PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 316–322 (1974)

# Changes in Plasma Zinc Related to Fasting and Dietary Protein Intake of Japanese Quail[1] (37801)

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A simple means of assessing zinc nutriture in man and domestic animals has not been developed. Under conditions of zinc deficiency, the concentration of zinc in the plasma is significantly decreased in a large number of species. When adequate diets were replaced by diets very low in zinc, the plasma zinc concentrations dropped to deficiency levels very rapidly, within 36 hr in calves and lambs (1) and within 24–36 hr in rats (2). Zinc in plasma has been found to decrease fairly consistently in many disease states and in response to certain therapeutic drug regimens (3).

There are relatively few studies of the effect upon plasma zinc caused by varying the major dietary components, protein, fat, and carbohydrate. Davies *et al.* reported marked decline of plasma zinc in normal human subjects within 30 and 60 min following an oral or intravenous dose of glucose (4); the subjects had been fasted overnight. In another study of ten adult subjects, plasma zinc concentrations after an overnight fast and 1 hr following a meal were not significantly different (5).

In the present study, the effects of fasting and supplementation with major dietary components upon plasma zinc level were investigated in young Japanese quail (*Coturnix coturnix japonica*).

*Methods and Materials.* Day-old coturnix of both sexes from our own stock colony were maintained in continuously lighted, heated batteries under conditions to minimize environmental zinc contamination. The birds were wing-banded at 7 days of age and were weighed at weekly intervals.

During the first 4 wk, the birds received a purified diet containing 35% soybean protein (RP-100, Ralston Purina Co., St. Louis, Mo.). It was identical to the principal basal diet described previously except that the content of ethoxyquin was decreased to 100 mg/kg (6). In the early experiments some of the birds received a total of either 7 or 75 mg of zinc per kg of diet. For these diets, 70 g of the soybean protein was extracted four times with disodium ethylenediaminetetraacetic acid ($Na_2EDTA$) by the procedure of Davis *et al.* (7). The remaining 280 g of soybean protein per kg of diet was not extracted. The total contaminant zinc in this diet was 7 mg/kg, an appropriate level for producing severe zinc deficiency without excessive mortality. Zinc carbonate was added to supply the higher level of dietary zinc.

In many of the subsequent experiments that did not involve zinc deficiency, the same level of extracted protein was fed during the first 4 wk. Statistical analysis showed that the presence of 70 g of extracted protein did not affect the plasma zinc level of a given dietary zinc concentration; therefore, use of extracted protein was discontinued. No extracted protein was fed during the initial 4-wk period to birds receiving 25 mg of zinc per kg of diet. Diet and demineralized drinking water were available at all times.

The effect upon plasma zinc level of 24-hr

[1] Taken in part from a dissertation submitted by B. H. in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Dairy Science (Nutrition), University of Maryland, 1971. A part of these data was presented at the annual meeting of the Federation of American Societies for Experimental Biology, April 13, 1971.

fasting followed by feeding various dietary supplements for 4 hr was measured when the birds were 4 wk of age. The supplements ranged from complete diets to single dietary components. All of the soybean protein in these supplements was extracted with $Na_2EDTA$. The soybean protein diet that was fed as a supplement was otherwise the same as the diets above, except that it contained no added zinc. The egg white diet had the same composition except that an equal weight of dried egg white (General Biochemicals, Chagrin Falls, Ohio) replaced the soybean protein. Also, in this diet the vitamins were omitted for simplicity of preparation, since it was not expected that they would affect zinc metabolism in normal birds during the 24-hr feeding period. To avoid the problem of stickiness, all of the egg white used in these supplements was autoclaved dry at 110° for 10 min, pulverized, and sieved. For some treatments the soybean protein and egg white were mixed with the same salts mixture minus zinc and at the same level of 6% salts as was used in all the diets.

In an attempt to obtain greater uniformity of response within a group, a 33% slurry of egg white in demineralized water was force-fed by slowly dispensing the slurry from a polyethylene pipet inserted into the back of the bird's mouth. After fasting for 24 hr, the birds received 2.48 g of egg white, force-fed in two divided doses at the beginning and middle of the 4-hr period. This was similar to the ad lib. intake of most groups.

Other supplements included glucose alone and corn oil mixed 445:555 (w/w) with finely ground cellulose (General Biochemicals, Chagrin Falls, Ohio). The cellulose had been washed several times with deionized water and ethanol to remove contaminants. The oil–cellulose mixture was paste-like and had the same caloric density as the purified diets. Most supplements contained low levels of contaminating zinc; zinc carbonate was added to a few supplements to supply 7 mg of zinc per kg of diet. Demineralized drinking water was available at all times.

Birds were weighed at the beginning and end of each fasting and supplement-feeding period. The amount of supplement consumed was weighed.

At the end of the experimental periods the birds were lightly anesthetized with sodium pentobarbital and ether. The thoracic cavity was opened and blood was withdrawn from the heart into a heparinized all-glass syringe. The blood was centrifuged and the separated plasma was stored at —18° until analysis. In most experiments, plasma from two to three birds was pooled for analysis. The plasma was wet-ashed with a 3:1 (v/v) mixture of nitric and perchloric acids and then assayed by atomic absorption spectrophotometry (model 303, Perkin-Elmer Corp., Norwalk, Conn., by the general procedure described in the manufacturer's manual No. 990-9461). All glassware and the polycarbonate storage tubes that came in contact with either the blood or plasma were soaked in acid, rinsed in deionized water, and dried to remove contaminating zinc before use. Diets and supplements were assayed for zinc by the same procedures.

All control groups and many experimental groups were replicated one or more times. Since there was good agreement between experiments, the data are presented in summary form by treatment. The data have been statistically evaluated by Student's $t$ test (8).

*Results*. At 4 wk the body weights averaged 46 and 82 g for birds receiving 7 and 75 mg of zinc per kg, respectively. The birds receiving the lower level of zinc exhibited the characteristic zinc deficiency syndrome (9). The plasma zinc level in the deficient birds was very low (62 $\mu$g/100 ml plasma) and was not altered by fasting (Table I). Normal birds fed 75 mg of zinc per kg diet had higher levels of zinc in the plasma than did the deficient birds. Fasting for 24 hr or feeding a low zinc soybean protein diet for 24 hr did not alter plasma zinc. There was a marked decrease in plasma zinc when the birds were fasted and then fed a low zinc diet for 4 hr.

In subsequent experiments, birds fed 75 mg of zinc per kg of diet had a higher non-fasting level of plasma zinc (Table II) than those in the first study (Table I). Birds that received 25 mg of zinc per kg of diet grew and developed normally; however, the non-fasted controls had lower plasma zinc levels than corresponding birds fed 75 mg of zinc

TABLE I. Effects of Fasting and Dietary Supplements on Plasma Zinc Level of Zinc-Deficient and Normal Coturnix at 4 Wk of Age (mean ± SE).

| Fasting period (hr) | Supplement[a] | | | | | Plasma Zn (μg/100 ml) |
|---|---|---|---|---|---|---|
| | Period (hr) | Description | Contaminant Zn (mg/kg) | No. birds | No. assays | |
| | | Zinc-deficient birds (7 mg Zn/kg diet to 4 wk age) | | | | |
| 0 | — | None | — | 10 | 3 | 62 ± 9 |
| 24 | — | None | — | 11 | 3 | 72 ± 16 |
| 0 | 24 | Soybean protein diet | 1.6 | 11 | 3 | 70 ± 15 |
| 24 | 4 | Soybean protein diet | 1.6 | 11 | 3 | 54 ± 18 |
| | | Normal birds (75 mg Zn/kg diet to 4 wk age) | | | | |
| 0 | — | None | — | 11 | 3 | 246 ± 30 |
| 24 | — | None | — | 11 | 3 | 279 ± 20 |
| 0 | 24 | Soybean protein diet | 1.6 | 11 | 3 | 234 ± 14 |
| 24 | 4 | Soybean protein diet | 1.6 | 11 | 3 | 133 ± 22[b] |

[a]Birds that were fasted only received no supplement. In birds that were both fasted and supplemented, the supplemental period immediately followed the fast. Plasma was collected for zinc assay at the end of the appropriate fast or supplement-feeding period.

[b]Significantly different from respective control group fasted 24 hr, $P < 0.01$.

($p<0.05$, Table II). Birds with either initial zinc intake had lower plasma levels after 24 hr of fasting or after receiving a low zinc diet or protein supplement for 4 hr following a 24 hr fast. When birds originally fed 75 mg zinc per kg of diet were then fed the low zinc dried egg white diet for 24 hr there was a marked drop in plasma zinc ($p<0.001$).

The effect of the 4-hr feeding of various dietary supplements was studied following a 24-hr fast in birds fed 75 mg of zinc per kg of diet to 4 wk of age (Table II). All of the supplements that contained protein, either alone or with other dietary components, caused a marked decline in plasma zinc when compared with the value obtained after a 24-hr fast alone. Dried egg white and soybean protein had similar effects. The plasma zinc level of zinc-deficient birds was not markedly changed by feeding the low zinc soybean protein diet either before or after fasting (Table I).

Glucose and corn oil–cellulose supplements did not cause marked decreases in plasma zinc compared to the control group fasted 24 hr, although the difference was statistically significant for the corn oil and cellulose mixture.

Several of the 4-hr supplements were also tested with zinc carbonate added to supply 75 mg of zinc per kg. These were all fed to fasted birds that had received 75 mg of zinc per kg of diet during the first 4 wk. Again, birds fed the supplements containing protein had levels of plasma zinc that were lower than those of birds fasted for 24 hr, and the addition of zinc to the supplement did not cause a significant increase over the birds receiving the corresponding low zinc supplement.

Birds that received either glucose plus zinc or corn oil–cellulose plus zinc had higher plasma levels than birds that received the corresponding low zinc supplements. Birds fed either of these supplements plus zinc had higher mean plasma zinc values than birds fasted 24 hr alone. The difference, however, was not statistically significant for the corn oil–cellulose group because of the high variation within this group.

*Discussion.* These studies show that plasma zinc level can be related to a prior constant dietary zinc concentration as reported by other workers (10). It was also shown that plasma zinc can be rather rapidly altered by fasting and by protein in supplements consumed after fasting.

The effect of fasting in normal quail differs from the observations of Spencer and Samachson who found no change in plasma zinc levels of obese men fasted for long periods of time (11). The men were probably utilizing cal-

TABLE II. Effects of Fasting and Dietary Supplements on Plasma Zinc Level of Normal Coturnix at 4 wk of Age (mean ± SE).

| Fasting period (hr) | Supplement[a] Period (hr) | Supplement[a] Description | Supplement[a] Contaminant Zn (mg/kg) | No Zn in supplement No. birds | No Zn in supplement No. assays | No Zn in supplement Plasma Zn (μg/100 ml) | 75 mg Zn/kg supplement No. birds | 75 mg Zn/kg supplement No. assays | 75 mg Zn/kg supplement Plasma Zn (μg/100 ml) |
|---|---|---|---|---|---|---|---|---|---|
| | | | Normal birds (25 mg Zn/kg diet to 4 wk age) | | | | | | |
| 0 | — | None | — | 10 | 5 | 321 ± 15[b] | — | — | — |
| 24 | — | None | — | 30 | 16 | 228 ± 8 | — | — | — |
| 24 | 4 | Dried egg white | 0.6 | 10 | 6 | 187 ± 8[b] | — | — | — |
| | | | Normal birds (75 mg Zn/kg diet 4 wk age) | | | | | | |
| 0 | — | None | — | 15 | 6 | 387 ± 20[b] | — | — | — |
| 24 | — | None | — | 19 | 11 | 264 ± 14 | — | — | — |
| 0 | 24 | Dried egg white diet | 1.0 | 7 | 3 | 231 ± 22 | — | — | — |
| 24 | 4 | Soybean protein diet | 1.6 | 22 | 10 | 150 ± 13[b] | 22 | 14 | 189 ± 17[b] |
| 24 | 4 | Soybean protein | 2.5 | 22 | 12 | 169 ± 11[b] | 16 | 8 | 194 ± 17[b] |
| 24 | 4 | Soybean protein + 6% salts (no Zn) | 2.4 | 4 | 4 | 143 ± 12[b] | — | — | — |
| 24 | 4 | Dried egg white | 0.6 | 16 | 7 | 130 ± 17[b] | 12 | 4 | 136 ± 29[b] |
| 24 | 4 | Dried egg white + 6% salts (no Zn) | 1.4 | 5 | 5 | 96 ± 11[b] | — | — | — |
| 24 | 4 | Dried egg white, force-fed | 0.6 | 12 | 3 | 146 ± 6[b] | — | — | — |
| 24 | 4 | Glucose | 0.2 | 10 | 10 | 226 ± 14 | 8 | 8 | 311 ± 18[b,c] |
| 24 | 4 | 44.5% corn oil + 55.5% cellulose | 0.5 | 10 | 10 | 220 ± 6[b] | 10 | 10 | 332 ± 40[c] |

[a]See footnote [a], Table I.
[b]Significantly different from respective control group fasted 24 hr, $P < 0.05$.
[c]Significantly different from corresponding group receiving no zinc in their supplement, $P < 0.05$.

ories primarily from depot fat, with fewer calories from breakdown of body protein. Anabolism of protein was probably small. From our data on the supplements, calories supplied by fat did not lower plasma zinc. A 24-hr fast for the zinc-supplemented birds resulted in a weight loss of approximately 11% of their prefasted weight. From gross observation, the fat depots in the birds at 4 wk of age were small; therefore, the birds were probably catabolizing body protein as well as fat. Again, the supplement data would suggest that utilization of body protein caused lowered plasma zinc.

The procedure of fasting for 24 hr followed by a 4-hr supplement-feeding period provided a simple means of focusing on the effects of individual macronutrients upon plasma zinc level. The time for food passage through the quail is reported to be 60–90 min (12). We have observed the same time intervals in zinc-deficient and normal birds (unpublished data). From observation, the birds ate at intervals throughout the 4-hr period and did not consume excessive amounts of diet at the beginning. Thus, similar but significant quantities of all supplements were consumed, digested, and absorbed during the 4-hr period. Force-feeding was feasible but somewhat time consuming. The within-group variation for plasma zinc of force-fed birds was among the smallest observed. Of a large number of birds examined from a variety of supplemental treatments, all had small amounts of diet in their crops when they were killed.

Contrary to the data of Davies *et al.* (4) glucose did not alter plasma zinc level significantly. The reason is not apparent, although many aspects of the experimental conditions are different.

The striking observation in these studies was that plasma zinc of normal birds was decreased by all supplements that contained protein (Tables I and II). Among the protein-containing supplements fed for 4 hr following the 24-hr fast, similar decreases in plasma zinc were obtained whether the supplement consisted of soybean protein alone, soybean protein plus 6% salts, or the diet containing 35% soybean protein. Dried egg white and soybean protein had similar quantitative effects upon plasma zinc. Additional studies are required to establish minimal levels of protein and of specific amino acids that could decrease plasma zinc concentration. Although not studied extensively, the effect of protein was not shown in zinc-deficient birds. The plasma zinc level in the deficient birds was less than half that of any other group in this study.

It seems unlikely that the fasted birds had difficulty in absorbing zinc, since inclusion of zinc in the glucose or fat–cellulose supplements did result in a higher plasma zinc level. It is unreasonable that protein would prevent zinc absorption. Under the conditions of protein supplement-feeding, one would expect a high rate of amino acid anabolism, possibly directed toward repletion of protein stores. The decrease of zinc in the plasma may be either incidentally or functionally related to these processes.

Zinc can bind to many amino acids; however, it binds most strongly to histidine and cysteine. It has been shown that feeding high levels of those amino acids and arginine can alter some aspects of zinc deficiency (13,14). It is possible that plasma amino acids have a functional role in the transport of zinc.

*In vitro* studies have shown that zinc can be removed from most plasma proteins by certain amino acids (15). If the turnover rate of zinc bound to plasma amino acids were rapid, such a mechanism could account for a significant movement of zinc via the plasma without a detectable change in the total zinc level. Under conditions where movement of amino acids from the plasma was more rapid, the level of plasma zinc might be reduced. The concentration of zinc in most tissues is almost an order of magnitude greater than the level in the plasma. Any condition that created a demand on a tissue could rapidly deplete the level of plasma zinc unless large amounts of zinc were being absorbed or moving out of another tissue.

It has been shown that there may be possible variations in plasma zinc level due to diurnal rhythm (16). We believe this effect to be minimal in our experiments since the birds were exposed to continuous artificial light. Also, in order to perform the feeding experiments and have enough time to collect blood for plasma, the times were staggered during the day. If there were a pronounced diurnal effect it would have been evident from variable results. We found close agreement when experiments were repeated, despite changes in the hours at which we collected the blood.

Urinary excretion of zinc was not measured. Decreases in plasma zinc due to losses by this route cannot be excluded.

Plasma zinc concentration is a useful measurement in evaluating zinc nutriture of an individual; however, cognizance must be taken of the numerous factors that can affect the measurement other than dietary zinc intake. The range between normal and deficient plasma zinc levels in man (3) is much smaller than that for experimental animals, as observed in this study and previously (10). In man the normal value is approximately 96 $\mu$g/100 ml plasma and 70 $\mu$g is generally regarded as the lower limit of normal. Even the severely deficient dwarfs had mean values of 68 $\mu$g/100 ml (17) and 48 $\mu$g (18). In the latter study the values for the dwarfs ranged from 29 to 83 $\mu$g. It appears that it would be impossible to produce changes in plasma zinc in man comparable to the magnitude of those shown here by graded zinc intake, fasting, and protein intake. Since there is considerable variability in plasma zinc levels of nor-human beings (3), part of this variability might be related to protein intake prior to plasma collection, particularly if the sample were collected soon after breakfast. This area seems to merit further investigation.

*Summary*. Day-old *Coturnix coturnix japonica* of both sexes were fed a purified diet containing deficient, requirement, or excess amount of zinc (7, 25, or 75 mg of zinc per kg of diet, respectively) for 4 wk. The plasma zinc concentrations reflected the level of zinc intake during this time. All birds except nonfasted controls and one group of supplemented birds were fasted for 24 hr. There was no decrease in plasma zinc in the deficient birds after fasting; however, there was a significant decrease in birds receiving 25 or 75 mg of zinc per kg of diet. Following the fast, supplements consisting of complete diets or dietary components were fed, with or without added zinc (75 mg of zinc per kg of diet) for 4 hr. In birds that had received normal or excess zinc, there was a marked decrease in plasma zinc from the 24-hr fasting value when the supplement contained either soybean or egg white protein. When zinc was added to the supplement, the effect of protein on plasma zinc level was unchanged. Supplements of glucose or corn oil plus cellulose did not result in the marked lowering of plasma zinc that was observed with protein. Plasma zinc was significantly increased when zinc was added to the supplement of glucose or corn oil plus cellulose.

Under conditions of rapid protein metabolism, it appears that one might expect a significant decrease in the plasma zinc level.

The authors wish to thank Jesse J. Gantt, Geneva Clark, Francenia V. Grinnage, and Maggie Smith, Food and Drug Administration, for excellent technical assistance and care of the birds; and F. E. Calvert, Ralston Purina Co., who supplied lots of soybean protein selected for their low zinc content.

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Received July 5, 1973. P.S.E.B.M., 1974, Vol. 145.