生活科学研究誌・Vol. 6 (2007) 《食品栄養分野》

雄性シリアンハムスターにおける血清指標および臓器における亜鉛含量におよぼす亜鉛の補給量および摂取間隔の影響について

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Effects of Quantity and Intake Interval of Zinc Supplement on Serum Indices and Zinc Content in Various Organs in Male Syrian Hamsters

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Summary

The present study examined male hamsters to establish the effect of the level and timing of dietary zinc intake on internal organs and serum levels of glucose, cholesterol, and triglyceride. The 20 hamsters were divided into four groups of five animals each. Group A was fed diet A every three days, Group B on diet B every two days, and Group C on diet C every day, while Group D was fed a low-zinc diet every day. The diet was designed so that the total zinc intake of Groups A, B, and C were equal. In group-D, a decrease in serum iron and the increase of iron in excretion substances were observed, suggesting that zinc has priority over iron in absorption from intestine. No significant difference was observed in the amounts of serum zinc of hamsters between the low zinc group of D and that of A, B and C groups. However, serum glucose level was increased significantly in group-D. Furthermore, we observed no significant difference in zinc concentration in serum, liver and kidney between hamster groups on a zinc-enriched diet despite different timing of ingestion (every day, every other day or every third day).

Keywords：*zinc ingestion, intake interval, serum glucose, iron, hamster*

Ⅰ. Introduction

Zinc is an essential but unstable trace element and is unevenly distributed throughout the body. The clinical manifestations of zinc deficiency include growth retardation, hypogonadism in males, neurosensory disorders, cell-mediated immunological dysfunction and skin changes[1)]. Zinc is essential for enzymes involved in DNA synthesis[2)], mitosis, cell division and protein synthesis[3)]. It is also a component of many transcription factors and proteins that control the cell cycle. Several hundreds of zinc-containing nucleoproteins are probably involved in gene expression of various proteins[4-6)].

Zinc deficiency can be due to inadequate dietary intake, decreased absorption, increased requirements, decreased utilization, increased loss, or genetic diseases[7-9)]. Zinc deficiency can be also observed in liver disease, sickle cell anemia, renal disease and gastrointestinal disorders[10)]. Moreover, various reports have described the consequences of zinc deficiency in the human body, such as bedsores which occur with a deficiency of zinc in elderly patients or bedridden patients receiving intravenous nutrition in hospitals or welfare-facilities[11-14].

The dietary habit of the Japanese has been changing from the Japanese style to the western style. This change causes lack of certain minerals for some Japanese, and it is therefore necessary to supplement such minerals. On the supply of minerals, it was reported[15] that two or three folds of the allowance for one day can be taken by every two or three days intake. However, this phenomenon is not clear.

The objectives of this study were to examine the effect of the quantity and intake interval of zinc supplement on the zinc, glucose, cholesterol and triglyceride contents in serum as well as contents in various organs in male Syrian hamsters.

Ⅱ. Materials and methods

2.1 Animals and treatments

Male Syrian hamsters were used in this experiment because the quantity of food consumed by a hamster per day is constant throughout the experimental period.

Five-week male Syrian hamsters were obtained from Japan SLC, Inc. and housed individually in stainless steel cages placed in a room with a controlled environment: temperature, 24-25℃ ; humidity, 50-75%; and a 12-h light-dark cycle. The hamsters were divided into four groups of five animals each, namely, the A, B, C and D groups. The A, B and C groups were the zinc ingestion group, and the D group was the low zinc ingestion group. The A group was placed on zinc-A diet (Z-A: 8.58 mg zinc/100 g of diet) every third day. The B group took in zinc-B diet (Z-B: 5.98 mg zinc/100 g of diet) every other day. Further, the A and B groups took in food of low zinc ingestion (low zinc diet) on the other days. The group C took in zinc-C diet (Z-C: 2.29 mg zinc/100 g of diet) every day. The D group of low zinc ingestion (Z-D: 0.54 mg zinc/100 g of diet) took in diet without adding more zinc (low zinc diet) every day. After preliminary breeding based on zinc-free AIN-93M diet (Oriental Yeast Co., Ltd.) to all groups, zinc chloride was added to the food of the A, B and C groups. Ten grams diet was given per day. Diet ingredients are shown in Table 1.

【Table 1】 Composition of experimental diets

	(%)			
Components	Z-A diet	Z-B diet	Z-C diet	Z-D diet
Cornstarch	46.57	46.57	46.57	46.57
α cornstarch	15.5	15.5	15.5	15.5
Casein	14.0	14.0	14.0	14.0
Sucrose	10.0	10.0	10.0	10.0
Cellulose powder	5.0	5.0	5.0	5.0
Soybean Oil	4.0	4.0	4.0	4.0
AIN-93M vitamin mixuture	1.0	1.0	1.0	1.0
Choline chloride	0.25	0.25	0.25	0.25
Methionine	0.18	0.18	0.18	0.18
AIN-93M mineral mix Without zinc	3.482	3.488	3.494	3.500
Zinc Chloride	0.018	0.012	0.006	0.000

2.2 Method of Mineral Analysis

1. Dry Ash Method: Food analysis of mineral concentration was made by the dry ash method for all foods. Samples (5-20 g) were weighted and then baked at 550℃ for 5-6 hours. The ashes were cooled and solved in 5 ml of 20% HCl. The solutions were evaporated and then filled up to 100ml by 1% HCl[16].

2. Wet Ash Method: Samples of organs were weighed, added 10ml concentrated HNO_3 and heated in the oven at 100℃. Then 2 ml of perchloric acid (60%) was added to the samples and heated at about 150℃, after cooled, samples were added 1ml HNO_3, and then heated at 150℃. Finally the samples solution was filled up to 25-100ml by 1% HCl[16].

Quantities of zinc, iron, and copper of their extracted organs were measured for by using an Atomic Absorption Flame Emission Spectrophotometer. (AA-640-13 Shimadzu, Kyoto, Japan)

2.3. Schedule for feeding

The schedule for feeding in each group is shown in Table 2. The dietary treatments of cycles of 6 days in A, B, C and D groups lasted for 6 times (in total 36 days). The animals were fed once a day at 10:00 a.m. and the food intake left over from the previous day were measured simultaneously. Moreover, the water intake was measured once a week. Body weight was recorded at the beginning of the experiment and weekly during the experimental period. Feces over a period of 6 days were collected at the final week. At the end of the 36 days experimental period, the hamsters were sacrificed, after the starvation for 24 hours. And their organs were weighed. Animal experiments followed our institution's criteria for the care and use of laboratory animals in research, which were in accordance with the guide for animal experimentation of Osaka City Institute of Public Health and Environmental Sciences.

2.4 Hematological and biochemical parameters

Food was with held 24 hours before the end of the experiment. Plasma from blood tubes containing heparin were obtained by centrifugation at 2000 rpm for 20 minutes, and stored at −20℃. The triglyceride, glucose, and total cholesterol levels of each group were measured with a kit (glucose B-test Wako, cholesterol E-test Wako and triglyceride G-test Wako, Wako Pure Chemical Industries, Osaka, Japan).

2.5 Statistical analysis

Data were represented by mean ± standard error, according to the method of ANOVA. Differences among groups of hamsters were compared with Tukey's multiple comparisons and considered significant at a level of $p < 0.05$.

Ⅲ. Results

3.1 Effect of zinc supplement on body and organ weight, food intake

As shown in Table 3, no significant difference in body weight gain among the 4 groups was observed. The total intake of diet during experimental period did not have the significant difference. Those are A: 285.6 g, B: 281.6 g, C: 284.5 g and D: 290.8 g. The weight of the liver and the kidney also were no significant difference among the 4 groups.

3.2 Effect of zinc supplement on zinc content in organs

Although the serum of group-D showed the tendency of a low value, there was no significant difference among groups A, B and C in the zinc content in serum (Table 4). But zinc content in testis of group A showed low value than that of group B ($p<0.05$), and low value than that of C and D ($p<0.01$). The iron content in serum of

【Table 2】 Schedule of feeding of 6 days

(continue 36 days)

Dietary groups	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
A	Z-A	Z-D	Z-D	Z-A	Z-D	Z-D
B	Z-B	Z-D	Z-B	Z-D	Z-B	Z-D
C	Z-C	Z-C	Z-C	Z-C	Z-C	Z-C
D	Z-D	Z-D	Z-D	Z-D	Z-D	Z-D

Zinc-A diet;(Z-A) 8.58 mg zinc/100 g, Zinc-B diet;(Z-B) 5.98 mg zinc/100 g
Zinc-C diet:(Z-C) 2.29 mg zinc/100 g, Zinc diet;(Z-D) 0.54 mg zinc/100 g

【Table 3】 Body and organ weight efficiency of food

		Dietary groups			
		A	B	C	D
Initial body weight	(g)	80.7±8.6	80.5±8.1	78.7±10.2	76.0±15.1
Final body weight	(g)	125.2±2.8	120.4±10.4	121.2±8.9	123.9±6.6
Body weight gain	(g/day)	7.4±1.9	6.6±1.1	7.1±1.4	8.0±3.2
Food intake	(g/day)	7.9±0.2	7.8±0.2	7.9±0.3	8.0±0.2
Testis weight	(g)	3.9±0.2	3.8±0.2	3.5±0.3	4.0±0.2
Liver weight	(g)	5.21±0.3	4.77±0.6	4.86±0.3	4.92±0.2
Kidney weight	(g)	0.88±0.1	0.88±0.1	0.90±0.1	0.92±0.1
Efficiency of food	(%)	2.3±0	2.4±0.2	2.4±0.1	2.4±0.1

Values are the means ±SD of five hamsters.
(on the average that be carried out twice, the trial becomes ten times

【Table 4】 Effect of dietary zinc supplement on the concentrations of zinc, iron and copper in serum, liver, kidney and testis

	Zn				Cu			
Group	serum(μg/ml)	liver(μg/g)	kidney(μg/g)	testis(μg/g)	serum(μg/ml)	liver(μg/g)	kidney(μg/g)	testis(μg/g)
A	2.40±0.07	2650±1210	30±5.2	48.0±4.4 a	0.54±0.04c	400±130	4.3±0.5	6.7±0.2
B	2.33±0.08	2090±300	25.1±6.6	60.0±2.1 c	0.52±0.03c	340±40	5.0±2.2	6.9±0.6
C	2.54±0.18	2380±1370	32.6±13.8	62.8±6.5 b	0.54±0.02 c	320±155	3.3±0.7	5.7±1.2
D	2.28±0.11	2110±710	25.3±7.2	64.2±8.6 b	0.64±0.12 a	370±30	3.5±0.8	7.3±0.5

Values are the means ±SD of five hamste (on the average that be carried out twice, the trial becomes ten times)
Data not sharing common alphabet are significantly different
(b: $p<0.01$ c: $p<0.05$)

group-D also showed a low value than those of groups A, B and C ($p<0.01$). But the serum copper content in group-D showed a higher value than that of group-A, B and C ($p<0.05$). The value of the zinc, copper and iron contents in the liver at group A was high tendency among 4 groups. There was no difference between the 4 groups in the zinc, copper and iron contents in the kidney.

3.3 Effect of zinc supplement on excretion of zinc

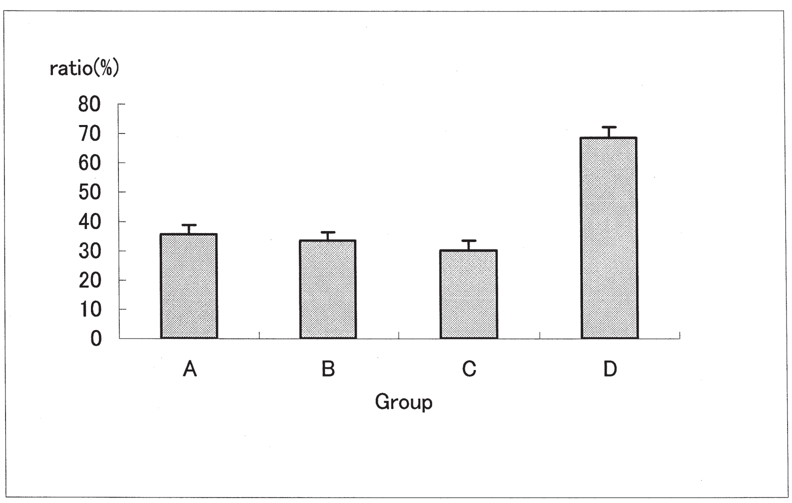
Significantly low zinc content in feces was seen in group-D, compared with groups A, B and C ($p<0.01$), although significant difference was not seen among group-A, B and C. As shown in Table 5, group-D showed significantly high iron content compared with group-A, B and C. In addition, copper among all groups, there were no differences.

The apparent absorption rate of the zinc is shown in Fig. 1. The apparent absorption rate was calculated as (%) = (a quantity of feed intake × zinc content in feed intake - feces weight × zinc content of feces) ÷ (a quantity of feed intake × zinc content in feed intake) × 100[17]. The apparent absorption rate was 35.6% in group-A, 33.5% in group-B, 30.2% in group-C, and

【Table 5】 Effect of dietary zinc supplement on the concentrations of zinc, iron, and copper in faces

Group	Zn(μg/g)	Cu(μg/g)	Fe(μg/g)	feces weight(g)
A	960±180b	210±30	490±40d	3.6±0.5
B	1000±240b	200±50	540±130d	3.6±0.7
C	840±160b	180±20	490±100d	2.7±0.4
D	77±30a	180±30	960±170c	4.3±0.4

Values are the means ±SD of five hamsters.
(on the average that be carried out twice, the trial becomes ten times)
Data not sharing common alphabet are significantly different
(b: $p<0.01$ d: $p<0.01$)



【Fig. 1】 The apparent absorption of zinc

Fe			
serum(μg/ml)	liver(μg/g)	kidney(μg/g)	testis(μg/g)
2.69±0.42 b	5710±2980	57.8±14.8	49.7±11.4
2.47±0.22 b	4350±.760	59.1±7.9	49.0±4.9
2.35±0.25 b	4440±2260	58.4±5.6	49.0±4.8
1.58±0.25 a	4600±790	61.1±7.1	57.1±11.9

【Table 6】 Effect of dietary zinc supplement on glucose, triacylglycerol and total choresterol concentration in serum

Group	glucose(mg/dl)	triacylglycerol(mg/dl)	total choresterol(mg/dl)
A	220.6±37.3 b	227.1±59.7	83.3±6.0
B	214.4±47.1 b	220.3±44.9	87.4±16.0
C	210.6±49.8 b	243.8±73.1	90.1±8.4
D	278.0 ±63.6 a	268.8±67.8	100.3±23.0

Values are the means ±SD of five hamsters.
(on the average that be carried out twice, the trial becomes ten times)
Data not sharing common alphabet are significantly different (p<0.05).

68.5% in group-D. Significant difference in the apparent absorption rate was not seen between group-A, B and C. Each of groups A, B and C showed a low apparent absorption rate over group-D (p<0.01). Apparent quantities of absorption (= total intake − quantity of an excreted) were A: 8.1 ± 0.2 mg, B: 8.0 ± 0.2 mg, C: 5.7 ± 0.2 mg and D: 1.5 ± 0.02 mg.

3.4 Effect of zinc supplement on glucose, triglyceride and total cholesterol contents in plasma

The serum glucose level of group-D showed a higher value compared with the other three groups (p<0.05). However, there was no significant difference in the triglyceride and total cholesterol levels of the 4 groups (Table 6).

Ⅳ. Discussion

Twenty experimental animals used in this study were allocated to four groups, namely, group-A, which ingested supplemented diet every third day; group-B, which ingested the supplemented diet every other day; group-C, which ingested the supplemented diet every day and group-D, which ingested a low zinc diet every day. Zinc chloride was not added to the diet of group D, but the diet had insufficient zinc which is derived from casein. Zinc concentration in serum, but not in liver and kidney, of zinc insufficient group (group D) was lower than those of zinc adequate groups. However, there was no statistically significant difference. Yanaga et al. have shown that the zinc concentrations in liver and serum of zinc deficient rats were lower than those of zinc adequate rats[18]. Furthermore, it has been reported that a low organ weight was observed for a low zinc-ingested group in rat[19]. It has also been reported that zinc deficiency resulted in skin disorders[20]. However we did not observe the decrease in organ weight and the skin disorder in the hamsters in our study. These results suggest that the zinc deficient in our study is mild but not severe.

The concentration of serum zinc of the group D did not significant decrease. Kauwell et al.[21] also reported that the zinc density out of plasma does not fall by intermediate zinc restrictions.

When zinc content of diet becomes low, the absorption rate increases[22]. In our study, apparent absorption rate of group-D showed a higher tendency. However no difference was seen among A-C groups. In low zinc intake group there may be an inherent mechanism to control zinc elimination to maintain homeostasis[23]. The zinc which was not absorbed is excreted in feces[24]. In our study, the amount of zinc in the feces of group-D was significantly lower (p< 0.01) compared with the other groups. On the other hand, the amount of iron excreted was significantly higher (p< 0.01) in group-D than the other groups. Some minerals, such as iron, copper, and cadmium, reportedly compete with zinc and affect the absorption from the intestinal tract.[25]. There was no remarkable difference in zinc content in serum and procedure was different (every other day, every third day, and every day). In this study, iron content in serum was significantly lower in the D group compared with the other three groups A, B and C (p<0.01). The quantity of excretion of iron at D group was higher than the other groups. It is known that the non-heme iron obstruct the absorption of zinc[26]. Tobia et al[27] have reported that serum glucose was significantly higher in rats fed with very low zinc than in pair-fed animals and that a linear relationship was observed between serum glucose and insulin in pair － fed animals but not in zinc-deficient groups. On the other hand, Tang and Shay[28] have shown that zinc promotes glucose transport into cells through the phosphoinositol- 3 -kinase signal transduction pathway. In this study we also observed the serum glucose level increased in lower zinc hamsters. As for the zinc and the relations of insulin, zinc acts on a catalyst of insulin by mutual relations with vitamins. In addition, it is not known that the dosage of zinc is effective for the prevention of diabetes[29, 30]. Sondergaard et al.[31] have shown that a zinc low intake results on the increase in serum glucose level. Furthermore, El Hendy et al.[32] reported that triglyceride and total cholesterol increased in zinc deficient rats. However, there was no difference in triglyceride and total cholesterol in serum between zinc-insufficient group and zinc-adequate groups in our study. Hughes[33] reports same result. Therefore, the relationship between diabetes and zinc is not clear.

From the results of this study it can be concluded that there is no significant difference in zinc concentration in serum and kidney between hamster groups on a zinc-suitable diet despite different timing of ingestion (every day, every other day or every third day). But iron concentration in the serum, timing of ingestion group-D seems to have high different from group-A, Band C. Furthermore, we observed and thus confirmed that serum glucose levels increase in lower zinc hamsters.

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雄性シリアンハムスターにおける血清指標および臓器における亜鉛含量におよぼす亜鉛の補給量および摂取間隔の影響について

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要旨：雄性ハムスターにおける亜鉛の摂取量および食餌タイミングの影響について、各臓器中のミネラル量と血清中のミネラル量、グルコース値、コレステロール値およびトリグリセリド値との関連性を検討した。20匹のハムスターを１）グループＡ(２日おきに食事を摂取)、２）グループＢ(１日おきに食事を摂取)、３）グループＣ(毎日食事を摂取)および４）グループＤ(毎日低亜鉛量の食事を摂取)の４グループに分け、１群５匹ずつとし、グループ化した。また、グループＡ、Ｂ、およびＣにおける実験期間中の亜鉛摂取量が等しくなるように考慮した。結果から、低亜鉛食を摂取した場合、血清鉄量の低下および吸収されずに排泄された糞中鉄量の増加が見られたことから、亜鉛と鉄の吸収において亜鉛の方が優先的に体内に吸収され鉄の取り込みが抑制されたことが示唆された。また、血清亜鉛量はほとんど変化しなかったにもかかわらず血清グルコース値の顕著な増加が認められたことから、低亜鉛食は高血糖を誘導することが示唆された。一方、食餌中の亜鉛量が充足されている場合、血清亜鉛量やグルコース値はほとんど変化しなかったことから、亜鉛の摂取方法の違いによる影響はないことが明らかとなった。