The Rise, the Fall and the Renaissance of Vitamin E

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Abstract

This review deals with the expectations of vitamin E ability of preventing or curing, as a potent antioxidant, alleged oxidative stress based ailments including cardiovascular disease, cancer, neurodegenerative diseases, cataracts, macular degeneration and more. The results obtained with clinical intervention studies have highly restricted the range of effectiveness of this vitamin. At the same time, new non-antioxidant mechanisms have been proposed. The new functions of vitamin E have been shown to affect cell signal transduction and gene expression, both *in vitro* and *in vivo*. Phosphorylation of vitamin E, which takes place *in vivo*, results in a molecule provided with functions that are in part stronger and in part different from those of the non-phosphorylated compound. The *in vivo* documented functions of vitamin E (preventing the vitamin E deficiency ataxia (AVED), slowing down the progression of non-alcoholic steato-hepatitis (NASH), decreasing inflammation and potentiating the immune response are apparently based on these new molecular mechanisms. It should be stressed however that vitamin E, when present at higher concentrations in the body, should exert antioxidant properties to the extent that its chromanol ring is unprotected or un-esterified.

Keywords

Vitamin E, tocopherols, non-antioxidant functions, polyphenols, signal transduction, gene expression

Preamble

This article is dedicated to Helmut Sies, a friend with whom I have had frequent scientific discussions but never conflicts; not being likeminded in all ideas possibly we have influenced each other. Seen from the outside we may have looked like enemies but this has never been the case. We have been together at scientific meetings, at New Year celebrations with our families, and at birthday parties. We have even being flying together in the occasion of Gordon Conferences or Keystone Symposia: Helmut was an excellent pilot and I tried to play the role of first officer. Helmut nominated me for as foreign member in the Nordrhein-Westfälische Akademie der Wissenschaften and I helped his Club in Dortmund to establish reciprocity with my club in Boston. It was my special pleasure to offer to Helmut, on behalf of a group of friends, an oil portrait of him with his family, made by Massimo Zuppelli, a prominent Italian painter.

[Insert Photograph 1]

The presentation has been immortalized in the following photograph.

[Insert photograph 2]

And finally, the real Sies family and the one in the background of the portrait are seen together.

[Insert photograph 3]

Henry A. Mattill in 1947, in an Annual Review of Biochemistry article, entitled “Antioxidants” [1] asked the question: “Is vitamin E solely a chemical antioxidant or does it, above and beyond that, also have a physiological role?” And continues “Several considerations do not favor the simple explanation that vitamin E is no more than a chemical antioxidant in living tissue.”

The original intuition of Mattill was not developed and the subsequent scientific research continued for almost 50 years, only in the direction of giving support to the “simple explanation”. Biochemistry, in the years following Mattill’s article, was essentially chemistry applied to biological systems and only later the biological component could grow with the advent of cell and molecular biology. In the chemistry era, free radicals have been intensively studied for their effects on nucleic acids, proteins and lipids and became an abundant source of research articles [2]. In the absence of cell pathophysiology information, chemical damage caused by free radical was set at the origin of all diseases, from cancer to atherosclerosis, to cataract, to neurodegeneration and, taking all this together, to aging. The recipe to fight free radicals was obviously found in the antioxidants which became then the remedy to all diseases and the fountain of youth [3], a concept that failed to be confirmed in most large epidemiological and clinical intervention studies. In the last two decades, alternative non-antioxidant molecular mechanisms were discovered that can explain the regulatory effects of vitamin E on signal transduction and gene expression.

Epidemiological and clinical intervention studies

The notion of vitamin E as a panacea was based at the beginning on animal studies and was later supported by a number of epidemiological studies. The latter, based on food intake analyses [4]

concluded that the intake of the natural antioxidant vitamin E protected against free radicals involved in cardiovascular disease [5, 6], cancer [7, 8], cataract [9] and neurodegeneration [10-12]. These conclusions became hypotheses for a number of intervention studies aimed at confirming them. Antioxidants like vitamin E, β -carotene, and selenium were tested in a large number of individuals with the expectation of great benefits. Although some of the initial results were positive [13] [14] later studies were largely negative [15-23] and even more disappointing were the derived meta-analyses that showed no benefit of vitamin E supplementation, but high doses vitamin E significantly increased overall mortality [24-30], although this conclusion has been challenged [31] [32]. Also the trial of a combination of vitamin E and selenium to prevent prostate cancer showed an increase of prostate cancer associated with vitamin E intake at higher doses [33]. The possibility that the antioxidants would not work as radical scavengers or that free radical damage was not at the basis of the diseases to be prevented by the antioxidant was rarely considered.

Similar to vitamin E, polyphenols biological functions have been attributed to their antioxidant properties, very potent *in vitro* but not when introduced into the body, due to their very low plasma and tissue concentrations, their inefficient uptake, their rapid modification and elimination [34-36]. Phenolic antioxidants response takes place at transcriptional level *in vitro* and *in vivo*. The transcription factor Nrf2 binds to the ARE (antioxidant responsive element) to activate gene transcription in response to tocopherols and polyphenols such as curcumin in specific tissues [37-39]. Transcribed genes are among others NAD(P)H quinone oxidoreductase 1, Glutamate-cysteine ligase, Sulfiredoxin 1 (SRXN1), Thioredoxin reductase 1 (TXNRD1), Heme oxygenase-1 (HMOX1, HO-1), Glutathione S-transferase (GST), UDP-glucuronosyltransferase (UGT) and Multidrug resistance-associated proteins (Mrps). The genes that are expressed under the effect of phenolic antioxidants can be grouped under the definition of cytoprotective genes although the more accepted teleological description is that of antioxidant genes. Vitamin E does not induce Nrf2 and cytoprotective genes, being apparently seen not as much as a foreign compound, like the phenolic antioxidants.

Documented *in vivo* effects of vitamin E

Not only vitamin E does not activate a cytoprotective response apt to produce its elimination but in fact its maintenance in the body is under the control of a specific transport protein, α -TTP, which selectively enriches only α -tocopherol [40]. α -TTP absence or mutation results in a neurological disease **ataxia with isolated vitamin E deficiency (AVED)** [41]. Vitamin E exerts a protective role against the neurological symptoms in this disease. Notwithstanding the general failure of vitamin E in protecting against disease, AVED remains a well-founded case of positive function of vitamin E. However, the molecular mechanism of this action has not yet been clarified. Few additional effects of vitamin E *in vivo* have survived its general misfortune. One of these is the effect of vitamin E in enhancing resistance to bacterial pneumonia by modulating the **innate immune response** [42-46]. Another established response is that on **non-alcoholic steato-hepatitis (NASH)**. This liver disease associated with obesity and diabetes is

progressive and can lead to cirrhosis and liver failure [47-49]. Vitamin E appears to be one of the best options including life-style modifications, weight loss and pioglitazone in non-diabetic patients with biopsy-proven NASH [48]. In NASH-patients vitamin E can also improve both Alanine transaminase (ALT) levels and histology with and without weight loss [50].

Another role of vitamin E that has survived most severe criticisms is that on **inflammation**. Inflammation biomarkers, studied *in vitro* and *in vivo* [51-53] as well as by human intervention studies and their meta-analyses [54-56] are significantly diminished by vitamin E.

How can an “antioxidant” *in vitro* become a “non-antioxidant” *in vivo*

The idea of Henry A. Mattill that vitamin E may not have solely chemical antioxidant properties *in vivo* [1] may be challenged by the following question: if vitamin E is a potent antioxidant in the test tube how can it not be an antioxidant *in vivo*? There are several possible answers [57]. In general, only very small amounts of polyphenols can be absorbed and become bioavailable and therefore they do not significantly contribute to the total antioxidant capacity of the body. During their uptake process in the intestines they can be methylated, acetylated, and glucuronized with loss of their antioxidant properties. After absorption antioxidant molecules can be rapidly metabolized and eliminated. Only one natural phenolic antioxidant, α -tocopherol is efficiently absorbed, in an unmodified form, is not rapidly removed by the drug metabolizing systems and is bioavailable in significant amounts in the body. The other tocopherol analogues (β -, γ -, δ - tocopherols, and the four tocotrienols), despite being of equal antioxidant potency, are not retained in the body and eliminated. Thus, with the exception of α -tocopherol, higher organism eliminate most of the nutritional antioxidants that would be available in the diet; apparently more free radical scavenging is not desired/required most likely because free radicals are important signaling molecules. Although α -tocopherol has been recently demonstrated to influence the oxidation of specific lipids in the brains of zebrafish, it is unclear why only a small fraction of the tested lipids (4 of 155) are protected despite being exposed to the same free radicals and why cellular symptoms of vitamin E deficiency are not mostly seen in tissues with highest free radical levels [58]. Moreover, oxidized vitamin E metabolites have rarely been detected and although vitamin C can regenerate vitamin E [59], this was recently not confirmed [60-62].

Chemical oxidation of Vitamin E is prevented by binding to a number of cellular proteins such as the tocopherol associated proteins (TAP)[63, 64] [65]. TAPs act as chaperone protein by masking the antioxidant hydroxyl residue of vitamin E and thus making of an antioxidant a non-antioxidant molecule. Similarly, specific binding proteins protect retinal [66] or estrogens [67, 68]: these molecules are antioxidants *in vitro* but binding to their specific proteins prevents them from being used as antioxidants. Thus, their physiological functions become highly independent of environmental events. Afamin and albumin can also bind vitamin E in significant amounts [69, 70], offering thus an additional protection mechanism by an extracellular molecule against vitamin E oxidation. An additional possibility for vitamin E

is to lose its antioxidant properties *in vivo* as a consequence of phosphorylation of its hydroxyl group [71]. This event will be discussed in a later chapter of this article.

Alternative molecular functions of vitamin E

Enzyme inhibition. Enzyme inhibition has been suggested to be at the basis of non-antioxidant properties of vitamin E. In particular protein kinase C- α [72][73-75], is inhibited by vitamin E *in vitro* at physiological doses. β -Tocopherol, which is not effective as inhibitor, prevents the inhibition by α -tocopherol [Figure 1]. This seminal finding provided the basis of a series of observations linking the effect of PKC inhibition to the inhibition of superoxide generation in macrophages and neutrophils [76, 77] [78, 79] [Figure 2]. Inhibition of PKC by vitamin E mediates, additionally, resistance to endothelial dysfunction as largely documented [80-82]. These findings clearly link molecular events to the anti-inflammatory effects of vitamin E documented in human studies [54-56, 83]. Another molecular event that may contribute to the anti-inflammatory properties of vitamin E is the direct inhibitory effect of cyclooxygenase [83]. Linked to the inhibition of PKC by vitamin E is its prevention of platelets aggregation [84]

Gene regulation *in vitro*. AP-1 inhibition by α -tocopherol represents the first indications of a gene regulation by vitamin E [85]. Subsequently, other genes have been described to be modulated by vitamin E (for reviews see [86-88]) including the α -tocopherol transfer protein (α -TTP), cytochrome P450 (CYP3A), CD36, SR-BI and SR-AI/II, tropomyosin, collagen(α 1), MMP-1, MMP-19 and connective tissue growth factor (CTGF). In most cases the non-antioxidant role of vitamin E was inferred from the unicity of the effects not shared by other antioxidants, including the vitamers β , γ and δ .

Gene regulation *in vivo*. In vitamin E deficient mice due to knockout of the α -TTP gene, the regulation of entire gene networks is altered, clearly showing a regulatory influence of vitamin E on gene expression *in vivo* [89] [90-92]. Gene expression profiles were also assessed by using μ array analysis on T cells, purified from splenocytes from young and old mice, fed with low or high amounts of Vitamin E. It was shown that aging had significant effects on genes involved in signal transduction, transcriptional regulation, and apoptosis pathways in T cells and that vitamin E had a significant effect on genes associated with the regulation of cell cycle [93]. It has been also shown that vitamin E regulates gene expression in liver, muscle and hypothalamus [94-96]. In subsequent studies, the difference was studied between natural RRR- α -tocopherol and the chemically synthesized racemic mixture of all S and R isomers. T cells from the spleen of mice fed with low or high amounts of either natural or racemic vitamin E were studied. Gene expression changes, assessed by gene array experiments, indicate significant qualitative and quantitative differences between the two vitamin forms. Marker genes were identified that responded uniquely to natural vitamin E and others to the synthetic form [97]. [Table 1] These data unequivocally show the non-antioxidant effect of vitamin E *in vivo*, given the fact that the different forms of vitamin E have the same radical scavenging properties but differentially affect gene expression. In a similar set of experiments the gene transcription differences produced by α - or γ -tocopherol were studied. The two vitamers that differ only by a methyl group on the chromane ring have essentially the same

antioxidant properties. Genes were found uniquely responding to either high α -tocopherol (e.g. induced: CD40 ligand, lymphotoxin A) or γ -tocopherol (e.g. repressed: poliovirus receptor-related-2) [98]. [Table 2 and 3] The observed changes in gene expression in T cells after high γ -tocopherol *in vivo* supplementation appear to be consistent with the anti-inflammatory functions of γ -tocopherol [99].

In conclusion, vitamin E exerts not only *in vitro* but also *in vivo* effects that cannot be attributed to the antioxidant properties of the molecule. Rather, it appears that vitamin E can act by binding to specific sites in enzymes and proteins [100, 101]. These functions, both enzyme regulation and gene transcription, may be implemented at physiological concentrations of the molecule. Supplementation with high amounts of vitamin E may result in the presence of free, unprotected molecules with the capacity of exerting radical scavenging functions. The latter, if uncontrolled, may interfere with the physiological cell signaling mediated by reactive oxygen species.

Tocopheryl phosphate

Phosphorylation of the hydroxyl group on the chromane ring of vitamin E represents a second answer to the question of how vitamin E, a potent antioxidant in the test tube, loses its chemical antioxidant properties *in vivo*.

[Figure 3]

Mammalian cells contain a tocopherol kinase activity competent to phosphorylate α -tocopherol to α -tocopheryl phosphate [71, 102]. Tocopheryl phosphate can be also synthesized *in vivo* [103] and, transported through the intestinal barrier, as indicated by supplementation data (Table 4) [104] At a cellular level tocopheryl phosphate crosses the cell membrane and its transport is inhibited by organic acid transporters inhibitors [105]. As observed with the tocopherols, the TAP proteins recognize tocopheryl phosphate and modulate its transport [106]. It is improbable that the phosphorylated vitamin is a form of storage, given its amount of three orders of magnitude less than that of the free vitamin. Rather, its increased activity suggests that tocopheryl phosphate is the active form of the vitamin. Phosphorylation is, analogously the reaction needed to activate vitamin B6 and B1. The effects of vitamin E, both at a cellular level and *in vivo* may be due to its phosphorylation, provided that the tocopherol kinase activity is present or that tocopherol phosphate is transported to the target cells or tissues. In the absence of the kinase or when the amount of vitamin E exceeds its capacity, antioxidant or other effects caused by the free vitamin can take place. In the proposed scheme [Figure 4] the existence of a tocopheryl phosphate phosphatase is also considered [107, 108]. The target(s) of tocopheryl phosphate and the molecular steps needed to exert the specific functions described above will be dealt with in the next paragraph.

Tocopheryl phosphate: a more active form of vitamin E

Phosphorylation of vitamin E results in increased activity as measured by inhibition of cell proliferation or gene expression. It has been shown that tocopheryl phosphate inhibits cell proliferation [102, 105] more efficiently than tocopherol. In the case of THP-1 monocytes tocopherol does not inhibit cell growth while tocopheryl phosphate shows a potent inhibitory effect [104, 105] indicating that the effect of tocopheryl phosphate is not due to its hydrolysis to tocopherol [Figure 5]. In fact, this experiment indicates that the criticism [108] that tocopheryl phosphate is taken in and hydrolyzed to tocopherol in cultured cells and in mice cannot be generalized. In fact, in the experiments presented by Nishio et al. [108], suggesting that some hydrolysis of tocopheryl phosphate can take place, the remaining, non-hydrolyzed tocopheryl phosphate in cells and mice is not measured.

Evidence of the effect of tocopheryl phosphate as such and not related to its potential hydrolysis to tocopherol has been documented in the study by Zingg et al [104] that shows important regulatory differences between tocopheryl phosphate and tocopherol in THP-1 monocytes. Most remarkable are the differences in gene expression by α -tocopherol and α -tocopheryl phosphate that uniquely up-regulate 11 and 60 genes, respectively. In the case of genes that were up-regulated by both molecules α -tocopheryl phosphate appeared to be more potent than α -tocopherol, up to 34-fold in the case of stanniocalcin 2 (this gene encodes a secreted, homodimeric glycoprotein that is expressed in a wide variety of tissues). Lirangi et al. [109] have also shown in NIH3T3-L1 α -tocopheryl phosphate transcriptionally activates a set of genes TRB3 (Tribbles Homolog 3), Sestrin-2 (SESN2), and Insulin-Induced Gene 1 (INSIG1) that potentially prevent fat accumulation in these cells. The opposite effect (transcriptional inhibition) has been seen with α -tocopherol. In contrast, in differentiated adipocytes, α -tocopheryl phosphate is responsible for the transcriptional inhibition of the same genes which are transcriptionally activated by tocopherol [109]. These molecular events may be significant in the regulation of surplus fat accumulation and in avoidance of lipotoxicity.

The CD36 scavenger receptor/fatty acid transporter was one of the first genes shown to be regulated by vitamin E in vitro and in vivo [110-114]. CD36 interacts with many ligands and mediates their uptake into cells [115]. In particular, oxLDL bind to CD36 and its uptake triggers the formation of foam cells [116]; this event is accelerated by stimulation of aP2 and CD36 expression by oxLDL [110, 117] and consequent acceleration of cholesterol and triglyceride accumulation during atherosclerosis [118]. The binding of oxLDL to CD36 induces the PI3K/Akt/PPAR signalling cascade leading to stimulation of CD36 expression in vascular smooth muscle cells and monocytes/macrophages, an event that can be inhibited by α -tocopherol [110, 113].

In HMC-1 mastocytoma cells, PI3K/Akt and cell proliferation are inhibited by α -, β -, γ -, and δ -tocopherol with different potency, involving inhibition of translocation of Akt to the plasma membrane [119], suggesting regulatory effects that are independent of the essential equal antioxidant activity of these vitamers. In prostate cancer cells, α - and γ -tocopherol inhibit cell proliferation and tumor growth by stimulating membrane translocation and activating the phosphatase PHLPP1, leading to inhibition of Akt [120]. An antagonistic effect between α -tocopherol and α -tocopheryl phosphate on the PI3K/Akt pathway

was observed in THP-1 monocytes, with α -tocopheryl phosphate stimulating Akt(Ser473) phosphorylation [104]. Stimulation of the PI3K γ /Akt pathway by α -tocopheryl phosphate leads to the induction of VEGF expression in these cells, whereas α -tocopherol does not, possibly indicating that α -tocopherol is not efficiently phosphorylated by the α -tocopherol kinase activity in these cells. The induction of VEGF by α -tocopheryl phosphate occurs at the promoter level and is due to up-regulation of the PI3K/Akt signalling pathway, a pathway that is down-regulated by α -tocopherol [107, 113, 121]. Recent research on HEK293 cells also suggests that the human tocopherol associated protein 1 (hTAP1/SEC14L2) enables differential induction of PI3K γ activity by mediating exchange of phosphatidylinositol with tocopherols and/or dissociation of an inactive complex leading to increased VEGF gene expression [71, 104, 113, 119, 122]. Taken together, α -tocopheryl phosphate and α -tocopherol differentially activate the PI3K/Akt pathway, with inhibition involving translocation of the PHLPP1 phosphatase and de-phosphorylation of pAkt, whereas activation involving dissociation of an inactive hTAP1/PI3K-heterodimer and hTAP1-mediated lipid exchange (Figure 6).

It is unknown at this time, whether the induction of VEGF expression by α -tocopheryl phosphate has a physiological function, and the molecular mechanisms of modulation of VEGF by vitamin E are yet to be understood (reviewed in [121]). Vitamin E has been demonstrated to have a regulatory role on VEGF expression in several *in vitro* and *in vivo* experimental systems, in which either activation [123, 124] or inhibition of VEGF [125-127] has been observed. We suggested that α -tocopheryl phosphate activates the PI3K/PKB pathway by acting as an active lipid mediator, and that the un-phosphorylated α -tocopherol inhibits its activation [113, 119, 122]. Based on these results we propose that α -tocopherol is converted locally to α -tocopheryl phosphate leading to the activation of the PI3K/PKB signaling pathway and VEGF production ultimately leading to increased angiogenesis and vasculogenesis (reviewed in [107]). In addition to the promotion of angiogenesis, VEGF plays an important role in the placenta and during embryonic development, where it is required for a successful pregnancy [128, 129]. Thus, in vitamin E deficient state, fetal resorption may be the consequence of decreased expression of VEGF as a result of insufficient production of VEGF what may lead to impaired formation of an extensive vascular net in the placenta, placental ischemia, and inadequate nutrient supply to the fetus [130]. Moreover, other effects of vitamin E can be linked to the induction of VEGF, such as the prevention of ischemia/reperfusion injury in the cardiovascular and nervous system [39, 131], the survival of muscle cells and neurons, the stimulation of neurite outgrowth and wound healing, and the prevention of neuro-degeneration [128, 132-135].

Conclusions

Since the discovery of vitamin E almost 100 years ago, most of the effects of vitamin E have been rationalized by its *in vitro* chemical ability to scavenge free radicals in the lipid phase, raising its status as prominent remedy for all kinds of ailments. However, when scrutinized *in vivo* in large-scale supplementation studies, it failed to reduce increased levels of free radicals as a causal component in

several diseases suggesting alternative mechanism of action. The signaling mechanism discovered during the last two decades offers explanations of the biological actions of vitamin E that go beyond the scavenging of free radicals, awakening its regulatory role as an active lipid mediator essential for signal transduction and gene expression. It appears mandatory to reconsider the role of vitamin E and its active phosphorylated derivative in number of pathological situations for which no explanation was provided by the antioxidant effects of the vitamin.

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Figure 1. Inhibition of protein kinase C by α -tocopherol but not β -tocopherol

Quiescent smooth muscle cells were re-stimulated with 10% FCS in the presence or absence of 50 μ M α -tocopherol or 50 μ M β -tocopherol. After 7 h, cells were permeabilized and PKC was measured. Only the PMA stimulated activity was considered. Control (100%) corresponds to 115.1 ± 24.0 pmol/min per 106 cells. Re-drawn from Ref [72] with permission.

Figure 2. Inhibition by α -tocopherol of the assembly and activity of NADPH oxidase

Diagram drawn according to [79, 136]

Figure 3. Molecular structure of tocopheryl phosphate

Figure 4. Diagram of phosphorylation and de-phosphorylation of tocopherol

According to Negis et al. [102] with permission.

Figure 5

Effect of α -tocopherol (α -T) and α -tocopheryl phosphate (α -TP) on the proliferation of THP-1 monocytes.

Cells were treated with increasing concentrations of α -T and α -TP for 48 h and cell proliferation was measured with an MTS assay kit. Control (C) refers to the 48 h value. The experiment was performed in quadruplicate and the mean and standard deviation were calculated for each treatment. A statistically significant difference of $p < 0.05$ (two-tailed unpaired Student's t-test) in the comparison of any given sample series to the one without added α -TP (control, C) is marked (*) From Ref. [105] with permission.

Figure 6. Mechanism of α -tocopherol and α -tocopheryl phosphate action

Molecular model in which α -tocopherol and α -tocopheryl phosphate can either inhibit or activate the PI3K γ /Akt pathway, depending on the activation state of the protein. Left: PI3K γ /Akt is activated and located at the plasma membrane; α -tocopherol and α -tocopheryl phosphate inhibit activation by activating the phosphatase PHLPP1 and de-phosphorylation of pAkt. Right: PI3K γ is not activated and complexed with hTAP1; α -tocopherol and α -tocopheryl phosphate activate PI3K γ by hTAP1-mediated lipid exchange and dissociation of the inactive hTAP1/PI3K γ heterodimer. Changes in the activity of PI3K γ /Akt will modulate cell proliferation and gene expression (e.g. VEGF, CD36) accordingly.

Table 1. Gene expression by side chain tocopherol isomers: Unique effects of natural vitamin E supplementation on gene up-regulation after ex vivo T cell stimulation

The listed genes were up-regulated only by natural vitamin E supplementation following cell stimulation by antiCD3/CD28. The values represent mean fold expression. N30= animal supplementation with natural vitamin E 30ppm. N500= animal supplementation with natural vitamin E 500ppm. From ref [97] with permission

N30	N500	GENE DESCRIPTION
26.4		Mouse Interleukin 3 (Il3), mRNA.
8.3		immunoglobulin kappa chain variable 28 (V28)
6.2		phosphoenolpyruvate carboxykinase 1, cytosolic
6.1		Mouse adipocyte complement related protein of 30 kDa (Acrp30), mRNA.
4.8		Mouse erythrocyte protein band 4.9 (Epb4.9), mRNA
4.6		Mouse proline rich protein MP5 (Prmp5), mRNA
4.1		Armadillo repeat gene deleted in velo-cardio-facial syndrome
4.0		Expressed sequence AU020229
3.5		Coagulation factor IX
3.4		Mouse clone MGC; 14049, IMAGE: 4188590, mRNA, complete cds.
3.3		Mouse glutamic acid decarboxylase 1(Gad1), mRNA
	7.6	Expressed sequence C79026
	7.3	Mouse adenosine deaminase, RNA-specific, B1 (Adarb1), mRNA
	6.0	Mouse, similar CCAAT enhancer binding protein (CE8P), γ , mRNA, cds
	5.4	ESTs
	4.0	Scgn10 like-protein
	4.0	Mouse thymus 8cl-xl mRNA, complete cds.
	4.0	Mouse coagulation factor V (F5), mRNA.
	3.9	FL=gb:8C016199.1
	3.7	Mouse-adult-male hippocampus cDNA, interferon-stim. Pr. (15 kDa), full insert
	3.2	Mouse serine hydroxymethyltransferase mRNA, complete cds.
	3.1	Mouse cytochrome P450, steroid inducible 3a11, mRNA, complete cds.
	3.1	Mouse zinc finger protein 146 (Zfp146), mRNA.
	3.0	Gap junction membrane channel protein α 1
	3.0	Mouse RIKEN cDNA 2010013B10 gene 2010013B10Rik). mRNA

Table 2. Transcription Potency of γ -tocopherol vs α -tocopherol (500ppm)

From Ref [98] with permission

γ -T/ α -T	Gene name
16.0	serine (or cysteine) peptidase inhibitor, clade H, member 1
13.6	homer homolog 1 (Drosophila)
13.5	nuclear receptor subfamily 4, group A, member 3
6.1	cysteine-rich PDZ-binding protein
3.9	fibroblast activation protein
3.6	atlastinGTPase 3
3.6	solute carrier family 9 (sodium hydrogen exchanger
3.4	early growth response 3
3.4	v-maf musculo aponeurotic fibrosarcoma oncogene family, protein F
3.2	metastasis associated lung adenocarcinoma transcript 1 (non-coding RNA)
3.1	angiotensin I converting enzyme (peptidyl-dipeptidase A)

Table 3. Transcription Potency of α -tocopherol vs γ -tocopherol (500ppm)

From Ref [98] with permission

α -T	γ -T	Gene name
10.8	1.2	CD40 ligand
8.6	0.4	Lymphotoxin A
7.6	0.4	A kinase (PRKA) anchor protein (yotiao) 9
7.3	1.0	Adenosine deaminase, RNA-specific, B1
6.0	1.0	CCAAT enhancer binding protein (CEBP), gamma
4.5	0.7	Signaling lymphocytic activation molecule family member 1
4.2	0.4	Reticuloendotheliosis oncogene
4.2	2.3	Regulator of G-protein signaling 16
4.0	1.0	Stathmin-like 3
4.0	1.0	BCL2-like 1
4.0	1.0	Coagulation factor V;
3.9	1.0	Nucleoredoxin-like 2
3.8	0.8	Glycoprotein 49 A; leukocyte immunoglobulin-like receptor

Table 4. Plasma concentrations of α -tocopheryl phosphate

From Ref [104] with permission

plasma	rats	minipigs	humans, unsupplemented	humans, supplemented with phospho-E (TPM)	
				200 IU, 12 weeks	400 IU, 12 weeks
ng/ml	92 $\pm$ 7 (n=8)	18 $\pm$ 11 (n=42)	15 $\pm$ 10 (n=16)	79 $\pm$ 10 (n=34)	160 $\pm$ 27 (n=35)
nM	173 $\pm$ 13 (n=8)	34 $\pm$ 21 (n=42)	28 $\pm$ 19 (n=16)	149 $\pm$ 19 (n=34)	301 $\pm$ 51 (n=35)

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