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Concentrations of lipids in plasma and lipoproteins and oxidative susceptibility of low-density lipoproteins in zinc-deficient rats fed linseed oil or olive oil

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The present study was performed to investigate the effect of zinc deficiency on concentrations of lipids in plasma and lipoproteins and the susceptibility of low-density lipoproteins (LDL) to lipid peroxidation in rats fed diets containing either linseed oil or olive oil as dietary fat, using a bifactorial experimental design. To ensure an adequate food intake, all the rats were force-fed by gastric tube. The dietary fat had a stronger effect on those parameters than the zinc supply. Rats fed linseed oil had lower concentrations of cholesterol, triglycerides, and phospholipids in plasma and lipoproteins but a higher susceptibility of LDL to lipid peroxidation than rats fed olive oil. The effect of zinc deficiency on those parameters were modified by the dietary fat. Zinc-deficient rats fed linseed oil had increased concentrations of total lipids, cholesterol, and phospholipids in plasma as well as in low- and high-density lipoproteins and an increased susceptibility of LDL to copper-induced lipid peroxidation compared to zinc-adequate rats fed linseed oil. The increased susceptibility of LDL in those rats might be attributable to increased levels of arachidonic acid and eicosapentaenoic acid in LDL. In contrast, in zinc-deficient rats fed olive oil, concentrations of lipids in plasma and lipoproteins were only slightly changed and the susceptibility of LDL to copper-induced lipid peroxidation was reduced compared to zinc-adequate rats fed olive oil. Tocopherol concentrations in plasma, expressed per mol lipid, were not influenced by zinc deficiency in the rats fed both types of fat. Therefore, a changed vitamin E status might not be involved in the effects of zinc deficiency on the susceptibility of LDL. In conclusion the study shows that the effects of zinc deficiency on the concentrations of plasma lipids and the susceptibility of LDL to lipid peroxidation depend on the type of fat. Therefore, the type of dietary fat must be given attention in the investigation of the effects of zinc deficiency on parameters of lipid metabolism. (J. Nutr. Biochem. 8:461–468, 1997) © *Elsevier Science Inc. 1997*

Keywords: zinc deficiency; lipids; lipoproteins; LDL peroxidation; rat; olive oil; linseed oil

Introduction

Oxidative damages are generally believed to be an important factor in many pathological processes.[1] In humans, in the pathogenesis of atherosclerosis, there is increasing evidence that lipid peroxidation in low-density lipoproteins (LDL) may be involved.[2] Several nutritional factors such as the intake of vitamin E as an antioxidant[3,4] or the fatty acid composition of dietary fats[5–7] ingested have been shown to influence the susceptibility of LDL to lipid peroxidation in humans and laboratory animals. Deficiencies of nutritionally essential minerals such as magnesium[8] or copper[9] also have been demonstrated to increase the susceptibility of LDL to lipid peroxidation in rats. Zinc deficiency has been shown to elevate lipid peroxidation in several tissues.[10–12] However, there is less information about the effects of zinc deficiency on the oxidative susceptibility of LDL. A recent study[13] demonstrating increased levels of highly polyunsaturated fatty acids in plasma lipids of zinc-deficient rats indicates an increased susceptibility to lipid peroxidation.

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Therefore, one aim of the present study was to investigate the oxidative susceptibility of LDL in zinc-deficient rats.

Concentrations of lipids in plasma, particularly that of cholesterol in low- and high-density lipoproteins are another important factor influencing the progress of atherosclerosis in humans.[14] Therefore, the second aim of the present study was to investigate the effect of zinc deficiency on concentrations of lipids in plasma and lipoproteins.

A general problem in zinc deficiency experiments is that rats largely reduce the food intake a few days after a zinc-deficient diet has been administered.[15] Therefore, rats fed a zinc-deficient diet suffer not only from zinc deficiency but generalized malnutrition. Thus, energy deficiency confounds the effects of zinc deficiency even in experiments including pair-fed control rats, particularly on parameters of lipid metabolism. For instance, concentrations of lipids in plasma of zinc-deficient and pair-fed control rats are much lower than in ad-libitum fed control rats.[16] To investigate the effect of zinc deficiency without distortion by the confounding effect of a very low food intake, we fed rats a zinc-deficient diet by gastric tube. The type of dietary fat is an important nutritionally factor influencing the concentrations of lipids in plasma and lipoproteins as well as the susceptibility of plasma lipids to lipid peroxidation. Fats with high amounts of polyunsaturated fatty acids (PUFA) reduce concentrations of lipids in plasma[17,18] and elevate the oxidative susceptibility of LDL[5–7] in comparison with fats containing high amounts of saturated or monounsaturated fatty acids. To investigate whether the effect of zinc deficiency on the oxidative susceptibility of LDL depends on the dietary fat, the present study used two different dietary fats: linseed oil with a high amount of PUFA and olive oil with a high amount of oleic acid.

Methods and materials

Animals and diets

Forty-four male Sprague-Dawley rats weighing 133 (±5) g were divided into four groups of 10 (zinc-adequate groups) and twelve (zinc-deficient groups) rats: (I) Zinc-adequate, olive oil; (II) zinc-deficient, olive oil; (III) zinc-adequate, linseed oil; and (IV) zinc-deficient, linseed oil. All the rats were fed three times (0800, 1500, 2200) per day by intragastric tube.[19] The diet consisted of (in g per kg): casein (200), corn starch (300), sugar (258), oil (olive oil or linseed oil, 150), cellulose (30), mineral mixture (40), vitamin mixture (20), and DL methionine (2). The fatty acid composition of the dietary fats is given in *Table 1*. The basal experimental diets with both types of dietary fat contained 0.5 mg Zn/kg; zinc-adequate diets were supplemented with 45 mg Zn/kg as zinc-sulfate heptahydrate. The basal tocopherol concentrations of the diets were: Linseed oil diet, 0.8 mg α-tocopherol and 74.7 mg γ-tocopherol per kg; olive oil diet, 26.6 mg α-tocopherol and 3.9 mg γ-tocopherol per kg; other tocopherols existed only in traces in both types of diet. All the diets were adjusted to a vitamin E activity of 75 I.U./kg by supplementation with all-*rac*-α-tocopherol acetate. Other vitamins and minerals were supplied in sufficient amounts as described previously.[20] Dietary slurries were prepared freshly before each feeding by mixing dry diet (85 g) with oil (15 g), and 55 mL double distilled water (with or without added zinc sulfate). The intragastric tube consisted of a 5-mL syringe connected with a slide catheter. During tube feeding the rat was hand-held, the catheter was inserted into the stomach of the rat, and the slurry was slowly injected. Each rat received 4.8 mL of slurry per feeding, representing 10.5 g of food dry matter per day. The rats had free access to drinking water (double distilled water, supplemented with 0.14 g/L sodium chloride to adapt osmolarity to that of tap water).

Table 1 Fatty acid composition of the dietary oils

Component	Olive oil (g/100 g fatty acids)	Linseed oil (g/100 g fatty acids)
16:0	9.6	5.1
16:1	0.9	—[1]
18:0	3.1	3.2
18:1	74.5	17.7
18:2	10.5	14.6
18:3 n-3	0.3	59.5
18:3 n-6	0.3	—[1]
20:1	0.7	—[1]

[1]Fatty acid exists only in traces (<0.1 gm/100 gm fatty acids).

After 12 days, rats fed both types of zinc-deficient diets showed symptoms of zinc deficiency such as sparse and coarse hair, and skin lesions around mouth, paws, and eyes. Therefore, the experiment was terminated after 13 days of feeding. Rats were overnight fasted, and killed by decapitation after a light anesthesia.

Preparation of lipoproteins and LDL oxidation

The fresh whole blood was collected in polyethylen tubes containing heparin to prevent clotting, and divided into two aliquots. One of those aliquots was supplemented with EDTA (1 mg/mL) and BHT (4.4 μg/mL).[21] This aliquot was used for most of the analyses performed (LDL oxidation experiments, fatty acid and tocopherol analyses, determination of thiobarbituric acid-reactive substances (TBARS). Plasma lipids were prepared by stepwise ultracentrifugation (230.000 g, 20 hr, 8°C). Plasma densities were adjusted by addition of potassium bromide. The lipoprotein classes (VLDL, $d < 1.019$ kg/L; LDL, $1.019 < d < 1.063$ kg/L; HDL, $1.063 < d < 1.21$ kg/L) were removed from each tube by suction with a pipette.

The concentrations of total cholesterol, triglycerides and phospholipids in plasma and lipoprotein fractions were measured enzymatically using an auto analyzer (model 704, Hitachi, Tokyo, Japan) and commercial available kit reagents (Boehringer, Mannheim, Germany).

Susceptibility of LDL to lipid peroxidation was performed as described by Esterbauer et al.[21] An aliquot of the LDL fraction was dialyzed overnight in the 100 fold volume of 0.01 M phosphate buffer (pH 7.4), 0.16 M NaCl, 0.1 μg/mL chloramphenicol. The buffer was changed four times. The dialyzed LDL fraction was diluted with the dialysis buffer and was then incubated in the presence of $CuSO_4$. The final conditions of all the samples were: 0.05 mg protein/mL, 1 μM $CuSO_4$, 25°C. The formation of conjugated dienes was measured by continuously monitoring the increase in absorbance at 234 nm. LDL samples from the rats fed the linseed oil diet were monitored for 6 hr, whereas those from the rats fed the olive oil diet were monitored for 24 hr because of their higher resistance against lipid peroxidation. For calculation of diene concentrations, an extinction coefficient E_{234nm} of 29,500 $mol^{-1} \cdot cm^{-1}$ was used.

Table 2 Body weight and zinc status of the rats

Parameter	Zn+, olive oil (10)	Zn−, olive oil (12)	Zn+, linseed oil (10)	Zn−, linseed oil (12)
Final body weight (g)[1]	193 ± 3[a]	180 ± 3[c]	195 ± 3[a]	186 ± 3[b]
Daily body weight gain (g)[1,2]	4.57 ± 0.11[ab]	3.60 ± 0.19[c]	4.73 ± 0.18[a]	4.10 ± 0.14[b]
Zinc concentration in plasma (μmol/L)[1]	19.4 ± 0.3[a]	7.8 ± 0.5[c]	17.6 ± 0.6[b]	7.5 ± 0.4[c]
Alkaline phosphatase in plasma (U/L)[1,2]	433 ± 24[a]	267 ± 21[b]	428 ± 41[a]	199 ± 10[c]

Results are means ± SEM. Means with different superscript letters (a,b,c) within the four treatment groups differ significantly by Fisher's multiple range test ($P < 0.05$). The number of analyses is given in parenthesis.
Results of ANOVA: 1, significant effect of factor zinc ($P < 0.05$); 2, significant effect of factor fat ($P < 0.05$).

Determination of fatty acids, tocopherols and TBARS

Lipids of plasma and LDL samples were extracted with a chloroform/methanol-mixture (2:1, v/v). Fatty acids of plasma and LDL total lipids were converted into methyl esters by transesterification with boron fluoride/methanol reagent.[22] Fatty acid methyl esters were separated by gas chromatography using a Hewlett-Packard HP 5890A gas chromatographic system (Hewlett-Packard, Taufkirchen, Germany), fitted with an automatic on-column injector, a flame ionization detector and a CP-Sil 88 capillary column (50 m × 0.25 mm internal diameter, film thickness 0.2 μm; Chrompack, Middleburg, The Netherlands). Fatty acid methyl esters were identified by comparing their retention times with those of individual purified standards and quantified with heptadecanoic acid methyl ester as an internal standard.[20]

Concentrations of α- and γ-tocopherol in plasma were determined by HPLC.[23] Plasma samples (100 μL) were mixed with 1 mL 1% pyrogallol solution (in ethanol, absolute) and 150 μL saturated sodium hydroxide solution. This mixture was heated for 30 min at 70°C. δ-Tocopherol was added as an internal standard, and tocopherols were extracted three times with n-hexane. Individual tocopherols of the extract were separated isocratically using a mixture of n-hexane and 1,4 dioxane (96:4, v/v) as mobile phase and a LiChrosorb Si 60 column (5 μm particle size, 250 mm length, 4 mm internal diameter; Merck, Germany) and detected by fluorescence (excitation wavelength: 295 nm; emission wavelength: 320 nm).

TBARS in plasma were determined according to Conti et al.[24] 50 μL plasma were mixed with 1 mL TBA reagent (10 mM TBA in 0.1 M phosphate buffer, pH 3) and 50 μL of 0.5% solution of butylated hydroxytoluene (in absolute ethanol). The mixture was heated for 1 hr at 100°C. Then TBARS were extracted with n-butanol and the fluorescence was measured (excitation wavelength: 515 nm; emission wavelength: 553 nm). 1,1,3,3 tetramethoxypropane was used as standard.

Zinc analysis and activity of alkaline phosphatase

Zinc concentration of plasma was determined directly by aspirating a dilute solution (1:5) into the flame of an atomic absorption spectrophotometer (model 303; Perkin Elmer, Überlingen, Germany). The activity of alkaline phosphatase in plasma was determined using a commercial reagent kit (Boehringer) in an auto analyzer.

Statistical analysis

Treatment effects were analyzed by two-way analysis of variance (ANOVA). Classification factors were zinc supply, dietary fat and the interaction between those factors. For statistical significant F-values ($P < 0.05$), means were compared by Fisher's multiple range test.

Results

Body weight gain and zinc status of the rats

Data about body weight gain and zinc status of the rats are shown in *Table 2*. Zinc-adequate rats had a higher final body weight, a higher daily body weight gain, a higher zinc concentration in plasma, and a higher activity of alkaline phosphatase in plasma than the equivalent zinc-deficient rats. According to ANOVA, the dietary fat also influenced daily body weight gains and the activity of alkaline phosphatase in plasma. Zinc-deficient rats fed the linseed oil diet had a higher final body weight and higher daily body weight gains but a lower activity of alkaline phosphatase in plasma than zinc-deficient rats fed the olive oil diet. Moreover, zinc-adequate rats fed the olive oil diet had a slightly higher zinc concentration in serum than zinc-adequate rats fed the linseed oil diet.

Concentrations of lipids in plasma and lipoproteins

Among the two experimental factors, the type of dietary fat had a far more pronounced effect on concentrations of lipids in plasma and lipoproteins than the zinc supply. Rats fed the olive oil diets had higher concentrations of all the lipids, particularly of triglycerides in plasma and all the lipoprotein fractions than the rats fed the linseed oil diet with the exception of cholesterol in high-density lipoproteins (HDL) (*Table 3*). The effect of zinc deficiency on concentrations of lipids in plasma and lipoproteins was more pronounced in the rats fed linseed oil than in the rats fed olive oil. Zinc-deficient rats fed the linseed oil diet had increased concentrations of cholesterol in plasma, HDL and LDL and increased concentrations of phospholipids in plasma and HDL compared with zinc-adequate rats fed the linseed oil diet. The concentration of total lipids in plasma was 22% higher in zinc-deficient rats than in zinc-adequate rats fed the linseed oil diet. In contrast, in the rats fed olive oil, zinc deficiency did not influence the concentration of total lipids in plasma. Nevertheless, there were some alterations of individual lipid concentrations in plasma and lipoproteins. Zinc-deficient rats fed the olive oil diet had higher concentrations of phospholipids in plasma, HDL and LDL and a higher concentration of cholesterol in HDL than zinc-adequate rats fed the olive oil diet. Moreover, zinc-deficient rats fed the olive oil diet had a higher concentration of total lipids in HDL and a lower concentration of total lipids in VLDL than zinc-adequate rats fed the olive oil diet. The

Table 3 Concentrations of lipids in plasma and lipoproteins

Parameter	Zn+, olive oil (10)	Zn−, olive oil (12)	Zn+, linseed oil (10)	Zn−, linseed oil (12)
Plasma (mmol/L)				
Total cholesterol[1,2]	2.46 ± 0.11[a]	2.52 ± 0.14[a]	1.46 ± 0.08[c]	1.82 ± 0.06[b]
Triglycerides[2]	2.48 ± 0.26[a]	2.24 ± 0.27[a]	0.69 ± 0.12[b]	0.81 ± 0.08[b]
Phospholipids[1,2]	1.84 ± 0.08[b]	2.14 ± 0.11[a]	1.13 ± 0.06[d]	1.38 ± 0.04[c]
Total[2]	6.78 ± 0.38[a]	6.90 ± 0.49[a]	3.29 ± 0.23[c]	4.01 ± 0.12[b]
HDL (mmol/L)				
Total cholesterol[1]	0.82 ± 0.04[b]	1.00 ± 0.04[a]	0.80 ± 0.03[b]	1.03 ± 0.04[a]
Triglycerides[2]	0.09 ± 0.01[a]	0.09 ± 0.01[a]	0.06 ± 0.01[b]	0.07 ± 0.01[b]
Phospholipids[1,2]	0.90 ± 0.04[b]	1.19 ± 0.04[a]	0.72 ± 0.03[c]	0.88 ± 0.03[b]
Total[1,2]	1.81 ± 0.07[b]	2.28 ± 0.08[a]	1.58 ± 0.07[c]	1.97 ± 0.08[ab]
LDL (mmol/L)				
Total cholesterol[2]	0.73 ± 0.06[ab]	0.85 ± 0.09[a]	0.54 ± 0.04[c]	0.64 ± 0.03[b]
Triglycerides[2]	0.47 ± 0.07[a]	0.67 ± 0.15[a]	0.21 ± 0.02[b]	0.19 ± 0.01[b]
Phospholipids[1,2]	0.48 ± 0.03[b]	0.65 ± 0.06[a]	0.38 ± 0.02[b]	0.44 ± 0.02[b]
Total[2]	1.68 ± 0.11[a]	2.17 ± 0.29[a]	1.13 ± 0.07[b]	1.27 ± 0.05[b]
VLDL (mmol/L)				
Total cholesterol[1,2]	0.91 ± 0.10[a]	0.67 ± 0.09[b]	0.12 ± 0.03[c]	0.16 ± 0.02[c]
Triglycerides[2,3]	1.92 ± 0.20[a]	1.47 ± 0.15[b]	0.42 ± 0.09[c]	0.55 ± 0.07[c]
Phospholipids[2,3]	0.45 ± 0.04[a]	0.30 ± 0.04[b]	0.04 ± 0.01[c]	0.06 ± 0.01[c]
Total[2,3]	3.28 ± 0.30[a]	2.44 ± 0.26[b]	0.58 ± 0.14[c]	0.77 ± 0.10[c]

Results are means ± SEM. Means with different superscript letters (a, b, c, d) within the four treatment groups differ significantly by Fisher's multiple range test ($P < 0.05$). The number of analyses is given in parenthesis. Results of ANOVA: 1, significant effect of factor zinc ($P < 0.05$); 2, significant effect of factor fat ($P < 0.05$); 3, significant interaction between factors zinc and fat ($P < 0.05$).

concentrations of triglycerides in plasma and lipoproteins were not influenced by the zinc supply in the rats fed both types of fat.

Susceptibility of LDL to lipid peroxidation and concentrations of TBARS and tocopherols in plasma

The rate of formation of conjugated dienes during incubation of dialyzed LDL fractions with 1 μM $CuSO_4$ is shown in *Figures 1* and *2*. The susceptibility of LDL to lipid peroxidation was predominately influenced by the dietary fat. LDL of rats fed linseed oil had a markedly lower lag-time and a higher rate of lipid peroxidation than LDL from rats fed olive oil. Average lag-times were 139 ± 10 min for the rats fed the linseed oil diet and 414 ± 46 min (means ± SEM) for the rats fed the olive diet.

Zinc deficiency also influenced the oxidative susceptibility of LDL. However, the direction of this effect depended on the type of fat. In the rats fed the linseed oil diet, zinc deficiency increased the susceptibility to LDL oxidation. Zinc-deficient rats fed the linseed oil diet had a significantly lower lag-time (118 ± 14 vs. 156 ± 11 min,

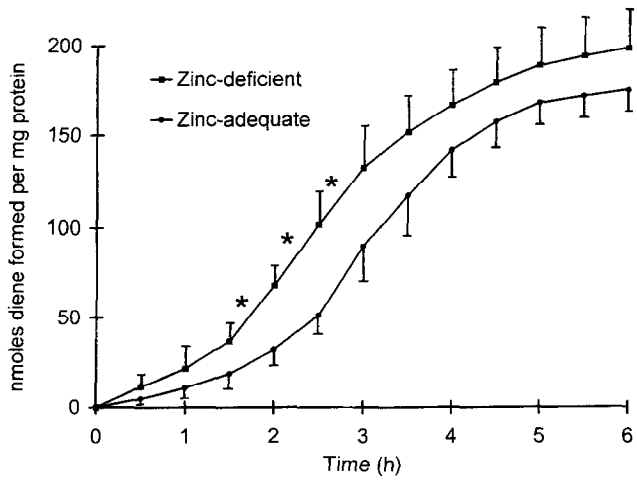

Figure 1 Rate of formation of conjugated dienes during Cu^{++} stimulated oxidation of LDL in the rats fed the linseed oil. Results are given as means and SEM of 10 (zinc-adequate) and 12 (zinc-deficient) samples. An asterisk indicates statistically significant differences ($P < 0.05$) between zinc-deficient and zinc-adequate rats.

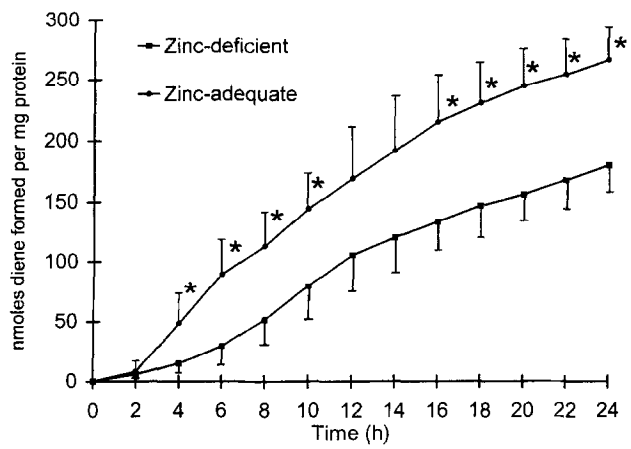

Figure 2 Rate of formation of conjugated dienes during Cu^{++} stimulated oxidation of LDL in the rats fed the olive oil diet. Results are given as means and SEM of 10 (zinc-adequate) and 12 (zinc-deficient) samples. An asterisk indicates statistically significant differences ($P < 0.05$) between zinc-deficient and zinc-adequate rats.

Table 4 Concentrations of TBARS and tocopherols in plasma

Parameter	Zn+, olive oil (10)	Zn−, olive oil (12)	Zn+, linseed oil (10)	Zn−, linseed oil (12)
TBARs				
μmol/L[1]	1.76 ± 0.08[a]	2.17 ± 0.08[b]	1.69 ± 0.10[a]	2.19 ± 0.17[b]
μmoles/mol lipid[2]	266 ± 18[b]	317 ± 22[b]	524 ± 33[a]	556 ± 42[a]
α-tocopherol				
μmol/L[1,2]	29.0 ± 1.4[a]	30.6 ± 1.7[a]	16.7 ± 1.1[c]	22.1 ± 2.2[b]
mmoles/mol lipid[2]	4.28 ± 0.12[b]	4.52 ± 0.22[b]	5.08 ± 0.18[a]	5.53 ± 0.20[a]
γ-tocopherol				
μmol/L[1,2]	—[d]	—[d]	0.70 ± 0.05[b]	1.00 ± 0.12[a]
mmoles/mol lipid[2]	—[d]	—[d]	0.23 ± 0.02	0.25 ± 0.02

Results are means ± SEM. Means with different superscript letters (a, b; c) within the four treatment groups differ significantly by Fisher's multiple range test ($P < 0.05$). The number of analyses is given in parenthesis.
Results of ANOVA: 1, significant effect of factor zinc ($P < 0.05$); 2, significant effect of factor fat ($P < 0.05$). d, exists only in traces ($P < 0.1$ μmol/L).

mean ± SEM, $P < 0.05$) and a higher maximum diene formation (203 ± 14 vs. 183 ± 13 nmol/mg protein, mean ± SEM, $P > 0.05$) than zinc-adequate rats fed the linseed oil diets. However, statistical significant differences in the increase of absorbance were observed only at the time points between 60 and 150 min because of the large standard deviations. In contrast, in the rats fed the olive oil diet, zinc deficiency lowered the susceptibility to lipid peroxidation in LDL. Zinc-deficient rats fed the olive oil diet had a significantly higher lag-time (455 ± 55 vs. 360 ± 84 min, mean ± SEM, $P > 0.05$) and a lower maximum diene formation (183 ± 27 vs. 264 ± 20 nmol/mg protein, mean ± SEM, $P < 0.05$) than zinc-adequate rats fed the olive oil diet.

Concentrations of TBARS and tocopherols in plasma are shown in *Table 4*. Zinc deficiency increased absolute concentrations of TBARS in the rats fed both types of fat. However, relative concentrations of TBARS expressed per mol plasma lipid were not different between zinc-deficient and zinc-adequate rats. In contrast, the dietary fat did not influence the absolute concentrations of TBARS. However, relative concentrations of TBARS (expressed per mol plasma lipid) were higher in the rats fed the linseed oil diet than in the rats fed the olive oil diet. α-tocopherol was the major tocopherol in plasma. Concentrations of γ-tocopherol were low compared with that of α-tocopherol. Rats fed the olive oil diet had higher absolute concentrations of α-tocopherol than those fed the linseed oil diet. However, relative concentrations (expressed per mol plasma lipid) of α-tocopherol were higher in the rats fed the linseed oil diet due to their lower plasma lipid concentrations. The effect of zinc deficiency on absolute tocopherol concentrations depended on the type of dietary fat. Zinc-deficient rats fed the linseed oil diet had higher concentrations of α- and γ-tocopherol than their zinc-adequate controls whereas there was no difference in the concentration of α-tocopherol between zinc-deficient and zinc-adequate rats fed the olive oil diet. However, when expressed per mol plasma lipid, there was no difference in tocopherol concentrations between zinc-deficient and zinc-adequate rats fed both types of fat.

Fatty acid composition of plasma and LDL total lipids

Fatty acid compositions of plasma and LDL total lipids are shown in *Table 5*. The fatty acid composition of plasma and LDL total lipids was markedly influenced by the dietary fat. Rats fed the olive oil diets had markedly higher levels of oleic acid and arachidonic acid but lower levels of linoleic acid, α-linolenic acid, eicosapentaenoic acid, and docosapentaenoic acid in plasma and LDL lipids than rats fed the linseed oil diet. In contrast, total saturated fatty acids and docosahexaenoic acid were not different between the rats fed linseed oil and rats fed olive oil. The average number of double bonds of fatty acids in plasma and LDL lipids was 1.38 and 1.44, respectively for the rats fed olive oil and 1.92 and 1.83, respectively, for the rats fed linseed oil. The effect of zinc deficiency on LDL and plasma fatty acids depended on the dietary fat. In the rats fed the olive oil diet, zinc deficiency had only slight effects on the fatty acid composition of plasma and LDL total lipids. The only changes caused by zinc deficiency was an increased level of arachidonic acid (in plasma and LDL) and a reduced level of linoleic acid (in LDL). Levels of total saturated fatty acids and other PUFAs remained unchanged by zinc deficiency. As a consequence of fatty acid alterations, the double bond index of fatty acids in plasma total lipids was slightly increased by zinc deficiency whereas that of LDL total lipids was not changed. In the rats fed the linseed oil diet, the effect of zinc deficiency on fatty acid composition was more pronounced. Zinc deficiency increased the levels of arachidonic acid and eicosapentaenoic acid (in plasma and LDL) and α-linolenic acid (in plasma) at the expense of linoleic acid (in plasma) or oleic acid and α-linolenic acid (in LDL). In contrast, the levels of total saturated fatty acids and other polyunsaturated fatty acids such as dihomo-γ-linolenic acid and docosahexaenoic acid were not changed by zinc deficiency. As a consequence of the increased levels of arachidonic acid and eicosapentaenoic acid, zinc-deficient rats fed linseed oil had a higher double bond index of fatty acids in plasma and LDL lipids than zinc-adequate rats fed linseed oil.

Table 5 Fatty acid composition of plasma and LDL total lipids

Fatty acid (mol/100 moles)	Zn+, olive oil (10)	Zn−, olive oil (12)	Zn+, linseed oil (10)	Zn−, linseed oil (12)
Plasma				
14:0[2]	0.68 ± 0.03[b]	0.72 ± 0.03[b]	0.95 ± 0.04[a]	0.85 ± 0.05[a]
16:0[1,2]	18.5 ± 0.3[a]	16.9 ± 0.3[b]	15.9 ± 0.3[c]	14.9 ± 0.3[d]
16:1 (n-7 + n-9)[2,3]	3.03 ± 0.08[b]	3.44 ± 0.15[a]	2.56 ± 0.08[c]	2.42 ± 0.12[c]
18:0[1,2]	6.62 ± 0.23[c]	8.17 ± 0.57[b]	9.09 ± 0.23[ab]	9.84 ± 0.32[a]
18:1 (n-7 + n-9)[2]	42.3 ± 0.8[a]	40.0 ± 1.0[b]	16.4 ± 0.3[c]	16.3 ± 0.3[c]
18:2 (n-6)[1,2,3]	13.6 ± 0.2[c]	12.6 ± 0.4[c]	25.4 ± 0.7[a]	19.9 ± 0.4[b]
18:3 (n-3)[2]	0.89 ± 0.11[c]	0.55 ± 0.03[c]	11.0 ± 0.8[b]	13.5 ± 1.2[a]
20:3 (n-6)[2]	0.56 ± 0.02[a]	0.56 ± 0.02[a]	0.50 ± 0.02[b]	0.42 ± 0.02[c]
20:4 (n-6)[1,2]	8.48 ± 0.50[b]	11.3 ± 0.8[a]	6.54 ± 0.21[c]	7.68 ± 0.43[bc]
20:5 (n-3)[1,2,3]	0.38 ± 0.05[c]	0.46 ± 0.05[c]	5.70 ± 0.32[b]	8.62 ± 0.47[a]
22:5 (n-3)[2]	0.22 ± 0.02[b]	0.20 ± 0.02[b]	1.05 ± 0.05[a]	1.12 ± 0.05[a]
22:6 (n-3)[1]	2.45 ± 0.07	2.78 ± 0.12	2.60 ± 0.12	2.81 ± 0.16
Double bond index*	1.33 ± 0.05[d]	1.42 ± 0.07[c]	1.84 ± 0.04[b]	1.99 ± 0.05[a]
LDL				
14:0[1]	1.11 ± 0.09[ab]	0.94 ± 0.08[b]	1.39 ± 0.15[a]	0.95 ± 0.08[b]
16:0[2,3]	15.2 ± 0.3[b]	16.3 ± 0.3[a]	13.7 ± 0.3[c]	12.9 ± 0.2[d]
16:1 (n-7 + n-9)[2]	3.33 ± 0.17[b]	3.81 ± 0.17[a]	2.65 ± 0.15[c]	2.53 ± 0.14[c]
18:0[2]	11.5 ± 0.4[bc]	10.9 ± 0.9[c]	13.0 ± 0.5[b]	14.9 ± 0.6[a]
18:1 (n-7 + n-9)[2]	31.1 ± 1.0[a]	31.5 ± 2.2[a]	15.1 ± 0.7[b]	12.9 ± 0.6[c]
18:2 (n-6)[1,2]	14.0 ± 0.4[b]	12.1 ± 0.4[c]	20.3 ± 0.6[a]	19.1 ± 0.6[a]
18:3 (n-3)[2]	0.51 ± 0.03[c]	0.37 ± 0.03[c]	9.40 ± 0.54[a]	8.45 ± 0.41[b]
20:3 (n-6)[1,2]	0.81 ± 0.04[a]	0.68 ± 0.03[b]	0.54 ± 0.03[c]	0.48 ± 0.02[c]
20:4 (n-6)[1,2]	15.0 ± 1.0[a]	16.5 ± 1.4[a]	7.69 ± 0.29[c]	9.40 ± 0.41[b]
20:5 (n-3)[1,2,3]	0.49 ± 0.03[c]	0.47 ± 0.04[c]	7.51 ± 0.28[b]	10.8 ± 0.5[a]
22:5 (n-3)[2]	0.30 ± 0.02[b]	0.28 ± 0.05[b]	1.57 ± 0.19[a]	1.24 ± 0.11[a]
22:6 (n-3)[2]	0.90 ± 0.03	1.02 ± 0.09	1.12 ± 0.10	1.14 ± 0.11
Double bond index*	1.43 ± 0.13[c]	1.44 ± 0.13[c]	1.76 ± 0.05[b]	1.89 ± 0.07[a]

Results are means ± SEM. Means with different superscript letters (a, b; c) within the four treatment groups differ significantly by Fisher's multiple range test ($P < 0.05$). The number of analyses is given in parenthesis.
Results of ANOVA: 1, significant effect of factor zinc ($P < 0.05$); 2, significant effect of factor fat ($P < 0.05$); 3, significant interaction between factors zinc and fat ($P < 0.05$).
*Average number of double bonds per fatty acid.

Discussion

In humans, concentrations of plasma lipids, particularly that of cholesterol in LDL and HDL as well as lipid peroxidation in LDL play an important role in the pathogenesis of atherosclerosis.[2,14] Zinc deficiency has been shown to affect the lipid metabolism in rats in several ways, and it was of interest whether zinc deficiency also influences those parameters that are known to be involved in the development of atherosclerosis in humans. For this purpose we used a model in which rats were fed zinc-deficient and zinc-adequate diets by gastric tube. Gastric tube feeding has been proved to be a convenient technique to induce a severe zinc deficiency without the confounding effects of low food intake typically observed in rats voluntarily consuming a zinc-deficient diet.[19,20] The food intake in the rats of the present study was lower than that of rats fed an adequate diet ad-libitum[25] but was approximately twice of that of rats fed a zinc-deficient diet ad-libitum.[15] Data about body weight gain as well as zinc concentration and activity of alkaline phosphatase in plasma clearly demonstrate that the rats fed the diets poor in zinc became severely zinc-deficient. Because the dietary fat, particularly its fatty acid composition modifies the lipid metabolism, a bifactorial design was used. The effect of zinc deficiency was investigated in rats fed a diet with high levels of monounsaturated fatty acids and in rats fed a diet with high levels of PUFAs.

The study clearly shows that all the parameters of lipid metabolism measured were markedly influenced by the type of fat. This agrees with several studies reported in literature. The strong hypolipidemic effect of (n-3) PUFA from linseed oil for instance has been reported by Stangl et al.[17] or Balasubramaniam et al.[18] In agreement with the present study, dietary fats with high levels of PUFA have been shown to increase the susceptibility of LDL to lipid peroxidation by an enrichment of PUFA in LDL. In contrast, diets rich in MUFA like in the present study have been shown to reduce the susceptibility of LDL by an enrichment of MUFA.[5–7] Absolute concentrations of tocopherols in plasma were lowered by linseed oil. However, this effect was attributable to the reduced concentrations of lipids in plasma. Other studies also demonstrated that plasma concentrations of tocopherols are correlated with plasma lipid concentrations.[26] In the present study, relative concentrations of tocopherols, expressed per mol lipid, were even higher in rats fed linseed oil. The study also demonstrates that zinc deficiency influences the parameters of lipid metabolism investigated. However, the effects of zinc deficiency were less pronounced than those of the dietary fat, and moreover the effects of zinc deficiency were modified by the type of dietary fat. In the rats fed linseed oil, zinc deficiency slightly increased concentrations of lipids in plasma and lipoproteins; in contrast, in the rats fed

olive oil, zinc deficiency did not change the concentration of total lipids in plasma. Former studies also demonstrated that zinc deficiency increases concentrations of lipids in plasma in rats force-fed sufficient quantities of diet. In those studies the effect of zinc-deficiency in this respect was also more pronounced in rats fed high-PUFA diets than in rats fed low-PUFA diets.[27] Zinc deficiency also has been shown to increase hepatic lipogenesis in force-fed rats.[28] However, a fatty liver as a consequence thereof has been reported only in rats fed low-PUFA diets but not in rats fed high-PUFA diets.[28,29] Therefore, it is likely that increased concentrations of lipids in plasma of zinc-deficient rats fed linseed oil are the consequence of an increased secretion of lipids from liver into the blood. In contrast, it seems that zinc-deficient rats fed diets with predominately saturated and monounsaturated fatty acids are incapable to enhance secretion of lipids from liver into blood, and therefore accumulate lipids in the liver. On the other hand, recent studies demonstrated that a hyperlipidemia can be the result of increased lipid peroxidation in liver.[30] Zinc deficiency causes increased oxidative stress, which may be more pronounced in rats fed linseed oil than in rats fed olive oil.[10–12] The hyperlipidemia observed in zinc-deficient rats fed linseed oil, therefore could be due to a marked oxidative stress in those rats.

The results of the present study concerning the effect of zinc deficiency on plasma lipids in force-fed rats are in contradiction with those observed in classical zinc deficiency studies in which rats were fed zinc-deficient diets ad-libitum. The results of those classical zinc deficiency studies are not quite uniform; however, most of those studies reported either hypolipidemia in zinc-deficient rats characterized by lowered concentrations of cholesterol and triglycerides.[31–33] This means that the effect of zinc deficiency on concentrations of plasma lipids depends not only on the type of dietary but also on the food intake.

Zinc deficiency also influenced the oxidative susceptibility of LDL. The increased susceptibility in the rats fed linseed oil might be attributable to increased levels of arachidonic acid and eicosapentaenoic acid in LDL. Increased levels of eicosapentaenoic acid in tissue lipids of zinc-deficient rats that have been also demonstrated in recent studies are attributed to an elevated incorporation of eicosapentaenoic acid into phosphatidylcholine, which is the major phospholipid in plasma.[13,34] Classical zinc deficiency experiments demonstrated an impaired desaturation of essential fatty acids, and consequently reduced levels of desaturation products in tissues of rats consuming low quantities of diet.[35,36] This effect was not observed in the present study and in recent zinc deficiency experiments with force-fed rats receiving diets with several types of fat.[13,29,34] The reason for this missing effect on fatty acid desaturation is that zinc deficiency impairs the desaturation of linoleic within the short experimental period only if the basal desaturation rate is stimulated by feeding a fat-free diet.[37]

Tocopherol concentrations in plasma, expressed per mol lipid, were not different between zinc-deficient and zinc-adequate rats, and therefore, a changed vitamin E status is unlikely to be responsible for the increased susceptibility of LDL to lipid peroxidation in zinc-deficient rats fed linseed oil. Zinc is an integral part of lipoproteins, and therefore the known role of zinc in preventing lipid peroxidation independent of its effect on metabolism of PUFA may be another possible mechanistic explanation of the increased susceptibility of LDL to lipid peroxidation in zinc-deficient rats fed linseed oil.[38] However, a possible reduced zinc concentration in LDL is unlikely to be the solely reason because in the rats fed olive oil, zinc deficiency did not increase but even lowered the susceptibility of LDL to lipid peroxidation. The increased resistance of LDL against lipid peroxidation in the rats fed olive oil caused by zinc deficiency can not be explained on the basis of fatty acid composition or vitamin E status. Probably other factors such as concentrations of antioxidants other than tocopherols may play a role in this effect. In general, it is likely that the intake of antioxidants with diet, particularly of vitamin E influences the effects of zinc deficiency on LDL peroxidation. The diets of the present study contained 75 I.U. vitamin E being just sufficient in diets with high levels of polyunsaturated fatty acids such as the linseed oil diet. Plasma levels of α-tocopherol, which were higher than 4 mmol per mol plasma lipid, indicate an adequate vitamin E status in all the rats, regardless of the dietary treatment.[39] Although zinc-deficient rats fed linseed oil had elevated levels of arachidonic acid and eicosapentaenoic acid in plasma compared to their zinc-adequate controls, concentrations of TBARS in plasma were not different between zinc-deficient and zinc-adequate rats fed both types of fat. This suggests that there were sufficient concentrations of lipid- and water-soluble antioxidants to prevent against increased lipid peroxidation in vivo.

An interesting effect of the present study was that zinc-deficient rats fed linseed oil had higher body weight gains than rats fed the identical basal diet containing olive oil. This result agrees with other studies,[32,35] which also demonstrated that the type of fat can modify the effect of zinc deficiency on growth and some of the symptoms observed in zinc deficiency. In this respect, those studies demonstrated a beneficial effect of γ-linolenic acid in zinc-deficient rats. The higher activity of alkaline phosphatase in zinc-deficient rats fed the olive oil diet compared with those fed the linseed oil diet might be due to their higher daily body weight gains. High body weight gains have been shown to accelerate zinc depletion in rats fed a zinc-deficient diet.[19]

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