Alopecia induced in young mice by exposure to excess dietary zinc

S.A. Mulhern, W.B. Stroube Jr and R.M. Jacobs

Division of Nutrition and Division of Chemical Technology, Food and Drug Administration, 200 C Street S.W., Washington (District of Columbia 20204, USA) 29 July 1985

Summary. Second generation mice were exposed to normal (50 ppm, Group I) or excess (2000 ppm, Group II) zinc in the maternal diet during gestation and lactation, then weaned and continued on the mother's diet until sacrifice at 8 weeks. Tibia zinc reflected dietary intake. Group II had reduced plasma copper, body weight, and hematocrit; the second coat of hair appeared late and was lighter in color than Group I, possibly as an effect of copper and pigmentation development and hair growth.
Key words. Alopecia; zinc excess; mice; copper deficiency.

In the course of our study of the effects of excess zinc on the immune response during ontogeny, we observed that mice exposed to excess dietary zinc during certain stages of development exhibited an abnormal hair loss at approximately 5 weeks of age. Because of the widespread use of excess zinc by humans in the form of supplements and in the treatment of coeliac disease[1] and Wilson's disease[2], as well as frequent administration to animals for the prevention of copper toxicosis in sheep[3] and increased growth rate in pigs[4], we report this abnormal hair growth pattern in the young mouse.

Diets. All mice were fed ad libitum (except the pair-fed group) a biotin-fortified egg white diet that contained either normal (50 ppm) or excess (2000 ppm) levels of zinc as zinc carbonate. The diets, which were formulated and prepared by Teklad Test Diets (Madison, Wisconsin), consisted of egg white solid, spray-dried, 200 g/kg; biotin, 0.004 g/kg; L-tryptophan, 0.6 g/kg; non-nutritive fiber (cellulose), 30 g/kg; and vitamin mixture AIN-76, 1%. Since this vitamin mixture did not include a source of choline, choline bitartrate was added at 10 g/kg. Salts were added to supply the mineral requirements of the mouse as recommended by the National Research Council[5]. Both diets were analyzed for zinc by atomic absorption spectrophotometry. Certified rodent chow 5002 was obtained from Ralston Purina Company, St. Louis, Missouri.

Animals. Female weanling C57BL/6J mice (first generation) were obtained from the Jackson Laboratory (Bar Harbor, Maine) at 21 days of age, matched by weight, and placed on a diet containing either 50 or 2000 ppm zinc. At approximately 6 weeks of age they were bred with age-matched C57BL/6J males. To study the effect of excess zinc during gestation/lactation/postweaning development, the dams and offspring (second generation) were distributed into 10 different dietary groups which received the following diets containing 50 or 2000 ppm zinc during gestation/lactation/postweaning development: 1) 50/50/50 (control); 2) 50/50/2000; 3) 2000/50/50; 4) 2000/2000/50; 5) 2000/50/2000; 6) 50/2000/50; 7) 50/2000/2000; 8) 2000/2000/2000; 9) 50/50/50 (pair-fed to group 8); 10) chow/chow/chow.

Table 1. Effect of excess dietary zinc on 8-week-old second generation C57BL/6J mouse tibia zinc levels, plasma copper levels, and hematocrit values (mean ± SD)[a]

	Group 1, 50 ppm zinc in diet	Group 8, 2000 ppm zinc in diet
Zinc, μg/g dry bone	179.29 ± 13.29	661.00 ± 29.38[b]
Copper, μg/100 ml	137.00 ± 29.55	47.67 ± 45.84[b]
Hematocrit, %	37.40 ± 4.13	22.25 ± 2.06[b]
b. wt, male, g	19.90 ± 2.04	10.13 ± 1.85[b]
b. wt, female, g	17.94 ± 1.02	10.21 ± 2.04[b]

[a] A minimum of six observations were used for each statistical analysis.
[b] Values significantly different from control values at $p \leq 0.05$.

Methods. At approximately 8 weeks of age the second generation mice from groups 1 and 8 were sacrificed by cervical dislocation. The tibias were removed and dried, and zinc was determined by neutron activation analysis[6]. Blood was collected from these mice, and plasma was separated by centrifugation. Plasma copper was determined by atomic absorption spectrophotometry (model 5000, flame absorption, equipped with a bead impact spoiler, Perkin-Elmer Corp., Norwalk, Connecticut). Micro-hematocrit determinations were made by using an Adams Micro-Hematocrit Centrifuge and reader.

Statistics. At least 6 observations were used for each statistical analysis. Student's t-test was performed to compare the mean of the experimental group with that of the control. Population

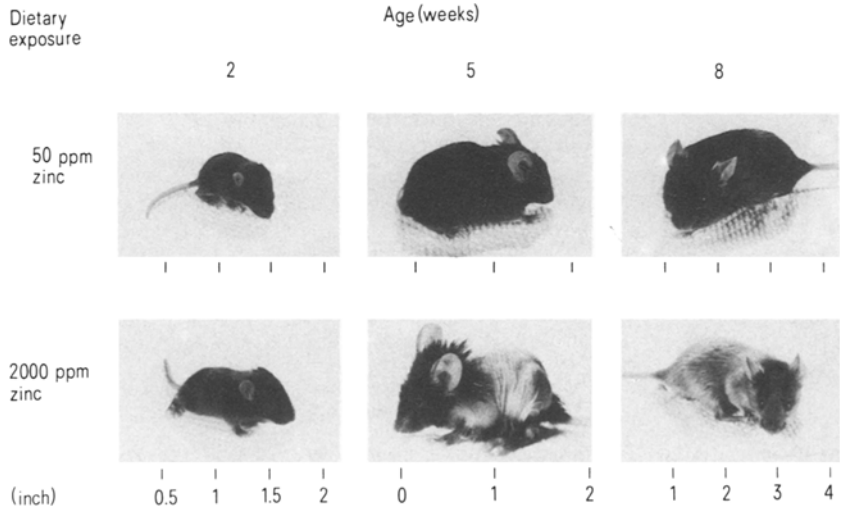

Fig. 1. Hair growth pattern of mice exposed to normal (50 ppm) and excess (2000 ppm) zinc at 2, 5, and 8 weeks of age. Five litters of mice were exposed to excess zinc. The photograph of the mouse exposed to 2000 ppm at 5 weeks of age has been enlarged. The other photographs are all natural size but were not all reproduced at the same magnification.

Table 2. Hair development of C57BL/6J mice exposed to zinc during different stages of ontogeny

Animal diet, gestation/lactation/postweaning	Hair description, age in weeks 2	5	8
1. 50/50/50	black	black	black
2. 50/50/2000	black	black	silver
3. 2000/50/50	black	black	black
4. 2000/2000/50	black	hairless	silver
5. 2000/50/2000	black	black	silver
6. 50/2000/50	black	hairless	silver
7. 50/2000/2000	black	hairless	silver
8. 2000/2000/2000	black	hairless	silver
9. 50/50/50 (pair-fed)	black	black	black
10. chow/chow/chow	black	black	black

variances were tested for equality by the F-test. Statements of difference were based on a one-tail value of ≤ 0.05.

Results. The data presented in table 1 indicate that mice exposed to excess zinc during gestation/lactation/postweaning development have reduced plasma copper, lowered hematocrit values, and reduced body weight ($p \leq 0.05$). The body weights from all other groups, except 1 (control), 2 (50/50/2000 ppm zinc), and 10 (chow/chow/chow), were reduced by varying amounts. Figure 1 shows that mice exposed to excess dietary zinc during gestation, lactation, and postweaning development have a normal coat of hair at 2 weeks of age. At approximately 2–4 weeks of age this group began to lose hair, so that at 5 weeks of age the alopecia was most severe. The remaining hair on the head did not evidence achromotrichia. The skin from these mice at 5 weeks of age was notably thinner than skin from normal mice. Figure 2 demonstrates that the skin folds of a normal mouse at the same age are much thicker. No histological examinations were performed to confirm this observation. Hair regrowth began at about 6 weeks and by 8 weeks of age hair had regrown but was lighter in color than that of control mice. The photograph of the mouse at 5 weeks of age is approximately 1–½ times the natural size. The photographs of the other mice are all natural size.

The data presented in table 2 demonstrate that mice exposed to excess dietary zinc at least during lactation, or lactation plus other developmental periods, develop alopecia by 5 weeks of age. All of the pups from groups 7 and 8 developed alopecia by 5 weeks of age. In all other groups where alopecia was observed, not all of the pups in any one litter exhibited this phenomenon and the alopecia was more moderate. The achromotrichia was variable among the groups and most pronounced in groups 7 (50/2000/2000 ppm zinc) and 8 (2000/2000/2000 ppm zinc).

Discussion. Abnormalities of hair and wool have been found in a number of species fed copper-deficient diets[7]. In humans, Menkes Kinky-Hair Syndrome is the result of a genetic defect which prevents the absorption of copper[8]. Other studies have shown that copper deficiency induced by excess zinc causes alopecia in both the monkey[9] and mink[10]. Our data demonstrate that mice which express severe symptoms of copper deficiency such as reduced plasma copper, lowered hematocrit, and achromotrichia also have an abnormal hair loss by 5 weeks of age. It is reasonable to assume that the abnormal hair growth observed in these mice was due to copper deficiency. Studies with copper supplementation will have to be done in order to confirm this. However, in support of this hypothesis it is interesting to note that the groups that had the most severe achromotrichia also had the most severe alopecia. Achromotrichia is thought to be due to the effect of copper deficiency on the activity of tyrosinase, the first enzyme in the melanin biosynthetic pathway[11]. Since pigmentation was normal in all groups at 2 weeks of age the dams may have had adequate copper to supply the fetus during gestation but not throughout lactation. In addition, all groups of nurslings could have received adequate nutrition at the beginning of lactation, but a reduction in both milk supply and lowered absorption of copper due to the excess zinc in the diet[12] could account for the alopecia in the different dietary groups. The regrowth of hair by 8 weeks of age may be the result of the lower copper requirement of the postweaning animal.

Alopecia was observed at the time of the second wave of hair growth, suggesting that excess dietary zinc interferes with some phase of the second hair growth cycle. Many drugs and chemicals lead to a characteristic anagen effluvium such as the antimitotic agents cyclophosphamide, methotrexate, vincristine, and colchicine[13]. Toxic levels of some metals exert their effect by causing the hair cycle to end prematurely[14]. Thallium[15] and anticoagulants induce telogen effluvium. Other medications, such as polyethylene alanine[16] and phenyl glycidyl ether[17], and polychlorinated biphenyls[18] cause hair loss of unknown type. In the current studies the observed alopecia probably was not due to the reduced feed intake since the pair-fed group did not exhibit alopecia at 5 weeks of age. Studies with mutant mice having a defect in copper metabolism show an abnormal and retarded development of the hair bulb. These effects can be ameliorated by copper supplementation[19], suggesting that copper is required for the normal development of the hair bulb. It follows that the mice in this study may have had an abnormal and retarded hair bulb development which would result in a delay in the second wave of hair growth. Since the anagen phase of hair growth is characterized by an increase in hair bulbs and a rapid cellular proliferation, it is possible that the excess dietary zinc caused a delay in the second anagen phase of the hair growth cycle. It is equally tenable that the excess zinc precipitated telogen development or directly poisoned the anagen root. Histological determinations are necessary to establish the pathomechanism of the delay of the second wave of hair growth in these mice.

These studies demonstrate that excess dietary zinc results in alopecia and achromotrichia when administered to the young mouse. It is known that excess zinc administered to adult humans results in hypocupremia[20] but there are no reports of alopecia development. Whether excess zinc will produce alopecia in humans when administered at certain stages of development and how alopecia is induced in these studies remain obvious matters for further investigation.

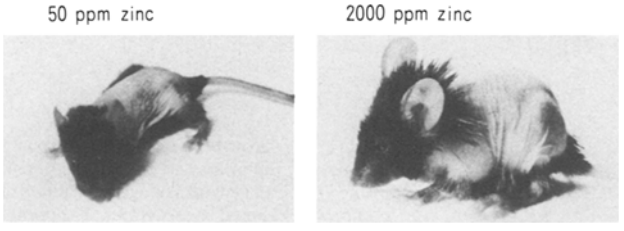

Fig. 2. Skin fold thickness of mice exposed to normal (50 ppm) and excess (2000 ppm) zinc at 5 weeks of age. The mouse exposed to normal zinc was shaved to reveal thickness of skin folds.

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The action of various vitamin D_3 metabolites on calcium and phosphorus metabolism in chick embryo calvariae

R. Brommage, L.E. Hart and H.F. DeLuca

Department of Biochemistry, University of Wisconsin-Madison, Madison (Wisconsin 53706, USA), 7 August 1985

Summary. Chick embryos from vitamin D-deficient hens given physiological doses of 1,25-dihydroxyvitamin D_3 or 24,25-dihydroxyvitamin D_3 or both become severely hypocalcemic, hyperphosphatemic and fail to hatch as compared to those derived from hens given 25-hydroxyvitamin D_3 or 24,25-difluoro-25-hydroxyvitamin D_3. Calvariae from the former contain less mineral and on incubation in vitro produce significantly lower calcium and higher phosphate concentration in the medium than do the calvariae derived from the embryos of hens supported on 25-hydroxyvitamin D_3 or 24,24-difluoro-25-hydroxyvitamin D_3.
Key words. Embryonic development; bone; vitamin D.

Embryos from hens receiving physiological doses of 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) and/or 24,25-$(OH)_2D_3$ as their sole source of vitamin D do not mobilize shell calcium, consequently they do not form a fully mineralized skeleton and become severely hypocalcemic[1]. It is now quite clear that 1,25-$(OH)_2D_3$ normally induces the active transport of calcium through the chorioallantoic membrane and that this metabolite is not available to the embryos during the latter stages of embryonic life unless the hen has received one of the metabolic precursors of 1,25-$(OH)_2D_3$, namely vitamin D_3 or 25-OH-D_3[2,3]. Under normal circumstances, vitamin D_3 and 25-OH-D_3 are provided to the embryo from the yolk and the embryo has the enzymatic machinery necessary to produce 1,25-$(OH)_2D_3$[4].

Of considerable interest is the possible role of 1,25-$(OH)_2D_3$ in skeletal metabolism. Although one report has claimed that 1,25-$(OH)_2D_3$ directly stimulates bone formation in embryonic chick femurs in organ culture[5], studies in rats indicate that no vitamin D metabolite is directly required for normal skeletal growth and mineralization[6,7]. 1,25-$(OH)_2D_3$ injections into chick embryos produce hypercalcemia[8], even in the absence of the shell[9]. Moreover, 1,25-$(OH)_2D_3$ stimulates the release of ^{45}Ca from prelabeled embryonic chick long bones in culture[10]. These last two findings indicate that, as is the case in hatched chicks and other species, 1,25-$(OH)_2D_3$ is capable of stimulating bone mineral mobilization in embryonic chicks.

The experiment described in this report further examines the role of 1,25-$(OH)_2D_3$ in skeletal homeostasis in the chick embryo.

Materials and methods. Chick embryos were obtained from hens treated with either 25-OH-D_3, 24,24-F_2-25-OH-D_3, 1,25-$(OH)_2D_3$, 24,25-$(OH)_2D_3$ or a combination of both 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ as their sole source of vitamin D. Extensive experimental details are provided in our two previous publications[3,4] and the doses of vitamin D_3 metabolites used are given in the table. Daily egg production was less than 5% in hens receiving no form of vitamin D and greater than 60% in hens receiving vitamin D_3 metabolites. Hatchability of fertile embryos from hens treated with 25-OH-D_3 or 24,24-F_2-25-OH-D_3 was greater than 90%, whereas fewer than 10% of the fertile embryos from hens treated with 1,25-$(OH)_2D_3$ and/or 24,25-$(OH)_2D_3$ hatched.

At 20 days of incubation, embryonic plasma was obtained and analyzed for calcium and inorganic phosphorus content[1]. Additional embryos were sacrificed at 20 days of incubation and their calvariae removed and incubated in 1.2 ml of medium in a shaker bath under an atmosphere of 95% O_2, 5% CO_2. The medium contained the following components: HEPES 30 mM, glucose 11.1 mM, NaCl 80 mM, KCl 5 mM, $MgSO_4$, 0.5 mM, $NaHCO_3$ 25 mM, $CaCl_2$ 0.96 mM, Na_2HPO_4 2.24 mM and a mixture of the 20 major natural amino acids except asparagine at a total concentration of 29.4 mM; in addition, the medium contained 50 mg/l ascorbic acid, penicillin, streptomycin, the modified Eagles' Medium concentrations of vitamins, 5% horse serum and sufficient NaOH to adjust the pH to 7.4.

After 6 h of incubation the calvariae were removed, lightly blotted, dried overnight at 100 °C, weighed and then extracted with 2 ml of 2N nitric acid. This extract was diluted with water and analyzed for calcium content by atomic absorption spectroscopy as previously described[1]. The medium concentrations of calcium and inorganic phosphate were determined by atomic absorption spectroscopy and the colorimetric assay of LeBel et al.[11], respectively. Since the changes in medium calcium concentration during the course of the incubation resulted in a net uptake of calcium into the calvariae that was always less than 2% of the initial calvarial calcium content, this uptake of calcium was ignored in presenting the data on calvarial calcium content.

Results. The results are shown in the table. The plasma calcium and phosphorus data have been presented and discussed previously[1] and are included here for comparison to the medium calcium and phosphate data. The data for the five groups of