Parameters of dietary selenium and vitamin E deficiency in growing rabbits

Andreas S. Müller, Josef Pallauf* and Erika Most

Institute of Animal Nutrition and Nutrition Physiology, Justus Liebig University Giessen, Giessen, Germany

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Abstract

4 x 5 growing female rabbits (New Zealand White) with an initial live weight of 610 ± 62 g were fed a torula yeast based semisynthetic diet low in selenium (<0.03 mg/kg diet) and containing <2 mg α-tocopherol per kg (group I). Group II received a vitamin E supplementation of 150 mg α-tocopherylacetate per kg diet, whereas for group III 0.40 mg Se as Na-selenite and for group IV both supplements were added. Selenium status and parameters of tissue damage were analyzed after 10 weeks on experiment (live weight 2355 ± 145 g). Selenium depletion of the Se deficient rabbits (groups I and II) was indicated by a significantly lower plasma Se content (group I: 38.3 ± 6.23 µg Se/mL plasma, group II: 42.6 ± 9.77, group III: 149 ± 33.4, group IV: 128 ± 6.45) and a significantly lower liver Se content (group I: 89.4 ± 18.2 µg/kg fresh matter, group II: 111 ± 26.2) as compared to the Se supplemented groups III (983 ± 204) and IV (926 ± 73.9). After 5 weeks on the experimental diets differences in the development of plasma glutathione peroxidase were observed. As compared to the initial status group (45.2 ± 4.50) pGPx activity in µL/mg protein was decreased in group I (19.1 ± 7.08), remained almost stable in the vitamin E supplemented group II (46.3 ± 11.2) whereas an elevated enzyme activity was measured in the Se supplemented groups III (62.4 ± 23.9) and IV (106 ± 19.9). In the rabbit organs investigated 10 weeks of Se deficiency caused a significant loss of Se dependent cellular glutathione peroxidase activity (GPx1) of 94% (liver), 80% (kidney), 50% (heart muscle) and 60% (musculus longissimus dorsi) in comparison to Se supplemented control animals. Damage of cellular lipids and proteins in the liver was due to either Se or vitamin E deficiency. However damage was most severe under conditions of a combined Se and vitamin E deficiency. It can be concluded that the activity of plasma glutathione peroxidase is a sensitive indicator of Se deficiency in rabbits. The loss of GPx1 activity indicates the selenium depletion in various rabbit organs. Both selenium and vitamin E are essential and highly efficient antioxidants which protect rabbits against lipid and protein oxidation.

Key words: selenium deficiency, vitamin E deficiency, rabbits, glutathione peroxidase, lipid oxidation, protein oxidation

Introduction

Selenium and vitamin E are two of the most important antioxidants protecting animal cells against oxidative injury. The biological activity of selenium is attained by maintenance of the activity of four different glutathione peroxidases (GPx1 = cellular glutathione peroxidase, GPx2 = gastrointestinal glutathione peroxidase, pGPx = plasmatic glutathione peroxidase, GPx4 = phospholipid-hydroperoxide glutathione peroxidase), a class of enzymes which catalyze the detoxification of hydrogen peroxide and a variety of organic hydroperoxides generated from the transformation of reactive oxygen species from cellular respiration and lipid peroxidation (12). Vitamin E mainly acts as a chain breaking antioxidant during lipid peroxidation (8). Dependent on the species, different tissues are affected in varying degrees by dietary selenium deficiency. In the literature liver necrosis, necrosis of the thyroid gland and multiple organ necrosis are described as...
in accordance with the NRC concept for purified diets for the aim of the present study (Table 1). The modifications of the NRC concept for rabbits so far. Consequently in the present study a dietary selenium- and/or vitamin E deficiency in rabbits was induced to examine the development of selenium and vitamin E status and to evaluate parameters to classify the role of selenium alone in protecting rabbits against damage to cellular lipids and proteins.

Materials and Methods

Experimental design, animals and diets
4 x 5 female 4 week old weanling New Zealand White rabbits (Hessische Landesanstalt für Tierzucht Homberg/Ohm, Neu-Ulmstein) with an initial live weight of 610 ± 62 g were fed selenium (Se)- and/or vitamin E-deficient diets for 10 weeks. The experimental diets were prepared in accordance with the NRC concept for purified diets for rabbits (30) taking into consideration modifications for the aim of the present study (Table 1). The modifications of the NRC concept were as follows:

- Torula yeast was used as a Se deficient protein source.
- Coconut oil and soy bean oil were added to the 4 experimental diets at a mixture ratio of 1:1 to minimize the vitamin E content of the basal diet but to provide sufficient amounts of unsaturated and essential fatty acids, respectively.
- Cellulose with a fibre length of 200 μm was admixed to the diets to obtain an optimum structure for fermentation in the hindgut of the rabbits.
- Mineral and vitamin mixtures for the Se and vitamin E deficient basal diet (group I: <0.030 mg Se and <2 mg α-tocopherol per kg diet) were prepared without addition of Se and vitamin E supplements. The diets for groups II (+E) and III (+Se) were supplemented with 150 mg/kg α-tocopherylacetate and 0.40 mg/kg Se as sodium selenite, respectively. Both supplements were added to diet IV.

Rabbits were allocated to four groups of five animals and housed individually in stainless steel cages under standard conditions (18 °C, 45% humidity, 12 h light:dark cycle). Over the whole experimental period diets were rationed to prevent osmotic diarrhoea as a consequence of the rather high mono- and disaccharide content of the experimental diets. The animals had free access to bidistilled water which was adjusted to the osmolality of tap water by the addition of 0.014% NaCl and 0.014% CaCl₂.

Collection of blood, preparation of plasma and preparation of tissue homogenates
During the experiment blood was taken after 2, 5 and 8 weeks from the rabbits' vena auricularis and collected in heparinized plastic tubes. Plasma was prepared by centrifugation at 2,540 x g for 15 minutes. The plasma in the supernatant was pipetted into sterile 2.0 mL Eppendorf cups and frozen until measurement of selenium content, α-tocopherol content and glutathione peroxidase activity at -80 °C. After ten weeks on experiment the rabbits had an average live weight of 2355 ± 145 g and were stunned with a captivebolt and subsequently killed by decapitation. Blood was collected in heparinized plastic tubes, and plasma was obtained and treated as described above. Liver, kidney, heart and musculus longissimus dorsi were removed immediately, placed in liquid nitrogen and stored at -80 °C until further analysis. To get an initial status for

| Table 1. Composition of the selenium and vitamin E deficient basal diet |
|-----------------------------|-----------------------------|
| Dietary components       | Content (g/kg diet) |
| Torula yeast (50% crude protein) | 300 |
| Cellulose FTC 200          | 160 |
| Glucose                    | 75  |
| Sucrose                    | 75  |
| Soybean oil                | 25  |
| Coconut oil                | 25  |
| DL-methionine              | 2   |
| Minerals and trace elements (without selenium) | 66 |
| Vitamin mixture (without Vitamin E) | 3  |
| Maize starch               | 269 |
| Total                      | 1000 |

1) Minerals and trace elements per kg diet
CaCO₃ | 25.0 g = 10.18 g Ca/kg diet
K₂HPO₄ | 22.0 g = 3.89 g P/kg diet
Na₂HPO₄ | 7.00 g = 1.52 g P/kg diet
MgSO₄ × 7 H₂O | 6.00 g = 590 mg Mg/kg diet
NaCl | 5.00 g = 1.96 g Na/kg diet
CuSO₄ × 5 H₂O | 40.0 mg = 10.2 mg Cu/kg diet
FeSO₄ × 7 H₂O | 500 mg = 100.4 mg Fe/kg diet
ZnSO₄ × 7 H₂O | 220 mg = 50.0 mg Zn/kg diet
MnSO₄ × 7 H₂O | 125 mg = 45.5 mg Mn/kg diet
CrCl₃ | 10.3 mg = 3.38 mg Cr/kg diet
NaF | 2.20 mg = 0.99 mg F/kg diet
KJ | 0.50 mg = 0.38 mg I/kg diet
Na₂MoO₄ × 2 H₂O | 1.20 mg = 0.25 mg Mo/kg diet
2) Vitamin mixture per kg diet
Vitamin A | 15000 I.U.
Vitamin D₃ | 1500 I.U.
Vitamin B₁₂ | 25.0 mg
Vitamin B₆ | 20.0 mg
Vitamin B₃ | 40.0 mg
Niacin | 0.02 mg
Pantothenic acid | 180 mg
Biotin | 5.00 mg
Vitamin K₃ | 5.00 mg
Cholinchloride | 150 mg
Vitamin C | 1200 mg

Determination of cellular glutathione peroxidase activity

All analyzed parameters five additional animals had been sacrificed at the beginning of the experiment. For the determination of cellular glutathione peroxidase activity (GPx) and thiobarbituric acid reactive substances (TBARS) samples of liver, kidney, heart and musculus longissimus dorsi were homogenized in 9 volumes of 10 mmol/L TRIS buffer (pH 7.4) under an atmosphere of nitrogen gas (1:10, w/v-homogenates). Liver homogenates for the protein carbonyl assay were prepared with phosphate buffer (50 mmol/L Na2HPO4) containing the antiproteases phenylmethyl-sulfonylfluoride (40 mg/mL), leupeptine (5 mg/mL), pepstatin (7 mg/mL), aprotinin (5 mg/L) and digitonin (1 g/L) (33).

Determination of selenium content in experimental diets, plasma and livers

Selenium content of experimental diets, plasma and liver was measured with an Atomic Absorption Spectrometer (AAS, Unicam PU 9400 X) with permanent deuterium background correction and Hydride Generation System (PU 9360 X) with continuous flow injection for digested samples (27, 42). For the microwave-supported digestion of samples either 0.3 g of the experimental diets and livers or 1 mL of plasma were put into acid resistant teflon digestion vessels. After the addition of 3 mL HNO3 (65%) and 1 mL H2O2 (30%) the vessels were closed (torsion: 14.5 Nm) and oxidative digestion of samples to the selenium oxidation state +IV was confirmed automatically by the continuous flow injection system of the AAS-apparatus (MLS 12000) with the following energy-time-program: 300 W-3 min, 0 W-3 min, 300 W-8 min, 0 W-3 min, 300 W-5 min, 0 W-3 min, 600 W-5 min, 0 W plus ventilation-30 min. After dilution of the digested samples with H2O bidest, to a total volume of 10 mL an aliquot of 4.5 mL of these diluted Se-(+VI)-solutions from each sample was carried over in a 20 mL volumetric flask. After addition of 1 mL amidosulfonic acid (1.4 mol/L) and 3.5 mL HCl (37%) a partial reduction of selenium to the oxidation state +IV was carried out by the incubation for 60 min in a waterbath at 70 °C. The final reduction of selenium to the hydride oxidation state –II was confirmed automatically by the continuous flow injection system of the AAS-apparatus with NaBH4 (0.2%) and NaOH (0.05%) immediately before measuring selenium content at a wavelength of 196 nm. Certified samples of bovine liver (NIST SRB 1577 b), serum (Seronorm TE Nr. 250 mg) was extracted with a mixture of methanol/dichloromethane (1:2, v/v). Lipid extraction and protein precipitation of plasma were carried out by the addition of 350 μL ethanol (96%) and 400 μL n-hexane to 100 μL of plasma. After concentration of the organic phase in an atmosphere of nitrogen at 45 °C the concentrated sample was dissolved in 500 μL methanol plus butylated hydroxytoluene (0.05%). Vitamin E content was measured by HPLC (Merck Hitachi) on a C-18 reversed-phase column (Lichrospher 100 RP-18, Merck) with a fluorescence detector (excitation: 295 nm, emission: 340 nm) referring to the method described by Bieri et al. (5). A mixture of methanol/H2O (98:2) was applied to elute the vitamin E derivatives at a flow rate of 2 mL/min for isocratic elution. The chromatographic peak was identified by comparison of retention time in the column with pure standards of α-tocopherol and α-tocopheryl acetate (Hoffmann-La Roche, Basel, Switzerland) in a concentration range from 0.15–1.5 μg/mL.

Determination of thiobarbituric acid reactive substances (TBA-RS) in the liver

Thiobarbituric acid reactive substances (TBA-RS) as an indicator of lipid peroxidation were determined fluorometrically (14). Additionally to this method a provocation of free radicals with Fe-(III)-ions was carried out before reaction with 2-thiobarbituric acid (7). Therefore 600 μL of a Fe-(III)-solution and 600 μL TRIS buffer (10 mmol/L, pH 7.4) were added to 1200 μL of the 15,000 × g supernatant from liver homogenates, mixed thoroughly and incubated for 60 min at 37 °C. Subsequently samples were diluted 1:1 (v/v) with trichloracetic acid (TCA) for protein precipitation and centrifuged for 5 min at 13,000 × g. After the addition of 500 μL of a TBA-solution (500μL) and 50 μL of a BHT-SDS-solution to 500 μL of the supernatant, samples were shaken and incubated for 10 min in a boiling water bath. Subsequently tubes were chilled on ice. The rose-colored trimethin-complex was extracted into 3 mL of n-butanol. After centrifugation for 5 minutes at 3,300 × g TBA-RS were measured fluorometrically (Perkin Elmer LS 50 B) in the butanol phase with an excitation wavelength of 532 nm and an emission wavelength of 553 nm. A standard curve for TBA-RS was prepared with

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1,1,3,3-tetraethoxypropanol in a concentration range of 0–1320 nM.

**Determination of protein carbonyls in the liver**

Several amino acids, especially arginine, histidine, methionine and cysteine tend to undergo oxidation under conditions of antioxidant deficiency. The reaction products are a variety of protein carbonyls. Protein carbonyls in the liver homogenates (1:10, w/v) were assayed spectrophotometrically (33). 2 mL dinitrophenylhydrazine (0.2% in 2.5 mol/L HCL) were added to 0.5 mL of liver homogenates, mixed thoroughly and incubated for 1 h at room temperature. 5 mL TCA solution (20%) were added to the samples for protein precipitation. The tubes were cooled in an ice bath for 10 min and subsequently centrifuged at 10,000 × g for 5 min to collect the protein fraction. The protein pellets were washed once with 4 mL TCA (10g/L) and three times with 4 mL of a mixture of ethanol/ethylacetate (1:1, v/v). Prior to the last washing process pellets were disrupted mechanically with a spatula and centrifuged at 15,000 × g for 10 min. The resulting protein precipitates were dissolved in guanidinium HCL (6 mol/L in 20 mmol/L potassium phosphate, pH 2.3) and incubated for 10 min at 37 °C. The carbonyl content was calculated from the peak absorbance (355–390 nm) using an absorption coefficient (e) of 22,000 [L/(mol·cm)]. Results are given as nmol protein carbonyl/mg protein.

Protein concentration was measured on the basis of 2,4-dinitrophenylhydrazine-free blank pellets from a standard curve prepared with pure glutamine hydroxamate. The mixture was incubated for 15 min at 37 °C. To stop the reaction for protein precipitation and for the change of glutamine hydroxamate into the siderochrome complex, 1 mL of a solution containing (0.37 mol/L FeCl₃, 0.3 mol/L trichloracetic acid and 0.6 mol/L HCl) was added to the incubation mixture. After centrifugation at 3,400 × g for 5 min, the supernatant was pipetted into macro-cuvettes and extinction was read spectrophotometrically at 505 nm. An additional sample, treated in the same way, but without addition of ADP served as the blank. Enzyme activity was estimated by a standard curve prepared with pure glutamine hydroxamate standards in the concentration range between 0 and 1000 μmol/L. One unit of glutamine synthetase activity was defined as 1 μmol glutamine hydroxamate generated per minute.

**Statistical analysis**

Statistical analysis of the experimental data was performed using the statistical package SPSS 8.0 for Windows. A one way analysis of variance (ANOVA) was performed after ascertainment of the normality of distribution (Kolmogorov-Smirnov-Test or Shapiro-Wilk-Test) and the homogeneity of variance (Levene-Test) of the experimental data. If both conditions were fulfilled differences between means for the experimental parameters were evaluated using the Scheffé-Test. If homogeneity of variance could not be ensured differences of means were examined using the Dunnett T3-Test. For plasma glutathione peroxidase where the time course was of interest, a multivariate analysis of variance (MANOVA) was additionally performed to examine the influences of the experimental factors selenium and vitamin E on pGPx activity over the time course.

**Table 2. Concentration of selenium and α-tocopherol in plasma and liver of growing rabbits after 10 weeks on Se and vitamin E deficient diets in comparison to Se and vitamin E supplemented animals and to the initial status group**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Initial status group</th>
<th>Group I OSe OE</th>
<th>Group II OSe 15 OE</th>
<th>Group III 0.4Se OE</th>
<th>Group IV 0.4Se 15 OE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Se (μg/L plasma)</td>
<td>104 ± 3.85b</td>
<td>38.3 ± 6.23a</td>
<td>42.6 ± 9.77a</td>
<td>149 ± 33.4b</td>
<td>126 ± 6.45b</td>
</tr>
<tr>
<td>Plasma α-tocopherol (μg/L plasma)</td>
<td>6.52 ± 0.97b</td>
<td>1.06 ± 0.40a</td>
<td>9.76 ± 3.84a</td>
<td>1.34 ± 0.24a</td>
<td>9.33 ± 3.36b</td>
</tr>
<tr>
<td>Liver Se (μg/kg fresh matter)</td>
<td>787 ± 98.9b</td>
<td>89.4 ± 18.2a</td>
<td>111 ± 26.2a</td>
<td>983 ± 204b</td>
<td>926 ± 73.9b</td>
</tr>
<tr>
<td>Liver α-tocopherol (μg/kg fresh matter)</td>
<td>6.95 ± 0.51a</td>
<td>1.25 ± 0.15a</td>
<td>32.2 ± 10.1b</td>
<td>2.34 ± 0.98a</td>
<td>34.6 ± 9.13b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. Different superscripts within a line indicate significant differences (Dunnett T3). Plasma Se: p < 0.001, plasma α-tocopherol: p < 0.01, liver Se: p < 0.001, liver α-tocopherol: p < 0.001

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Results

After 10 weeks of Se and vitamin E deficiency mean live weight of the rabbits was 2355 ± 104 g and no significant differences in live weight between the rabbits of the four dietary groups could be observed. The mean daily gain during the experiment was 24.8 ± 2.35 g in all experimental groups at a feed efficiency (g feed per g gain) of 3.40. The Se and vitamin E depletion of the rabbits in groups I and II as compared to the Se supplemented groups III and IV was indicated by significantly lower plasma Se concentrations (Table 2). Likewise significantly lower tocopherol contents were measured in the plasma of the vitamin E deficient groups I and III than in the vitamin E added groups II and IV. Selenium deficiency of the rabbits was also reflected by a significantly lower Se content in the livers of groups I and II. A significantly higher tocopherol concentration was achieved in the livers of the vitamin E supplemented groups II and IV as compared to the vitamin E deficient groups I and III (Table 2).

Development of plasma glutathione peroxidase (pGPx) is shown in Table 3. At the beginning of the experiment pGPx activity in the initial status group was 45.2 ± 4.50 mU/mg protein. After 2 weeks on the experimental diets pGPx activity was almost stable in all experimental groups. While in group I a significantly decreased pGPx activity was measured after 5 weeks on Se deficiency, pGPx activity at this point of time remained nearly constant in group II and was increased in the Se supplemented groups III and IV. Until week 8 of the experiment pGPx activity in the Se deficient groups I and II declined to about 50% of the values measured after 5 weeks. For the Se supplemented groups III and IV however a further increase in pGPx activity was obtained. After 10 weeks significantly lower pGPx activities were detected in the plasma of both Se deficient groups I and II as compared to the Se sufficient groups III and IV. Thereby significant influences of the experimental time and significant interactions for the factors experimental time, selenium and vitamin E on the development of pGPx activity could be noted (Table 3, MANOVA).

According to a different expression in various rabbit organs 10 weeks of selenium deficiency (groups I and II) caused a significant loss of selenium dependent cellular glutathione peroxidase (GPx1) in all examined organs as compared to the initial status group and to the Se supplemented groups III and IV (Table 4). The loss of GPx1 activity was most obvious in liver and kidney. In these organs the activity was decreased to about 6% and 15% of the control values in the Se supplemented groups III and IV, respectively. In the heart muscle and in the musculus longissimus dorsi about 50% higher GPx1 activities were measured in the Se supplemented groups III and IV as compared to the Se deficient groups I and II.

The highest rate of radical induced lipid peroxidation was measured in the livers of the Se and vitamin E deficient rabbits (Table 5). Single vitamin E supplementation (group II) and single Se supplementation effected a pro-

Table 3. Development of plasma glutathione peroxidase (mU/mg protein) in growing rabbits during 10 weeks of selenium and vitamin E deficiency

<table>
<thead>
<tr>
<th>Initial status group:</th>
<th>45.2 ± 4.50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeks on experimental diets</td>
<td>Group I</td>
</tr>
<tr>
<td>0Se 0E</td>
<td>0Se 15OE</td>
</tr>
<tr>
<td>2</td>
<td>46.0 ± 6.78a</td>
</tr>
<tr>
<td>5</td>
<td>19.1 ± 7.08a</td>
</tr>
<tr>
<td>8</td>
<td>9.05 ± 6.85a</td>
</tr>
<tr>
<td>10</td>
<td>5.54 ± 1.09a</td>
</tr>
</tbody>
</table>

Influences of the different experimental factors on plasma GPx activity (MANOVA):
Week 0.004, Week × Se < 0.001, Week × vit. E < 0.001, Week × Se × vit. E 0.015
Values are expressed as mean ± SD. Different superscripts within a line indicate significant differences (Scheffé).
Week 2: p < 0.05, week 5: p < 0.05, week 8: p < 0.01, week 10: p < 0.001

Table 4. Activity of selenium dependent cellular glutathione peroxidase (mU/mg protein) in various tissues of growing rabbits after 10 weeks on selenium and/or vitamin E deficient diets in comparison to animals with selenium and/or vitamin E supplementation and to the initial status group

<table>
<thead>
<tr>
<th>Organ</th>
<th>Initial status</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>group</td>
<td>0Se 0E</td>
<td>0Se 15OE</td>
<td>0.45Se 0E</td>
<td>0.45Se 15OE</td>
</tr>
<tr>
<td>Liver</td>
<td>367 ± 55.0b</td>
<td>26.6 ± 9.40a</td>
<td>33.1 ± 13.0a</td>
<td>417 ± 45.4b</td>
<td>488 ± 33.0b</td>
</tr>
<tr>
<td>Kidney</td>
<td>233 ± 14.3b</td>
<td>55.9 ± 11.9c</td>
<td>82.5 ± 14.5c</td>
<td>350 ± 24.6c</td>
<td>343 ± 36.3c</td>
</tr>
<tr>
<td>Heart muscle</td>
<td>35.5 ± 4.15b</td>
<td>18.5 ± 4.00a</td>
<td>20.6 ± 3.80b</td>
<td>36.4 ± 6.90b</td>
<td>35.3 ± 3.94b</td>
</tr>
<tr>
<td>M. long. dorsi</td>
<td>18.4 ± 3.25ab</td>
<td>10.5 ± 2.39a</td>
<td>10.6 ± 2.22a</td>
<td>26.0 ± 3.34a</td>
<td>24.1 ± 3.70b</td>
</tr>
</tbody>
</table>

Different superscripts within the line indicate significant differences (Dunnett T3). Values are expressed as mean ± SD. Different superscripts within the line indicate significant differences (Dunnett T3). Liver: p<0.001, kidney: p<0.001, heart muscle: p<0.01, musculus longissimus dorsi: p<0.01
Table 5. Parameters of lipid and protein oxidation in the liver of growing rabbits after 10 weeks on selenium and/or vitamin E deficient diets in comparison to animals with selenium and/or vitamin E supplementation and to the initial status group

<table>
<thead>
<tr>
<th>Liver parameter</th>
<th>Initial status group</th>
<th>Group I 0Se 0E</th>
<th>Group II 0Se 15OE</th>
<th>Group III 0.4Se 0E</th>
<th>Group IV 0.4Se 15OE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBA-RS (nmol/g fresh matter)</td>
<td>62.2 ± 42.0 c</td>
<td>195 ± 38.1 d</td>
<td>2.59 ± 0.75 a</td>
<td>19.1 ± 5.56 b</td>
<td>1.50 ± 0.28 a</td>
</tr>
<tr>
<td>Protein carbonyls (nmol/mg protein)</td>
<td>1.23 ± 0.33 a</td>
<td>3.00 ± 0.51 b</td>
<td>1.69 ± 0.11 a</td>
<td>1.42 ± 0.12 a</td>
<td>1.16 ± 0.15 a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. Different superscripts within the line indicate significant differences (Scheffé). TBA-RS: p<0.001, protein carbonyls: p<0.01

Table 6. Activity of glutamine synthetase (mU/mg protein) in various rabbit tissues of growing rabbits after 10 weeks on selenium and/or vitamin E deficient diets in comparison to animals with selenium and/or vitamin E supplementation and to the initial status group

<table>
<thead>
<tr>
<th>Organ</th>
<th>Initial status group</th>
<th>Group I 0Se 0E</th>
<th>Group II 0Se 15OE</th>
<th>Group III 0.4Se 0E</th>
<th>Group IV 0.4Se 15OE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>783 ± 98.1 c</td>
<td>298 ± 48.8 a</td>
<td>701 ± 101 c</td>
<td>472 ± 97.8 b</td>
<td>657 ± 36.1 c</td>
</tr>
<tr>
<td>Kidney</td>
<td>784 ± 94.0 a</td>
<td>1372 ± 307 b</td>
<td>1573 ± 304 b</td>
<td>1315 ± 49.8 b</td>
<td>1359 ± 203 b</td>
</tr>
<tr>
<td>Heart muscle</td>
<td>122 ± 17.3 a</td>
<td>149 ± 21.6 a</td>
<td>224 ± 20.5 b</td>
<td>200 ± 31.8 b</td>
<td>284 ± 19.6 a</td>
</tr>
<tr>
<td>M. long. dorsi</td>
<td>115 ± 20.0 b</td>
<td>60.9 ± 4.77 a</td>
<td>55.0 ± 6.43 a</td>
<td>59.1 ± 11.8 b</td>
<td>46.5 ± 6.71 a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. Different superscripts within the line indicate significant differences (Dunnett T3). Liver, kidney, heart muscle and musculus longissimus dorsi: p<0.05

Discussion

According to the experimental design in the present study an extensive depletion of body Se and vitamin E stores of the rabbits was obtained. Comparable liver Se concentrations as in the initial status group and the Se supplemented groups III and IV of the present study were measured in the livers of New Zealand White rabbits (range 950–1300 ng selenium/g fresh matter) which received commercial diets (0.3 mg Se/kg diet) in studies on selenite toxicity (4, 43, 44). No data for the Liver Se content of Se deficient rabbits could be found in the literature. The rabbits of the vitamin E deficient groups I and III in the present study exhibited an α-tocopherol content in the liver close to values reported for New Zealand White rabbits (5.73 μg/g fresh matter) from a breeding colony (45), which unintentionally received a spoiled diet for 4 months with an α-tocopherol content less than 2 mg/kg diet.

Total plasma selenium consists of three main components:
1. A variety of anorganic and organic selenium compounds which circulate in plasma after the intestinal absorption (3).
2. Selenium from the plasmatic form of glutathione peroxidase (37).

Therefore the decreasing plasma Se content during an alimentary selenium deficiency could be proved to be the result of a diminished selenium absorption on the one hand and of a lowered synthesis of the functional selenoproteins pGpx and selenoprotein P in the main synthesis organs, liver and kidney on the other hand (17). In the present study significantly lower plasma Se concentrations in the Se deficient groups I and II (range: 26.6–55.1 μg/L) in comparison to the Se supplemented animals of groups III and IV (range: 109.5–206.8) pointed to a lowering of total plasma Se content in all the above mentioned components. In a histological study and in a trial on changes
in bone density due to Se deficiency a less marked selenium depletion in New Zealand White rabbits was indicated by plasma Se content (40, 41). In these studies after 7 weeks on Se deficient torula yeast based diets, mean Se contents of 79.9 ng selenium/mL plasma were measured. Moreover much higher mean contents for plasma selenium (100.4 ± 2.7 µg/L) in contrast to our data were achieved for selenium (+0.5 mg Se/kg diet) and vitamin E (+150 mg α-tocopherylacetate) supplemented rabbits (40, 41). A possible explanation for this deviation could be a consequence of a later onset of Se depletion or Se supplementation in these experiments (40, 41). However results for the development of plasma Se content similar to our findings were obtained in a study with rats (25). Nearly 150 µg selenium/L were achieved in the plasma of Se supplemented rats (+0.5 mg Se/kg diet, +50 mg vitamin E/kg diet). After 8 weeks on Se depleted diets rats in the same trial showed a plasma Se content (30 µg/L) close to the values obtained in our study. In the present study the hypothesis of a lowered synthesis of functional plasma selenoproteins during Se deficiency was underlined by the development of pGPx activity. The pGPx activity seems to be a sensitive indicator to illustrate the onset and the course of selenium deficiency. The differentiated development of pGPx activity with a decrease in groups I and II and a distinct increase in groups III and IV after 5 weeks on experiment elucidates the retarded relation between body selenium stores and pGPx activity. For rabbits no further results for the development of pGPx activity could be found in the literature but a nearly parallel course for pGPx activity during Se deficiency was observed in studies with rats (25, 29). The severe selenium deficiency in groups I and II was also confirmed by the significant decrease of GPx1 activity in the various rabbit organs. In liver, kidney, heart and musculus longissimus dorsi GPx1 activity in the initial status group and in the Se supplemented groups III and IV was in agreement with results for rabbits described in the literature (38). No data for GPx1 activity in the various organs of selenium deficient rabbits seem to be available in the literature. Lipid peroxidation was examined after the generation of hydroxyl radicals by adding Fe-(III)-ions to the incubation buffer of duodeneal epithelial cells which were incubated with Fe-(III)-ions and ascorbic acid for radical genesis (13). A similar strong protective effect of vitamin E against lipid peroxidation (92%) was also found for rabbits in homogenates of musculus longissimus dorsi after the animals were fed diets enriched with polyen fatty acids (23). In an experiment to study the differentiated action of selenium and vitamin E against lipid peroxidation, rats were fed a selenium and vitamin E deficient diet for 36 weeks (22). The diet of a second group was supplemented with 30 mg α-tocopherylacetate/kg diet. Both supplements were added to the diet of the positive control group. Without additional chemical provocation of radicals in the liver homogenates impact factors against lipid peroxidation of 5% (for selenium), 27% (for vitamin E) and 31% (for selenium + vitamin E) in comparison to the negative control group were achieved (22). In other studies (39, 16) a protective effect for selenium of 22% and for vitamin E of 91% was demonstrated in rat liver homogenates.

During cellular protein catabolism degradation of proteins is introduced by a variety of marker mechanisms. The most important posttranslational modifications include:

- The phosphorylation of serine and threonine residues
- The conjugation of the lysine-c amino-group with ubiquitin
- The oxidation of methionine residues to methionine sulfoxide
- The oxidation of histidine, arginine and proline residues by mixed function oxidation (11)

In the present study two assays were used to classify oxidative damage to cellular proteins:

1. The more general photometrical protein carbonyl assay (33). The most important disadvantage of this method is due to an additional recording of other carbonyl compounds from lipid and carbohydrate soiLings in the homogenates (6, 32).

2. The loss of glutamine synthetase activity represents a more sensitive assay to examine oxidative protein damage. Glutamine synthetase is a dodecamer enzyme with 16 histidine residues per subunit. The oxidation of only one histidine residue in the active centre of the enzyme with the hydrophile sequence Met-His-Cys-His-Met by mixed function oxidation leads to the loss of enzyme activity (28, 9).

In the case of both methods damage to the cellular protein fraction could be unequivocally detected in the livers of selenium and vitamin E deficient rabbits (group I). As for lipid peroxidation it is also difficult to find comparable data in the literature for the protective role of selenium and vitamin E against protein damage. In most cases data only exist for pure in vitro-systems. In the present study on protein carbonyl formation the two single dietary supplementations of vitamin E and of selenium resulted in an inhibition of protein oxidation of 50% as compared to the negative control group. Supplementation of both nutrients gave the most effective protection (62%). A two to three-fold induction of specific proteases in the heart muscle
and the musculus longissimus dorsi of vitamin E deficient rabbits (19) is in accordance with our results for glutamine synthetase activity in the liver and in the heart muscle. In vitro-studies a loss of glutamine synthetase activity of 40-95% could be demonstrated after incubation of the purified enzyme with different mixed function oxidases or iron-(III)-ions and ascobic acid (36, 28). In these studies damage to the glutamine synthetase protein could be inhibited nearly completely by the addition of metal chelators (e.g. EDTA), the substrate of the enzyme (glutamate) or cofactors of the reaction (Mg$^{2+}$, Mn$^{2+}$). The maintenance of a high glutamine synthetase activity in the liver and the heart muscle of groups II and IV points to an overall protection of liver and heart tissue by dietary vitamin E supplementation. Furthermore these results possibly give evidence for an inhibited lipid mediated protein oxidation in the vitamin E supplemented groups (6). The protective effect of selenium in the liver and heart muscle with regard to glutamine synthetase was not quite complete and therefore it can be hypothesized that the action of selenium seems to be mainly restricted to the cellular protein fraction. This hypothesis is additionally confirmed by the results for liver protein carbonyl content where the efficiency of selenium alone against protein oxidation was stronger in tendency than the efficiency of vitamin E alone.

In conclusion the results show that dietary selenium is necessary to maintain optimum activities of GPx1 in various rabbit tissues. Additionally an essential role of selenium in the prevention of lipid and protein oxidation could be clearly demonstrated in rabbits. This antioxidative effect of selenium is especially important during periods of vitamin E deficiency. Therefore dietary selenium supplementation seems to be suitable for rabbits in addition to vitamin E supply.

References

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