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ORIGINAL CONTRIBUTION

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Supplementation of vitamins C and E increases the vitamin E status but does not prevent the formation of oxysterols in the liver of guinea pigs fed an oxidised fat

Summary *Background* Dietary oxidised fats are a source of oxidative stress. They cause deleterious effects in animal organism by lowering the antioxidant status of tissues and enhancement of the formation of lipid oxidation products. The vitamins E and C might be useful to prevent the formation of oxidation products by dietary oxidised fats. *Aim of the study* The purpose of this study was to investigate whether or not supplementation of diets with vitamins E and C is able to prevent oxidative stress and the formation of lipid oxidation products caused by dietary oxidised fats. Among lipid oxidation products, oxysterols should be particularly considered because of their high pathophysiological effects. *Methods* Male guinea pigs were divided into five groups. Four groups were fed diets with an oxidised fat supplemented with 35 or 175 mg α-tocopherol equivalents/kg and 300 or 1000 mg of vitamin C/kg for 29 days. One group, used as a control, was fed the same basal diet with fresh fat with 35 mg α-tocopherol equivalents/kg and 300 mg of vitamin C/kg. *Results* The guinea pigs fed the oxidised fat diet with 35 mg α-tocopherol equivalents/kg and 300 mg vitamin C/kg had significantly lower concentrations of tocopherols in various tissues, higher concentrations of various oxysterols and thiobarbituric acid-reactive substances in the liver, higher concentrations of glutathione in the liver and lower concentrations of glutathione in erythrocytes than the control animals fed the fresh fat. Increasing the dietary vitamin E concentration from 35 to 175 mg α-tocopherol equivalents/kg and/or the dietary vitamin C concentration from 300 to 1000 mg/kg increased tissue tocopherol concentrations in guinea pigs fed the oxidised fat but did not influence concentrations of oxidation products in the liver and glutathione concentrations in liver and erythrocytes. *Conclusions* The results demonstrated that supplementation of vitamins E and C improves the vitamin E status but does not prevent the formation of lipid oxidation products in the liver of guinea pigs fed oxidised fats.

Key words guinea pigs – oxidised fat – vitamin E – vitamin C – lipid oxidation products – antioxidant status

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Introduction

In developed countries, fried foods are an important source of fat. Oxidised fats as components of heated and fried foods affect the metabolism in several ways and play an important role in the development of various diseases [1]. The pathophysiologic effects of dietary oxidised fats are mainly due to deleterious effects of the adducts or oxidised products present in the oil. Part of the primary and secondary lipid peroxidation products which are formed during heating of oils are absorbed; many of them promote the generation of free radicals and cause oxidative stress in tissues [2]. The vitamins E and C, major antioxidants both in foods and in the body, have been shown to reduce the deleterious effects of oxidised fats in animals due to the reduction of oxidative stress [3–5]. Vitamin C is able to regenerate the tocopheryl radical which has been produced by the reaction of tocopherols with lipid peroxides [6]. The interaction

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between both vitamins under conditions of oxidative stress has not yet been investigated. Therefore, we conducted an experiment with growing guinea pigs fed oxidised fat diets with nutritionally adequate or excess concentrations of vitamins E and C. In most of the studies dealing with oxidised fats in animals, the generation of TBARS is a preferred method for estimation of lipid peroxidation. But besides polyunsaturated fatty acids (PUFA), cholesterol may also undergo oxidative modifications in the course of lipid peroxidation. Concentrations of oxysterols derived from non-enzymatic reactions such as 7β-hydroxycholesterol, 7-ketocholesterol or cholestan-3β, 5α, 6β-triol are highly predictive markers of in vivo lipid peroxidation [7, 8]. We also planned to determine the concentrations of oxidised (GSSG) and reduced (GSH) glutathione and the activities of antioxidative enzymes in liver and erythrocytes because these parameters also react sensitively to oxidative stress [9]. As model animals, guinea pigs were used which are – like humans – not able to synthesise ascorbic acid. The study, therefore, should be considered as a model study to assess potential beneficial effects of supplementation of vitamins E and C in dietary regimes containing oxidised fats.

Materials and methods

Animals and diets

Fifty male weanling guinea pigs with an average initial body weight of 218 (±9 g, SD), obtained from Charles River GmbH (Sulzfeld, Germany), were randomly assigned to one of five groups of ten each. They were housed individually in Macrolon cages in a room controlled for temperature (22±2 °C), humidity and light (12 hour light: 12 hour dark cycle). All animal procedures described followed established guidelines for the care and handling of laboratory animals and were approved by the regional council of Saxony-Anhalt.

Five diets were used which were based on a semisynthetic diet which consisted of (in g/kg): casein [300], sucrose [241], corn starch [160], cellulose [130], fat [100], mineral mixture [40], vitamin mixture [20], L-arginine [9]. Minerals and vitamins, with the exception of vitamins E and C, were supplemented according to recommendations for guinea pig diets [10]. One of the diets (diet 1) contained fresh fat, with a vitamin E concentration of 35 mg α-tocopherol equivalents/kg and a vitamin C concentration of 300 mg/kg. The other four diets (diets 2, 3, 4, 5) contained an oxidised fat (see "Preparation of the test fats") and varying concentrations of vitamin E (35 or 175 mg α-tocopherol equivalents/kg) and vitamin C (300 or 1000 mg/kg). Based on the native concentrations of the fats, diets were supplemented individually with all-rac-α-tocopheryl acetate (the biopotency of all-rac-α-tocopheryl acetate is considered to be 67 % of that of α-tocopherol).

The diets were prepared by mixing the dry components with fat and water and subsequent freeze drying. Afterwards the diets were stored at –20 °C. Diets were administered in restricted amounts to standardise the food intake. Feeding took place once daily at 08.00 h. Water was freely available from nipple drinkers. The diets were fed for 29 d.

Preparation of the test fats

The oxidised fat was prepared by heating a mixture of sunflower oil and lard (1:1, w/w) at a temperature of 55 °C for a period of 49 d. 5 kg of sunflower oil (Buttela, Brökelmann & Co., Hamm, Germany) and 5 kg of lard (Laru, Langensiepen & Ruckebier, Bottrop, Germany) were filled into a quartz glass beaker which was placed into a drying oven set at the intended temperature. Throughout the heating process, air was continuously bubbled through the fats at a flow rate of 650 ml/min. The resulting oxidised fat was stored at –20° C until the test diets were prepared. The heat treatment of the fat mixture caused a loss of PUFA. To equalize the fatty acid composition of fresh and oxidised fats, the fresh fat was composed of sunflower oil and lard at a ratio of 19:81 (w/w).

Before inclusion into the diets, the fats were analysed for fatty acid composition (see "lipid analysis"), peroxide value (POV) [11], concentration of carbonyls [12] and percentage of polar compounds [13]. To assess the concentrations of lipid peroxidation products of the fats after they have been included into the diets, the fats were extracted from aliquots of the diets with a mixture of hexane and isopropanol (3:2, v/v) and analysed for peroxide values, the concentrations of conjugated dienes [14] and TBARS [15].

Sample collection

In week 5 of the feeding period, faeces of each animal were collected individually over a period of seven days for the determination of the tocopherol absorption rate. After completion of the feeding periods the guinea pigs were starved for 12 h and killed by decapitation under light anaesthesia with diethyl ether. Blood was collected into heparinised polyethylene tubes (Sarstedt, Nümbrecht, Germany). Erythrocytes were obtained by centrifugation (1,100 g, 10 min) and washed three times with sodium chloride solution (145 mmol/L) by centrifugation (1,100 g, 10 min). Erythrocyte cytosol was prepared by centrifugation (1,100 g, 10 min) of the frozen erythrocytes. Low density-lipoproteins (LDL) were isolated from plasma by sequential ultracentrifu-

gation (900,000 g, 1.5 h) [16]. Liver and abdominal adipose tissue from mesenteric depots were excised immediately and frozen in liquid nitrogen. Liver samples were stored at –80 °C pending analysis; adipose tissue, plasma, LDL, erythrocytes and faeces samples were stored at –20 °C.

Analyses

Concentrations of α-tocopherol in liver, plasma, LDL, adipose tissue, erythrocytes and dried faeces samples were determined by HPLC [17]. The concentrations of α-tocopherol in liver, plasma and LDL were expressed per mol of lipids (triacylglycerols + total cholesterol). The absorption of vitamin E was calculated as 100 × (moles of α-tocopherol consumed within one week – moles of α-tocopherols excreted by faeces within one week)/moles of α-tocopherol consumed within one week.

Activities of antioxidant enzymes and concentrations of glutathione were determined in liver homogenate and in haemolysate. Catalase (EC 1.11.1.6) activity was determined at 25 °C using hydrogen peroxide as substrate [18]. Total superoxide dismutase (SOD; EC 1.1.1.15) activity was determined with pyrogallol as the substrate [19]. Glutathione peroxidase (GSH-Px; EC 1.11.1.9) was determined with t-butyl hydroperoxide as substrate [20]. Glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) activity was determined according to [21]. Concentrations of GSH and GSSG were determined in protein-free liver homogenates [22]. Ascorbic acid in plasma was determined by HPLC [23].

TBARS were measured in the liver according to a standard assay [24]. To determine the concentrations of oxysterols in the liver, liver lipids were extracted with a mixture of n-hexane and isopropanol (3:2, v/v). Oxysterols from lipid extracts and dietary fats were derivatised with bis-(trimethyl-silyl)-trifluoroacetamide (BSTFA, Sigma-Aldrich, Steinheim, Germany) and pyridine by heating samples at 60 °C for 60 min. Trimethylsilyl ethers were separated and quantified by gas chromatography/mass spectrometry in selected ion monitoring mode as described recently [24].

The fatty acid composition of the dietary fats was determined by gas chromatography [25]. To determine the concentrations of triacylglycerols and total cholesterol in the liver, an aliquot of the lipid extract was dried and the lipids were dissolved in a small volume of Triton X-100 [24]. Concentrations of triacylglycerols and total cholesterol in liver lipid extract, plasma and LDL were determined using enzymatic reagent kits (Merck, Darmstadt, Germany).

Statistics

To evaluate the effect of the diet, data were subjected to ONEWAY with diet as a treatment factor. For statistical significant F values, individual means were compared by Fisher's multiple range test. To evaluate the effect of dietary vitamin E and vitamin C concentrations, data within the four groups fed the oxidised fat were additionally subjected to ANOVA with vitamin E and vitamin C and their interactions as factor levels. Treatment effects were considered significantly different for $P < 0.05$.

Results

Characterisation of the experimental fats

The fatty acid composition of the fresh fat and the oxidised fat was similar (Table 1). The fresh fat had a higher concentration of total cholesterol than the oxidised fat. In the oxidised fat, approximately 4mol/100mol of total cholesterol was oxidised; 7β-hydroxycholesterol and 7-ketocholesterol were the main oxidation products. In the fresh fat, the concentrations of all oxysterols were below the detection limit. Before inclusion into the diet, the POV of the oxidised fat was 200 times higher than that of the fresh fat; the concentrations of total carbonyls was

Table 1 Characteristics of the experimental fats

	Fresh fat (Sunflower oil/lard, 19:81)	Oxidised fat (Sunflower oil/lard, 1:1)[1]
Major fatty acids, g/100 g fatty acids		
Myristic acid (14:0)	1.3	1.0
Palmitic acid (16:0)	22.5	20.9
Stearic acid (18:0)	13.8	12.8
Oleic acid (18:1)	35.5	36.4
Linoleic acid (18:2 n-6)	20.6	20.2
Sterols, µmol/kg		
Total cholesterol	1800	1120
7β-hydroxycholesterol	< 0.1	20
7-ketocholesterol	< 0.1	24
cholestan-3β, 5α, 6β-triol	< 0.1	0.5
Peroxidation products		
Before inclusion in the diet		
Peroxide value, mEq O_2/kg	3.2	666
Carbonyls, mmol/kg	< 0.1	479
Polar compounds, %	3.2	53
After inclusion in the diet		
Peroxide value, mEq O_2/kg	23	1533
Conjugate dienes, mmol/kg	10	196
TBARS,[2] mmol/kg	3.6	14

[1] Prepared by heating at a temperature of 55 °C for 49 days
[2] TBARS, thiobarbituric acid-reactive substances

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even more than 5000 times higher in the oxidised fat than in the fresh fat. The percentage of total polar compounds of the oxidised fat was 18 times higher than that of the fresh fat. Inclusion of the fats into the diet increased the POV in both types of fat. After inclusion in the diet, the POV was 67 times higher in the oxidised fat than in the fresh fat; concentrations of conjugated dienes and TBARS were 20 and 4 times higher, respectively in the oxidised fat than in the fresh fat.

Diet and nutrient intake and body weights of the animals

Within the groups fed the oxidised fat diet, eight animals (two animals of the group fed diet 2, four animals of the group fed diet 3, two animals of the group fed diet 4) did not completely consume the diet administered. These animals were removed from the experiment. The diet consumption of all the animals that stayed in the experiment was identical, being 14.0 g per day. The calculated intake of physiological energy (218 kJ/d), protein (4.2 g/d) and fat (1.4 g/d) was identical in all treatment groups; animals of groups 2 to 5 ingested less cholesterol (1.4 vs. 2.3 µmoles/d) but more peroxides (1.94 vs. 0.03 µmoles/d), 7β-hydroxycholesterol (28 vs. < 0.1 nmoles/d), 7-ketocholesterol (34 vs. < 0.1 nmoles/d) and cholestan-3ß,5α,6ß-triol (0.7 vs. < 0.1 nmoles/d) than animals of group 1. The daily intake of vitamin E was 49 µg in animals of groups 1, 2, 3 and 245 µg in animals of groups 4, 5. The daily intake of vitamin C was 420 µg in animals of groups 1, 2, 4 and 1400 µg in animals of groups 3, 5. Body weight gains and final body weight gains of the guinea pigs did not differ between the five groups: body weight gains during the feeding period were 33 ± 13 g, and final body weights were 245 ± 36 g (means ± SD, n = 42).

Tissue α-tocopherol concentrations

Guinea pigs fed the oxidised fat diet with 35 mg α-tocopherol equivalents and 300 mg vitamin C/kg (diet 2) had lower α-tocopherol concentrations in liver, plasma, LDL, adipose tissue and erythrocytes than guinea pigs fed the fresh fat diet with equivalent vitamin E and vitamin C concentrations (diet 1) (Table 2). Within the groups fed the oxidised fat diets, the dietary vitamin E concentration influenced the α-tocopherol concentrations in all tissues analysed. Guinea pigs fed the oxidised fat diets with 175 mg α-tocopherol equivalents/kg (diets 4, 5) had 3 to 4 times higher α-tocopherol concentrations in liver,

Table 2 Concentrations of α-tocopherol, glutathione, ascorbic acid, thiobarbituric acid-reactive substances (TBARS) and some oxysterols in tissues or plasma of guinea pigs fed a diet with fresh fat or diets with oxidised fat containing 35 or 175 mg α-tocopherol equivalents/kg and 300 or 1000 mg vitamin C/kg for 29 days

Diet	1	2	3	4	5	ANOVA Vitamin		
Fat	Fresh	Oxidised	Oxidised	Oxidised	Oxidised			
Vitamin E (mg α-Toc/kg)	35	35	35	175	175	E	C	E x C
Vitamin C (mg/kg)	300	300	1000	300	1000			
	$n = 10$	$n = 8$	$n = 6$	$n = 8$	$n = 10$			
α-tocopherol								
Liver, mmol/mol lipids	2.10 ± 0.68^{b}	0.77 ± 0.32^{d}	1.06 ± 0.48^{c}	2.63 ± 0.69^{b}	4.42 ± 1.48^{a}	< 0.001	< 0.001	< 0.05
Plasma, mmol/mol lipids	3.60 ± 0.76^{c}	1.55 ± 0.32^{d}	1.84 ± 0.54^{d}	5.07 ± 0.98^{b}	6.60 ± 1.40^{a}	< 0.001	< 0.001	NS
LDL, mmol/mol lipids	4.73 ± 1.58^{b}	2.31 ± 0.56^{c}	2.94 ± 0.93^{bc}	7.18 ± 0.89^{a}	7.80 ± 2.40^{a}	< 0.001	NS	NS
Adipose tissue, nmol/g	28.8 ± 8.1^{b}	16.1 ± 8.0^{c}	16.2 ± 9.3^{c}	37.3 ± 9.6^{b}	82.1 ± 10.4^{a}	< 0.001	< 0.001	< 0.001
Erythrocytes, µmol/g	8.60 ± 5.25^{b}	1.67 ± 1.25^{c}	3.91 ± 3.51^{bc}	7.01 ± 3.13^{bc}	14.5 ± 10.0^{a}	< 0.001	< 0.05	NS
Ascorbic acid								
Plasma, µmol/L	9.93 ± 5.72	6.97 ± 5.79	9.22 ± 3.68	12.7 ± 8.6	15.0 ± 5.2	< 0.05	NS	NS
Glutathione, liver, µmol/g								
GSH	2.39 ± 0.53^{b}	3.91 ± 0.53^{a}	3.91 ± 0.62^{a}	4.13 ± 1.03^{a}	4.41 ± 0.59^{a}	NS	NS	NS
GSSG	0.22 ± 0.14^{b}	0.54 ± 0.18^{a}	0.46 ± 0.15^{a}	0.47 ± 0.11^{a}	0.59 ± 0.18^{a}	NS	NS	NS
Glutathione, erythrocytes, µmol/g								
GSH	1.98 ± 0.58^{a}	1.18 ± 0.48^{b}	0.97 ± 0.43^{b}	1.27 ± 0.44^{b}	0.93 ± 0.39^{b}	NS	NS	NS
GSSG	0.09 ± 0.03^{a}	0.04 ± 0.02^{b}	0.05 ± 0.03^{b}	0.06 ± 0.02^{ab}	0.04 ± 0.02^{b}	NS	NS	NS
Oxidation products, liver, mmol/mol lipids								
TBARS	7.4 ± 3.8^{b}	16.4 ± 7.5^{a}	12.1 ± 5.3^{ab}	13.4 ± 2.5^{a}	12.7 ± 3.7^{a}	NS	NS	NS
7β-hydroxycholesterol	76 ± 16^{b}	113 ± 15^{a}	90 ± 22^{ab}	134 ± 75^{a}	127 ± 61^{a}	NS	NS	NS
7-ketocholesterol	68 ± 22^{b}	128 ± 31^{ab}	106 ± 48^{ab}	104 ± 8^{ab}	161 ± 103^{a}	NS	NS	NS
Cholestantriol	58 ± 19^{b}	123 ± 18^{a}	134 ± 11^{a}	108 ± 49^{ab}	128 ± 50^{a}	NS	NS	NS

Values are means ± SD; Means sharing the same letter do not differ significantly; ANOVA has been performed within oxidised fat level; *NS* nonsignificant ($P < 0.05$)

plasma, LDL, adipose tissue and erythrocytes than guinea pigs fed the oxidised fat diet with 35 mg α-tocopherol equivalents/kg (diets 2, 3). The dietary vitamin C concentration influenced the concentrations of α-tocopherols in liver, plasma, adipose tissue and erythrocytes. Guinea pigs fed the oxidised fat diets with 1000 mg vitamin C/kg (diets 3, 5) had 1.6, 1.3, 1.8, and 2.1 times higher α-tocopherol concentrations in liver, plasma, adipose tissue and erythrocytes than guinea pigs fed the oxidised fat diet with 300 mg vitamin C/kg (diets 2, 4). The tocopherol concentration of LDL was not influenced by the dietary vitamin C concentration.

The absorption of α-tocopherol from the diet was not different between the five treatment groups. In the five treatment groups, it was 83 ± 4 % on average.

Concentration of ascorbic acid in plasma

The plasma ascorbic acid concentration of guinea pigs fed the oxidised fat diet with 35 mg α-tocopherol equivalents and 300 mg vitamin C/kg was 30 % lower than that of the guinea pigs fed the fresh fat diet with equivalent vitamin E and vitamin C concentrations (Table 2). The difference, however, was not statistically significant due to large standard deviations. Plasma ascorbic acid concentrations within the groups fed the oxidised fat diets were significantly influenced by the dietary vitamin E concentration and in tendency ($P < 0.10$) by the dietary vitamin C concentration. Guinea pigs fed the oxidised fat diets with 175 mg α-tocopherol equivalents/kg (diets 4, 5) had 1.7 times higher plasma ascorbic acid concentrations than guinea pigs fed the oxidised fat diet with 35 mg α-tocopherol equivalents/kg (diets 2, 3). Guinea pigs fed the oxidised fat diets with 1000 mg vitamin C/kg (diets 3, 5) had 1.2 times higher plasma ascorbic acid concentrations than guinea pigs fed the oxidised fat diet with 300 mg vitamin C/kg (diets 2, 4).

Concentrations of GSH and GSSG in liver and erythrocytes

Guinea pigs fed the oxidised fat diet with 35 mg α-tocopherol equivalents and 300 mg vitamin C/kg (diet 2) had higher concentrations of GSH and GSSG in the liver than guinea pigs fed the fresh fat diet with equivalent vitamin E and vitamin C concentrations (diet 1) (Table 2). Within the groups fed the oxidised fat diets, neither the dietary vitamin E concentration nor the dietary vitamin C influenced the concentration of GSH and GSSG in the liver. In erythrocytes, the concentrations of GSH and GSSG were lower in guinea pigs fed the oxidised fat diet with 35 mg α-tocopherol equivalents and 300 mg vitamin C/kg than in guinea pigs fed the fresh fat diet with equivalent vitamin E and vitamin C concentrations (diet 1). Within the groups fed the oxidised fat diets, the concentrations of GSH and GSSG in erythrocytes were also not influenced by the dietary vitamin E and vitamin C concentration.

Activities of antioxidant enzymes in liver and erythrocytes

The activities of SOD and GSH-Px in the liver were not different between the five groups (data not shown). The activity of catalase in the liver was significantly higher in the rats fed the oxidised fat than in the rats fed the fresh fat. Within the groups fed the oxidised fat diets, the activity of this enzyme was not influenced by dietary vitamin E and vitamin C concentrations. In rats fed the fresh fat (diet 1), the activity of catalase was 7.2 ± 1.7 μkat/g protein (mean ± SD, n = 10); in the rats fed the oxidised fat diets (diets 2 to 5) it was 12.5 ± 2.3 μkat/g protein (mean ± SD, n = 32), on average for the four groups.

The activities of SOD, catalase and G6PDH in erythrocytes were not different between the five groups (data not shown).

Concentrations of oxidation products in the liver

Guinea pigs fed the oxidised fat diet with 35 mg α-tocopherol equivalents and 300 mg vitamin C/kg (diet 2) had higher concentrations of TBARS and oxysterols (7β-hydroxycholesterol, 7-ketocholesterol, cholestan-3β, 5α, 6β-triol) in the liver than guinea pigs fed the fresh fat diet with equivalent vitamin E and vitamin C concentrations (diet 1) (Table 2). Within the guinea pigs fed the oxidised fat diets, the concentrations of these oxidation products were not influenced by the dietary vitamin E and the dietary vitamin C concentration.

Discussion

The comparison of the group fed the fresh fat diet (diet 1) and the group fed the oxidised fat with equivalent vitamin E and vitamin C concentration shows that the oxidised fat used in this study lowered the vitamin E status of the animals. Because the absorption of vitamin E was not affected by dietary oxidised fats, strongly reduced α-tocopherol concentrations in tissues of guinea pigs fed the oxidised fat might be predominately due to an enhanced vitamin E turnover caused by dietary lipid peroxidation products [25]. As expected, supplementation of the diets with vitamin E increased the tocopherol concentrations in tissues of guinea pigs fed an oxidised fat. The observation that vitamin C also increased the tocopherol concentration in tissues suggests that vitamin C is able to spare vitamin E under

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conditions of oxidative stress. Moreover, this study shows that supplemental vitamin E increases plasma concentrations of ascorbic acid in guinea pigs fed an oxidised fat. This suggests that vitamin E is also able to spare ascorbic acid under conditions of oxidative stress.

Most of 7β-hydroxycholesterol, 7β-ketocholesterol or cholestan-3β, 5α, 6β-triol in the liver is formed from cholesterol by non-enzymatic oxidation, predominately in the course of oxidation of PUFA [7, 8]. Therefore, we assume that the increased concentrations of those oxysterols in guinea pigs fed the oxidised fat were mainly due to oxidative stress caused by the oxidised fat. Increased concentrations of TBARS which derive from oxidation of polyunsaturated fatty acids support this suggestion. However, a part of the oxysterols in the liver of guinea pigs fed the oxidised fat may also derive from the diet. The analysis of the fats showed that the oxidised fat had a considerable concentration of oxysterols. Recent studies have shown that oxysterols in the diets can be absorbed and are incorporated into animal tissues [26, 27]. Increased concentrations of oxysterols in the liver are critical because of several unfavourable effects of oxysterols. They are associated with mutagenic and carcinogenic events [28] and are involved in the pathogenesis of atherosclerosis [29]. Oxysterols are transported as constituents of lipoproteins and may therefore leave the liver. Once they have entered the bloodstream they could exert their toxic effects on various cell types, as described in *in vitro* studies for endothelial cells [30] and smooth muscle cells [31].

The finding that supplementation of vitamins E and C does not prevent the formation of lipid oxidation products in the liver caused by dietary oxidised fats was unexpected. Other studies [4–5] found inverse correlations between the concentrations of tocopherols and ascorbic acid and lipid peroxidation in several tissues of animals fed oxidised fats. The distinction of our results with those of others could be due to the lipid peroxidation products considered. Other studies dealing with the effects of vitamins E and C on the formation of lipid peroxides in animals fed oxidised fats considered TBARS only as an indicator of lipid peroxidation [4, 5]. The concentration of TBARS is a relatively unspecific parameter for estimation of lipid peroxidation, especially as the majority of the measured lipid peroxidation products are only formed during the test [32]. In a recent study, which investigated the effects of various dietary vitamin E levels on the concentrations of hepatic oxysterols in rats fed fish oil or coconut oil, high dietary vitamin E levels also failed to completely prevent the formation of oxysterols in rats fed fish oil [33]. These findings show that dietary tocopherol concentrations in excess of the requirement do not reduce the concentrations of oxysterols in the liver of animals under conditions of oxidative stress.

GSH is an important component of the water-soluble antioxidant capacity. It has been shown that nutritive oxidative stress, such as feeding diets with fish oil, enhances the formation of GSH in the liver but causes depletion of GSH in erythrocytes which are lacking the capacity to synthesise glutathione [34]. Increased concentrations of GSH in the liver and reduced concentrations in erythrocytes observed in animals fed oxidised fats, therefore, are also indicative of oxidative stress in guinea pigs fed the oxidised fat. The finding that the concentrations of glutathione in liver and erythrocytes were similar within the four groups fed the oxidised fat supports the finding that supplemental vitamin E and vitamin C could not considerably reduce the stress of dietary oxidation products on the antioxidant system due to the oxidised fat.

Antioxidant enzymes play a crucial role in the defence against reactive oxygen species such as superoxide anions or peroxides. The finding that the activities of SOD and GSH-Px in the liver and of SOD, G6PDH and catalase in erythrocytes were not different between guinea pigs fed fresh fat and those fed oxidised fat suggests that the dietary oxidised fat did not stimulate the expression of antioxidant enzymes. This agrees with some other studies, in which feeding of oxidised fats did also not increase activities of SOD, GSH-Px and glutathione reductase in the liver of rats [35] or chicken [36]. These findings suggest that the organism is not able to prevent the deleterious effects of lipid peroxidation products by an enhanced expression of antioxidant enzymes.

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