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Zinc deficiency and the activities of lipoprotein lipase in plasma and tissues of rats force-fed diets with coconut oil or fish oil

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The present study was performed to investigate the effect of zinc deficiency on the activities of lipoprotein lipase in postheparin serum and tissues of rats fed diets containing either coconut oil or fish oil as dietary fat, using a bifactorial experimental design. To ensure an adequate food intake, all the rats were force-fed by gastric tube. Experimental diets contained either 0.8 mg zinc/kg (zinc-deficient diets) or 40 mg zinc/kg (zinc-adequate diets). The effects of zinc deficiency on the activities of lipoprotein lipase in postheparin serum and postprandial triglyceride concentrations and distribution of apolipoproteins in serum lipoproteins depended on the type of dietary fat. Zinc-deficient rats fed the coconut oil diet exhibited a reduced activity of lipoprotein lipase in postheparin serum and adipose tissue, markedly increased concentrations of triglycerides in serum, and a markedly reduced content of apolipoprotein C in triglyceride-rich lipoproteins and high density lipoproteins compared with zinc-adequate rats fed coconut oil. By contrast, zinc-deficient rats fed the fish oil diet did not exhibit reduced activities of lipoprotein lipase in postheparin serum and adipose tissue and increased concentrations of serum lipids compared with zinc-adequate rats fed the fish oil diet. This study suggests that a reduced activity of lipoprotein lipase might contribute to increased postprandial concentrations of serum triglycerides observed in zinc-deficient animals. However, it also demonstrates that the effects of zinc deficiency on lipoprotein metabolism are influenced by dietary fatty acids. (J. Nutr. Biochem. 11:132–138, 2000)

Keywords: zinc deficiency; lipoprotein lipase; lipoproteins; rats; serum triglycerides

Introduction

Zinc deficiency produces profound alterations of lipid metabolism. With regard to serum lipids, zinc deficiency has been shown to increase concentrations of triglycerides.[1,2] This effect, according to studies by Koo et al.,[3,4] might be the result of impaired plasma clearance of triglyceride-rich lipoproteins in zinc-deficient rats as a consequence of a reduced activity of lipoprotein lipase in muscle tissue. A more recent study, however, demonstrated that the effect of zinc deficiency on serum triglyceride concentrations is different for various types of dietary fat sources.[1] Zinc-deficient rats fed a diet containing predominantly coconut oil exhibited markedly increased concentrations of serum triglycerides compared with zinc-adequate controls, whereas zinc-deficient rats fed the same basal diet containing linseed oil as a source of fat did not exhibit increased serum triglycerides.[1] This observation suggests that dietary fatty acids modify the effect of zinc deficiency on the rate of serum triglyceride clearance. The present study was undertaken to determine the activities of lipoprotein lipases and hepatic lipase in zinc-deficient rats fed diets with either coconut oil, which contains predominately saturated fatty acids, as a source of fat or fish oil, which contains high levels of highly unsaturated fatty acids, as a source of fat. A general problem when investigating the effects of zinc deficiency is that animals reduce their food intake sharply within a few days of being put on a zinc-deficient diet.[5] To

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Table 1 Composition of the basal diets

Ingredient	Amount g/kg diet
Casein	200
Corn starch	300
Saccharose	258
Oil*	150
Mineral mixture†	40
Cellulose	30
Vitamin mixture‡	20
DL methionine	2

*Fish oil/soybean oil (85:15, w/w) or coconut oil/soybean oil (85:15, w/w).
†Mineral mixture supplied the following (per kg diet): 13.6 g $CaCO_3$; 10.7 g $Na_2HPO_4 \times 2\ H_2O$; 8.2 g KH_2PO_4; 6.0 g KCl; 3.4 g $MgCl_2 \times 6\ H_2O$; 249 mg $FeSO_4 \times 7\ H_2O$; 47 mg $CuSO_4 \times 7\ H_2O$; 46 mg $MnSO_4 \times 5\ H_2O$; 9.0 mg KJ; 4.5 mg $NiSO_4 \times 6\ H_2O$; 1.5 mg $Na_2SiO_3 \times 5\ H_2O$; 0.67 mg $Na_2SeO_3 \times 5\ H_2O$; 0.57 mg $SnCl_2 \times 2H_2O$; 0.51 mg $CrCl_3 \times 6\ H_2O$; 0.50 mg $NaMoO_4 \times 2\ H_2O$; 0.23 mg NH_4VO_3; saccharose to 40 g.
‡Vitamin mixture supplied the following (per kg diet): 1.7 mg all-*trans*-retinol; 25 μg cholecalciferol; 140 mg all-*rac*-α-tocopherol acetate; 1 mg menadione sodium bisulfate; 5 mg thiamin-HCl; 6 mg riboflavin; 6 mg pyridoxine-HCl; 15 mg Ca pantothenate; 30 mg nicotinic acid; 1,000 mg choline chloride; 0.2 mg folic acid; 0.025 mg cyanocobalamine; saccharose to 20 g.

avoid the confounding effects of a very low food intake in this experiment, all rats were force-fed by gastric tube. This technique has been shown to be a practical approach for supplying rats with sufficient energy and nutrients while inducing severe zinc deficiency.[1,2,6–8] It is known that the activity of lipoprotein lipase in vivo is regulated by apolipoproteins of triglyceride-rich lipoproteins and high density lipoproteins (HDL).[9,10] To elucidate whether possible alterations of serum clearance of triglycerides is due to an altered composition of apolipoproteins, the distribution of the major apolipoproteins was determined in individual lipoprotein fractions.

Methods and materials

Animals and diets

Forty-eight male Sprague-Dawley rats weighing 123 ± 4 g were divided into four groups of 12 rats each: (1) zinc-adequate, coconut oil; (2) zinc-deficient, coconut oil; (3) zinc-adequate, fish oil; and (4) zinc-deficient, fish oil. All rats were fed four times (8:00 AM, 1:00 PM, 6:00 PM, and 11:00 PM) a day by intragastric tube.[8] The composition of the experimental diet is shown in *Table 1*. The basal experimental diet contained 0.8 mg Zn/kg; zinc-adequate diets were supplemented with 40 mg Zn/kg as zinc-sulfate heptahydrate. The dietary fats used were based predominately on coconut oil or salmon oil. Because coconut oil contains insufficient quantities of essential fatty acids (linoleic acid and α-linoleic acid), soybean oil was added at 150 g/kg of total dietary fat. The fatty acid composition of both dietary fats is shown in *Table 2*. Fish oil contains a high percentage of highly unsaturated fatty acids, which increases the requirement of vitamin E. Therefore, all diets were supplemented with relatively high levels of vitamin E (140 mg all-*rac*-a-tocopherol acetate/kg diet). Dietary slurries were prepared fresh before each feeding by mixing dry diet (85 g) with oil (15 g) and 55 mL deionized water (with or without added zinc sulfate). The intragastric tube consisted of a 5 mL syringe connected to a slide catheter. During tube feeding, the rat was hand-held and the catheter inserted into the rat's stomach and the slurry slowly injected. Each rat received 4.0 mL of slurry per feeding, representing 11.0 g of dietary dry matter per day. Food rejection by vomiting was completely avoided by feeding the animals at 5-hour intervals. The rats had free access to drinking water (deionized water, supplemented with 0.14 g/L sodium chloride to adjust osmolarity to that of tap water).

Table 2 Fatty acid composition of the dietary oils*

Component	Coconut oil/Soybean oil (g/100 g fatty acids)	Fish oil/Soybean oil (g/100 g fatty acids)
8:0	6.6	—†
10:0	5.0	—
12:0	38.2	—
14:0	14.6	5.9
16:0	10.1	13.4
16:1 (n − 7)	—	8.5
18:0	4.0	1.7
18:1 (n − 9)	9.5	14.3
18:2 (n − 6)	10.6	9.8
18:3 (n − 3)	1.2	10.0
18:4 (n − 3)	—	3.5
20:5 (n − 3)	—	9.9
22:1	—	10.8
22:5 (n − 3)	—	1.2
22:6 (n − 3)	—	9.6

*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.

All rats were housed individually in Macrolon cages. A diurnal 12-hour light/dark cycle, with a temperature of 23°C, and 60% humidity were maintained. After 9 days, rats fed the zinc-deficient diets showed symptoms of zinc deficiency such as sparse and coarse hair; skin lesions around the mouth, eyes, and paws; ataxy; and lethargy. Five rats from the groups fed the zinc-deficient diets died during the experiment and one of the control rats was removed from the experiment.

Analyses

All rats received their last regular meal on the morning of day 11; exactly 3 hours later, the rats were slightly anesthetized with diethyl ether and injected with 150 IU heparin/kg body weight.[11] Exactly 15 minutes after that, the rats were sacrificed by decapitation. Blood was collected from the neck into glass tubes, and liver, heart, and epididymal adipose tissue were excised. Serum was obtained by centrifugation at 1,100 g for 15 minutes. Serum and tissue samples were then stored at −80°C pending analysis. Lipoproteins were separated by stepwise ultracentrifugation (230,000 × g, 20 h, 8°C). Serum densities (δ) were adjusted by the addition of potassium bromide. The lipoprotein classes (VLDL + chylomicrons, $\delta < 1.019$ kg/L; LDL, $1.019 < \delta < 1.063$ kg/L; HDL, $1.063 < \delta < 1.21$ kg/L, where VLDL is very low density lipoprotein, LDL is low density lipoprotein, and HDL is high density lipoprotein) were removed from each tube by suction with a pipette. The concentrations of total cholesterol, triglycerides, and phospholipids in serum and lipoprotein fractions were measured enzymatically using an autoanalyzer (model 704, Hitachi, Tokyo, Japan) and commercially available kit reagents (Boehringer, Mannheim, Germany, and Merck, Darmstadt, Germany).

Activities of total lipase and hepatic lipase were determined according to the methods of Assman and Jabs[12] and Krauss et al.[13]

Table 3 The effect of dietary treatment on body weight gains and zinc status of rats

Parameter	Zn+, coconut oil (n = 12)	Zn−, coconut oil (n = 10)	Zn+, fish oil (n = 11)	Zn−, fish oil (n = 9)
Initial body weight (g)	122 ± 4	123 ± 4	122 ± 5	124 ± 3
Final body weight at day 11 (g)*	159 ± 5	148 ± 6†	161 ± 4	151 ± 7†
Body weight gain from day 1 to day 11 (g)*	36.2 ± 2.9	24.8 ± 7.2†	38.6 ± 3.3	26.5 ± 6.6†
Zinc concentration in serum at day 11 (μmol/L)*	15.1 ± 2.3	4.9 ± 1.2†	14.9 ± 1.5	4.5 ± 1.1†
Alkaline phosphatase in serum at day 11 (U/L)*	844 ± 187	439 ± 171†	1,021 ± 311	465 ± 196†

Results are means ± SD.
*Results of analysis of variance: significant effect of factor zinc ($P < 0.05$).
†Significantly different means between zinc-adequate and zinc-deficient rats within one fat level by Student's t-test ($P < 0.05$).
Zn+–zinc-adequate. Zn−–zinc-deficient.

with modifications. Liver and adipose tissues were homogenized in a buffer consisting of 50 mM tris-[hydroxymethyl]aminomethane, 5 IU heparin/mL, and 2 μg leupeptine/mL, adjusted to pH 8.4. The homogenate was centrifuged (1,000 g, 15 minutes) and the intranatant fraction used for enzyme assay. The measurement of lipase activities was based on the release of [1-^{14}C]-oleate from glycerol-[1-^{14}C]-trioleate during incubation with serum or tissue sample preparations. The activity of lipoprotein lipase in postheparin serum was defined as the difference between total activity and hepatic lipase activity. For preparing the substrate mixture for total lipase assay, a mixture of 80 μL glycerol-[1-^{14}C]-trioleate (92.5 kBq/mL, in hexane:isopropanol, 3:2, v/v) and 800 μL trioleate solution (62.5 mM, in hexane:isopropanol, 3:2, v/v) was dried and then suspended in a solution consisting of 1 mL gum arabic (50 g/L), 2 mL bovine serum albumin (90 g/L), 1 mL buffer (0.99 M Tris, 0.75 M NaCl, pH 8.4), and 0.8 mL activator serum. Activator serum was prepared from fresh pig's blood by heating at 56°C for 1 hour to inactivate lipolytic activity. This mixture was vigorously shaken, incubated at 37°C for 15 minutes and was then ready for enzyme assay. The assay mixture for the determination of hepatic lipase did not include activator serum but contained protamine sulfate (100 μL, 10 g/L). The reaction was started by adding 100 μL of postheparin serum or tissue preparation with 480 μL of substrate mixture; the incubation was carried out at a temperature of 37°C over a period of 1 hour. Then 1.7 mL Dole reagent (isopropanol:n-hexane-1 M sulfuric acid, 40:10:2, v/v), 1 mL n-hexane, and 1 mL water were added. The mixture was partitioned by vortexing and centrifugation. The upper phase was used for separation of the lipids with amino-bonded silica packs (ICT, Bad Homburg, Germany) according to Kaluzny et al.[14] In the first step triglycerides, diglycerides, and monoglycerides were eluted from the column with a mixture of chloroform and isopropanol (2:1, v/v). The selectivity of the separation was assessed using ^{14}C-radiolabelled lipid standards. The chloroform/isopropanol mixture eluted 92.6% of a mixture of mono-, di-, and triglycerides and 0.8% of oleic acid applied to the columns. In the second step free fatty acids were eluted from the columns with a mixture of diethyl ether and glacial acetic acid (98:2, v/v). This mixture eluted 82.5% of ^{14}C-oleate and 0.1% of ^{14}C-glycerides applied to the columns. The radioactivity was measured in both fractions using a liquid scintillation spectrometer (Kontron Instruments, Eching, Germany). Blanks using heat-inactivated serum and tissue homogenates were used to correct for nonenzymatic hydrolysis. Recoveries of ^{14}C-radiolabelled standards were used to correct for incomplete recovery of the lipids.

Apolipoproteins of the individual lipoprotein fractions were separated by gel electrophoresis. Because the apolipoprotein bands from nondelipidated lipoprotein fractions applied to gel electrophoresis were less sharply defined, subsequent electrophoresis was performed on partially delipidated samples.[15] The individual lipoproteins were mixed with ice-cold diethyl ether. Further delipidation was achieved by the addition of 0.5 mL of an ethanol-diethyl ether mixture (3:1, v/v) and vigorous mixing of the samples. The resultant protein suspension was centrifuged and lyophilized with a vacuum concentrator; the protein was resuspended in phosphate buffered saline and desalinated by ultrafiltration using 3 kD ultrafilters (Schleicher & Schuell, Dassel, Germany). To estimate the apolipoprotein content in the purified lipoprotein fractions, the samples were mixed with sample buffer containing sodium dodecyl sulphate (SDS) and dithiothreitol, then heated at 95°C for 5 minutes and applied to a discontinous SDS polyacrylamide gel (12%).[16] The gel was visualized with silver staining.[17] The resultant band intensities were scanned with a video documentation system (Video-Doc, Pharmacia, Freiburg, Germany) and analyzed with the Image Master 1D-program (Pharmacia). The apolipoprotein bands were identified with reference to various molecular weight standards.

Statistical analysis

A two-factor analysis of variance was used to analyze the data. The model included the following as factors: zinc supply (zinc-adequate or zinc-deficient), type of fat (fish oil or coconut oil), and the interaction between those factors. Within the main factors, pairwise comparisons (zinc-adequate vs. zinc-deficient; fish oil vs. coconut oil) were performed using a t-test with the error estimated from the two-factor model. Additionally, means of the zinc-adequate and zinc-deficient groups were compared within the two fat levels using Student's t-test.

Results

Body weight gain, zinc status, and serum lipids

Table 3 shows the data for body weight gain and zinc status of the rats. Zinc-deficient rats fed both types of fat had lower final body weights and body weight gains, lower serum zinc concentrations, and lower activities of alkaline phosphatase in serum than the equivalent zinc-adequate rats. The type of fat did not influence body weight gains or zinc status of the rats.

Table 4 shows the concentrations of lipids in serum and individual lipoprotein fractions. The effect of zinc deficiency on the concentrations of triglycerides in serum and individual lipoproteins was determined by the type of fat. Zinc-deficient rats fed the coconut oil diet had markedly higher concentrations of triglycerides in serum (4.8-fold), chylomicrons and VLDL (6.4-fold), HDL (3-fold), and

Table 4 The effect of dietary treatment on concentrations of lipids in serum and lipoproteins

Parameter	Zn+, coconut oil (n = 12)	Zn−, coconut oil (n = 10)	Zn+, fish oil (n = 11)	Zn−, fish oil (n = 9)
Triglycerides (mmol/L)				
Serum*†‡	0.56 ± 0.29	2.69 ± 3.45§	0.46 ± 0.14	0.47 ± 0.13
Chylomicrons + VLDL*†	0.26 ± 0.14	1.69 ± 2.34§	0.20 ± 0.08	0.21 ± 0.08
HDL*†	0.15 ± 0.06	0.45 ± 0.43§	0.12 ± 0.04	0.13 ± 0.04
LDL*†	0.12 ± 0.06	0.64 ± 0.78§	0.09 ± 0.03	0.10 ± 0.04
Total cholesterol (mmol/L)				
Serum*†	1.91 ± 0.27	2.61 ± 0.52§	1.35 ± 0.41	1.99 ± 0.56§
Chylomicrons + VLDL*	0.13 ± 0.13	0.33 ± 0.29§	0.05 ± 0.03	0.21 ± 0.18§
HDL*†	1.29 ± 0.15	1.49 ± 0.19§	1.02 ± 0.19	1.24 ± 0.18§
LDL*†	0.39 ± 0.09	0.66 ± 0.25§	0.32 ± 0.09	0.43 ± 0.21§
Phospholipids				
Serum*†	1.98 ± 0.25	2.65 ± 0.57§	1.60 ± 0.18	2.07 ± 0.41§
Chylomicrons + VLDL*†	0.19 ± 0.10	0.45 ± 0.37§	0.13 ± 0.03	0.25 ± 0.14§
HDL*†	1.37 ± 0.17	1.73 ± 0.23§	1.14 ± 0.12	1.38 ± 0.16§
LDL*†	0.33 ± 0.11	0.56 ± 0.24§	0.27 ± 0.06	0.35 ± 0.15§

Results are means ± SD.
Results of analysis of variance: *Significant effect of factor zinc ($P < 0.05$); †Significant effect of factor fat ($P < 0.05$); ‡ significant interaction between factors zinc and fat ($P < 0.05$); §Significantly different means between zinc-adequate and zinc-deficient rats within one fat level by student's t-test ($P < 0.05$).
Zn+–zinc-adequate. Zn−–zinc-deficient. VLDL–very low density lipoproteins. HDL–high density lipoproteins. LDL–low density lipoproteins.

LDL (5.3-fold) than zinc-adequate rats fed coconut oil. By contrast, zinc-deficient rats fed fish oil did not exhibit increased levels of triglycerides compared with zinc-adequate rats fed fish oil. The concentrations of cholesterol and phospholipids in serum and lipoproteins were generally higher in rats fed the coconut oil diet than in rats fed the fish oil diet. Zinc deficiency elevated the concentrations of cholesterol and phospholipids in serum and all the individual lipoproteins, regardless of the dietary fat.

Activity of lipase and apolipoprotein distribution of serum lipoprotein fractions

Table 5 shows the activities of lipoprotein lipase and hepatic lipase in postheparin serum and tissues. Because the intravenous injection of heparin is known to release both endothelial lipoprotein lipase and hepatic lipase, lipoprotein lipase activity in serum was measured by subtracting hepatic lipase activity from the total postheparin triglyceride hydrolase activity. In general, the activity of postheparin serum lipases did not differ between zinc-adequate rats fed fish oil and zinc-adequate rats fed coconut oil, which suggests that the activity of these enzymes is not influenced by dietary fatty acids. The effect of zinc deficiency on the activities of these enzymes depended on the type of fat. Zinc-deficient rats fed coconut oil had a lower total lipase activity in postheparin serum than did zinc-adequate rats fed coconut oil. The decrease was due to a significant reduction of lipoprotein lipase, whereas the activity of hepatic lipase was not affected by zinc deficiency. In the rats fed the fish oil diets, zinc-deficiency did not affect the activities of total lipase, lipoprotein lipase, and hepatic lipase in postheparin serum.

The lipoprotein lipase activity in adipose tissue was also reduced by zinc deficiency in rats fed coconut oil but was not affected by zinc deficiency in rats fed the fish oil diet. By contrast, the activity of lipoprotein lipase in cardiac

Table 5 The effect of dietary treatment on activities of lipases in postheparin serum and tissues

Parameter	Zn+, coconut oil (n = 12)	Zn−, coconut oil (n = 10)	Zn+, fish oil (n = 11)	Zn−, Fish oil (n = 9)
Postheparin serum				
Total lipase activity (U)	24.2 ± 11.1	15.7 ± 10.3§	30.1 ± 16.6	30.0 ± 10.5
Lipoprotein lipase (U)*†	14.8 ± 8.7	6.2 ± 5.1§	17.2 ± 9.7	18.7 ± 6.2
Hepatic lipase (U)	9.4 ± 7.4	9.5 ± 7.4	13.0 ± 9.3	11.2 ± 6.3
Adipose tissue				
Lipoprotein lipase (U)*†	1.65 ± 0.59	1.12 ± 0.44§	1.93 ± 0.82	1.64 ± 0.29
Cardiac tissue				
Lipoprotein lipase (U)*‡	0.37 ± 0.15	0.84 ± 0.17§	0.47 ± 0.15	0.72 ± 0.21§

Results are means ± SD.
Results of analysis of variance: *Significant effect of factor zinc ($P < 0.05$); †Significant effect of factor fat ($P < 0.05$); ‡Significant interaction between factors zinc and fat ($P < 0.05$). Units (U) are expressed as μmol oleic acid released/h/mL (serum) and μmol oleic acid released/h/mg protein (adipose tissue, cardiac tissue).
Zn+–zinc-adequate. Zn−–zinc-deficient.

Table 6 The effect of dietary treatment on the composition of apolipoproteins (apo) of serum lipoprotein fractions

Parameter	Zn+, coconut oil (n = 12)	Zn−, coconut oil (n = 10)	Zn+, fish oil (n = 11)	Zn−, Fish oil (n = 9)
VLDL + chylomicrons				
Apo AI	40.3 ± 12.6	38.5 ± 14.7	33.5 ± 16.3	30.8 ± 14.6
Apo AIV	22.7 ± 10.9	32.4 ± 13.3	28.1 ± 12.1	32.0 ± 14.6
Apo C*	27.0 ± 11.6	8.9 ± 5.0†	28.8 ± 14.1	17.0 ± 9.4†
Apo E*	10.2 ± 4.7	16.2 ± 4.8†	8.3 ± 2.9	12.7 ± 5.5†
HDL				
Apo AI*	48.8 ± 4.0	56.5 ± 4.1†	51.1 ± 4.1	57.1 ± 8.2‡
Apo AIV	13.8 ± 4.6	15.6 ± 4.3	12.1 ± 3.9	12.9 ± 5.8
Apo C*	19.2 ± 3.0	10.5 ± 1.7†	18.2 ± 3.6	10.2 ± 1.8†
Apo E*	18.3 ± 2.7	17.5 ± 2.8	18.6 ± 2.0	19.7 ± 4.0
LDL				
Apo B100	58.0 ± 4.1	57.3 ± 6.2	57.2 ± 6.1	56.3 ± 3.0
Apo B48	42.0 ± 4.1	42.7 ± 62	42.9 ± 6.1	43.7 ± 3.0

Results are means ± SD.
Results of analysis of variance: *Significant effect of factor zinc ($P < 0.05$). †Significantly different means between zinc-adequate and zinc-deficient rats within one fat level by Student's t-test ($P < 0.05$).
Zn+–zinc-adequate. Zn−–zinc-deficient. VLDL–very low density lipoproteins. HDL–high density lipoproteins. LDL–low density lipoproteins.

tissue was elevated by zinc deficiency in the rats fed both types of fat.

Table 6 shows the percentage distribution of apolipoproteins in the different lipoprotein fractions. In rats fed both types of fat, zinc deficiency caused an increase of apolipoprotein (apo) E at the expense of apo C in the triglyceride-rich fraction and an increase of apo AI at the expense of apo C in HDL. The levels of major apolipoproteins in LDL (B48 and B100) were not influenced by zinc deficiency, regardless of the type of dietary fat.

Discussion

This study was conducted to determine the effect of zinc deficiency on parameters of lipoprotein metabolism in rats in interaction with various fatty acids. The animals used in this model were fed a zinc-deficient diet by gastric tube. Using this technique, we were able to prevent the reduction in food intake that normally occurs after feeding a zinc-deficient diet. The severity of the zinc deficiency was evident from the sharp reduction in the zinc concentration and the activity of alkaline phosphatase in the plasma. These parameters indicated that the severity of zinc deficiency is not influenced by the type of dietary fat.

This study showed that the effects of zinc deficiency on the metabolism of triglyceride-rich lipoproteins are related to the type of fat. Zinc-deficient animals whose diet contained predominantly saturated fatty acids exhibited reduced activities of total lipase and lipoprotein lipase in postheparin serum and greatly increased concentrations of triglycerides in triglyceride-rich lipoproteins. Because triglyceride-rich lipoproteins are catabolized by endothelial lipoprotein lipase, it may be assumed that the two parameters are causally linked. These results are in agreement with a previous study by Koo et al.,[4] who also noted a marked reduction in lipoprotein lipase activity in zinc deficiency. These authors also showed that the reduction in the activity of this enzyme is attributable not to reduced enzyme synthesis but to changes in the composition of lipoprotein.[4] The present study, like that of Koo et al.,[3] and Koo and Lee,[18] also showed altered compositions of apolipoproteins in zinc deficiency. The reduced levels of apo C in triglyceride-rich lipoproteins and HDL are presumably due to a reduced synthesis of this protein in the liver. Zinc is known to play a major role in gene expression and protein synthesis.[19] Unlike the activity of lipoprotein lipase, the effect of zinc deficiency on the apolipoprotein composition of the lipoproteins was also observed in animals fed the fish oil diet. This observation suggests that this effect is generally due to zinc depletion in the liver. Studies by other authors using a similar experimental technique showed that zinc concentrations in the liver decline within a few days of feeding a zinc-deficient diet.[7,20] Apo C plays a special role in the activation of lipoprotein lipase. It is known that apo CII activates the enzyme, whereas apo CIII inhibits it.[21] The various subfractions of apo C were not determined in this study. A reduced content of apo C in lipoproteins might cause a lowered activity of the enzyme in vivo, which would explain the elevated postprandial triglyceride concentrations. The activity of lipoprotein lipase in this study was investigated using porcine serum as an activator and an artificial substrate. Therefore, we can assume that the zinc deficient animals also had fewer enzyme molecules, or that the specific activity of the enzyme was reduced. The activity of lipoprotein lipase in serum that can be released with heparin represents the activity of the endothelial surface of different tissues. Therefore, it was conjectured that the reduced activity of this enzyme in postheparin plasma might be the consequence of a reduced body mass.[4] However, the fact that the activity of the enzyme in zinc deficiency was diminished in the animals fed coconut oil, but not in the animals fed fish oil, argues against this hypothesis. Body mass in the two zinc-deficient groups was reduced by a similar extent as that in the control groups. Unlike triglyceride-rich lipoproteins and HDL, the relationships between apo B100 and apo B48, the principal apolipoproteins in LDL, were unchanged in zinc deficiency. This is consistent with the observations of other authors.[22]

An interesting observation of this study is that the activity of postheparin lipoprotein lipase in zinc deficiency was not reduced and the postprandial triglyceride concentration not increased when feeding a diet containing fish oil. An earlier study also showed elevated postprandial concentrations of triglycerides in zinc deficiency on a diet with coconut oil, whereas a linseed oil diet was not associated with increased postprandial triglyceride concentrations in zinc deficiency.[1] This suggests that the reduced activity of the enzyme in the zinc-deficient animals on the coconut oil diet is not a primary consequence of a lowered zinc status, because the zinc status was reduced to a similar extent in both zinc-deficient groups, but is a secondary consequence of other defects of the lipid metabolism. These secondary consequences can evidently be prevented by n-3 fatty acids in the diet. Recent studies demonstrated that n-3 fatty acids can also prevent other effects of zinc deficiency on the lipid metabolism. Zinc-deficient animals fed a diet with coconut oil, for example, developed a fatty liver, whereas this did not occur in animals receiving a diet with fish oil or linseed oil.[1,23] The relationship between zinc deficiency, dietary fat, and lipoprotein metabolism is still poorly understood. An earlier study showed that a concomitant deficiency of essential fatty acids aggravates the effects of zinc deficiency on various symptoms.[24,25] A link between zinc deficiency and shortage of essential fatty acids seems unlikely in the present study because all diets contained adequate amounts of essential fatty acids. However, the ratio of n-6 to n-3 fatty acids may play a role in the effects of zinc deficiency on lipid metabolism. Arachidonic acid and eicosapentanoic acid are known to produce eicosanoids with varying activities and different functions.[26] There are several studies in the literature that suggest that reduced formation of eicosanoids plays a role in the pathogenesis of zinc deficiency.[27–29]

The eicosanoids emanating from eicosapentanoic acid may potentially inhibit the effects of zinc deficiency on lipid metabolism. Other possible explanations might be found in endogenous lipid peroxidation. Highly unsaturated fatty acids of fish oil, in particular eicosapentanoic acid and docosahexanoic acid, are incorporated in phospholipids of cell membranes, thereby increasing their oxidative sensitivity.[30] This leads to increased concentrations of lipid peroxidation products in tissues and membranes, even if the vitamin E supply of the experimental animals is high as in this study.[31] Endogenously formed lipid peroxidation products could by secondary action modify the effects of zinc deficiency because even exogenous lipid peroxidation products ingested with the diet have a very strong effect on lipid metabolism.[32]

Further factors may be implicated in the different effect of zinc deficiency on lipoprotein lipase in diets with coconut oil and fish oil. The size and composition of the chylomicrons may play a role in the observed effects. It is well known that the fatty acid profile of the diet is reflected in the fatty acid profile of the chylomicrons.[33] In addition, diets with high concentrations of polyunsaturated fatty acids lead to larger chylomicrons than do diets with low levels of polyunsaturated fatty acids, which can then be degraded more rapidly.[34,35] This observation might be of significance because Koo et al.[36] noted that not only the composition of the apolipoproteins but also the size of the chylomicrons was altered in zinc deficiency. It was interesting that lipoprotein activity was increased in the cardiac muscle of zinc-deficient animals but decreased in adipose tissue. This suggests that the effects of zinc deficiency depend not only on the type of dietary fat but also show organ-specific variations. Compensatory effects may play a role here.

In conclusion, this study confirms previous work that showed clearly that zinc deficiency influences the lipid metabolism in a variety of ways. However, the study also shows that the type of dietary fat is an important determinant of the effects of zinc deficiency. Highly unsaturated fatty acids from fish oil evidently provide some protection against certain effects of zinc deficiency on the lipid metabolism.

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