Phytosterols (PS) such as β-sitosterol (SitOL) are found at high concentrations in functional food designed to reduce serum cholesterol (ChOL) levels. Likewise, ChOL is oxidized chemically in food and probably also by biotransformation in vivo. The oxidation products of sterols have cytotoxic and probably genotoxic activities. Due to this adverse property, oxysterols are associated with the adverse effects of ChOL in the human body.

In order to investigate the effect of high dosage of PS in the diet on sterol, PS and oxo-ChOL levels in the body, levels of sterols and oxysterols were determined (i) in the livers of female guinea pigs fed different diets containing PS (SitOL and camposterol (CampOL) or 1/and ChOL for 2 weeks as well as (ii) in the diet. In addition the frequency of micronucleated erythrocytes (MNE) was determined as a marker for genotoxic stress. PS were used in different formulations in functional food, so we also investigated the influence of different particle size of PS on ChOL levels in the liver and on MNE rate.

**Methods**

**Genotoxicity in vivo**

Peripheral blood was fixed on a slide and stained with giemsa and may-greenwald dye. MNE were quantified microscopically.

**Isolation of the sterols and oxysterols from liver tissue and diet**

Sterols and oxysterols were extracted with lipophilic solvents, followed by cold saponification, purified and separated from each other by solid phase extraction (SPE), derivatized with N,O-bis(trimethylsilyl)-trifluoroacetamide+1% trimethylchlorosilane, separated by gas chromatography (GC) and detected by mass spectrometry (MS) using a SLB 5 MS column. The temperature was as following: 200°C for 2 min, 4°C/min heating up to 300°C for 22 min. Mass-selective detection (70 eV, electron impact) was performed in the scan mode. Sterols and oxysterols were identified by their characteristic fragmentation ions.

**Quantification**

Internal standards and calibration curves were used for quantification of sterols and oxysterols in the livers.

**Internal standards**

Oxysterols were quantified using deuterium labeled compounds which were obtained as follows:

ChOL labeled with 6 deuterium atoms (D6-ChOL) was heated at 160°C for 4 h. Then, oxidation products were separated by SPE.

The internal o xo-D6-ChOL standard mixture contained the relevant oxidation products, 7α-HO-D-ChOL (8.0±0.1 ng/100 μl), 7β-HO-D-ChOL (12.7±1.0 ng/100 μl), 7-keto-D-ChOL (47.9±1.9 ng/100 μl), 5α-epoxy-D-ChOL (20.3±0.9 ng/100 μl) and 5α-epoxy-D-ChOL (19.2±0.2 ng/100 μl).

ChOL was quantified using 40 μg 5α-cholestan-3β-ol and both SitOL and CampOL using 8.2 μg SitOL. Internal standards were added in the liver samples prior to isolation of (oxy)sterols.

**Calibration curves**

For calibration, known concentrations of commercially available reference compounds were analyzed by GC/MS together with the internal standards mentioned above. Absolute concentrations of oxo-SitOLs were estimated using calibration curves of the respective o xo-ChOLS.

**Results**

Analysis of diets confirmed equal ChOL concentrations in all high ChOL supplemented and equal PS concentrations in all high supplemented diets (Fig. 1).

**Hepatic sterol levels**

ChOL (1821–3963 μg/g liver), SitOL (15.3–53.5 μg/g liver) and CampOL (38.7–93.3 μg/g liver) were detected in the livers (Fig. 1, upper panel). High levels of ChOL in the diet increased ChOL levels in the group. As expected, in the groups +ChOL+PS: lower tissue levels of ChOL were observed. A ChOL-reduced effect of PS was observed.

High levels of PS in the diet increased PS-level in the liver, PS increase in the livers was less than ChOL reduction. Thus, PS have also a sterol-reduced effect.

**Hepatic oxysterol levels**

Moreover, in the livers of the guinea pigs not only oxy-ChOLs, but also 7-HO-SitOLs (0.6±0.4 μg/g liver, α±β-epoxy-ChOL (0.6±0.4 μg/g liver, depending on the diet, Fig. 1, low panel). The tissue levels of oxy-ChOLs were significantly increased in the groups +ChOL and +ChOL+PS. After addition of PS to ChOL in the diet, lower tissue levels of oxy-ChOLs but higher 7-HO-SitOL levels were observed. Due to the higher decrease in oxy-ChOL levels than the respective increase in 7-HO-SitOLs, PS led to the reduction of total oxysterol levels.

**Micronucleus test**

In the +ChOL and +ChOL+PS groups, a significantly increase of MNE was observed (Fig. 1, middle panel). After addition of PS to ChOL in the diet, the MNE rate was significantly reduced compared to the +ChOL group. The significant increase of the MNE rate in both groups, fed ChOL only, indicated that rather oxy-ChOLs than ChOL itself were associated with the induction of MNE.

**Influence of particle size**

Dietary uptake of oxysterols is reflected in their tissue levels. Oxidation products of ChOL may affect genetic stability and may thus have an impact on human health whereas PS seem to reduce not only hepatic ChOL but also oxo-ChOL levels.

**Summary**

Dietary intake of oxysterols is reflected in their tissue levels. Oxidation products of ChOL may affect genetic stability and may thus have an impact on human health whereas PS seem to reduce not only hepatic ChOL but also oxo-ChOL levels.