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ABSORPTION AND ORGAN RETENTION OF ZINC FROM A DIET OF COOKED AND RAW, LEAN AND MEDIUM FAT FISH FILLET

By

EVA ROSENDAHL KNUDSEN, AMUND MAAGE and KAARE JULSHAMN
Institute of Nutrition
Directorate of Fisheries
N-5024 Bergen, Norway

ABSTRACT

Two biological studies were carried out for 14 days each. Eight groups of 8–9 rats, were given diets based on lean fish (Cod), medium fat fish (Redfish), and albumin. The diets were given with or without zinc supplementation. Both fish species were given as raw and boiled, freeze dried fillet. Growth, excretion of zinc in urine and feces, and retention of zinc in liver and femur were measured. All analyzes of zinc were done with flame atomic absorption (AAS). The zinc concentration of femur was shown to be a better parameter than liver for zinc status in the body. We found a better absorption and retention of zinc in femur in the rats given medium fat fish than in those given lean fish. The rats given raw or boiled fish showed no differences in zinc absorption or organ retention. We suggest that the availability of zinc is not altered as the protein is coagulated, but that fish fat increase the bioavailability of zinc.

INTRODUCTION

Dietary surveys in several countries have shown that the mean zinc intake of adult populations are below recommended daily allowances (RDA) (Prasad, 1982; Norwegian Council of Nutrition, 1986). Infants, adolescents and pregnant women are likely to be zinc deficient on a normal diet (Jameson, 1976). Zinc deficiency is also prevalent in old age and in cases of protein-calorie malnutrition and alcoholism (Prasad, 1982).

It is generally accepted that dietary animal protein has a positive effect on the intestinal absorption of zinc (Solomons, 1982) while the availability of zinc from a cereal diet is poor, because of its high phosphate and phytate content (Naevert et al., 1985). Fish is an excellent animal protein source, but contain rather low levels of zinc (approximately 3–6 mg/kg freshweight).

Since increased fish consumption is now recommended in many countries, it is of value to achieve knowledge about zinc absorption from fish diets.

This study was carried out to investigate the zinc biovailability from diets containing fish fillets differing in fat contents, compared to albumin.

MATERIALS AND METHODS

Animals and experimental design

Two experiments (Exp. 1 and 2) were carried out using male albino rats of Møll-Wistar breed (Møllegaard, Denmark), with initial weight 76 ± 5 g (Exp. 1) and 51 ± 3 g (Exp. 2). In Exp. 1, 45 rats were divided into 5 groups. In Exp. 2, 48 rats were divided into 6 groups. Five randomly selected rats were killed at the beginning of each experiment for reference data. The rats were caged individually in plexiglass cages with stainless steel wire-mesh tops and bottoms, in a room maintained at 20 ± 2 °C, with an ambient humidity about 61% and a 12 hour light-dark cycle. The feeding period was 14 days. The feed consumption was recorded daily and was approximately 8 g/d per rat at the start of the experiment, increasing gradually to 14 g/d. The animals reached their food through a tunnel. This arrangement kept feed loss at a minimum. The animals had access to deionized, distilled water. Urine and feces were collected during the feeding periods. The rats were weighed every third day. At the end of the experiment the rats were killed by intraperitoneal injection of Mebumal 0.1 ml/100 g (Exp. 1), or with carbondioxide gas (Exp. 2). Liver, kidneys and femur were dissected, weighed and frozen at − 20 °C.

Diets

The composition of the diets is shown in Table 1. Fish was obtained at the local market. The diets were based on cod fillet *(Gadus morhua)* (Exp. 1) or redfish fillet *(Sebastes marinus)* (Exp. 2) as the protein source. The fillets were minced and freeze dried either raw or after autoclaving for 20 minutes. Eggalbumin (from a.s. Svenske eggprodukter, Sweden) served as the protein source in the control diets.

In each experiment four different fish-diets were used. Two diets were made from raw fish, and the other two diets from cooked fish. One of each diet was supplemented with zinc sulphate to approx. 20 mg Zn/kg. Exp. 1 had one albumin diet, and Exp. 2 had two albumin diets, one supplemented with zinc sulphate. The zinc concentrations of all diets, as determined by analysis, are shown in Table 2. All diets had approx. 950 g/kg dry matter, 220 g/kg protein, and the fat contents were 50 g/kg in the diets of Exp. 1 and 85 g/kg in Expt. 2.

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

Protein source[a])	250
Dextrinized potato starch	500
Sucrose	100
Cellulose	50
Corn oil	50
Mineral mixture[b])	40
Vitamin mixture	10

[a]) Diets in exp. 1:
- C: Cod fillet, minced, raw, freeze dried.
- C+: Cod fillet, minced, raw, freeze dried, Zn-supplemented.
- CB: Cod fillet, minced, boiled, freeze dried.
- CB+: Cod fillet, minced, boiled, freeze died, Zn-supplemented.
- Alb+: Albumin, Zn-supplemented.

Diets in exp. 2:
- R: Redfish fillet, minced, raw, freeze dried.
- R+: Redfish fillet, minced, raw, freeze dried, Zn-supplemented.
- RB: Redfish fillet, minced, boiled, freeze dried.
- RB+: Redfish fillet, minced, boiled, freeze dried, Zn-supplemented.
- Alb: Albumin.
- Alb+: Albumin, Zn-supplemented.

[b]) Eggum (1973), but without zinc.

Analytical methods

Liver, feces and diets were freeze dried, homogenized, and stored in air-tight polyethylene boxes. Femurs were thoroughly cleaned. Zinc was analyzed by flame atomic spectrophotometry (AAS). The samples (approx. 0.1 g) were digested in a mixture of 2 ml concentrated nitric- and perchloric acid (Merck, suprapure quality, 9 : 1) as described by Julshamn et al., (1982). The digested samples were diluted 5 times. The accuracy of the method was tested by analyzing the SRM 1577a Bovine Liver and 1566 Oyster Tissue from National Bureau of Standards. Significant differences between groups were tested by Student t-test on Luxor ABC's IDA-800 statistic pack.

RESULTS

Feed intake and growth

The feed and zinc intake of all groups in both experiments are given in Table 3. The total feed intakes were equal in all groups of Exp. 1, and there were no significant differences in weight gain (Table 3). In Exp. 2 the groups

Table 2. Mean dietary zinc levels in the feeds (± S.D., n = 5).

	Exp. 1					Exp. 2					
	C	C+	CB	CB+	Alb+	R	R+	RB	RB+	Alb.	Alb+
Zn concentration (mg/kg)	6.8	20.6	6.9	20.4	12.4	4.8	17.6	4.6	20.2	2.7	15.1
S.D.	1.0	2.7	0.6	2.0	0.6	0.2	1.5	0.4	2.4	0.4	1.0

Table 3. Feed intakes, percent growth, calculated zinc intakes and excretion, and % absorption of zinc.

	Exp. 1					Exp. 2					
	C	C+	CB	CB+	Alb+	R	R+	RB	RB+	Alb.	Alb+
Total feed intake (g)............	138	139	138	139	137	93	111	90	111	79	111
S.D.	0.4	0.6	1.4	1.4	2.1	9.5	1.2	11.4	2.3	9.3	3.3
Growth, %..........................	100	102	110	107	101	111	155*	103	147*	67	139*
S.D.	9	12	13	12	3	19	15	14	11	11	16
Total Zn intake (μg)	937	2860	984	2845	1706	445	1948	410	2230	210	1675
S.D.	3	12	16	29	25	46	21	52	47	25	49
Zn Excretion (μg)	304	1068	338	1041	489	83	595	83	679	54	219
S.D.	150	247	129	655	184	17	68	14	145	10	91
Absorption (%)[1]................	68	63	65	60	73	78	70**	80	70**	74	87***
S.D.	16	9	13	9	11	7	3	3	6	4	6

[1]) % Absorption = Zn intake – Zn excreted in feces / Zn intake × 100.
* Rats fed zinc supplemented diets showed significantly better growth ($p < 0.001$) than rats given unsupplemented diets.
** Zinc supplemented rats significantly different from unsupplemented at $p < 0.01$.
*** Zinc supplemented rats significantly different from unsupplemented at $p < 0.001$.

given the highest dietary levels of zinc, had significantly higher feed intakes as well as higher growth rates ($p < 0.001$), than the groups given low dietary zinc.

Parallell groups given raw and cooked fish had no significant differences in feed intake or growth.

Absorption

The zinc intakes, excretion in feces, and apparent absorption as determined as the difference between zinc intake and excretion of zinc in feces in percent of intakes, are shown in Table 3. The zinc absorption values were not corrected for endogenous loss and are therefore "apparent" absorption.

The groups given unsupplemented diets showed the highest absorption in both experiments (Table 3). The control groups fed albumin diets (Alb, Alb+) showed a higher zinc absorption than the groups fed the fish diets. The apparent absorption were higher in all groups of Exp. 2 compared to Exp. 1, while the intakes were lower.

No significant differences in absorption were found between the groups given raw or cooked fish, in either of the experiments.

Tissue retention

The zinc concentrations in liver and femur of the rats are given in Table 4. The initial liver zinc levels were higher than the zinc levels at the end of the experiments, due to a dilution effect of growth of the animals. In both experiments, the liver zinc concentrations of the rats given the high zinc diet, were significantly higher than the rats given the unsupplemented diet ($p < 0.01$). The high zinc groups of Exp. 2 had higher liver zinc levels than the corresponding groups of Exp. 1 (Table 4).

The femur zinc concentrations reflected the dietary levels of zinc better than the liver zinc concentrations. The high and low zinc groups showed significantly different zinc levels in femur at $p < 0.001$ in both experiments.

The highest zinc levels in femur were found in the groups fed zinc supplemented albumin diets (Alb+), followed by the zinc supplemented fish groups of Exp. 2. The non supplemented fish groups of Exp. 2, had lower zinc concentration in femur than the corresponding groups in Exp. 1. No statistical differences were found between the raw or cooked fish groups in either of the experiments.

Table 4. Zinc concentration (mg Zn/kg wet weight) and total zinc in liver (n = 8) and in femur (n = 4) of rats fed the experimental diets (Table 1) for 14 days (Mean ± S.D.).

	Exp. 1					Exp. 2					
	C	C+	CB	CB+	Alb+	R	R+	RB	RB+	Alb.	Alb+
Liver (mg/kg)[1]	26.9	28.7**	26.2	29.7*	25.8	27.0	32.4*	26.3	30.5**	25.9	27.0
S.D.	2.2	1.0	1.7	2.4	2.1	2.0	3.0	1.8	3.4	0.9	2.4
(μg)	162.2	177.0	160.7	185.6	163.4	113.5	171.0	115.3	160.9	85.5	142.7
S.D.	13.7	14.7	11.3	8.6	7.8	14.2	26.7	14.6	9.3	9.9	11.5
Femur (mg/kg)[2]	69.2	116.6*	72.0	103.6*	122.8	57.6	120.9*	55.8	125.7*	56.3	142.7*
S.D.	5.1	13.1	9.5	4.9	4.1	8.2	11.1	5.4	14.9	2.2	15.7
(μg)	23.4	38.0	24.2	36.7	39.8	10.4	27.3	10.5	27.3	9.5	30.3
S.D.	2.7	3.8	3.6	1.8	1.3	1.6	1.4	1.4	2.7	1.1	3.1

[1]) Zinc concentration of 0-groups 39.5 ± 1 (Exp. 1) and 39.6 ± 7.2 (Exp. 2).

[2]) Zinc concentration of 0-groups 95.6 ± 13.0 (Exp. 1) and 105.1 ± 17.0 (Exp. 2).

* Zinc supplemented groups significantly different from unsupplemented at $p < 0.001$.

** Zinc supplemented groups significantly different from unsupplemented at $p < 0.05$.

DISCUSSION

No significant differences between the measured parameters were found in these experiments between the groups given raw or boiled fish, neither for the low nor for the high zinc groups. Therefore coagulation of the protein did not influence the availability of zinc in the rat from the diets given.

The lower feed and zinc intakes observed in the low zinc groups in Exp. 2, could be due to anorexia. Animals on a low zinc intake have previously been shown to develop anorexia (Prasad et al., 1967; Miller et al., 1968). Pedersen and Eggum (1983) have proposed marginal zinc deficiency to be a limiting factor in the utilization of protein in food. They found a distinct effect on growth when the zinc concentration in the feed was less than 3 mg Zn/kg diet. In these experiments a clear growth retardation was seen in diets having 4–6 mg Zn/kg.

In both experiments, the albumin diets gave lower growth rates than the fish diets. In spite of this, the zinc concentrations in the zinc supplemented albumin groups (Table 2) should be sufficient for optimal growth, as determined by the NRC (NRC, 1978). Other experiments have shown that at least 20 mg Zn/kg feed is necessary for optimal growth (Weigand and Kirchgessner, 1977), and this seems more in accordance with our results, as the zinc concentrations in the supplemented albumin diets were 12.4 and 15.1 (Exp. 1 and 2, respectively).

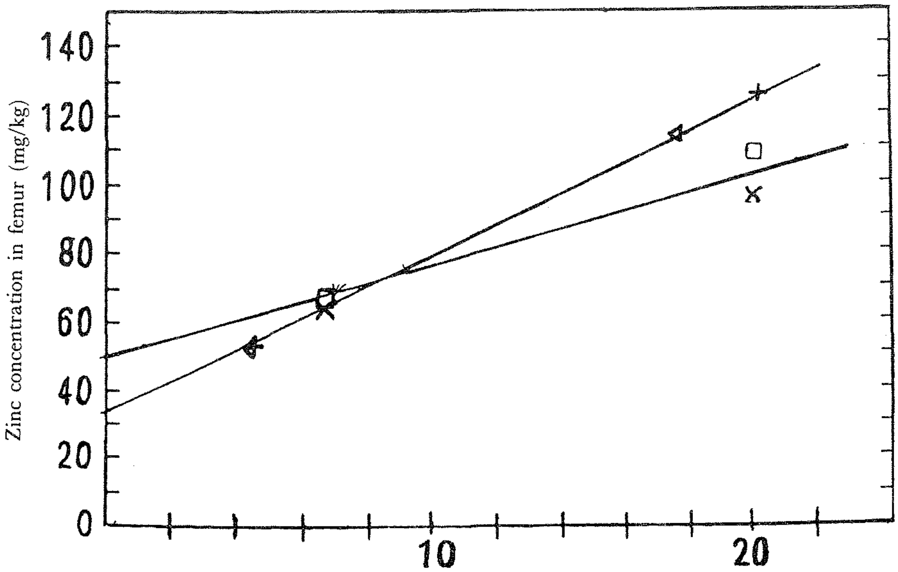
Apparent absorption of zinc from the cod diets in Exp. 1 was approximately 66 and 62% (mean low and high zinc diets, respectively). The absorption from the redfish diets of Exp. 2 was approximately 79 and 70% (mean low and high zinc diets, respectively). Thus the absorption of zinc from the redfish diets were respectively 13 and 8% higher, but one has to take into consideration that the initial weight of the rats in this group was lower, and the rats had a higher growth rate.

Many studies have aimed at finding a good parameter for the zinc status of the body. The zinc concentration in femur seems to give the best picture of the zinc status in rats, followed by liver and kidneys (Momcilovic et al., 1975, Henry et al., 1987). We also found femur to be the best indicator of the bioavailability of zinc from these experiments.

Using femur as the criterion for zinc status, zinc from the albumin diets showed better bioavailability than from fish diets, while giving lower growth and low zinc concentrations in the liver. Other authors (Turnlund and Margen, 1979; Franz et al., 1980; Pedersen and Eggum, 1983) have stated that femur zinc as parameter for zinc availability, is strongly influenced by the type of protein, making it difficult to compare fish diets to albumin diets.

Comparing growth, percent absorption and zinc concentrations in the tissues between the two experiments, the biovailability of zinc seems to be

Figure 1. Effect of zinc intake from diets based on cod- or redfish fillets on the zinc concentration in femur.



Zinc concentration in feed (mg/kg)

◁ +: Redfish diets.
y = 35.2 + 4.6x, r = 1.0

□ ×: Cod diets.
y = 50.7 + 2.9x, r = 0.98

greater from the redfish diets, than from the cod diets. This may be seen from a regression curve were the zinc concentration in femur is plotted against the zinc concentration in the feed (Henry et al., 1987), giving the regression coefficients 2.9 and 4.6 for cod and redfish diets, respectively (Fig. 1).

In experiments with herring meal it was found a greater zinc absorption from diets with 4.2 than 2.4% fat (Maage et al., 1987). Polyunsaturated, essential fatty acids, especially of the linolenic acid group, can stimulate zinc absorption (Bettger et al., 1979). This is thought to be of significance for the better availability of zinc from human milk compared to cows milk (Cunnane, 1982). It has been suggested that essential fatty acids influence the cation absorption in general, probably by modifying the lipid composition in the brushborder membrane, thereby changing the permeability of the membrane (Cunnane and Horrobin, 1980).

The fat contents of the redfish diets were 3% higher than the cod diets and as the fish lipids are highly unsaturated, this could be one reason for a better biovailability of zinc from the redfish diets in these experiments.

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