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Development of zinc deficiency in ^{65}Zn labeled, fully grown rats as a model for adult individuals

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Abstract

The development of zinc deficiency in adults was studied in a metabolism experiment involving 31 adult, female rats labeled homogenously with ^{65}Zn. The animals were fed restricted amounts (8 g/day) of a semisynthetic diet containing either 58 µg Zn/g (control, n = 7) or 2 µg Zn/g (Zn deficiency, n = 24). Control animals were sacrificed at day 0 (n = 3) and day 29 (n = 4). Zinc deficient animals were sacrificed at day 1, 2, 4, 7, 11, 16, 22, and 29 (3 animals per group). The development of zinc deficiency comprised 4 phases: (I) Fecal Zn excretion needed several days to adjust to the low level of Zn intake. The high initial Zn loss via feces was counterbalanced mainly by Zn mobilization from the skeleton. (II) During the 2nd week of deficiency Zn mobilization from tissue storage changed transiently to soft tissues (mainly muscle and fat tissue). (III) After the 2nd week the skeleton resumed to mobilize Zn. (IV) At the end of the study the skeleton Zn storage was exhausted and alkaline phosphatase activity indicated severe Zn deficiency. Urinary Zn excretion was too small to contribute quantitatively to changes in Zn metabolism during any phase of Zn deficiency. In conclusion, adults may compensate a deficient Zn supply by mobilizing tissue Zn for several weeks. The skeleton revealed to be the major short-term as well as long-term source of whole body tissue Zn that can be mobilized.

Key words: zinc deficency, adult, ^{65}Zn, metabolism, mobilization

Introduction

Model studies on zinc deficiency are usually performed with fast growing laboratory animals (e.g. rats). But the intensive anabolic situation may cause severe interferences between the effects of zinc deficiency *per se* and the metabolism *in toto*, like the cyclic depression of food intake and the stagnation of growth. Furthermore, the increase in body mass raises the pool size of tissue Zn and complicates measurements on Zn exchange, redistribution and mobilization within tissues. In bones for example, it is difficult to distinguish true mobilization of Zn from indirect "dilution" due to a standstill of Zn incorporation into the growing tissue mass. These disadvantages may restrict the transferability of results retrieved from experiments with fast growing model animals to the situation of adult individuals who are living at maintenance metabolism, as it is the most common case e.g. in humans. On the other hand, it is difficult to induce Zn deficiency in adults because the metabolic Zn requirement for maintenance is low (1, 2). Recently, however, an animal model to induce Zn deficiency of controlled severity in adults has been established on the base of a purified diet enriched with phytate (3, 4, 5). The use of phytate in a purified diet depresses absorption of dietary Zn as well as reabsorption of intestinally secreted Zn of endogenous origin and may thus induce Zn deficiency to an extent which depends on the dose of dietary phytate contents (3, 6, 7, 8). On base of such an animal model the present experiment was designed to study the development of Zn deficiency in the adult organism with special emphasis on the time sequence of changes in Zn metabolism and the extent to which Zn is mobilized from storage tissues.

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Material and methods

Experimental design

The present study comprised two parts, a pre-experimental period of 10 weeks and a subsequent experimental period of 29 days, where 31 female, adult rats were subjected to zinc deficiency.

Pre-experimental period

During the pre-experimental period 31 young, female Sprague-Dawley rats weighing 49 g were subjected to an alimentary labeling procedure (2, 6). According to this method, a ^{65}Zn labeled diet is fed to young, growing animals for several weeks. By incorporating the tracer into the growing body mass, all tissue compartments are labeled with uniform specific ^{65}Zn activity. In the present experiment the animals were reared with a semisynthetic diet based on purified casein and addition of 8 mg pure phytate per g (dodecasodium phytate, Sigma Aldrich, Deisenhofen, Germany) which mirrors phytate contents of common cereal based meals (Table 1). The diet contained 2 µg/g native Zn and 56 µg/g Zn from $ZnSO_4$. Radioactive ^{65}Zn was added to the diet at 13 Bq ^{65}Zn per µg of total Zn (Isotope Products Laboratories, Burbank, California, USA). Other macro and trace minerals as well as vitamins were supplemented in amounts meeting the recommendations for growing rats (9).

The animals were housed in conventional plexiglas cages and were fed the labeled diet ad libitum for 9 weeks. During this time the female rats completed their juvenile growth and reached a body weight of 212 g and thus may be considered as young adult for the breed used in the present study. The animals were then transferred into individual metabolism cages and were fed the labeled diet for another week. Food supply was restricted to 8.0 g per head and day (equivalent to maintenance requirement for energy) and was offered in one portion until fully consumed. The animals had free access to deionized water supplemented with 0.014% NaCl. Environmental conditions were standardized (25 °C, relative humidity of 60%, light/dark cycle of 11:13 h).

Table 1. Composition of the diet

Component	Content
Casein (mg/g)	200
Sucrose (mg/g)	280
Corn starch (mg/g)	347
Sunflower oil (mg/g)	10
Coconut fat (mg/g)	75
DL Methionine (mg/g)	2
Cellulose (mg/g)	30
Na phytate (mg/g)	8
Vitamins, minerals (mg/g)[1)]	46
$ZnSO_4 \cdot 7H_2O$ (µg/g)	183

[1)] per kg diet: 5000 IU all-trans retinol, 1000 IU cholecalciferol, 150 mg α-tocopherolacetate; 5 mg menadione-Na-bisulfite; 5 mg thiaminmononitrate; 10 mg riboflavin; 6 mg pyridoxine-hydrochloride; 20 mg Ca-D-pantothenate; 50 mg nicotinic acid; 1000 mg choline chloride; 1000 µg folic acid; 200 µg biotine; 25 µg cyanocobalamine; 2.00 g $Na_2HPO_4 \cdot 2H_2O$; 17.6 g KH_2PO_4; 1.73 g KCl; 16.2 g $CaCO_3$; 8.4 g $MgCl_2 \cdot 2H_2O$; 0.496 g $FeSO_4 \cdot 7H_2O$; 0.117 g $CuSO_4 \cdot 5H_2O$; 0.308 g $MnSO_4 \cdot H_2O$; 2.62 mg KI; 1.20 mg NaF; 4.48 mg $NiSO_4 \cdot 6H_2O$; 0.504 mg $Na_2MoO_4 \cdot 2H_2O$; 0.667 mg $NaSeO_3 \cdot 6H_2O$; 0.513 mg $CrCl_3 \cdot 6H_2O$

Experimental period

After 10 weeks of alimentary labeling the study proceeded to the experimental period. The dietary inclusion of ^{65}Zn was stopped and the diet was split into two types: (A) a control diet with the same composition as during the pre-experimental period, and (B) a Zn deficient diet, which was identical to the control diet except for supplemented $ZnSO_4$. The Zn contents of the two diets accounted for 58 µg/g and 2 µg/g, respectively. At the onset of the experimental period (day 0), 3 animals were sacrificed as reference control group. The other animals were allotted either to the control diet (n = 4) or to the Zn deficient diet (n = 24). On day 1, 2, 4, 7, 11, 16, 22 and 29 after the start of the experimental period, 3 Zn-deficient animals were sacrificed respectively and on day 29 the remaining control animals were also sacrificed. Daily fecal and urinary excretion was collected individually.

Sample preparation and analytical procedures

The animals were sacrificed by decapitation under anaesthesia (diethyl ether) after not being fed for 22 h. The blood was collected into heparinized tubes and separated into blood plasma and blood cells by centrifugation (1000 g). The animals were dissected as follows: liver, spleen, brain, lung, kidneys, a sample of pancreas, gastrointestinal (GI) tract (oesophagus, stomach, small intestine, colon, caecum), and the complete coat, which was separated into skin and hair by shaving. The carcass was heated for 2 h at 140 °C and all bones were removed. The remaining tissues of the carcass were ascribed to 'muscle and fat tissue' and the removed bones to 'skeleton'.

Blood plasma was analyzed for activity of alkaline phosphatase (commercial test kit, Boehringer, Mannheim, Germany). Food, feces, urine and tissues were mineralized in platinum vessels in a muffle furnace at 480 °C for 48 h. The ash was transferred into 0.3 mol/L HCl solution and analyzed for Zn (AAS 300, Perkin Elmer, Beaconsfield, UK). Standards were prepared from a certified Zn standard solution of 1000 mg/L (Merck, Darmstadt, Germany). All aqueous solutions were made with double-distilled deionized water. Excreta and tissues were also analyzed for ^{65}Zn activity (1828 Compugamma CS, Berthold, Munich, Germany). Calibration was performed by using appropriate dilutions and geometries of the same ^{65}Zn solution, which was used to label the diet during the pre-experimental period. All ^{65}Zn activity was corrected for radioactive decay to a common reference day (start of the study). ^{65}Zn activity was analyzed in the fresh samples as well as in the final ash solutions. Recovery of ^{65}Zn was used to correct losses of total Zn along the entire process of sample preparation.

Calculations and statistics

Individual Zn balance (intake, excretion, retention) was calculated on the basis of excreta collected during each animals last day alive. True absorption of dietary Zn and endogenous fecal excretion of Zn were quantified by the isotope-dilution method as adapted to the alimentary labeling technique (2). This method requires a minimum time lag of 3 days after the end of the ^{65}Zn labeling procedure. Respective calculations were therefore performed not before day 4 of Zn deficiency.

The data was divided into 5 treatment groups: the control group (control animals of day 0 and 29) and four Zn deficiency groups: day 1–2, day 3–7, day 8–16 and day 17–29. A one-factorial analysis of variance was performed with the treatment group as factor level. The respective mean values were tested by a multiple comparison procedure (Student-Newman-Keuls test). The Tables 2–4 present the mean values and the pooled standard error (S.E.) as derived from the analysis of variance. Significant differences among means ($p < 0.05$) are marked by superscripts. Additionally, linear contrasts were performed in some cases to test selected subgroups of mean values for statistically significant differences.

Results

The outer appearance of the animals remained normal during the entire experiment. Food intake and body weight accounted for 8.0 g/day and 212 g regardless of treatment.

As shown in Fig. 1a, alkaline phosphatase activity in blood plasma did not differ systematically from the baseline level (69 U/L) for up to 3 weeks of Zn deficiency but then dropped to about half of normal values at the end of the study (39 U/L). Blood plasma Zn was reduced already

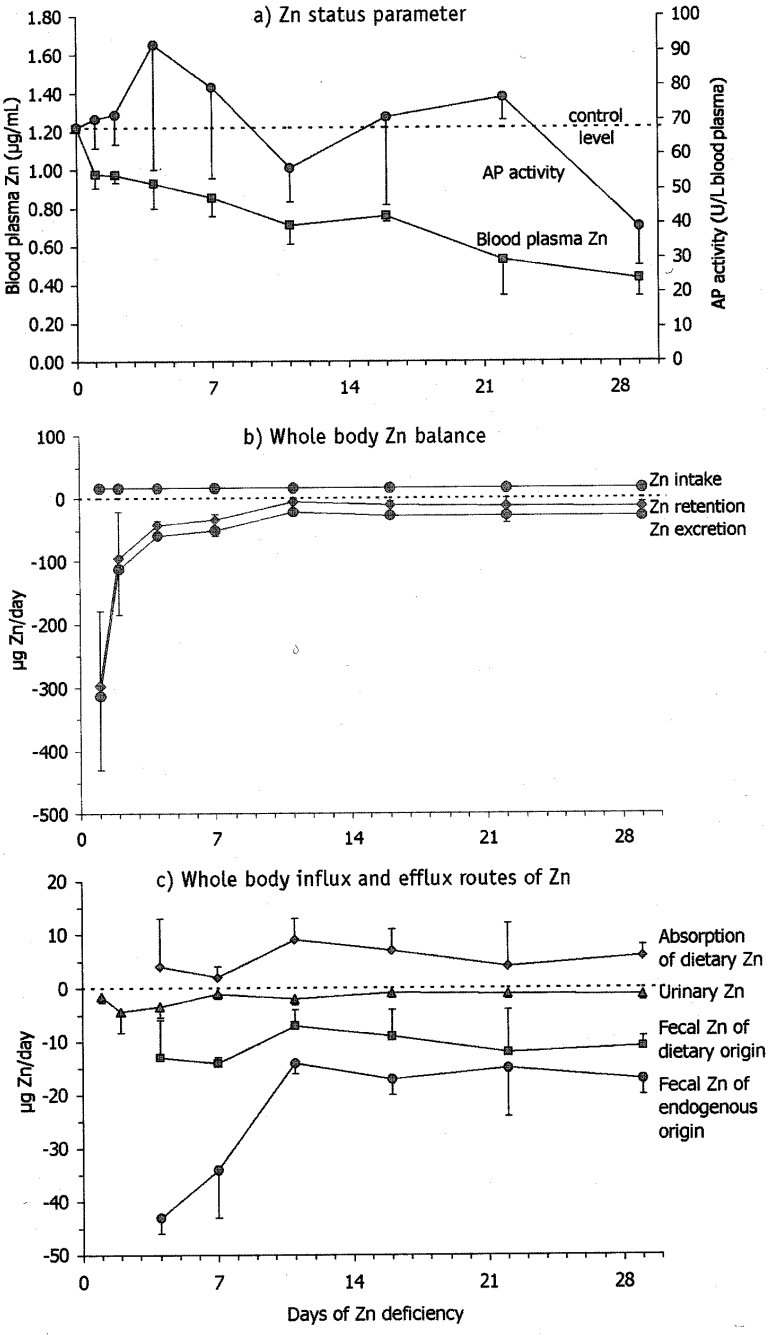

Fig. 1. Reaction of (**a**) Zn status parameters, (**b**) whole body Zn balance and (**c**) whole body Zn influx and efflux to Zn deficiency (excretion and efflux are expressed in negative values)

Table 4. ^{65}Zn activities in major tissue compartments (% of initial whole body activity[1])

	Control[2]	Days of zinc deficiency				S.E.
		1–2	3–7	8–16	17–29	
Muscle and fat tissue	33.6^{a}	33.6^{a}	32.2^{a}	29.4^{b}	28.8^{b}	1.9
Skeleton	36.8^{a}	33.1^{ab}	31.9^{b}	33.5^{ab}	29.7^{b}	2.6
Skin	9.3^{a}	7.7^{b}	7.1^{b}	7.2^{b}	7.1^{b}	1.1
Organs and blood	9.3^{a}	8.1^{b}	7.0^{c}	7.0^{c}	5.8^{d}	0.5
Hair	11.0	10.0	10.2	10.5	8.8	1.9

[1] Mean whole body activity at day 0 of the experiment = 75.2 kBq ^{65}Zn.
[2] Reference control group (day 0).
[a, b, c, d] Means in a row not sharing a superscript are statistically different ($p < 0.05$).

tained in muscle and fat tissue but was released from the skeleton. The following week was characterized by a significant loss of ^{65}Zn from muscle and fat tissue and in contrary a numerical gain of ^{65}Zn in the skeleton. From the onset of the 3rd week of deficiency the skeleton returned to release ^{65}Zn, while muscle and fat tissue showed only minor changes. ^{65}Zn activity in the skin was reduced mainly at the onset of Zn deficiency, while organs and blood released ^{65}Zn continuously during the entire experiment. Also the hair tended to lose ^{65}Zn, however without statistical evidence. In total, the changes in ^{65}Zn activity were quantitatively most relevant in the muscle and fat tissue and in the skeleton.

Discussion

It was a major methodological aim of the present study to induce Zn deficiency without interferences from the overall metabolic situation of the organism. Therefore, the animals were treated with Zn deficiency at maintenance level in a neither anabolic nor catabolic situation, which is evident from the fact that the body weights remained the same throughout the experimental period, and baseline Zn retention was close to zero and blood plasma levels of key players of the somatotropic axis in all animals stayed the same (growth hormone, insulin-like growth factor 1 and receptors, free fatty acids, glucose) (10). Also the levels of plasma alkaline phosphatase were typical of adult rats (2, 3, 5, 6).

Fecal Zn excretion needed several days to adjust to the abrupt onset of Zn deficiency. A similar delay in the regulatory answer of Zn homeostasis has also been observed in former studies (3, 11). Time patterns of 3 to 4 days could be attributed also to the idiorhythmics of Zn metabolism (12) and the oscillation of food intake in Zn deficient rats (13). Obviously the reaction of Zn homeostasis to a change in Zn supply is driven by a feed back system with a time lag of several days. This time lag caused considerable Zn losses via feces at the beginning of the study. It explains for the marked drop in skeleton Zn content during the first days of Zn deficiency. Similar rates of initial Zn losses due to a delayed adaptation of fecal Zn excretion to Zn deficiency were also observed in previous studies (3, 4, 11, 14).

True absorption of dietary Zn was adjusted to Zn deficiency already at day 4, while endogenous fecal Zn excretion required more than one week to reach the low levels, which are known from long-term Zn deficiency studies (3, 5). This gives rise to the hypothesis that the organism attempts to maintain a certain secretion of endogenous Zn into the digestive tract, e.g. because it is involved in digestive functions. In the case of Zn deficiency, however, endogenous Zn secretion has to be supplied by mobilizing tissue Zn. In this context, the approach of endogenous fecal Zn excretion to a minimum plateau during the first week of Zn deficiency might indicate that Zn mobilization shifted from exhausting labile sources to slow-releasing tissue Zn stores.

Zn deficiency reduced the concentrations and contents of Zn in various tissues. In general, the reactions were similar to observations of numerous previous studies (e.g. 4, 5, 14, 15, 16, 17). However, the Zn contents of most of these tissues were too small and/or their reactions on Zn deficiency were too less pronounced to contribute to whole body Zn mobilization to a significant extent. Quantitatively relevant amounts of Zn were provided only by muscle and fat tissue, the skeleton and partially by the skin.

At the onset of Zn deficiency the high losses of fecal Zn were counterbalanced mainly by mobilizing Zn from the skeleton. It has been shown by previous experiments with adult rats that about $^{1}/_{4}$ of skeleton Zn is involved in the whole body Zn turnover (7) and that about 20% of bone Zn may be mobilized during Zn deficiency (4, 14). As has been demonstrated in recent depletion-repletion studies, the mobile subcompartment of the skeleton Zn is quickly exchangeable (5). Obviously, the skeleton Zn contributes significantly in compensating short-term variations in dietary Zn supply. In contrast to bones, mobilization of Zn from soft tissues (mainly muscle and fat tissues) occurred mainly around the second week of Zn deficiency. At the same time, the skeleton Zn content transiently recovered, presumably due to a partial redistribution of Zn from soft tissues towards the mobile subcompartment of the bones. However, the soft tissue Zn storage appeared to be exhausted within the second week of Zn deficiency. After this point, the skeleton resumed to mobilize Zn. The onset of the second phase of bone Zn release corresponded to the final settings of endogenous fecal Zn excretion and clearly reflects the long-term saving mode of Zn metabolism during Zn deficiency. Nevertheless, the amounts of mobilized bone Zn seemed to provide enough Zn to maintain basic metabolic functions at least until day 22, since alkaline phosphatase activity remained normal. At day 29, however, alkaline phosphatase activities were severely reduced and the skeleton Zn of the corresponding subgroup of Zn deficient animals was already 20% below normal levels. This loss of bone Zn is already as high as the pool size of mobile skeleton Zn (7). Obviously, the skeleton Zn storage was exhausted at the end of the study.

The time course of ^{65}Zn activity in major tissue compartments provides additional information about mobilization and redistribution of whole body Zn stores. Con-

stant ^{65}Zn activity in muscle and fat tissue and decreasing levels in the skeleton during the 1st week of Zn deficiency support the hypothesis that the skeleton served as major Zn buffer to compensate high initial Zn loss via feces at the onset of Zn deficiency. The following week was characterized by an inverse behavior of these tissues, where bone ^{65}Zn activity recovered slightly, while at the same time significant amounts of ^{65}Zn were released from the muscle and fat tissue. This gives further evidence to the assumption that a part of mobilized Zn from muscle and fat tissue was transiently redistributed to the skeleton.

In all it may be concluded that adult individuals are able to compensate Zn deficiency for several weeks without major metabolic disorders by mobilization of Zn from tissue stores. This capability may maintain common parameters of Zn status like alkaline phosphatase activity at rather normal levels and may thus mask the actual severity of the dietary Zn supply (see also 18). Zn stores are localized mainly in the skeleton and in the muscle and fat tissue. The skeleton, however, proved to be the major Zn storage due to its dominating contribution to mobilizable whole body Zn and its flexibility to serve as both short-term as well as long-term Zn storage.

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