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Abstracts

Guest Editor

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Abstracts

1 Mature Breast Milk Vitamin E and Fat Content in Exclusively Breastfeeding Greek Women
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Introduction: Vitamin E demonstrates a multiple role in the functions of human body as it prevents oxidative damage due to sudden exposure to higher oxygen levels than those in intrauterine environment. For this reason, dietary provision of adequate vitamin E is of evident importance. This study aims to determine tocopherol vitamin E and fat content of Greek exclusive breastfeeding mother’s mature breast milk at 20–30 days postpartum and correlate with maternal diet characteristics.

Method: This is a prospective observational study of lactating women (n = 64, mean age 32.5 ± 3.1 years) who delivered healthy full-term neonates. Milk samples and 3-day dietary records were obtained at 20–30 days postpartum. Milk tocopherol content was determined by high-performance liquid chromatography method (HPLC) and fat content by the crematocrit method. Energy intake and macronutrient intake was estimated by using the Nutritionist PRO diet analysis software.

Results: Mature milk’s α-tocopherol content was 8.3 ± 3.4 μmol/L, (β+γ)-tocopherol was 0.59 ± 0.33 μmol/L, δ-tocopherol was 0.018 ± 0.05 μmol/L and total vitamin E was 8.9 ± 3.6 μmol/L. Total fat at first month postpartum was 31.7 ± 16.42 g/L while the ratio Vitamin E/ Total Fat was 0.14 ± 0.06 mg/g. Maternal vitamin E dietary intake was 7.2 ± 3.7 mg/day. Though vitamin E dietary intake was less than recommended, vitamin E content in breast milk was considered sufficient for infant needs. Milk tocopherol content correlated only with mothers’ total fat dietary intake.

Conclusion: This study is among a few in literature to determine tocopherol content of mature breast milk in Mediterranean women and to detect dietary factors which may influence its values. The only maternal dietary characteristic to affect breast milk tocopherol content was mothers’ total fat intake, while tocopherol intake seems to have no effect. Establishment of dietary parameters which affect vitamin E content in human milk helps design health-care strategies, so as to improve human milk quality and therefore the infants’ health status.

References

2 Comparison of Antioxidant Activities of Carotenoids Measured by Photochemiluminescence
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Clinical trials and epidemiological studies have indicated that the incidence of diseases such as inflammation, cardiovascular diseases and cancer becomes lower by the consumption of fruits and vegetables [1]. These chronic and degenerative diseases can be caused by reactive oxygen species (ROS), which can damage biological molecules such as proteins, lipids and DNA [2]. The main compounds, which are able to reduce the oxidation of cellular components in organism, are the antioxidant compounds. The protective effects could be due to their properties as free radical scavengers, hydrogen-donating compounds, singlet oxygen quenchers and metal ion chelators [3]. For these reasons we are interested to analyse the antioxidant activities of foods, of single substances and of biological systems.

In the past, the focus was on the investigation of antioxidant secondary plant products, such as the water-soluble polyphenols and the fat-soluble carotenoids. To determine the total antioxidant capacity (TAC) or rather activity it is important to use different convenient methods for simple and quick quantification. But to date these methods are rarely available for lipophilic substances. The aim of this study was to measure the antioxidant activities of carotenoids and xanthophylls by using a photochemiluminescence (PCL) method in comparison to the antioxidant activity of α-tocopherol, which is used for calibration. The measured antioxidant activities using the PCL method are compared to the results obtained by using other established methods. Our results will be discussed regarding a relation between the antioxidant activities of the carotenoids and their structures. The next step will be the determination of the antioxidant capacity of food products by using this method.

References
3 Vitamin K and Vitamin K-Dependent Proteins in Health and Disease

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Vitamin K or vitamin “Koagulation” (German spelling for coagulation) was discovered by the 1943 Nobel Prize winners Henrik Dam and Edwards A. Doisy, as a fat soluble substance, the deficiency of which caused bleeding disorders. Vitamin K in its reduced form, vitamin K hydroquinone, is required as a cofactor for the γ-glutamyl carboxylase enzyme that catalyses the γ-carboxylation of specific glutamyl residues to γ-carboxyglutamic acid residues. The γ-carboxyglutamate reaction generates γ-carboxyglutamate and vitamin K 2,3-epoxide which is then recycled back to the hydroquinone form by a vitamin K reductase system. Vitamin K-dependent proteins (VKDP) are a family of proteins characterized by such vitamin K-dependent post-translational modifications. Warfarin and its derivatives inhibit the vitamin K epoxide reductase, blocking thereby the gamma-carboxylation reaction. VKDP synthesized in the presence of warfarin are under-gamma-carboxylated and are either not secreted but degraded intracellularly or have impaired biological activities. Since VKDP are mainly secreted factors most of which regulate blood coagulation, the specificity and efficiency of warfarin in inhibiting the gamma-carboxylation reaction, has led to its widespread use in oral anticoagulant therapy. Processes regulated by VKDP are referred to as vitamin K-dependent mechanisms and warfarin treatment as anti-vitamin K therapy or AVK. Until recently, interest in vitamin K- was mostly restricted to the field of hematology. However, the discovery that some VKDPs are ligands for a family of related tyrosine kinase receptors has opened up a new area of research. Moreover, the phenotypes associated with the invalidation of genes encoding VKDP or their receptors revealed the implication of VKDP in regulating retino genesis, neurogenesis, osteogenesis, and spermatogenesis. Therefore, the elucidation of the molecular mechanisms underlying the role of vitamin K and VKDP in regulating apoptotic cell phagocytosis may lead could form the framework of new therapeutic strategies aiming at a selective targeting of VKDP associated pathologies.

References
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4 Natural Food Pigments and Eye Health

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Carotenoids are highly conjugated pigments (yellow to red) ubiquitous in nature. More than 750 compounds have been discovered until now, belonging either to the carotenes (hydrocarbon caro-tenoids) or to the xanthophylls (oxygenated carotenoids). Carotenoids are epidemiologically linked to several chronic, degenerative diseases. One of these diseases, age-related macular degenera-tion (AMD) impairs the visual performance of the central field of vision and preferably occurs after the age of 50. Aging can be considered as the main risk factor besides cigarette smoking, arterial hypertension and genetic predisposition. Due to the demographic changes with people getting older and older and the lack of clear prevention and therapy strategies, the macular carotenoids lutein and zeaxanthin moved into the focus of research.

The detection of the yellow spot (macula lutea) in the retina and the finding that it is solely com-posed of lutein and zeaxanthin suggest that it plays a special role in visual performance. Lutein and zeaxanthin accumulate in the Henle fibre layer of the central retina (fovea). They are entirely of dietary origin and found mainly in green vegetables (e.g. kale, spinach). The absorption maximum is at about 460 nm. Lutein and zeaxanthin act as blue light filter: after the transit of light through the macula lutea, only about 5% of short-wave light (400–500 nm) reaches functional structures. Besides optical mechanisms, both xanthophylls also possess biological functions including lipophilic antioxidation capacity. As oxidative stress causes photoreceptor cell death and retinal degeneration, a protective role of lutein and zeaxanthin seems plausible.

Several recently conducted and ongoing human intervention studies in Jena demonstrated that an uptake of lutein and zeaxanthin, either as supplement or as complex food, significantly elevated antioxidant capacity in plasma, circulating xanthophyll levels in plasma as well as the volume of the macular pigment. Therefore, food rich in lutein and zeaxanthin can be recommended as advantageous to AMD patients.

5 Lycopene Influences Tissue Remodelling Through Regulation of MMP-9 in Macrophages and Fibroblasts Exposed to Cigarette Smoke

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Matrix metalloproteinase-9 (MMP-9) is a neutral proteinase involved in the breakdown and remodelling of the extracellular matrix, a process that is mediated by an enzyme cascade in which MMP-9 plays a key role. The detection of the yellow spot (macula lutea) in the retina and the finding that it is solely composed of lutein and zeaxanthin suggest that it plays a special role in visual performance. Lutein and zeaxanthin accumulate in the Henle fibre layer of the central retina (fovea). They are entirely of dietary origin and found mainly in green vegetables (e.g. kale, spinach). The absorption maximum is at about 460 nm. Lutein and zeaxanthin act as blue light filter: after the transit of light through the macula lutea, only about 5% of short-wave light (400–500 nm) reaches functional structures. Besides optical mechanisms, both xanthophylls also possess biological functions including lipophilic antioxidation capacity. As oxidative stress causes photoreceptor cell death and retinal degeneration, a protective role of lutein and zeaxanthin seems plausible.

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matrix (ECM) under a variety of physiological and pathological conditions. Increasing evidence shows that MMP-9 may be up-regulated by the exposition to cigarette smoke and that lycopene may counteract several signal pathways affected by cigarette smoke exposure. However, at the moment, it is unknown if this carotenoid may inhibit cigarette smoke-induced MMP-9 expression. Presently, we examined the inhibitory mechanism of lycopene on MMP-9 induction in cultured human macrophages (THP-1 cells), in isolated rat alveolar macrophages (AMs) and in cultured RAT-1 fibroblasts, all cellular sources of MMP-9, exposed to cigarette smoke extract (CSE). CSE induced a marked increase in MMP-9 expression in cultured as well as in isolated cells. Lycopene pre-treatment (0.5–2 μM) reduced CSE-mediated MMP-9 induction in a dose- and time-dependent manner. Lycopene attenuated CSE-mediated activation of Ras, enhancing the levels of this protein in the cytosolic fraction. Moreover, lycopene inhibited CSE-induced ERK1/2 and NF-κB activation in a dose-dependent manner. Lycopene-mediated inhibition of MMP-9 was reversed by mevalonate and associated with a reduced expression of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. Taken together, these results suggest that lycopene may inhibit CSE-mediated MMP-9 induction, primarily by blocking prenylation of Ras in a signaling pathway, in which MEK1/2-ERK1/2 and NF-κB are involved.

A high prevalence of vitamin D deficiency has been reported in infants, children, and adolescents from diverse countries around the world, including Greece. Some of the factors responsible are changes in BMI, milk intake and sun protection. Overweight and obesity in adolescence has been found to increase the risk for vitamin D deficiency.

There is accumulating evidence that optimal vitamin D status throughout the lifespan, even in utero, may be important not only in maintaining bone health, but also in protecting against many chronic conditions, including autoimmune diseases, diabetes, hypertension, cardiovascular diseases and cancer.

A marked geographic variation in incidence of Type 1 Diabetes Mellitus (T1DM), with higher rates in regions more distant from the equator than in those being closer, has been attributed to the differences in UVB radiation.

Results from the 2001–2004 National Nutrition and Health Survey in the United States indicated that metabolic syndrome prevalence was 3.8 fold higher among obese adolescents with lower 25(OH)D levels compared to those with higher. Also independently of adiposity, a strong association between low vitamin D level and metabolic syndrome as well as an association of low vitamin D level with hypertension and hyperglycaemia was observed.

There is some evidence that supplementation with vitamin D (usually high dose) and in some cases in combination with calcium; may beneficially influence blood glucose and insulin levels. A 10 ng/mL increment in 25(OH)D level has been shown to mildly decrease fasting blood glucose and insulin resistance.

References

midized placebo-controlled interventional trials have failed to verify a consistent benefit.

8 Carotenoids Derived from Algae as Nutraceutical Ingredients

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Carotenoids are isoprenoid molecules which may be the first naturally occurring pigments. They are synthesized de novo by photosynthetic plants, fungi and algae and are responsible for the bright colors of various fruits and vegetables. Carotenoids are fat-soluble compounds, some of which are provitamins A. These compounds can be chemically distinguished into xanthophylls (oxygenated molecules), such as lutein, cryptoxanthin, zeaxanthin, astaxanthin and fucoxanthin, as well as into carotenes (hydrocarbons lacking oxygen), such as α-carotene, β-carotene and lycopene [1, 2]. Many macroalgae and microalgae are rich in carotenoids. For example the microalgae Spirulina spp is the richest β-carotene source known. Also, some kinds of macroalgae such as Ascophyllum spp are the main sources of the carotenoid pigments astaxanthine and fucoxanthine. These compounds in the algae aid in sunlight absorption. Individually these carotenoids are used as food pigments (in dairy products, beverages, etc), as feed additives, in cosmetic and in pharmaceuticals, especially nowadays when there is an increasing demand by consumers for natural products. Compared to other sources, the production of pigments from algae has many advantages like cheaper and easy production, easier extraction, higher yields, no lack of raw materials and no seasonal variation [2, 3].

Recently, there has been considerable interest in dietary carotenoids with respect to their antioxidant properties and their ability to reduce the incidence of some chronic diseases where free radicals are involved like aging, atherosclerosis, some types of cancer and some neurodegenerative diseases. Furthermore, carotenoids have many benefits such as anti-inflammatory and immune enhancing properties in humans and animals. Possibly carotenoids protect cells from oxidative stress by quenching singlet oxygen damage with various mechanisms [4].

Therefore, carotenoids derived from algae could be a leading natural resource in the research for potential functional ingredients.

References

9 Mechanisms Mediating the Multiple Actions of Vitamin D

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Vitamin D is a principal factor that maintains calcium homeostasis and is required for bone development and maintenance. Recent evidence has indicated an interrelationship between vitamin D and health beyond bone including effects on protecting against certain autoimmune diseases including diabetes and multiple sclerosis and inhibition of proliferation of a number of malignant cells including breast and prostate cancer cells. In spite of the importance of vitamin D, the exact mechanisms involved in vitamin D action remain to be defined. Studies in our laboratory of intestinal calcium absorption using calbindin-D9k and TRPV6 null mutant mice suggest that 1,25(OH)2D3 mediated calcium absorption is more complex than the traditional transcellular model. With regard to non-classical actions, we found that C/EBPa, considered a potential tumor suppressor gene in breast cancer, is induced by 1,25(OH)2D3 in MCF-7 breast cancer cells and enhances the transcription of the vitamin D receptor (VDR). Since levels of VDR correlate with the antiproliferative effects of 1,25(OH)2D3, these findings suggest mechanisms whereby 1,25(OH)2D3 acts to inhibit the growth of breast cancer cells. Additional non-classical actions of 1,25(OH)2D3 include effects on the immune system. Recent studies in our lab have indicated that 1,25(OH)2D3 has a direct repressive effect on the expression of IL-17, a cytokine that plays a critical role in the pathogenesis of autoimmune inflammation. The mechanism involves, at least in part, a competition of VDR with NFAT for binding to the NFAT element. 1,25(OH)2D3 also regulates innate immunity. 1,25(OH)2D3 induces the antimicrobial peptide cathelicidin (CAMP) with a subsequent killing of bacteria. We found that C/EBPa functionally cooperates with VDR in the regulation of CAMP transcription. Thus there is increasing evidence that C/EBP isoforms may be key mediators of 1,25(OH)2D3 action. In summary, our findings define novel mechanisms involved in classical as well as non classical actions of 1,25(OH)2D3 and suggest new therapeutic targets with potential for preventing and treating disease.

10 Simultaneous Analysis of Vitamin E Isoforms in Food by LC-MS/MS

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Vitamin E consists of α-, β-, γ-, δ-tocopherols (T) and their respective tocotrienols (T3), lipophilic compounds naturally occurring in plants. It plays an important role for human health due to vitaminic and antioxidant activities. Dietary intake of vitamin E mainly derives
from vegetable fats and oils, even though cereals and meat products contribute too. The most common analytical methods employ normal phase chromatographic separation for the eight vitamin E isoforms, despite the weakness of the MS detection sensitivity [1].

The aim of the present study was to develop an extremely simple, rapid and sensitive LC-MS/MS method for the simultaneous determination of the eight isoforms of Vitamin E in food samples, using reverse phase chromatographic separation [2]. Tocopherols and tocotrienols were determined in pistachio, margarine, pork fillet and ventricina, a typical Italian cured meat.

The chromatographic separation was carried out on a pentafluorophenyl (PFP) column and was achieved by an isocratic elution with MeOH/H2O acidified with TCA. The MS detection was performed on a QQQ instrument interfaced with APCI source operating in positive mode. A multiple reaction monitoring (MRM) method was employed to increase the sensitivity. Remarkable features of the method are: low detection (1–3 ngmL⁻¹) and quantification limits (4–10 ngmL⁻¹), wide linear ranges (5–15000 ngmL⁻¹) and fastness of analysis (only 15 min). All the Vitamin E isoforms were found in pistachio raw nuts, margarine, pork fillet, and ventricina samples. Alpha-T was the most abundant isoform in pork fillet, ventricina and margarine, whereas, γ-T was main vitamin E constituent in pistachio raw nuts. The proposed LC–MS/MS method is suitable to expand the composition knowledge of the comprehensive vitamin E homologues in foodstuffs.

References

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Serum Vitamin A and Vitamin E Concentrations After Parenteral Vitamin A Administration in Sheep
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Twenty primiparous dairy sheep of Mytilene breed that were fed with a ration deficient in vitamin A and carotenoids, after 2 months adaptation period were divided in 2 groups of 10 animals each. The animals of group A were administered vitamin A palmitate by intramuscular injection, while the animals of group B were used as controls and received only the vehicle of the preparation without vitamin A. Serum vitamin A concentration increased significantly in the animals of group A compared to animals of group B (P < 0.01) from the first 24 h post injection and remained significantly high for 8 days, while at 10 days post injection they reached the pre-injection levels (P > 0.01).

Serum vitamin E concentration declined significantly (P < 0.05) in animals of group A compared to animals of group B for 8 days, when they reached the pre-injection levels (P > 0.05). No changes in serum vitamin A and E levels in the animals of the 2 groups were observed 20 days after the injection of vitamin A.

12
Retinoid Metabolism in Autoimmune Liver Disease
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Retinol and retinoic acids have been shown to participate in immunity [1–3] but their role in autoimmunity is still under research.

The aim of this study was to examine the metabolism of retinol in autoimmune liver diseases and to investigate underlying pathophysiological mechanisms. Serum concentrations of retinol, 13-cis and all-trans retinoic acid were estimated in 48 patients with Autoimmune Cholestatic Liver Disease (group 1) and 15 patients with Autoimmune Hepatitis (group 2) with an HPLC-DAD method. The results were compared with healthy controls matched for age and sex. Retinol was increased in both groups compared to controls with statistically significant difference observed only in group 1 (489 ± 159 ng/ml vs 356 ± 215 ng/ml, p = 0.002). All-trans and 13-cis retinoic acid levels were decreased significantly compared to controls in group 1 (all trans: 2.07 ± 0.71 vs 3.9 ± 1.4 ng/ml, p < 0.0001, 13-cis: 1.47 ± 1 vs 2.8 ± 2.3 ng/ml, p < 0.0006) and group 2 (all trans: 2.5 ± 0.8 vs 3.9 ± 1.4 ng/ml, p < 0.0001, 13-cis: 1.03 ± 0.47 vs 2.8 ± 2.3 ng/ml, p = 0.0006). Cirrhotic patients from both groups had decreased levels of retinol and retinoic acids compared to controls and to non-cirrhotics. Retinol levels were correlated negatively with Child-Pugh Score. It has been shown for the first time that retinoids and especially retinoic acids levels are correlated negatively with disease severity.

References
Hydroponically Cultured Tomato Fruit Phenolics, Lycopene and Antioxidant Activity at Different Ripening Stages

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Five ripening stages from mature green to deep red stage of hydroponically cultured tomato were studied for their total phenols, flavonoids, nonflavonoids and lycopene content. Furthermore for those ripening stages antiradical activity (DPPH) and the ferric reducing antioxidant power (FRAP) were assessed. The content of all chemicals as well the antiradical activity and the ferric reducing antioxidant power were increased during fruit maturation (70–242 mg (GAE)/kg for total phenols, 32.3–44.16 mg (GAE)/kg for flavonoids, 137.7–198.4 mg/Kg (GAE)/kg for nonflavonoids, 5–132 mg/Kg (GAE)/kg for lycopene, 70–840 μmol/kg for DPPH and 210–4120 μmol/g for FRAP). There was correlation between lycopene and DPPH ($r^2=0.6798$) and lycopene and FRAP ($r^2=0.7048$) but not between total phenols and DPPH or FRAP.

Low Vitamin E Plasma Levels Are Associated with Cerebrovascular Events and Mortality in Hemodialysis Patients

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Background and Objective: Hemodialysis patients experience an exceedingly high mortality compared to the general population, which might be linked among other things to the increased oxidative burden of these patients. In this context the antioxidant vitamin E might be beneficial although trials in the general population failed. Therefore, it was the aim of the present study to analyze the association of vitamin E plasma concentration and specific clinical outcomes in hemodialysis patients.

Methods and Study Design: Alpha-tocopherol was determined in plasma of 1046 diabetic hemodialysis patients by rp-HPLC. Clinical outcomes of the patients were monitored during the follow-up period (mean 4 years) and hazard ratios were determined by Cox regression analyses for pre-specified endpoints according the baseline alpha-tocopherol quartiles: sudden cardiac death (n = 134), stroke (n = 89), combined cardiovascular events (n = 398), and all-cause mortality (n = 508).

Results: The median age of the study population was 66 (30–83) years and the median alpha-tocopherol level was 22.8 (3.4–87.1) μmol/L. Patients with low alpha-tocopherol levels (first quartile with $<18.3$ μmol/L) revealed a 2 fold increased risk for stroke in comparison to those with high levels (fourth quartile with $>29.1$ μmol/L) (HR 2.00, 95% CI 1.11–3.61). Especially patients without a history of cerebrovascular events in the past were affected (HR 3.01, 95% CI 1.43–6.33) and particularly the risk of ischemic stroke was increased (HR 2.95, 95% CI 1.43–6.08). Furthermore, all-cause mortality was significantly increased in patients with low alpha-tocopherol levels in comparison to those with high levels (HR 1.32, 95% CI 1.02–1.69).

Conclusions: Low alpha-tocopherol levels were strongly associated with an increased risk for stroke, particularly ischemic stroke, and all-cause mortality in hemodialysis patients with diabetes mellitus. Nevertheless, whether vitamin E supplementation would be beneficial in decreasing the occurrence of (ischemic) stroke requires further investigations.

Vitamin D and Pre-Eclampsia: A Systematic Review and Meta-Analysis

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Vitamin D may protect from pre-eclampsia due to its influences on immune modulation and vascular function. We carried out a systematic review and meta-analysis to assess the evidence for a role of vitamin D supplementation or status (measured by circulating 25-hydroxyvitamin D, 25(OH)D) in the development of pre-eclampsia.

Initial search of the PubMed central database, and references within published papers identified 103 potentially relevant papers, out of which 70 were excluded based on abstract only (mostly reviews/not relevant). After examination of full text, 18 were excluded due to lack of relevant outcome or exposure data, one had no comparison group and one duplicated findings from another study. One trial was excluded due to characteristics in the design. One recent trial was identified through a presentation in a professional meeting. Studies were assessed for quality and stratified to 1) interventions, 2) prospective and 3) cross-sectional observational studies.

There were 4 randomised trials, out of which only one was blinded. Vitamin D dosages in the treatment group varied between 450 IU/d and 4000 IU/d, and all except one had used other micronutrients (all calcium, one + vitamin A, one multivitamin). Odds ratios (ORs) from the trials were 0.44 to 0.69, with meta-analysed OR 0.66 (95% CI 0.52–0.84, I² 0.0%). There were 9 observational studies, with two studies excluded as only comparisons between mean 25(OH)D was presented. Of the remaining, 5 were prospective, one cross-sectional and one reported dietary intake. The comparison of vitamin D sufficient vs. insufficient was based on 25(OH)D concentrations with study specific ORs ranging from 0.19 to 0.79; meta-analysed OR 0.45 (95% CI 0.29–0.71, I² 29.7%).

Abstracts
Evidence is remarkably consistent in supporting the association between vitamin D and pre-eclampsia. However, many of the studies are low quality. A well-designed randomised controlled trial is required to demonstrate or refute the association between vitamin D and pre-eclampsia.

16 Reduced γ-Carboxylase Activity in Uremia – A Possible Mechanism of Uremic Vascular Calcification

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Vascular Calcification (VC) is present in chronic kidney diseases. This can be inhibited by matrix gla protein (MGP), which achieves full activity by carboxylation by the vitamin K dependent γ-carboxylase. Its inhibition by warfarin leads to augmented vascular calcification. The vitamin K regeneration cycle is formed by DT-diaphorase, VKOR and γ-carboxylase. Vitamin K deficiency is present in dialysis patients and so we investigated whether uremia reduces enzyme activities.

10 Wistar rats in each group were fed a) standard diet b) 100 mg/kg vitamin K2 c) 0.75% adenine or d) 0.75% adenine + 100 mg/kg vitamin K2. Finally, serum parameters, extent of VC, uncarboxylated (uc)MGP and in kidney an liver activities of diaphorase, VKOR and γ-carboxylase were measured.

After 4 weeks of treatment, creatinine, urea (7-fold) and phosphate were higher in adenine groups (c+d) than in controls (a+b). Calcium was unchanged. Aortic calcium content was higher in c+d than in controls. Systolic blood pressure was unaltered in all groups. DT-diaphorase activity was significantly higher (70, 72%) in groups c+d. VKOR activity was unchanged; γ-Carboxylase was more active in groups c+d, significantly. This also led to significant higher levels of ucMGP (12.2 vs 8.6, 7.4 μM).

Experimental uremia inhibits the key enzyme of the vitamin K dependent carboxylation, γ-Carboxylase. This is accompanied by higher levels of ucMGP and calcium deposition in the aorta. Even though there is reduced enzyme activity, vascular calcification and ucMGP can be reduced by dietary vitamin K2 (group d). Our data identifies a new mechanism of uremic vascular calcification and supports the rationale of our vitamin K2 interventional study VitaVasK.

17 Effect of Plant Sterols on Vitamin D Absorption

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Cholecalciferol (vitamin D3) is either obtained from the diet or produced photochemically in the skin from 7-dehydrocholesterol, which is produced in relatively large quantities from cholesterol in the gut1 and is accumulated in the skin2. Since plant sterols and stanols alter micelle formation and decrease absorption of cholesterol, it is possible that absorption of fat-soluble nutrients including fat soluble vitamins is also affected.

The objective was to examine if plant sterols interfere with the absorption and possibly the synthesis of vitamin D3. A randomized study was conducted in 40 apparently healthy adult volunteers aged 18–60 years who received orally either 25000 IU vitamin D3 (group A, n = 20) or 25000 IU vitamin D3 together with 2 g plant sterols (Group B, n = 20). Levels of Vitamin D3, 25-hydroxyvitamin D3, calcium, phosphorus, magnesium, cholesterol and parathyroid hormone were measured in blood taken immediately before consumption of Vitamin D3 and at 12 h, 24 h and 168 h (7 days) after administration of Vitamin D3. Serum vitamin D3 concentration increased significantly in both groups at 12 h and 24 h and 25-hydroxyvitamin D3 at 168 h after the consumption of vitamin D. Group B had lower vitamin D3 levels 12 hours and 24 hours after administration and lower 25-hydroxyvitamin D3 levels at 168 h after administration.

Hence, inhibiting the absorption of cholesterol may also affect the absorption of vitamin D3. Long term (>4 weeks) administration of sterols is needed to investigate the impact on Vitamin D absorption and consequently its metabolism.

Reference


18 Development of a Pedagogic Game for Nutrition Education

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Nutrition education can be a tool for improving dietary habits in children and may help them to make healthier food choices1. Interactive games based on digital technology can add value over traditional educational approaches2.

An interactive pedagogic game for the promotion of knowledge on healthy nutrition with emphasis in local food and Mediterranean
Nutrition was developed. Title of the game is “Invulnerable”. The character of the game is young king Achilles, a name that refers to the mythical invulnerable hero. Achilles aims to get back his palace and throne, stolen by “Hub” the king of bad nutrition. In his way to the palace, he must avoid the nutritional temptations and defeat the enemies sent by Hub: decay, fat and microbes. In his way the hero must consume healthy foods. For every healthy choice the character’s defense increases and for every nutritional temptation it decreases. After a predefined number of right choices, the character becomes “invulnerable”, jumps higher and beats the enemies easier. The player identifies with the hero thus making easier the adoption of the correct choices.

The educational goals are the recognition of the healthy local food and of the foods that are not healthy and the comprehension of the importance of healthy nutrition for the body defense against disease. The game was evaluated in a pilot study including 11 school children aged 12 years old and it was shown to improve the knowledge on healthy and non healthy foods. Further investigation is needed to determine if the application can contribute to attitude change and to adoption of healthier nutritional habits.

The game is accessible at http://atrotos.teilar.gr. The project was financed by the Hellenic Secretariat of New Generation.

References

Fortification of Buttermilk Drink by Fat-Soluble Vitamins A and D
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The purpose of this research was to examine the fortification methods and retention of fat-soluble vitamins A, D in fermented buttermilk drink during storage and processing. Fortification of fermented buttermilk drink with vitamins A and D was tested using different addition methods at the different stages of technological process: before pasteurization, pre-emulsified with whey proteins, after fermentation to the buttermilk, after fermentation to the juice of sea buckthorn, and during storage at different conditions.

The composition of fermented buttermilk drink for the fortification with vitamins was tested. The products with different composition were evaluated by physical, chemical and sensory properties. It was determined that the best textural and sensory properties were characteristic to the fermented buttermilk drink with the addition of 2% of whey protein concentrate and when it was mixed with 10% of juice of sea buckthorn.

The analysis of vitamins content in the buttermilk showed that the content of vitamins A and D changed a little during pasteurization. It was shown that milk proteins had protective effect to vitamins A and D during technological process of fermented buttermilk drink. The retention of cholecalciferol in fermented buttermilk drink was higher in comparison with the retention of retinol. Storage stability of the vitamins in the fermented buttermilk drinks was different as well: the lost of cholecalciferol was 54.53–59.02% in the light and 53.52–64.28% in the dark; the lost of retinol 77.06–83.51% and 77.88–81.25% respectively.

The best results of vitamins retention were obtained when vitamins were added to the juice of sea buckthorn and then mixed with the fermented buttermilk drink. It could be explained due to low pH of sea buckthorn juice. Fermented buttermilk drink with the addition of juice of sea buckthorn appears to be suitable product for the fortification with vitamins A and D.

Analytical Methods for Fat Soluble Vitamins
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The determination of fat soluble vitamins (FSVs) in biological matrices remains an analytical challenge although significant progress has been accomplished in instrumentation and methods during the last years. The FSVs usually exist in animal and plant tissues and in food in relatively low quantities and other compounds of similar structure that usually exist in much larger amounts may interfere in the determination. Additionally the fat soluble vitamins exist in several forms and in isomers with different biological activity. In most nutritional and metabolic studies it is necessary to determine different metabolites of the vitamins.

Several methods exist for the determination of fat soluble vitamins including spectrophotometric, immunologic, radioimmunologic, capillary electrophoresis and chromatographic. Classical spectrophotometric methods usually involve extraction, saponification and detection in the UV or visible. These methods tend to be abandoned in favor of the more accurate and specific High Performance Liquid Chromatography (HPLC) methods. HPLC methods can determine different chemical forms of the vitamins, metabolites and their isomers. For example for vitamin A retinol, retinoic acid and retinyl esters and for vitamin D hydroxylated metabolites can be assayed. Most methods involve one or more sample preparation steps. Liquid, supercritical fluid and solid phase extraction are commonly applied. Modern instrumentation allows automation and online sample preparation. UV, diode array, fluorescence, electrochemical, and MS detection are used. Although the very common UV detection is adequate in some cases, MS and specially MS/MS detection is gaining wide approval due to increased sensitivity and specificity.
21 Regulatory Functions of Tomato Lycopene in Cholesterol Metabolism: Implications in Atherosclerosis and Cancer

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Alterations in cholesterol metabolism are implicated in atherosclerosis and cancer. Increased ingestion of tomatoes and tomato products, containing lycopene, has been associated with decreased risk of such chronic diseases, although the exact molecular mechanism is still unknown. We show new evidence that lycopene may exert its antiatherosclerotic and antitumoral effects through changes in mevalonate pathway and in cholesterol metabolism. In normal macrophages, lycopene dose-dependently reduced intracellular total cholesterol. Such an effect was associated with a decrease in cholesterol synthesis through a reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase expression and with an increase in cholesterol efflux through an enhancement of ABCA1 and caveolin-1 expression. On the other hand, in prostatic, colon and lung cancer cells, the carotenoid inhibited tumor cell growth by a mechanism involving a reduction in HMG-CoA reductase expression and an inactivation of Ras, NF-κB and MAPK cascade. Lycopene and statins, applied together, reduced proinflammatory cytokine levels, suggesting that simultaneous inhibition of tumor cell growth by a mechanism involving a reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase expression and an inactivation of Ras, NF-κB and MAPK cascade. Lycopene and statins, applied together, reduced proinflammatory cytokine levels, suggesting that simultaneous inhibition of tumor cell growth by a mechanism involving a reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase expression and an inactivation of Ras, NF-κB and MAPK cascade.

22 Dermatopharmacokinetics of α-Tocopherol, α-Tocotrienol and γ-Tocotrienol in Hairless Mouse Skin In Vivo

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Skin is a protective organ susceptible to oxidative stress. To diminish the oxidative injury topicaly applied antioxidants must reach susceptible cells in sufficient for their action concentrations. Ingredients of vitamin E, as α-tocopherol, α-tocotrienol and γ-tocotrienol are potent antioxidants that may protect skin. As their pharmacological effect depends on its pharmacokinetic data, their cutaneous and subcutis penetration kinetics were determined in vivo, in hairless mice. An approximately 5% w/v solution of each was applied topically for 0.5 to 4 hours. The skin was washed, the mice killed, then froma punch biopsy, stratum corneum, epidermis, dermis, and subcutis were cryosectionned. Homogenised slices were extracted and above molecules measured by H.P.L.C. analysis with electrochemical detection. All the molecules were rapidly absorbed. α-Tocopherol was better absorbed at the early application times (0.5, 1 hour) while the γ-tocotrienol was less absorbed than the other two. The non statistical difference (p > 0.1) between the penetration of all substances into the stratum corneum, with the exception of two time points, shows strong evidence of early reaching steady state absorption. The kinetic pattern of penetration of the molecules into the stratum corneum, and in the different skin compartments as the capillary and reticular dermis seems to be the same as for the absorption into the living skin and subcutis. The mass penetrating into stratum corneum seems related to that absorbed into the living cutaneous tissue and the subcutaneous fat (p < 10⁻³). α- and γ-tocotrienols act as penetration enhancers for the α-tocopherol. All tested molecules seem to be candidates for topical use against the oxidative stress of the skin.

23 Effect of Vitamin E on the Stress-Induced Changes in Rats

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Biologic stress (BS), the homeostatic response of the organism to any demand, is mediated via an adaptive regulation of the nervous, immune and endocrine systems. It is manifested by morphologic, biochemical and functional alterations, which can be used as markers of BS. There is an extensive overlap between diseases attributed to BS and Oxidative Stress (OS), suggestive of their relationship. Many of the changes due to BS, i.e. the activation of hypothalamus-pituitary-adrenals axis, indicate the important role of the brain in the defence against stressful conditions. Yet, intensive BS causes tissue injury, while the molecular mechanisms have not been fully clarified. We, therefore, study the influence of α-tocopherol (α-Toc) on the development of BS biomarkers, on biomarkers of OS in brain and liver, as well as on drug metabolism.

In our experiments, two groups of rats are exposed to stress, e.g. cold and starvation. Before stress and during its application, one group is treated with α-Toc, 0.42 mmol/kg, p.o., once daily for 20 days.

Our results show that oxidative injury accompanies the development of BS, while treatment with α-Toc prevents stress-induced radical attack, reduces stress indices, such as plasma corticosterone and uroepesinogen, and ameliorate the affected morphological changes.

BS increases drug metabolic activity (total P450, CYP2E1, CYP3A1). Administration of α-Toc combined with BS further increases erythromycin N-demethylation (CYP3A1), compared with stress control.
group, while 4-nitrophenol aromatic hydroxylation (CYP2E1) is not affected significantly.

It is concluded that there is an interrelation between biologic stress and the ensuing oxidative insult. Treatment with α-tocopherol completely eliminates radical damage in brain and liver. Vitamin E reduces greatly stress responses. These findings reinforce the strategic role of brain in the development of biologic stress. Furthermore, our results suggest new pathways in the rational design to lead compounds for overcoming stress and conditions related to this.

Tomatoes and Prostate Cancer: Focus on the Respective Effect of Tomato Matrix and Lycopene

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Numerous epidemiological studies have highlighted the role of fruits and vegetables in the nutritional prevention of major chronic diseases in the western countries. Among the plant foods, many attempts have been made to establish a relationship between tomato consumption and reduced risk of prostate cancer. Tomato and its derivatives are the main source of lycopene, the carotenoid that gives the red colour to tomatoes. Therefore the hypothesis that lycopene is primarily responsible for reduction the risk occurred in the prostate cancer is the most prevalent.

The aim of this work is the establishment of an experimental approach on cell lines to highlight the potential role of whole tomato compared to isolated lycopene. A clinical intervention experimental design was implemented using the yellow tomato, a tomato variety devoid of lycopene. A nutritional approach has been undertaken on healthy volunteers, whose the usual diet has been supplemented with lycopene (LY) or placebo (P) and red tomatoes (TR) on the one hand or with yellow tomatoes (TY) on the other hand. After each one week-period supplementation, the sera naturally enriched with exogenous metabolites brought by endogenous metabolites induced by TR, TY, LY and P have been collected and studied against the one obtained after the first washout period. The impact of these sera on healthy volunteers, whose the usual diet has been supplemented with lycopene (LY) or placebo (P) and red tomatoes (TR) on the one hand or with yellow tomatoes (TY) on the other hand. After each one week-period supplementation, the serum naturally enriched with exogenous metabolites brought by endogenous metabolites induced by TR, TY, LY and P have been collected and studied against the one obtained after the first washout period. The impact of these sera on cell proliferation. Among the genes differentially expressed after tomatoes and lycopene intake, the sera obtained after the supplementation with tomatoes (red or yellow) leads to a decrease in the expression of cyclin D1 gene linked to cell cycle arrest, and an increase Bax/Bcl2 ratio a marker of pro-apoptotic effect.

From this original ex-vivo approach, and based on the aforementioned molecular mechanisms, it can be suggested that tomatoes rather than the sole lycopene may account for the relationship between tomato consumption and its potential effect on decreasing the risk factor for prostate cancer. Metabolic studies on rats fed tomatoes and lycopene support this hypothesis.

References


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Development and Evaluation of a Novel Membrane Mimic System (PC/CHOL Liposome-β-Lg Formulation) for Vitamin E Delivery

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Vitamin E (VE) or α-tocopherol is the major fat soluble antioxidant in the human body, since it protects cellular membranes and other lipids against oxidative damage. It reduces the formation of hydroperoxides and delays the initial phase of the oxidative process [1]. Vitamin E is a sensitive, easily oxidized in the air molecule, so must be preserved from pro-oxidant elements which could affect its chemical integrity and decrease its physiological benefits [2, 3].

Encapsulation constitutes a promising approach to preserve VE native properties over time and increase its concentration in aqueous media. There has been an increasing interest in the development of a carrier or delivery system for VE. Liposomes have been studied as sustained delivery systems. They have advantages over other delivery systems, being biodegradable, non-toxic and non-immunogenic [4].

A new liposome/β-lactoglobulin formulation has been developed and characterized as a possible stable delivery system for VE. β-lactoglobulin has been studied extensively and has shown to bind a variety of hydrophobic molecules including fat soluble vitamins in vitro [5].

The aim of this study was the preparation and characterization of β-lactoglobulin-liposome formulation and the determination of VE encapsulation efficiency, in order to develop a new more efficient carrier for VE in aqueous media. Mixtures of β-lactoglobulin, phosphatidylcholine and cholesterol in the form of liposomes have been investigated by Fourier Transform Infrared Spectroscopy (FTIR), SDS-page and other biochemical techniques. A high encapsulation percentage of vitamin E was observed, in addition to a very promising stability behaviour.

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Abstracts

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Yogurt Fortified with Vitamin D: A Study for Bioavailability of Vitamin D in a Dairy Product

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Fortification of foods with vitamin D has been proposed as a way to address vitamin D deficiency [1, 2]. Yogurt was selected for fortification as a product of high consumption by all ages with increasing consumption in Europe [3].

Apparently healthy volunteers were divided in two matched groups of 20 persons and consumed 100 g yogurt fortified with 25,000 IU vitamin D3 or a dispersion with the same amount of vitamin D3. Blood samples were collected at 0 hours (before consumption), 12 h, 24 h and 168 h (7 days) after consumption and serum levels of vitamin D3 and 25(OH)D were measured. In both groups peak serum vitamin D3 concentration increased significantly at 12 hours, started decreasing after 24 h and returned to nearly basal levels at 7 days. No significant differences were observed in serum vitamin D3 and 25(OH)D levels between the two groups.

In a second long-term study for the bioavailability of vitamin D in yogurt, 60 volunteers were randomly separated into two matched groups of 30 persons. Group A was consuming 200 g yogurt fortified with 200 IU of vitamin D3 every day for thirty days and group B was consuming 200 g non-fortified yogurt for the same period. Serum 25(OH)D levels at the beginning of the study were similar in the two groups (20.1 ± 10.8 ng/mL vs 21.4 ± 16.4 ng/mL respectively). At the end of the study, serum 25(OH)D levels increased significantly in both groups, but the level in the fortified yogurt group A (33.2 ± 24.1 ng/mL) was higher than group B (26.6 ± 16.3 ng/mL).

In conclusion yogurt is a product suitable for fortification with vitamin D as it effectively increases serum vitamin D3 and 25(OH)D levels in adults.

References

Calcium and the Natural History of Prostate Cancer

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Recent research has fueled interest in the role of vitamin D in prostate cancer [1]. Although many epidemiologic studies have examined prostate cancer risk in relation to metabolites of vitamin D, serum calcium has been little studied. This is at first surprising, given that numerous epidemiologic studies implicate dietary calcium as a risk factor for prostate cancer. However, it likely reflects the belief that serum calcium levels are little affected by dietary calcium intake.

We examined prostate cancer risk in relation to serum calcium in two National Health and Nutrition Examination Surveys (NHANES). In NHANES I, twenty five prostate cancer deaths occurred over 46,188 person-years of follow-up. Serum calcium was measured an average of 9.9 years prior to the diagnosis of prostate cancer. When we compared men in the top tertile to men in the bottom tertile of total serum calcium, the relative hazard for fatal prostate cancers was 2.68 (95% CI 1.02–6.99; Ptrend = 0.04). In order to confirm this association, we examined mortality in NHANES III which also included measurements of ionized serum calcium. Twenty five prostate cancer deaths occurred over 56,625 person-years of follow-up. Serum calcium was measured an average of 5.3 years prior to death. Compared to men in the lowest tertile of total serum calcium, the multivariate-adjusted relative risk for death from prostate cancer for men in the highest tertile was 2.07 (95% CI 1.06–4.04). For ionized serum calcium, men in the highest tertile had a relative risk of 3.18 (95% CI 1.09–9.28) [2]. These results are unlikely to be the result of confounding by metastatic prostate cancer because metastatic prostate cancer causes serum calcium levels to fall [3]. Alternately, it is possible that early stage prostate cancers act to increase calcium levels in serum. If this is true, serum calcium levels may aid in the early detection of prostate cancer.

We recently showed that men who genetically are high absorbers of calcium have an increased risk of prostate cancer. This may harmonize the dietary and serum findings. Prostate cancer cells express the calcium-sensing receptor as well as calcium-dependent channels that regulate prostate cancer cell proliferation. Thus, high levels of calcium in serum may promote the growth of potentially fatal cancers.

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Association of Vitamin K with Bone and Energy Metabolism

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Background: Osteoporosis is characterized by reduced bone mass resulting in increased fracture risk. Osteocalcin (OC) is a vitamin K-dependent protein involved in bone mineralization. Vitamin K status of bone, i.e. the carboxylation degree of OC, can be improved by increased vitamin K intake, and high-dose supplementation with vitamin K1 or the short-chain K2 vitamin menaquinone-4 (MK-4) were shown to reduce the age-related loss of femoral neck BMD or strength. We were therefore interested to see whether low-dose intake of the long-chain K2 vitamin menaquinone-7 (MK-7) improves osteocalcin carboxylation and bone quality. Next to its role in bone metabolism, OC has been associated with the regulation of glucose and insulin metabolism. Therefore, we also studied the association between (changes in) vitamin K status of bone and energy metabolism.

Methods: In 244 healthy postmenopausal women, long-term effects of low-dose MK-7 (natural MK-7 as MenaQ7, NattoPharma) intake were studied on bone health. We also studied possible associations of vitamin K status with body composition and circulating adiponectin. Using archived samples, we investigated short-term effects of natural MK-7 supplementation on circulating adiponectin in 42 healthy adults. Long-term effects of high-dose MK-4 intake on BMI and circulating adiponectin were studied in archived samples of 164 postmenopausal women.

Results: Long-term, low-dose MK-7 intake significantly improved OC carboxylation and bone quality. Cross-sectionally, both circulating carboxylated OC and adiponectin showed an inverse correlation with measures of body composition. However, carboxylated OC did not correlate with circulating adiponectin. Both short-term low-dose MK-7 and long-term high-dose MK-4 supplementation significantly increased OC carboxylation, but did not affect circulating adiponectin. Next to a beneficial effect on bone strength, high-dose MK-4 intake was associated with small but significant decreases in body weight and BMI.

Conclusion: Both long-term low-dose MK-7 and high-dose MK-4 supplementation can improve bone quality. Long-term vitamin K2 intake also stabilized body weight, which rejects the hypothesis that a higher vitamin K status of bone negatively affects energy metabolism in healthy people.

References

Antioxidative and Non-Antioxidative Effects of Tocopherol Metabolites on Atherogenesis

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Vitamin E is a mixture of isomers which differ by methylation patterns of the hydroxychromanol ring and saturation of the side-chain, and of which α-tocopherol (α-TOH) is assigned the most active vitamer [1–2]. In the liver, α-TOH is metabolized by side-chain truncation initiated by CYP3A4-dependent ω-hydroxylation which results in the formation of the alcohol derivate α-13’-OH. Subsequent β-oxidation in peroxisomes forms the acid derivate α-13’-COOH [3–4]. Aside from hepatic metabolism of α-TOH, knowledge about transport and metabolism of vitamin E after dietary or supplementary intake is yet incomplete [5]. Using LC-MS, we were able to detect α-13’-COOH in human plasma after a one-week supplementation with α-TOH. Vitamin E, especially α-TOH, is the most important lipid antioxidant which is widely used to prevent age-associated diseases [6]. We therefore wanted to know if the physiological long-chain metabolites (LCMs) promote antioxidative or non-antioxidative effects comparable to α-TOH. Antioxidative effects were investigated by analyzing LPS-induced NO production and the expression of inflammatory mediators by macrophages. Additionally we investigated the effect of physiological LCMs on macrophage foam cell formation, a hallmark of atherogenesis. We have found that, contrary to α-TOH, its LCMs increase CD36 expression. Unexpectedly, the accumulation of neutral lipids was decreased by α-13’-OH and α-13’-COOH similarly to α-TOH because phagocytic activity was impaired by these LCMs. Furthermore, we wanted to know whether cholesterol homeostasis in macrophages is influenced by the LCMs. Therefore we investigated mRNA expression of genes involved in cholesterol metabolism and determined the amount of total cholesterol and intermediates of cholesterol biosynthesis as well as formation of oxysterols. Compared with α-TOH, its LCMs were more active in regulating macrophage function. We therefore hypothesize that α-TOH and its LCMs α-13’-OH and α-13’-COOH may regulate different and sometimes even contrary signaling pathways.

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