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REVIEW

α-Tocopherol incorporation in mitochondria and microsomes upon supranutritional vitamin E supplementation

Charlotte Lauridsen · Søren Krogh Jensen

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Abstract Vitamin E (α-tocopherol) is a major lipid-soluble chain-breaking antioxidant in humans and mammals and plays an important role in normal development and physiology. The localization of α-tocopherol within the highly unsaturated phospholipid bilayer of cell membranes provides a means of controlling lipid oxidation at the initiation site. Mitochondria are the site for major oxidative processes and are important in fat oxidation and energy production, but a side effect is leakage of reactive oxygen species. Thus, incorporation of α-tocopherol and other antioxidants into mitochondria and other cellular compartments is important in order to maintain oxidative stability of the membrane-bound lipids and prevent damage from the reactive oxygen species. Many studies regarding mitochondrial disease and dysfunction have been performed in relation to deficiency of vitamin E and other antioxidants, whereas relatively sparse information is available regarding the eventual beneficial effects of antioxidant-enriched mitochondria in terms of health and function. This may be due to the fact that only little scientific information is available concerning the effect of supranutritional supplementation with antioxidants on their incorporation into mitochondria and other cellular membranes. The purpose of this review is therefore to briefly summarize experimental data performed with dietary vitamin E treatments in relation to the deposition of α-tocopherol in mitochondria and microsomes.

C. Lauridsen (✉) · S. K. Jensen
Department of Animal Science, Faculty of Science and Technology, Aarhus University, P.O. Box 50, 8830 Tjele, Denmark
e-mail: Charlotte.Lauridsen@agrsci.dk

S. K. Jensen
e-mail: SorenKrogh.Jensen@agrsci.dk

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Introduction

Vitamin E (α-tocopherol) is an essential nutrient for humans and animals and functions as an essential lipid-soluble antioxidant, scavenging lipid peroxyl radicals in a lipid milieu. Novel functions of vitamin E also comprise the regulation of gene activity (Nell et al. 2007). The lipids associated with the subcellular organelles (e.g., mitochondria and microsomes) are especially susceptible to oxidation by virtue of their high contents of phospholipids containing relatively large amounts of polyunsaturated fatty acids (Pearson et al. 1977). Oxidative stress, characterized by increased generation of reactive oxygen species (ROS), and subsequent damage to mitochondria, has been proposed to cause a wide range of metabolic disorders (Mao et al. 2011). It has been estimated that as much as 1–2% of all oxygen consumed may result in the formation of ROS, with the vast majority of ROS being generated in the mitochondria (Gille and Sigler 1995; Ischiropoulos and Beckman 2003). Vitamin E prevents the propagation of free radicals in membranes, and when peroxyl radicals are formed, these react 1,000 times faster with vitamin E than with polyunsaturated fatty acids (Buettner 1993). Interest in preventing oxidant-induced pathologies and reduced stabilization of polyunsaturated fatty acids in the mitochondria and other subcellular fractions has therefore led to studies on antioxidant therapy. Recently, it was claimed (Mao et al. 2011) that the effectiveness of antioxidant therapy seems to be largely limited by the poor capacity to accumulate specifically within the mitochondria. However, there are actually limited amount of data available on the

incorporation of vitamin E and other antioxidants into supranutritional dietary levels. The purpose of this review is therefore to briefly summarize experimental data performed with vitamin E treatments provided via the diet in relation to the deposition of α-tocopherol in mitochondria and microsomes. It is beyond the scope of the present study to describe behavior and location of tocopherols and tocotrienols in membranes, as these topics are already covered in the recent excellent reviews by Atkinson et al. (2008, 2010).

Vitamin E form and function

Vitamin E comprises a group of compounds possessing vitamin E activity, and the most abundant sources of vitamin E are vegetable oils, nuts, and green leafy vegetables. Molecules having vitamin E antioxidant activity include four tocopherols (α-, β-, γ-, and δ-) and four tocotrienols (α-, β-, γ-, and δ-). α-Tocopherol is the compound demonstrating the highest vitamin E activity, and the natural form available in plants is always RRR-α-tocopherol, while the more common used form is synthetic manufactured all-rac-α-tocopheryl acetate. Due to the occurrence of three chiral carbons in the phytyl part, 2^3 possible confirmations of α-tocopherol exist. Thus, the commercial available α-tocopherol consists of a racemic mixture of all eight possible stereoisomers (RRR, RSR, RRS, RSS, SSS, SRS, SSR, and SRR) differing in configuration of the side chain (Fig. 1). The antioxidant activity of these eight stereoisomer forms is equal, but those with 2R-configuration have the highest biological activity.

Animal feed is commonly supplemented with all-rac-α-tocopheryl acetate although other esterified forms such as tocopheryl succinate are available as well. Tocopherols (vitamin E) are absorbed in the small intestine as free alcohols alone or in combination with emulsified fat products; these commercially available forms must be hydrolyzed by the bile acid-activated enzyme carboxylic ester hydrolase before absorption, a process that may be limited in young animals due to limited activity of carboxylic ester hydrolase (Lauridsen et al. 2001).

Once absorbed in the intestine, vitamin E enters the circulation via the lymphatic system. It is absorbed together with lipids, packed into chylomicrons, and transported to the liver with the chylomicrons and the remnants derived thereof. No differences among the different vitamin E forms appear at this stage. However, the liver is playing an essential role in the vitamin E metabolism due to the presence of the α-tocopherol transfer protein, α-TTP, whereby the α-tocopherol, not β-, γ-, δ-tocopherols, is maintained in human plasma and tissues. Most of the ingested β-, γ-, δ-tocopherols are secreted into the bile or not taken up and excreted in the feces. Likewise, α-TTP preferentially discriminates between the 8 stereoisomer forms of α-tocopherol, that is, the 2S-forms exert very little importance for the vitamin E activity due to their limited bioavailability. Whereas nutritive vitamin E deficiency is rare in humans, defects in the gene for α-TTP lead to severe general vitamin E deficiency with characteristics neurological disorders. In patients suffering from familial isolated vitamin E deficiency having defective α-TTP (Traber et al. 1993), the default pathway results in lysosomal accumulation of α-tocopherol and its ultimative excretion rather than the secretion of α-tocopherol into plasma. In addition, we have found notable differences between animal species with regard to the biodiscrimination between the 2R-forms (Jensen and Lauridsen 2007), and symptoms of vitamin E deficiency also depend on the animal species, that is, rodents and calves develop myocardial necroses, rodents and poultry encephalomalacia, and myopathy (such as Mulberrys heart disease in swine) *retinophatia pigmentosa* are often observed phenomena.

The relative bioavailability of the different forms of vitamin E varies between tissues as well as with dose and time after dosing and duration of dosing as demonstrated in a review by Blatt et al. (2004). With regard to enrichment of plasma and the further incorporation into cellular membranes, Mino et al. (1985) injected vitamin E-deficient rats with 10 mg/kg of all-rac-α-tocopherol intramuscularly and observed that the tocopherol concentration in plasma promptly increased and reached a maximum level at 3–6 h, while that in red blood cells (RBC) reached a maximum after 12 h. The changing pattern of RBC tocopherol concentrations coincided with that of tocopherol concentrations in total liver homogenates and in subcellular fractions of the liver, except for the microsomal fractions, which had a maximum concentration at 6 h. Irrespective of supplementation or control, close correlation between RBC α-tocopherol and liver subcellular fraction α-tocopherol was obtained (supplemented rats: mitochondrial fraction, 0.85, $p < 0.001$; microsomal fraction, 0.75, $p < 0.001$; deficient rats: mitochondrial fraction, 0.97, $p < 0.001$; microsomal fraction, 0.98, $p < 0.001$), whereas with regard to plasma, no correlation to liver subcellular fractions was obtained (Mino et al. 1985). When vitamin E was provided by dietary means to rats, it was furthermore demonstrated that mitochondrial and microsomal fractions were the

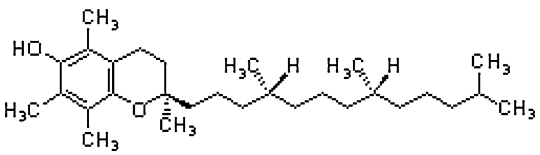

Fig. 1 Chemical structure of α-tocopherol

major tocopherol-containing fractions of liver subcellular membrane fractions and that both inner and outer mitochondrial fractions contained substantial amounts of α-tocopherol (Buttriss and Diplock 1988a). Buttriss and Diplock (1988b) furthermore reported that in different intracellular membranes, there are different proportions of tocopherol not only with respect to total lipids but also to individual polyunsaturated fatty acids. Recently, it has been hypothesized that α-tocopherol partitions into domains of membranes that are enriched in polyunsaturated phospholipids, amplifying the concentration of the vitamin in the place where it is most needed (Atkinson et al. 2010).

Due to the great interest in the antioxidant function of vitamin E, studies of metabolism have concentrated on metabolites resulting from oxidation of the chroman moiety. Besides degradation in the liver by side chain reaction, the main hepatic oxidation product is the α-tocopheryl quinone (Liebler 1993). The distribution of α-tocopherol and α-tocopheryl quinone was investigated in mitochondrial inner and outer membranes of liver mitochondria of rats receiving excessive tocopherol, and the ratio between quinone and tocopherol tended to be higher in the mitochondrial inner membrane than in the outer membrane (Gregor et al. 2006). The data furthermore showed that the α-tocopheryl quinone arising from excessive oxidative degradation of α-tocopherol can potentially interfere with mitochondrial electron transfer (Gregor et al. 2006). Searching for urinary metabolites of vitamin E, another metabolite of α-tocopherol, 2,5,7,8-tetramethyl-2(2′-carboxyethyl)-6-hydroxychroman (α-CEHC), was identified (Schultz et al. 1995). The metabolites of vitamin E are the CEHC products of the respective forms of vitamin E, that is, α-, β-, γ-, and δ-CEHC, and the various non-α-tocopherol forms are metabolized in preference to α-tocopherol (Swanson et al. 1999; Sontag and Parker 2002). Recently, it has been indicated that mitochondria play a role in the metabolism of α-tocopherol, as the end product, α-CEHC, was found almost exclusively in mitochondria (Mustacich et al. 2010). Thus, mitochondria play a role in α-tocopherol β-oxidation at both basal dietary and high hepatic levels of α-tocopherol.

Dietary enrichment of mitochondria and microsomes with α-tocopherol

In relation to mitochondrial health and disease, most interest has been given to the importance in case of deficiency of vitamin E and/or lack of other antioxidants. However, since vitamin E is widely ingested via food or as food supplement, studies on the enrichment of mitochondria through supranutritional vitamin E supplementation are an important issue. In fact, little scientific information is available on the possibilities of enriching mitochondria with antioxidants. The amount of vitamin E in membranes is reported in different ways, that is, μg/mg total lipid, μg/mg total protein, or as the ratio of the number of phospholipid molecules per tocol molecule (Atkinson et al. 2008). With regard to vitamin E, most interest in the capability of increasing the concentration of α-tocopherol in mitochondria and microsomes has arisen due to the impact in relation to increased oxidative stability of muscle food. Thus, most data on the effectiveness of dietary vitamin E on enrichment of α-tocopherol in subcellular fractions have originated from experiments performed on skeletal muscle of livestock.

Experiments with muscle membranes isolated from livestock

Lipid oxidation is one of the primary mechanisms of quality deterioration in cooked meat systems during storage (Pearson et al. 1977, Tichivangana and Morrissey 1985; Gray and Pearson 1987). It is generally believed that lipid oxidation in muscle foods is initiated in the highly unsaturated phospholipids fraction of subcellular membranes (Gray and Pearson 1987). α-Tocopherol functions as a free radical quencher in biological cells (Machlin 1984); its localization within the phospholipids bilayer of cell membranes provides a means of controlling lipid oxidation at a likely initiation site (Hafeman and Hoekstra 1977). α-Tocopherol from the diets of animals is probably preferentially incorporated into the plasma membranes of mitochondria and microsomes (Arnold et al. 1993), that is, compared to muscle homogenate, α-tocopherol has been found to be approximately 6.0 and 8.2 times higher in muscle mitochondria and microsomes, respectively (Monahan et al. 1990a, b). It appears that cytosolic binding proteins facilitate the transport of α-tocopherol into the mitochondrial (Mowri et al. 1981) and microsomal (Murphy and Mavis 1981) membranes. Thus, α-tocopherol is most concentrated in cell fractions such as mitochondria and microsomes. Table 1 summarizes the different in vivo experiments performed to study the positive effect of dietary vitamin E on the increase in α-tocopherol in mitochondria and microsomes of different animal species. In brief, the α-tocopherol content of the subcellular membranes of porcine and chicken muscle can be elevated by supplementation of α-tocopherol in the diets of these animals. The increase can be obtained both by using long term lower dietary doses and by short-term supplementation with high levels of the vitamin in the diet (Wen et al. 1997) (Table 1). It appeared also that the mechanism of α-tocopherol deposition in the subcellular membranes may be species specific as the microsomal concentrations in cattle/beef were higher than the mitochondrial content

Table 1 Experiments showing influence of dietary vitamin E supplementation on α-tocopherol levels in muscle membranal fractions of livestock

Animal species	Muscle	Subcellular fractions	Dietary treatments	Results	References
Pig	Longissimus dorsi	Mitochondria, microsomes	40 and 160 mg/kg diet	2.7- and 2.6-fold increase of mitochondria and microsomes, respectively	Monahan et al. (1990b)
Pig	Loin	Mitochondria, microsomes	10, 100, 200 mg vitamin E/kg feed	Mitochondria: 8.3- and 12.0-fold increase with 100 and 200 mg/kg, respectively Microsomes: 2.5- and 4.4-fold increase with 100 and 200 mg/kg, respectively	Asghar et al. (1991)
Pig	Muscle	Microsomes	10 and 200 mg all-rac-α-tocopheryl acetate/kg	6.6-fold increase	Monahan et al. (1993)
Cattle	Longissimus lumborum	Mitochondria, microsomes	0 and 2,000 IU/day all-rac-α-tocopheryl acetate/kg	4.4-fold increase for mitochondria and microsomes	Arnold et al. (1993)
Cattle	Leg muscle (semitendinosus, semimembranosus, adductor and biceps femoris)	Mitochondria, microsomes	0 and 500 mg	8- and 5.1-fold increase for mitochondria and microsomes	Engeseth et al. (1993)
Chicken	Breast and thigh	Mitochondria, microsomes	20 and 200 mg all-rac-α-tocopheryl acetate/kg feed and either olive oil or tallow	Mitochondria: 3.2-fold increase for breast and muscle Microsomes: 4.7- and 6-fold increase for breast and thigh, respectively (tallow), and 2.4- and 2.6–fold for breast and thigh, respectively, for olive oil	Lauridsen et al. (1997)
Pig	Gluteo biceps	Mitochondria, microsomes	30, 200, and 1,000 mg vitamin E/kg feed (duration 4 weeks)	Mitochondria: 3.6- and 6.1-fold increase with 200 and 1,000, respectively Microsomes: 2.9- and 5.6-fold increase w 200 and 1,000 mg, respectively	Wen et al. (1997)
Pig	Longissimus dorsi (ld) and psoas major (pm)	Mitochondria, microsomes	20 and 200 mg all-rac-α-tocopheryl acetate/kg feed	Mitochondria: 3.1- and 2.4-fold increase for pm and ld, respectively Microsomes: 2.1- and 2.2-fold increase for pm and ld, respectively	Lauridsen et al. (2000)
Pig	Semimembranosus	Mitochondria, microsomes	0, 200, and 500 mg all-rac-α-tocopheryl acetate/kg feed	Mitochondria: 1.8- and 2.0-fold increase with 200 and 500 mg/kg, respectively Microsomes: 1.9- and 2.9-fold increase with 200 and 500 mg/kg, respectively	Onibi et al. (2000)
Pig	Muscle (biceps femori and/or gluteus biceps)	Mitochondria, microsomes	70, 150, and 250 mg all-rac-α-tocopheryl acetate/kg feed to sows [muscles of piglets obtained at weaning (d 28 of age)]	Total α-tocopherol levels increased 1.6- and 1.8-fold increase in mitochondria and microsomes, respectively (70 vs. 250 mg/kg)	Lauridsen and Jensen (2005)

(Engeseth et al. 1993; Arnold et al. 1993), whereas this is generally not the case for pig subcellular membranes, that is, in the study by Lauridsen et al. (2000), the concentration of α-tocopherol in the mitochondrial fraction was found to be more sensitive to the feed level than was the concentration in the microsomes.

With regard to chicken, Asghar et al. (1989, 1990) found that supplementation of the diet with α-tocopherol

(200 mg/kg) only appeared to increase the α-tocopherol concentration in the microsomal membranes in the dark meat of chicken and did not increase, to any extent, the concentration of α-tocopherol in the mitochondrial fractions isolated from dark and white meat as compared to those isolated from the control group.

In most of the experiments mentioned in Table 1, the differences in the concentrations of α-tocopherol in the subcellular fractions were evident in the enhanced oxidative stability of the enriched membranes, that is, reduced rates of Fe^{2+}-catalyzed lipid peroxidation (Monahan et al. 1990a), lower peroxidation rates when subcellular fractions were exposed to metmyoglobin/hydrogen peroxide (Asghar et al. 1991), and lower rate of formation of free radicals in subcellular fractions as determined by ESR-spectroscopy using spin-trapping technique (Lauridsen et al. 2000).

The pig is generally accepted as a large animal model in human research due to its similarity to human size, physiology, organ development, and disease progression (Lunney 2007) and is especially a good human model in terms of lipid digestion and absorption (Innis 1993), and the obtained dietary effects may therefore be extrapolated to human membranes. Hence, the effectiveness in α-tocopherol accumulation in mitochondria and microsomes by dietary means seems rather ineffective, underlining that the interest in developing mitochondria-targeted antioxidants, which can accumulate 100- to 1,000-fold within the mitochondria, has arisen (Murphy and Smith 2007).

Other dietary factors to enhance deposition of α-tocopherol in membranal fractions

Although the effect is far as significant as relatively to dietary vitamin E, it should be noted that other dietary components may increase the subcellular concentration of α-tocopherol. In the study by Lauridsen et al. (2000), an additive influence of dietary all-rac-α-tocopheryl acetate (200 mg/kg feed) and copper (175 mg/kg feed) was observed on the concentration of α-tocopherol in mitochondria and microsomes. In addition, the deposition of α-tocopherol in the mitochondrial fraction of the dark (thigh) muscle was higher when chickens were fed olive oil than animal fat; however, no effect of dietary oil treatments was obtained on the deposition of α-tocopherol in mitochondria of light (breast) or microsomal fractions of the chicken muscle (Lauridsen et al. 1997). Furthermore, a sparing effect of coenzyme Q, which is also located in the inner membranes of mitochondria, on vitamin E was observed in a liver mitochondria study with rats (Ibrahim et al. 2000). Thus, both the presence of antioxidant enzymes such as copper-dependent superoxide dismutase and the proportion of unsaturated fatty acids and antioxidants such as coenzyme Q may affect the deposition of α-tocopherol in subcellular membranes. Another factor may be the eventual additive effect of dietary vitamin C on the deposition of α-tocopherol in subcellular fractions. Thus, in pigs, we have shown that vitamin C supplementation increased liver concentration of α-tocopherol, and in immune cells, the proportion of the RRR-α-tocopherol was increased with vitamin C supplementation on the expense of the RRS-stereoisomer form (Fig. 2) (Lauridsen and Jensen 2005). The interaction of these two vitamins in vivo probably indicates an increased regeneration of α-tocopherol (Hamilton et al. 2000). It would be interesting to study the impact of high dietary levels of vitamin C on the deposition of α-tocopherol as well as the distribution of its stereoisomer forms in mitochondria and other subcellular fractions. We have observed differences between animal species with regard to the biodiscrimination between α-tocopherol stereoisomer forms when provided dietary supplements of all-rac-α-tocopheryl acetate (Jensen and Lauridsen 2007), and it is likely that this species influence would also be reflected in the distribution α-tocopherol stereoisomer forms in cellular fractions. It may be expected that RRR-α-tocopherol and the all-rac-stereoisomers behave differently in biological membranes where the protein content is high and stereoselective tocopherol-protein binding may occur (Atkinson et al. 2008).

Experiments with rat liver mitochondria and cultured cells

Injections with α-tocopherol seem to be more efficient in enrichment of subcellular fractions with α-tocopherol compared with dietary vitamin E. Table 2 summarizes experiments with rats given daily injections (or dietary supplements) with α-tocopherol on the subcellular deposition of α-tocopherol in the liver. The strategy of provision

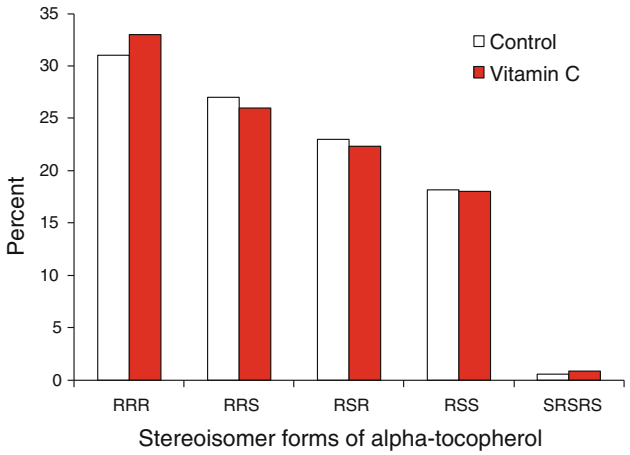

Fig. 2 Distribution of α-tocopherol stereoisomer forms of immune cells (Modified after Lauridsen and Jensen 2005)

Table 2 Experiments showing influence of α-tocopherol provision on α-tocopherol levels in liver membranal fractions of rats

Subcellular fractions	Treatments	Results	References
Mitochondria (inner and outer), microsomes, and lysosomal and nuclear fraction	Depletion versus 100 mg vitamin E (all-rac-α-tocopheryl acetate)/kg diet	α-Tocopherol levels were 16.2-, 8.3-, and 2.9-fold higher in whole washed, inner, and outer mitochondrial fractions, respectively, and 21.6-, 10.2-, and 302-fold higher in nuclear, lysosomal, and microsomal fractions, respectively (supplemented versus deficient rats)	Buttriss and Diplock (1988b)
Mitochondria	10, 110, and 1,320 IU/vitamin E (RRR-α-tocopherol-equivalent/kg diet) and coenzyme Q	App. 1.8- and 2.9-fold increase in mitochondria after 14 and 28 days of dietary treatment with 1,320 IU/kg diet, respectively. Treatment with 110 IU/kg resulted in app. 0.8- and 0.9-fold increase after 14 and 28 days of dietary treatment	Ibrahim et al. (2000)
Mitochondria, microsomes	Daily injections for 9 days with α-tocopherol (subcutaneous) with 10 mg RRR-α-tocopherol/100 g body wt	Total α-tocopherol levels increased 30- and 29-fold in mitochondria and microsomes, respectively (treated vs. control rats)	Gumpricht et al. (2004)
Mitochondria, microsomes, peroxisomes	3 daily α-tocopherol injections (subcutaneous) with 10 mg RRR-α-tocopherol/100 g body wt	Total α-tocopherol levels increased 28-fold in microsomes, and 8-fold and 3-fold in mitochondria and peroxisomes, respectively (treated versus control rats)	Mustacich et al. (2010)

of vitamin E to rats in the study by Gumpricht et al. (2004) was achievable by dietary supplementation. In addition, when endothelial cells from human umbilical vein were cultured with RRR-α-tocopherol, incorporated tocopherol was found (in a dose- and time-dependant manner) to associate predominantly with membrane fractions of the cell, and when expressed on the basis of organelle protein, the highest amount of tocopherol was found in the plasma membrane followed by mitochondria > endoplasmic reticulum > cytosol (Can and Tran 1990). Likewise in human monocytes, it was found (Baoutina et al. 1998) that most (app. 88%) of the α-tocopherol partitioned into the membrane fractions (plasma membrane app. 41%, mitochondria and lysosomes app. 26%, and endosomes plus endoplasmic reticulum app. 21%).

Tocopherol analogues forms

Fariss et al. (2001), Fariss and Zhang (2003) have demonstrated that compared with unesterified α-tocopherol or α-tocopheryl acetate, α-tocopheryl succinate may provide a form of the antioxidant that more rapidly accumulates in liver and liver mitochondria (and hence may be a more effective hepatoprotective agent under ROS-generating conditions). In contrast, trolox, which is a structural analog of α-CEHC, is not taken up by subcellular fractions (microsomes, peroxisomes, or mitochondria) (Mustacich et al. 2010), although app. 100% of the liver trolox was recovered in the liver homogenates.

Recently, Murphy and co-workers developed a series of mitochondria-targeted antioxidants by conjugation with triphenylphosphonium cations, which can permeate lipid bilayers easily and accumulate manyfold within the mitochondria. One of these mitochondria-targeted antioxidants is a mitochondria-targeted vitamin E derivative (Mao et al. 2011). The molecule rapidly permeates lipid bilayers and because of the large mitochondrial membrane potential (negative inside) accumulates several hundredfold inside isolated mitochondria and within mitochondria in cultured cells (Smith et al. 2003). However, the biological response of this molecule (or its potential oxidation products) in comparison with α-tocopherol is yet not known. The in vivo preference for α-tocopherol in comparison with other natural occurring tocopherol analogues forms may also rest on the potential "toxic" activity of the analogues, for example, non-α-tocopherols may generate cytotoxic adducts (Cornwell et al. 2002; Wang et al. 2006).

Importance of function and health of mitochondria

As seen above, it is possible to enrich mitochondria and microsomes of livestock and animal models with α-tocopherol through supranutritional dietary levels of all-rac-α-tocopheryl acetate. However, daily injections with α-tocopherol or provision of mitochondria-targeted vitamin E derivatives seems to be more efficient in provision of α-tocopherol to mitochondria. There is a lack of knowledge, however, regarding the composition of α-tocopherol stereoisomer forms in mitochondria after dietary all-rac-α-tocopheryl acetate supplementation. The reviewed studies have furthermore shown that increased deposition of α-tocopherol would lead to increased stability of mitochondria and microsomes. In some experiments, the impact

of increased membrane α-tocopherol concentration has been studied with respect to the activity and concentration of other antioxidants in the compartment. Although antioxidant therapy has been intensively studied to prevent or treat diseases associated with oxidative damage of mitochondria, the enrichment of α-tocopherol or other antioxidants by mitochondria has not been investigated in parallel with measurements on the function and health of mitochondria. According to the literature (for review see Traber and Atkinson 2007), it seems likely that incorporated α-tocopherol can alter membrane properties by protecting oxidizable lipids and thereby modulate receptors and signaling pathways that are dependant on insertion in specific membrane regions. It was furthermore concluded by the authors (Traber and Atkinson 2007) that virtually all of the variation and scope of vitamin E's biological activity could be seen and understood in light of protection of polyunsaturated fatty acids and membrane qualities that polyunsaturated fatty acids bring about. Although vitamin E is generally not considered as having any toxic effects at high dietary levels, it should be mentioned that some possible harmful outcomes have been reported (Traber and Atkinson 2007).

Hence, when addressing the biologic impact of antioxidant therapy such as vitamin E supplementation in relation to mitochondrial function and health, it may be recommended in future studies to take into consideration the fatty acid composition of the cellular membranes and the parallel effect on gene expression analyses. Both diet and biosynthesis are informatic codes for membranes fatty acid composition, which is also prone to genetic variation. Studies on incorporation of vitamin E into mitochondria and the impact on membrane function in terms of lipid stabilization actually show that the response is influenced by dietary fatty acid treatments (Lauridsen et al. 1997; Onibi et al. 2000). The possibility that modulation of gene transcription may be indirectly linked through tocopherol's effects on membranes as discussed (Nell et al. (2007); Atkinson et al. 2008) may open for further research of the vitamin in relation to mitochondrial alterations and human health.

Conclusion

α-Tocopherol is most concentrated in membrane-rich fractions such as mitochondria and microsomes, and it is possible by dietary means to further increase the concentration. Injection with α-tocopherol may provide efficient mitochondrial deposition of the vitamin. However, the optimal level of vitamin E supplementation for mitochondrial health still has to be determined. In addition, its deposition in mitochondria and other membranal fractions may be further influenced by the presence of fatty acids and other antioxidants. Hence, future experiments studying antioxidant therapy for increased mitochondrial performance should also consider the impact of the variation in membranal fatty acid composition and the relationship with gene regulation.

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References

Arnold RN, Arp SC, Scheller KK, Williams SN, Schaefer DM (1993) Tissue equilibration and subcellular distribution of vitamin E relative to myoglobin and lipid oxidation in displayed beef. J Anim Sci 71:105–118

Asghar A, Lin CF, Gray JI, Buckley DJ, Booren AM, Crackel RL, Flegal CJ (1989) Influence of oxidized dietary oil and antioxidant supplementation on membrane-bound lipid stability in broiler meat. Br Poult Sci 30:815–823

Asghar A, Lin CF, Gray JI, Buckley DJ, Booren AM, Flegal CJ (1990) Effects of dietary oils and α-tocopherol supplementation on membranal lipid oxidation in broiler meat. J Food Sci 55:46–50

Asghar A, Gray JI, Booren AM, Gomaa EA, Abouzied MM, Miller ER (1991) Effects of supranutritional dietary vitamin E levels on subcellular deposition of α-tocopherol in muscle and on pork quality. J Sci Food Agric 57:31–41

Atkinson J, Epand RF, Epand RM (2008) Tocopherols and tocotrienols in membranes: a critical review. Free Radic Biol Med 44:739–764

Atkinson J, Harroun T, Wassall ST, Stillwell W, Katsaras J (2010) The location and behaviour of α-tocopherol in membranes. Mol Nutr Food Res 54:641–651

Baoutina A, Dean RT, Jessup W (1998) Alpha-tocopherol supplementation of macrophages does not influence their ability to oxidize LDL. J Lipid Res 39:114–130

Blatt DH, Pryor WA, Mata JE, Rodriguez-Proteau R (2004) Re-evaluation of the relative potency of synthetic and natural α-tocopherol: experimental and clinical observations. J Nutr Biochem 15:380–395

Buettner GR (1993) The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. Arch Biochemica Biophys 300:535–543

Buttriss JL, Diplock AT (1988a) The relationship between α-tocopherol and phospholipid fatty acids in rat liver subcellular membrane fractions. Biochim Biophys Acta 962:81–90

Buttriss JL, Diplock AT (1988b) The α-tocopherol and phospholipid fatty acid content of rat liver subcellular membranes in vitamin E and selenium deficiency. Biochim Biophys Acta 963:61–69

Can AC, Tran K (1990) The uptake of (R, R, R,) α-tocopherol by human endothelial cells in culture. Lipids 25:17–21

Cornwell DG, Williams MV, Wani AA, Wani G, Shen E, Jones KH (2002) Mutagenicity of tocopheryl quinines: evolutionary advantage of selective accumulation of dietary alpha-tocopherol. Nutr Cancer 43:111–118

Engeseth NJ, Gray JI, Booren AM, Asghar A (1993) Improved oxidative stability of veal lipids and cholesterol through dietary vitamin E supplementation. Meat Sci 35:1–15

Fariss MW, Zhang JG (2003) Vitamin E therapy in Parkinson's disease. Toxicology 189:129–146

Fariss MW, Nicholls-Grzemski FA, Timenstein MA, Zhang JG (2001) Enhanced antioxidant and cytoprotective abilities of

vitamin E succinate is associated with a rapid uptake advantage in rat hepatocytes and mitochondria. Free Radic Biol Med 31:530–541

Gille G, Sigler K (1995) Oxidative stress and living cells. Folia Microbiol 40:131–152

Gray JI, Pearson AM (1987) Rancidity and warmed-over flavour. In: Pearson AM, Dutson TR (eds) Advances in meat research, 3: restructured meat and poultry products. Van Nostrand Reinhold, New York, pp 221–269

Gregor W, Staniek K, Nohl H, Gille L (2006) Distribution of tocopheryl quinone in mitochondrial membranes and interference with ubiquinone-mediated electron transfer. Biochem Phamarcol 71:1589–1601

Gumpricht E, Devereaux MW, Traber M, Sokol RJ (2004) Enrichment of rat hepatic organelles by vitamin E administered subcutaneously. Free Radic Biol Med 37:1712–1717

Hafeman DG, Hoekstra WG (1977) Lipid peroxidation in vivo during vitamin E and selenium deficiency in the rat as monitored by ethane evolution. J Nutr 107:666–672

Hamilton IMJ, Gilmore WS, Benzie IFF, Mulholland CW, Strain JJ (2000) Interactions between vitamin C and E in human subjects. Br J Nutr 84:261–267

Ibrahim WH, Bhagavan HN, Chopra RK, Chow CK (2000) Dietary coenzyme Q10 and vitamin E alter the status of these compounds in rat tissues and mitochondria. J Nutr 130:2343–2348

Innis SM (1993) The colostrum deprived piglet as a model for study of infant nutrition. J Nutr 123:386–390

Ischiropoulos H, Beckman JS (2003) Oxidative stress and nitration in neurodegeneration: cause, effect, or association? J Clin Invest 111:163–169

Jensen SK, Lauridsen C (2007) α-Tocopherol stereoisomers. Vitam Horm 76:281–308

Lauridsen C, Jensen SK (2005) Influence of supplementation of all-rac-α-tocopheryl acetate preweaning and vitamin C postweaning on α-tocopherol and immune responses of piglets. J Anim Sci 83:1274–1286

Lauridsen C, Morrissey PA, Buckley DJ (1997) Influence of dietary fat and vitamin E supplementation on α-tocopherol levels and fatty acid profiles in chicken muscle membranal fractions and on susceptibility to lipid peroxidation. Meat Sci 46:9–22

Lauridsen C, Jensen SK, Skibsted LH, Bertelsen G (2000) Influence of supranutritional vitamin E and copper on α-tocopherol deposition and susceptibility to lipid oxidation of porcine membranal fractions of M. Psoas major and M. Longissimus dorsi. Meat Sci 54:377–384

Lauridsen C, Hedemann MS, Jensen SK (2001) Hydrolysis of tocopheryl and retinyl esters by porcine carboxyl ester hydrolase is affected by their carboxylate moiety and bile acids. J Nutr Biochem 12:219–224

Liebler DC (1993) The role of metabolism in the antioxidant function of vitamin E. Crit Rev Toxicol 23:147–169

Lunney JK (2007) Advances in swine biomedical model genomics. Int J Biol Sci 3:179–184

Machlin LJ (1984) Vitamin E. In: Machlin LJ (ed) Handbook of vitamins: nutritional, biochemical and clinical aspects. Marcel Dekker, New York, pp 99–145

Mao G, Kraus GA, Kim I, Spurlock ME, Bailey TB, Beitz DC (2011) Effect of mitochondria-targeted vitamin E derivative on mitochondrial alteration and systemic oxidative stress in mice. Br J Nutr 106:87–95

Mino M, Kasugai O, Nagita A (1985) Relationship between red blood cell and liver tocopherol concentration after administration and depletion of vitamin E. Int J Vitam Nutr Res 55:47–51

Monahan FJ, Buckley DJ, Morrissey PA, Asghar A, Hanrahan TJ, Lynch PB (1990a) Effect of dietary vitamin E on the stability of raw and cooked pork. Meat Sci 27:99–108

Monahan FJ, Buckley DJ, Morrissey PA, Lynch PB, Gray JI (1990b) Effect of dietary α-tocopherol levels in porcine tissues and on susceptibility to lipid peroxidation. Food Sci Nutr 42F:203–212

Monahan FJ, Gray JI, Asghar A, Haug A, Shi B, Buckley DJ (1993) Effect of dietary lipid and vitamin E supplementation on free radical production and lipid oxidation in porcine muscle microsomal fractions. Food Chem 46:1–6

Mowri H, Nakagawa Y, Inoue K, Nojima S (1981) Enhancement of the transfer of α-tocopherol between liposomes and mitochondria by rat-liver protein. Eur J Biochem 117:537–542

Murphy DJ, Mavis RD (1981) Membrane transfer of α-tocopherol. J Biol Chem 256:10464–10468

Murphy MP, Smith RA (2007) Targeting antioxidants to mitochondria by conjugation to lipophilic cations. Annu Rev Pharmacol Toxicol 47:629–656

Mustacich DJ, Leonard SW, Patel NK, Traber MG (2010) α-Tocopherol β-oxidation localized to rat liver mitochondria. Free Radic Biol Med 48:73–81

Nell S, Bahtz R, Boβecker A, Kipp A, Lamndes N, Bumke-Vogt C, Halligan E, Lunec J, Brigelius-Flohe R (2007) PCR-verified microarray analysis and functional in vitro studies indicate a role of α-tocopherol in vesicular transport. Free Radic Res 41:930–942

Onibi GE, Scaife JR, Murray I, Fowler VR (2000) Supplementary alpha-tocopherol acetate in full-fat rapeseed-based diets for pigs: effects on the alpha-tocopherol content and fatty acid composition of pig muscle microsomal and mitochondrial membranes and membranes lipid peroxidation. J Sci Food Agric 80:1633–1639

Pearson AM, Love JD, Shorland FB (1977) Warmed over flavour in meat, poultry and fish. Adv Food Res 23:1–74

Schultz M, Leist M, Petrzika M, Gassmann B, Brigelius-Flohė R (1995) Novel urinary metabolite of alpha-tocopherol, 2,5,7,8-tetramethyl-2(2′-carboxyethyl)-6-hydroxychroman, as an indicator of an adequate vitamin E supply? Am J Clin Nutr 62(Suppl):152S–1534S

Smith RAJ, Porteous CM, Gane AM, Murphy MP (2003) Delivery of bioactive molecules to mitochondria in vivo. Proc Natl Acad Sci Med Sci 100:5407–5412

Sontag TJ, Parker RS (2002) Cytochrome P450 omega-hydroxylase pathway pf tocopherol catabolism: novel mechanism of regulation of vitamin E status. J Biol Chem 277:25290–25296

Swanson JE, Ben RN, Burton GW, Parker RS (1999) Urinary excretion of 2,7,8-trimethyl-2-(beta-carboxyethyl)-6-hydroxychroman is a major route of elimination of gamma-tocopherol in humans. J Lipid Res 40:665–671

Tichivangana JZ, Morrissey PA (1985) Metmyoglobin and inorganic metals as prooxidants in raw and cooked muscle systems. Meat Sci 15:107–116

Traber MG, Atkinson J (2007) Vitamin E, antioxidant and nothing more. Free Radic Biol Med 43:4–15

Traber MG, Sokol RJ, Kohlschütter A, Yokota T, Muller DPR, Dufour R, Kayden HJ (1993) Impaired discrimination between stereoisomers of α-tocopherol in patients with familial isolated vitamin E deficiency. J Lipid Res 34:201–210

Wang X, Thomas B, Sachdeva R, Arterburn L, Frye L, Hatcher PG, Cornwell DG, Ma J (2006) Mechanism of arylating quinone toxicity involving Michael adduct formation and induction of endoplasmic reticulum stress. Proc Natl Acad Sci USA 103: 3604–3609

Wen J, Morrissey PA, Buckley DJ, Sheehy PJA (1997) Supranutritional vitamin E supplementation in pigs: Influence on subcellular deposition of α-tocopherol and on oxidative stability by conventional and derivative spectrophotometry. Meat Sci 47: 301–310