ELSEVIER

Journal of Nutritional Biochemistry 15 (2004) 103–111

THE JOURNAL OF Nutritional Biochemistry

Dietary oxidized cholesterol decreases expression of hepatic microsomal triglyceride transfer protein in rats

Robert Ringseis, Klaus Eder*

Institut für Ernährungswissenschaften, Martin-Luther-Universität Halle-Wittenberg, Emil-Abderhaldenstraße 26, D-06108 Halle/Saale, Germany

Received 21 January 2003; received in revised form 20 September 2003; accepted 26 September 2003

Abstract

The aim of this study was to compare the effects of dietary oxidized cholesterol and pure cholesterol on plasma and very low density lipoprotein (VLDL) lipids and on some parameters of VLDL assembly and secretion in rats fed two different dietary fats. Four groups of male growing Sprague-Dawley rats were fed diets containing pure or oxidized cholesterol (5 g/kg diet) with either coconut oil or salmon oil as dietary fat (100 g/kg diet) for 35 days. Rats fed oxidized cholesterol supplemented diets had significantly lower concentrations of triglycerides and cholesterol in plasma and VLDL than rats fed pure cholesterol supplemented diets irrespective of the type of fat. In addition, rats fed oxidized cholesterol supplemented diets had significantly lower relative concentrations of microsomal triglyceride transfer protein messenger ribonucleic acid (mRNA) than rats fed pure cholesterol supplemented diets. In contrast, hepatic lipid concentrations and the relative concentration of apolipoprotein B mRNA were not influenced by the dietary factors investigated. Parameters of hepatic lipogenesis (relative mRNA concentration of sterol regulatory element binding protein-1c and activity of glucose-6-phosphat dehydrogenase) were significantly reduced by feeding fish oil compared to coconut oil, but were not affected by the type of cholesterol. In conclusion, the data of this study suggest, that dietary oxidized cholesterol affects VLDL assembly and/or secretion by reducing the synthesis of MTP but not by impairing hepatic lipogenesis or synthesis of apolipoprotein B.

1. Introduction

Oxidized cholesterols or oxysterols are oxygenated derivatives of cholesterol which are inevitable components of cholesterol-containing foods such as egg-containing products, dairy products, meat products, and marine foods [1]. Although, estimates concerning the absorption rates of oxysterols vary greatly, it is well known that oxysterols are readily absorbed from the intestine and transported in chylomicrons to the liver [2]. Results from animal studies exhibited that dietary oxidized cholesterol disturbed cholesterol and fatty acid metabolism as shown by reduced activities of key enzymes involved in cholesterol homeostasis and an increased activity of Δ6-desaturase [3–5]. This disturbance of cholesterol homeostasis and the promotion of eicosanoid formation from arachidonic acid is possibly related to the atherogenic effects observed by dietary oxysterols, although it is still discussed controversial whether oxysterols act pro- or anti-atherogenic [2]. The perturbation of lipid metabolism by oxysterols is also indicated by the observation that dietary oxidized cholesterol leads to decreased concentrations of triglycerides and/or cholesterol in plasma as compared to pure cholesterol [5–7].

Plasma lipids mainly derive from the liver, which are secreted by the liver within apolipoprotein B (apoB) containing lipoproteins (very low density lipoprotein, VLDL). Dietary cholesterol enhances VLDL synthesis and secretion through an increase in the transcription of microsomal triglycerid transfer protein (MTP) [8]. MTP is essential for the assembly of apoB containing lipoproteins by translocation of apoB and component lipids (cholesterol, cholesterol esters, triglycerides, and phospholipids) across the endoplasmatic reticulum [9]. Transcription of the MTP gene is regulated by cholesterol through a sterol response element located in its promoter [10]. Specific oxysterols are potent regulators of lipid metabolism through activation of liver X

Abbreviations: PUFA, polyunsaturated fatty acids; α-toc, α-tocopherol equivalents; MTP, microsomal triglyceride transfer protein; apoB, apolipoprotein B; SREBP-1c, sterol regulatory element binding protein 1c; G-6-PDH, glucose-6-phosphat dehydrogenase; VLDL, very low density lipoprotein; mRNA, messenger ribonucleic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TG, triglycerides; PE, phosphatidyl ethanolamine; PC phosphatidyl choline; PI, phosphatidyl inositol; SM, sphingomyelin; CL, cardiolipin.

* Corresponding author. Fax: +345 552714.

E-mail address: eder@landw.uni-halle.de (K. Eder).

0955-2863/04/$ – see front matter
doi:10.1016/j.jnutbio.2003.09.011

receptor α (LXRα), a nuclear transcription factor involved in the regulation of genes important for cholesterol homeostasis [11]. Therefore, it appears to be possible that dietary oxidized cholesterol alters the synthesis and secretion of VLDL by affecting the transcription of MTP and/or apoB, too. However, the effects of dietary oxidized cholesterol on VLDL synthesis or secretion have not yet been investigated. Hence, this study was conducted to determine the effects of oxidized cholesterol on plasma and VLDL lipids and the gene expression of apoB and MTP in rats. Rats fed pure cholesterol were used as controls.

Concentrations of VLDL lipids are strongly influenced by hepatic lipid concentrations, too. Concerning, that dietary oxidized cholesterol was shown to affect hepatic cholesterol biosynthesis and lipogenesis [3, 4, 7], we proposed to measure the concentrations of cholesterol, triglycerides and phospholipids in the liver. As parameters of hepatic lipogenesis we determined the gene expression of the sterol regulatory element binding protein-1c (SREBP-1c) and the activity of the lipogenic enzyme glucose-6-phosphate dehydrogenase (G-6-PDH). SREBP-1c, a target gene of LXRα, is a transcription factor that regulates expression of various lipogenic genes. Therefore, SREBP-1c not only coordinates the regulation of hepatic lipogenesis but also controls the assembly and secretion of apoB-containing lipoproteins [12]. As the type of fat shows a marked influence on hepatic lipid synthesis and plasma lipid concentrations we planned to use two dietary fats, salmon oil and coconut oil, with a very different fatty acid composition. Fish oil shows a suppressive effect on hepatic lipogenesis, which is mediated by a reduced transcription of lipogenic enzymes, which are controlled by SREBP-1c [13]. Dietary oxidized cholesterol was shown to modulate desaturation of fatty acids within liver phospholipids [3, 4, 14], which might influence the concentration of VLDL component lipids. Therefore, we further determined the fatty acid composition of hepatic phospholipids and triglycerides to assess possible effects of oxidized cholesterol on the desaturation of fatty acids.

2. Materials and methods

2.1. Animals and diets

36 male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) with an initial body weight of 72 g ($\pm$ 7 g, SD) were assigned to four groups of nine rats each. The animals were kept individually in Macrolon cages in a room maintained at a temperature of 23°C and 50 to 60% relative humidity with lighting from 0700 to 1900 h. All experimental procedures described followed established guidelines for the care and handling of laboratory animals and were approved by the council of Saxony-Anhalt.

Semisynthetic diets were used. The composition of the basal diets is shown in Table 1. Minerals and vitamins with the exception of vitamin E were supplemented in accordance with recommendations by the American Institute of Nutrition AIN-93G [15] for rat diets. The dietary fat (coconut oil vs. salmon oil) and cholesterol obtained from Sigma-Aldrich (Steinheim, Germany) (5 g pure cholesterol per kg vs. 5 g oxidized cholesterol per kg) were varied according to a two-factorial design. Oxidized cholesterol was prepared by heating cholesterol (Sigma-Aldrich, Steinheim, Germany) placed as a thin film on a glass Petri dish at 115°C for 48 hr in an electric oven. Oxysterols (Sigma-Aldrich, Steinheim, Germany) in the diets were determined using a quantitative GC-MS method with selective ion monitoring [16], which has been described in detail in a previous paper [17].

To equalize the vitamin E concentrations of the diets irrespective of the dietary fats used, the native tocopherol concentrations of the two fats were analyzed. Based on these native concentrations, diets were supplemented individually with all-*rac*-α-tocopheryl acetate (Merck Eurolab, Darmstadt, Germany), allowing for a biopotency of 67% compared to α-tocopherol. The final vitamin E concentrations of both types of diets were 40 mg α-tocopherol equivalents/kg. The fatty acid composition of coconut oil (Palmin, Hamburg, Germany) and salmon oil (Caelo, Hilden, Germany) is shown in Table 2.

The peroxide values of the dietary fats, which were

Table 1
Composition of the basal diets

Ingredient	Cholesterol (g/kg diet)	Oxidized Cholesterol (g/kg diet)
Corn starch	398	398
Casein	200	200
Saccharose	200	200
Fat*	100	100
Mineral mixture[†]	40	40
Vitamin mixture[‡]	20	20
Cellulose	35	35
Cholesterol	5	—
Oxidized Cholesterol	—	5
DL methionine	2	2
	(mg/kg diet)	
All-*rac*-α-tocopheryl acetate (supplemented)	59.5*/49.5[#]	59.5*/49.5[#]

* 100 g/kg coconut oil or 100 g/kg salmon oil.

[†] Mineral mixture supplied the following (per kg diet): 7.56 g calcium carbonate; 8.67 g dicalcium phosphate; 6.87 g potassium chloride; 3.77 g sodium bicarbonate; 1.01 g magnesium oxide; 0.116 g ferrous sulfate hydrate; 0.038 g zinc oxide; 0.016 g manganese oxide; 0.024 g copper sulfate pentahydrate; 0.0032 g calcium iodate; 0.0033 g sodium selenite pentahydrate; saccharose to 40 g.

[‡] Vitamin mixture supplied the following (per kg diet): 1.34 mg all-*trans*-retinol; 25 μg cholecalciferol; 7.5 mg menadion sodium bisulfite; 5 mg thiamine hydrochloride; 6 mg riboflavine; 6 mg pyridoxine hydrochloride; 15 mg calcium pantothenate; 30 mg nicotinic acid; 1,000 mg choline chloride; 2 mg folic acid; 0.2 mg biotin; 0.025 mg vitamin B12; saccharose to 20 g.

* All-*rac*-α-tocopheryl acetate (mg/kg diet) supplemented to the coconut oil diets.

[#] All-*rac*-α-tocopheryl acetate (mg/kg diet) supplemented to the salmon oil diets.

Table 2
Fatty acid composition of the dietary fats*

Fatty acid	Coconut oil (g/100 g fatty acids)	Salmon oil (g/100 g fatty acids)
8:0	8.1	—†
10:0	6.3	—†
12:0	45.6	—†
14:0	17.0	6.1
16:0	9.7	14.5
16:1	—†	8.2
18:0	4.5	3.0
18:1 (n − 9)	6.3	12.9
18:1 (n − 7)	—†	2.7
18:2 (n − 6)	1.9	2.2
20:1	—†	4.3
20:4 (n − 3)	—†	1.4
20:5 (n − 3)	—†	12.7
22:1	—†	4.5
22:5 (n − 3)	—†	3.1
22:6 (n − 3)	—†	10.0

* The table contains fatty acids with amounts greater than 1 g/100 g fatty acids only.

† Fatty acid exists in amounts smaller than 1 g/100 g fatty acids.

extracted from the diets with a mixture of hexane and isopropanol (3:2, as described in [18]) and measured according to official methods [19], were 3.9 and <0.1 mEq O_2 per kg salmon oil and coconut oil, respectively.

The diets were prepared weekly by solubilizing the all-*rac*-α-tocopheryl acetate and pure cholesterol/oxidized cholesterol preparation in the fat and mixing it with the dry components and water. The diets were then freeze dried and stored at –20°C to prevent autoxidation of lipids, e.g., polyunsaturated fatty acids and cholesterol. The water content after freeze drying was below 5 g per 100 g of diet.

To standardize the feed intake, the diets were fed daily in restricted amounts at 0800 h. The feed intake was increased from 7.0 g per day to 15.0 g per day during the experiment resulting in an average daily feed intake of 14.4 g. Water was provided ad libitum from nipple drinkers. The experimental diets were fed for 35 days.

2.2. Sample collection

At the end of the feeding period the rats were starved overnight, anesthetized with diethyl ether and killed by decapitation. The liver was excised immediately, frozen with liquid nitrogen and stored at −80°C until analysis. Plasma was separated from blood by centrifugation (1,100 g, 10 min) at 4°C. For separation of VLDL the plasma density was adjusted to $\delta = 1006$ g/L by adding 0.3 mL of a solution containing 0.195 mol/L sodium chloride and 2.44 mol/L sodium bromide to 0.6 mL of plasma and centrifuged using a Mikro-Ultracentrifuge (Sorvall Products, Bad Homburg, Germany) at 900,000 g for 1.5 h. Plasma and VLDL were stored at –80°C until analysis.

2.3. Lipid analysis

Liver lipids were extracted with a mixture of hexane and isopropanol (3:2, v/v) [18]. Total cholesterol and triglyceride concentrations of liver, plasma, and VLDL were determined using enzymatic reagent kits obtained from Merck Eurolab (Darmstadt, Germany). For the measurement of liver total cholesterol and liver triglycerides, lipids of the extract were dissolved in Triton X-100 before enzymatic measurement as described by De Hoff et al. [20]. Hepatic cardiolipin (CL), phosphatidyl ethanolamine (PE), phosphatidyl inositol (PI), phosphatidyl choline (PC) and sphingomyelin (SM) were separated using a HP 1050 HPLC station (Hewlett Packard, Waldbronn, Germany) fitted with a quarternary pump and a Supelcosil column (25 cm, 4.6 mm, 5 μm; Supelco, Bellefonte, USA) and quantified using an evaporative light scattering detector (Sedex 55; SEDERE, Alfortville Cedex, France) and external standards. Separation was achieved using a gradient of two solvent mixtures, A and B, as mobile phases [A: chloroform, methanol, double-distilled water and ammonia solution (30%) (60/34/5.5/0.5; v,v,v,v); B: chloroform, methanol, ammonia solution (30%) (80/19.5/0.5; v,v,v)] at flow rate of 1.0 mL/min (total run time was 42 min). Triglycerides (TG) could be also separated, but were not quantified. For analysis of fatty acids of liver lipids TG, PE and PC were collected with a fraction collector (FC 203B; Abimed, Langenfeld, Germany) using the same chromatographic system and gradient system as used for quantification of liver lipids. The fatty acid composition of experimental fats and liver TG, PE and PC was determined by gas chromatography (GC) of fatty acid methyl esters (FAME) as described previously in detail [21]. Briefly, fats were methylated with trimethylsulfonium hydroxide according to [22]. FAME were separated using a GC system (HP 5890, Hewlett-Packard GmbH, Böblingen, Germany) fitted with an automatic on-column injector, a polar capillary column (30 m FFAP, 0.53 mm i.d., Macherey and Nagel, Düren, Germany) and a flame ionization detector.

2.4. Measurement of G-6-PDH activity

The activity of glucose-6-phosphate dehydrogenase (G-6-PDH) in the liver was determined by the method of Deutsch [23]. One unit of G-6-PDH is defined as one μmol reduced β-nicotinamide adenine dinucleotide phosphate oxidized per min.

2.5. Measurement of relative mRNA concentrations

To quantify the gene expression of apoB, MTP and SREBP-1c the total RNA was isolated from liver by Trizol™ reagent (Invitrogen, Karlsruhe, Germany). The relative quantities of apoB mRNA, MTP mRNA and SREBP-1c mRNA compared to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA were determined by

Table 3
Body weights, body weight gain and concentrations of plasma and VLDL lipids of rats fed diets with two different dietary fats (coconut oil vs. salmon oil) and with 5 g/kg of either pure or oxidized cholesterol

Dietary Fat	Coconut oil		Salmon oil	
Dietary Cholesterol Parameters	Pure Cholesterol (n = 9)	Oxidized Cholesterol (n = 9)	Pure Cholesterol (n = 9)	Oxidized Cholesterol (n = 9)
Initial body weight (g)	72.0 ± 8.2	72.2 ± 8.0	72.3 ± 8.3	71.9 ± 9.1
Final body weight (g)*	261 ± 13^{b}	265 ± 10^{b}	284 ± 10^{a}	283 ± 11^{a}
Body weight gain (g/d)*	5.41 ± 0.34^{b}	5.52 ± 0.21^{b}	6.05 ± 0.22^{a}	6.04 ± 0.36^{a}
Triglycerides (mmol/L)				
Plasma*†	0.79 ± 0.25^{a}	0.59 ± 0.12^{b}	0.54 ± 0.13^{b}	0.44 ± 0.19^{b}
VLDL*†	0.21 ± 0.08^{a}	0.13 ± 0.04^{b}	0.12 ± 0.04^{bc}	0.07 ± 0.03^{c}
Cholesterol (mmol/L)				
Plasma*†	4.80 ± 0.47^{a}	4.45 ± 0.63^{a}	1.94 ± 0.25^{b}	1.68 ± 0.17^{b}
VLDL*†‡	3.18 ± 0.38^{a}	2.40 ± 0.66^{b}	0.45 ± 0.14^{c}	0.34 ± 0.16^{c}

Results are expressed as means ± SD.

Results of analysis of variance: * Significant effect of factor fat ($P < 0.05$); † Significant effect of factor cholesterol ($P < 0.05$); ‡ Significant interaction between factors fat and cholesterol ($P < 0.05$). Mean values in a row without a common superscript letter differ ($P < 0.05$).

VLDL-very low density lipoprotein.

quantitative real-time RT-PCR. First strand cDNA was prepared using the Omniscript RT Kit (200) from QIAGEN (Hilden, Germany) and Oligo dT-Primer pd(T)$_{12\ to\ 18}$ from Amersham Pharmacia Biotech (Freiburg, Germany). The real-time PCR was performed with a Taq DNA Polymerase from Promega (Mannheim, Germany) and specific primers coding for GAPDH, apoB, MTP and SREBP-1c. Amplification of first strand cDNA was performed using a real-time RCR cycler (Rotorgene, Corbett Research, LTF Labortechnik, Wasserburg, Germany). The primers used for PCR were as follows: 5′-GCATGGCCTTCCGTGTTCC-3′ (sense) and 5′-GGGTGGTCCAGGGTTTCTTACTC-3′ (antisense) for rat GAPDH; 5′-GGAAAGGGGAGG-GAAAAGGTT-3′ (sense) and 5′-TTAGGTAGGGGCT-CACATTATTGG-3′ (antisense) for rat apoB; 5′-GGAGC-CATGGATTGCACATT-3′ (sense) and 5′-AGGAAGGCTTCCAGAGAGGA-3′ (antisense) for rat SREBP-1c; mouse specific 5′-CGCGAGTCTAAAAC-CCGAGTG-3′ (sense) and 5′-CCCTGCCTGTAGATAGC-CTTTCAT-3′ (antisense) for rat MTP.

2.5. Statistical analysis

Data were subjected to ANOVA using the Minitab Statistical Software (Minitab, State College, PA, USA). Classification factors were dietary fat, cholesterol and the interaction of both factors. In cases where the differences between variances and means were large, data were transformed to logarithms prior to ANOVA. For statistically significant F-values, individual means of the treatment groups were compared by Fisher′s multiple range test. Means were considered significantly different for $P < 0.05$.

3. Results

Concentrations of oxysterols in the diets. The diets supplemented with 5 g oxidized cholesterol/kg contained the following oxysterols (mg/kg) which are known to be formed at the highest concentrations during heating of cholesterol: 7β-OH, 2.7; α-epoxycholesterol, 2.6; β-epoxycholesterol, 6.9; cholestanetriol, 0.1; 25-hydroxycholesterol, 9.4; 7-ketocholesterol, 1.7. Other minor, unidentified oxysterols have not been quanified. In the diets containing pure cholesterol the concentrations of all oxysterols were below the limit of detection of 0.025 mg/kg.

Body weights of the rats. The initial body weights of the rats were similar within the four treatment groups (Table 3). However, rats fed the salmon oil diets gained significantly more body weight during the feeding period and had significantly higher final body weights than the rats fed coconut oil. The type of cholesterol (oxidized vs. pure cholesterol) did not affect the body weight gain and the final body weights of the rats.

Plasma and VLDL lipids. The lipid concentrations of plasma and VLDL were significantly influenced by both dietary factors, the type of cholesterol and the type of fat (Table 3). The results of ANOVA showed that rats fed the diets containing oxidized cholesterol had significantly lower concentrations of triglycerides and cholesterol in plasma and VLDL than rats fed diets containing pure cholesterol. Rats whose diets contained coconut oil had significantly higher concentrations of triglycerides and cholesterol in plasma and VLDL than rats whose diets contained salmon oil.

Parameters of hepatic lipogenesis and concentrations of liver lipids. The relative concentrations of SREBP-1c mRNA and the activities of G-6-PDH were both affected by the type of dietary fat (Table 4); rats fed the salmon oil diets had markedly lower relative concentrations of SREBP-1c

Table 4
Parameters of hepatic lipogenesis and concentrations of liver lipids of rats fed diets with two different dietary fats (coconut oil vs. salmon oil) and with 5 g/kg of either pure or oxidized cholesterol

Dietary Fat	Coconut oil		Salmon oil	
Dietary Cholesterol Parameter	Pure Cholesterol (n = 9)	Oxidized Cholesterol (n = 9)	Pure Cholesterol (n = 9)	Oxidized Cholesterol (n = 9)
SREBP-1c mRNA (% of GAPDH*100)*	8.15 ± 4.03[a]	7.26 ± 3.70[a]	1.90 ± 0.91[b]	1.27 ± 0.45[b]
G-6-PDH activity (U/mg protein)*	6.94 ± 2.38[a]	8.21 ± 3.93[a]	1.07 ± 0.24[b]	1.22 ± 0.28[b]
		(μmol/g)		
Total cholesterol	103 ± 28	111 ± 24	125 ± 22	133 ± 25
Triglycerides	26.6 ± 5.6	35.6 ± 11.4	38.0 ± 11.5	41.7 ± 19.7
Cardiolipin	0.77 ± 0.15	0.80 ± 0.12	0.68 ± 0.10	0.69 ± 0.09
PE	11.9 ± 0.7	12.3 ± 0.6	11.7 ± 1.1	12.0 ± 1.2
PI	4.98 ± 0.41	4.72 ± 0.46	4.62 ± 0.50	4.34 ± 0.77
PC	17.7 ± 1.5	17.6 ± 1.2	18.5 ± 1.4	17.5 ± 2.0
Sphingomyelin	3.07 ± 1.05	2.86 ± 0.44	2.33 ± 0.32	2.65 ± 0.65

Results are expressed as means ± SD.
Results of analysis of variance: * Significant effect of factor fat ($P < 0.05$). Mean values in a row without a common superscript letter differ ($P < 0.05$).
SREBP-1c-sterol regulatory element binding protein-1c. G-6-PDH-glucose-6-phosphat dehydrogenase. PE-phosphatidyl ethanolamine. PI-phosphatidyl inositol. PC-phosphatidyl choline.

mRNA and activities of G-6-PDH than rats fed the coconut oil diets. The type of cholesterol did not influence the relative concentrations of SREBP-1c mRNA and the activities of G-6-PDH. In contrast, neither the type of fat nor the type of cholesterol influenced the concentrations of total cholesterol, triglycerides and phospholipids in the liver.

Fatty acid composition of liver lipids. The fatty acid compositions of liver TG, PE and PC were strongly influenced by the type of dietary fat (Tables 5–7). Rats fed salmon oil diets had significantly higher proportions of long chain n-3 PUFA and total PUFA, but significantly lower proportions of total MUFA in TG, PE and PC than the rats fed coconut oil diets. Proportions of total SFA were slightly elevated in PE and PC, but strongly reduced in TG of the rats fed salmon oil compared to the rats fed coconut oil. Oxidized cholesterol showed only slight effects on the fatty acid composition of hepatic TG, PE and PC as compared to pure cholesterol. The only notable effect of dietary oxidized cholesterol was exhibited on the proportion of 20:4 n-6 in hepatic TG, PE and PC. Rats fed oxidized cholesterol had

Table 5
Fatty acid composition of liver triglycerides of rats fed diets with two different dietary fats (coconut oil vs. salmon oil) and with 5 g/kg of either pure or oxidized cholesterol

Dietary Fat	Coconut oil		Salmon oil	
Dietary Cholesterol Parameter	Pure Cholesterol (n = 9)	Oxidized Cholesterol (n = 9)	Pure Cholesterol (n = 9)	Oxidized Cholesterol (n = 9)
Total SFA*	33.7 ± 2.0[a]	33.3 ± 1.1[a]	16.0 ± 1.3[b]	16.6 ± 1.3[b]
16:0*	28.1 ± 2.0[a]	27.5 ± 1.4[a]	13.1 ± 1.4[b]	13.8 ± 1.3[b]
18:0*	1.76 ± 0.28[a]	1.71 ± 0.17[ab]	1.50 ± 0.15[b]	1.48 ± 0.22[b]
Total MUFA*	46.9 ± 2.9[a]	48.9 ± 1.7[a]	34.7 ± 1.2[b]	34.1 ± 1.8[b]
16:1*	12.0 ± 1.3[a]	12.9 ± 0.6[a]	7.84 ± 1.15[b]	7.53 ± 0.99[b]
18:1*[†‡]	34.1 ± 1.8[b]	37.0 ± 2.5[a]	25.8 ± 1.4[c]	25.7 ± 1.2[c]
Total PUFA*	2.94 ± 0.50[b]	2.98 ± 0.36[b]	35.3 ± 1.3[a]	34.2 ± 1.3[a]
18:2n-6*[†]	1.35 ± 0.25[c]	1.57 ± 0.16[c]	4.15 ± 0.43[b]	4.63 ± 0.34[a]
20:2n-6*	0.36 ± 0.08[a]	0.34 ± 0.05[a]	0.13 ± 0.10[b]	0.14 ± 0.11[b]
20:3n-6*	0.09 ± 0.03[b]	0.09 ± 0.03[b]	0.22 ± 0.03[a]	0.24 ± 0.04[a]
20:4n-6*[†]	0.41 ± 0.06[b]	0.47 ± 0.09[b]	0.62 ± 0.07[a]	0.69 ± 0.12[a]
20:5n-3*	0.14 ± 0.07[b]	0.10 ± 0.05[b]	6.61 ± 0.86[a]	6.87 ± 1.01[a]
22:5n-3*	0.21 ± 0.12[b]	0.13 ± 0.05[b]	4.81 ± 0.69[a]	4.50 ± 0.60[a]
22:6n-3*	0.26 ± 0.14[b]	0.12 ± 0.03[b]	18.2 ± 1.0[a]	17.4 ± 1.4[a]

Results are expressed as means ± SD.
Results of analysis of variance: * Significant effect of factor fat ($P < 0.05$); [†] Significant effect of factor cholesterol ($P < 0.05$); [‡] Significant interaction between factors fat and cholesterol ($P < 0.05$). Mean values in a row without a common superscript letter differ ($P < 0.05$).
SFA-saturated fatty acids. MUFA-unsaturated fatty acids. PUFA-polyunsaturated fatty acids.

Table 6
Fatty acid composition of liver phosphatidyl ethanolamine of rats fed diets with two different dietary fats (coconut oil vs. salmon oil) and with 5 g/kg of either pure or oxidized cholesterol

Dietary Fat	Coconut oil		Salmon oil	
Dietary Cholesterol Parameter	Pure Cholesterol (n = 9)	Oxidized Cholesterol (n = 9)	Pure Cholesterol (n = 9)	Oxidized Cholesterol (n = 9)
Total SFA*†	31.8 ± 1.0^{b}	32.4 ± 0.7^{b}	32.2 ± 1.6^{b}	33.7 ± 1.0^{a}
16:0	14.4 ± 0.8	14.5 ± 0.9	15.5 ± 1.05	14.9 ± 0.9
18:0	16.9 ± 1.3	17.5 ± 1.2	16.9 ± 1.5	18.4 ± 1.0
Total MUFA*†	13.3 ± 0.8^{a}	12.8 ± 1.2^{a}	11.5 ± 0.7^{b}	10.4 ± 1.2^{c}
16:1*†	1.95 ± 0.25^{a}	1.73 ± 0.13^{b}	1.38 ± 0.17^{c}	1.15 ± 0.20^{d}
18:1*	5.46 ± 0.57^{a}	5.38 ± 0.64^{a}	4.94 ± 0.24^{ab}	4.62 ± 0.74^{b}
Total PUFA*†	39.8 ± 0.7^{c}	40.6 ± 0.9^{bc}	41.5 ± 1.6^{b}	42.7 ± 0.7^{a}
18:2n-6*	5.15 ± 0.53^{a}	5.30 ± 0.43^{a}	2.29 ± 0.27^{b}	2.53 ± 0.21^{b}
20:2n-6*	2.91 ± 0.55^{a}	3.11 ± 0.45^{a}	0.25 ± 0.22^{b}	0.22 ± 0.22^{b}
20:3n-6*	1.21 ± 0.13^{a}	1.24 ± 0.13^{a}	0.47 ± 0.07^{b}	0.51 ± 0.07^{b}
20:4n-6*†	20.6 ± 1.4^{b}	21.9 ± 0.6^{a}	7.18 ± 0.73^{d}	8.09 ± 0.79^{c}
20:5n-3*	0.60 ± 0.09^{b}	0.66 ± 0.11^{b}	11.2 ± 0.60^{a}	11.5 ± 0.93^{a}
22:5n-3*	0.34 ± 0.15^{b}	0.33 ± 0.07^{b}	3.01 ± 0.33^{a}	2.86 ± 0.48^{a}
22:6n-3*	7.59 ± 0.78^{b}	7.16 ± 0.47^{b}	16.4 ± 1.50^{a}	15.8 ± 1.04^{a}

Results are expressed as means ± SD.

Results of analysis of variance: * Significant effect of factor fat ($P < 0.05$); † Significant effect of factor cholesterol ($P < 0.05$). Mean values in a row without a common superscript letter differ ($P < 0.05$).

SFA-saturated fatty acids. MUFA-unsaturated fatty acids. PUFA-polyunsaturated fatty acids.

significantly higher proportions of 20:4 n-6 in TG, PE and PC than rats fed pure cholesterol.

Relative mRNA concentrations of apoB and MTP. According to ANOVA, the relative concentrations of MTP mRNA were significantly influenced by the factor cholesterol (Fig. 1). Rats receiving oxidized cholesterol had lower relative concentrations of MTP mRNA than rats receiving pure cholesterol. The type of fat showed no effect on the relative concentrations of MTP mRNA. The relative concentrations of apoB mRNA were not affected by both dietary factors investigated, the type of fat and type of cholesterol (Fig. 2).

Table 7
Fatty acid composition of liver phosphatidyl choline of rats fed diets with two different dietary fats (coconut oil vs. salmon oil) and with 5 g/kg of either pure or oxidized cholesterol

Dietary Fat	Coconut oil		Salmon oil	
Dietary Cholesterol Parameter	Pure Cholesterol (n = 9)	Oxidized Cholesterol (n = 9)	Pure Cholesterol (n = 9)	Oxidized Cholesterol (n = 9)
Total SFA*	36.8 ± 0.9^{b}	37.2 ± 1.1^{b}	38.0 ± 1.2^{ab}	38.3 ± 0.8^{a}
16:0*†‡	16.7 ± 0.7^{c}	17.1 ± 0.9^{c}	21.7 ± 1.1^{a}	20.3 ± 0.9^{b}
18:0*†‡	19.5 ± 1.2^{a}	19.4 ± 1.2^{a}	15.6 ± 1.2^{c}	17.4 ± 1.0^{b}
Total MUFA*†	20.0 ± 1.2^{a}	19.3 ± 1.2^{a}	17.4 ± 0.8^{b}	16.0 ± 1.2^{c}
16:1*†	3.77 ± 0.46^{a}	3.44 ± 0.39^{ab}	3.16 ± 0.37^{b}	2.66 ± 0.35^{c}
18:1*	10.3 ± 0.5^{a}	10.5 ± 0.4^{a}	9.43 ± 0.52^{b}	9.21 ± 0.93^{b}
Total PUFA*	33.1 ± 0.7^{b}	33.8 ± 1.0^{b}	37.2 ± 0.8^{a}	37.5 ± 0.8^{a}
18:2n-6*	9.43 ± 0.65^{a}	9.53 ± 0.71^{a}	3.98 ± 0.41^{b}	4.32 ± 0.29^{b}
20:2n-6*	6.20 ± 0.88^{a}	6.29 ± 0.66^{a}	0.26 ± 0.12^{b}	0.27 ± 0.12^{b}
20:3n-6*	2.56 ± 0.19^{a}	2.50 ± 0.22^{a}	1.21 ± 0.12^{b}	1.28 ± 0.11^{b}
20:4n-6*†	11.5 ± 0.5^{ab}	12.0 ± 1.1^{a}	9.83 ± 0.50^{c}	11.1 ± 0.3^{b}
20:5n-3*†‡	0.31 ± 0.10^{c}	0.34 ± 0.05^{c}	11.3 ± 0.6^{a}	10.3 ± 0.7^{b}
22:5n-3*	0.11 ± 0.02^{b}	0.13 ± 0.02^{b}	2.06 ± 0.19^{a}	1.90 ± 0.26^{a}
22:6n-3*	2.44 ± 0.31^{b}	2.34 ± 0.09^{b}	7.80 ± 0.49^{a}	7.61 ± 0.51^{a}

Results are expressed as means ± SD.

Results of analysis of variance: * Significant effect of factor fat ($P < 0.05$); † Significant effect of factor cholesterol ($P < 0.05$); ‡ Significant interaction between factors fat and cholesterol ($P < 0.05$). Mean values in a row without a common superscript letter differ ($P < 0.05$).

SFA-saturated fatty acids. MUFA-unsaturated fatty acids. PUFA-polyunsaturated fatty acids.

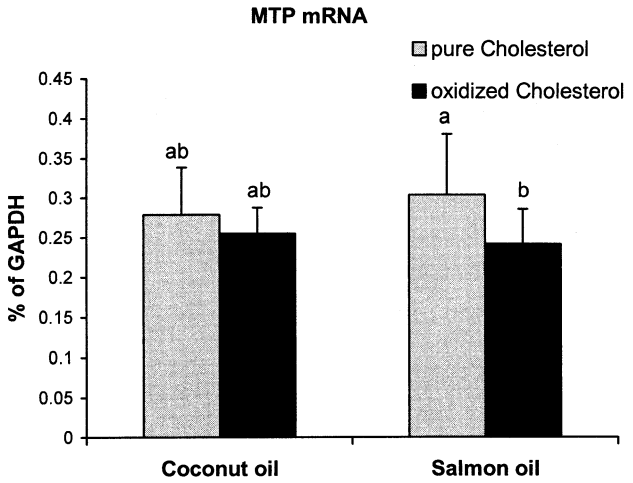

Fig. 1. Relative concentration of microsomal triglyceride transfer protein (MTP) mRNA (MTP mRNA in % of GAPDH) of rats fed diets with two different dietary fats (coconut oil vs. salmon oil) and with 5 g/kg of either pure or oxidized cholesterol. Results are means ± SD of nine rats per group. Results of analysis of variance: Significant effect of factor cholesterol ($P < 0.05$). Bars marked without a common superscript letter differ ($P < 0.05$).

4. Discussion

In this study the effect of dietary oxidized cholesterol compared to pure cholesterol on lipid metabolism in rats was investigated with special regard to parameters of VLDL synthesis and secretion. To gain insight into the composition and the degree of oxidation of the oxidized cholesterol preparation we measured six of the dominating oxysterols knowing to be formed under the applied oxidizing conditions. The measured oxysterols in the oxidized cholesterol preparation are the quantitatively dominating oxysterols in Western foods [1]. Regarding the low concentrations of those oxysterols suggests that we used a moderately oxidized preparation. This is relevant with respect to human diets concerning the estimation that about 1% of the cholesterol consumed in a mixed Western diet is oxidized [1]. The experimental animals were fed a restrictive diet in order to exclude secondary effects which might result from different feed intakes. However, the rats fed salmon oil showed a higher body weight gain than the rats fed coconut oil. This effect might be attributed to the low concentrations of essential fatty acids (linoleic, α-linolenic acid) in the coconut oil, although we could not observe any signs of a deficiency of essential fatty acids e.g., raised concentrations of 20:3 (n-9). The differences in body weights between the rats are however unlikely to jeopardize the results of our study with regard to the effects of oxidized cholesterol.

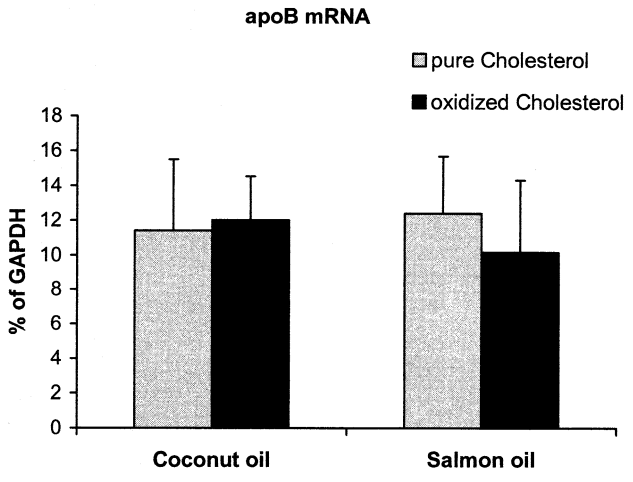

Fig. 2. Relative concentration of apolipoprotein B (apoB) mRNA (apoB mRNA in % of GAPDH) of rats fed diets with two different dietary fats (coconut oil vs. salmon oil) and with 5 g/kg of either pure or oxidized cholesterol. Results are means ± SD of nine rats per group.

It is well documented in rats and other animal species that oxysterols of an oxidized cholesterol preparation are readily absorbed from the intestine and transported to the liver, where they exert various biological effects [6, 24]. Our study showed that feeding oxidized cholesterol lowered the concentrations of plasma cholesterol and triglycerides as observed in feeding experiments from other investigators [5–7]. The experiment further revealed that dietary oxysterols reduced the concentrations of VLDL cholesterol and triglycerides possibly indicating a reduced VLDL synthesis and/or secretion. It is known that a decreased expression of apoB and MTP reduces VLDL assembly and secretion [9]. Thus, we measured the expression of these two genes and found that the expression of MTP was significantly reduced in the rats fed oxidized cholesterol, whereas the expression of apoB was not influenced. In patients with the recessive disorder abetalipoproteinemia the genetic loss of the expression of the MTP gene is responsible for the abnormally lowered VLDL concentrations [25]. Therefore, it appears to be possible, that the reduced transcription of the MTP gene is at least in part responsible for the lowered plasma and VLDL lipid concentrations in the rats fed oxidized cholesterol.

Dietary fish oil, as compared to coconut oil, is a source of oxidative stress due to the incorporation of highly oxidation susceptible n-3 PUFA into liver lipids [26]. According to ANOVA, the effect of oxidized cholesterol on the relative gene transcription of MTP was independent of the dietary fats used. This suggests that the effect of oxidized cholesterol on MTP gene transcription is not due to the induction of oxidative stress.

The observation that hepatic concentrations of lipids and the parameters of hepatic lipogenesis (SREBP-1c mRNA, activity of G-6-PDH) were not influenced by the dietary oxidized cholesterol varies from results from other investigators [3, 4, 7]. However, this difference might be attributed to the fact that we, in contrast to those investigators, used a moderately oxidized cholesterol preparation. Oxysterols are potent inhibitors of hepatic HMG-CoA reductase and the high amount of oxysterols in the diets from those investigators might have strongly suppressed cholesterol biosyn-

thesis in the liver [27]. The increased proportion of 20:4 n-6 in liver TG, PE and PC in the rats fed oxidized cholesterol possibly indicates a raised desaturation of linoleic acid. This is consistent with the observation from other studies, that dietary oxidized cholesterol increases the activity of Δ6-desaturase [3, 4, 14].

Therefore, the data of our study suggest, that dietary oxidized cholesterol possibly affects VLDL assembly and/or secretion by reducing the transcription of MTP but does not impair the synthesis of component lipids. The reduced transcription of MTP after feeding oxidized cholesterol may be in part explained by the results from in vitro studies, which showed that the expression of cholesterol-7α-hydroxylase (CYP7A1) also affects VLDL assembly and secretion due to a positive correlation between CYP7A1 mRNA and MTP mRNA [12]. The activity of CYP7A1 was reported to be decreased by dietary oxidized cholesterol as compared to pure cholesterol [3, 4, 7]. As the activity of CYP7A1 is highly correlated with the abundance of CYP7A1 mRNA it seems possible that a decreased transcription of CYP7A1 is responsible for the reduced concentrations of MTP mRNA in this study. In addition, results from in vitro study showed, that the oxysterols 25-hydroxycholesterol and 7-ketocholesterol, both of which were found in the oxidized cholesterol-supplemented diets, reversed the CYP7A1-induced changes on VLDL assembly and secretion [28]. However, this explanation is speculative and has to be confirmed by following studies.

In agreement with results from other animal studies we could demonstrate that dietary oxidized cholesterol influences lipid metabolism [3 to 5]. Although, the observed reduction of plasma and VLDL lipid concentrations possibly indicates a beneficial effect of dietary oxysterols with regard to the development of atherosclerosis it should be rather interpreted as sign of a perturbed lipid metabolism. Cell culture studies could demonstrate that 25-hydroxycholesterol enhanced the release of several prostaglandins from endothelial and smooth muscle cells by increasing the amount of prostaglandin G/ hr synthase-2 protein in a manner that was similar to characteristics of smooth muscle cell activation secondary to intravascular injury [29, 30]. With respect to this observation from in vitro studies the increased desaturation of linoleic acid to arachidonic acid must be considered critically due to the possible formation of eicosanoids which are related to cardiovascular diseases.

In conclusion, the study showed that dietary oxidized cholesterol significantly reduced plasma and VLDL lipid concentrations. It was further shown that dietary oxidized cholesterol lowered the expression of MTP, which is essential for VLDL assembly and secretion. However, further studies are necessary to confirm a causal linkage between dietary oxidized cholesterol and a decreased expression of MTP and lipid lowering in plasma.

References

[1] Van de Bovenkamp P, Kosmeijer-Schuil TG, Katan MB. Quantification of oxysterols in Dutch foods: egg products and mixed diets. Lipids 1988;23:1079–85.
[2] Brown AJ, Jessup W. Oxysterols and atherosclerosis. Atherosclerosis 1999;142:1–28.
[3] Osada K, Kodama T, Yamada K, Nakamura S, Sugano M. Dietary oxidized cholesterol modulates cholesterol metabolism and linoleic acid desaturation in rats fed high-cholesterol diets. Lipids 1998;33:757–64.
[4] Osada K, Kodama T, Noda S, Yamada K, Sugano M. Oxidized cholesterol modulates age-related change in lipid metabolism in rats. Lipids 1995;30:405–13.
[5] Cho BHS, Egwim PO, Fahey GC. Effects of pure and auto-oxidized forms of cholesterol on plasma, liver lipids and hepatic lipogenesis in chicks. Comp Biochem Physiol 1986;83B:767–70.
[6] Vine DF, Mamo JCL, Beilin LJ, Mori TA, Croft KD. Dietary oxysterols are incorporated in plasma triglyceride-rich lipoproteins, increase their susceptibility to oxidation and increase aortic cholesterol concentration of rabbits. J Lipid Res 1998;39:1995–2004.
[7] Osada K, Kodama T, Cui L, Ito Y, Sugano M. Effects of dietary oxidized cholesterol on lipid metabolism in differently aged rats. Biosci Biotech Biochem 1994;58:1062–9.
[8] Bennett AJ, Bruce JS, Salter AM, White DA, Billett MA. Hepatic microsomal triglyceride transfer protein messenger RNA concentrations are increased by dietary cholesterol in hamsters. FEBS Lett 1996;394:247–50.
[9] Wang S, McLeod RS, Gordon DA, Yao Z. The Microsomal triglyceride transfer protein facilitates assembly and secretion of apolipoprotein B-containing lipoproteins and decreases cotranslational degradation of apolipoprotein B in transfected COS-7 cells. J Biol Chem 1996;271:14124–33.
[10] Hagan DL, Kienzle B, Jamil H, Hariharan N. Transcriptional regulation of human and hamster microsomal triglyceride transfer protein genes. Cell type-specific expression and response to metabolic regulators. J Biol Chem 1994;269:28737–44.
[11] Janowski BA, Willy PJ, Devi TR, Falck JR, Mangelsdorf DJ. An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. Nature 1996;383:728–31.
[12] Wang S-L, Du EZ, Martin D, Davis RA. Coordinate regulation of lipogenesis, the assembly and secretion of apolipoprotein B-containing lipoproteins by sterol response element binding protein 1. J Biol Chem 1997;272:19351–8.
[13] Kim HJ, Takahashi M, Ezaki O. Fish oil feeding decreases mature sterol regulatory element-binding protein 1 (SREBP-1) by down-regulation of SREBP-1c mRNA in mouse liver. A possible mechanism for down-regulation of lipogenic enzyme mRNAs. J Biol Chem 1999;274:25892–8.
[14] Osada K, Kodama T, Minehira K, Yamada K, Sugano M. Dietary protein modifies oxidized cholesterol-induced alterations of linoleic acid and cholesterol metabolism in rats. J Nutr 1996;126:1635–43.
[15] Reeves PG, Nielsen FH, Fahey GC. AIN-93 Purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc Writing Committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939–51.
[16] Mori TA, Croft KD, Puddey IB, Beilin LJ. Analysis of native and oxidized low-density lipoprotein oxysterols using gas chromatography-mass spectrometry with selective ion monitoring. Redox Report 1996;2:25–34.
[17] Brandsch C, Ringseis R, Eder K. High dietary iron concentrations enhance the formation of cholesterol oxidation products in the liver of adult rats fed salmon oil with minimal effects on antioxidant status. J Nutr 2002;132:2263–9.
[18] Hara A, Radin NS. Lipid extraction of tissues with a low toxicity solvent. Anal Biochem 1978;90:420–6.

[19] Association of Official Analytical Chemists. In: Horwitz H. editor. Official Methods of Analysis. 13th ed. Airlington: VA. 1980. pp. 440–441.
[20] De Hoff JL, Davidson JH, Kritchevsky V. An enzymatic assay for determining free and total cholesterol in tissues. Clin Chem 1978;24: 433–5.
[21] Brandsch C, Ringseis R, Eder K. High dietary iron concentrations enhance the formation of cholesterol oxidation products in the liver of adult rats fed salmon oil with minimal effects on antioxidant status. J Nutr 2002;132:2263–9.
[22] Butte W. Rapid method for the determination of fatty acid profiles from fats and oils using trimethylsulfonium hydroxide for transesterification. J Chromatogr 1983;261:142–5.
[23] Deutsch J. Glucose-6-Phosphat-Dehydrogenase. In: Bergmeyer HU, editor. Methods of enzymatic analysis. Vol. 3. Weinheim: VCH, 1985. pp. 190–197.
[24] Vine DF, Croft KD, Beilin LJ, Mamo JCL. Absorption of dietary cholesterol oxidation products and incorporation into rat lymph chylomicrons. Lipids 1997;32:887–93.
[25] Wetterau JR, Aggerbeck LP, Bouma ME, Eisenberg C, Munck A, Hermier M, Schmitz J, Gay G, Rader DJ, Gregg RE. Absence of microsomal triglyceride transfer protein in individuals with abetalipoproteinemia. Science 1992;258:999–1001.
[26] D'Aquino M, Benedetti PC, Di Felice M, Gentili V, Tomassi G, Maiorino M, Ursini F. Effect of fish oil and coconut oil on antioxidant defence system and lipid peroxidation in rat liver. Free Radic Res Commun 1991;1:147–52.
[27] Johnson KA, Morrow CJ, Knight GD, Scallen TJ. In vivo formation of 25-hydroxycholesterol from endogenous cholesterol after a single meal, dietary cholesterol challenge. J Lipid Res 1994;35:2241–53.
[28] Du EZ, Fleming JF, Wang S-L, Spitsen GM, Davis RA. Translocation-arrested apolipoprotein B evades proteasome degradation via a sterol-sensitive block in ubiquitin conjugation. J Biol Chem 1999; 274:1856–62.
[29] Wohlfeil ER, Campbell WB. 25-hydroxycholesterol increases eicosanoids and alters morphology in cultured pulmonary artery smooth muscle and endothelial cells. Arterioscler Thromb Vasc Biol 1999; 19:2901–8.
[30] Wohlfeil ER, Campbell WB. 25-Hydroxycholesterol enhances eicosanoid production in cultured bovine coronary artery endothelial cells by increasing prostaglandin G/H synthase-2. Biochim Biophys Acta 1997;1345:109–20.