophys.* 1988, *263*, 137–149.

[58] McArdle, A., van der Meulen, J. H., Catapano, M., Symons, M. C. *et al.*, Free radical activity following contraction-induced injury to the extensor digitorum longus muscles of rats. *Free Radic. Biol. Med.* 1999, *26*, 1085–1091.

[59] Gohil, K., Viguie, C., Stanley, W. C., Brooks, G. A., Packer, L., Blood glutathione oxidation during human exercise. *J. Appl. Physiol.* 1988, *64*, 115–119.

[60] Viguie, C. A., Frei, B., Shigenaga, M. K., Ames, B. N. *et al.*, Antioxidant status and indexes of oxidative stress during consecutive days of exercise. *J. Appl. Physiol.* 1993, *75*, 566–572.

[61] Misra, H. P., Fridovich, I., The generation of superoxide radical during the autoxidation of hemoglobin. *J. Biol. Chem.* 1972, *247*, 6960–6962.

[62] Winterbourn, C. C., Oxidative reactions of hemoglobin. *Methods Enzymol.* 1990, *186*, 265–272.

[63] Walters, C. L., Casselden, R. J., Taylor, A. M., Nitrite metabolism by skeletal muscle mitochondria in relation to haem pigments. *Biochim. Biophys. Acta* 1967, *143*, 310–318.

[64] Rassaf, T., Flogel, U., Drexhage, C., Hendgen-Cotta, U. *et al.*, Nitrite reductase function of deoxymyoglobin: oxygen sensor and regulator of cardiac energetics and function. *Circ. Res.* 2007, *100*, 1749–1754.

[65] Shiva, S., Huang, Z., Grubina, R., Sun, J. *et al.*, Deoxymyoglobin is a nitrite reductase that generates nitric oxide and regulates mitochondrial respiration. *Circ. Res.* 2007, *100*, 654–661.

[66] Cossins, A., Berenbrink, M., Physiology: Myoglobin's new clothes. *Nature* 2008, *454*, 416–417.

[67] Hendgen-Cotta, U. B., Merx, M. W., Shiva, S., Schmitz, J. *et al.*, Nitrite reductase activity of myoglobin regulates respiration and cellular viability in myocardial ischemia-reperfusion injury. *Proc. Natl. Acad. Sci. USA* 2008, *105*, 10256–10261.

[68] Beinert, H., Ackrell, B. A., Kearney, E. B., Singer, T. P., Iron-sulfur components of succinate dehydrogenase: stoichiometry and kinetic behavior in activated preparations. *Eur. J. Biochem./FEBS* 1975, *54*, 185–194.

[69] Loschen, G., Azzi, A., Richter, C., Flohe, L., Superoxide radicals as precursors of mitochondrial hydrogen peroxide. *FEBS Lett.* 1974, *42*, 68–72.

[70] Jackson, M. J., Redox regulation of adaptive responses in skeletal muscle to contractile activity. *Free Radic. Biol. Med.* 2009, *47*, 1267–1275.

[71] Kobzik, L., Reid, M. B., Bredt, D. S., Stamler, J. S., Nitric oxide in skeletal muscle. *Nature* 1994, *372*, 546–548.

[72] Reid, M. B., Haack, K. E., Franchek, K. M., Valberg, P. A. *et al.*, Reactive oxygen in skeletal muscle. I. Intracellular oxidant kinetics and fatigue in vitro. *J. Appl. Physiol.* 1992, *73*, 1797–1804.

[73] Bailey, D. M., Young, I. S., McEneny, J., Lawrenson, L. *et al.*, Regulation of free radical outflow from an isolated muscle bed in exercising humans. *Am. J. Physiol.* 2004, *287*, H1689–H1699.

[74] Vina, J., Gimeno, A., Sastre, J., Desco, C. *et al.*, Mechanism of free radical production in exhaustive exercise in humans and rats; role of xanthine oxidase and protection by allopurinol. *IUBMB Life* 2000, *49*, 539–544.

[75] Alvarez, R., Vazquez, P., Perez, F., Jimenez, A. *et al.*, Regulation of fast skeletal muscle activity by SERCA1 vicinal-cysteines. *J. Muscle Res. Cell Motil.* 2009, *30*, 5–16.

[76] Novelli, G. P., Bracciotti, G., Falsini, S., Spin-trappers and vitamin E prolong endurance to muscle fatigue in mice. *Free Radic. Biol. Med.* 1990, *8*, 9–13.

[77] Barclay, J. K., Hansel, M., Free radicals may contribute to oxidative skeletal muscle fatigue. *Can. J. Physiol. Pharmacol.* 1991, *69*, 279–284.

[78] Almagor, M., Kahane, I., Gilon, C., Yatziv, S., Protective effects of the glutathione redox cycle and vitamin E on cultured fibroblasts infected by Mycoplasma pneumoniae. *Infect. Immun.* 1986, *52*, 240–244.

[79] Le, C. T., Hollaar, L., Van der Valk, E. J., Franken, N. A. *et al.*, Protection of myocytes against free radical-induced damage by accelerated turnover of the glutathione redox cycle. *Eur. Heart J.* 1995, *16*, 553–562.

[80] Gohil, K., Oommen, S., Quach, H. T., Vasu, V. T. *et al.*, Mice lacking alpha-tocopherol transfer protein gene have severe alpha-tocopherol deficiency in multiple regions of the central nervous system. *Brain Res.* 2008, *1201*, 167–176.

[81] Swift, J. N., Jr., Kehrer, J. P., Seiler, K. S., Starnes, J. W., Vitamin E concentration in rat skeletal muscle and liver after exercise. *Int. J. Sport Nutr.* 1998, *8*, 105–112.

[82] Birringer, M., Pfluger, P., Kluth, D., Landes, N., Brigelius-Flohe, R., Identities and differences in the metabolism of tocotrienols and tocopherols in HepG2 cells. *J. Nutr.* 2002, *132*, 3113–3118.

[83] Mustacich, D. J., Leonard, S. W., Patel, N. K., Traber, M. G., alpha-tocopherol beta-Oxidation localized to rat liver mitochondria. *Free Radic. Biol. Med.* 2009, *48*, 73–81.

[84] Sontag, T. J., Parker, R. S., Cytochrome P450 omega-hydroxylase pathway of tocopherol catabolism. Novel mechanism of regulation of vitamin E status. *J. Biol. Chem.* 2002, *277*, 25290–25296.

[85] Salminen, A., Vihko, V., Pilstrom, L., Effect of endurance training on the capacity of red and white skeletal muscle of mouse to oxidize carboxyl-14C-labelled palmitate. *Acta Physiol. Scand.* 1977, *101*, 318–328.

[86] Gohil, K., Vasu, V. T., Cross, C. E., Gene expression profiles and markers of oxidant stress in tissues of a-TTP-KO mice. unpublished.

[87] Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G., Gluud, C., Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database Syst. Rev.* 2008, CD007176.

[88] Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G., Gluud, C., Mortality in randomized trials of antioxidant

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

supplements for primary and secondary prevention: systematic review and meta-analysis. *J. Am. Med. Assoc.* 2007, *297*, 842–857.

[89] May, J. M., Qu, Z. C., Mendiratta, S., Protection and recycling of alpha-tocopherol in human erythrocytes by intracellular ascorbic acid. *Arch. Biochem. Biophys.* 1998, *349*, 281–289.

[90] Cornwell, D. G., Ma, J., Studies in vitamin E: biochemistry and molecular biology of tocopherol quinones. *Vitam. Horm.* 2007, *76*, 99–134.

[91] Mehlhorn, R. J., Sumida, S., Packer, L., Tocopheroxyl radical persistence and tocopherol consumption in liposomes and in vitamin E-enriched rat liver mitochondria and microsomes. *J. Biol. Chem.* 1989, *264*, 13448–13452.

[92] Green, J., Diplock, A. T., Bunyan, J., McHale, D., Muthy, I. R., Vitamin E and stress. I. Dietary unsaturated fatty acid stress and the metabolism of alpha-tocopherol in the rat. *Br. J. Nutr.* 1967, *21*, 69–101.

[93] Muller, D. R., Harries, J. T., Lloyd, J. K., Vitamin E therapy in abetalipoproteinaemia. *Arch. Dis. Child.* 1970, *45*, 715.

[94] Muller, D. P., Lloyd, J. K., Wolff, O. H., Vitamin E and neurological function. *Lancet* 1983, *1*, 225–228.

[95] Machlin, L. J., Clinical uses of vitamin E. *Acta Vitaminol. Enzymol.* 1985, *7*, 33–43.

[96] Kayden, H. J., Traber, M. G., Neuropathies in adults with or without fat malabsorption. *Ann. N. Y. Acad. Sci.* 1989, *570*, 170–175.

[97] Elias, E., Muller, D. P., Scott, J., Association of spinocerebellar disorders with cystic fibrosis or chronic childhood cholestasis and very low serum vitamin E. *Lancet* 1981, *2*, 1319–1321.

[98] Muller, D. P., Lloyd, J. K., Effect of large oral doses of vitamin E on the neurological sequelae of patients with abetalipoproteinemia. *Ann. N. Y. Acad. Sci.* 1982, *393*, 133–144.

[99] Harding, A. E., Muller, D. P., Thomas, P. K., Willison, H. J., Spinocerebellar degeneration secondary to chronic intestinal malabsorption: a vitamin E deficiency syndrome. *Ann. Neurol.* 1982, *12*, 419–424.

[100] Zamel, R., Khan, R., Pollex, R. L., Hegele, R. A., Abetalipoproteinemia: two case reports and literature review. *Orphanet J. Rare Dis.* 2008, *3*, 19.

[101] Bjorneboe, A., Bjorneboe, G. E., Bodd, E., Hagen, B. F. *et al.*, Transport and distribution of alpha-tocopherol in lymph, serum and liver cells in rats. *Biochim. Biophys. Acta* 1986, *889*, 310–315.

[102] Traber, M. G., Sokol, R. J., Burton, G. W., Ingold, K. U. *et al.*, Impaired ability of patients with familial isolated vitamin E deficiency to incorporate alpha-tocopherol into lipoproteins secreted by the liver. *J. Clin. Invest.* 1990, *85*, 397–407.

[103] Cohn, W., Gross, P., Grun, H., Loechleiter, F. *et al.*, Tocopherol transport and absorption. *Proc. Nutr. Soc.* 1992, *51*, 179–188.

[104] Kayden, H. J., Hatam, L. J., Traber, M. G., The measurement of nanograms of tocopherol from needle aspiration biopsies of adipose tissue: normal and abetalipoproteinemic subjects. *J. Lipid Res.* 1983, *24*, 652–656.

[105] Traber, M. G., Sokol, R. J., Ringel, S. P., Neville, H. E. *et al.*, Lack of tocopherol in peripheral nerves of vitamin E-deficient patients with peripheral neuropathy. *N. Engl. J. Med.* 1987, *317*, 262–265.

[106] Traber, M. G., Sokol, R. J., Kohlschutter, A., Yokota, T. *et al.*, Impaired discrimination between stereoisomers of alpha-tocopherol in patients with familial isolated vitamin E deficiency. *J. Lipid Res.* 1993, *34*, 201–210.

[107] Rajaram, O. V., Fatterpaker, P., Sreenivasan, A., Involvement of binding lipoproteins in the absorption and transport of alpha-tocopherol in the rat. *Biochem. J.* 1974, *140*, 509–516.

[108] Minehira-Castelli, K., Leonard, S. W., Walker, Q. M., Traber, M. G., Young, S. G., Absence of VLDL secretion does not affect alpha-tocopherol content in peripheral tissues. *J. Lipid Res.* 2006, *47*, 1733–1738.

[109] Rajaram, O. V., Fatterpaker, P., Sreenivasan, A., Occurrence of -tocopherol binding protein in rat liver cell sap. *Biochem. Biophys. Res. Commun.* 1973, *52*, 459–465.

[110] Catignani, G. L., An alpha-tocopherol binding protein in rat liver cytoplasm. *Biochem. Biophys. Res. Commun.* 1975, *67*, 66–72.

[111] Catignani, G. L., Bieri, J. G., Rat liver alpha-tocopherol binding protein. *Biochim. Biophys. Acta* 1977, *497*, 349–357.

[112] Murphy, D. J., Mavis, R. D., Membrane transfer of alpha-tocopherol. Influence of soluble alpha-tocopherol-binding factors from the liver, lung, heart, and brain of the rat. *J. Biol. Chem.* 1981, *256*, 10464–10468.

[113] Yoshida, H., Yusin, M., Ren, I., Kuhlenkamp, J. *et al.*, Identification, purification, and immunochemical characterization of a tocopherol-binding protein in rat liver cytosol. *J. Lipid Res.* 1992, *33*, 343–350.

[114] Tamaru, Y., Hirano, M., Kusaka, H., Ito, H. *et al.*, alpha-Tocopherol transfer protein gene: exon skipping of all transcripts causes ataxia. *Neurology* 1997, *49*, 584–588.

[115] Hoshino, M., Masuda, N., Ito, Y., Murata, M. *et al.*, Ataxia with isolated vitamin E deficiency: a Japanese family carrying a novel mutation in the alpha-tocopherol transfer protein gene. *Ann. Neurol.* 1999, *45*, 809–812.

[116] Cavalier, L., Ouahchi, K., Kayden, H. J., Di Donato, S. *et al.*, Ataxia with isolated vitamin E deficiency: heterogeneity of mutations and phenotypic variability in a large number of families. *Am. Journal Hum. Genet.* 1998, *62*, 301–310.

[117] Mariotti, C., Gellera, C., Rimoldi, M., Mineri, R. *et al.*, Ataxia with isolated vitamin E deficiency: neurological phenotype, clinical follow-up and novel mutations in TTPA gene in Italian families. *Neurol. Sci.* 2004, *25*, 130–137.

[118] Qian, J., Atkinson, J., Manor, D., Biochemical consequences of heritable mutations in the alpha-tocopherol transfer protein. *Biochemistry* 2006, *45*, 8236–8242.

[119] Morley, S., Panagabko, C., Shineman, D., Mani, B. *et al.*, Molecular determinants of heritable vitamin E deficiency. *Biochemistry* 2004, *43*, 4143–4149.

[120] Terasawa, Y., Ladha, Z., Leonard, S. W., Morrow, J. D. *et al.*, Increased atherosclerosis in hyperlipidemic mice

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

deficient in alpha -tocopherol transfer protein and vitamin E. *Proc. Natl. Acad. Sci. USA* 2000, *97*, 13830–13834.

[121] Jishage, K., Arita, M., Igarashi, K., Iwata, T. *et al.*, Alpha-tocopherol transfer protein is important for the normal development of placental labyrinthine trophoblasts in mice. *J.Biol. Chem.* 2001, *276*, 1669–1672.

[122] Gordon, J. W., Scangos, G. A., Plotkin, D. J., Barbosa, J. A., Ruddle, F. H., Genetic transformation of mouse embryos by microinjection of purified DNA. *Proc. Natl. Acad. Sci. USA* 1980, *77*, 7380–7384.

[123] Smithies, O., Gregg, R. G., Boggs, S. S., Koralewski, M. A., Kucherlapati, R. S., Insertion of DNA sequences into the human chromosomal beta-globin locus by homologous recombination. *Nature* 1985, *317*, 230–234.

[124] Sedivy, J. M., Sharp, P. A., Positive genetic selection for gene disruption in mammalian cells by homologous recombination. *Proc.Natl. Acad. Sci. USA* 1989, *86*, 227–231.

[125] Baribault, H., Kemler, R., Embryonic stem cell culture and gene targeting in transgenic mice. *Mol. Biol. Med.* 1989, *6*, 481–492.

[126] Khanna, S., Patel, V., Rink, C., Roy, S., Sen, C. K., Delivery of orally supplemented alpha-tocotrienol to vital organs of rats and tocopherol-transport protein deficient mice. *Free Radic. Biol. Med.* 2005, *39*, 1310–1319.

[127] Traber, M. G., Vitamin E regulatory mechanisms. *Annu. Rev. Nutr.* 2007, *27*, 347–362.

[128] Uehara, Y., Minowa, O., Mori, C., Shiota, K. *et al.*, Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor. *Nature* 1995, *373*, 702–705.

[129] Azzi, A., Molecular mechanism of alpha-tocopherol action. *Free Radic. Biol. Med.* 2007, *43*, 16–21.

[130] Brigelius-Flohé, R., Davies, K. J., Is vitamin E an antioxidant, a regulator of signal transduction and gene expression, or a 'junk' food? Comments on the two accompanying papers: ''Molecular mechanism of alpha-tocopherol action'' by A. Azzi and ''Vitamin E, antioxidant and nothing more'' by M. Traber and J. Atkinson. *Free Radic. Biol. Med.* 2007, *43*, 2–3.

[131] Traber, M. G., Atkinson J, Vitamin E, antioxidant and nothing more. *Free Radic. Biol. Med.* 2007, *43*, 4–15.

[132] Brigelius-Flohé, R., Vitamin E: the shrew waiting to be tamed. *Free Radic. Biol. Med.* 2009, *46*, 543–554.

[133] El-Khatib, S., Chenau, U. A., Carpenter, M. P., Trucco, R. E., Caputto, R., Possible presence of lipid peroxides in tissue and tocopherol deficient animals. *Nature* 1964, *201*, 188–189.

[134] Roderuck, C. E., Analysis of certain components of skeletal muscle during vitamin E deficiency. *J. Biol. Chem.* 1949, *181*, 11–16.

[135] Quinn, P. J., Is the distribution of alpha-tocopherol in membranes consistent with its putative functions? *Biochemistry* 2004, *69*, 58–66.

[136] Worman, H. J., Inner nuclear membrane and signal transduction. *J. Cell. Biochem.* 2005, *96*, 1185–1192.

[137] Patel, T. B., Single transmembrane spanning heterotrimeric g protein-coupled receptors and their signaling cascades. *Pharmacol. Rev.* 2004, *56*, 371–385.

[138] Okura, Y., Tawara, S., Kikusui, T., Takenaka, A., Dietary vitamin E deficiency increases anxiety-related behavior in rats under stress of social isolation. *BioFactors* 2009, *35*, 273–278.

[139] Yoshida, Y., Itoh, N., Hayakawa, M., Habuchi, Y. *et al.*, The role of alpha-tocopherol in motor hypofunction with aging in alpha-tocopherol transfer protein knockout mice as assessed by oxidative stress biomarkers. *J. Nutr. Biochem.* 2009, *21*, 66–77.

[140] Tanito, M., Yoshida, Y., Kaidzu, S., Chen, Z. H. *et al.*, Acceleration of age-related changes in the retina in alpha-tocopherol transfer protein null mice fed a Vitamin E-deficient diet. *Invest. Ophthal. Vis. Sci.* 2007, *48*, 396–404.

[141] Mardones, P., Strobel, P., Miranda, S., Leighton, F. *et al.*, Alpha-tocopherol metabolism is abnormal in scavenger receptor class B type I (SR-BI)-deficient mice. *J. Nutr.* 2002, *132*, 443–449.

[142] Chang, E. H., Rigotti, A., Huerta, P. T., Age-related influence of the HDL receptor SR-BI on synaptic plasticity and cognition. *Neurobiol. Aging* 2009, *30*, 407–419.

[143] Yoshida, Y., Hayakawa, M., Cynshi, O., Jishage, K., Niki, E., Acceleration of lipid peroxidation in alpha-tocopherol transfer protein-knockout mice following the consumption of drinking water containing a radical initiator. *J. Oleo Sci.* 2008, *57*, 577–583.

[144] Adams, J. D., Jr., Odunze, I. N., Biochemical mechanisms of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity. Could oxidative stress be involved in the brain? *Biochem. Pharmacol.* 1991, *41*, 1099–1105.

[145] Nakabeppu, Y., Tsuchimoto, D., Yamaguchi, H., Sakumi, K., Oxidative damage in nucleic acids and Parkinson's disease. *J. Neurosci. Res.* 2007, *85*, 919–934.

[146] Cuddihy, S. L., Ali, S. S., Musiek, E. S., Lucero, J. *et al.*, Prolonged alpha-tocopherol deficiency decreases oxidative stress and unmasks alpha-tocopherol-dependent regulation of mitochondrial function in the brain. *J. Biol. Chem.* 2008, *283*, 6915–6924.

[147] Musiek, E. S., Cha, J. K., Yin, H., Zackert, W. E. *et al.*, Quantification of F-ring isoprostane-like compounds (F4-neuroprostanes) derived from docosahexaenoic acid in vivo in humans by a stable isotope dilution mass spectrometric assay. *J. Chromatogr.* 2004, *799*, 95–102.

[148] Hill, A. V., A challenge to biochemists. *Biochim. Biophys. Acta* 1950, *4*, 4–11.

[149] Qian, J., Morley, S., Wilson, K., Nava, P. *et al.*, Intracellular trafficking of vitamin E in hepatocytes: the role of tocopherol transfer protein. *J. Lipid Res.* 2005, *46*, 2072–2082.

[150] Meier, R., Tomizaki, T., Schulze-Briese, C., Baumann, U., Stocker, A., The molecular basis of vitamin E retention: structure of human alpha-tocopherol transfer protein. *J. Mol. Biol.* 2003, *331*, 725–734.

[151] Min, K. C., Kovall, R. A., Hendrickson, W. A., Crystal structure of human alpha-tocopherol transfer protein bound to

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

its ligand: implications for ataxia with vitamin E deficiency. *Proc. Natl. Acad. Sci. USA* 2003, *100*, 14713–14718.

[152] Stocker, A., Molecular mechanisms of vitamin E transport. *Ann. N. Y. Acad. Sci.* 2004, *1031*, 44–59.

[153] Hosomi, A., Goto, K., Kondo, H., Iwatsubo, T. *et al.*, Localization of alpha-tocopherol transfer protein in rat brain. *Neurosci. Lett.* 1998, *256*, 159–162.

[154] Copp, R. P., Wisniewski, T., Hentati, F., Larnaout, A. *et al.*, Localization of alpha-tocopherol transfer protein in the brains of patients with ataxia with vitamin E deficiency and other oxidative stress related neurodegenerative disorders. *Brain Res.* 1999, *822*, 80–87.

[155] Brigelius-Flohé, R., Traber, M. G., Vitamin E: function and metabolism. *FASEB J.* 1999, *13*, 1145–1155.

[156] Vasu, V. T., Oommen, S., Lim, Y., Valacchi, G. *et al.*, Modulation of ozone-sensitive genes in alpha-tocopherol transfer protein null mice. *Inhalation Toxicol.* 2009, *22*, 1–16.

[157] Oommen, S., Vasu, V. T., Leonard, S. W., Traber, M. G. *et al.*, Genome wide responses of murine lungs to dietary alpha-tocopherol. *Free Radic. Res.* 2007, *41*, 98–109.

[158] Gohil, K., Oommen, S., Vasu, V. T., Aung, H. H., Cross, C. E., Tocopherol transfer protein deficiency modifies nuclear receptor transcriptional networks in lungs: modulation by cigarette smoke in vivo. *Mol. Aspects Med.* 2007, *28*, 453–480.

[159] Bechet, D., Tassa, A., Combaret, L., Taillandier, D., Attaix, D., Regulation of skeletal muscle proteolysis by amino acids. *J. Ren. Nutr.* 2005, *15*, 18–22.

[160] Mikhailov, A. T., Torrado, M., The enigmatic role of the ankyrin repeat domain 1 gene in heart development and disease. *Int. J. Dev. Biol.* 2008, *52*, 811–821.

[161] Nell, S., Bahtz, R., Bossecker, A., Kipp, A. *et al.*, PCR-verified microarray analysis and functional in vitro studies indicate a role of alpha-tocopherol in vesicular transport. *Free Radic. Res.* 2007, *41*, 930–942.

[162] Azzi, A., Stocker, A., Vitamin E: non-antioxidant roles. *Prog. Lipid Res.* 2000, *39*, 231–255.

[163] Stocker, A., Azzi, A., Tocopherol-binding proteins: their function and physiological significance. *Antioxid. Redox Signal.* 2000, *2*, 397–404.

[164] Zingg, J. M., Kempna, P., Paris, M., Reiter, E. *et al.*, Characterization of three human sec14p-like proteins: alpha-tocopherol transport activity and expression pattern in tissues. *Biochimie* 2008, *90*, 1703–1715.

[165] Zimmer, S., Stocker, A., Sarbolouki, M. N., Spycher, S. E. *et al.*, A novel human tocopherol-associated protein: cloning, in vitro expression, and characterization. *J. Biol. Chem.* 2000, *275*, 25672–25680.

[166] Stocker, A., Zimmer, S., Spycher, S. E., Azzi, A., Identification of a novel cytosolic tocopherol-binding protein: structure, specificity, and tissue distribution. *IUBMB Life* 1999, *48*, 49–55.

[167] Patnaik, R. N., Nair, P. P., Binding of D-alpha-tocopherol to rat liver nuclear components. *Experientia* 1975, *31*, 1023–1024.

[168] Guarnieri, C., Flamigni, F., Caldarera, C. M., A possible role of rabbit heart cytosol tocopherol binding in the transfer of tocopherol into nuclei. *Biochem. J.* 1980, *190*, 469–471.

[169] Guarnieri, C., Flamigni, F., Caldarera, C. R., Subcellular localization of alpha-tocopherol and its effect on RNA synthesis in perfused rabbit heart. *Ital. J. Biochem.* 1980, *29*, 176–184.

[170] Yamauchi, J., Iwamoto, T., Kida, S., Masushige, S. *et al.*, Tocopherol-associated protein is a ligand-dependent transcriptional activator. *Biochem. Biophys. Res. Commun.* 2001, *285*, 295–299.

[171] Evans, W. J., Campbell, W. W., Sarcopenia and age-related changes in body composition and functional capacity. *J. Nutr.* 1993, *123*, 465–468.

[172] Kim, J. S., Wilson, J. M., Lee, S. R., Dietary implications on mechanisms of sarcopenia: roles of protein, amino acids and antioxidants. *J. Nutr. Biochem.* 2010, *21*, 1–13.

[173] Raj, I. S., Bird, S. R., Shield, A. J., Aging and the force-velocity relationship of muscles. *Exp. Gerontol.* 2010, *45*, 81–90.

[174] Johnson, E. J., Age-related macular degeneration and antioxidant vitamins: recent findings. *Curr. Opin. Clin. Nutr. Metab. Care* 2010, *13*, 28–33.

[175] Ting, A. Y., Lee, T. K., MacDonald, I. M., Genetics of age-related macular degeneration. *Curr. Opin. Ophthalmol.* 2009, *20*, 369–376.

[176] Petersen, R. C., Early diagnosis of Alzheimer's disease: is MCI too late? *Curr. Alzheimer Res.* 2009, *6*, 324–330.

[177] Reitz, C., Mayeux, R., Use of genetic variation as biomarkers for mild cognitive impairment and progression of mild cognitive impairment to dementia. *J. Alzheimers Dis.* 2010, *19*, 229–251.

[178] Cellini, E., Piacentini, S., Nacmias, B., Forleo, P. *et al.*, A family with spinocerebellar ataxia type 8 expansion and vitamin E deficiency ataxia. *Arch. Neurol.* 2002, *59*, 1952–1953.

[179] Cohen-Tannoudji, M., Babinet, C., Beyond 'knock-out' mice: new perspectives for the programmed modification of the mammalian genome. *Mol. Hum. Reprod.* 1998, *4*, 929–938.

[180] Aguzzi, A., Brandner, S., Sure, U., Ruedi, D., Isenmann, S., Transgenic and knock-out mice: models of neurological disease. *Brain Pathol.* 1994, *4*, 3–20.

[181] Campana, W. M., Schwann cells, activated peripheral glia and their role in neuropathic pain. *Brain, Behav. Immun.* 2007, *21*, 522–527.

[182] Gerits, N., Kostenko, S., Moens, U., In vivo functions of mitogen-activated protein kinases: conclusions from knock-in and knock-out mice. *Transgenic Res.* 2007, *16*, 281–314.

[183] Jacquier, A., The complex eukaryotic transcriptome: unexpected pervasive transcription and novel small RNAs. *Nat. Rev. Genet.* 2009, *10*, 833–844.

[184] Munro, K. M., Perreau, V. M., Current and future applications of transcriptomics for discovery in CNS disease and injury. *Neurosignals* 2009, *17*, 311–327.

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

[185] Ward, M., Guntert, A., Campbell, J., Pike, I., Proteomics for brain disorders--the promise for biomarkers. *Ann. N. Y. Acad. Sci.* 2009, *1180*, 68–74.

[186] Franklin, T. B., Mansuy, I. M., Epigenetic inheritance in mammals: evidence for the impact of adverse environmental effects. *Neurobiol. Dis.* 2009, in press.

[187] Backhed, F., Crawford, P. A., Coordinated regulation of the metabolome and lipidome at the host-microbial interface. *Biochim. Biophys. Acta* 2009, in press.

[188] Ishii, T., Itoh, K., Sato, H., Bannai, S., Oxidative stress-inducible proteins in macrophages. *Free Radic. Res.* 1999, *31*, 351–355.

[189] Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T. *et al.*, Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev.* 1999, *13*, 76–86.

[190] Venugopal, R., Jaiswal, A. K., Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes. *Oncogene* 1998, *17*, 3145–3156.

[191] Koch, S., Volkmar, C. M., Kolb-Bachofen, V., Korth, H. G. *et al.*, A new redox-dependent mechanism of MMP-1 activity control comprising reduced low-molecular-weight thiols and oxidizing radicals. *J. Mol. Med.* 2009, *87*, 261–272.

[192] Spiess, P. C., Morin, D., Jewell, W. T., Buckpitt, A. R., Measurement of protein sulfhydryls in response to cellular oxidative stress using gel electrophoresis and multiplexed fluorescent imaging analysis. *Chem. Res. Toxicol.* 2008, *21*, 1074–1085.

[193] Passarelli, C., Petrini, S., Pastore, A., Bonetto, V. *et al.*, Myosin as a potential redox-sensor: an in vitro study. *J. Muscle Res. Cell Motil.* 2008, *29*, 119–126.

© 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim