

**THE RELATIONSHIP BETWEEN ZINC AND PROTEIN
IN PREGNANT WOMEN**

by

Elizabeth A. Cross

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APPROVED:

S. J. Ritchey, Chairman

L. J. Taper

R. W. Young

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Introduction

The need for zinc in animal growth and reproduction was discovered in the 1930's (1). Subsequent studies have shown that deficiencies were more harmful in the times of development. Rapid growth accompanied by a subnormal amount of zinc intake impaired development. Young rats on restricted oral zinc had poor appetite, low weight gain, poor growth and delayed sexual development (2, 3). When severe zinc restriction was imposed on rats at anytime during pregnancy, fewer pups were born and those who were born had abnormalities of bone and brain, as well as low birth weights (4-8).

The essentiality of zinc for humans has been established in the past 25 years. Retarded growth and sexual development were discovered in Egypt and Iran (9). Growth and sexual development have been stimulated by oral zinc supplementation. Absorption/metabolism problems, which alter the body's zinc stores and usage are evident even in developed countries (10-14).

Thus, it seemed that in the U. S., zinc deficiencies would not be evident in the general populace. But in 1972 middle class school children in Denver were found to have poor appetite and growth regression (15). These problems were corrected by increased zinc intake.

Other reports have indicated that some older people had decreased taste acuity which may be improved by supplementation with zinc (16). Accelerated wound healing with zinc supplementation has also been noted (17). Results of these findings emphasized the need for further study of other risk groups within the population.

Based on the accumulation of zinc in the fetus and other products of conception, estimations have been made for increased human needs during pregnancy (18). These increased needs are reflected in the 1980 Recommended Dietary Allowance (RDA) as an intake of 20 milligrams per day (19) for the pregnant female.

Intake of zinc in the total population has been less than the RDA (20). With the normal zinc intake low in the general populace, would the recommended zinc intake of pregnant women be met on self-selected diets? If not, would this lower intake result in smaller size or other abnormalities to the infant? At the present time these are unanswered questions.

Zinc is linked with protein in accessibility and action in species other than the human (21-24). Increased need for nitrogen is, like zinc, very evident in the growth process of pregnancy (25-27). The RDA reflects this increase also. Problems with a low protein intake are not of as much concern in the United States as elsewhere in the world. In the U. S. the general population consumes nearly twice the protein it needs (28). High protein intakes apparently affect the retention of some minerals such as calcium (29). Does the high protein of our diets affect zinc retention? The present study was designed to provide some information relevant to the relationship between dietary zinc and protein in pregnant women, a group usually at high risk.

Review of Literature

Zinc

Zinc in Serum or Plasma

Studies with rats show lower zinc serum and plasma values for pregnant females than for non-pregnant females on the same level of zinc intake (30, 31). While zinc deficient gravid rats have lower zinc plasma levels as pregnancy progresses, there are further decreases when the zinc intake is limited.

Human studies on plasma and serum zinc show lower zinc in the serum and plasma for contraceptive users and pregnant women than for women who are neither taking oral contraceptives nor are pregnant (20; 32-36). Serum and plasma zinc levels decrease also with the duration of pregnancy (33-36). Deficiency in zinc intake cannot be imposed on pregnant human subjects but observed zinc deficiency did cause lowered zinc plasma levels in non-pregnant human subjects (37). Apparently, the serum zinc changes are the same during pregnancy for both the rat and human.

Turkish women of middle class status were an exception to the progressively lower zinc serum levels as pregnancy progressed, unlike their counterparts from rural areas (38). Only maintenance of a constant zinc level, in the serum, during pregnancy was recorded in this study. Healthy, middle-class, first parity women in Colorado followed the same zinc pattern that the poorer Turkish women followed. Their plasma zinc decreased as pregnancy progressed (33).

Lower concentration of zinc in the serum and plasma has more than

one causitive factor. It has been postulated that lower zinc serum or plasma is a partial result of the higher volume of blood that accompanies pregnancy (39, 41). Another study, measuring the volumes and affinity of albumin and α -2-macroglobulin for zinc, showed that as pregnancy progresses there is a decrease in the binding of zinc to these molecules. Less zinc is carried by these blood proteins, and the zinc that is carried is more easily released (41).

Lowered intakes of zinc decrease the plasma zinc levels in rats (31). Women on very low zinc intake have a drop in serum zinc (20). It has been postulated that lowered intake of zinc may contribute to the decreases in blood values in pregnant humans (35, 36, 38, 41). Jamison's study correlated lower serum zinc during pregnancy with fetal deformity and maternal complications thus demonstrating the potential severity of the problem (35).

Zinc Balances

Interest in the amount of zinc needed for adequate nutrition in the human has fostered studies on zinc retention. Intake differences (5.61, 8.61, 11.61, 14.61 milligrams per day) did not make a significant difference in zinc retention, even though a general increase in zinc retention was observed (42). In this balance study with pre-adolescent subjects, body sweat losses were calculated as part of the loss of zinc from the body.

In Tipton's study with adult men, sweat loss was not calculated, yet in these men on self-selected diets and normal to high intakes of zinc (11, 18 milligrams per day), one was in a low positive balance,

while the other was in negative zinc balance (43). The reason for this lack of positive balance is not explained.

Some other nutrients that are found in normal diets have been studied for their affect on zinc retention. Phytate has for some time been known to adversely affect mineral absorption, including zinc absorption (44). The calcium content of the gut, when high, lowers the amount of zinc absorbed also (44). Even the ratios of percentages of protein to zinc seem to influence the amount of zinc retained (45). Increases in zinc retention are observed with increased protein/zinc ratios, but retention is not continuous throughout. When the protein/zinc ratio increases beyond this point, both zinc absorption and balance decrease.

Few zinc balance studies have been done with adult women as subjects, with even less done with pregnant subjects. However, Sandstead has estimated the retention of zinc for human pregnancy at 750 milligrams per day, basing his calculation on the amount of zinc retained in the fetus, availability of zinc and other factors (18).

In studies with adult women, oral contraceptive users have been compared with women of the same age who are not on hormones. One investigator used normal (U. S.) intakes of zinc in his study (9 milligrams per day), while another gave his subjects very little zinc (0.17 milligrams per day). All zinc balances were negative with the very low zinc intake, but the oral contraceptive users had lost less than the others (20). As skin losses were recorded also, it was noted that women who were not on the oral contraceptives had cyclic changes in

skin excretion of zinc. These losses of zinc were much heavier in the luteal part of the cycle than in the menstrual part. Higher skin losses could account for some of the differences in zinc balance between those on oral contraceptives and those not taking hormones.

In the study done by Crews (46), subjects on normal zinc intake did not show high positive balances. Although there was no significant difference between zinc balances, the oral contraceptive users had higher zinc balances than those not on oral contraceptives.

The balance study done with pregnant subjects was done in a metabolic unit. The subjects were teenagers with the youngest sixteen years old (47). In this study protein and zinc were fed in three levels. Protein was fed at 10.5, 15.0 and 19.5 gm nitrogen, while zinc was fed at 26, 29 and 32 mg. Zinc balance was not positive for all the subjects until zinc intake was above twenty-six milligrams a day. With the highest protein intake the zinc balance, even at a higher zinc intake, was not as positive as at the next lower zinc and protein intake. When subjects were fed the 15.0/29 the average zinc balance was 6 mg per day. When 19.5/32 ratio was fed, balances averaged 2.8 mg per day.

With the increased need during pregnancy, or a simulated pregnancy, there is an increased capacity to conserve the zinc that is absorbed, or so it seems from human studies with oral contraceptives (20, 47). Changes found in gravid animals may indicate a compensatory mechanism for retaining zinc in the body. When gravid rats were sacrificed, near term, the latter contained more zinc than the non-pregnant rats in

the same experiment (48).

Other body changes have also been observed in animals. Davis et al (49) noted an increase in duodenum weight during pregnancy, with an increase in zinc concentration. The increases in the duodenum were parallel to increased zinc absorption. Other compensatory factors may also be present. Loss of dermal zinc may vary with hormone changes as described by Hess et al (20).

Zinc Deficiencies - Animals

Weanling laboratory rats subjected to a diet very low in zinc (.33-.46 milligrams) showed a slowing in growth in the first week. The cause for the retarded growth seemed to be anorexia. Total food efficiency decreased also, from 2.79 to 9.09 grams food per gram of body weight gained (2). After several weeks on this deficient diet, skin erosion appeared on the paws also. When repletion of some (2) of the rats was performed, anorexia disappeared quickly while hair conditions took eight weeks to return to normal.

Other manifestations of zinc deficiency are reported in studies with young animals. Swenerton et al (3) reports that fissures at the corner of the mouth, abnormal estrous, dermal lesions and edema resulted when young rats were fed a zero to two parts per million zinc level (3).

Experiments with zinc deficiencies in gravid rats are much more plentiful than balance studies with pregnant humans. Apgar has published many articles on various zinc intake levels with pregnant rats. In 1968 rats fed diets containing only 6 ppm zinc displayed anorexia

and difficult delivery of pups (4). In the same year another article, dealing with an experiment feeding 1 mcg. of zinc per gram of food, anorexia was proven not to be the sole cause of pregnancy problems. With pair-fed controls it was found that lowered zinc intake level was the cause of greater difficulty than lowered food intake alone (50).

Apgar (51) has reported also that rats when fed only one part per million (1 ppm) zinc, had difficulty even mating. Only one-half of the female rats mated and all the fetuses were reabsorbed by day twenty-one. The next point of exploration was the critical time factor for zinc deficiency. It was found that depletion late in gestation (day 18) caused only abnormal delivery. While, when depletion was from day twelve or fifteen, there was a loss of females. If, on the other hand, deficiency was from day one (at 1 ppm Zn), there were visible malformations of the pups (52). Hurley et al (53) found the same gross malformations.

Repletion of the gravid rats was next explored by Apgar. Different levels of zinc (15 or 30 milligrams zinc acetate) were given on day fifteen, eighteen, nineteen, twenty and twenty-one. The most benefit was obtained when the zinc intake was highest and repletion earliest (54). Again Hurley et al (7) had the same results.

Apgar reported three other experiments with pregnant rats. One study suggests the level for zinc needed during gestation to be 60 or more micrograms zinc per gram of total food eaten (50, 55). Another test contrasted and compared thiamin, another nutrient whose absence induces anorexia, with zinc (56). The pups from the zinc deficient

group died shortly after birth. The final study of the three compared two different strains of rats and found no difference in tolerance to zinc intake levels of 300 mg, 600 mg and 900 mg given in a single injection (57).

Closer examination of the pups of zinc deficient dams showed that, even if there were not gross malformations, there were still abnormalities. Hickory et al (6) found skeletal abnormalities which ranged from bone structure malformation to lack of mineralization in cranial bones, ribs, vertebrae and digits. Cox et al (30) found that other minerals were not in normal balance with zinc depletion. In this experiment results show that the calcium level of the whole fetus was higher than normal, while the level of iron in the brain and serum were lower. In both these studies there were no gross abnormalities which could be discerned by looking at the pups.

The most serious of the teratogenic problems is the malformation of the central nervous system, in pups whose dams were on a zinc deficient diet. Malformations were exencephaly and hydrocephaly among others. Even when gross malformations were not evident, microscopic tissue abnormalities were sometimes present (8, 58). Results of this type, as well as the others found in deficient animals, increase the need for knowledge of the human requirement for zinc.

Zinc Deficiencies - Human

Zinc deficiencies in humans are not so easily proven. Buerk et al (38) induced zinc deficiency in four adult humans. Some of the subjects were more resistant to zinc depletion than others, as

measured by balances, but all developed erythematous vesicular skin lesions in the groin and over joints. All the lesions disappeared soon after zinc supplementation began.

To discover conditions of disease states that might include or predispose to zinc deficiency is more difficult than producing a deficiency in humans. Sandstead lists the conditions that could or do cause zinc deficiency (59). Among those listed are acrodermatitis enteropathica, intestinal malfunction, alcoholism and pregnancy. Studies have been done in these areas which help clarify the need for zinc.

Acrodermatitis enteropathica is an inherited human zinc deficiency disorder, which is often apparent in infancy. Failure to thrive and depression are some of the symptoms which disappear when the person is supplemented with zinc (13, 14). The zinc lowering mechanism seems to be a protein excreted from the pancreas which chelates zinc. People with acrodermatitis enteropathica do not seem to absorb this combination.

Another disease which seems to cause zinc deficiencies, on normal zinc intakes, by malabsorption or excessive excretion, is Crohn's Disease. People who have Crohn's Disease more often have depressed hair and serum zinc and lowered taste acuity (11). Other types of gut irritation could also be causitive factors in zinc malabsorption, initiating a zinc deficiency. Alcohol consumption, in excess, would fit this description.

Alcoholics which have progressed to the stage of cirrhosis also

had physiological problems, which could be lessened by supplementation with zinc. Cirrhotics with abnormal dark adaption returned to normal dark adaption when zinc was given them along with Vitamin A (12), while Vitamin A supplementation alone did not give a significant improvement.

Normal middle-class Americans with no disease or conditional problems would not be expected to have zinc deficiencies. Yet in a study to assess and standardize hair as a biopsy material for zinc, middle-class children were discovered with low zinc levels. When these subjects, from Colorado, were examined for taste acuity, more than eighty percent were found to have decreased acuity (15). Supplementation with zinc returned these children to near normal acuity.

The deficiency of zinc directly associated with teratogenic effects in the human infant is the most dreaded aspect of zinc deficiency. Direct abnormalities to pups in zinc deficient dams have been proven (6, 8, 30). Although teratogenicity has never been proven in humans, it was suggested to Jamison's study (35). Also adult women who have the inherited disorder acrodermatitis enteropathica, do not become pregnant easily or often do not carry the infant to term (60, 61). If these women do give birth to a living child, it often has congenital malformations. These malformations are sometimes of the central nervous system.

This information, plus the epidemiological studies which show higher incidences of some types of central nervous system malformations in countries already known for their zinc deficiencies, has alerted

some researchers to link zinc deficiency with the increase in these forms of teratogenicity (62).

Protein

Nitrogen Balance

In the process of establishing a base line from which protein needs for maintenance and growth could be calculated, Scrimshaw documented obligatory nitrogen loss (63). On a non-protein diet, the lowest loss was on the fourteenth day. The loss at that time was 2.6 grams of nitrogen a day. Even this loss may be underestimated because intergumentary losses were not included in this balance calculation.

Adding to this base line knowledge is a study done with male subjects. Intakes of nitrogen paralleled the apparent nitrogen balances for these adult males (64). Cutaneous losses were measured in this study. The added nitrogen loss thus accounted for may account for the lack of positive nitrogen balances of most of these subjects on an intake of four grams of nitrogen a day. The protein was of a high biological quality (egg) and so should have been well utilized (64).

Kohler et al (65) found that the quality of protein affected the excretion of nitrogen and thus the nitrogen balance. Not only is there constant obligatory loss, but the protein source affects the amount of nitrogen retained by the body.

The growth process requires more of all nutrients than a maintenance state. Faster growth periods demand more of the nutrients than less rapid growth. Yet, when gravid rats were studied for nitrogen

retention over the whole gestation period, maternal nitrogen retention was greater for the first half of gestation (65). Higher nitrogen in the dams' diet did not increase maternal nitrogen storage as much as the time of maternal gestation (66, 67). At fourteen days the carcass of the pregnant dams had higher levels of nitrogen than at twenty-one days. In the rat at least, nitrogen seems to be stored during early gestation for use during the last part.

Nitrogen needs for human pregnancy, based on growth of the fetus, other non-maternal growth, obligatory loss and utilization of nitrogen from foods, is estimated at thirty extra grams of protein per day (65). It was also suggested that pregnant women need at least seventy-six grams of protein a day in at least the last two trimesters of pregnancy. Calloway, though, suggests the nitrogen intake level may be more flexible if adequate calories, from sources other than protein, are included in the diet (25).

Nitrogen balance studies with pregnant human subjects are not common. In one balance study comparing pregnant women in the first half of pregnancy with non-pregnant women, lower nitrogen excretion was found for the pregnant women (68). These two groups of women were fed the Recommended Dietary Allowances (19) for pregnant women. The pregnant women had higher nitrogen retention and greater weight gain than their non-pregnant counterparts. Pregnant women seem to utilize nitrogen differently than non-pregnant women.

The researchers of a nitrogen balance study with pregnant teenagers, treated their subjects as mature because they were at least four

years past menarche when they became pregnant (26). This research was conducted during the girls' third trimester of pregnancy. Nitrogen intake was from 9.3 to 20.0 grams per day with a retention average of 2.4 grams a day. Five of the subjects gained thirty grams per day more lean body mass as measured by total body and weight gain, than was predicted. The reason for this late pregnancy lean body mass increase is not explained. Increases in the pregnant human lean body mass seem to parallel the pregnant rat. Research indicates that there may be storage of lean body mass in earlier pregnancy with possible use of this reserve in the last trimester (69, 70).

Johnstone (72) and Marine (73) who published since 1980, both show increased nitrogen needs as pregnancy progresses. In Johnstone's study mean nitrogen retention in the third trimester was 1.2 grams per day or 12.11 grams intake. This level met the calculated need of extra maternal tissues. No comparison to the same or different subjects was made at any other time of pregnancy. Marine's (73) research was a comparison between subjects in mid-trimester, last trimester and non-pregnant women. Significantly higher nitrogen balances were found in the women in late pregnancy.

Higher intakes of protein may be needed in pregnant women in third world countries, but pregnant women in developed nations do not seem to be in need of additional nitrogen (28). The intake of nitrogen in the general non-pregnant female may be adequate for pregnancy.

Protein and Zinc Interaction

Deficiencies of either protein or zinc cause abnormal growth.

Meiners et al (72) in research with preadolescent human subjects, found that different levels of zinc intake did not affect the nitrogen retention or balance. Conversely, does the levels or types of protein consumed affect the zinc retention or balance? When Reinhold et al (44) fed protein to rats at a low level (7.4%) and zinc at two to four parts per million, he found no effect on tissue zinc attributable to the low protein intake.

On a five percent casein diet, rats had lower zinc concentration in the kidney and feces after an intramuscular dose of labeled zinc (71 Zn), than similar rats fed extra tryptophan or picolinic acid (73). True total zinc absorption was less with the added nitrogen components, than on the five percent casein diet.

Van Campen and House, feeding low (5%) or medium (15%) levels of protein to rats found that with two dietary (9 and 33 ppm) levels of zinc, the absorption and retention of zinc were lower on the low protein intake (44). Retention of a single dose of zinc (11 Mgm) was influenced by both the dietary levels of protein and zinc. The group of rats fed the highest protein and lowest zinc levels, retained the most zinc from that single dose. Differences in zinc plasma were also noted. In the rats with both the highest intakes of protein and zinc, plasma zinc was lower than for any of the other groups (47).

When the protein/zinc interaction was approached from the experimental animal choice of the pig, the only conclusion drawn was that secondary zinc deficiency may be induced by low protein intake (74). Zinc was fed at 7 mg per day, but the low protein diet was supplemented

with extra carbohydrates in some of the experimental animals. When a recovery diet was fed, all the animals had higher zinc serum levels than were found on the low protein intake.

Hurley et al (75) suggests that high calcium levels in the diet restrict bone reabsorption and deprive the fetus of the zinc that could be obtained from that source. This experimentor also states, as the result of an experiment with rats, that very low protein (5%) intake would cause soft tissue reabsorption, thus freeing zinc for gestation purposes.

When humans were fed two levels of protein (8 or 16%) and two levels of added fiber (12 to 22 grams, non-specific source), a difference was found in the apparent need for zinc (76). All the high protein diets, with or without added fiber had higher apparent zinc needs than the low protein diets.

When moderate (1,010 milligrams) and high (2,525 milligrams) levels of phosphorus were incorporated in a human diet with two levels of nitrogen (8 and 21 grams), there were differences in zinc utilization. Fecal zinc was less on the high nitrogen/moderate phosphorus combination than on any of the other diets (77). Subjects on the high nitrogen combinations lost significantly more zinc in the urine than those on low nitrogen intakes. Zinc balance was still highest for the group on high nitrogen/moderate phosphorous diet. Dermal loss was not assessed and may have made a difference.

In a zinc balance study with pregnant teenage subjects, performed on a metabolic ward, three levels of protein were fed. At

sixty-six grams of protein a day only half of the teens were in positive zinc balance at an intake of twenty-six milligrams of zinc (47). All the subjects were in positive zinc balance on an intake of twenty-nine milligrams and ninety-four grams protein. On the diet with the highest levels of zinc (32 milligrams) and protein (122 grams), all the teenage subjects were in positive zinc balance, but not as high a balance as on the next lower intake levels. Little fiber was included in these diets. These studies suggest an interaction between protein and zinc.

Materials and Methods

Procedure for Recruiting and Instructing Subjects

Twenty-four pregnant women, between the fourth and sixth months of pregnancy, were recruited from the Montgomery County area of Virginia. Notices posted on the university campus and ads in the campus and local city newspapers were used to recruit subjects.

(Appendix 1)

Subjects consumed a self-selected diet and used only dietary vitamin/mineral supplements of their choice or as prescribed by their physician.

The study was conducted under guidelines of the institution for research involving human subjects. Each subject was provided a clear written and vocal explanation of the study and signed a consent form (Appendix 4) prior to beginning.

Containers for sample collection, a small scale, recording sheet (Appendix 2) and instructions in the experimental requirements were given to each subject before the first collection period started.

Containers, labeled with a number and the subject's last name, consisted of gallon zip-lock, zinc-free plastic bags (food), small, white, zinc-free plastic garbage bags, pint-sized cardboard ice cream containers (feces), and acid-washed, screw-topped, one-liter plastic bottles (liquid and urine).

Subjects were on the study for four weeks. During the four weeks, a written record was kept of all food, liquid and supplement intake.

During the first and fourth weeks all food and excreta were collected.

Weights of the subjects were taken at the end of each collection week.

Collections of food and drink were started with the first intake of day one and included all the intake for that day. Urine collections started with the second voiding of the day and included the first voiding of the next day, to form a 24-hour composite. Fecal excretions were collected over the same time span as the food and drink. The food record was returned with each day's samples.

Procedures for Collecting and Compositing Samples

Samples were collected from the subjects daily, sometimes every second day for the convenience of the subjects and experimentors. Containers were stored in the refrigerator (food and urine) for 24 hours or freezer (feces) for one week, until composited.

Samples collected daily by subjects were:

Food and drink--portions equal in volume or weight, to actual consumption. Duplicate portions were weighed or measured; one was eaten; one was provided for collection.

Urine - total excretion

Feces - total excretion

Samples collected once during study:

Supplements consumed during study; a record was kept, samples were collected for analysis.

A 15 ml venous blood sample, at end of study, was collected by a licensed laboratory technician.

Plastic disposable containers were used to avoid zinc

contamination.

Compositing to generate weekly samples was necessary for all the samples collected on a daily basis. All weekly samples were stored in acid-washed containers in the freezer until analyzed.

Food was composited using the daily sample of both food and liquid. The weight was recorded and deionized water added to give a weight to the next even 100 grams. After blending, an aliquot consisting of five percent of the total weight was kept frozen in an acid-washed plastic container. Liter sized containers were used to hold the week's samples.

Urine volume was measured and recorded each day. The volume was then brought to 2000 milliliters with deionized water. After mixing in a two-liter, acid-washed glass jug, two aliquots were removed, one sample of 150 milliliters (7.5%) for the weekly composite and the other sample for a daily creatinine test.

Feces were held frozen until the collection period had ended. At this time they were thawed, combined for the week, weighed and diluted with an equal portion of deionized water. Total weight was recorded before blending in zinc-free blender and sampling. A sample of approximately 200 milliliters was collected in acid-washed, plastic bottles and refrozen until analysis.

A few crystals of Sodium heparin and five plastic beads were added to each blood sample and immediately centrifuged for 12 minutes at 2500 rpm's. The plasma was then carefully removed from the packed cells by pasteur pipets and refrozen in 5 ml plastic test tubes.

Analysis of Samples for Zinc

Digestion of samples for zinc analysis was accomplished by the wet ash procedure. The thawed sample was homogenized, if needed, and approximately 5 grams weighed into a 250 milliliters acid-washed beaker. The exact weight was recorded to four decimal places. Ten milliliters of nitric acid was added to the sample and let set until the sample was dissolved. Oxidization of the dissolved sample was accomplished with 5 milliliters of hydrogen peroxide (H_2O_2). After adding 10 milliliters of HNO_3 , the samples were placed over a very low heat for two hours. The temperature was increased and refluxing begun. Refluxing to dryness took from five to six hours. A second time nitric acid (5 milliliters) and hydrogen peroxide (1 milliliter) were refluxed until dry to ensure total ashing. The ash was diluted by adding two milliliters of 50% Hydrochloric acid and 25 milliliters of deionized water. This sample was then used to determine the zinc by atomic absorption spectrophotometry. Urine and plasma samples were not wet ashed. Urine was diluted 1:25 before using the atomic absorption spectrophotometer for zinc determination. Plasma was heated to 37°c in a hot water bath, diluted 1:5 before being measured by the atomic spectrophotometer. The atomic absorption spectrophotometer Perkins-Elmer 503 was used to establish a zinc level for every sample (74). The atomic absorption spectrophotometer was calibrated to known zinc standards of .1, .2 and .5 micrograms before each sampling.

Analysis of Samples for Nitrogen

Nitrogen content was assayed by the macro Kjeldahl method for food

and feces. The well-mixed sample was weighed, with the weight recorded to two decimal places. Samples were then added to Kjeldahl approximately 5 gm flasks which contained boiling chips and salts (NaSO_4 - CuSO_4) in a 100/2 ratio. Twenty milliliters of sulfuric acid were added to each flask and the flasks heated gently until the contents became clear. The clear solution was then refluxed, with higher heat, for thirty minutes. After the flasks had cooled, 250 milliliters of deionized water was added and the flasks allowed to cool again. Sixty-five to seventy milliliters of sodium Hydroxide was then added to each flask. After the flasks were stoppered, the samples were mixed with sodium hydroxide. Samples were then distilled into a container with an acid-base marker, holding 25 milliliters each deionized water and boric acid. When the distillate volume had reached 250 milliliters, the flasks were removed and tritiated with hydrochloric acid.

Urine was assayed for nitrogen content by the Technicon auto-analyzer (75).

Statistical Analysis of the Results

Correlation between the zinc and nitrogen balance was computed on the Texas Instrument 55 Calculator. Students' "T" test was used to determine possible differences between nitrogen balances and zinc balances based on zinc supplementation. Also calculated by this method was the possible differences between nitrogen intake levels and nitrogen and zinc balance. One way ANOVA was used to determine possible differences between month of pregnancy and zinc and nitrogen balance.

Results and Discussion

Description of Subjects

Description of the subjects appear in Table 1. Infant birth weight ranged from six pounds ten ounces, to nine pounds eight ounces, with a mean weight of seven pounds and fourteen ounces. The sexes of the infants were seven males and eleven females.

Nineteen subjects' data were used for final analysis. Four of the subjects were eliminated from the final statistical analysis because of suspected incomplete collections. One other subject's data was dropped because of incorrect laboratory analysis of the mineral supplement.

Zinc Retention

Zinc, in the food and liquid, was analyzed as intake separate from the supplement the subject was taking on the advice of her physician. Total intake of zinc, for these nineteen subjects, ranged from 6.98 to 28.36 milligrams per day, with a mean of 19.14 ± 6.90 milligrams per day. Zinc excretion was measured in both urine and feces. Excretion ranges from 5.57 to 35.18 milligrams were present. The mean was 18.43 ± 8.37 .

Excretion of zinc was primarily through the gut. In this study there is no difference made between zinc which was excreted by the body and zinc which was not absorbed from the food. The contents of the food have an influence on the absorption of zinc from the gut. A negative influence on zinc absorption is exerted by high phytate, iron and calcium levels (80-82). Differences in the ingestion of these

TABLE 1
Description of Subjects

Subject No.	No. Pregnancy	Mo. Began Study	<u>Baby's</u>			Baby's Sex	Zinc in Plasma mg/ml	Average Creatnir
			Mother's Wt. Wk. 1 (lb.)	Birth Wt. Lb.	Oz.			
<u>High Zinc Supplementation</u>								
1	1	6	154	7	12	M	83.5	1.10
2	2	6	131.75	6	14	F	76.7	1.00
3	1	4	146.5	8	.75	F	92.0	.95
4	2		135.5				53.5	.96
5	1	5	146.5	7	14	F	79.5	.81
6	1	6	133.25	8	5	F	74.5	1.20
7	2	6	164.5	8	0	M	84.5	1.01
8	1	5	161.5	7	12	M	73.7	.93
9	1	5	175			F	72.0	.70
10	2	4	136	9	8	M	76.7	1.10
11	1	5	138	6	10	F	42.5	.65
12	3	5	122.5	7	13.5	M	81.5	.96
13	1	4	165	8	8.5	F	67.0	1.17
<u>Low Zinc Supplementation</u>								
14	1	6	132	8	2.5	M	72.0	.97
15	1	5	135.75	6	15.5	F	57.5	.85
16	1	6	152.5	6	14	M	53.5	.96
17	1	5	140	8	15	F	77.5	1.08
18	1	6	124.5	8	4	F	58.5	.91
19	1	5	205	7	7	?	62.2	1.4

nutrients undoubtedly affected the zinc gut absorption and excretion levels. On higher zinc intake levels than these subjects were on, Schraer et al (47) found that eighty percent of the zinc intake of those pregnant teens was accounted for in fecal content of zinc. Some subjects in this study lost more zinc than they ingested.

Calculations of zinc balances indicated a range from -26.42 to 13.77 milligrams of zinc per day. Mean zinc balance was 0.705 milligrams per day \pm 8.69. Zinc intake, excretion, source, amount and balance data are in Table 2.

Balance data will have higher values than would have been found if integumentary zinc losses had been measured and calculated into the zinc losses. When Schraer et al (47), measured zinc loss through the skin, they found that it comprised eleven percent of the total loss.

Zinc average balance was less than one milligram per day (.705) but not far from the daily retention of zinc calculated by Sandstead. The calculated zinc retention was 750 micrograms per day (18). This closeness in balance with the calculated pregnancy need may explain the lack of any overt signs of zinc malnutrition in either the mothers or the infants.

Thirteen of the nineteen subjects were on a high zinc supplement, one which has over twelve milligrams of zinc. The other six subjects were on less. Without zinc supplementation, less than half of the subjects were ingesting half of the Recommended Dietary Allowances for pregnant women (19).

Self-selected diets investigated by Holden (80, 81) show levels

TABLE 2
Zinc retention (mg/day) by subjects consuming a range of zinc from food and supplements

Subject #	Zinc Intake			Zinc Excretion			Balance
	Food	Supplement	Total	Urine	Feces	Total	
<u>High Zinc Supplement</u>							
1	23.82	*	23.82	.268	21.43	21.69	2.13
2	9.30	14.75	24.05	.409	11.16	11.56	12.49
3	10.58	14.73	25.31	.160	28.25	28.41	-3.10
4	9.99	13.75	23.74	.181	15.79	15.97	7.77
5	8.49	12.21	20.70	.214	14.95	15.16	5.54
6	10.26	13.35	23.61	.385	27.64	28.02	-4.41
7	11.91	11.84	23.75	.627	23.09	23.72	0.03
8	14.61	13.75	28.36	.361	21.52	21.88	6.48
9	5.69	12.35	18.04	.193	20.97	21.16	-3.12
10	10.19	14.92	25.11	.553	24.97	25.52	-0.41
11	6.70	13.75	20.45	.150	10.81	10.97	9.48
12	9.99	14.73	24.72	.336	27.89	28.22	-3.50
13	6.94	15.12	22.06	.504	7.79	8.29	13.77

$$\begin{array}{ll} \text{Intake} & \bar{X} = 23.36 \pm 2.59 \\ \text{Excretion} & \bar{X} = 20.04 \pm 6.98 \end{array} \qquad \begin{array}{ll} \text{Balance} & \bar{X} = 3.32 \pm 6.33 \end{array}$$

<u>Low Zinc Supplement</u>							
14	8.77	.02	8.79	.285	10.81	11.09	-2.30
15	8.62	6.82	15.44	.292	17.48	17.77	-2.33
16	6.88	.10	6.98	.205	10.29	10.50	-3.52
17	9.64	*	9.64	.192	9.34	9.53	0.11
18	10.26	.01	10.27	.283	5.29	5.57	4.70
19	8.74	.02	8.76	.419	34.76	35.18	-26.42

$$\begin{array}{ll} \text{Intake} & \bar{X} = 9.98 \pm 2.90 \\ \text{Excretion} & \bar{X} = 14.94 \pm 10.67 \end{array} \qquad \begin{array}{ll} \text{Balance} & \bar{X} = 4.96 \pm 10.91 \end{array}$$

of intake in humans with a daily intake well below the 1974 RDA for zinc (8.6 ± 0.5 mg). Jamison also emphasizes that northern European diets may be too low in zinc for pregnancy stress (35). This suggests that zinc intake in a normal diet may not be high enough for the demands of pregnancy.

Even though the above studies are valid, there was no significant difference in zinc balances between the zinc supplemented group and the non-supplemented group. Supplementation seems to make no significant difference in zinc retention.

Nitrogen Retention

Protein intakes for this group of women varied widely. Averages ranged from some few (2) eating approximately forty grams, to one subject with 122 grams per day. Most subjects were taking in about seventy-five grams of protein a day (SD 3.25). The suggested intake in pregnancy is not less than seventy-five grams of protein a day (47). Only eight subjects were ingesting at or above the suggested protein intake.

Nitrogen intake, excretion and balance are tabulated in Table 3. Nitrogen intake from food and drink ranged from 6.59 to 19.59 grams per day, with a mean of 11.02 grams. Fecal excretion of nitrogen had average levels from .55 to 1.57 grams per day. Mean was 1.05 grams of nitrogen per day. Urine nitrogen was higher than the fecal nitrogen, 4.14 to 9.91 grams of nitrogen was the range with a mean being 7.18 grams. Correlation between nitrogen in the intake and nitrogen in the urine was $r=.578$ ($p < .01$). Total nitrogen excretion varied from 4.66

TABLE 3
Nitrogen Retention (g/day) of subjects

<u>Subject #</u>	<u>N Intake</u>	<u>Nitrogen Excretion</u>			<u>Balance</u>
		<u>Feces</u>	<u>Urine</u>	<u>Total</u>	
<u>High Zinc Supplement</u>					
1	14.88	.964	7.07	8.03	6.45
2	10.31	1.10	7.46	8.56	1.75
3	12.87	.879	6.45	7.33	5.54
4	9.42	.996	6.09	7.09	2.33
5	9.04	1.00	6.20	7.20	1.84
6	12.50	1.12	9.91	11.03	1.47
7	14.22	1.27	8.39	9.66	4.56
8	6.59	1.02	5.60	6.62	-.03
9	6.78	1.02	5.20	6.22	.56
10	13.95	1.11	9.83	10.94	3.01
11	8.14	.520	4.14	4.66	3.48
12	11.58	.750	9.10	9.85	1.73
13	7.19	.550	7.82	8.37	-1.18
<i>Intake</i>	$\bar{X} = 10.57 \pm 2.95$				<i>Balance</i> $\bar{X} = 2.42 \pm 2.17$
<i>Excretion</i>	$\bar{X} = 8.12 \pm 1.88$				

<u>Low Zinc Supplement</u>					
14	9.50	1.07	7.83	8.90	.60
15	10.95	1.44	7.31	8.74	2.22
16	8.55	1.52	5.81	7.33	1.22
17	12.35	1.35	7.00	8.35	4.00
18	10.96	.668	6.90	7.57	3.39
19	19.58	1.57	8.29	9.86	9.72
<i>Intake</i>	$\bar{X} = 11.98 \pm 3.95$				<i>Balance</i> $\bar{X} = 3.44 \pm 3.39$
<i>Excretion</i>	$\bar{X} = 8.46 \pm 0.93$				

to 11.03 grams per day with $8.23 \pm SD 1.62$ the mean. Total intake of nitrogen was correlated to total excretion of nitrogen by $r=0.63$ ($p < .01$). Nitrogen balances ranged from -1.18 to 9.72 with 2.77 grams the mean daily average.

Nitrogen losses were higher for this group of pregnant women than for men on a low protein intake (62). This would be expected because of increased protein intake in the women. Even in some subjects whose fecal losses were low as Calloway et al's study, the urinary losses were not as low as the subjects in this study. This is undoubtedly because of the higher nitrogen intake of these pregnant subjects.

Average nitrogen balance was 2.77 grams per day. This balance is much like the balance found for third trimester subjects and may reflect the trimester these subjects were in (85). This balance appears higher than it is because of no measurement of integumentary loss done on the pregnant women. Subjects on low nitrogen intakes had body surfaces loss of nitrogen that was low (.129 grams) (61). Lack of uniformity of nitrogen intake in this study's pregnant subjects negates any form of calculating supposed loss of nitrogen from the skin because higher nitrogen intakes increase skin nitrogen loss.

If nitrogen retention increased significantly as pregnancy progressed, the nitrogen data of the subjects who were four, five and six months pregnant could not be compared except within each month. With no significant difference found over these months in nitrogen balance, comparison could be made across all the pregnant subjects. The increased storage of nitrogen, in tissues, with a decrease in body

nitrogen at the termination of pregnancy, in rats is discussed by Naismith et al (66, 67, 71). King and co-workers reported the same type of increase in early nitrogen retention findings with human subjects (70).

Marino (85) and Johnstone (87) did not find that there was no difference in nitrogen balance between the second and third trimester of pregnancy. Their research showed definite increases in retention in the last trimester. The factors causing these discrepancies in studies need further investigation.

Nitrogen balances were not significantly different based on zinc supplementation. Meiners et al (72) found that zinc intake did not significantly affect the nitrogen balance.

The students' "T" test was used to determine if there were differences in nitrogen retentions at different levels of nitrogen intake. The subjects were divided into two groups, with an intake of 10.00 grams of nitrogen used as the point of division. The point of 10.00 grams of nitrogen was chosen as a convenient division factor (Table 4). Eleven subjects were above this intake of nitrogen while eight were below. It was found that there is a difference in nitrogen balance based on nitrogen intake ($p < .01$). The higher the nitrogen intake, the higher the nitrogen balance. An abstract from an article in Germany suggests that the type of protein ingested influenced the amount of retention. A higher biological value protein gives higher nitrogen retention (65).

Subjects in this study ingested varying amounts and types of

TABLE 4
Nitrogen Retention (g/day) of subjects

<u>Subject #</u>	<u>N Intake</u>	<u>Nitrogen Excretion</u>			<u>Balance</u>
		<u>Feces</u>	<u>Urine</u>	<u>Total</u>	
<u>Average Intake of Nitrogen Above 10 gm per Day</u>					
1	14.88	.964	7.07	8.03	6.45
2	10.31	1.10	7.46	8.56	1.75
3	12.87	.879	6.45	7.33	5.54
6	12.50	1.12	9.91	11.03	1.47
7	14.22	1.27	8.39	9.66	4.56
10	13.95	1.11	9.83	10.94	3.01
12	11.58	.750	9.10	9.85	1.73
15	10.95	1.44	7.31	8.74	2.22
17	12.35	1.35	7.00	8.35	4.00
18	10.96	.668	6.90	7.57	3.39
19	19.58	1.57	8.29	9.86	9.72
<i>Intake</i>	$\bar{X} = 13.10 \pm 2.60$				
<i>Excretion</i>	$\bar{X} = 9.03 \pm 1.27$				
<u>Balance</u> $\bar{X} = 3.99 \pm 2.50$					

Average Intake of Nitrogen Below 10 gm per Day

4	9.42	.996	6.09	7.09	2.33
5	9.04	1.00	6.20	7.20	1.84
8	6.59	1.02	5.60	6.62	-.03
9	6.78	1.02	5.20	6.22	.56
11	8.14	.520	4.14	4.66	3.48
13	7.19	.550	7.82	8.37	-1.18
14	9.50	1.07	7.83	8.90	.60
16	8.55	1.52	5.81	7.33	1.22
<i>Intake</i>	$\bar{X} = 8.15 \pm 1.17$				
<i>Excretion</i>	$\bar{X} = 7.05 \pm 1.30$				
<u>Balance</u> $\bar{X} = 1.10 \pm 1.45$					

protein. All the subjects consumed more than half their protein from animal sources during the mid two weeks (86). Thus, the higher biological value of the protein may have affected the nitrogen balance, increasing it.

Zinc and Nitrogen Interaction

Zinc and nitrogen intake, from the food source alone, did not correlate well. Although high protein foods may be the best source of zinc, other factors may be influencing this lack of correlation. This might be explained by the level of zinc added to the solid food and liquid, by the glass and other items touching the cooked and served food in the home. Even galvanized pipes that carry the water in older homes, as well as the inside of some of the metal cans used in canning, could have added zinc to the subjects' intake.

When correlation was performed between the balances 0.606 ($p < .01$) was the result (Figure 1). This negative relationship showed when the students' "T" test was used on these same balances (Table 5). The balances were not found to be equal ($p < .001$). In the higher nitrogen intake group the zinc balances were lower. Greger and Snedeker found that although higher protein increased zinc serum it also increased urinary zinc excretion (77). Their subjects retained more zinc on a high protein diet than on a low protein diet. Sandstead et al (76) found that zinc requirement was increased with protein intake. This may be the factor influencing this study. Other confounding variables may be influencing the results. Further research is indicated to clarify what is actually happening.

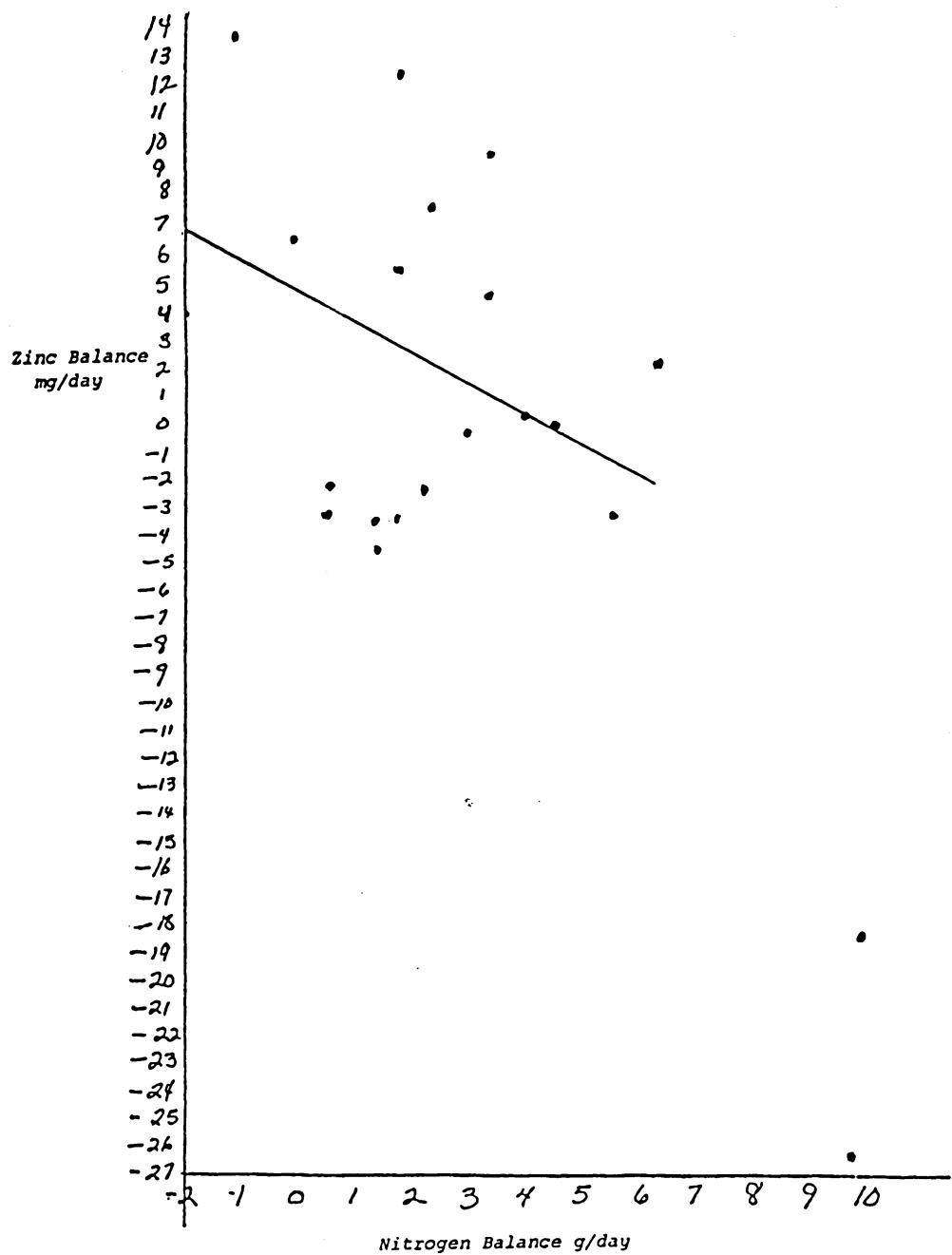


FIGURE 1
Graph of Zinc and Nitrogen Balance Correlation

TABLE 5
Zinc Retention mg/day by subjects consuming a range of
Zinc from food and supplements

<u>Subject #</u>	<u>Zinc Intake</u>			<u>Zinc Excretion</u>			<u>Balance</u>
	<u>Food</u>	<u>Supplement</u>	<u>Total</u>	<u>Urine</u>	<u>Feces</u>	<u>Total</u>	
<u>Average Intake of Nitrogen Above 10 gm per day</u>							
1	23.82	*	23.82	.268	21.43	21.69	2.13
2	9.30	14.75	24.05	.409	11.16	11.56	12.49
3	10.58	14.73	25.31	.160	28.25	28.41	-3.10
6	10.26	13.35	23.61	.385	27.64	28.02	-4.41
7	11.91	11.84	23.75	.627	23.09	23.72	0.03
10	10.19	14.92	25.11	.553	24.97	25.52	-0.41
12	9.99	14.73	24.72	.336	27.89	28.22	-3.50
15	8.62	6.82	15.44	.292	17.48	17.77	-2.33
17	9.64	*	9.64	.192	9.34	9.53	0.11
18	10.26	.01	10.27	.283	5.29	5.57	4.70
19	8.74	.02	8.76	.419	34.76	35.18	-26.42
Intake		$\bar{X} = 19.50 \pm 6.94$		Balance		$\bar{X} = 1.88 \pm 9.43$	
Excretion		$X = 21.38 \pm 9.24$					

<u>Average Intake of Nitrogen Below 10 gm per day</u>							
4	9.99	13.75	23.74	.181	15.79	15.97	7.77
5	8.49	12.21	20.70	.214	14.95	15.16	5.54
8	14.61	13.75	28.36	.361	21.52	21.88	6.48
9	5.69	12.35	18.04	.193	20.97	21.16	-3.12
11	6.70	13.75	20.45	.150	10.81	10.97	9.48
13	6.94	15.12	22.06	.504	7.79	8.29	13.77
14	8.77	.02	8.79	.285	10.81	11.09	-2.30
16	6.88	.10	6.98	.205	10.29	10.50	-3.52
Intake		$\bar{X} = 18.64 \pm 7.30$		Balance		$\bar{X} = 4.26 \pm 6.49$	
Excretion		$X = 14.38 \pm 5.07$					

Outcome of Pregnancy

All the babies in this study were without visible malformation. Two of the infants were jaundiced at the time of the mothers' release from the hospital. Neither of these infants were from mothers with low zinc supplementation or plasma levels. One mother had higher than average positive balances of both zinc and nitrogen while the other was lower in both areas.

One infant, although over six pounds, was judged premature because of food intake ability and time she was born. The mother was on zinc supplementation, but her zinc plasma was the lowest in the research group (42.5). Her zinc balance was one of the highest (9.48), while her nitrogen balance was above average. This agrees with the conclusions drawn by Jamison that low serum zinc has a connection to infant prematurity (35).

Summary

Nineteen adult, pregnant women were subjects for a home balance study, from February through May of 1980. Each recruit was on a four-week study. The first and last weeks were collection weeks, while the middle two weeks were weeks of intake recording only. No manipulation of either the diet or supplements was attempted. Collection of input included all the liquid consumed as well as food.

The month of pregnancy the subject was in at the start of the study, when tested, showed no significant difference for either zinc or nitrogen balance. When the women were grouped by their zinc intake (supplementation) no significant differences were found in either zinc or nitrogen balances. Regrouping the women by their high (over >10g) or low (under <10g) intake of nitrogen did result in significant differences in either zinc and nitrogen balances. The group with higher nitrogen intake also had significantly higher nitrogen balances ($p < .01$). The same group also had significantly lower zinc balances ($p < .001$).

Correlation between the zinc and nitrogen balances gave a significant figure also $r = -.606$ ($p < .01$). It would thus appear that nitrogen intake has (when higher than 10 grams per day) a negative effect on zinc balance.

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APPENDIX 1

NEEDED: SUBJECTS FOR A NUTRITION STUDY

Subjects: Thirty healthy young adult females in their 4th to 5th month of pregnancy.

Purpose: To evaluate utilization of zinc during pregnancy.

When: For a four week period between February 1, 1980 and May 1, 1980

Experimental Regime: All subjects will consume their regular self-selected diet over the four week period.

Excreta (urine and feces) will be collected each day during the first and fourth week of the study.

All food and drink consumed will be weighed and collected during the first and fourth week of the study.

Accurate records of food and drink consumed will be kept during the second and third week of the study.

Samples of blood will be taken once during the study.

Hair samples will be taken at the beginning of the study.

Remuneration: Each participant will be paid \$136 upon successful completion of the study.

For Additional Information:

Call Dr. L. Janette Taper - 961-5549 week days before 5

or

Jean Oliva - 951-1794
Liz Cross - 953-1816

evenings and weekends

APPENDIX 2

Twenty-four hour recall

Name _____

Please record each day's food intake as you eat each meal. Write down all food, beverages, and vitamin/mineral supplements taken. (For collection weeks only — collect a weighed sample of all food and beverages consumed. We are asking that you do not add any vitamin/mineral supplements to your daily food collection but please provide us with one sample of any vitamin/mineral supplement you take, if any).

	ITEM NAME	SERVING SIZE
EXAMPLE:	Lean Ground Beef	4 oz. patty
BREAKFAST		
LUNCH		
DINNER		
SNACKS		

APPENDIX 3

Instructions for Zinc Study Participants

All food, feces, and urine are to be collected for two 7-day periods -- during the first week of the four week study period and again during the fourth week. Food intake is also to be recorded during these collection periods. During the second and third (or two middle) weeks of the four week study, only food intake will be recorded. No collections are required during this time. Participants will be weighed twice -- once during the first collection period and once during the second collection period. The first weighing will be done in Solitude House at a time convenient to the participant and investigator. The second weighing will be done in Wallace Hall, Room 306. Again the time will be arranged. This will be an early morning time (7:30 a.m.) as a fasting blood sample and hair sample will be taken at the same time. Donuts and orange juice will be served at this second session.

Food Collection - Please weigh out an equal portion of all food and drink consumed. Place the food in the ziplock bags provided and drink in the plastic bottle marked water. Please place in the containers food prepared as you eat it -- no uncooked spaghetti, no bones, pits, skins (unless eaten), etc. Do not put food or drink in any other containers you may have around the house eg. glass. If you need more containers, call us. We will get extras to you.

Dr. Taper 961-5549
Jean 951-1794
Liz 953-1816

Urine Collection - Start collecting with the second voiding on Sunday through the first voiding the following Sunday. One day's collection consists of the second voiding of the day through the (and including) first voiding of the next day. Please keep all 24-hour collections separate.

Fecal Collection - Start collecting with the first bowel movement on the first day (Sunday) of the collection period and end with the last movement on the seventh day (Saturday) of the collection period. Please do not tie knots in the plastic collection bags. Instead use the twist ties provided.

All collections (food, urine, and fecal) will be picked up after each 24-hour period by a student at a time and place convenient to both the participant and the student.

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THE RELATIONSHIP BETWEEN
ZINC AND PROTEIN IN PREGNANT
WOMEN

by

Elizabeth A. Cross

(ABSTRACT)

A balance study was conducted for twenty-eight days on pregnant women consuming self-selected diets. Subjects were divided into the following groups: low zinc (9.98 ± 2.90 , no supplement), high zinc (23.36 ± 2.59 , on supplement), low nitrogen (below 10 gm/day) or high nitrogen (above 10 gm/day). Zinc and nitrogen data were determined from food, beverage and supplement intakes and urine and fecal excretions. Zinc retentions were 3.32 ± 6.33 in the supplemented group, 4.94 ± 10.91 in the non-supplemented group. Zinc retention in the nitrogen divided groups was -1.88 ± 9.43 for the group with intake above 10 gm/day, 4.26 ± 6.49 for the group with intake below 10 gm/day.

No significant difference was found between the zinc supplemented group and the non-zinc supplemented group for either zinc or nitrogen retention.

Nitrogen retentions were 2.42 ± 2.17 mg/day for the zinc supplemented group and 3.53 ± 3.29 mg/day for the non-zinc supplemented group. Nitrogen retention for the group with intake above 10 gm/day was 3.99 ± 2.50 mg/day for the group with intake below 10 gm/day was 1.10 ± 1.45 mg/day.

Significant differences were found for nitrogen retention in the

groups based on nitrogen intake. Higher nitrogen intake was associated with higher nitrogen retentions ($p < .01$). Significant differences were also found in zinc retention based on nitrogen consumption. Higher nitrogen intake was associated with lower zinc retention ($p < .001$).

Plasma zinc was also measured. The mean plasma zinc was 70.46 ± 12.78 micrograms of zinc per 100 ml. The subject with the lowest zinc plasma level in the group also had a premature infant.