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Effects of Higher Doses of Vitamin E on Toxicity and Inflammation

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Authors' contributions

This work was carried out in collaboration between all authors. Author EJ was the principle investigator, designed the study together with author DV and wrote the initial manuscript. Authors PB and EG proposed and performed the biomarker measurements, the data collection and the statistical analysis. Authors DV and LI designed, coordinated and performed the mice study, including section. All authors contributed to the text, read and approved the final manuscript.

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ABSTRACT

High doses of vitamin E close to the upper limit of toxicity (UL) but still recommended and considered as harmless and beneficial, can possibly cause a number of adverse effects.
In a 14-day study, in which male mice were exposed to intra peritoneal doses of 100 and 200 mg vitamin E (alpha-tocopherol)/kg bw/day, biomarkers of oxidative stress related processes (ROM, TTL), and biomakers of tissue toxicity (ALP, ALT, AST, LDH) and inflammation (MCP-1, IL-6, TNF-α, PAI-1 and resistin) were determined.
Oxidative stress parameters in plasma did not change, whereas some biomarkers of inflammation were statistically significantly higher on exposure to vitamin E. In the liver, beneficial effects on tissue biomarkers were observed, whereas the inflammation biomarkers showed an U-shaped relation with the dose of vitamin E. In the kidney, the biomarkers of tissue damage showed mixed effects, whereas a substantial increase in the inflammation biomarkers was observed. In the brain, the biomarkers of tissue toxicity showed beneficial effects, whereas the inflammatory biomarkers showed an increase or an U-shaped behaviour with increasing doses of vitamin E.
Especially, a dose of 200 mg of vitamin E/kg bw/day showed a number of adverse effects in several tissues. These results confirm our previous study in male mice with exposure of vitamin E by feed. Since the dose of 200 mg of vitamin E/kg bw/day is lower than the upper limit for vitamin E, the UL should be re-evaluated, in view of the effects in kidney and brain.

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Keywords: Vitamin E; redox status; inflammation; liver; kidney; brain.

ABBREVIATIONS

UL : Upper limit of toxicity
BAP : Biological antioxidant potential
IL-6 : Interleukin-6
MCP-1 : Monocyte chemoattractant protein-1
tPAI-1 : Total plasminogen activator inhibitor
ROM : Reactive oxygen metabolites
TNF alpha : Tumor necrosis factor-alpha
TTP : Total thiols in proteins

1. INTRODUCTION

In many reports, vitamin E is considered as having beneficial effects on several physiological processes in the body because of its antioxidant properties [1,2,3]. In addition, the risk of developing chronic diseases can be reduced by an optimal vitamin E status in tissues [4,5,6,7,8]. As a result, vitamin E supplementation is very popular in general population, being one of the most used vitamins in supplements [9,10,11]. In a previous study, however, we have reported both the beneficial and the adverse effects of higher doses of vitamin E in mice, administered by feed of 550 mg/kg diet, corresponding with 25 times the recommended daily intake [12]. In the kidney of male mice, tissue enzymes along with inflammation biomarkers were increased by higher vitamin E doses [12]. Also, some recent human studies reported that the intake of high doses of vitamin E can have detrimental effects, such as a higher risk for prostate cancer [13,14] and even a higher mortality [15,16,17,18,19]. Therefore, the intake of supplements with high doses of vitamin E is questioned, because vitamin E can act as an antioxidant and as a pro-oxidant as well [20,21].

In this study, we investigated another way of administration of vitamin E to see whether this is important for the toxicological effects observed earlier. Therefore, vitamin E was administered by intra peritoneal by two relatively high but still below the upper level of toxicity doses of vitamin E in male mice.

2. MATERIALS AND METHODS

2.1 Experimental Animals and Exposure Protocol

Male balb/c mice (N=22) weighing 20-25 g were exposed to vitamin E (Medana Pharma, Kaunas, Lithuania) by intra peritoneal injection for 14 days. The two experimental groups (N=8 in each group) received 100 and 200 mg alpha-tocopherol/kg body weight (bw)/day in peanut oil (0.1 mL). The control group (N=6) received peanut oil only (0.1 mL). The peanut oil contained 0.15 mg vitamin E/g oil. The mice were kept in separate cages. 16 Hours after the last injection, the mice were anaesthetized and terminated. The organs (liver, kidney and brain) were removed and immediately cooled on ice.

All procedures were performed according to the Republic of Lithuania Law on the Care, Keeping and Use of Animals (License of State Veterinary Service for working with laboratory animals No 0200).

2.2 Preparation of Organ Samples

Liver, kidney and brain samples were weighed and homogenized in three volumes (w/w) of homogenization buffer (50 mM Tris-HCl, pH 7.6; 5 mM $MgCl_2$; 60 mM KCl; 25 mM sucrose). Tissue homogenates were centrifuged at 3000 rpm for 10 min (1000×g) in a Beckman J2-21 centrifuge. The supernatant was immediately frozen at -80°C and the pellet was discarded. All the samples were shipped from Kaunas to Bilthoven on dry ice. The samples were received frozen and stored at -80°C until analysis.

2.3 Preparation of Blood Samples

Whole blood was collected after mouse decapitation into Eppendorf test tubes. Blood was centrifuged at 4000 rpm for 10 min (1600×g). Heparin plasma samples were immediately frozen at -80°C. All samples were shipped from Kaunas to Bilthoven on dry ice. The samples were received frozen and stored at -80°C until analysis.

2.4 Biochemical Analysis

Reactive oxygen metabolites (ROM), biological antioxidant potential (BAP) and total thiols in proteins (TTP) were determined with an auto-analyzer (LX-20 Pro, Beckman-Coulter, Mijdrecht, the Netherlands). ROM and BAP were determined using kits from Diacron, Grosseto, Italy, and TTP was determined with a kit from Rel Assay, Gaziantep, Turkey. These three assays were adapted for use with the LX20 auto-analyzer [22] and checked for long-term storage stability [23]. Intra assay coefficients of variation

were 2.1% for ROM, 1.5% for BAP and 2.7% for TTP.

Enzyme activities of ALP, ALT, AST and LDH were measured with dedicated kits from Beckman-Coulter for the LX-20 Pro auto-analyzer. Intra assay coefficients of variation were 2.4% for ALP, 1.6% for ALT, 1.2% for AST and 2.9% for LDH.

Interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), plasminogen activator inhibitor-1 (PAI-1), MCP-1 and resistin were determined with a Mouse Adipokine kit (Millipore, Amsterdam, The Netherlands) using the Luminex technique. Intra assay coefficients of variation were 6.4% for IL-6, 4.9% for TNF-α, 7.4% for PAI-1, 10.0% for MCP-1 and 5.2% for resistin.

2.5 Statistical Analysis

The data were analyzed by means univariate ANOVA using Excel. Results were expressed as the mean ± standard error of mean. Statistical significance was set at $P<0.05$.

3. RESULTS

3.1 Plasma

The effect of exposure to relatively high doses of vitamin E was studied by measuring plasma biomarkers of redox status. Because of the limited amount of plasma that was available, the following biomarkers were selected: ROM for the assessment of oxidative stress by measuring hydro peroxides, BAP for the evaluation of total antioxidant status and TTP for the total thiols in plasma. In addition, five inflammation biomarkers with the Luminex technique.

The effects of exposure to vitamin E on the biomarkers ROM, BAP and TTP are shown in Fig. 1. The exposure to both doses of vitamin E did not have any statistically significant effects on these biomarkers. All P-values were in the range of 0.188-0.934.

The effects of vitamin E on inflammatory parameters are shown in Fig. 2. The treatment with vitamin E resulted in substantially higher concentrations of MCP-1, total PAI-1 and resistin. The increase in these inflammation parameters was statistically significant with a P-value of 0.044 for MCP-1 (200 mg dose vs. control), P=0.017 and P= 9*E-6 for PAI-1 (100 mg and 200 mg doses vs. control, respectively) and P=1*10-5 for resistin (200 mg dose vs. control). Concentrations of IL-6 and TNF-α did not change statistically significantly under exposure to vitamin E. The ANOVA results were P=0.0034, 0.798, 0.593, 0.0014 and 1.46*E-6 for MCP-1, IL-6, TNF-α, PAI-1 and resistin, respectively.

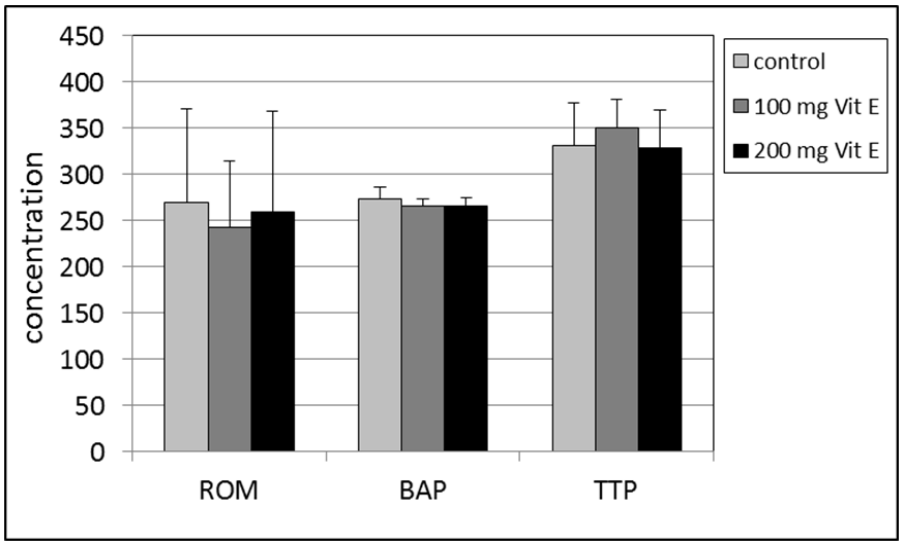

Fig. 1. Plasma concentrations of ROM (expressed in U/L), BAP (expressed in mEq/L) and TTP (expressed in µmol/L) after administration of vitamin E. The concentration of BAP was divided by a factor of 10 to fit in the figure

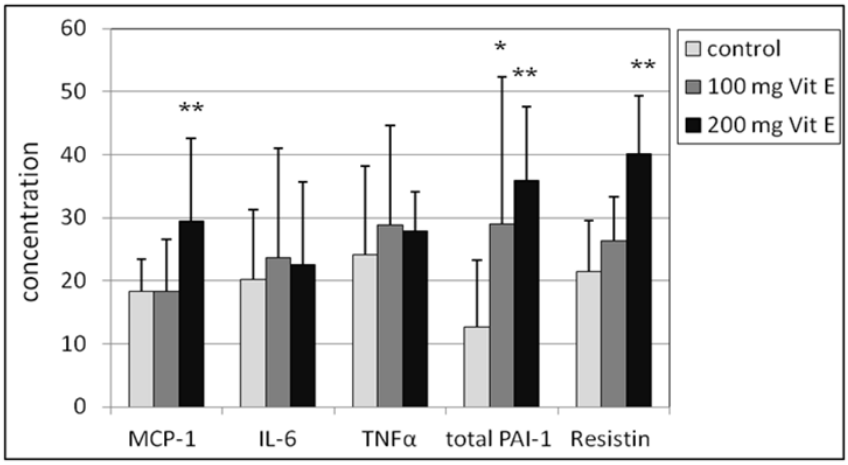

Fig. 2. Plasma concentrations of MCP-1, IL-6, TNF-α, resistin (expressed in pg/mL), and PAI-1 (expressed in µg/mL). The concentrations of MCP-1, PAI-1 and resistin were divided by factors 2, 200 and 50, respectively, to fit in the figure. Statistics: * p<0.05 vs. control group; ** p<0.005 vs. control group

3.2 Liver

Effects of vitamin E on the liver were investigated by measuring activities of enzymes ALP, ALT, AST and LDH, the total antioxidant status (BAP), total thiols (TTP), and biomarkers of inflammation.

In the liver, the general view is that the exposure to vitamin E has a beneficial effect on most of these biomarkers, as is shown in Fig. 3. A dose 200 mg of vitamin E caused a decrease in the activity of ALP (P=0.01), AST (not statistically significant) and LDH (P=0.012) In addition, the total thiols, reflecting redox status, increased with the higher doses of vitamin E, with P-values of 0.004 and 0.0001 after exposure to 100 and 200 mg vitamin E, respectively. The total antioxidant status decreased unexpectedly with P=0.0011 and 0.0010 after exposure to 100 and 200 mg vitamin E, respectively.

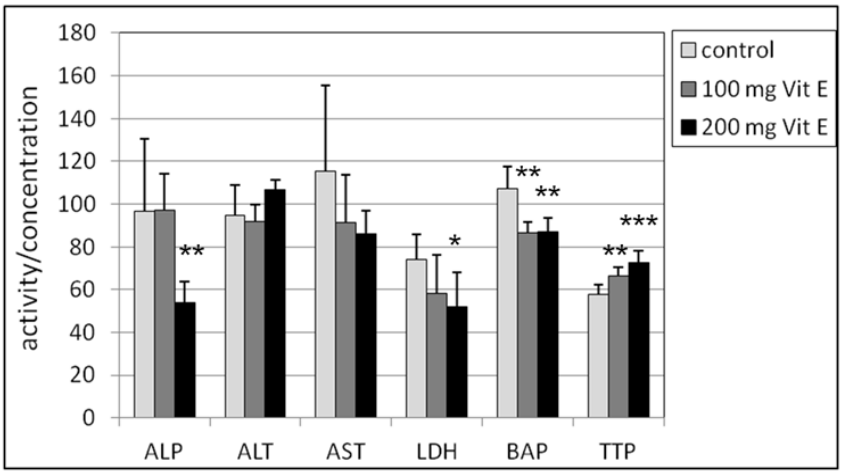

Fig. 3. Liver supernatant activities of ALP, ALT, AST, LDH (expressed in IU/g protein) and concentrations of BAP (expressed in mEq/g protein) and TTP (expressed in µmol/g protein). The activities of ALP, ALT, AST and LDH and the concentration of BAP were divided by factors 0.2, 10, 10, 100 and 2, respectively to fit in the figure. Statistics: * P<0.05 vs. control group; ** P<0.01 vs. control group; *** P<0.001 vs. control group

In the liver, the 200 mg dose of vitamin E caused an increase in the inflammation biomarkers compared to the effects of the 100 mg vitamin E dose, which was statistically significant for MCP-1 (P=0.002), IL-6 (P=9*E-5) and TNF-α (P=0.029.

Although there were no statistically significant effects of the 100 mg group with the control group, the inflammation biomarkers seems to behave in a biphasic phase with increasing vitamin E doses, except for total PAI-1 (Fig. 4).

3.3 Kidney

In the kidneys, the activities of enzymes ALP and AST decreased upon exposure to vitamin E with P-values of 0.0005 and 0.0085 (for ALP) and 5*10-4 and 2.7*10-6 (for AST). However, the activity of LDH increased with P-values of 0.0014 and 0.0002, for 100 and 200 mg vitamin E, respectively. BAP decreased with statistical significance for 200 g vitamin E (P=0.036) and TTP increased statistically significantly with 100 mg vitamin E (P=0.002) but not with 200 mg vitamin E.

The concentrations of inflammation biomarkers in the kidney were higher for higher doses of vitamin E. Statistical significance was observed for MCP-1 (P=0.047) and IL-6 (P=0.0003) in the 100 mg vitamin E group. In the 200 mg vitamin E group, all biomarkers of inflammation were statistically significantly higher than in the control group, with P-values of 0.012, 3.1*E-5, 0.016, 0.043 and 8.3*E-5 for MCP-1, IL-6, TNF- α, total PAI-1 and resistin, respectively.

3.4 Brain

In the brain, the activities of enzymes ALP, ALT and AST decreased upon exposure to vitamin E with P-values of 4.6*10-6 and 1.0*10-7 (for ALP), 0.029 (for ALT, the highest dose of vitamin E only), and 0.0036 and 0.0003 (for AST). The activity of LDH also increased in the brain with P-values of 0.0014 and 0.0023 for 100 and 200 mg vitamin E, respectively. BAP decreased with statistical significance for a dose of 200 mg vitamin E (P=0.025) and TTP increased statistically significantly for 100 and 200 mg vitamin E (P=1.7*10-4 and 3*10-4, respectively), as in the liver and kidney.

The biomarkers of inflammation in the brain tissue showed a tendency to increase with the higher doses of vitamin E, except for MCP-1 and total PAI-1. IL-6 concentrations increased in a dose-related manner for both doses of vitamin E; 200 mg of vitamin E caused a statistically significant increase with a P-value of 0.033. Both TNF-a and resistin showed a biphasic behaviour with vitamin E: i) a statistically significant decrease of resistin concentration for 100 mg of vitamin E (P=0.0012); ii) an increase in TNF-a and resistin for the 200 mg of vitamin E (P-values of 0.035 and 0.024, respectively).

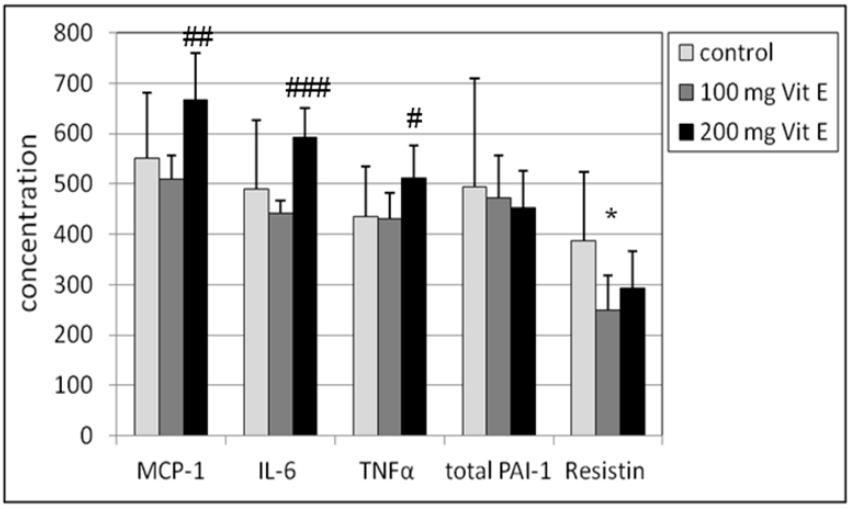

Fig. 4. Liver supernatant concentrations of MCP-1, IL-6, TNF-α, resistin (expressed in pg/mL), and PAI-1 (expressed in µg/mL). The concentrations of TNF-α and total PAI-1 were divided by a factor of 0.5 and 2, respectively, to fit in the figure. Statistics: * P<0.05 vs. control group; # P<0.05 vs. 100 mg vitamin E group; ## P<0.005 vs. 100 mg vitamin E group; ### P<0.001 vs. 100 mg vitamin E group

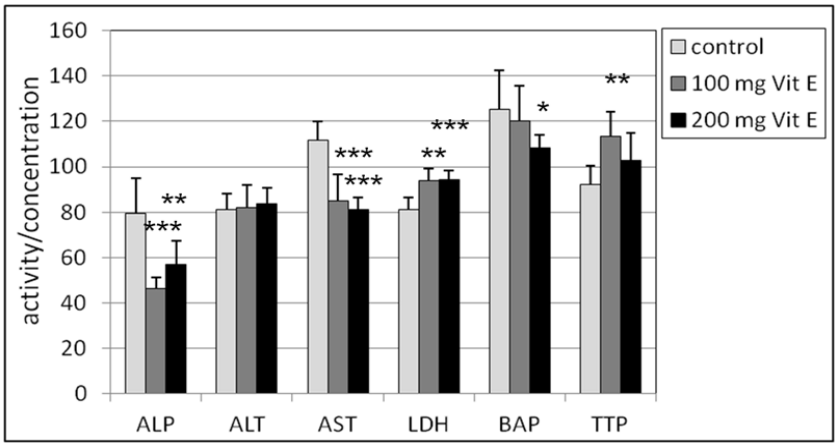

Fig. 5. Kidney supernatant activities of ALP, ALT, AST, LDH (expressed in IU/gprotein) and concentrations of BAP (expressed in mEq/g protein) and TTP (expressed in µmol/g protein). The activities of ALP, AST and LDH and the concentrations of BAP and TTP were divided by a factor of 20, 10, 100, 2.5 and 0.4, respectively, to fit in the figure. Statistics: * p<0.05 vs. control group; ** p<0.01 vs. control group; *** p<0.001 vs. control group

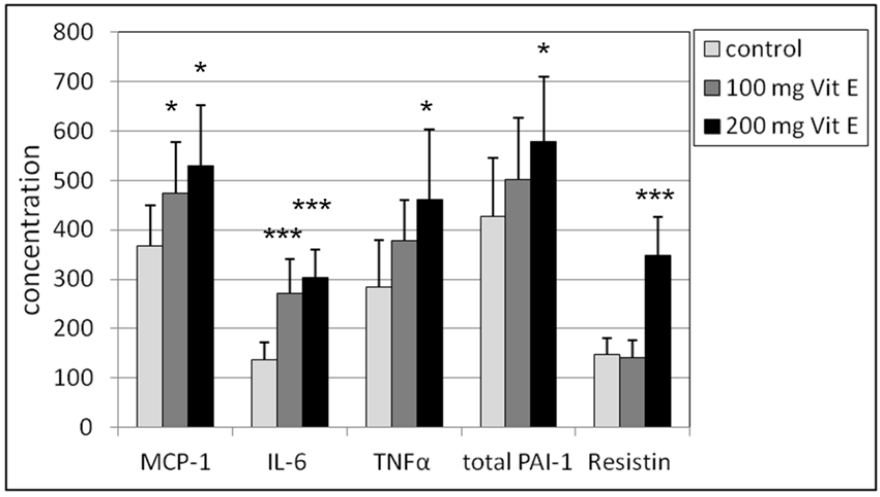

Fig. 6. Kidney supernatant concentrations of MCP-1, IL-6, TNF-α, resistin (expressed in pg/mL), and PAI-1 (expressed in µg/mL). The concentrations of TNF-α, total PAI-1 and resistin were divided by a factor of 0.5, 2 and 4, respectively, to fit in the figure. Statistics: * P<0.05 vs. control group; *** p<0.001 vs. control group

In summary, the measured biomarkers with their effects on plasma, liver, kidney and brain have been summarized in Table 1. The general view is that the biochemical biomarkers (ROM, ALT, AST, LDH) showed no consistent effects on vitamin E supplementation, although BAP and TTP showed small, but consisted effects in all tissues, whereas ALP showed a consistent decrease in all tissues. In contrast, most of the inflammation biomarkers (MCP-1, IL-6, TNF-α, PAI-1, resistin) showed an increase in all tissues, especially in the kidney.

4. DISCUSSION

In the present sub-acute study with male mice, the effects of relatively high doses of vitamin E were investigated in plasma, liver, kidney and brain tissue extracts. In plasma, biomarkers of oxidative stress processes, such as hydroperoxides (ROM) and total thiols (TTP), and biomarkers of inflammation were measured. Hydroperoxides and total thiols have been proven to represent several physiological processes, in which oxidation is involved. These

were measured in several large-scale human studies on total mortality, cardiovascular disease, cancer [24,25,26,27,28,29] and frailty [30], and also in mice studies [12,31]. In the present study, these biomarkers (ROM and TTP) in plasma were not affected by 14-day exposure to 100 and 200 mg of vitamin E.

Besides these oxidation-related biomarkers, also the inflammation biomarkers were determined. The cytokines IL-6 and TNF-α were not affected, which is in agreement with the observation that the oxidative stress biomarkers also did not change. Oxidative stress and inflammation processes are closely related, as was shown in our previous studies on aluminium exposed mice, where we indeed observed a concomitant increase of ROM, IL-6 and TNF-a [32]. However, the concentrations of three other inflammation parameters, MCP-1, PAI-1 and resistin, were statistically significantly higher after exposure to 100 and 200 mg vitamin E/kg bw/day.

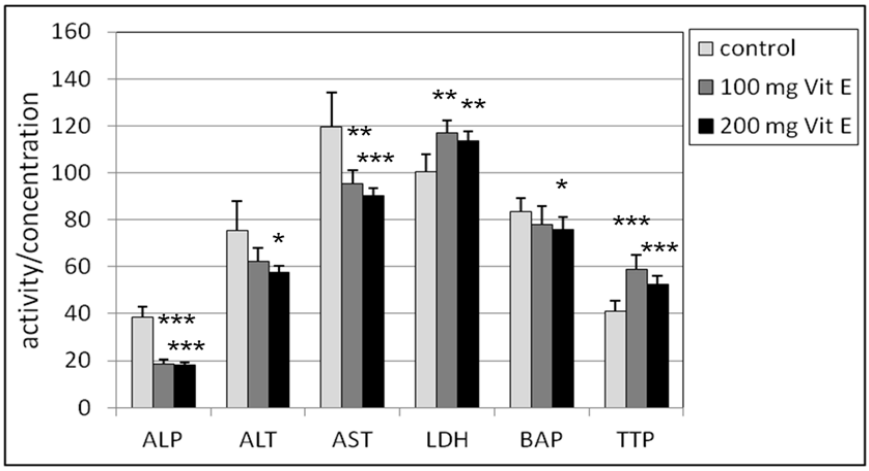

Fig. 7. Brain supernatant activities of ALP, ALT, AST, LDH (expressed in IU/gprotein), and concentrations of BAP (expressed in mEq/g protein) and TTP (expressed in expressed in µmol/g protein). The activities of AST and LDH and the concentration of BAP were divided by a factor of 25, 50 and 5, respectively, to fit in the figure. Statistics: * P<0.05 vs. control group; ** P<0.01 vs. control group; *** P<0.001 vs. control group

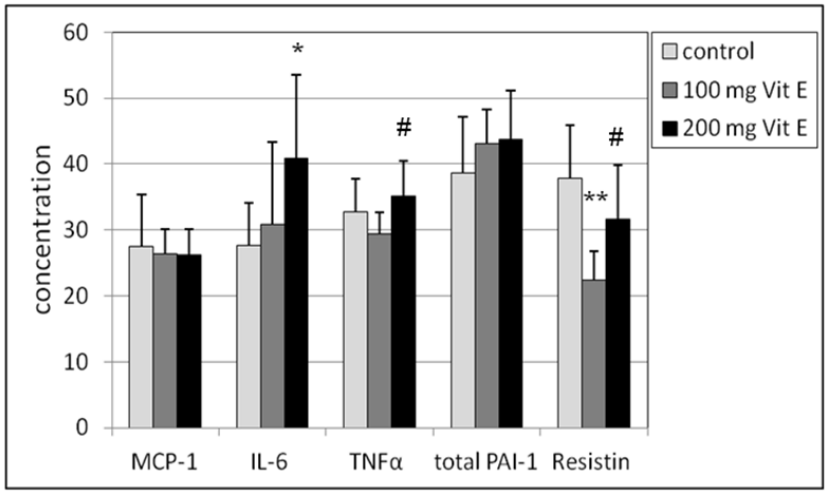

Fig. 8. Brain supernatant concentrations of MCP-1, IL-6, TNF-α, resistin (expressed in pg/mL), and PAI-1 (expressed in µg/mL). The concentrations of MCP-1, TNF-α, and total PAI-1 were divided by a factor 2, 0.5, and 10, respectively to fit in the figure. Statistics: * P<0.05 vs. control group; ** P<0.005 vs. control group; # P<0.05 vs. 100 mg vitamin E group

Table 1. The effects of vitamin E supplementation of the various tissues

	Plasma	Liver	Kidney	Brain
ROM	0			
BAP	0	-	-	-
TTP	0	+	+	+
ALP		-	-	-
ALT		0	0	-
AST		0	-	-
LDH		-	+	+
MCP-1	+	+	+	0
IL-6	0	+	+	+
TNF-α	0	+	+	+
PAI-1	+	0	+	0
resistin	+	0	+	0

"0" means no statistically significant effect, "+" means an increase of the biomarker and "-"means a decrease of the biomarker on supplementation of vitamin E. No data means that the biomarker was not measured

In the tissues of liver, kidney and brain, we looked at the biomarkers of enzyme induction (ALP, ALT, AST and LDH) the total antioxidant status (BAP) and the redox status (TTP). The enzymes are usually measured in liver toxicity studies, but damage in other tissues are also reflected by an induction of these enzymes, although at a lower level. Exposure to vitamin E showed in general that the tissue enzymes had lower activity at a higher dose of vitamin E, indicating a lower toxicity. An exception is the LDH activity in the kidney and brain, which increased on exposure to both doses of vitamin E. BAP decreased in all tissues, which was somewhat unexpected in the face of anti-oxidative properties of vitamin E. The concentration of total thiols (TTL) was higher on exposure to vitamin E, which means a favorable effect of this vitamin. However, exposure to a higher dose of vitamin E (200 mg) caused lowering of the redox status, which was reflected by a possible biphasic behavior in kidney and brain tissue, although not statistically significant. Because of the limited number of mice in the exposed groups (N=8), a possible statistical significance of the observed effects is possibly prevented and the borderline statistical significance can be questioned. However, we think that most of the effects observed in this study are large enough to draw conclusions on vitamin E toxicity. At least the effects we observe with the 200 mg dose of vitamin E in kidney tissue (all inflammation biomarkers) and in brain tissue (IL-6) are very substantial and statistically sound.

In addition, the biomarkers of inflammation (MCP-1, IL-6, TNF-α, total PAI-1, resistin) were determined. In all tissues, the high dose of vitamin E (200 mg) caused an increase in most of the inflammation biomarkers, whereas the lower dose of 100 mg caused an increase mainly in kidney tissue. Apparently the kidney is more sensitive to vitamin E exposure then liver and brain. This observation confirms our previous study with oral expose of vitamin E [12]. The increase of inflammation biomarkers (IL-6 and TNF-α) in the tissues was not reflected in the circulation (serum). Probably the extend of induction in the tissues is not large enough to observe also an increase in serum.

The increase in inflammation biomarkers can be considered as adverse because they are linked to unwanted effects related to several diseases, such as neurological complaints [33,34,35], cardiovascular diseases [36,37,38], diabetes [39,40,41,42,43], and injuries of the liver [44,45,46], kidney [47] or lung [48].

The beneficial effect of the recommended dietary allowance (RDA) doses of vitamin E on these parameters, often observed as a decrease of inflammation status, becomes completely opposite when higher doses of vitamin E are used. In addition, the increase in inflammation parameters is dose-dependent, being a strong evidence of these adverse effects.

The Food and Nutrition Board of the National Academy of Sciences specified the RDA of vitamin E as 15 mg/day and established the upper intake level of toxicity (UL) as 1000 mg/day [49,50]. The UL is the upper level that is unlikely to pose risk of adverse health effects to 95% of the general population. This UL was established in rodent studies starting from a lowest observed adverse effect level (LOAEL) of 500 mg vitamin E/kg bw/day [51]. This dose is much higher than the doses of 100 mg and 200 mg vitamin E/kg bw/day that were used in the present study.

There are some concerns about a long-term safety of the established UL 1000 mg/day of vitamin E [52,53]. In Miller's study [52], a possible increase in mortality and in the incidence of heart failure was observed at a dose of 900 IU/day (about 600 mg) of vitamin E. Therefore, an UL of vitamin E toxicity of 1000 mg/day may be too high.

The European Food Safety Authority (EFSA) also evaluated the UL for vitamin E. They considered the study of Meydani et al. [54] as the most relevant study and deduced an UL for vitamin E at 300 mg/day for human adults [55].

There are conflicting reports on the beneficial or adverse effects of vitamin E supplementation. Some studies have found positive or no effect [7], or are inconclusive [6,8], whereas others showed toxic effects on prostate cancer [13] and hemorrhagic stroke [16].

In the present study, we observed adverse effects of vitamin E as the increase of inflammation biomarkers in the circulation (MCP-1, PAI-1 and resistin) and in tissues (in addition also IL-6 and TNF-α). The findings of the previous mice study in which vitamin E was supplied in the feed, being the adverse effects observed in the kidney [12], were confirmed by the present study in which vitamin E was supplied by intraperitoneal injection in oil. Therefore, a possible re-evaluation of the UL for vitamin E should be considered.

5. CONCLUSIONS

In a short-term study (14 days), male mice were exposed to an intra peritoneal dose of 100 and 200 mg vitamin E (alpha-tocopherol)/kg bw/day. Biomarkers of oxidative stress related processes, and biomakers of tissue toxicity and inflammation were determined.

In the liver, some beneficial effects on biomarkers of enzyme induction were observed, whereas the inflammation biomarkers showed an U-shaped relation with the dose of vitamin E. In the kidney, the largest effects of toxicity were observed by a substantial increase in all inflammation biomarkers. This confirms the observed effects in the kidney of an oral study with vitamin E. In brain tissue, inflammatory biomarkers IL-6, TNF-α and resistin showed also an increase or an U-shaped behaviour with increasing doses of vitamin E.

Especially, the dose of 200 mg of vitamin E/kg bw/day which is lower than the upper limit for vitamin E, caused a number of adverse effects in all tissues, but most pronounced in kidney and brain. Also in view of the results in other reported studies, the upper limit of toxicity of vitamin E should be re-evaluated.

ETHICS

This study was performed according to the Republic of Lithuania Law on the Care, Keeping and Use of Animals (License of State Veterinary Service for working with laboratory animals No 0200).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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