Research Article

Nanoscale and customary non-esterified sitosterols are equally enriched in different body compartments of the guinea pig

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The impact of sitosterol formulation particle size on the intestinal sterol absorption and the sterol status in various tissues in Dunkin Hartley guinea pigs was investigated. Three groups of animals (six each) were fed a basal diet ("control") or a basal diet containing either customary sitosterol ("customary", particle size: 10 000 – 90 000 nm) or nanoscale sitosterol ("nanoscale", particle size: 200 – 300 nm). The average daily sitosterol intake was 21 ± 7 mg (control), 154 ± 8 mg (customary), and 127 ± 18 mg (nanoscale) for 2 weeks. Sitosterol and cholesterol were analyzed in samples of plasma, blood cells, bile, liver, kidney, jejunal mucosa/serosa, cecum, colon and feces. Concentrations of sitosterol in all analyzed matrices increased significantly in the supplemented groups when compared to control group. No differences in the sitosterol concentrations in analyzed matrices occurred between nanoscale and customary group. The cholesterol concentrations in tissues remained unchanged. Fecal fatty acid and sterol distributions were modified during sitosterol intervention. Both particle sizes equally increased sitosterol levels in cholesterol-metabolizing compartments in the guinea pig. No differences in body compartment accumulation and intestinal absorption of the different sitosterol particle sizes were observed.

Keywords: Cholesterol / Guinea pig / Nanoscale particle / Sitosterol / Tissue

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1 Introduction

As a result of a high incidence of coronary heart diseases in Western countries, several risk factors like an elevated serum LDL cholesterol concentration, the oxidation of LDL, the lifestyle (smoking, exercise), and also increased plasma plant sterol concentrations are discussed [1, 2]. Plant sterols are of wide interest as food ingredients, because they present one out of many prospects to reduce serum LDL cholesterol concentrations since plant sterols and cholesterol interfere with each other regarding their transport, absorption and metabolism [3]. In healthy humans, plant sterols are poorly absorbed with an absorption rate between 0.5 and 15% of the ingested plant sterols [4, 5]. Based on a Western diet, typically consumed in industrial nations [6], the daily plant sterol intake is approximately 150 – 300 mg on average with sitosterol as the major plant sterol [7, 8]. The daily consumption of at least 1.3 g of plant sterol esters or 3.4 g of plant stanol esters in two meals in combination with a low-fat diet can reduce the risk of heart diseases according to the health claim § 101.83 of the U.S. Food and Drug Administration [9]. In contrast, the European Commission recommends avoiding intakes exceeding 3 g of plant sterols per day [10]. A simulation study emphasized that a replacement of three common foods with plant sterol-enriched products may lead to a daily consumption of more than 8.6 g plant sterols [11].

The importance of studies dealing with the plant sterol bioavailability can be demonstrated by the genetic disease phytosterolemia. Mutation in genes encoding ATP-binding cassette transporters (ABCG5 and ABCG8) for sterol discharging into the intestine results in an accumulation of plant sterols and cholesterol in plasma [12, 13]. Elevated plant sterol plasma concentrations in phytosterolemic patients may lead to changes in membrane fluidity, xanthomatosis, early arteriosclerosis and cardiovascular death [2,

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Abbreviations: FA, fatty acids; **FAME**, fatty acid methyl esters; **MUFA**, mono-unsaturated fatty acids; **PUFA**, poly-unsaturated fatty acids; **SFA**, saturated fatty acids

14, 15]. This potential raises the question about proatherogenic properties of plant sterols [16]. The serious consequences of this disease indicate that a large plant sterol accumulation in the body also needs to be prevented in healthy subjects.

The intestinal absorption of the plant sterols is possibly influenced by the manner in which the plant sterols are prepared and administered [17]. Some different absorption routes of nanoscale particles into the lymphatic/blood system are described. Possible plant sterol absorption mechanisms include the nanoscale particles entry via the M-cells of the Peyer's patches at the gut-associated lymphoid tissue, a transcellular pathway through the intestinal enterocytes and finally a paracellular entry along the tight junctions [18]. As a result of these mechanisms, an enhancement of absorption of the smaller nanoscale plant sterols may occur when compared to the customary plant sterols.

The objective of this study was to investigate the influence of nanoscale non-esterified sitosterol and customary non-esterified sitosterol on the sitosterol and cholesterol concentrations in cholesterol-metabolizing compartments of the guinea pig to evaluate possible effects regarding the intestinal absorbance and accumulation in the body. One of the main focus points of this study was the gut epithelium, which is a crucial site for sterol absorption by the body.

2 Materials and methods

2.1 Test substance

The customary, non-esterified plant sterol containing 83% sitosterol, 11% sitostanol, and 6% campesterol was purchased from Fluka (Munich, Germany). From this customary supply of plant sterol, the nanoscale plant sterol particles were generated by the rapid expansion of supercritical solution (RESS) process. Particle sizes were determined with the scanning electron micrograph pictures [19]. The nanoscale plant sterol composition was identical to the customary plant sterol supply. In order to prevent a subsequent agglutination, both sterol preparations were mixed with a powdered albuminous matrix. The particle size of the nanoscale formulation ranged between 200 – 300 nm, and that of the customary plant sterol ranged between 10 000 – 90 000 nm.

2.2 Study design

The Animal Welfare Commission of the Thuringian State Office of Food Safety and Consumer Protection approved this study. Eighteen female 5-week-old Dunkin Hartley guinea pigs were purchased from Harlan Winkelmann (Borchen, Germany) and were randomly divided into three study groups with six animals each. The three groups were fed different diets for 2 weeks; with a common basal diet ("control"), or with the basal diet containing either a customary sitosterol supplementation ("customary") or a nanoscale sitosterol supplementation ("nanoscale") (details of basal diet: nutrients: metabolizable energy: 10.5 MJ/kg, protein: 170 g/kg, fat: 30 g/kg, fiber: 130 g/kg, ash: 66 g/kg, nitrogen-free extract: 496 g/kg; fatty acids (g/kg): C16:0: 5.2, C18:0: 9, C18:1: 5.1, C18:2: 15.1, C18:3: 3.2; amino acids (g/kg): lysine: 10.8, methionine + cysteine: 6.9, threonine: 6.3, trytophane: 2.2, valine: 8.4, isoleucine: 7.0, leucine: 12.4, phenylalanine + tyrosine: 12.3; vitamins: Vitamin A: 15 000 IE/kg, Vitamin D_3: 1000 IE/kg, Vitamin E: 0.12 g/kg, Vitamin C: 2.4 g/kg; minerals and trace elements (g/kg): calcium: 10, phosphorus: 6, iron: 0.26, manganese: 0.07, zinc: 0.09, copper: 0.02). Nanoscale and customary sitosterols were incorporated into the common feed pellets (Ssniff, Soest, Germany) during fabrication of the customary and nanoscale diets. Water and feed were provided *ad libitum.* The animals were housed in single cages in an air-conditioned room with a light-dark cycle of 12 h. The animal's weight and their feed consumption were determined daily. After 2 weeks, the guinea pigs were anesthetized with gaseous carbon dioxide and killed by exsanguination after aorta puncture.

Based on the means and variances of the plasma LDL cholesterol concentrations reported by Ramjiganesh *et al.* [20] (guinea pigs, sitostanol and cholesterol feeding), the use of six animals per group, and the univariate analysis of variance (ANOVA), the power of the study amounted to 99.9% ($p < 0.05$).

2.3 Sample collection and preparation

Samples of liver, kidney, aorta blood, bile and segments of jejunum (mucosa and serosa), of cecum, and of colon were taken. Furthermore, the gut contents were collected and sampled for each intestinal segment. All tissues and organs were washed with isotonic sodium chloride solution (0.9% w/w) and were weighed. The total daily fecal excrement for each animal was collected quantitatively during the second study week, and was combined producing a sample feces pool of one week for each animal. Tissues, gut contents, and feces were lyophilized, homogenized and sampled. The bile of the six animals of each treatment group was pooled, because of the large variation in the volume of the gallbladder contents of the individual animals. The blood was centrifuged at $1700 \times g$ for 10 min at room temperature to separate plasma using tri-potassium ethylene diamine tetraacetate as anticoagulant. The residues were washed with isotonic sodium chloride solution (0.9% w/w) to obtain the blood cells. The samples were frozen at $-20^{\circ}C$ and stored until analysis.

2.4 Sterol analysis

The sterol analysis was performed as described previously [21]. Briefly, in duplicate 200 mg of lyophilized sample containing the 5α-cholestane (internal standard) was hydro-

lyzed with ethanolic sodium hydroxide (1 mol/L, 90% ethanol v/v) and the sterols were extracted with cyclohexane. The extract was concentrated and re-dissolved in 250 µL decane. The determination of the sterols was performed with a GC-FID 17A instrument (Shimadzu, Kyoto, Japan), equipped with a capillary column (Optima d3; 30-m length; 0.25-mm id; 0.25-µm film thickness; Macherey-Nagel, Dueren, Germany). Component identification was conducted by comparison of the retention times with those of the standards. All sterol standard substances were purchased from Sigma (Munich, Germany). Coprostanol, epicoprostanol, coprostanone, and cholestanol were summarized as cholesterol metabolites in feces. The limits of detection were 5.01 ppm (parts per million) for cholesterol and 4.21 ppm for sitosterol.

2.5 Fatty acid analysis

The fat content of feces was extracted following the method of Folch *et al*. [22]. An aliquot of 50 mg fecal fat was derivatized with sodium methoxide (Merck, Darmstadt, Germany) to form fatty acid methyl esters (FAME). FAME were purified by TLC. The FAME fraction was diluted in hexane and analyzed by GC-FID (GC-FID17A, Shimadzu) provided with a fused silica capillary column (DB 225 ms; 30 m, 0.25 mm, 0.25 µm; Agilent Technologies, Palo Alto, USA). The fatty acids (FA) were divided in saturated fatty acids (SFA; C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0, C25:0), monounsaturated fatty acids (MUFA; C15:1Δ10c, C16:1Δ7c, C18:1Δ9t, C18:1Δ9c, C18:1Δ11c, C20:1Δ11c, C22:1Δ13c, C24:1Δ15c), poly-unsaturated fatty acids (PUFA; C18:2Δ9t,12t, C18:2Δ9c,12c, C18:3Δ6c,9c,12c, C18:3Δ9c,12c,15c, CLA, C20:2Δ11c,14c, C20:4Δ5c, 8c,11c,14c) and iso/ante iso branched-chain fatty acids (iso/ante iso FA; C13:0i, C13:0ai; C14:0i, C15:0i, C15:0ai, C16:0i, C17:0i, C17:0ai, C18:0i).

2.6 Data handling and statistical methods

Chromatogram analysis was conducted employing LabSolutions Software Class 5000® and GCMSsolution® (Shimadzu). Power analysis was performed using software PASS2000® (NCSS, Kaysvillen, Utah, USA). Statistical analyses of the experimental results were accomplished utilizing SPSS® for Windows® (version 11.5.1, SPSS Inc., Chicago, Illinois, USA) with the general linear model using univariate ANOVA. Scheffe's test was used for post hoc comparisons of the means. The statistical test criteria of a probability level (p) less than 0.05 was necessary for sample concentration differences to be considered significant when group sample comparison was performed. The results are presented as mean ± SD.

3 Results

3.1 Animals

The weight increase of the animals was constant from the beginning (body weight control: 327 ± 14 g, customary: 340 ± 16 g, nanoscale: 338 ± 21 g) until the end (body weight control: 459 ± 40 g, customary: 467 ± 18 g, nanoscale: 451 ± 49 g) of the study. There were no differences in the daily feed intake of the various groups (Table 1). The mean feed intake of all animals was 35.6 ± 5.9 g/day during the study. Feed consumption per day rose parallel with weight increase. The daily sitosterol intake was significantly higher by sitosterol supplementation in the customary and nanoscale group animals when they were compared to the control animals. No cholesterol was detected in the diet. Furthermore, there were no differences in the mass of daily-excreted feces between the different groups. However, daily sitosterol and cholesterol excretions were significantly increased in the customary and nanoscale groups compared to the control group.

3.2 Gut content

The gut contents of the guinea pigs fed the supplemented diets (customary and nanoscale) demonstrated elevated concentrations of sitosterol in all intestinal segments when compared to the sitosterol concentrations in the guinea pigs fed the control diet (Table 2). Additionally, there was a reduction in the sitosterol concentration of the jejunal content in the nanoscale group when it was compared with the customary group. However, a general tendency for increasing sitosterol concentration in the gut contents during intestinal passage for both supplement groups was observed.

Table 1. Sitosterol intake and fecal excretion

	Control (n = 6)	Customary (n = 6)	Nanoscale (n = 6)
Feed intake, g/d	31.2 ± 10.7	39.6 ± 2.1	36.2 ± 5.0
Sitosterol intake, mg/d	21 ± 7	154 ± 8[a)]	127 ± 18[a)]
Feces, g/d	11.3 ± 1.4	12.5 ± 2.8	12.8 ± 3.2
Sitosterol excretion, mg/d	5.0 ± 0.8	80.9 ± 16.6[a)]	84.4 ± 16.4[a)]
Cholesterol excretion, mg/d	2.3 ± 0.4	3.3 ± 0.8[a)]	3.1 ± 0.4[a)]

a) Significantly different from control, $p < 0.05$ (univariate ANOVA, Scheffe's test).

Table 2. Sterol concentration in the different gut contents and in feces

		Sitosterol μmol/g dry weight	Cholesterol μmol/g dry weight
Jejunum	Control ($n = 6$)	1.93 ± 0.51	2.85 ± 1.06
	Customary ($n = 6$)	16.7 ± 8.0[a)]	2.10 ± 1.09
	Nanoscale ($n = 6$)	15.2 ± 4.5[a,b)]	1.97 ± 1.24
Cecum	Control ($n = 6$)	0.92 ± 0.24	0.47 ± 0.10
	Customary ($n = 6$)	17.8 ± 6.0[a)]	0.54 ± 0.05
	Nanoscale ($n = 6$)	18.8 ± 4.5[a)]	0.52 ± 0.05
Colon	Control ($n = 6$)	0.99 ± 0.29	0.83 ± 0.18
	Customary ($n = 6$)	20.8 ± 6.8[a)]	0.70 ± 0.03
	Nanoscale ($n = 6$)	17.9 ± 1.7[a)]	0.83 ± 0.31
Feces	Control ($n = 6$)	1.28 ± 0.29	0.52 ± 0.03
	Customary ($n = 6$)	20.0 ± 4.3[a)]	0.70 ± 0.05[a)]
	Nanoscale ($n = 6$)	20.7 ± 4.1[a)]	0.67 ± 0.05[a)]

a) Significantly different from control, $p < 0.05$ (univariate ANOVA, Scheffe's test).
b) Significantly different from customary, $p < 0.05$ (univariate ANOVA, Scheffe's test).

This concentrating effect is not clearly apparent in the control group. In contrast, the sitosterol concentration in gut contents of the control group was elevated in the jejunum compared to the sitosterol concentration in the cecum. These trends were also verifiable in the cholesterol concentrations of the different gut contents for all study groups. Furthermore, cholesterol concentrations of all gut contents for the supplemented groups were similar to those observed in the control group. In contrast, fecal cholesterol concentration was significantly increased in both intervention groups compared to the control group.

3.3 Gut tissue

The comparison of sitosterol concentrations in the gut tissue samples revealed no significant differences for the nanoscale and customary groups (Table 3). However, the sitosterol concentration in all gut tissue samples was significantly elevated by sitosterol supplementation compared to control group. Testing the jejunum separately as mucosa and serosa the mucosal sitosterol concentration was higher than the serosal sitosterol concentration for all groups. Furthermore, there was a distal decline in the sitosterol gut tissue concentration for each segment. No changes in cholesterol concentration of the gut tissues were observed during this intervention when compared to control group. It should be noticed that the cholesterol concentration in the tissues of the large intestine of the customary and nanoscale groups tended to be higher than in the control group.

3.4 Body tissues and blood

As a result of the sitosterol treatments, the plasma sitosterol concentrations were significantly increased for both supplemented groups, whereas the cholesterol concentrations were slightly, but not significantly, decreased (Table 4). The same sitosterol and cholesterol concentration conditions were observed in the blood cell and liver samples of the supplemented groups. In addition, a sitosterol enrichment was also observed in the kidney tissue samples of the supplemented groups but to a lesser extent than that detected in the liver samples. The relative sitosterol contents of the group-pooled bile increased from 8% of total sterols in the control group to 45% and 43% in the customary and nanoscale group, respectively (Fig. 1).

Table 3. Sterol concentration in gut tissues

		Sitosterol μmol/g dry weight	Cholesterol μmol/g dry weight
Mucosa	Control ($n = 6$)	2.34 ± 0.89	13.6 ± 3.1
	Customary ($n = 6$)	7.00 ± 3.29[a)]	13.9 ± 1.8
	Nanoscale ($n = 6$)	6.81 ± 2.54[a)]	14.4 ± 1.8
Serosa	Control ($n = 6$)	1.26 ± 0.48	25.6 ± 7.5
	Customary ($n = 6$)	2.80 ± 1.43[a)]	23.6 ± 2.8
	Nanoscale ($n = 6$)	2.83 ± 1.04[a)]	23.4 ± 2.3
Cecum	Control ($n = 6$)	0.29 ± 0.12	27.5 ± 7.7
	Customary ($n = 6$)	0.63 ± 0.17[a)]	34.4 ± 9.4
	Nanoscale ($n = 6$)	0.63 ± 0.12[a)]	31.8 ± 8.2
Colon	Control ($n = 6$)	0.19 ± 0.02	18.0 ± 8.0
	Customary ($n = 6$)	0.39 ± 0.07[a)]	24.5 ± 3.7
	Nanoscale ($n = 6$)	0.41 ± 0.17[a)]	25.1 ± 7.3

a) Significantly different from control, $p < 0.05$ (univariate ANOVA, Scheffe's test).

Table 4. Sterol concentration in body tissues

		Sitosterol	Cholesterol
		μmol/L	mmol/L
Plasma	Control ($n = 6$)	32.4 ± 3.6	1.87 ± 0.34
	Customary ($n = 6$)	55.8 ± 25.1[a)]	1.71 ± 0.80
	Nanoscale ($n = 6$)	58.9 ± 9.4[a)]	1.71 ± 0.21
Blood cells	Control ($n = 6$)	38.2 ± 8.0	2.64 ± 0.44
	Customary ($n = 6$)	69.1 ± 18.8[a)]	2.41 ± 0.36
	Nanoscale ($n = 6$)	71.3 ± 11.6[a)]	2.33 ± 0.31
		μmol/g dry weight	μmol/g dry weight
Liver	Control ($n = 6$)	0.22 ± 0.02	16.0 ± 3.5
	Customary ($n = 6$)	0.40 ± 0.08[a)]	14.7 ± 1.6
	Nanoscale ($n = 6$)	0.45 ± 0.08[a)]	14.5 ± 1.7
Kidney	Control ($n = 6$)	0.25 ± 0.07	42.5 ± 13.7
	Customary ($n = 6$)	0.36 ± 0.08[a)]	42.0 ± 11.4
	Nanoscale ($n = 6$)	0.33 ± 0.06[a)]	42.4 ± 9.1

a) Significantly different from control, $p < 0.05$ (univariate ANOVA, Scheffe's test).

3.5 Fecal lipids

The supplemented groups demonstrated similar FA distributions in the feces of the guinea pigs when compared to the control group, but increased SFA levels in the customary

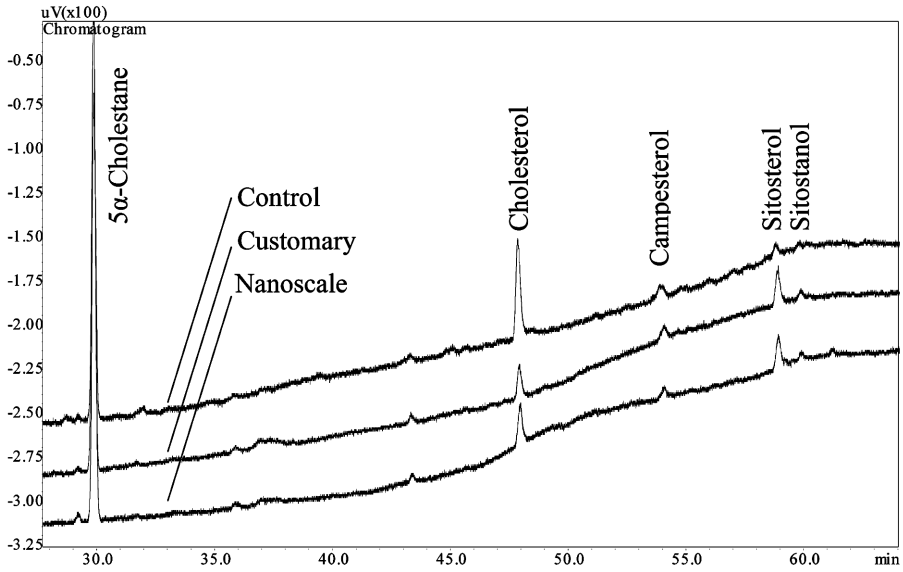

Figure 1. Chromatograms of the biliary sterols.

Table 5. Fecal lipid excretion

	Control ($n = 6$)	Customary ($n = 6$)	Nanoscale ($n = 6$)
Fatty acid distribution,% of total FAME			
SFA	42.2 ± 2.0	45.9 ± 3.7[a)]	43.1 ± 0.9
MUFA	15.5 ± 1.1	15.7 ± 1.3	15.5 ± 1.5
PUFA	20.6 ± 3.7	19.8 ± 4.5	21.8 ± 1.5
Iso/ante iso FA	21.2 ± 2.4	18.1 ± 2.5	18.9 ± 1.8
Cholesterol metabolites, μmol/g dry weight			
Cholesterol + metabolites[b)]	1.92 ± 0.12	1.78 ± 0.19	1.94 ± 0.23
Metabolites	1.40 ± 0.10	1.08 ± 0.18[a)]	1.27 ± 0.19
Cholesterol conversion,%	73 ± 1	60 ± 5[a)]	65 ± 3[a)]

a) Significantly different from control, $p < 0.05$ (univariate ANOVA, Scheffe's test).
b) Metabolites: sum of coprostanol, epicoprostanol, coprostanone, cholestanol.

group were observed (Table 5). Yet, a slight decrease in the intestinal iso/ante iso FA concentrations, which is usually synthesized by gut flora, occurred when the supplemented groups were compared to the control group. The total excretion of the cholesterol-based sterols that include cholesterol plus its bacterial metabolites formed similar overall concentrations when all study groups were compared. However, the conversion of cholesterol into its metabolites was diminished in the supplemented groups compared to the control group. This trend was supported by a lower fecal cholesterol metabolites concentration (Table 5) and a heightened fecal cholesterol concentration (Table 2) in the supplemented groups compared to the control group.

4 Discussion

With respect to the cholesterol metabolism, the guinea pig is highly comparable to human unlike other laboratory animals (enzyme equipment, hepatic cholesterol and lipoprotein metabolisms) [23]. Regardless of the dietary treatment, all guinea pigs had comparable weight gain. In all of the tissues and gut contents sampled, an enrichment of sitosterol was observed by the sitosterol application. In addition, there were only slight differences in the amounts of cholesterol in the investigated tissues and gut contents of the supplemented groups compared to the control group. No significant differences in sitosterol accumulation and absorption were found regarding to the particle size of the plant sterols.

During the absorption, sitosterol is luminal embedded in mixed micelles. After transport into the enterocyte, the majority of the plant sterols will be discharged back into the lumen by ATP-binding cassette transporters ABCG5 and ABCG8 [24]. By nanoscaling of the plant sterol particles additional trans- and paracellular absorption pathways are possible [18]. These specific absorption routes could lead to a higher intestinal permeability for the smaller sized plant sterol particles that would finally contribute to an increased accumulation of plant sterols in the body. However, in our study neither differences in the sitosterol con-

centrations of intestinal mucosa and serosa nor discrepancies in the sitosterol accumulation in liver tissue or blood cells between the nanoscale and customary group of the guinea pigs were demonstrated.

In several investigations, sitosterol has often been utilized to decrease plasma cholesterol concentrations [25]. In contrast, the findings of this study demonstrated only a slight diminution of the plasma cholesterol concentration by sitosterol intervention. The minute decrease of plasma cholesterol concentrations following the intervention could result from the application of a cholesterol-free diet. In different guinea pig studies showing the hypocholesterolemic effect of plant sterols, the diet was additionally supplemented with cholesterol to enhance the cholesterol plasma baseline level [20, 26]. It was also shown, if the dietary intake of cholesterol is extended, an abundant reduction in plasma cholesterol concentration occurred by a plant sterol-supplemented diet [27]. Further factors to consider for the almost unchanged plasma cholesterol concentration might be the age of animals utilized in this investigation (5 weeks old at the start of the study) and the duration of the study. The effects of aging on intestinal absorption and related metabolic processes are largely unknown. Age-related changes could affect the efficiency or process involved in the cholesterol interference mechanisms of sitosterol supplementation in both animal and human. In a meta-analysis of different plant sterol studies an age-dependent reduction of LDL cholesterol was found in human subjects. The serum LDL cholesterol reduction appeared most intensively in older adults (50–59 years old) [28]. This change in cholesterol metabolism might be a result of an aging effect. The short-time design of this study (2 weeks) might be also a reason for the non-hypocholesterolemic effect of the supplemented sitosterol in opposite to other investigations showing a hypocholesterolemic effect after 4 weeks [20, 26]. However, the accumulation effect was evaluated after a 2-week sitosterol supplementation, because the sitosterol interaction with cholesterol starts with the first administration of sitosterol.

In the liver and blood cells of the guinea pigs an enrichment of sitosterol resulted from the sitosterol intervention and was accompanied by a slight decrease in the cholesterol concentrations in these tissues. The secretion of sitosterol via bile was increased, while cholesterol secretion was reduced, contrary to the findings of Miettinen *et al*. [29]. The decreased biliary cholesterol efflux was supported by the slightly reduced cholesterol concentration in the jejunum contents observed in the sitosterol supplemented groups. Interestingly, by supplementation the reduction in cholesterol secretion via bile and in the cholesterol concentration of the jejunal contents did not alter the cholesterol concentration in the jejunal mucosa, where the main absorption of cholesterol takes place. The jejunal sitosterol concentration was significantly increased by about 200% in the mucosa and by approximately 100% in the serosa when compared to the control group. Unabsorbed and via ABCG5 and ABCG8 re-transported sitosterol might cause the increased sitosterol concentrations in all gut tissues and their contents.

Generally, the appearance of sitosterol in tissues is affiliated with the appearance of cholesterol. Therefore, the gut bacteria metabolism may also be affected by high sitosterol doses. A change in enzyme activities of the gut flora could result. Amongst others, the enzymes of the gut flora metabolize non-branched FA into iso/ante iso FA, unsaturated FA into SFA, and form coprostanol out of cholesterol [30–32]. Some results of this study indicated a change in gut enzyme activities during sitosterol supplementation. Thus, the fecal portion of SFA was raised in feces of the group receiving customary supplement of sitosterol and the amounts of iso/ante iso FA tended to decrease. The fecal cholesterol concentration was surprisingly increased by sitosterol intervention in spite of unchanged cholesterol conditions in the colon contents. The analysis of the fecal cholesterol metabolites showed a reduction in the conversion of cholesterol reflected by a decrease in fecal cholesterol metabolites concentrations. Therefore, the total excretion of cholesterol including its metabolites remained unchanged, but the distribution of the sterols was affected during sitosterol intervention.

In summary, the oral application of nanoscale and customary sitosterol particles induced an increase of the sitosterol concentration in cholesterol-metabolizing compartments in the guinea pig. No differences in the absorption or accumulation of nanoscale sitosterol were observed when it was compared with customary sitosterol. Both forms of sitosterol may influence the enzyme activities of gut bacteria.

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