BioMetals 1997, **10**, 271–276

Effect of nicotinic acid on zinc and iron metabolism

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Received 4 September 1996; accepted for publication 3 February 1997

Nicotinic acid has functional groups capable of forming complexes with trace metals. The present study examines the effect of nicotinic acid supplementation on absorption and utilization of zinc and iron. *In vitro* zinc uptake by human erythrocytes was studied using blood samples of 10 healthy subjects. It was found that 8 μmoles nicotinic acid or NADP increased ^{65}Zn uptake by 38.9% and 43.1% in fasting, and by 70.9% and 28.1% in postprandial conditions. In animal experiments, nicotinic acid supplementation to finger millet based diet resulted in significant enhancement of percent zinc absorption, liver zinc and growth of weanling mice ($P < 0.05$). When mice were fed with nicotinic acid-deficient, -adequate and -excess synthetic diets for four weeks it was observed that, in comparison with the nicotinic acid-deficient diet, percent zinc absorption, intestinal zinc, percent haeomoglobin and liver iron increased significantly under nicotinic acid-adequate and -excess conditions. The results obtained suggested that nicotinic acid, in addition to its known effect on growth and metabolism, may be playing an important role in enhancing zinc and iron utilization.

Keywords: in vitro zinc uptake, nicotinic acid, zinc and iron bioavailability

Introduction

Bioavailability of trace metals such as zinc refers to the sum total of the processes of absorption across the gastro-intestinal tract, transport via blood, and utilization of the metals by various organs. These processes are known to be influenced by a number of factors, but the complex mechanisms of their interactions are poorly understood especially in case of composite diets and meals (IAEA Report 1994). Calcium, phytate, fibre, iron and copper affect zinc absorption adversely while citrate, picolinate and the amino acids are known to have an enhancing effect (Solomans 1982). The release of zinc from intracellular protein ligands and its transfer to portal blood is not well understood (Cunnane 1988), although the effects of various factors on red cell uptake of ^{65}Zn have been reported (Wouwe *et al*. 1990).

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Among the non-protein ligands of dietary origin, members of the B-complex group of vitamins, such as nicotinic acid and riboflavin containing –NH and –OH groups, are promising in terms of forming complexes with zinc. It has been reported that riboflavin deficiency results in decreased absorption of iron both in animals and in humans (Powers *et al*. 1993). Likewise, riboflavin and its active form, FAD, showed an enhancing effect on zinc bioavailability from whole wheat bread in an *in vitro* system (Agte *et al*. 1992). It remains to be seen, however, if nicotinic acid exerts any effect on zinc and iron bioavailability. The work reported in this paper had the following objectives:

(a) to study the effect of nicotinic acid and NADP on zinc uptake in an *in vitro* system comprising a suspension of erythrocytes; and

(b) to study the interactive effect of nicotinic acid on zinc absorption and its utilization using an animal model.

Methods

In vitro *uptake of ^{65}Zn by human erythrocytes*

In vitro uptake of ^{65}Zn by erythrocytes can be measured in samples of 0.4 ml packed cells under nearly physiological conditions (Wouwe *et al*. 1990). Use of erythrocytes as a model has been validated by their use of zinc binders such as histidine. Blood samples of 10 healthy, active men were collected in citrated bulbs under both fasting and postprandial conditions at a sports centre in Pune. After separation of plasma by centrifugation, the erythrocytes were washed with cold physiological saline and finally suspended in 0.9% saline in 1:1 proportion in separate tubes. In all, 10 sets of nine preweighed test tubes each were used in the experiment. A suspension of erythrocytes prepared from a blood sample was added (3 ml per tube) in all nine tubes of a set. Of these, three tubes each were randomly selected as controls, treatment 1 (nicotinic acid) and treatment 2 (NADP). Buffered saline (1 ml) was added in each of the control test tubes. In treatment 1 tubes, 1 ml of a solution of nicotinic acid (8 μmoles) was added and in treatment 2 tubes, NADP (8 μmoles) was added. A 1 ml solution of ^{65}Zn (specific activity 280 mCi g^{-1}; activity per tube 2.1 μCi) at pH 7.4 was added in all the tubes of 10 sets. The tubes were incubated for 30 min and then centrifuged at 2500 rpm. After removing the supernatant solution, tubes were dried in the oven at 60°C to constant weight and the dry cell mass (representing cell membrane and cytoplasm together) was noted. Gamma activity of the tubes was recorded on a gamma counter (Electronic Corporation of India) having a well-type detector of NaI–Th crystal. The average ^{65}Zn counts per g dry cells were taken as estimates of zinc uptakes with a counting efficiency of about 56% at 0.511 energy level of ^{65}Zn.

Interactive effect of nicotinic acid on zinc absorption in mice

Study design. Weanling mice have been shown to be sensitive to changes in dietary zinc intakes in terms of growth, protein gain, organ weights, and zinc contents of organs such as liver, the effect being more prominent in male than in female mice (Morgan *et al*. 1988a,b). Further, when the interaction of essential micronutrients with zinc is to be studied, the effect could be better appreciated by comparing the responses to deficient and adequate levels (Powers *et al*. 1993). Considering this fact, two experiments using mice models were undertaken.

In *Experiment 1*, 12 weanling male mice (21 day old) were used as a model and were randomly allocated into two equal groups. A control group of six mice was given basal low nicotinic acid diet *ad lib*. (gross intake 10.8 mg kg^{-1}), while the other group of six mice were given nicotinic acid-supplemented basal diet *ad lib*. (30 mg kg^{-1} diet).

In *Experiment 2*, 18 mice were fed on a synthetic diet (AIN-76) at three levels of nicotinic acid. A nicotinic acid-adequate group of six mice (10 mg kg^{-1}) was pairfed with a nicotinic acid-deficient group of six mice (1.8 mg kg^{-1}) the remaining six mice were fed on the same diet with a nicotinic acid level of 30 mg kg^{-1}.

Mice were housed individually in metabolic cages made of polypropylene with stainless steel wire bottoms. Animals were provided with double-distilled water from polypropylene bottles having stainless steel spouts. Stainless steel cups were used for providing food. Mice were observed for body weight, and zinc and iron absorption for three weeks.

Diet. The composition of the low nicotinic acid diet for Experiment 1 is given in Table 1. Reported diets deficient in nicotinic acid use maize and soybean combinations (Carter & Carpenter 1982, Carpenter *et al*. 1988). In the present experiments, however, finger millet was used owing to its lower contents of nicotinic acid and tryptophan as compared with maize. It also represents the staple habitually consumed in several parts of southern India. Diet was supplemented with all other nutrients except nicotinic acid and zinc to meet AIN-76 recommendations. Table 1 also gives the composition of the synthetic diet of Experiment 2.

Zinc and iron absorption. Faecal collection was for each week, separately for each animal. Faeces were dried, and seven days pooled samples were weighed and 1 g aliquots used for the estimation of zinc. Apparent absorption was calculated as:

$$\text{Apparent absorption (\%)} = (\text{intake} - \text{faecal output}) \times 100/\text{intake}$$

Organ levels of zinc and iron. At the end of the experiment, all the mice in fasting condition were sacrificed by cervical dislocation. Cardiac blood was collected into heparinized tubes. The liver and kidneys were removed, washed with saline and weighed. Organs were dried to constant weight in an oven at 60°C and digested using the triacid mixture. Due to inadequate quantity of blood (< 1 ml) whole blood was analysed and separate estimations of plasma and erythrocyte zinc contents were not made.

Analytical methods. Zinc contents of diet, faeces, organs and blood samples were estimated by atomic absorption spectrophotometry using specific hollow cathode lamps (Perkin-Elmer, USA model 2380). The samples were digested using the triacid mixture (HNO_3:$HClO_4$:H_2SO_4 in 3:2:1 proportion), diluted to known volume with double-distilled water in metal-free glassware and analysed using an atomic absorption spectrophotometer. Rice flour (NIES 10) was used as a biological standard for quality control. The observed values of trace metals deviated by ± 5% from the reported values. Body weights of mice were recorded twice a week on an electronic balance with a sensitivity of 0.1 g.

Table 1. Ingredients and nutrients of basal diet

Experiment 1				Experiment 2		
Ingredients (g kg^{-1})		Nutrients*			Ingredients (g kg^{-1})	
Ragi (Finger millet)	600	Protein	(g kg^{-1})	200	Casein	60
Soyabean	115	Fat	(g kg^{-1})	53	Gelatin	60
Casein	80	Fibre	(g kg^{-1})	49.2	Sucrose	620
Cellulose	20	Niacin	(mg kg^{-1})	10.8	Starch	150
Sugar	180	Zinc	(mg kg^{-1})	21	Cellulose	50
Vitamin mixture**	5				Corn oil	50
					Vitamin and mineral mixture without niacin as per AIN-76	10

*Observed values.

**Vitamins and minerals as per AIN-76 standard, without adding zinc or nicotinic acid. For treatment group 30 mg kg^{-1} nicotinic acid has been added during preparation of diet.

Table 2. ^{65}Zn uptake by erythrocytes in the presence of nicotinic acid or NADP. Values expressed as counts per g dry cells

Physiological state	Number of samples	Control	Nicotinic acid	Paired *t* test	NADP	Paired *t* test
Fasting	10	2086 ± 441	2896 ± 972	2.76*	2985 ± 744	5.58**
Postprandial	10	2089 ± 501	3553 ± 1550	4.77**	2675 ± 777	1.09 (NS)

Values given as mean ± SE.

* $P < 0.05$.

** $P < 0.01$.

Statistical methods. Mean and standard deviations were calculated for the data from the erythrocyte uptake experiment and the mice experiments. Student's *t* test was used to compare the differences between treatments at the 5% level of significance.

Results

In vitro *uptake of ^{65}Zn by human erythrocytes*

Table 2 gives the values of zinc uptake by erythrocytes under fasting and postprandial conditions for each subject. Considering these as basal values, percent change in zinc uptake in the presence of nicotinic acid or NADP was calculated. As seen in Table 2, the increase in zinc uptake by nicotinic acid addition was found to be 38.9% in fasting and 70.9% in the postprandial state. Both increases were statistically significant. NADP was found to enhance the uptake of zinc by 43.1% in fasting and 28.1% in the postprandial state. In postprandial conditions the enhancing effect of NADP on zinc uptake was less pronounced than that of nicotinic acid.

Interactive effect of nicotinic acid on zinc absorption in mice

The observed growth rates of mice in Experiment 1 were 0.28 and 0.36 g day^{-1} for the control and nicotinic acid-supplemented groups, with gain-to-feed ratios of 0.134 and 0.155, respectively (Figure 1). Differences in these parameters were more conspicuous during the second week, when the average growth rate of the nicotinic acid-supplemented group was 188% of the control group, and the average ratio of gain-to-feed was 128% of the control group.

When body compositions of both the experimental groups were compared (Table 3), the control group was found to contain a considerably higher percentage of fat ($P < 0.05$) and a lower percentage of fat-free body mass than the nicotinic acid-supplemented group ($P < 0.05$). Differences in percent body water between both groups were small and nonsignificant. This indicated that nicotinic acid supplementation increased body protein and bone skeleton.

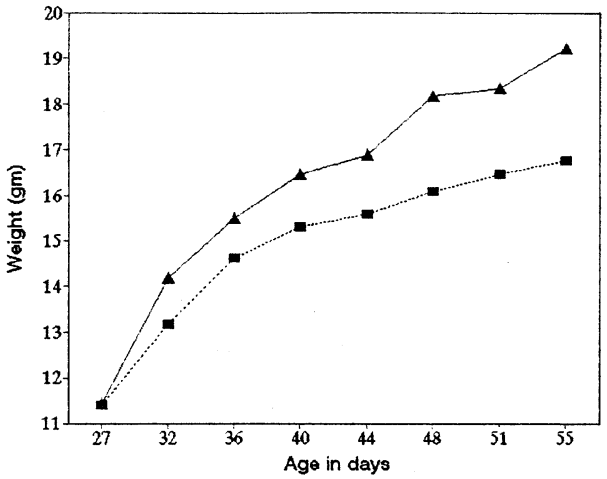

Figure 1. Weights of weanling mice in response to different diets. Comparison of niacin-supplemented diet (triangles) with basal diet (squares).

It was further observed (Table 3) that the nicotinic acid-supplemented group had higher values of zinc and iron intakes in the first two weeks, probably due to increased food intake. However, the percent absorption and the total bioavailable zinc intake were significantly higher only during the second week. In the case of iron, both percent absorption and total bioavailability were higher for the nicotinic acid-supplemented group but the differences were not statistically significant.

In order to examine the effect of nicotinic acid supplementation on utilization of zinc and iron, blood samples and organs such as liver and kidney were tested for their zinc and iron contents. The results obtained (Table 4a) indicated that the liver contents of both zinc and iron were significantly higher in the nicotinic acid-supplemented group than in the control group. However, only the iron content was found to be significantly higher in the kidneys of the nicotinic acid-supplemented group. It was further seen that the zinc concentrations in the whole blood samples of the nicotinic acid-supplemented group were 20% lower, while the iron concentrations were 3.5% higher compared with the control group. The differences were, however, not statistically significant.

It was noticed in Experiment 2 (Table 4b) that, in spite of the pairfeeding of the two experimental groups of mice, the nicotinic acid-supplemented group showed increased percent absorption of zinc, blood haemoglobin levels and iron content in liver, indicating the enhancing effect of nicotinic acid on iron and zinc bioavailability.

Discussion

Both riboflavin and nicotinic acid contain heterocyclic nitrogen-containing ring structures in addition to carboxylic acid groups. The complex of nicotinamide with transition metals such as chromium in the form of glucose tolerance factor is well known (Mertz 1986). Nicotinic acid is essentially pyridine-3-carboxylic acid and complexes of zinc with pyridine are reported (Dawson *et al.* 1986). Further, in many enzymatic redox reactions, metals such as calcium, magnesium and zinc co-exist with nicotinamide as cofactor. These facts formed the conceptual basis for the present work.

Zinc deficiency has not been a specific problem amongst the poor in the Middle East but is now known to occur in children and adolescents from widely diverse areas including the USA. Major manifestations include retarded growth and development and increased incidence of pregnancy complications. Other manifestations include suppressed

Table 3. Feed intake, body fat, body protein, zinc uptake and % zinc absorption during two observation periods at the age of 21–35 days and 35–49 days, respectively. (Values expressed as mean ± SD for control group and nicotinic acid supplemented group)

	Period I			Period II		
	Control	Nicotinic acid	Significance of difference	Control	Nicotinic acid	Significance of difference
Feed intake (g)	29.1 ± 4.2	32.7 ± 2.6	$P < 0.1$	31.1 ± 4.1	35.2 ± 2.9	$P < 0.05$
Zinc intake (mg)	0.62 ± 0.09	6.9 ± 0.06	$P < 0.1$	0.7 ± 0.06	0.75 ± 0.06	NS
% Zinc absorption	16.3 ± 6.8	21.0 ± 6.3	NS	16.7 ± 2.5	21.4 ± 2.8	$P < 0.05$
% Iron absorption	19.7 ± 8.3	20.6 ± 7.6	NS	25.9 ± 4.9	29.3 ± 4.2	NS
% Body fat	–	–	–	10.0 ± 1.2	7.9 ± 1.1	$P < 0.05$
% Body protein	–	–	–	16.6 ± 1.1	18.2 ± 0.7	$P < 0.05$

Table 4a. Concentrations of zinc and iron in liver, kidney and blood in control and nicotinic acid supplemented mice* during Experiment 1

	Control ($\mu g\ g^{-1}$ weight)	Nicotinic acid supplemented ($\mu g\ g^{-1}$ weight)	% change	*P* value
Liver				
Zinc	32 ± 2.5	47 ± 3.7	46.9	0.01
Iron	258 ± 40.8	438 ± 40.8	69.8	0.05
Kidney				
Zinc	75 ± 15.5	85 ± 9.8	13.3	NS
Iron	145 ± 35.5	248 ± 18.4	71.1	0.05
Blood				
Zinc	12 ± 2.0	15 ± 2.5	20.0	NS
Iron	468 ± 28.6	485 ± 70.2	3.5	NS

* Six control and six nicotinic acid supplemented mice were used.

Table 4b. Concentrations of zinc and iron in liver and blood, and zinc absorption in three groups during Experiment 2

	Group I NA deficient	Group II NA adequate	Group III NA excess
Liver			
Zinc ($\mu g\ g^{-1}$)	19.0 ± 4	21.0 ± 3	24.0 ± 5
Iron ($\mu g\ g^{-1}$)	61.0 ± 11	68.0 ± 10	78.0 ± 17
Haemoglobin (%)	12.2 ± 1.6	14.3 ± 1.2	13.7 ± 1.6
Zinc absorption (%)	33.8 ± 11.7	63.4 ± 3.3	57.3 ± 3.9

immunity, poor healing, dermatitis, and impairment of neuropsychological functions (Sandstead 1991).

Earlier studies of *in vitro* erythrocyte zinc uptakes have shown that exchangeable erythrocyte zinc can be assessed in samples of 0.4 ml packed cells using ^{65}Zn. Further, the presence of zinc binders such as histidine amplified ^{65}Zn uptake by cells (Wouwe *et al*. 1990). The results of our *in vitro* experiments with erythrocytes showed that addition of both nicotinic acid and NADP resulted in increased uptake of zinc in fasting conditions as well as in the postprandial state. When two physiological states are compared, namely fasting and postprandial, the interactive effect of nicotinic acid was found to be higher in the postprandial state than in the fasting state. Secondly, NADP, which contains adenine and phosphate groups, showed less enhancement than nicotinic acid, suggesting that these functional groups diminished the net zinc uptake by erythrocytes.

It is a well established fact that in vegetarian diets most of the niacin is in the bound form, having low bioavailability, although gross levels of niacin in the diet may be adequate (Carter & Carpenter 1982). Gross niacin content of finger millet (1.1 mg per 100 g) is lower than that of corn (1.8 mg per 100 g). The latter is used to formulate niacin-deficient diets in animal experiments.

It has been reported that niacin deficiency at weaning age manifests in growth retardation (Carpenter *et al*. 1988). Low levels of bioavailable zinc also show similar effects. In the present study, mice fed with a basal diet containing zinc in the natural form, showed lower rates of growth (0.14 g day^{-1}) as compared with the reported rate of about 0.2 g day^{-1} on a synthetic diet (Morgan *et al*. 1988a,b). In Experiment 1, the nicotinic acid-supplemented group of mice exhibited nearly a two fold increase in the growth rate. Further, during Experiment 2 changes in percent zinc absorption and intestinal zinc contents showed significant differences. In the case of iron, percent haemoglobin and liver iron showed significant increases following supplementation with nicotinic acid. As regards the liver, kidneys and blood, the average zinc and iron contents increased but the differences were not statistically significant.

The results obtained in the present study suggest that nicotinic acid, in addition to its known effect on growth and metabolism, may be playing an important role in enhancing zinc and iron utilization.

Acknowledgements

The authors are grateful to the Director, Agharkar Research Institute, Pune for providing facilities for this work. They also thank Dr Mrs Rajurkar, Department of Nuclear Chemistry, University of Poona for providing the gamma counter facility, Mr S. Girigosavi, Ms Seema Khot and Mr Dawre for their technical assistance and Major S.B. Chaudhari, Armed Forces Sports Centre, Pune for providing human blood samples.

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