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The effect of dietary fat on activities of lipogenic enzymes in liver and adipose tissue of zinc-adequate and zinc-deficient rats

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The aim of the present study was to investigate if zinc-deficiency influences the regulation of lipogenic enzymes by dietary polyunsaturated fatty acids. Therefore, rats were fed a fat-free diet with either adequate or deficient zinc supply for 6 days. After that period the groups were divided; half of the rats were given the fat-free zinc-adequate and zinc-deficient diets for another 3 days, whereas the other half was given the same diets supplemented with 5% safflower oil. To control food intake, all the rats were force-fed by gastric tube. At the end of the experiment, zinc-deficient rats fed both, the fat-free diet and the 5% safflower oil diet had largely reduced zinc concentrations and activities of alkaline phosphatase in serum proving their zinc-deficient state. Zinc-deficient rats fed both the fat-free and the 5% safflower oil diet had markedly increased concentrations of triglycerides in liver compared with zinc-adequate rats. Zinc-deficient rats fed both the fat-free diet and the 5% safflower oil diet had increased activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase compared with their controls. In contrast, activities of fatty acid synthase and malic enzyme were not changed by zinc deficiency. This suggests that fatty liver is not mainly due to increased lipogenesis but to other factors such as impaired exclusion of lipids from liver. The addition of safflower oil to the fat-free diet suppressed activities of hepatic lipogenic enzymes in both, zinc-adequate and zinc-deficient rats. However, the suppression was more pronounced in zinc-adequate rats than in zinc-deficient rats. In adipose tissue, addition of safflower oil elevated activities of lipogenic enzymes in zinc-adequate rats but lowered activities in zinc-deficient rats. Those data suggest that zinc-deficiency affects regulation of lipogenic enzymes in liver and adipose tissue. (J. Nutr. Biochem. 7:190–195, 1996.)

Keywords: zinc deficiency; lipogenic enzymes; liver; adipose tissue; force-feeding; rats

Introduction

A general problem in the investigation of the effects of zinc deficiency is that a few days after a zinc deficient diet has been administered, animals largely reduce their food intake. Therefore, the effects of zinc deficiency are confounded by the effects of low food intake. To avoid the confounding effects of low food intake, in a series of experiments the effects of zinc deficiency were investigated in rats fed sufficient amounts of food by gastric tube.[1–5] Those studies revealed that zinc deficiency causes markedly elevated activities of hepatic lipogenic enzymes and a fatty liver when the dietary fat consists of a coconut oil/safflower oil mixture (7:1, w/w).[3] In contrast, when linseed oil or fish oil was used as source of dietary fat, zinc deficiency did not produce a fatty liver, and activities on lipogenic enzymes were only slightly increased compared with zinc-adequate rats.[1,3] Those studies suggested that the dietary fat is involved in the effects of zinc deficiency on lipogenic enzymes. Several studies have shown that the dietary fat plays a role for the regulation of hepatic lipogenic enzymes. Feeding fat-free diets elevates the activities of hepatic lipogenic enzymes,

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whereas feeding diets with polyunsaturated fatty acids (PUFAs), particularly linoleic acid or α-linolenic acid markedly lowers the activities of hepatic lipogenic enzymes by suppression of mRNA levels.[6–8] The mechanism underlying enzyme suppression is not completely understood.[9–11] However, it is very likely that PUFAs alone do not suppress lipogenic enzymes but a metabolite, possibly an eicosanoid. This was demonstrated by the observation that the addition of safflower oil to a fat-free diet failed to suppress lipogenic enzymes if eicosatetraynoic acid, an inhibitor of cyclooxygenase, lipoxygenase, and cytochrome P-450 metabolism was added.[9,10]

We hypothesized that zinc deficiency affects the suppression of hepatic lipogenic enzymes by dietary fatty acids.[3] The present experiment was conducted to test this hypothesis. For this purpose, a model described by Clarke et al.[9] and Tomlinson et al.[10] was used. Zinc-adequate and zinc-deficient rats were initially fed a fat-free diet to elevate activities of hepatic lipogenic enzymes. After 6 days, the rats were switched to the same diet supplemented with 5% safflower oil. After terminating the experiment, activities of lipogenic enzymes in liver and adipose tissue were determined.

Table 1 Composition of the fat-free basal diet

Ingredient	Amount
	g/kg diet
Casein (EDTA purified)	200
Sugar	408
Corn starch	300
Fiber (cellulose)	30
Mineral mixture[1]	40
Vitamin mixture[2]	20
DL-Methionine	2

[1]Mineral mixture supplied the following (per kg diet): 10.74 g $Na_2HPO_4 \cdot 2H_2O$; 8.20 g KH_2PO_4; 6.00 g KCl; 3.40 g $MgCl_2 \cdot 6H_2O$; 13.6 g $CaCO_3$; 248.8 mg $FeSO_4 \cdot 7H_2O$; 47.2 mg $CuSO_4 \cdot 7H_2O$; 46.1 mg $MnSO_4 \cdot 5H_2O$; 9.0 mg KI; 4.48 mg $NiSO_4 \cdot 6H_2O$; 0.50 mg $NaMoO_4 \cdot 2H_2O$; 0.57 mg $SnCl_2 \cdot 2H_2O$; 0.67 mg $Na_2SeO_3 \cdot 5H_2O$; 0.51 mg $CrCl_3 \cdot 6H_2O$; 0.23 mg NH_4VO_3; 1.51 mg $NaSiO_3 \cdot 5H_2O$; sugar to 40 g.
[2]Vitamin mixture supplied the following (per kg diet): 1.7 mg all-*trans*-retinol; 7.5 μg cholecalciferol; 150 mg all-*rac*-α-tocopherol acetate; 5 mg menadione sodium bisulfite; 5 mg thiamin · HCl; 10 mg riboflavine; 6 mg pyridoxine · HCl; 20 mg Ca pantothenate; 50 mg nicotinic acid; 1000 mg choline chloride; 0.2 mg folic acid; 0.025 mg cyanocobalamine; sugar to 20 g.

Methods and materials

Animals and diets

Forty-four male Sprague-Dawley rats weighing 122 g (±5 g) were divided into two groups containing 20 and 24 rats. Throughout the experiment, all the rats were fed four times per day (0800, 1300, 1800, 2300) by intragastric tube.[1–3,12] The group consisting of 20 rats received a semisynthetic basal fat-free (FF) diet supplemented with 40 mg zinc per kg (as zinc sulfate, zinc-adequate group) for 6 days. The second group received the same diet without zinc supplementation (zinc-deficient group). This group included a higher number of animals because of the higher risk of mortality during the experiment. At the morning of day 7, both groups were divided in two groups of 10 (zinc-adequate group) resp. 12 rats (zinc-deficient group). One of the groups remained on the fat-free diet, whereas the other was switched to the same diet added with 50 g safflower oil per kg diet. This feeding period included 3 days in which the rats were fed four times daily according to the regular feeding schedule. The rats were then additionally fed at 0500 and 1000 to stimulate activity of lipogenic enzymes, and were then killed exactly 3 hours after the last feeding.

The zinc concentration of the basal diet was 0.5 mg/kg diet, the concentration of total lipids was 1.3 g/kg diet. The composition of the basal diet is shown in *Table 1*. The rats were housed individually in Macrolon cages. A daily 12-hr light/dark cycle, a temperature of 23°C and 60% humidity were maintained. Diet slurries were freshly prepared before each feeding by mixing 100 g of dry diet with 60 mL of double distilled water (fat-free diet) and 5 g of safflower oil (5% safflower oil diet). The safflower oil used was composed (in g/100 g fatty acids) of palmitic acid (16:0) 7.5, stearic acid (18:0) 2.5, oleic acid (18:1) 13.7 and linoleic acid (18:2 n-6) 75.2; other fatty acids existed only in traces (<0.2 g/100 g fatty acids). Immediately before feeding, the slurry was warmed in a glass bottle at 50°C for a few minutes. The intragastric tube consisted of a 5-mL syringe connected with a slide catheter. During tube feeding, the conscious rat was hand-held. The catheter was then inserted into the stomach of the rat, and the slurry was slowly injected. To avoid contamination, zinc-deficient rats were always fed before zinc-adequate rats. Each rat was fed 4 mL of slurry per feeding (fat-free diet, representing 12.8 g of dry matter per day) resp. 4.16 mL (5% safflower oil diet, representing 13.4 g of 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).

Lipid analyses

Liver lipids were extracted with a hexane-isopranol mixture (3:2, vol/vol, containing butylated hydroxytoluene as antioxidant).[13] For determination of total liver lipids, the solvent of a portion of the extract was evaporated, and the lipid mass was determined gravimetrically. For determination of liver total cholesterol and triglycerides, extract was dissolved in Triton-100 as described by De Hoff et al.[14] Total cholesterol and triglycerides were determined using enzymatic reagent kits obtained from Boehringer (Mannheim, Germany).

Fatty acids of liver total lipids were converted into methyl esters by transesterification with boron fluoride/methanol reagent.[15] 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 automatical 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). The oven temperature program was as follows: 75°C, initial temperature; raised to 160°C with 30°C/min; 160°C held for 1 min; raised to 200°C with 15°C/min; 200°C held for 1 min; raised to 225°C with 10°C/min. The detector temperature was 300°C. FAMEs were identified by comparing their retention times with those of individual purified standards and quantified with heptadecanoic acid methyl ester as an internal standard. Serum lipids were determined using an auto analyzer (Model 704, Hitachi, Tokyo, Japan) and commercial available kit reagents (Boehringer).

Activities of lipogenic enzymes

Approximately 4 g of liver resp. 1 g of adipose tissue from the mesentery of small intestine were homogenized in 10 mL of 0.25 M Sucrose buffer (in 0.1 M phosphate buffer, pH 7.4) using a

Potter-Elvehjem-homogenizer. Homogenates were centrifuged (105,000 g for 1 hr at 4°C), and the supernatants were used for enzyme assays. All the enzymes were assayed by spectrophotometric methods. Glucose-6-phosphate dehydrogenase (G6PDH),[16] 6-phosphogluconate dehydrogenase (6PGDH),[17] and malic enzyme (ME)[18] were determined by the rate of NADP reduction. In the assay of G6PDH, activity of 6PGDH was inhibited by maleimide.[16] Fatty acid synthase (FAS) was determined from the rate of malonyl CoA-dependent NADPH oxidation.[19] Citrate cleavage enzyme (CCE) was determined from NADH oxidation in an assay coupled with malate dehydrogenase.[20]

Zinc analysis and activity of alkaline phosphatase

Zinc concentration of serum 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 serum 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 and addition of safflower oil, as well as interaction between those factors. Means were compared by pairs within the two fat levels using Student's *t*-test.

Results

Weight gain and zinc status of the rats

The initial body weight was 122 g (±5 g) for both, the zinc-deficient and zinc-adequate group. During the first 6 days of the experiment, feeding the zinc-deficient diet did not affect growth of the rats. Body weight gain during this period, in which both groups were fed the fat-free diets was 28.7 g (±4.9 g) in the zinc-adequate rats and 27.3 g (±4.5 g) in the zinc-deficient rats. During the remaining 3 days of the experiment growth was significantly affected by zinc deficiency. Body weight gain during this period was (means ± SD): zinc-adequate rats on fat-free diet, $18.0g^{A}$ (±3.3 g); zinc-deficient rats on the fat-free diet, $2.6g^{B}$ (±5.1 g); zinc-adequate rats on the 5% safflower oil diet, $19.7g^{a}$ (±1.9 g); zinc-deficient rats on the 5% safflower oil diet, $8.7g^{b}$(±8.6 g). Activity of alkaline phosphatase as well as zinc concentration in serum were markedly decreased by zinc deficiency in both, the rats fed the fat-free diet and the rats fed the diet supplemented with 5% safflower oil (*Table 2*).

Table 2 Activity of alkaline phosphatase and zinc concentration in serum

Treatment	Alkaline phosphatase (U/L)[c]	Zinc concentration (μmol/L)[c]
Zn+, FF (10)	428 ± 95[A]	18.8 ± 1.5[A]
Zn−, FF (9)	246 ± 83[B]	3.91 ± 0.46[B]
Zn+, 5% SO (9)	431 ± 107[a]	17.9 ± 1.8[a]
Zn−, 5% SO (12)	311 ± 70[b]	3.63 ± 0.66[b]

Results are means ± SD. Means with different superscript letters (A, B; a, b) differ significantly by Student's *t*-test ($P < 0.05$). The number of analyses is given in parenthesis. Results of ANOVA: c, significant effect of factor zinc ($P < 0.05$).
Abbreviations: FF, fat-free; SO, safflower oil.

Liver and serum lipids

Zinc-deficient rats fed both the fat-free or the 5% safflower oil diet had markedly increased concentrations of total lipids and triglycerides in the liver compared with the equivalent zinc-adequate rats (*Table 3*). Addition of 5% safflower oil did not influence concentrations of total lipids and triglycerides in the liver. The concentration of hepatic cholesterol was not influenced by the zinc supply in both, the rats fed the fat-free diet or the diet supplemented with 5% safflower oil. In serum, zinc deficiency elevated concentrations of triglycerides, cholesterol, and phospholipids in the rats fed both types of diet. Addition of 5% safflower oil had no significant effect on concentrations of triglycerides, cholesterol, and phospholipids in serum lipids in the rats fed zinc-deficient or zinc-adequate diets.

Concentrations of fatty acids in liver are shown in *Table 4*. Zinc deficiency markedly increased concentrations of hepatic total fatty acids, saturated and monounsaturated fatty acids in the rats fed both the fat-free as well as the 5% safflower oil diet. Addition of 5% safflower oil had no effect on concentrations of total, saturated and monounsaturated fatty acids in liver of both zinc-deficient and zinc-adequate rats. The concentrations of (*n*-6) PUFAs independent of the zinc status of the rats were elevated by addition of 5% safflower oil whereas those of (*n*-3) PUFAs were lowered by addition of 5% safflower oil. The zinc supply had no significant effect on concentrations of total (*n*-6) and (*n*-3) PUFAs. However, some of the individual (*n*-6) PUFAs (18:2, 18:3, 20:2, 20:3) were elevated by zinc deficiency, others (20:4, 22:5) were lowered by zinc deficiency.

Activities of lipogenic enzymes in liver and adipose tissue

Activities of hepatic lipogenic enzymes are shown in *Table 5*. Zinc-deficient rats fed the fat-free diets had higher activities of glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) than zinc-adequate rats fed the fat-free diets; in contrast the activities of fatty acid synthase (FAS), malic enzyme (ME), and citrate cleavage enzyme (CCE) were not different between those two groups. Zinc-deficient rats fed the 5% safflower oil diet had higher activities of G6PDH, 6PGDH and CCE than zinc-adequate rats fed the 5% safflower oil whereas the activities of FAS and ME were not different between those two groups of rats. Activities of lipogenic enzymes in adipose tissue are shown in *Table 6*. Zinc-deficient and zinc-adequate rats fed the fat-free diet had similar activities of all the lipogenic enzymes measured in adipose tissue. In contrast, zinc-deficient rats fed the 5% safflower oil had significantly lower activities of all the lipogenic enzymes than zinc-adequate rats fed the 5% safflower oil diet.

Table 7 shows the effect of adding 5% safflower oil to a fat-free diet on the activities of lipogenic enzymes in liver and adipose tissue. Adding safflower oil lowered activities

Table 3 Concentrations of lipids in liver and serum

Parameter	Zn+, FF (10)	Zn−, FF (9)	Zn+, 5% SO (9)	Zn−, 5% SO (12)
Liver				
Total lipids (mg/g)[c]	77.3 ± 15.5[B]	125 ± 33[A]	75.6 ± 20.3[b]	140 ± 38[a]
Triglycerides (μmol/g)[c]	43.0 ± 21.8[B]	96.4 ± 37.4[A]	42.0 ± 19.3[b]	115 ± 46[a]
Cholesterol (μmol/g)	7.61 ± 2.08	7.59 ± 1.70	7.23 ± 1.77	7.84 ± 2.44
Serum				
Triglycerides (mmol/l)[c,d]	0.91 ± 0.35[B]	1.91 ± 0.54[A]	0.73 ± 0.21[b]	2.56 ± 1.41[a]
Cholesterol (mmol/l)[c]	1.54 ± 0.31[B]	2.18 ± 0.46[A]	1.69 ± 0.41[b]	2.24 ± 0.53[a]
Phospholipids (mmol/l)[c]	1.76 ± 0.33[B]	2.46 ± 0.39[A]	1.84 ± 0.37[b]	2.83 ± 0.69[a]

The results are means ± SD. Means with different superscript letters (A, B; a, b) differ significantly by Student's *t*-test ($P < 0.05$). The number of analyses is given in parenthesis. Results of ANOVA: c, significant effect of factor zinc ($P < 0.05$); d, significant interaction between factors fat and zinc ($P < 0.05$).
Abbreviations: FF, fat-free; SO, safflower oil.

in hepatic lipogenic enzymes in both zinc-adequate and zinc-deficient rats. However, the rate of decrease was higher in zinc-adequate than in zinc-deficient rats. In the adipose tissue, adding safflower oil increased activities of lipogenic enzymes in zinc-adequate rats, whereas it decreased the activities in zinc-deficient rats.

Discussion

The present study was performed to investigate the effect of zinc deficiency on regulation of lipogenic enzymes by dietary PUFAs. For this purpose, a fat-free diet was fed for 6 days and thereafter 5% safflower oil was added. The same experimental design has recently been used by other investigators.[9,10] A potential problem of this feeding scheme is that the caloric values of both diets are different by adding the oil. On the other hand, preparing isocaloric diets with and without fat would necessitate varying the amount of carbohydrates. However, when investigating activities of hepatic lipogenic enzymes it is necessary to keep the carbohydrate intake constant because the carbohydrate intake is a major factor influencing the activities of hepatic lipogenic enzymes.[11]

In the present study the addition of safflower oil to a fat-free diet decreased activities of hepatic lipogenic enzymes within 3 days in zinc-adequate rats by 11 to 37%. This observation agrees with several other studies showing that dietary PUFAs suppress hepatic lipogenic enzymes.[6–10] The study also shows that the suppression of hepatic lipogenic enzymes by PUFAs was lower in zinc-deficient rats

Table 4 Concentrations of fatty acids in liver

Fatty acid	Zn+, FF (10)	Zn−, FF (9)	Zn+, 5% SO (9)	Zn−, 5% SO (12)
			(μmol/g)	
Total[c]	196 ± 64[B]	339 ± 100[A]	197 ± 56[b]	374 ± 132[a]
SFA[c]	91.3 ± 32.6[B]	169 ± 51[A]	90.3 ± 28.2[b]	190 ± 74[a]
14:0[c]	2.88 ± 1.54[B]	6.13 ± 2.25[A]	2.87 ± 1.05[b]	6.99 ± 3.38[a]
16:0[c]	69.8 ± 29.2[B]	139 ± 46[A]	68.8 ± 24.2[b]	156 ± 65[a]
18:0[c]	17.8 ± 2.2[B]	22.4 ± 2.8[A]	18.0 ± 3.1[b]	25.5 ± 6.1[a]
MUFA[c]	75.2 ± 32.1[B]	142 ± 53[A]	62.5 ± 25.7[b]	137 ± 52[a]
14:1 (n − 5)[c]	0.32 ± 0.22[B]	0.83 ± 0.41[A]	0.32 ± 0.13[b]	0.91 ± 0.47[a]
16:1 (n − 7 + n − 9)[c]	19.0 ± 9.3[B]	39.1 ± 14.7[A]	16.8 ± 5.5[b]	37.8 ± 16.0[a]
18:1 (n − 7 + n − 9)[c]	55.4 ± 22.6[B]	102 ± 38[A]	44.9 ± 20.3[b]	97.2 ± 37.1[a]
(*n* − 6) PUFA[d]	20.7 ± 1.4	20.0 ± 2.3	38.2 ± 3.9	41.8 ± 9.2
18:2 (*n* − 6)[c,d]	5.37 ± 0.54	5.99 ± 1.13	16.5 ± 3.2[b]	23.7 ± 9.0[a]
18:3 (*n* − 6)[c,d]	0.18 ± 0.04[B]	0.30 ± 0.11[A]	0.53 ± 0.24[b]	1.03 ± 0.78[a]
20:3 (*n* − 6)[c,d,e]	1.03 ± 0.09	1.03 ± 0.15	0.86 ± 0.15[b]	1.46 ± 0.24[a]
20:4 (*n* − 6)[c,d,e]	12.1 ± 1.1[A]	10.3 ± 1.1[B]	17.1 ± 1.1[a]	13.0 ± 1.3[b]
22:4 (*n* − 6)[d]	0.30 ± 0.02	0.33 ± 0.04	0.49 ± 0.19	0.45 ± 0.03
22:5 (*n* − 6)[c,d,e]	1.21 ± 0.19	1.28 ± 0.22	2.11 ± 0.57[a]	1.25 ± 0.31[b]
(*n* − 3) PUFAs[d,e]	5.81 ± 0.59	5.59 ± 0.69	4.21 ± 0.36[b]	4.80 ± 0.68[a]
20:5 (*n* − 3)	0.43 ± 0.03	0.46 ± 0.04	0.46 ± 0.03	0.47 ± 0.04
22:5 (*n* − 3)	0.20 ± 0.05	0.20 ± 0.04	0.15 ± 0.03[b]	0.21 ± 0.06[a]
22:6 (*n* − 3)[d,e]	5.00 ± 0.58	4.73 ± 0.64	3.45 ± 0.33	3.93 ± 0.56
20:3 (*n* − 9)[d,e]	2.24 ± 0.45[A]	1.65 ± 0.52[B]	0.30 ± 0.06[b]	0.45 ± 0.13[a]

Results are means ± SD. Means with different superscript letters (A, B; a, b) differ significantly by Student's *t*-test ($P < 0.05$). The number of analyses is given in parenthesis. Results of ANOVA: c, significant effect of factor zinc ($P < 0.05$); d, significant effect of factor fat ($P < 0.05$); e, significant interaction between factors zinc and fat ($P < 0.05$).
Abbreviations: FF, fat-free; SO, safflower oil.

Table 5 Activities of lipogenic enzymes in liver*

Enzyme	Zn+, FF (10)	Zn−, FF (9)	Zn+, 5% SO (9)	Zn−, 5% SO (12)
FAS[d]	21.3 ± 4.7	22.8 ± 2.8	17.0 ± 3.9	19.7 ± 3.6
G6PDH[c,d]	125 ± 25[B]	155 ± 33[A]	78.3 ± 19.8[b]	132 ± 26[a]
6PGDH[c]	112 ± 16[B]	143 ± 22[A]	99.7 ± 18.6[b]	135 ± 26[a]
ME[d]	99.6 ± 17.8	100 ± 22	69.9 ± 14.1	76.8 ± 18.6
CCE[c]	54.9 ± 7.8	62.5 ± 12.6	45.4 ± 11.4[b]	59.6 ± 14.4[a]

Results are means ± SD. *Activities are expressed as nmoles NADPH oxidized per min · mg protein at 37°C (FAS), nmoles NAOP reduced per min · mg protein at 37°C, (G6PDH, 6PGDH, ME), nmoles NAD reduced per min · mg protein at 37°C (CCE). Means with different superscript letters (A, B; a, b) differ significantly by Student's *t*-test ($P < 0.05$). The number of analyses is given in parenthesis.
Results of ANOVA: c, significant effect of factor zinc ($P < 0.05$); d, significant effect of factor fat ($P < 0.05$).
Abbreviations: FF, fat-free; SO, safflower oil; FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; ME, malic enzyme; CCE, citrate cleavage enzyme.

than in zinc-adequate rats. Therefore, it can be speculated that zinc deficiency may influence the regulation of lipogenic enzymes by dietary PUFAs. It was suggested that the active form of PUFAs that suppresses activities of hepatic lipogenic enzymes may be an eicosanoid.[9,10] Other studies synthesis in adipose tissue, in contrast to liver, is not primarily regulated by dietary fatty acids but by other modulators. Therefore, the distinct influence of dietary PUFAs on lipogenic enzymes in adipose tissue of zinc-deficient and zinc-adequate rats may be explained by other factors such as

Table 6 Activities of lipogenic enzymes in adipose tissue*

Enzyme	Zn+, FF (10)	Zn−, FF (9)	Zn+, 5% SO (9)	Zn−, 5% SO (12)
FAS[c,d]	0.61 ± 0.25	0.53 ± 0.12	0.85 ± 0.10[a]	0.44 ± 0.28[b]
G6PDH[c,d]	4.00 ± 1.23	3.57 ± 0.50	4.80 ± 0.87[a]	2.74 ± 1.20[b]
6PGDH[c,d]	1.66 ± 0.51	1.58 ± 0.28	2.18 ± 0.52[a]	1.33 ± 0.69[b]
ME[c,d]	9.42 ± 3.09	8.70 ± 1.34	11.3 ± 2.7[a]	6.34 ± 2.94[b]
CCE[c]	1.49 ± 0.53	1.14 ± 0.18	1.81 ± 0.30[a]	1.03 ± 0.44[b]

Results are means ± SD. *Activities are expressed as nmoles NADPH oxidized per min · mg adipose tissue at 37°C (FAS), nmoles NADP reduced per minute · mg adipose tissue at 37°C (G6PDH, 6PGDH, ME), nmoles NAD reduced per min · mg adipose tissue at 37°C (CCE). Means with different superscript letters (a, b) differ significantly by Student's *t*-test ($P < 0.05$). The number of analyses is given in parenthesis.
Results of ANOVA: c, significant effect of factor zinc ($P < 0.05$); d, significant interaction between factors fat and zinc ($P < 0.05$).
Abbreviations: FF, fat-free; SO, safflower oil; FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; ME, malic enzyme; CCE, citrate cleavage enzyme.

indicate that polyunsaturated fatty acids exert their suppressive effect after they have been incorporated into hepatic nuclear phospholipids.[21]

An interesting phenomenon observed in the present study was that the activities of lipogenic enzymes in adipose tissue were elevated by addition of safflower oil in zinc-adequate rats, whereas they were lowered in zinc-deficient rats. Data from several studies[22–25] suggest that fatty acid impaired activity of lipoprotein lipase in zinc-deficient rats.[26] Endocrinological changes, particularly lowered concentrations of insulin described in zinc deficiency[12,27] also may be involved in this phenomenon.

The present study, like former studies[1,3,5] shows that zinc-deficient rats force-fed higher amounts of food than they would voluntary consume gain a fatty liver. Experimental data in zinc-deficient rats fed a diet with coconut oil

Table 7 The effect of adding 5% safflower oil to a fat-free diet on activities of lipogenic enzymes in liver and adipose tissue of zinc-adequate and zinc-deficient rats

Enzyme	Liver		Adipose tissue	
	Zinc-adequate	Zinc-deficient	Zinc-adequate	Zinc-deficient
	Change of activity (%)			
FAS	−21	−14	+39	−17
G6PDH	−37	−15	+20	−23
6PGDH	−11	−5	+31	−16
ME	−30	−23	+20	−27
CCE	−21	−5	+22	−10

as the predominate dietary fat suggested that the fatty liver is due to increased hepatic lipogenesis.[3] However, in the present study zinc-deficient rats developed a fatty liver without having clearly increased activities of all the lipogenic enzymes. This demonstrates that other factors than increased lipogenesis are responsible for fatty liver in zinc-deficient rats. Most probable, the fatty liver in zinc-deficient individuals is due to impaired exclusion of lipids from the liver by very low-density lipoproteins because zinc deficiency impairs synthesis of apoproteins and changes structure and diameter of lipoproteins.[28] The increased levels of triglycerides in serum of zinc-deficient rats observed in the present study may be due to impaired activity of lipoprotein lipase[26] and to molecular alterations of lipoproteins.[28]

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References

1 Eder, K. and Kirchgessner, M. (1994). The dietary fat influences the effects of zinc deficiency on liver lipids and fatty acids in rats force-fed equal quantities of diet. *J. Nutr.* **124,** 1917–1926

2 Eder, K. and Kirchgessner, M. (1994). Levels of polyunsaturated fatty acids in tissues from zinc deficient rats fed a linseed oil diet. *Lipids* **29,** 839–844

3 Eder, K. and Kirchgessner, M. (1995). Zinc deficiency and activities of lipogenic enzymes in liver of rats fed coconut oil or linseed oil. *Lipids* **30,** 63–69

4 Eder, K. and Kirchgessner, M. (1995). Activities of liver microsomal fatty acid desaturases in zinc deficient rats force-fed a dict with a coconut oil/safflower oil mixture or linseed oil. *Biol. Trace Elem. Res.* **48,** 215–230

5 Eder, K. and Kirchgessner, M. (1993). Zum Einfluß von Zinkdepletion auf den Fettgehalt und die Fettsäurezusammensetzung von Leber und Gehirn bei zwangsernährten Ratten. *Z. Ernaehrungswiss.* **32,** 187–197

6 Herzberg, G.R. and Rogerson, M. (1988). Hepatic fatty acid synthesis and triglyceride secretion in rats fed fructose- or glucose-based diets containing corn oil, tallow or marine oil. *J. Nutr.* **118,** 1061–1067

7 Wilson, M.D., Blake, W.L., Salati, L.M., and Clarke, S.D. (1990). Potency of polyunsaturated and saturated fatty acids as short-term inhibitors of hepatic lipogenesis in rats. *J. Nutr.* **120,** 544–552

8 Musch, K., Ojakian, M.A., and Williams, M.A. (1974). Comparison of α-linolenate and oleate in lowering activity of lipogenic enzymes in rat liver: evidence for a greater effect of dietary linolenate independent of food and carbohydrate intake. *Biochim. Biophys. Acta* **337,** 343–348

9 Clarke, B.A. and Clarke, S.D. (1982). Suppression of rat liver fatty acid synthesis by eicosa-5,8,11,14-tetraynoic acid without a reduction in lipogenic enzymes. *J. Nutr.* **112,** 1212–1219

10 Tomlinson, J.E., Nakayama, R., and Holten, D. (1988). Repression of pentose phosphate pathway dehydrogenase synthesis and mRNA by dietary fat in rats. *J. Nutr.* **118,** 408–415

11 Iritani, N. (1992). Nutritional and hormonal regulation of lipogenic-enzyme gene expression in rat liver. *Eur. J. Biochem.* **205,** 433–442

12 Schülein, A., Kirchgessner, M., and Roth, H.P. (1992). Auswirkungen eines alimentären Zinkmangels bei zwangsernährten Ratten auf Wachstum, Zinkstatus und Serumkonzentrationen von Insulin und Glukagon. *J. Anim. Physiol. a. Anim. Nutr.* **67,** 157–169

13 Hara, A. and Radin, N.S. (1978). Lipid extraction of tissues with a low-toxicity solvent. *Anal. Biochem.* **90,** 420–426

14 De Hoff, J.L., Davidson, L.H., and Kritchevsky, V. (1978). An enzymatic assay for determining free and total cholesterol in tissue. *Clin. Chem.* **24,** 433–435

15 Morrison, W.R. and Smith, L.M. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J. Lipid. Res.* **5,** 600–608

16 Deutsch, J. (1983). Glucose-6-Phosphat Dehydrogenase. In *Methods of Enzymatic Analysis* (H.U. Bergmeyer, ed.) Verlag Chemie, Weinheim, pp. 190–197

17 Horecker, B.L. and Smyrniotis, P.Z. (1955). 6-Phosphogluconic Dehydrogenase. *Methods Enzymol.* **1,** 323–327

18 Hsu, R.Y. and Lardy, H.A. (1969). Malic enzyme. *Methods Enzymol.* **13,** 230–235

19 Nepokroeff, C.M., Lakshmanan, M.R., and Porter, J.W. (1975). Fatty acid synthase from rat liver. *Methods Enzymol.* **35,** 37–44

20 Takeda, Y., Suzuki, F., and Inoue, H. (1969). ATP citrate lyase (citrate-cleavage enzyme). *Methods Enzymol.* **13,** 153–163

21 Fukuda, H., Tatsumi, H., Iritani, N., Katsurada, A., Kinoshita, C., Fujita, H., and Tanaka, T. (1988). Lipogenic enzyme induction and incorporation of exogenous fatty acid into liver and liver nuclei. *Biochim. Biophys. Acta* **960,** 19–25

22 Nelson, G.J., Kelley, D.S., Schmidt, P.C., and Serrato, C.M. (1987). The influence of dietary fat on the lipogenic activity and fatty acid composition of rat white adipose tissue. *Lipids* **22,** 338–344

23 Michaelis, O.E. IV, Scholfield, D.J., Nace, C.S., and Reiser, S. (1978). Demonstration of the disaccharide effect in nutritionally stressed rats. *J. Nutr.* **108,** 919–925

24 Nace, C.S. Szepesi, B., and Michaelis, O.E. IV. (1979). Regulation of glucose-6-phosphate dehydrogenase and malic enzyme in liver and adipose tissue: Effect of dietary trilinolein level in starved-refed and ad libitum-fed rats. *J. Nutr.* **109,** 1094–1102

25 Becker, W. and Mansson, J.E. (1985). Incorporation of ^{14}C into tissue lipids after oral administration of [1-^{14}C]linoleic acid in rats fed different levels of essential fatty acids. *J. Nutr.* **115,** 1248–1258

26 Koo, S.I. and Lee, C.C. (1989). Effect of marginal zinc deficiency on lipoprotein lipase activities in postheparin plasma, skeletal muscle and adipose tissues in the rat. *Lipids* **24,** 132–136

27 Roth, H.-P. and Kirchgessner, M. (1994). Influence of alimentary zinc deficiency on concentration of growth hormone (GH), insulin-like growth factor I (IGF-I) and insulin in the serum of force-fed rats. *Horm. Metab. Res.* **26,** 404–408

28 Koo, S.I., Algilani, M.S., Norvell, J.E., and Henderson, D.A. (1986). Delayed plasma clearance and hepatic uptake of lymph chylomicron 14C-cholesterol in marginally zinc-deficient rats. *Am. J. Clin. Nutr.* **43,** 429–437