Fisk. Dir. Skr., Ser. Ernæring, Vol. III, No 2, 37-45 (1990)

EFFECT OF UNSATURATED FAT ON ZINC ABSORPTION IN RATS

By

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ABSTRACT

Six groups of rats of normal zinc status were fed six different diets with hens egg albumen as the protein source. The zinc contents of the diets were 12 and 30 mg/kg. The diets contained either fish oil (highly unsaturated fat), lard (saturated fat), or a mixture of these two fat sources. Zinc intake and excretion via faeces and urine were recorded for 15 days. Samples of liver, femur and blood serum were collected for zinc determinations. The activity of the zinc dependent enzyme alkaline phosphatase was measured in blood serum. There were no differences in the food intake or in the weight gain among the groups. The groups given unsaturated fat and low zinc in the diet (12 mg Zn/kg) had the highest percentage retention of zinc. At both dietary zinc levels the groups given unsaturated fat showed the highest hepatic zinc level. The highest activity of alkaline phosphatase was recorded in the serum of the group given unsaturated fat and 30 mg Zn/kg diet. The results indicate that fish oil may have a positive influence on zinc absorption.

INTRODUCTION

Reports from different parts of the world have shown nutritional deficiency of zinc in humans to be fairly prevalent, both in developing and affluent societies (Prasad, 1982). The Norwegian Council of Nutrition has found that zinc is one of the nutrients that may fall below the recommended daily intake in certain population groups. The recommended daily intake of zinc is 15 mg for young males and pregnant women, and 12 mg for adolescents in the Nordic countries, whereas the mean daily intake in Norway is estimated to 11.1 mg (Statens Ernæringsråd, 1986).

In a previous work with rats, the bioavailability of zinc from diets containing raw and boiled fillets from different fish species were compared (Knudsen et al., 1989). The study showed no difference in availability of zinc from raw or boiled fish. But a better absorption of zinc from medium fat fish (redfish) than from lean fish (cod) was observed. We suggested that marine fish fat might enhance the absorption of zinc.

The aim of this study was to investigate the retention of zinc in rats fed diets differing in fat sources at two zinc levels.

A preliminary account of this work was given at the 4th Nordic Nutritional Congress, August 14-17, 1988, Odense, Denmark.

MATERIALS AND METHODS

Diets

The composition of the 6 diets is shown in Table 1. Except for the fat sources and zinc levels, the diets were equal. The diets contained capelin oil (highly unsaturated fat), lard (saturated fat), or 1:1 capelin oil - lard, at 12 mg Zn/kg (low zinc groups) or 30 mg Zn/kg (high zinc groups), supplemented as zinc sulphate ($ZnSO_4 \cdot 7H_2O$). Antioxidant was added to the fat sources, 200 mg Etoxiquin/kg fat. The diets were stored three weeks at -20°C before start of the study, and were kept frozen until the day of feeding.

Animals and experimental design

Forty male albino rats of Møll-Wistar breed, obtained from Møllegård, Denmark, weighing 50-55 g, were randomly divided into six groups with 6 animals in each group. The rats were caged individually in plexiglass cages with stainless steel wire-mesh tops and bottoms, at a room temperature of 20±2°C, with an ambient humidity about 60 %, and a 12 hour light-dark cycle. The cages were designed to separate faeces and urine.

Table 1. Composition of the experimental diets (g/kg).

Ingredients	g/kg
Albumen from eggs	250
Dextrinized potato starch	500
Sucrose	100
Cellulose	50
Fat*	50
Mineralmix**	40
Vitaminmix	10

*: The different fat sources in the experimental diets: UF = capelin oil (capelin = Mallotus villosus) UF/SF = capelin oil/lard (1:1) SF = lard.

**: The mineral mixture was made after recipe from Eggum (1973), and was made without zinc. Zinc was added to the diets as $ZnSO_4x7H_2O$ to approximately 12 (low zinc groups) and 30 mg/kg (high zinc groups).

The feeding period was 15 days. The diets were given dry. The feed consumption was recorded daily and was approximately 8 g/day at start of the experiment, increasing gradually to 12 g/day. The animals reached their food through a tunnel, an arrangement that kept feed loss at a minimum. Deionized distilled water was given *ad libitum*. Faeces and urine were collected daily during the experimental period. The rats were weighed after 5, 10 and 15 days. At the end of the experiment, the animals were anaesthetized by intraperitoneal injection of Mebumal, 0.1 ml/100 g body weight, and exsanguinized by heart puncture.

Analytical methods

Blood samples taken directly from the heart were centrifuged twice at 3000 RPM for 7 minutes, and the serum was collected and stored at -80°C until analysis. Alkaline phosphatase activities in serum were analyzed colorimetrically according to the method described by Tietz (1976). Liver and femur were dissected, weighed and frozen at -20°C. The tissue samples were freeze-dried until constant weight and homogenized.

The samples (approx. 0.1 g) were digested in a 9:1 mixture of 2 ml concentrated nitric and perchloric acid (Merck, suprapure quality) as described by Julshamn et al.(1982). Zinc, copper and iron were analyzed by flame atomic absorption spectrophotometry (AAS). Selenium and phosphorus were analyzed by graphite furnace AAS using nickel as matrix modifier (Lin and Julshamn, 1984).

The accuracy and precision of the AAS methods were tested by analyzing the SRM 1577a Bovine Liver and 1566 Oyster Tissue from National Institute of Standards and Technology (NIST).

All the methods used were found to be satisfactory with regard to the tests.

All data were analysed by a 2-way analysis of variance (ANOVA), followed by single degree-of-freedom comparisons to assess treatment differences between groups.

RESULTS

The analysis of variance showed significant effects of zinc level of the diets on zinc retention, total liver zinc, total femur zinc and femur zinc concentration. For fat sources there were significant effects on zinc retention, total liver zinc, liver copper concentration and on the activity of alkaline phosphatase in serum. By ANOVA there were no significant interactions between zink level and fat source for any of the parameters studied.

Table 2. Feed (g/15-days) and zinc intakes (mg/15 days) of the experimental animals, and weight gain during the experiment (N = 6).

	Low zinc groups			*High zinc groups*		
	UF	UF/SF	SF	UF	UF/SF	SF
Feed intake	111	115	119	110	115	120
SEM	2.0	4.5	2.0	2.9	3.3	2.0
Zinc intake	1.41	1.38	1.32	3.43	3.31	3.62
SEM	0.03	0.05	0.02	0.09	0.10	0.06
Total weight gain	68.2	68.7	72.5	71.7	69.7	72.3
SEM	0.9	2.3	1.6	1.4	3.2	2.5

1) Results are means + SEM (Standard error of means).

It was of interest, however, to study whether there were different effects of fat sources at high and low levels of zinc. The results from the low Zn and the high Zn groups were analyzed separately in one-way analysis of variance.

Feed intake and weight gain

The feed and zinc intakes of the experimental groups are shown in Table 2. The intake of feed was slightly higher in the groups given saturated fat. There were slight differences in zinc intakes between the groups given the same zinc level which were due to small variations in the analysed zinc concentrations of the diets. The analysed zinc concentrations of the diets were: 12.7, 12.0 and 11.1 mg/kg at the low zinc level, and 31.2, 28.8 and 30.2 at the high zinc level in UF, UF/SF and SF respectively.

The weight gains of the experimental animals are also shown in Table 2. The average weight of the rats at start was 53±2 g, and the weight gains during the experimental period were not significantly different.

Retention

As the difference between apparent absorption (zinc intake - zinc excretion in faeces) and retention (zinc intake - (zinc in faeces + zinc in urine)) is small, only the retention values are given in Table 3.

There was a significant effect due to zinc level of the diets on the apparent retention of zinc. The groups given unsaturated fat and mixed fat and low zinc level, showed a significantly higher retention of zinc than the group given saturated fat (1.24, 1.21 and 1.10 μg respectively). At the high zinc level the group given mixed fat retained significantly more zinc than the other groups (1.64, 1.97 and 1.70 μg respectively).

Table 3. Apparent retention [1] of zinc (mg) in growing rats fed different fat sources and zinc levels (N = 6) [2].

	Low zinc groups			High zinc groups		
	UF	UF/SF	SF	UF	UF/SF	SF
Apparent retention						
Mean	1.24 [c]	1.21 [c]	1.10	1.64 [d]	1.97	1.70 [d]
SEM	0.03	0.06	0.03	0.06	0.09	0.09
%	88.4	87.8	83.4	47.8	54.0	46.7

[1] Apparent retention = Zn intake - (Zn in faeces + Zn in urine).
[2] Results with same superscript are not significantly different ($p > 0.05$).

Organ retention of zinc and copper

Liver

There was a significant effect from dietary fat on liver zinc retention at both zinc levels. As shown in Table 4 total zinc in the liver was highest in the groups given unsaturated fat. At both zinc levels, the groups given unsaturated fat

Table 4. The concentrations of zinc and copper (mg/kg dry weight) in the liver [1] and femur [2], and total amount of zinc (μ g) in liver and femur in rats after 15 days on the experimental diets (N = 6).

	Low zinc groups			High zinc groups		
	UF	UF/SF	SF	UF	UF/SF	SF
Total liver Zn	167 [a]	155 [a]	137 [b]	181 [a]	169 [a]	145 [b]
SEM	5.3	12.2	3.8	5.9	6.9	3.8
Liver Zn conc.	114 [c]	115 [c]	108 [c]	112 [c]	124 [c]	116 [c]
SEM	3.0	6.8	2.3	4.1	4.3	3.2
Liver Cu conc.	9.2 [e]	9.2 [e]	13.4 [f]	8.9 [e]	9.9 [e]	11.9 [f]
SEM	0.6	0.7	0.9	0.4	0.9	1.0
Total femur Zn	22.7 [g]	21.7 [g]	23.0 [g]	34.8 [h]	32.2 [h]	35.5 [h]
SEM	0.6	0.6	1.1	0.9	1.6	1.2
Femur Zn conc.	157 [j]	155 [j]	160 [j]	249 [k]	239 [k]	245 [k]
SEM	3.8	4.4	6.5	6.5	5.6	7.0
Femur Cu conc.	6.1 [l]	7.9 [l]	7.8 [l]	6.3 [m]	6.5 [m]	6.5 [m]
SEM	0.3	1.0	1.0	.04	0.08	0.08

[1] Dry matter of liver: 292 ± 5 g/kg.
[2] Dry matter of femur: 502 ± 21 g/kg.
[3] Results with same superscript are not significantly different ($p > 0.05$).

(UF) and mixed fat (UF/SF) had significantly higher amount of hepatic zinc than the groups given saturated fat (SF) (167, 155 and 137 μg at the low zinc level, and 181, 169 and 145 at the high zinc level in the UF, UF/SF and SF groups respectively)

There were no significant effects of dietary zinc levels or fat sources on the zinc concentration in the liver.

There was a significant effect from the dietary fat source on the copper concentration in the livers. The concentration of copper was significantly higher in the livers of the groups given only saturated fat compared to unsaturated fat and mixed fat, at both zinc levels (9.2, 9.2 and 13.4 mg Cu/kg dry weight at the low zinc level, and 8.9, 9.9 and 11.9 at the high zinc level, in the UF, UF/SF and SF groups respectively).

Femur

There was a clear response in femur to increased amounts of zinc ($p<0.05$). However, there was no significant effect on femur zinc concentrations due to the different fat sources, neither in the low zinc groups nor in the high zinc groups.

In femur there was no significant difference in copper concentration at any zinc level or fat regime.

Serum

There was no difference in the zinc levels in serum that could be related to type of fat, or to increased intake of zinc.

The activities of the enzyme alkaline phosphatase were highest in the serum of rats given capelin oil (UF). This difference was statistically significant at the high zinc level ($p<0.05$).

Table 5. Concentration of zinc and activity of alkaline phosphatase in serum in rats fed the experimental diets for 15 days (N = 6). [1)]

	Low zinc groups			*High zinc groups*		
	UF	UF/SF	SF	UF	UF/SF	SF
Serum Zn (mg/L)	1.7^a	1.6^a	1.5^a	1.8^a	1.7^a	1.7^a
SEM	0.1	0.1	0.1	0.2	0.3	0.2
Alkaline phosphatase (U/L)	665^b	575^b	515^b	899	540^b	556^b
SEM	80	29	39	106	23	45

[1)] Results with same superscript are not significantly different ($p > 0.05$).

DISCUSSION

There was no effect of the different dietary treatments on growth, probably because of a good zinc status of the rats. In the present study 12 mg Zn/kg diet was chosen for the low zinc diets because this level is found to be sufficient for optimal growth (NRC, 1978). Bettger et al. (1979) found restricted growth and skin lesions in zinc deficient rats fed a diet of 7 mg Zn/kg and hydrogenated coconut oil. In a previous study on zinc absorption in rats from fish diets with cornoil, we found a lower growth at 5 mg Zn/kg diet than in the control group, but not at 7 mg Zn/kg (Knudsen et al., 1989). All rats in the present experiment gained weight at a normal rate.

Literature data state that the main part of zinc is excreted via faeces, and less than 1 % via urine (Methfessel and Spencer, 1973; Underwood, 1977). The excretion of zinc is shown to be fairly constant and independent of the zinc intake (Weigand and Kirchgessner, 1976). In this study about 3-6 % of the ingested zinc was found in the urine.

The fat source of the experimental diets influenced the apparent retention of zinc as measured by balance study. This is seen in the low zinc groups where the rats fed fish oil (UF) retained most zinc (88.4 %), the mixed fat group (UF/SF) retained slightly less (87.8 %), and the group given lard (SF) retained significantly less than the other groups (83.4 %).

No such clear pattern was found among the high zinc groups even though the mixed fat group retained significantly more than the other groups.

It is possible that the unsaturated fat may influence the absorption of zinc from the gut. This is supported by findings of Hamilton et al. (1981) who indicated that essential fatty acids (EFA) had positive influence on zinc absorption from the gut, and the findings of Cunnane (1982) who found enhanced absorption of zinc in children with the inherited lethal disease acrodermatitis enteropatica (failure of zinc absorption) who were given breastmilk in comparison with cow milk. The higher amounts of linoleic acid or its metabolites in breast milk than in cow milk was thought to influence the absorption of zinc.

From this study it is indicated that unsaturated fat has an effect on apparent retention of zinc at low dietary zinc levels.

The liver has been reported to be a storage organ for zinc (Henry et al., 1987; Momĉiloviĉ et al., 1975). In this study we did not find a significant effect of increased zinc intake on the hepatic zinc concentration. But there was found a pattern towards higher total hepatic zinc with increasing amount of fish oil in the diets.

An increasing number of studies report a negative effect on copper uptake when the zinc intake is increased (Fosmire, 1990). It is suggested that copper in its divalent form is competing with zinc for binding to metallothionein

(Evans et al., 1975). Sandstead (1982) showed in balance studies with men that as the amount of zinc ingested increased, the amount of copper required to maintain balance increased. Sandstead's observations were made at modest zinc levels, near the Recommended Daily Allowances (RDA).

The present study showed no significantly lower copper concentration in the organs at high dietary zinc (30 mg/kg) compared to the low level (12 mg Zn/kg). But there was an effect from fat on the copper concentration in the livers: the groups given only saturated fat had higher copper concentrations than the other groups, at both zinc levels.

Several studies have shown femur to be a more sensitive parameter in measuring the zinc status than the liver (Henry et al., 1987; Knudsen, 1987). Momĉiloviĉ et al. (1975) found that total femur zinc relative to body weight to be the best parameter for the study of zinc bioavailability in rats. In our study the concentration of zinc in the femur was substantially affected by dietary zinc concentration. By increasing the dietary zinc concentration from 12 to 30 mg/kg, the zinc concentration in femur was increased from 160 to about 245 mg/kg dry weight. On the other hand only minor differences were found between the groups fed different fat regimes.

In humans the concentration of zinc in serum or plasma are the most common parameter used for assessing the zinc status (Everett and Apgar, 1987). From our study no significant differences were found in serum zinc concentrations due to dietary treatments. The activity of alkaline phosphatase has also been widely used as a zinc status indicator, and has proved to be a sensitive indicator in rats with zinc deficiency (Dib and Carreau, 1987). In our study where rats were fed diets containing sufficient zinc for normal growth, there were only minor differences in the activity of alkaline phosphatase between the rats fed 12 mg Zn/kg and the rats fed 30 mg Zn/kg. The highest alkaline phosphatase activity was found in the rats fed unsaturated fat and 30 mg Zn/kg diet.

Even though the data are not fully conclusive it is tentatively concluded from the balance study data and the organ retention data that there is a general trend towards better zinc retention in the rats fed fish oil compared to diets with lard. For population groups with marginal zinc intakes, a dietary intake of unsaturated fat may have a positive effect on the bioavailability of zinc. This will need further investigation.

ACKNOWLEDGEMENT

This work was supported by the Norwegian Fishery Research Council, grant no.III 711.047. Cand.scient. Rune Waagbø is thanked for his assistance in analyzing alkaline phosphatase.

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