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13. ABSTRACT ***(Maximum 200 Words)***

Zinc and iron deficiencies are common among young women who seldom consume red meat. It seemed likely that iron stores (measured by serum ferritin concentration) were related to zinc nutriture (measured by metabolically active zinc pools), and that food frequency would reflect these relationships. Zinc and iron are essential for brain function. It therefore, seemed likely that the low zinc and iron nutriture would adversely affect neuropsychological performance. To test these hypotheses, iron deficient young women without anemia were recruited for measurements of zinc kinetics, and for participation in a double blind randomized controlled trial of zinc and iron treatment on neuropsychological function. Serum ferritin and the rapidly exchangeable tissue zinc pool correlated significantly, as did plasma zinc and the rapidly exchangeable zinc, and serum ferritin and exchangeable zinc were predicted by a focused food frequency questionnaire. Zinc and iron repletion were both efficacious for aspects of neuropsychological function. Zinc was especially efficacious for eye-hand coordination, reasoning and short-term visual memory. The neuropsychological findings were consistent with observations in other populations.

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Introduction

The National Health and Nutrition Examination Survey # 2 (NHANES-II) found that many women choose diets low in iron (Fe) and zinc (Zn), and that the 25th percentile for serum ferritin of premenopausal women was about 14 ng/mL, a level consistent with absent bone marrow iron (1). Low serum ferritin concentrations (low Fe status) and low Zn status tend to be associated (2, 3). Zn and Fe are essential for cognition (4). Therefore we conducted a preliminary research that found Fe and Zn repletion of deficient women significantly improved short-term verbal and visual memory about 20% (5), and Zn repletion of experimentally Zn deprived men significantly improved cognition, including short-term visual memory (6).

Our hypothesis for this project was: "Repletion of Zn and Fe deficiencies will improve cognition of premenopausal women."

Body of the Report

Design. We tested our hypothesis through a 16-week double blind randomized controlled trial with a cross over of the Zn and Fe treatments and a pseudo crossover of the control treatment after 8 weeks. Power calculations showed we needed at least 16 subjects per group to detect a 20 % effect with a coefficient of variation of 15 %, at $\alpha = 0.05$ and $\beta = 0.2$. To be conservative we studied 20 subjects per experimental group.

Subjects. Sixty premenopausal women of various ethnic backgrounds, aged 19-39 years, with a regular menstrual cycle of 24-34 days, good health, a body mass index ±10 % of desirable, completion of 12 grades of school, apparent good understanding of the information provided during the informed consent process, normal screening laboratory tests, a history consistent with mild Fe and Zn deficiencies, hemoglobin concentration $\geq$ 110 g/L and serum ferritin concentration $\geq$ 5 and $\leq$ 18 μg/L were studied. In addition 20 normal women of the same age with serum ferritin concentrations $\geq$ 30-60 μg/L were studied. Individuals with recurrent or chronic illness, eating disorders, chronic medication, nutritional supplements that contain Fe or Zn in the past 60 days, failure to menstruate the previous month, daily consumption of more than two ounces of ethanol or 10 cigarettes, and recreational use of controlled substances were excluded.

The subjects were selected from 708 individuals of various ethnic backgrounds who expressed potential interest through their telephone response to advertisements (Appendix 1). The selection process included an initial screening by phone interview, an invitation to participate in direct screening, informed consent, interviews to obtain demographic, medical and food frequency information, physical examination, and laboratory screening designed to exclude persons with illness.

Baseline Fe status was evaluated by erythrocyte indices, serum Fe, transferrin saturation and ferritin concentration. Baseline Zn status was evaluated by measurements of fasting plasma, leukocyte, lymphocyte, platelet, urine and hair Zn, Zn kinetics, and taste acuity.

Treatment. Subjects were numbered consecutively and the treatment prearranged by a formal scheme of restrictive randomization. Capsules containing respective treatments were uniquely color coded, with at least 2 colors representing a treatment. Subjects received one color capsule the first 8 weeks and another color capsule the second 8 weeks.

After enrollment, at least 7 days before measurement of Zn kinetics, all subjects were administered a daily capsule containing a mixture of micronutrients (M), not including Zn or Fe. Thus all subjects consumed M throughout the experiment. M was designed based on the 1989 RDA (7) and the fact subjects ate their usual diets. M provided 50 % of the RDA or 50 % of the Estimated Safe and Adequate Daily Dietary Intake of micronutrients listed in the RDA, with the following exceptions. Zn and Fe were excluded because they are the topic of the research. Calcium, magnesium, and phosphate were excluded because they are potential inhibitors of Zn and Fe retention (8). To minimize potential interference with Zn metabolism (9-13) folate was included at 25 % of the RDA. M cured latent micronutrient deficiencies that might interfere with responses to Zn or Fe, and provided a control for the effect of being in the study.

After completion of baseline measurements, the 60 deficient subjects were administered the assigned treatment double blind. The three treatments were: M, 30 mg Zn with M (ZnM), or 30 mg Fe with M (FeM). The treatments were administered for 8 weeks, then treatments of the Zn and Fe groups were switched (cross over) and treatment was administered an additional 8 weeks. The 20 deficient and 20 normal subjects administered M underwent a pseudo cross over at 8 weeks.

Treatments were provided in batches of 35 capsules. Subjects were interviewed weekly by phone to ascertain progress and the number of capsules remaining. Subjects were seen monthly and health, diet and the number of capsules remaining determined. Subjects who did not take the treatment $\geq$ 5 x/week, who developed a febrile illness or accident that caused severe stress, who substantially changed their diet, or failed to menstruate, or were otherwise unable to comply with study requirements were discharged from the study.

Outcomes. The main baseline outcomes relevant to our hypothesis were food frequency, and indices of Fe and Zn status, including Zn kinetics that were assessed on day 8-12 of the menstrual cycle. The main treatment outcome, neuropsychological function, was measured on days 8-12 of the menstrual cycle at baseline, after 8 weeks of the first treatment, and then after 8 weeks of the second (cross over) treatment. Effects of Fe or Zn alone, Fe and Zn together, and of the order of treatment were measured.

Zn kinetics and related measures. Zn kinetics were measured in 50 subjects. The procedure was discontinued (with permission) when it appeared the procedure was deterring subjects from continuing in the study to participate in the treatment trial.

Subjects were admitted to the Clinical Research Center. Meals were designed to provide < 6 mg Zn per 24 hours. After an overnight fast of at least 8 hours a 24-h urine collection was started. Urine was collected using a plastic "hat" and plastic bottles that were pre-washed with 1% nitric

acid and deionized water. Aliquots were stored at -208°C. A hair sample was collected from the occipital scalp and stored in a disposable Falcon polypropylene test tube.

Height (nearest mm) and weight (nearest 0.1 kg) were measured with subjects barefoot and dressed in a hospital gown. Subjects were then positioned supine with their arms and legs abducted for measurement of impedance and reactance using a BIA 101A analyzer (RJL systems, Clinton Twp, MI) and a current of 800 μA at 50 kHz. Signal introducing electrodes were applied to the dorsal surface of the proximal phalanx of the right middle finger and just proximal to the middle toes of the dorsal surface of the right foot. Detecting electrodes were applied to the dorsal surface of the right wrist between the prominences of radius and ulna and on the dorsal side of the right ankle between the maleoli. Fat free mass (nearest 0.1kg) was calculated using software (Weight Manager version 2.05a) provided by the manufacturer.

After completion of the above Zn kinetics were measured. The protocol followed our previous procedure (14) with minor modification. ^{67}Zn chloride was prepared from ^{67}Zn oxide (^{67}Zn natural abundance 4.11%; enrichment, 93.11%, Oak Ridge National Laboratories, Oak Ridge, TN), dissolved in saline, sterilized by passage through Millipore (Fisher Scientific Co., Houston, TX) filter (0.2 μM pore size), and placed in sterile glass vials containing saline, total volume 10.0 mL (3). The solutions in the vials were tested for sterility (Department of Clinical Microbiology and Immunology, UTMB) and pyrogenicity (Scientific Associates Inc., St. Louis, Missouri).

Short Teflon catheters were placed in both antecubital veins of the subject. The catheters were attached to a slow drip of normal saline by a three-way-stop-cock. A baseline blood sample was taken for measurement of the background ^{67}Zn/^{68}Zn ratio and the plasma, platelet, and white blood cell Zn contents. Then 2 mg of sterile, pyrogen-free, ^{67}Zn dissolved in normal saline was administered over 3 min through the stopcock. This was followed by rapid administration of saline for 1 min. Blood samples were taken from the other arm at 5, 15, 30, 40, 50, 60 and 90 min, 2, 6, 12 and 24 hours after the administration of ^{67}Zn. Volumes of blood taken at each time point were at least 10 mL. Before each blood collection, about 2 mL blood was drawn to remove saline and diluted blood from the catheter. Blood samples were taken in a "Monovette" syringe containing lithium heparin (10 U/mL blood) obtained from the Sarstedt Company (Germany). Blood samples were surrounded with ice after collection until delivery to the laboratory for processing.

Chemical analysis was done on batches of samples. To remove contaminants hair samples were washed with acetone, threefold with purified water (Milli Q^{TM}, Millipore Co., Bedford, Massachusetts) and again with acetone (14). The hair, plasma and urine samples were digested with hydrogen peroxide (15) and dissolved in 1% nitric acid. Plasma Zn and hair Zn were analyzed by flame atomic absorption spectrometry (15). Platelet Zn was analyzed by flameless atomic absorption spectrometry (16). ^{67}Zn/^{68}Zn ratios in plasma were measured by using an inductively coupled plasma- mass spectrometer VG PlasmaQuad-1, upgraded to a PlasmaQuad-2 plus status (VG Instruments, Winsford, England, UK) (14). The coefficient of variation (CV) of the isotope ratio measurement was 0.2-0.6%.

The tracer-to-tracee ratio was calculated based on Cobelli et al's approach (17).

$$TTR = (A_{n68} \times IR - A_{n67}) / (A_{s67} - A_{s68} \times IR)$$

TTR: Tracer-to-tracee ratio
IR: Isotope ratio of ^{67}Zn to ^{68}Zn
A_{n67}: Natural abundance of ^{67}Zn
A_{n68}: Natural abundance of ^{68}Zn
A_{s67}: Abundance of ^{67}Zn in the enriched preparation
A_{s68}: Abundance of ^{68}Zn in the enriched preparation

The disappearance of Zn tracer from plasma was described by a four-exponential function (18, 19):

$$TTR = H_1 e^{-g_1 t} + H_2 e^{-g_2 t} + H_3 e^{-g_3 t} + H_4 e^{-g_4 t}$$

where $g_1 > g_2 > g_3 > g_4$.

If $g_1 >> g_2 >> g_3 >> g_4$ as is generally found in zinc kinetics (19), the truncated form of the polyexponential function (the bi-exponential function with a constant term) can be substituted for the complete form when the observation period is shorter than the half-life of the third term. To avoid hyper-parameterization in curve fitting, the truncated form was used instead of the complete form.

In Miller et al's (19) model based on Wastney (20), g_1, g_2, g_3 and g_4 are 137.6, 3.564, 0.1106 and 0.00232, respectively. The corresponding half-lives are 7.25 min, 4.67 h, 6.26 d and 298.7 d.

For the 24 hr observation, the truncated tri-exponential model (the bi-exponential function with a constant term) was used because the change in the third term was relatively small after 24 hours.

$$TTR = H_1 e^{-g_1 t} + H_2 e^{-g_2 t} + H_3$$

There are two possible three compartment models corresponding to the above function, i.e., closed mammillary model and closed catenary model. We selected the closed mammillary model so as to accommodate the simpler biological interpretation (Figure 1). The model consists of compartments, transfer rate constants k_{ij}, which represent fractional transfer into *compartment* i from *compartment* j per unit of time, fluxes F_{ij}, which represent the mass of Zn transferred into *compartment* i from *compartment* j per unit of time. A subscript "o" denotes the outside of the system. The transfer rate constants k_{ii} represent the sum of the outward transfer rate constants of *compartment* i.

The central Zn compartment pool size Q_1, rapidly exchangeable Zn pool (EZP), plasma Zn turnover rate (TR) and k_{11}, sum of the transfer rate constants from *compartment* 1 are common for the mammillary and catenary models.

The precise calculation of kinetic parameters in the closed mammillary model from the coefficients in the truncated tri-exponential function was done according to Landaw et al (21).

$$k_{11} = k_{01} + k_{21} + k_{31} = \frac{\sum_{i=1}^{3} H_i g_i}{\sum_{i=1}^{3} H_i}$$

Roots of the numerator of the Laplace transformation of tri-exponential function, i.e., the solutions of the following quadratic equation give k_{22} ($= k_{12} + k_{02}$) and k_{33} ($=k_{13} + k_{03}$).

$$s^2 \sum_i H_i + s \sum_{i \neq j} H_i g_j + \sum_{i \neq j \neq k} H_i g_j g_k = 0$$

Then $\square_2$ and $\square_3$are calculated as follows.

$$\gamma_j = k_{ij}\, k_{ji} = \frac{\sum_{j=1}^{3} H_j}{\sum_{j=1}^{3} \frac{H_j}{\left(k_{jj} - g_j\right)^2}}$$

For the single outlet model, $k_{02} = 0$ and $k_{03} = 0$. Subsequently, Q_2, Q_3, and the transfer rate constants k_{ij} are uniquely determined:

$$k_{12} = k_{22}$$

$$k_{13} = k_{33}$$

$$k_{21} = \frac{\gamma_2}{k_{12}}$$

$$k_{31} = \frac{\gamma_3}{k_{13}}$$

$$k_{01} = k_{11} - (k_{21} + k_{31})$$

$$Q_1 = \frac{D_o}{H_1 + H_2 + H_3}$$

$$Q_2 = \frac{\gamma_2}{k_{12}} Q_1$$

$$Q_3 = \frac{\gamma_3}{k_{13}} Q_1$$

$$\text{EZP} = Q_1 + Q_2 + Q_3$$

$$F_{12} = F_{21} = k_{12}\, Q_2$$

$$F_{13} = F_{31} = k_{13}\, Q_3$$

$$TR = F_{21} + F_{31} = k_{11}\, Q_1$$

where Q_1: plasma Zn compartment (central compartment);
D_0: dose of tracer administered;
EZP: rapidly exchangeable Zn pool;
TR: plasma Zn turnover rate.

For the closed mammillary model, k_{01} equals to 0. When the coefficients in the polyexponential function are given, all parameters in the mammillary model can be solved arithmetically and the common tabulation software is enough for this purpose.

The urinary Zn excretion rate constant was calculated based on the assumption that excreted Zn originated from plasma (20, 22). The pool size of the central plasma compartment Q_1 was derived from the Zn kinetics. Independent from the tracer kinetics, urinary Zn excretion rate was determined by using total Zn in the 24-hour urine sample. Subsequently, the urinary Zn excretion rate constant was calculated as urinary Zn excretion rate divided by Q_1

Computations and statistics were done using a Macintosh PowerBook 165C (Apple), Power Macintosh 7300/166 and PowerBook 1400cs/166 and SYSTAT 5 for Macintosh, version 5.2.1 software (SYSTAT, Inc., Evanston, IL) and Prism version 2.0 for Macintosh (GraphPad Software, Inc., San Diego, CA). At the curve fitting to the disappearance data, TTR was

logarithmically transformed to stabilize the random variation (23). Logarithmically transformed TTR was fit to the truncated tri-exponential function by nonlinear regression procedure with the simplex method in the software SYATAT 5 for Macintosh, version 5.2.1 software (SYSTAT, Inc., Evanston, IL). Using the determined coefficients in the truncated tri-exponential function, the kinetic parameters in the closed mammillary model were calculated with the tabulation software Microsoft Excel version. 4.0 and 5.0.

The subjects were divided into quartiles based on the plasma Zn concentration. From the lowest to the highest plasma Zn, quartiles were designated as Quartile I, Quartile II, Quartile III and Quartile IV. Differences among quartiles were tested by one-way ANOVA. Then, the difference from Quartile IV as control was evaluated by the post hoc test of Dunnett's simultaneous comparison. For the selected parameters, i.e., nutritional indicators of iron and taste thresholds, the subjects were divided into those with plasma Zn $\geq$700 ng/mL and those with plasma Zn <700 ng/mL. Differences in the continuous variables (iron parameters) between the plasma Zn categories were determined by Welch's t-test; difference in the discrete variables (taste thresholds) were determined by the Mann-Whitney U-test. Pearson's correlation coefficients were calculated between the Zn kinetic parameters and body weight or fat-free mass. Difference in the urinary Zn excretion rate and the urinary Zn excretion rate constant between subjects with or without a history of urinary tract infections were determined by Welch's t-test. P-values < 0.05 were considered statistically significant.

Measurement of Taste Acuity. Subsequent to measurement of Zn kinetics taste acuity was measured (24) using an electrogustometer (Rion TR-06, Tokyo, Japan). Areas of the tongue innervated by the chorda tympani (2 cm from the tip of the tongue on the edge) and glossopharyngeal nerve (circumvallate papilla) were studied. Response to direct current was measured and a logarithmic scale used to indicate the intensity of response. Response to the tastants was measured using filter-paper discs. Wet discs containing graded concentrations of tastant were applied to the tongue. Threshold of the lowest concentration for correct identification was determined. For "sweet" (sucrose) the concentrations were 0.3, 2.5, 10, 20, and 80-%; for "salty" (sodium chloride) the concentrations were 0.3, 1.25, 5, 10, and 20-%; for "sour" (tartaric acid) the concentrations were 0.02, 0.2, 2, 4, and 8-%; for "bitter" (quinine hydrochloride) the concentrations were 0.001, 0.02, 0.1, 0.5, and 4-%. For each tastant the respective concentrations were designated level 1, 2, 3, 4, and 5, indicated progressively decreasing concentrations of an identifiable taste sensation, and level 6 indicating no taste sensation.

Measurement of neuropsychological function. Performance was measured by computerized tasks of known validity and reproducibility. The tasks were from a library compiled by Co-Investigator James G Penland, PhD of the USDA Agricultural Research Service Grand Forks Human Nutrition Research Center, Grand Forks, ND.

Testing was done in the morning after breakfast, in a quiet room. The subjects were alone and the door to the room was closed. Preceding the test, subjects were directed to have a good nights rest and to not consume ethanol or other psychoactive substances. Test administration required about 90 minutes. Therefore the subjects were scheduled for 2 hours. This allowed time for a

short rest break halfway through the session. Subjects were oriented to the test procedure and content before collection of baseline data. This procedure decreased contamination by variability related to learning and practice. Testing was done on the day 8-12 of the menstrual cycle before treatment and after 8 and 16 weeks of treatment.

Attention was measured by a vigilance task (25) for sustained attention; an orienting task (26) for the effects of tonic arousal on attention; a color word naming task (27) for the degree of interference in a simple attention task caused by the presentation of conflicting but irrelevant stimulus information; by a perceptual processing task (28) similar to the "Stroop" task, but which emphasizes size or visual extent as a source of conflicting stimulus information; and by a continuous vigilance task (29) for sustained attention in a stimulus rich environment.

Perception was measured by a letter-matching task (30) for the depth of perceptual processing involved in pattern recognition; an automatization task (31) for effortful versus automatic processing in a perception-memory paradigm; a short term memory task of characters displayed on a screen (32) for perceptual processes and memory, and a time estimation task (33).

Higher cognitive processes were measured by a symbol digit task (34) for encoding skills and short-term memory for symbolic information; a mathematics task (35) for performance on integer addition, subtraction, multiplication and/or division problems; a shape recognition task (36) for perception and recognition of common geometric figures varying from one to three dimensions: shape, size and color; a verbal learning task (37) for learning and memory of verbal material; and a concept formation task (38, 39) for processes involved in developing or learning concepts.

Spatial orientation was measured by a maze task (40) for spatial orientation and memory as the subject navigates simple and complex mazes; a cube recognition task (41, 42) for reproduction of spatial information in memory; and a cube recognition task for additional assessment of subject processing of spatial information.

Psychomotor (sensory-motor) skills were measured by a tapping task (38, 43) often used in neuropsychological assessment to assess response to feedback; a steadiness task (35, 44) for sensory fine motor control; a pursuit task (45) for sensory-motor organization; a trails task (43, 46, 47) for visual motor skills and ability to concentrate; and a direction task for preparation in the performance of a sensory motor task.

Neuropsychological data were copied on backup disks for storage, and transmitted directly to Dr Penland's FTP site via the Internet.

Associations between subjects' histories and iron and Zn status indices. Correlations between subjects' histories (food frequencies and gynecological histories), and Fe status indices (serum ferritin, serum Fe, total Fe binding capacity, saturation of Fe binding capacity, erythrocyte protoporphyrin, hemoglobin, hematocrit, red cell count and red cell indices) and Zn indices (plasma Zn, platelet Zn, hair Zn, and Zn kinetic parameters) were determined by multiple regression analysis. Excluded from the analyses were subjects taking oral contraceptives (total

number 15) and subjects whose saturation of Fe binding capacity was abnormally high (83%, n = 1). Thus, the number of subjects used for the analyses was 34.

Stepwise multiple regression analysis with backward variable selection found in the SYSTAT software was employed for the statistical analyses. The initial explanatory variables were a history of bleeding through menstrual pads and frequencies of consumption per week of 12 food items, i.e., vitamin C-fortified drink, coffee, tea, lettuce, beans, bran breakfast cereals, beef, chicken, egg, yogurt, milk and orange juice, that included the possible sources of bioavailable Fe and Zn, and absorption enhancers and inhibitors of Fe and Zn. To incorporate a history of bleeding through menstrual pads in regression analyses, "0" or "1" was given to the corresponding variable respectively when the subject did or did not have the history. P value for removal of variables from the model was 0.10.

Associations between serum ferritin and indices of Zn status. Correlations between serum ferritin and indices of Zn status were determined by regression analysis. The subjects used for this analysis were the same as those used for the regression analysis of subjects' histories and indices of Fe and Zn status. The subjects who were taking oral contraceptives and whose saturation of Fe binding capacity was abnormal (83%) were excluded. First, the simple linear regression between serum ferritin and Zn status indices was calculated. Based on the linear regression, p-values for the studentized residual adjusted by Bonferroni's correction were calculated. Subjects whose p-value was < 0.05 were treated as statistical outliers. Statistical outliers were successively removed from the data set. When no outlier was detected, simple linear regression was completed. If nonlinearity was observed by the inspection, the data were fitted to several equations (second-order and third-order polynomial equations, four-parameter logistic equation, and broken-line equation) using the Prism (GraphPad) software. The best equation was selected by F ratio test and comparison of R^2.

Results.

Measurements of Zn kinetics and related findings (Appendix 2). Table 1 shows the characteristics of the 50 subjects in which Zn kinetics were measured. Two subjects who habitually took dietary supplements containing Fe and Zn were excluded from statistical analyses. The subjects were all premenopausal women living in Texas and comprised of 33 whites, 10 Hispanics, 5 blacks, 1 Native American, and 1 Asian. Because individuals with apparent Fe deficiency anemia were excluded at the screening, the hemoglobin concentration was above 11 g/dL. The number of subjects with serum ferritin concentration < 20 ng/mL was 31. The number of subjects with Fe-deficient erythropoesis, erythrocyte protoporphyrin >100 μg/dL, was 3. All subjects with Fe-deficient erythropoesis had serum ferritin < 20 ng/L. Twenty subjects had plasma Zn concentrations < 700 ng/mL and 33 subjects had plasma Zn concentrations < 750 ng/mL.

Table 2 shows the Zn kinetic parameters determined by the closed mammillary model (Figure 1). Two subjects were not included because of incomplete sample collection. Therefore, the data represent 48 subjects. The median coefficient of determination (R^2) was 0.9988 with a range from 0.9893 to 0.9998. The variations of the rate constants k_{12} and k_{31} were larger than k_{21} and k_{13}. The variation of the Zn pool size Q_1 was larger than Q_2 and Q_3. Since the pool size Q_3 was much larger than Q_1 and Q_2, EZP was virtually same as Q_3.

Table 3 shows the correlation coefficients between Zn pools or fluxes and body weight, fat-free mass and plasma Zn concentration. The Zn pool Q_3 and EZP significantly and positively correlated with body weight. The correlation between the Zn pool Q_1 and body weight was marginal ($p = 0.067$). The Zn pools Q_1 and Q_3 and EZP significantly and positively correlated with fat-free mass. The Zn flux F_{31} and turnover rate significantly and positively correlated with body weight and fat-free mass. Plasma Zn significantly and positively correlated with the Zn pools Q_1 and Q_2 and the Zn flux F_{21}. The correlation between plasma Zn and Q_2 was stronger than that between plasma Zn and Q_1, i.e. ($R^2 = 0.415$ vs 0.253). Plasma Zn was not significantly correlated with body weight or fat-free mass.

The variation of Q_3 per body weight, EZP per body weight, F_{31} per body weight and turnover rate per body weight was smaller than without correction by body weight. The variation in Q_3, EZP, F_{31} and turnover rate per fat-free mass was smaller than without correction by fat-free mass (Table 2).

Relationships between plasma Zn quartiles and Zn kinetic parameters were determined. Plasma Zn concentration (ng/mL) ranged from 492 to 650 in Quartile I; 653 to 715 in Quartile II; 718 to 765 in Quartile III; 781 to 939 in Quartile IV. Quartiles I, II and III were significantly lower than Quartile IV. There were no significant differences among the quartiles in age, body weight, body height, body mass index, fat free mass and ethnic composition (Table 4).

The first section of Table 5 shows the coefficients in the truncated triexponential function (bi-exponential with a constant term). H_1, g_1, H_2 and g_2 in Quartiles I, II and III were higher than Quartile IV. H_3 in Quartile I was higher than Quartile IV and H_3 in Quartile II and III tended to

be greater than in Quartile IV. An increase in g_1 and g_2 of the lower quartiles was consistent with the rate of Zn disappearance being inversely correlated to the background plasma Zn concentration.

The second section of Table 5 shows the comparison among the quartiles of the rate constants in the closed mammillary Zn kinetic model. The k_{11} in Quartiles II and III was significantly higher than Quartile IV. The k_{12} in Quartiles I, II and III was significantly higher than Quartile IV. The k_{21} did not vary among the quartiles. The k_{13} in Quartile I was significantly higher than Quartile IV. The k_{31} in Quartiles I, II and III was significantly higher than Quartile IV.

The third section of Table 5 shows the comparison among the quartiles of Zn pool sizes and fluxes in the closed mammillary model. The pool size Q_1 in Quartiles I, II and III was significantly lower than Quartile IV. The pool size Q_2 in Quartiles I, II and III was significantly lower than Quartile IV. The pool sizes Q_3 and EZP (sum of Q_1, Q_2 and Q_3) were not different among the quartiles. Pool sizes of Q_1 in Quartiles I, II and III were respectively 73, 66, and 70 % of that in Quartile IV. Pool sizes of Q_2 in Quartiles I, II and III were respectively 39, 43, and 50 % of that in Quartile IV. The pool size Q_2 (mg) ranged from 2.62 to 9.50 in Quartile I; 3.12 to 12.44 in Quartile II; 2.93 to 12.97 in Quartile III, 8.52 to 20.12 in Quartile IV. There was no statistical difference in Q_2 among Quartiles I, II and III. The Zn flux F_{21} represents the flux between the central Zn pool (Pool 1) and the lesser peripheral Zn pool (Pool 2). Because of the steady state assumption in the closed mammillary Zn kinetic model, F_{21} (the flux from Pool 1 to Pool 2) and F_{12} (the flux from Pool 2 to Pool 1) are equal. F_{31} (the flux from Pool 1 to Pool 3) is equal to F_{13} (the flux from Pool 3 to Pool 1). The Zn flux F_{21} in Quartiles I, II and III was significantly lower than Quartile IV, while F_{31} did not vary among the quartiles. The difference in turnover rates between Quartile III and IV was marginal ($p = 0.06$ by Dunnett's multiple comparison test; $p = 0.04$ by Welch's t-test); Quartiles I, II and III were not statistically different. The turnover rate in the combined Quartiles I, II and III was 310±75 mg/day (mean ± SD, $n = 36$) and significantly lower than that in Quartile IV ($p = 0.044$ by Welch's t-test).

The fourth section of Table 5 shows the comparison among the quartiles of Zn pool sizes and fluxes per body weight. Zn pool sizes and fluxes per body weight gave the similar results in comparison among quartiles to those without correction by body weight. Because the Zn pool size Q_3 and EZP and the Zn fluxes F_{31} and turnover rate were highly correlated with body weight, the statistical power for those parameters generally became stronger compared to the not corrected ones. In addition to the significant changes found in the raw parameters, the turnover rate in Quartile III was significantly lower than Quartile IV.

The fifth section of Table 5 shows the comparison among the quartiles of the Zn pool sizes and fluxes per fat free mass. Zn pool sizes and fluxes per fat free mass gave the similar results in comparison among quartiles to those with correction by body weight.

Associations between plasma Zn quartiles and serum electrolytes are shown in Table 6. Serum calcium in Quartile III was slightly and significantly lower than Quartile IV. Serum sodium in Quartile II was slightly and significantly higher than Quartile IV. Serum potassium in Quartile

III was significantly lower than Quartile IV. Serum chloride did not change among the quartiles. Serum bicarbonate in Quartile I was significantly lower than Quartile IV. The anion gap had a decreasing trend depending on plasma Zn concentration; in Quartile I it was significantly higher than Quartile I.

Relationships between plasma Zn quartiles and other clinical laboratory indices were also examined (data are not shown). Platelet Zn and hair Zn did not vary among the quartiles. The menstrual histories, i.e., the age of menarche, menstrual cycle, menstrual bleeding period, and an incidence of history of bleeding through menstrual pads were not different among the quartiles. Vital signs, i.e., body temperature, frequency of respiration, frequency of pulse and blood pressure did not differ among the quartiles. No difference among the quartiles was observed in serum creatine phosphokinase, blood urea nitrogen, serum creatinine, serum total cholesterol, serum total cholesterol, serum triglyceride, serum alkaline phosphatase, serum lactate dehydrogenase, serum aspartate transaminase, serum alanine transaminase, serum □glutamyl transpeptidase, serum total protein, serum albumin, serum uric acid, serum glucose, serum inorganic phosphorus and serum magnesium. Difference among the quartiles was not found in hematological indices, i.e., total blood counts, red cell indices, reticulocyte counts, prothrombin time, and APTT. Urinalyses did not vary among groups, i.e., appearance, pH, urinary protein, glucose, ketone bodies, bilirubin, urobilinogen, nitrite and urine sediments.

Table 7 shows the comparison among the quartiles of urinary zinc excretion rate (ZnExR), ZnExR per body weight or fat-free mass, and ZnExR constant (ZnExR divided by Q_1). ZnExR had a trend to decrease depending on plasma Zn concentration. ZnExR, ZnExR per body weight and ZnExR per fat free mass in Quartiles I and II were significantly lower than Quartile IV. The decrease in ZnExR constant of Quartile I compared to Quartile IV was marginal ($p = 0.12$ by Dunnett's multiple comparison test; $p = 0.048$ by Welch's t test). ZnExR constant in Quartile II, III and IV was similar. ZnExR constant in Quartile I was significantly lower than that for a composite of Quartiles II, III and IV ($p = 0.0002$ by Welch's t test).

The relationship of past history of urinary tract infections and urinary Zn excretion is shown in Table 8. Subjects with a history of urinary tract infections (UTI) had significantly lower urinary Zn excretion rate, urinary Zn excretion rate per body weight, urinary Zn excretion rate per fat-free mass and urinary Zn excretion rate constant than the subjects with no history of UTI. The central Zn pool Q_1 was not different between the subjects with and without a history of UTI, (mean ± SD) 2.10 ± 0.58 mg vs 2.21 ± 0.60 mg. Other Zn indices were unrelated to history of UTI.

Relationships between plasma Zn and Fe status indices are shown in Table 9. Serum ferritin, serum Fe and saturation of Fe binding capacity in subjects with plasma Zn < 700 ng/mL were significantly lower than in subjects with plasma Zn ≥ 700 ng/mL. Table 10 shows the comparison among the quartiles of Fe status indices. There was an increasing trend of serum ferritin, serum Fe and saturation of Fe binding capacity depending on plasma Zn. Erythrocyte protoporphyrin was higher in Quartile II than in Quartile IV.

Associations of subjects' food frequencies to Fe status indices (Appendix 2). Food frequency characteristics of the study population are shown in Table 11. Most of the food items were consumed by $\geq$ 70% of the subjects. Twenty-four and 32 % consumed vitamin-C fortified drinks and bran breakfast cereals respectively. Five of 34 subjects had a history of bleeding through menstrual pads (15%).

Associations of serum ferritin with food frequency and menstrual bleeding are shown in Table 12. After the other variables were controlled for, the significant positive predictor of serum ferritin was beef ($p = 0.002$). The negative predictor was history of bleeding through menstrual pads ($p = 0.021$). Each time of beef consumption per week was associated with greater serum ferritin by 3.84 ng/mL. Each event of bleeding menstrual pads was associated with lower serum ferritin by 15.1 ng/mL.

Associations of erythrocyte protoporphyrin concentrations with food frequencies and history of bleeding through menstrual pads are shown in Table 13. After the other variables were controlled for, the significant negative predictor was egg ($p = 0.027$). The positive predictors were milk ($p = 0.004$) and a history of bleeding through menstrual pads ($p = 0.006$). The contribution of beans was marginal ($p = 0.052$).

Associations of hemoglobin concentrations with food frequencies and history of bleeding through menstrual pads are shown in Table 14. After the other variables were controlled for, the significant positive predictors were beef ($p = 0.003$) and coffee ($p = 0.028$).

Associations of hematocrit percentage with food frequencies and history of bleeding through menstrual pads are shown in Table 15. After the other variables were controlled for, the significant positive predictors were orange juice ($p = 0.001$), beef ($p = 0.002$), and lettuce ($p = 0.002$). The negative predictor was yogurt ($p = 0.030$).

Associations of red blood cell count with food frequencies and history of bleeding through menstrual pads are shown in Table 16. After the other variables were controlled for, the significant positive predictor was orange juice ($p = 0.030$).

Coffee consumption was the only explanatory variable by step wise multiple regression for mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), serum iron and saturation of iron binding capacity (Table 17). Therefore, the outcome of the stepwise multiple regression analysis was same as that of the simple linear regression. Coffee consumption was the positive predictor of MCV, MCH, MCHC, serum iron and saturation of iron binding capacity. These indices were highly correlated with each other. Thus the correlation coefficient was similar among those items (0.448 to 0.487). In contrast food frequency and bleeding through menstrual pads were unrelated to total Fe binding capacity and red blood cell distribution width.

Associations between the central Zn pool size Q_1 per fat-free mass with food frequencies and bleeding through menstrual pads are shown in Table 18. After the other variables were controlled for, the significant positive predictor of Q_1 per fat-free mass was beef ($p = 0.042$).

Negative predictors were history of bleeding through menstrual pads ($p = 0.009$) and bran breakfast cereals ($p = 0.024$). Each time of beef consumption per week was associated with greater Q_1 per fat-free mass by 1.84 μg/kg. Each time of bran breakfast cereals consumption per week was associated with lower Q_1 per fat-free mass by 2.65 μg/kg. Each event of bleeding through menstrual pads was associated with lower Q_1 per fat-free mass by 13.0 μg/kg.

Associations of the lesser peripheral Zn pool size Q_2 (mg) with food frequencies and history of bleeding through menstrual pads are shown in Table 19. After the other variables were controlled for, the significant positive predictors of Q_2 were yogurt ($p = 0.003$) and beef ($p = 0.001$). The significant negative predictor was bran breakfast cereals ($p = 0.020$).

Associations of the greater peripheral Zn pool size Q_3 per fat-free mass (mg/kg) with food frequencies and history of bleeding through menstrual pads are shown in Table 20. After the other variables were controlled for, the significant positive predictors were beef ($p = 0.001$), lettuce ($p = 0.003$) and tea ($p = 0.049$). The significant negative predictors were bleeding through menstrual pads ($p = 0.001$) and yogurt ($p = 0.035$).

Associations of the rapidly exchangeable Zn pool size (EZP, i.e., sum of Q_1. Q_2 and Q_3) per fat-free mass (mg/kg) with food frequencies and history of bleeding through menstrual pads are shown in Table 21. After the other variables were controlled for, the significant positive predictor was beef ($p = 0.003$). The significant negative predictor was history of bleeding through menstrual pads ($p = 0.010$).

Associations of the Zn turnover rate (sum of fluxes from the central Zn compartment) per fat-free mass (mg/kg) with food frequencies and history of bleeding through menstrual pads are shown in Table 22. After the other variables were controlled for, the significant positive predictor was beef ($p = 0.008$). The significant negative predictors were history of bleeding through menstrual pads ($p = 0.036$) and bran breakfast cereals ($p = 0.039$).

Associations between serum ferritin and Zn status indices (Appendix 2). The association between serum ferritin concentration and the central Zn pool size Q_1 per fat-free mass was highly significant ($n = 31$, $R^2 = o.394$, $p < 0.0001$), as shown in Figure 2. The subject ID 156 was found to be an outlier from the regression. The studentized residual of the subject ID 156 was -3.69.

The association between serum ferritin concentration and the lesser peripheral Zn pool size Q_2 was highly significant ($n = 27$, $R^2 = 0.685$, $p < 0.0001$), as shown by Figure 3. Five subjects identified as outliers by the sequential outlier detection protocol were not included in the analysis. For these subjects, ID 104, 159, 114, 156 and 312, the studentized residual was 3.01, 4.19, 3.69, -3.08, and -3.09 respectively. Inspection of the plot suggested nonlinearity. After removal of the statistical outliers several possible curves were fitted to the data. R^2 was 0.685, 0.726, 0.777, 0.778 and 0.756 respectively for the first order polynomial equation (straight line), the second order polynomial equation (quadratic curve), the third order polynomial equation (cubic curve), the four-parameter logistic equation (logistic sigmoidal curve) and the broken-line equation. F ratio tests were performed to determine the best fit among the equations with different degrees of freedom. The p-value obtained from the comparison of the broken-line

equation to the first order polynomial equation was 0.015, showing the broken-line equation gave a better fit. The p-values obtained from the comparison of the third order polynomial and the four-parameter logistic equations with the broken-line equation were respectively 0.158 and 0.141, indicating the broken-line equation was the better fit. It was obvious that the broken-line equation gave a better fit than the second order polynomial equation (R^2 0.756 vs 0.726 with the same degree of freedom). Therefore, the broken-line equation was selected as the best fit. The highly significant nonlinear relationship represented by the broken-line equation between serum ferritin concentration and Q_2 is shown in Figure 4. The parameter estimates in the broken-line equation were as follows:

$$Q_2 = a\,(x - b) + a\,|x - b| + c$$

$a = 0.112 \pm 0.016$ (estimate ± asymptotic standard error) (mg/ng/mL);
half value of the slope at the proportional section of the line
$b = 17.8 \pm 2.7$ (ng/mL);
break point between the proportional and horizontal sections of the line
$c = 4.69 \pm 0.34$ (mg);
the value for Q_2 at the horizontal section of the line
$|\,X\,|$; absolute value of X
Standard error of estimate = 1.28
$R^2 = 0.756$ ($n = 27$, $P < 0.0001$).

The broken-line equation indicated that when serum ferritin concentration was less than the break point 17.8 ng/mL, average Q_2 stayed constant (4.69 mg); when serum ferritin exceeded the break point, average Q_2 increased proportionally with serum ferritin; and the standard deviation around the regression line, i.e., standard error of estimate, was 1.28 mg.

The association between serum ferritin concentration and the greater peripheral Zn pool size Q_3 per fat-free mass is shown in Figure 5. No outliers were identified. The level of significance was less than that between serum ferritin concentration and Q_1 per fat-free mass or Q_2.

Characteristics of the outliers were as follows. In the subject ID 104, 114 and 159, Q_2 was higher than the estimate from serum ferritin concentration. Subject 104 had normal serum ferritin (31 ng/mL), normal plasma Zn (876 ng/mL), high frequency of consumption of animal products such as beef (3 times/wk), bacon (1 time/wk), pork (0.5 time/wk), and chicken (3 times/wk); low consumption of vitamin C-rich foods, such as orange juice (0.5 time/wk), other fruit juice (0 time/wk), orange (0 time/wk), and vitamin C-fortified drinks (0 time/wk). Subject ID 114 had low serum ferritin (15 ng/mL), borderline plasma Zn (741 ng/mL), marginal hair Zn (119.3 μg/g), and low urinary Zn excretion rate constant (0.0645 day^{-1}). Subject ID 159 had low serum ferritin (19 ng/mL), normal plasma Zn (848 ng/mL), marginal hair Zn (119.1 μg/g), high consumption of breakfast cereals (14 times/wk but no bran cereals) and brown bread (14 times/wk), high consumption of yogurt (7 times/wk); low consumption of beef (0.5 time/wk), bacon (0 time/wk) and pork (0 time/wk); low consumption of milk (0 time/wk), and low consumption of vitamin C-rich foods such as orange juice (0 time/wk), orange (0.5 time/wk), mix juice (0 time/wk), other fruit juice (0.5 time/wk) and vitamin C-fortified drinks (0 time/wk).

In subjects ID 156 and 312, Q_2 was lower than the estimate from serum ferritin concentration. In subject ID 156, Q_1 per fat-free mass was also lower than the estimate from serum ferritin concentration. Subject ID 156 had high serum ferritin (61 ng/mL), borderline plasma Zn (720 ng/mL); high consumption of animal products such as beef (3 times/wk), pork (0.5 time/wk), fish (3 times/wk), and chicken (3 times/wk); higher consumption of green beans (5 times/wk); and higher consumption of vitamin C-rich foods such as orange juice (5 times/wk), mix juice (7 times/wk), other fruit juice (3 times/wk) and vitamin C-fortified drinks (0.5 times/wk). Subject ID 312 had high serum ferritin (39 ng/mL), low plasma Zn (600 ng/mL), high consumption of animal products such as beef (3 times/wk), beef (3 times/wk), and pork (3 times/wk), and high vitamin C-rich foods such as orange juice (7 times/wk), mix juice (3 times/wk), peaches (21 times/wk), and vitamin C-fortified drinks (0.5 time/wk).

Effects of oral contraceptives on Zn kinetics and other indices.. Use of oral contraceptives (OC) was associated with differences in Zn kinetics and other indices (Table 23). Subjects who took OC had higher Q_1 per fat free-mass, higher F_{21} per fat-free mass and higher k_{21}. In addition, they were younger and thinner, had higher serum TIBC, and lower serum alkaline phosphatase activity in serum and APTT. OC use was associated with a significant and positive correlation between APTT and Q_2 (Spearman's rank correlation coefficient = 0.657, n = 13, p = 0.015), but not with Q_1, Q_3 and EZP per fat-free mass, plasma Zn, urinary Zn excretion rate and urinary Zn excretion rate constant. No OC use was associated with a positive correlation between APTT and Q_1 per fat-free mass (Spearman's rank correlation coefficient 0.599, n = 29, p = 0.0006) and negative correlation between APTT and urinary Zn excretion rate constant (Spearman's rank correlation coefficient -0.442, n = 22, p = 0.039), while APTT was not significantly correlated with Q_3, EZP per fat-free mass, Q_2, plasma Zn or urinary Zn excretion rate.

Taking OC changed relationships between serum ferritin concentration and Zn kinetics. The correlation between serum ferritin concentrations and Q_1 per fat-free mass was negative and significant (r = -0.735, p = 0.004), the correlation between serum ferritin concentration was negative and marginal (r = -0.456, p = 0.088), while correlations between serum ferritin concentration and Q_3 per fat-free mass (r = -0.125, p =0.685), EZP per fat-free mass (r = -0.211, P = 0.490) and Zn turnover rate per fat-free mass (r = -0.430, P = 0.142) were not significant.

Associations between plasma Zn and taste thresholds are shown in Table 24. Electric taste thresholds of subjects with plasma Zn > or < 700 ng/mL are compared. Because the total number of tested subjects was limited to 35 and the obtained data were discrete variables, the subjects were divided into two groups based on lower limit of normal for plasma Zn (700 ng/mL). Overall median (25 percentile, 75 percentile) was 2 (-5, 8) for the left side of chorda tympani; 2 (-5, 7) for the right side of chorda tympani; 10 (1, 17) for the left side of glossopharyngeal nerve, 9 (-0.5, 20) for the right side of glossopharyngeal nerve. Subjects with plasma Zn < 700 ng/mL had lower thresholds in electric tastes for both sides of chorda tympani and glossopharyngeal nerve. When the electrical taste detection thresholds less than 0 dB and 6 dB were considered to be hypersensitivities respectively in chorda tympani and glossopharyngeal nerve, in both the right and left sides of chorda tympani, 14 normal and 3 hypersensitive subjects had plasma Zn $\geq$ 700 ng/mL, and 6 normal and 12 hypersensitive subjects had plasma Zn < 700 ng/mL (p = 0.004 of Fisher's exact probability). Stimulation of the right side of glossopharyngeal nerve found 14

normal and 3 hypersensitive subjects with plasma Zn $\geq$ 700 ng/mL, and 8 normal and 10 hypersensitive subjects with plasma Zn < 700 ng/mL ($p = 0.023$ of Fisher's exact probability). Stimulation of the left side of glossopharyngeal nerve found 13 normal and 3 hypersensitive subjects with plasma Zn $\geq$ 700 ng/mL, and 6 normal and 10 hypersensitive subjects with plasma Zn < 700 ng/mL ($p = 0.015$ of Fisher's exact probability).

The threshold for bitter of the right glossopharyngeal nerve innervations was significantly higher ($p = 0.027$ by Mann-Whitney U test) in subjects with plasma Zn < 700 ng/mL (median 3; 25^{th} percentile 2; 75^{th} percentile 3.5) compared to subjects with plasma Zn $\geq$ 700 ng/mL (median 2; 25^{th} percentile 2; 75^{th} percentile 3). Taste thresholds for bitter at another region and another tastants, i.e., sweet, salty and sour in any region were not related to plasma Zn concentration (data not shown).

Neuropsychological Findings. Preliminary analysis of effects of treatments in 58 deficient & 20 normal subjects found the percentage improvement in eye-hand coordination measured by percentage time-on-target during a tracking task was greater after treatment with ZnM than after treatment with M ($p<0.002$). Similarly the percentage improvement in performance of a reasoning task, measured by the reaction time required for performance of the oddity task, was greater after treatment with ZnM than after treatment with M ($p<0.008$). In addition, ZnM was more efficacious than FeM for accuracy of short-term recollection of objects presented during a visual memory task ($p<0.02$). FeM was more efficacious than M for eye-hand coordination (tracking; $p<0.02$) & visual perception, as measured by accuracy during a comparison task ($p<0.02$). When the data analysis are complete the results will be forwarded for addition to the record.

Discussion

Zn kinetics and related findings. Subjects with low plasma Zn concentrations had accelerated plasma Zn disappearance. Both g_1 and g_2 were increased. The value of g_2 in Quartile I was nearly twice that in Quartile IV. Rapid plasma Zn disappearance is characteristic of Zn deficiency (3, 18, 48). The comparable results of the model-based Zn kinetic analysis were found from the report of King et al (3, 18, 48), who measured Zn kinetic indices in five healthy men before and after 5-6 wk of severe zinc depletion (< 5 μmol/d). The k_{11} (translated to our notation) increased from ~150 day^{-1} at baseline to 200 day^{-1} at the end of depletion.

In spite of the increased transfer rate constant k_{12}, the Zn flux F_{21} and turnover rate in subjects with low plasma Zn (Quartiles I, II and III) were decreased compared to Quartile IV. King et al (48) found lower plasma Zn concentrations and turnover rate, the increased k_{11} (in our notation) and the onset of the clinical symptoms of Zn depletion in 5 men who were severely deprived of Zn for 5-6 weeks. The Zn kinetic parameters in Quartiles I, II, and III were indicative homeostatic adjustments, i.e., a reduction of urinary zinc excretion, an increase in plasma fractional turnover rates and avid retention of zinc released from selected tissues, called "homeostatic measures" by King et al (48).

The nature of *compartment 1* is obvious as the central compartment, i.e., plasma Zn compartment. A measurable peripheral compartment by tracer study is a composite of sub-compartments with similar kinetic properties. Kinetic parameters do not provide information concerning the anatomy of the compartment, such as kinds of organs. Although the nature or physical existence of *compartment 2* can not be defined from the data, a certain portion of liver Zn is presumably represented by *compartment* 2. Wallwork et al (49) observed increases and decreases in the concentrations of Zn in liver and plasma that fluctuated during the feeding cycle of Zn deprived rats. In association with anorexia Zn increased in plasma, presumably from tissue catabolism. The relative amplitude of the feeding cycle to the median Zn levels was 65 % and 26 % for plasma and liver, respectively. Aamodt et al (50) found that mean radioactivity at liver region in 17 smell/taste dysfunction patients increased very rapidly to 50 % of the total injected amount of ^{69m}Zn within 15 min. Foster et al (51) designated the fastest exchanging peripheral compartment to be a part of liver.

The differences in the transfer rate constants among the quartiles suggest the nature of Zn compartments. Compared to Quartile IV, Quartiles I, II and III had the higher k_{12} and k_{31}, lower Q_2 and unchanged Q_3. In addition, Q_3 and F_{31} correlated with body weight and fat-free mass, while Q_2 and F_{21} did not. This suggests a preferential adaptation mechanism in *compartment 2* to provide Zn to *compartment 3*, and/or stronger binding of Zn in *compartment 3*. One interpretation is that *compartment 2* is involved in the regulatory aspects of Zn metabolism while *compartment 3* is more involved with constitutional aspects of Zn metabolism.

Among Quartiles II, III and IV, the urinary Zn excretion rate constant was not different, and the urinary Zn excretion rate was related to plasma Zn concentration. Because of the linearity of the renal Zn excretion system, lower plasma Zn concentration *per se* can act as a primary

homeostatic measure. When plasma Zn was ~600 ng/mL (Quartile I), the urinary Zn excretion rate constant also decreased, thus conserving Zn.

Taste acuity and zinc. Our subjects displayed gustatory hypersensitivity to the electric stimulus without apparent abnormality of taste perception. Zn deficiency slows motor and sensory nerve conduction velocity in guinea pigs, mediated at least in part by decreased Na^+, K^+- ATPase activity and decreased myo-inositol concentrations in peripheral nerves with minimal morphologic abnormalities (52, 53). In humans, dysesthesia, dysgeusia/hypogeusia, dysosmia/hyposmia, and muscle weakness may be associated with Zn deficiency (54-57). Zn deficient patients do not always recognize Dysgeusia, and our subjects did not complain of dysgeusia. We suggest that hypersensitivity of the gustatory nerves to electrical stimulation might be used to detect mild Zn deficiency (and other causes of the phenomenon). Provisionally, until further verification, the gustatory response to 0 dB and 6 dB electric stimuli may serve as indicators of hypersensitivity of the chorda tympani and glossopharyngeal nerves, respectively.

Urinary Zn excretion and urinary tract infection. Urinary Zn excretion is one of the important regulators of Zn metabolism (20). Our analysis of Babcock et al's (58) data found that the urinary Zn excretion, rate constant, and size of the central plasma Zn compartment (Q_1 in our notation) increased respectively in six free-living women with taste or smell dysfunction when they were administered 100 mg Zn daily for about 300 days: urinary excretion, (mean ± SD) from 378 ± 162 to 1542 ± 472 μg Zn daily, urinary Zn excretion rate constant, 0.168 ± 0.088 day^{-1} to 0.513 ± 0.213 day^{-1}; and Q_1, 2.37 ± 0.50 to 3.20 ± 0.77 mg. In comparison, our subjects with a history of urinary tract infection (UTI) excreted 353 ± 232 μg Zn daily; had a Zn excretion rate constant of 0.168 ± 0.100 day^{-1}; and a Q_1 of 2.10 ± 0.58 mg. In contrast, our subjects with no history of UTI excreted 615 ± 479 μg Zn daily, and had a urinary Zn rate constant of 0.313 ± 0.251 day^{-1}, levels that were between the before and after Zn treatment values of Babcock et al's (58) subjects. Note that Babcock's subjects were considered low in Zn because of the presence of taste or smell dysfunction. Their daily Zn excretion and the rate constant prior to Zn supplementation should be considered "abnormal". The Zn excretion and the rate constant of our subjects with a history of UTI were similar to these "abnormal" values.

Our finding of an association between previous UTI and level of Zn excretion in urine might indicate that renal conservation of Zn is realized at the expense of increased susceptibility to UTI. This suggestion is attractive in view of Zn's essentiality for immunity (59) and resistance to infection (60, 61). Prasad et al. (62) found that Zn deficient sickle-cell disease patients had higher incidence of infections involving respiratory and urinary tracts, and that Zn supplementation to the Zn deficient patients decreased the incidence of infections. Delivery of Zn via secreta or excreta *per se* may be important for mucosal health. Zn in prostatic secretions is antibacterial (63) (64) and antitrichomonal (65). The high zinc concentration of prostatic secretions is presumed to be an important defense of the urinary tract against infection in men, whose UTI incidence is lower than women (66, 67). A Zn-containing salivary growth factor (gustin/carbonic anhydrase VI) is considered responsible for maintenance of taste bud function (51). However we did not find differences in plasma, platelet, and hair concentrations or Zn kinetic parameters (except urinary Zn excretion and its rate constant) that might indicate an intrinsic abnormality in Zn metabolism.

Common factors that determine iron and Zn nutriture. The analyses of gynecological and dietary histories confirmed that beef consumption, hence red meat consumption, is one of the important determinants of iron nutriture (68, 69). Beef consumption was positively associated with iron nutriture, i.e., positive association with serum ferritin, hemoglobin, and hematocrit. A history of bleeding through menstrual pads was negatively associated with serum ferritin and positively associated with erythrocyte proptoporhyrin. A history of bleeding through menstrual pads is the objective sign of heavy menstruation. In this study a history of bleeding through menstrual pads decreased serum ferritin by 15.1 ng/mL, which was close to the value (14.0 ng/mL) found before by us (3). Milk consumption increased erythrocyte protoporphyrin, consistent with the Fe-deficient erythropoesis. Milk casein and calcium in milk inhibit non-heme Fe absorption (70). Orange juice consumption was positively associated with hematocrit and red cell count. Effect of bran breakfast cereals on serum ferritin was negative and marginal. It is notable that coffee consumption was positively associated with MCV, MCH, MCHC, serum iron and saturation of Fe binding capacity, but not with serum ferritin. Coffee contains polyphenols that inhibit Fe absorption. Coffee of weak strength, which is common in the US, is however reported not to inhibit Fe absorption (71). The effect of coffee might be artifact. Another possible, but unproven explanation is that coffee stimulated erythropoiesis and Fe-release from the liver.

Similar to Fe, beef was the important determinant of Zn indices. Beef consumption was positively associated with Q_1, Q_3, EZP and Zn turnover rate per fat-free mass and Q_2. Zn in beef and other red meat is highly bioavailable (72). A history of bleeding through menstrual pads was negatively associated with Q_1, Q_3, EZP and Zn turnover rate per fat-free mass, indicating that excessive menstrual blood loss decreases body Zn pool sizes, although Zn loss by menstrual blood was not closely examined. The Zn content in blood is about 10 μg/mL does not completely account for Zn loss associated with menstruation. A possible mechanism is loss of Zn via exudates and endometrial exfoliations that increase when menstruation is heavy. Consumption of bran breakfast cereals was negatively associated with Q_1, Zn turnover rate per fat-free mass, and Q_2. Bran is a potent inhibitor of Zn absorption because of its high phytate and dietary fiber content (8, 74). Effect of yogurt was not consistent; a positive correlation was found with Q_2 while a negative correlation was found with Q_3 per fat-free mass. We have no reasonable explanation for these associations.

Beef consumption and a history of bleeding through menstrual pads were common determinants for both Fe and Zn nutriture. These dietary associations may predict the association between Fe and Zn nutritional indices. Consistent with this expectation correlations were found between serum ferritin and Zn kinetic parameters. They included linear correlations between serum ferritin, and Q_1 and Q_3 per fat-free mass and a nonlinear association between serum ferritin and Q_2. Although the association between serum ferritin and Q_2 was strong, there were statistical outliers. Subjects ID 104, 114 and 159 had higher Q_2 than predicted from serum ferritin. Subjects ID 104 and 159 were characterized by dietary habits consistent with low vitamin C consumption. Subject ID 114 had low urinary Zn excretion rate constant. Subjects ID 114 and 159 had low hair Zn. In Subjects ID 156 and 312, Q_2 was lower than predicted by the serum ferritin concentration. Both subjects had dietary habits consistent with high vitamin C consumption. High intakes of vitamin C can apparently inhibit Zn retention (73). Thus extremely low and high vitamin C may affect Zn absorption and Zn pool sizes. For Subjects ID

114 and 159, low Zn loss due to low urinary Zn and/or low hair Zn may also explain the higher Zn pool sizes. Statistical outliers were not observed in Q_3 per fat-free mass. This suggests that Q_3 per fat-free mass is not as sensitive as Q_2 for detection of outliers. The correlation between serum ferritin and Q_3 per fat-free mass *per se* was weaker than the correlation between serum ferritin and Q_1 per fat-free mass, and Q_2.

Relationship of plasma Zn to Zn status. Traditionally, the critical value for plasma Zn was set at 700 ng/mL. Because Q_2 was found to be changed with plasma Zn, the critical value of plasma Zn to detect mild Zn deficiency was examined based on the relationship between plasma Zn and Q_2. To elucidate the delimiting concentration of plasma Zn, mathematical examination was done. Inspection of the plot found nonlinearity between plasma Zn and Q_2. Several possible curves were fitted to the data. The four-parameter logistic equation (logistic sigmoidal curve) did not converge and was removed from the further analyses. R^2 was 0.415, 0.452, 0.474, and 0.478 respectively for the first order polynomial equation (straight line), the second order polynomial equation (quadratic curve), the third order polynomial equation (cubic curve), and the broken-line equation. The F ratio test was performed to determine the best fit between equations with different degrees of freedom. The p-value obtained from the comparison of the broken-line equation over the first order polynomial equation was 0.025, indicating the broken-line equation gave a better fit. It was obvious that the broken-line equation gave the better fit than the second order polynomial equation (R^2 = 0.478 vs 0.452 with the same degree of freedom); and the broken-line equation gave the better fit than the third order polynomial equation (R^2 = 0.478 vs 0.474 in spite of the higher degree of freedom in the third order polynomial equation). Therefore, the broken-line equation was selected as the best fit.

Figure 6 shows the nonlinear relationship represented by the broken-line equation between plasma Zn and Q_2. The parameter estimates in the broken-line equation were as follows:

$$Q_2 = a\,(x - b) + a\,|x - b| + c$$

$a = 0.0228 \pm 0.0045$ (estimate ± asymptotic standard error, ASE) (mg/ng/mL);
half value of the slope at the proportional section of the line

$b = 696.3 \pm 27.46$ (ng/mL);
break point between the proportional and horizontal sections of the line

$c = 5.53 \pm 0.76$ (mg); the value for Q_2 at the horizontal section of the line

$|\,X\,|$; absolute of X

Standard error of estimate = 3.40

$R^2 = 0.478$ ($n = 48$, $P < 0.0001$).

If the estimate of break point is directly used, the critical value for plasma Zn becomes 696.3 ng/mL. The round number is 700 ng/mL. The broken-line relationship tells that Q_2 stays at minimum when plasma Zn is less than the break point 696.3 ng/mL. If the estimate + 2 ASE is used for safety, the critical value becomes 751.2 ng/mL (=696.3 + 2 • 27.46). The round number is 750 ng/mL.

The critical value for the upper limit of the "abnormal" Q_2. The critical value for the "abnormal" Q_2 was determined as the mean + 2SD for the subjects whose plasma Zn was less than the break point in the plasma Zn-Q_2 relationship. The number of subjects whose plasma Zn was less than the break point (696.3 ng/mL) was 20. Subjects whose values were above mean+2SD were serially removed. After the removal of three outliers, the mean ± SD of Q_2 was 4.76 ± 1.17 mg (n = 17). The critical value for the "abnormal" Q_2 was tentatively determined to be 7.1 mg (= 4.76 + 2 • 1.17). The critical value for the "abnormal" Q_2 that was determined from the serum ferritin-Q_2 plot was 7.3 mg, as is shown in the following section. The critical value for the "abnormal" Q_2 was decided to be 7.3 mg, although the further verification is required.

Table 25 shows the results when those critical numbers were applied to classify 48 subjects. Twenty-seven of 33 subjects with plasma Zn concentration less than 750 ng/mL were low in Q_2. Three of 15 subjects with plasma Zn concentration above 750 ng/mL were low in Q_2. The association was strongly significant (P = 0.0001, Odds ratio 18.0). The sensitivity (percentage of true positive) to detect low Q_2 by low plasma Zn was 82% (= 27/(27+6)). The specificity (percentage of true negative) for low Q_2 was 80% (= 12/(3+12)). However, those figures must be considered preliminary because of the small number of subjects.

Serum ferritin as a possible Zn nutritional indicator. Serum ferritin is commonly measured during health care. Measurement of plasma Zn is less common and measurement of Zn kinetics is limited to clinical research. If serum ferritin is indeed an indirect indicator of Zn status it offers a readily available way to detect an increased risk of Zn deficiency. We found a nonlinear relationship between serum ferritin and Q_2 using a broken-line equation and determined delimiting numbers. The estimate of Q_2 at the horizontal section was 4.69 mg represented by the coefficient "c". The standard error of estimate (SEE), i.e., the standard deviation of residuals, was 1.28 mg. Thus, the lower limit of normal Q_2 was tentatively defined to be 7.3 mg (= 4.69 + 2 • 1.28). A similar number was found by a somewhat different approach. The critical value for the "abnormal" Q_2 was also determined as the mean+2SD for the subjects whose serum ferritin was less than the break point in the serum ferritin-Q_2 relationship. The number of subjects whose serum ferritin was less than the break point (17.8 ng/mL) was 14. The mean ± SD of Q_2 of those subjects was 4.69 ± 1.28 (n = 14). The critical value for the "abnormal" Q_2 was tentatively determined to be 7.3 mg (= 4.69 + 2 • 1.28). The break point was 17.8 ng/mL represented by "b" with 2.7 ng/mL of asymptotic standard error. Thus, the critical number for serum ferritin was tentatively defined to be 23 ng/mL (= 17.8 + 2 • 2.7).

Table 26 shows the results when those critical numbers were applied to classify 35 subjects who did not take oral contraceptives. Twenty of 23 subjects with serum ferritin less than 23 ng/mL were low in Q_2. Four of 12 subjects with serum ferritin above 23 ng/mL were low in Q_2. The association was strongly significant (P = 0.0022, Odds ratio 13.3). The sensitivity (percentage of true positive) to detect low Q_2 was 83% (=20/(20+4)). The specificity (percentage of true negative) for low Q_2 was 67% (= 8/(4+8)). However, those figures must be considered preliminary because of the small number of subjects.

Effect of oral contraceptives on Zn kinetic parameters. Effect of oral contraceptives (OC) on Zn kinetics was conspicuous. OC increased flux between *compartment* 1 and *compartment* 2, and

the transfer rate constant k_{21}, from *compartment* 1 to 2. Assuming *compartment* 2 represents liver, OC seems to increase the Zn flux to liver. OC, especially ethinyl estradiol, is known to increase liver Fe, and liver and erythrocyte Zn (74) in rats. Administration of 17 beta-estradiol reciprocally decreases liver copper (Cu) and increases plasma Cu in rats (75). Lei et al (74) suggested that ethinyl estradiol, i.e., mestranol, induced a shift of minerals from one pool to another. Prasad et al (76) found that ethinyl estradiol increased total Fe binding capacity, plasma Cu and erythrocyte Zn and decreased plasma Zn concentration in women. Our finding in total Fe binding capacity was consistent with their finding. On the other hand we did not find the change in plasma Zn concentration. However, the Zn kinetic findings, especially increased k_{21}, were consistent with a shift of Zn from one pool to another.

The number of subjects who were taking oral contraceptives was 15. Therefore the statistical power was lower than in the group that were not taking oral contraceptives ($n = 35$). However, the difference in the relationship between serum ferritin and Zn kinetic parameters was not explained by the lower power. The signs of the correlation coefficients were all negative. The correlation between serum ferritin and Q_1 per fat-free mass was significant. There was a positive relationship between Fe and Zn nutriture when oral contraceptives are not taken. Therefore, the outcomes for subjects who took oral contraceptives were considered to be a mixture of the dietary association of Fe and Zn, and the mineral shifts caused by the oral contraceptives. The lower serum alkaline phosphatase activity may be a reflection of a shift in Zn; the enzyme requires Zn and magnesium as cofactors. Our findings suggest knowledge of oral contraceptive usage is important when one is assessing the Fe and Zn status of premenopausal women. Our findings also suggest that more knowledge is needed of associations between the menstrual cycle and Zn metabolism.

Apart from Zn metabolism, the decreased activated partial thromboplastin time (APTT) found in the subjects taking oral contraceptives was considered to show the increased blood coagulation tendency caused by oral contraceptives. OC is a risk factor of thrombosis in women. Shortened APTT was also found in other populations (77-79). One wonders about contributions of Zn, Cu and other trace element nutriture to this phenomenon. In the subjects taking OC, APTT positively correlated with Q_2. In the subjects not taking OC, APTT positively correlated with Q_1 per fat-free mass and negatively correlated with urinary Zn excretion rate constant. These findings suggest that premenopausal women with low Zn nutriture who are taking OC are at increased risk of thrombosis.

Serum electrolyte changes relevant to Zn deficiency. Serum bicarbonate was depressed and anion gap was elevated in Quartile I compared to Quartile IV. These changes are relevant to the mild or marginal Zn deprivation. Long-term Zn deprivation in rhesus monkeys decreased serum bicarbonate (total carbon dioxide) (80). Carbonic anhydrase requires Zn as a cofactor and the apoenzyme is activated by Zn in vitro (81). Zn-deprived rats were reported to have low carbonic anhydrase activity in tongue epithelium, submandibular gland (82) and erythrocytes (83), but not in kidney (84). Carbonic anhydrase converts carbon dioxide into proton and bicarbonate anion to facilitate normal pH (85). The low serum bicarbonate and high anion gap found in the hypozincemic subjects may be derived from the changes in carbonic anhydrase activity.

Effects of treatments on neuropsychological functions. Treatment with ZnM caused a highly significant improvement in eye-hand coordination ($p<0.002$) and performance of a reasoning task ($p<0.008$) compared to the effect of M. ZnM also improved short-term visual memory ($p<0.02$) more than FeM. These findings were consistent with results of double blind randomized controlled trials of ZnM treatment compared to M treatment on neuropsychological function of children (86), and findings from a pilot study of effects of ZnM on short term visual memory of sideropenic women (5). In addition, FeM was more efficacious than M for eye-hand coordination ($p<0.02$) and visual perception ($p<0.02$). These findings were consistent with findings in men (87, 88) and college students (89) and adolescent girls (90) that found Fe status related to neuropsychological function.

Key Research Accomplishments.

1. Demonstration of the correlation between zinc and iron nutriture. We confirm our earlier studies of this topic (3). Our work is the first to show that the rapidly turning over tissue pool of Zn and serum ferritin concentration is highly related (that we know of).
2. Demonstration of the correlation between the rapidly turning over tissue pool of Zn and plasma Zn concentration. The "safe cutoff" for plasma Zn is likely to be 750 ng/mL, not 700 ng/mL. Prior to our work the cutoff between normal and low was arbitrary. Our work is the first to provide a biological basis for the cut off (that we know of).
3. Demonstration for the first time (that we know of) of the positive correlation between the rapidly turning over tissue pool of Zn and lean body mass. This finding is consistent with the intracellular location of Zn.
4. Demonstration of correlations of food frequency with Fe and Zn status. Our findings confirm our previous report of these relationships (3). Inclusion of the food frequency as part of the medical history would be a cost effective way of identifying patients at risk of Fe and Zn deficiencies.
5. Discovery of the association between electrical nerve stimulation threshold and Zn status in humans.
6. Discovery the association between previous history of urinary tract infections and low urinary excretion of Zn. The finding suggests that low urinary Zn predisposes for UTI.
7. Demonstration that relatively mild Zn deficiency adversely affects neuropsychological function of young women. This finding is consistent with our pilot findings in women (5) and with observations in children (86).

Reportable Outcomes.

All of the Key Research Accomplishments.

Conclusions.

1. Low Fe status related to diet is highly correlated with low Zn status.
2. The "safe cutoff" for plasma Zn is likely to be 750 ng/mL, not 700 ng/mL.
3. Zn in the rapidly turning over tissue pool is highly correlated with lean body mass.
4. Food frequency data indicate risk of Fe and Zn deficiencies.
5. Nerve conduction of humans is related to Zn status.
6. Mild deficiencies of Zn and Fe adversely affect neuropsychological function of young women.

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Appendix 1. Course of Respondents and Outcomes

Course of Respondents

708 Responded to advertisements
319 Completed phase 1
138 Completed phase 2
111 Completed phase 3
91 started new treatment (cross over)
83 completed the study, 63 low Zn/Fe subjects and 20 normal subjects. This number was sufficient to fulfill the plan.

Outcomes of All Participants

Subject Number	Birthday	Phase I Screening Completed	Iron status nl vs low	Phase 2 Zn status	Phase 3 Baseline Completed	Phase 3 Crossover Completed	Phase 4 Completed	Comments † †
440	7/17/78	12/1/97	-----	-----	-----	-----	-----	Not eligible
142	6/10/70	NE	-----	-----	-----	-----	-----	Not eligible
458	6/7/75	NE	-----	-----	-----	-----	-----	Not eligible
072	7/27/71	5/17/96	-----	-----	-----	-----	-----	Dropped out
539	3/1/66	6/3/98						Dropped out
294	6/10/54	NE	-----	-----	-----	-----	-----	Not eligible
330	12/23/65	-----	-----	-----	-----	-----	-----	Dropped out
311	8/7/69	6/13/97	-----	-----	-----	-----	-----	Not eligible
233	3/31/71	3/13/97	Normal	4/18/97	5/9/97	7/3/97		Moved away
528	6/12/66	-----	-----	-----	-----	-----	-----	Dropped out
401	1/3/74	10/8/97	-----	-----	-----	-----	-----	Not eligible
483	8/31/71	2/6/98	-----	-----	-----	-----	-----	Dropped out
004	9/9/63	2/16/96	-----	-----	-------	------	-----	Not eligible
121	5/5/59	11/7/96	-----	-----	-----	-----	-----	Not eligible
234	3/27/68	NE	-----	-----	-----	-----	-----	Not eligible
323	9/29/75	9/18/97	-----	-----	-----	-----	-----	Not eligible
237	9/7/78	-----	-----	-----	-----	-----	-----	Dropped out
266	6/8/76	4/10/97	-----	-----	-----	-----	-----	Not eligible
130	10/3/55	9/20/96	-----	-----	-----	-----	-----	Not eligible
489	10/1/69	2/27/98	Low					Dropped out
174	12/31/62	NE	-----	-----	-----	-----	-----	Not eligible
385	4/22/59	NE	-----	-----	-----	-----	-----	Not eligible
137	1/16/76	9/24/96	-----	-----	-----	-----	-----	Dropped out
202	1/2/68	2/10/97	Low	3/13/97	4/28/97	7/7/97	9/16/97	Completed
358	10/10/75	8/20/97	-----	-----	-----	-----	-----	Not eligible
490	9/21/65	NE	-----	-----	-----	-----	-----	Not eligible
247	7/24/59	------	-----	-----	-----	-----	-----	Dropped out
206	4/18/65	NE	-----	-----	-----	-----	-----	Not eligible
391	5/27/73	9/26/97	Low	10/10/97	11/1/97	12/16/97	2/20/98	Completed
219	5/22/71	NE	-----	-----	-----	-----	-----	Not eligible
240	8/31/68	NE	-----	-----	-----	-----	-----	Not eligible
438	10/3/60							Dropped out

155	8/30/72	-----	-----	-----	-----	-----	-----	Dropped out
532	1/8/69	-----	-----	-----	-----	-----	-----	Dropped out
411	2/10/58							Dropped out
125	9/9/64	9/16/96	Low	10/7/96	1/16/97	3/15/97	6/20/97	Completed
513	2/23/74	4/6/98	Low	5/4/98	6/17/98			Dropped out
253	12/8/77	NE	-----	-----	-----	-----	-----	Not eligible
509	4/16/73	NE	-----	-----	-----	-----	-----	Not eligible
067	10/20/72	NE	-----	-----	-----	-----	-----	Not eligible
433	7/26/78	11/1/97	-----	-----	-----	-----	-----	Dropped out
051	12/27/65	NE	-----	-----	-----	-----	-----	Not eligible
403	6/8/64	2/24/97	Low	3/19/98	4/9/98	6/6/98		Dropped out
073	12/21/70	NE	-----	-----	-----	-----	-----	Not eligible
096	11/14/67	-----	-----	-----	----	----	----	Dropped out
278	4/19/73	NE	-----	-----	-----	-----	-----	Not eligible
337	3/15/69	8/8/97	-----	-----	-----	-----	-----	Dropped out
201	7/28/64	2/10/97	Low	2/28/97	3/26/97	5/14/97	-----	Dropped out
331	11/28/66	7/31/97	-----	-----	-----	-----	-----	Not eligible
387	12/16/65	4/2/98	Low					Dropped out
066	10/18/67	NE	-----	-----	-----	-----	-----	Not eligible
359	6/12/72							Dropped out
200	2/7/69	-----	-----	-----	-----	-----	-----	Dropped out
154	8/22/70	-----	-----	-----	-----	-----	-----	Dropped out
227	2/14/78	------	-----	-----	-----	-----	-----	Dropped out
426	9/30/66	-----	-----	-----	-----	-----	-----	Dropped out
419	12/29/62	NE	-----	-----	-----	-----	-----	Not eligible
138	7/9/57	NE	-----	-----	-----	-----	-----	Not eligible
231	11/29/72	5/7/97	-----	-----	-----	-----	-----	Dropped out
113	7/11/72	-----	-----	-----	-----	-----	-----	Dropped out
375	1/20/72	-----	-----	-----	-----	-----	-----	Dropped out
101	12/23/58	8/5/96	-----	-----	-----	-----	-----	Not eligible
092	1/22/64	NE	-----	-----	----	----	----	Not eligible
274	11/27/67	-----	-----	-----	-----	-----	-----	Dropped out
123	10/28/58	9/16/96	-----	-----	-----	-----	-----	Dropped out
461	8/14/61	NE	-----	-----	-----	-----	-----	Not eligible
485	12/24/59	2/27/98	Low	4/9/98	5/7/98	7/3/98		Dropped out
335	9/17/69	8/12/97	-----	-----	-----	-----	-----	Not eligible
350	5/20/62	NE	-----	-----	-----	-----	-----	Not eligible
132	2/27/56	NE	-----	-----	-----	-----	-----	Not eligible
160	1/27/58	NE	-----	-----	-----	-----	-----	Not eligible
235	12/7/64	NE	-----	-----	-----	-----	-----	Not eligible
161	1/28/63	NE	-----	-----	-----	-----	-----	Not eligible
422	6/29/61	10/21/97	-----	-----	-----	-----	-----	Not eligible
376	5/10/63	9/29/97	Low	10/23/97	11/7/97	1/5/98	3/2/98	Completed
084	1/2/74	7/19/96	-----	-----	-----	-----	-----	Not eligible
027	12/24/66	NE	-----	-----	-----	-----	-----	Not eligible
306	8/31/56	5/27/97	-----	-----	-----	-----	-----	Not eligible
469	1/3/64	-----	-----	-----	-----	-----	-----	Dropped out
163	9/1/66	NE	-----	-----	-----	-----	-----	Not eligible

348	5/22/69	------	-----	-----	-----	-----	-----	Dropped out
517	12/28/67							Dropped out
203	1/14/97	2/17/97	Low	3/13/97	4/28/97	7/23/97	9/19/97	Completed
162	3/7/61	10/30/96	Low	10/3/96	1/30/97	3/26/97	5/28/97	Completed
189	7/2/74	4/11/97	Low	5/13/97	-----	-----	-----	Dropped out
374	12/5/59	9/11/97	Normal	9/25/97	10/9/97	12/17/97	2/9/98	Completed
472	1/30/60	1/12/98	Normal	1/16/98	1/29/98	3/17/98	5/13/98	Completed
352	9/30/62	9/4/97	Low	11/17/97	-----	-----	-----	Dropped out
264	9/15/67							Dropped out
414	10/7/77	-----	-----	-----	-----	-----	-----	Dropped out
147	5/13/52	NE	-----	-----	-----	-----	-----	Not eligible
135	3/21/57	9/28/96	-----	-----	-----	-----	-----	Dropped out
261	11/22/60	4/2/97	-----	-----	-----	-----	-----	Dropped out
194	5/5/64	2/14/97	Low	3/15/97	4/24/97	6/23/97	8/21/97	Completed
442	6/24/58	12/16/97	-----	-----	-----	-----	-----	Not eligible
071	12/18/69	5/16/96	-----	-----	-----	-----	-----	Dropped out
140	12/15/63	9/18/96	-----	-----	-----	-----	-----	Not eligible
046	8/23/63	NE	-----	-----	-----	-----	-----	Not eligible
315	3/11/69	6/2/97	Low	7/22/97	11/11/97	1/9/98	2/27/98	Completed
076	7/11/71	9/20/96	-----	-----	-----	-----	-----	Dropped out
463	1/30/63	2/27/98	Normal	3/17/98	-----	-----	-----	Dropped out
384	7/3/61	10/1/97	-----	-----	-----	-----	-----	Not eligible
535	8/27/64	-----	-----	-----	-----	-----	-----	Dropped out
144	4/21/65	NE	-----	-----	-----	-----	-----	Not eligible
297	4/3/62	6/2/97	Normal	10/13/97	10/28/97	-----	-----	Dropped out
515	2/18/64	4/23/98	-----	-----	-----	-----	-----	Not eligible
459	4/25/72	-----	-----	-----	-----	-----	-----	Dropped out
304	2/12/70	5/21/97	-----	-----	-----	-----	-----	Not eligible
013	10/17/68	NE	-----	-----	-----	-----	-----	Not eligible
068	2/28/70	NE	-----	-----	-----	-----	-----	Not eligible
248	9/2/60	5/16/97	-----	-----	-----	-----	-----	Not eligible
088	5/14/68	NE	-----	-----	-----	-----	-----	Not eligible
095	1/17/67	NE	-----	-----	----	----	----	Not eligible
157	1/17/67	NE	-----	-----	-----	-----	-----	Not eligible
382	6/2/60	NE	-----	-----	-----	-----	-----	Not eligible
430	5/8/65	11/6/97	-----	-----	-----	-----	-----	Dropped out
002	6/22/77	NE	-----	-----	-------	------	-----	Not eligible
320	11/11/71	6/13/97	-----	-----	-----	-----	-----	Not eligible
244	10/30/59	-----	-----	-----	-----	-----	-----	Dropped out
477	12/9/72	1/28/98	-----	-----	-----	-----	-----	Not eligible
346	12/25/70	-----	-----	-----	-----	-----	-----	Dropped out
345	9/21/75	NE	-----	-----	-----	-----	-----	Not eligible
423	12/23/69	10/28/97	-----	-----	-----	-----	-----	Not eligible
213	9/16/63	----	-----	-----	-----	-----	-----	Dropped out
159	1/8/69	10/14/96	Low	10/30/96	11/13/96	1/8/97	3/11/97	Completed
021	6/3/71	NE	-----	-----	-----	-----	-----	Not eligible
425	4/27/72	-----	-----	-----	-----	-----	-----	Dropped out
372	7/20/68							Dropped out

069	5/21/69	5/14/96	-----	-----	-----	-----	-----	Not eligible
115	9/15/60	NE	-----	-----	-----	-----	-----	Not eligible
351	5/12/59	------	-----	-----	-----	-----	-----	Dropped out
128	3/5/57	9/19/96	-----	-----	-----	-----	-----	Not eligible
270	7/27/62	-----	-----	-----	-----	-----	-----	Dropped out
011	7/28/68	-----	-----	-----	-----	-----	-----	Dropped out
421	3/6/73	-----	-----	-----	-----	-----	-----	Dropped out
001	3/2/70	3/8/96	Low	5/15/98	-------	------	-----	Dropped out
275	9/11/72	7/17/97	-----	-----	-----	-----	-----	Not eligible
435	4/22/78	-----	-----	-----	-----	-----	-----	Dropped out
120	11/11/58	NE	-----	-----	-----	-----	-----	Not eligible
406	10/22/70	NE	-----	-----	-----	-----	-----	Not eligible
063	1/17/66	NE	-----	-----	-----	-----	-----	Not eligible
312	12/22/	6/3/97	Normal	8/5/97	8/18/97	10/7/97	12/2/97	Completed
065	10/13/59	NE	-----	-----	-----	-----	-----	Not eligible
336	9/11/56	NE	-----	-----	-----	-----	-----	Not eligible
087	7/14/59	NE	-----	-----	-----	-----	-----	Not eligible
379	1/10/73	NE	-----	-----	-----	-----	-----	Not eligible
103	3/17/74	NE	-----	-----	-----	-----	-----	Not eligible
044	7/4/66	3/11/96	-----	-----	-----	-----	-----	Dropped out
024	3/6/71	NE	-----	-----	-----	-----	-----	Not eligible
105	11/7/59	NE	-----	-----	-----	-----	-----	Not eligible
060	9/21/73	5/15/96	Low	8/22/96	9/20/96	2/7/97	4/4/97	Completed
141	10/1/65	NE	-----	-----	-----	-----	-----	Not eligible
272	5/29/59	4/17/97	Normal	6/24/97	7/11/97	10/9/97	11/26/97	Completed
427	10/15/73	11/11/97	-----	-----	-----	-----	-----	Not eligible
378	10/11/68	-----	-----	-----	-----	-----	-----	Dropped out
226	3/17/65	3/12/97	Normal	4/30/97	5/15/97	8/14/97	10/23/97	Completed
106	5/27/57	-----	-----	-----	-----	-----	-----	Dropped out
500	1/10/61							Dropped out
050	2/17/65	NE	-----	-----	-----	-----	-----	Not eligible
118	3/8/66	12/10/96	Normal	12/10/96	1/8/97	3/14/97	-----	Dropped out
107	4/3/70	8/7/96	-----	-----	-----	-----	-----	Dropped out
486	12/22/65	NE	-----	-----	-----	-----	-----	Not eligible
409	1/18/58	11/21/97	Normal	1/15/98	1/22/98	3/17/98	5/23/98	Completed
418	10/25/58	11/10/97	-----	-----	-----	-----	-----	Dropped out
432	10/31/74	11/18/97	-----	-----	-----	-----	-----	Not eligible
251	2/5/67	4/11/97	Low	8/7/97	9/25/97	12/1/97	3/5/98	Completed
007	3/23/75	NE	-----	-----	-------	------	-----	Not eligible
324	8/14/56	8/6/97	Low	9/26/97	10/14/97	12/5/97	2/4/98	Completed
551	3/23/72							Dropped out
153	11/4/59	NE	-----	-----	-----	-----	-----	Not eligible
277	9/24/73	NE	-----	-----	-----	-----	-----	Not eligible
310	8/18/63	5/23/97	-----	-----	-----	-----	-----	Not eligible
424	4/21/74	-----	-----	-----	-----	-----	-----	Dropped out
420	2/6/60	-----	-----	-----	-----	-----	-----	Dropped out
300	12/26/61	6/17/97	-----	-----	-----	-----	-----	Not eligible
269	12/28/63	4/16/97	Normal	6/18/97	7/1/97	10/14/97	-----	Dropped out

116	7/5/73	NE	-----	-----	-----	-----	-----	Not eligible
340	5/3/77							Dropped out
059	12/13/64	NE	-----	-----	-----	-----	-----	Not eligible
446	8/28/75	12/17/97	-----	-----	-----	-----	-----	Not eligible
119	12/11/70	9/16/96	-----	-----	-----	-----	-----	Not eligible
501	9/26/74	4/8/98	Low	7/3/98				Dropped out
460	3/7/73	1/5/98	-----	-----	-----	-----	-----	Not eligible
034	11/30/64	NE	-----	-----	-----	-----	-----	Not eligible
496	1/30/76	7/7/98						†Dropped out
145	10/30/72	NE	-----	-----	-----	-----	-----	Not eligible
195	7/21/74	3/10/97	-----	-----	-----	-----	-----	Dropped out
207	3/1/70	NE	-----	-----	-----	-----	-----	Not eligible
525	10/27/60							Dropped out
025	10/9/62	NE	-----	-----	-----	-----	-----	Not eligible
268	8/19/69	-----	-----	-----	-----	-----	-----	Dropped out
514	12/25/69	-----	-----	-----	-----	-----	-----	Dropped out
218	6/30/66	-----	-----	-----	-----	-----	-----	Dropped out
198	6/7/75	4/15/97	-----	-----	-----	-----	-----	Dropped out
547	11/2/76							Dropped out
282	8/15/68	-----	-----	-----	-----	-----	-----	Dropped out
041	7/27/72	NE	-----	-----	-----	-----	-----	Not eligible
519	7/27/72	NE	-----	-----	-----	-----	-----	Not eligible
019	11/4/69	2/14/96	Normal	5/16/96	-----	-----	-----	Dropped out
030	9/7/73	3/4/96	-----	-----	-----	-----	-----	Dropped out
026	9/14/68	NE	-----	-----	-----	-----	-----	Not eligible
319	9/14/68	-----	-----	-----	-----	-----	-----	Dropped out
246	11/25/65	3/27/97	-----	-----	-----	-----	-----	Not eligible
267	11/2/58	6/20/97	-----	-----	-----	-----	-----	Not eligible
186	5/6/66	6/25/97	-----	-----	-----	-----	-----	Not eligible
301	9/26/77	6/4/97	Low	7/15/97	-----	-----	-----	Moved away
250	3/26/78	-----	-----	-----	-----	-----	-----	Dropped out
279	6/13/63	NE	-----	-----	-----	-----	-----	Not eligible
314	2/4/59	-----	-----	-----	-----	-----	-----	Dropped out
139	11/13/57	NE	-----	-----	-----	-----	-----	Not eligible
143	9/2/75	NE	-----	-----	-----	-----	-----	Not eligible
431	6/12/75	11/14/97	-----	-----	-----	-----	-----	Not eligible
366	3/9/64	9/4/97	Low	3/27/98	4/13/98	6/10/98		Dropped out
010	10/16/60	NE	-----	-----	-----	-----	-----	Not eligible
364	3/12/73	9/5/97	Normal	9/26/97	10/30/97	12/22/97	2/23/98	Completed
151	2/13/73	10/1/96	-----	-----	-----	-----	-----	Not eligible
299	6/21/65	1/7/98	-----	-----	-----	-----	-----	Not eligible
022	6/21/65	NE	-----	-----	-----	-----	-----	Not eligible
129	6/21/65	NE	-----	-----	-----	-----	-----	Not eligible
098	8/17/74	NE	-----	-----	----	----	----	Not eligible
289	1/6/64	-----	-----	-----	-----	-----	-----	Dropped out
032	10/25/67	3/5/96	-----	-----	-----	-----	-----	Not eligible
506	1/29/74	4/8/98	Low					Dropped out
286	11/18/70	-----	-----	-----	-----	-----	-----	Dropped out

471	1/25/58	-----	-----	-----	-----	-----	-----	Dropped out
354	12/5/59	-----	-----	-----	-----	-----	-----	Dropped out
498	12/6/58	NE	-----	-----	-----	-----	-----	Not eligible
317	2/3/75	6/18/97	-----	-----	-----	-----	-----	Dropped out
215	11/15/61	3/11/97	Low	7/31/97	8/12/97	10/8/97	12/3/97	Completed
394	7/19/65	-----	-----	-----	-----	-----	-----	Dropped out
249	10/9/76	------	-----	-----	-----	-----	-----	Dropped out
230	12/4/76	------	-----	-----	-----	-----	-----	Dropped out
169	3/25/74	11/21/96	-----	-----	-----	-----	-----	Dropped out
439	12/1/71	1/2/98	-----	-----	-----	-----	-----	Not eligible
156	1/20/63	10/11/96	Normal	6/13/97	-----	-----	-----	Dropped out
211	2/11/59	NE	-----	-----	-----	-----	-----	Not eligible
190	8/10/64	NE	-----	-----	-----	-----	-----	Not eligible
012	3/30/60	-----	-----	-----	-----	-----	-----	Dropped out
538	8/20/72	-----	-----	-----	-----	-----	-----	Dropped out
015	10/20/62	NE	-----	-----	-----	-----	-----	Not eligible
260	9/14/72							Dropped out
408	8/18/60	-----	-----	-----	-----	-----	-----	Dropped out
373	1/12/60	-----	-----	-----	-----	-----	-----	Dropped out
276	6/12/72	6/25/97	-----	-----	-----	-----	-----	Not eligible
039	12/2/66	3/1/96	-----	-----	-----	-----	-----	Dropped out
291	12/3/52	NE	-----	-----	-----	-----	-----	Not eligible
074	9/5/71	5/24/96	-----	-----	-----	-----	-----	Dropped out
236	3/3/97	NE	-----	-----	-----	-----	-----	Not eligible
079	4/25/56	6/27/96	-----	-----	-----	-----	-----	Not eligible
467	5/26/65	1/16/98	-----	-----	-----	-----	-----	Dropped out
389	3/23/53	NE	-----	-----	-----	-----	-----	Not eligible
429	10/6/63	10/28/97	-----	-----	-----	-----	-----	Not eligible
552	10/7/72							Dropped out
136	7/25/72	9/18/96	Low	10/3/96	1/21/97	3/19/97	-----	Moved away
082	2/24/58	-----	-----	-----	-----	-----	-----	Dropped out
516	4/29/76	-----	-----	-----	-----	-----	-----	Dropped out
006	8/5/60	NE	-----	-----	-------	------	-----	Not eligible
450	4/1/61	1/16/98	Low	2/12/98	2/18/98	-----	-----	Dropped out
110	2/1/57	NE	-----	-----	-----	-----	-----	Not eligible
117	3/5/67	8/23/96	-----	-----	-----	-----	-----	Dropped out
131	10/5/65	NE	-----	-----	-----	-----	-----	Not eligible
033	6/21/71	NE	-----	-----	-----	-----	-----	Not eligible
292	9/6/59	-----	-----	-----	-----	-----	-----	Dropped out
124	8/5/57	NE	-----	-----	-----	-----	-----	Not eligible
075	9/16/73	NE	-----	-----	-----	-----	-----	Not eligible
322	1/26/64	-----	-----	-----	-----	-----	-----	Dropped out
127	4/29/70	NE	-----	-----	-----	-----	-----	Not eligible
191	4/8/70	1/30/97	-----	-----	-----	-----	-----	Dropped out
090	3/4/57	7/19/96	Low	8/15/96	10/17/96	1/10/97	4/10/97	Completed
541	12/27/70	NE	-----	-----	-----	-----	-----	Not eligible
175	11/26/68	NE	-----	-----	-----	-----	-----	Not eligible
047	6/19/69	4/3/96	-----	-----	-----	-----	-----	Dropped out

017	6/26/73	3/7/96	-----	-----	-----	-----	-----	Dropped out
444	9/4/71	12/8/97	Normal	1/13/98	2/1/98	4/7/98	5/27/98	Completed
448	5/19/73	12/8/97	Normal	1/28/98	5/26/98			Dropped out
271	12/28/59	4/22/97	Low	6/11/97	8/4/97	10/20/97	12/17/97	Completed
457	4/26/73	-----	-----	-----	-----	-----	-----	Dropped out
480	7/13/60	4/3/98	Low	4/17/98	4/23/98	6/15/98		Dropped out
255	9/17/71	NE	-----	-----	-----	-----	-----	Not eligible
523	10/31/60	7/7/98						Dropped out
508	3/17/63	4/3/98	Low	4/23/98	5/6/98	6/24/98		Dropped out
549	9/21/51	NE	-----	-----	-----	-----	-----	Not eligible
520	11/9/73	NE	-----	-----	-----	-----	-----	Not eligible
293	10/6/59	-----	-----	-----	-----	-----	-----	Dropped out
057	4/3/71	NE	-----	-----	-----	-----	-----	Not eligible
371	8/8/76	NE	-----	-----	-----	-----	-----	Not eligible
241	12/27/60	NE	-----	-----	-----	-----	-----	Not eligible
355	8/26/71	NE	-----	-----	-----	-----	-----	Not eligible
404	6/3/64							Dropped out
339	2/13/78	----	-----	-----	-----	-----	-----	Dropped out
224	5/12/59	------	-----	-----	-----	-----	-----	Dropped out
305	8/19/67	NE	-----	-----	-----	-----	-----	Not eligible
334	6/17/76							Dropped out
546	8/7/77	NE	-----	-----	-----	-----	-----	Not eligible
545	4/2/65	6/15/98	-----	-----	-----	-----	-----	Not eligible
503	1/31/60	4/9/98	-----	-----	-----	-----	-----	Dropped out
357	8/22/67	9/9/97	-----	-----	-----	-----	-----	Not eligible
505	4/14/61	4/3/98	Low	4/16/98	6/3/98			Dropped out
386	2/28/73	10/2/97	Normal	10/17/97	10/31/97	12/30/97	2/16/98	Completed
040	3/13/71	5/21/97	Normal	6/13/97	6/27/97	9/4/97	11/6/97	Completed
507	12/18/62	4/2/98	-----	-----	-----	-----	-----	Not eligible
329	6/25/59	NE	-----	-----	-----	-----	-----	Not eligible
397	1/6/72	-----	-----	-----	-----	-----	-----	Dropped out
487	5/25/57	-----	-----	-----	-----	-----	-----	Dropped out
482	7/2/57	NE	-----	-----	-----	-----	-----	Not eligible
185	6/17/78	NE	-----	-----	-----	-----	-----	Not eligible
018	4/21/65	3/5/96	Normal	5/19/96	-----	-----	-----	Dropped out
005	11/12/71	NE	-----	-----	-------	------	-----	Not eligible
199	11/16/67	7/14/97	-----	-----	-----	-----	-----	Not eligible
220	8/27/65	----	-----	-----	-----	-----	-----	Dropped out
091	8/2/67	7/19/96	Normal	7/24/96	----	----	----	Dropped out
497	12/27/57	-----	-----	-----	-----	-----	-----	Dropped out
008	1/5/74	-----	-----	-----	-------	------	-----	Dropped out
239	9/3/61	3/17/97						Dropped out
370	6/8/70	9/4/97	Low	11/3/97	11/14/97	1/7/98	2/27/98	Completed
338	6/4/68	8/8/97	-----	-----	-----	-----	-----	Not eligible
453	3/15/72	-----	-----	-----	-----	-----	-----	Dropped out
481	11/26/72	NE	-----	-----	-----	-----	-----	Not eligible
263	1/8/59	------	-----	-----	-----	-----	-----	Dropped out
003	5/17/61	2/15/96	-----	-----	-------	------	-----	Not eligible

197	9/3/73	2/26/97	Low	5/1/97	5/27/97	-----	-----	Dropped out
043	12/30/66	NE	-----	-----	-----	-----	-----	Not eligible
009	8/14/70	2/20/96	Normal	7/1/97	8/14/97	10/13/97	12/8/98	Completed
326	1/6/71	8/6/97	-----	-----	-----	-----	-----	Dropped out
465	12/3/73	1/19/98	-----	-----	-----	-----	-----	Not eligible
083	5/27/58	-----	-----	-----	-----	-----	-----	Un Dropped ou
064	11/27/61	NE	-----	-----	-----	-----	-----	Not eligible
181	8/26/63	-----	-----	-----	-----	-----	-----	Dropped out
316	2/3/60	6/10/97	-----	-----	-----	-----	-----	Not eligible
349	4/28/78	8/14/97	Low	9/8/97	9/30/97	11/21/97	1/17/98	Completed
188	8/12/75	NE	-----	-----	-----	-----	-----	Not eligible
479	2/26/60	-----	-----	-----	-----	-----	-----	Dropped out
225	9/23/76	3/18/97	Low	6/6/97	-----	-----	-----	Dropped out
524	4/25/59	-----	-----	-----	-----	-----	-----	Dropped out
540	8/20/65	7/6/98						Dropped out
526	12/14/61	-----	-----	-----	-----	-----	-----	Dropped out
165	3/9/57	11/22/96	Normal	1/3/97	1/21/97	3/20/97	5/17/97	Completed
238	8/25/78	NE	-----	-----	-----	-----	-----	Not eligible
214	11/9/60	-----	-----	-----	-----	-----	-----	Dropped out
413	1/24/60	10/1/97	-----	-----	-----	-----	-----	Not eligible
534	1/12/65							Dropped out
054	6/23/72	3/27/96	-----	-----	-----	-----	-----	Dropped out
052	4/14/77	NE	-----	-----	-----	-----	-----	Not eligible
085	10/1/57	7/12/96	-----	-----	-----	-----	-----	Dropped out
164	10/10/60	11/21/96	Low	12/20/96	2/14/97	5/10/97	7/4/97	Completed
449	9/15/73	-----	-----	-----	-----	-----	-----	Dropped out
407	8/20/97	9/25/97	Normal					Dropped out
466	1/19/58	NE	-----	-----	-----	-----	-----	Not eligible
045	5/26/73	-----	-----	-----	-----	-----	-----	Dropped out
527	2/28/79	5/15/98	Normal					Dropped out
475	11/5/65	----	-----	-----	-----	-----	-----	Dropped out
148	7/30/58	NE	-----	-----	-----	-----	-----	Not eligible
434	1/14/65	-----	-----	-----	-----	-----	-----	Dropped out
410	6/29/61	NE	-----	-----	-----	-----	-----	Not eligible
158	12/25/69	-----	-----	-----	-----	-----	-----	Dropped out
530	4/19/69							Dropped out
360	7/1/66	-----	-----	-----	-----	-----	-----	Dropped out
150	7/27/71	9/25/96	Normal	10/15/96	10/22/96	12/9/96	2/11/97	Completed
176	1/12/58	NE	-----	-----	-----	-----	-----	Not eligible
210	3/3/58	-----	-----	-----	-----	-----	-----	Dropped out
196	7/19/75	-----	-----	-----	-----	-----	-----	Dropped out
398	11/2/74	-----	-----	-----	-----	-----	-----	Dropped out
031	10/7/71	NE	-----	-----	-----	-----	-----	Not eligible
193	1/27/65	2/19/97	-----	-----	-----	-----	-----	Dropped out
029	7/20/67	NE	-----	-----	-----	-----	-----	Not eligible
393	7/15/58	NE	-----	-----	-----	-----	-----	Not eligible
325	10/11/59	7/14/97	-----	-----	-----	-----	-----	Dropped out
473	6/20/68	-----	-----	-----	-----	-----	-----	Dropped out

445	2/24/60	3/23/98	Low					Dropped out
295	12/4/69	-----	-----	-----	-----	-----	-----	Dropped out
023	10/26/62	NE	-----	-----	-----	-----	-----	Not eligible
014	1/13/63	-----	-----	-----	-----	-----	-----	Dropped out
177	4/18/62	1/13/97	Low	2/27/97	4/19/97	6/7/97	8/30/97	Completed
356	9/19/64	NE	-----	-----	-----	-----	-----	Not eligible
455	10/27/65	-----	-----	-----	-----	-----	-----	Dropped out
441	8/3/73	12/1/97	-----	-----	-----	-----	-----	Dropped out
049	4/26/70	NE	-----	-----	-----	-----	-----	Not eligible
504	9/26/70	3/27/98	Low					Dropped out
361	10/23/62	NE	-----	-----	-----	-----	-----	Not eligible
499	2/12/26	NE	-----	-----	-----	-----	-----	Not eligible
383	5/31/63	NE	-----	-----	-----	-----	-----	Not eligible
493	8/5/65	-----	-----	-----	-----	-----	-----	Dropped out
016	8/6/71	NE	-----	-----	-----	-----	-----	Not eligible
380	8/1/71	-----	-----	-----	-----	-----	-----	Dropped out
099	5/25/72	7/24/96	Normal	8/13/96	9/13/96	11/8/96	12/30/96	Completed
436	1/6/78	-----	-----	-----	-----	-----	-----	Dropped out
179	4/21/77	NE	-----	-----	-----	-----	-----	Not eligible
028	5/22/71	NE	-----	-----	-----	-----	-----	Not eligible
343	3/15/74	8/15/97	-----	-----	-----	-----	-----	Not eligible
474	2/8/65	NE	-----	-----	-----	-----	-----	Not eligible
170	4/30/74	NE	-----	-----	-----	-----	-----	Not eligible
512	7/29/59	5/18/98	Low	6/25/98				Dropped out
388	7/1/60	NE	-----	-----	-----	-----	-----	Not eligible
308	11/7/73	NE	-----	-----	-----	-----	-----	Not eligible
296	6/4/67	5/16/97	-----	-----	-----	-----	-----	Not eligible
341	3/14/74	3/27/98	Low	6/12/98				Dropped out
327	5/10/60	NE	-----	-----	-----	-----	-----	Not eligible
303	1/13/71	5/30/97	-----	-----	-----	-----	-----	Not eligible
149	2/8/70	-----	-----	-----	-----	-----	-----	Dropped out
223	2/10/64	4/1/98	-----	-----	-----	-----	-----	Not eligible
209	8/5/58	-----	-----	-----	-----	-----	-----	Dropped out
521	5/15/53	-----	-----	-----	-----	-----	-----	Dropped out
462	5/27/72	NE	-----	-----	-----	-----	-----	Not eligible
365	2/17/72	-----	-----	-----	-----	-----	-----	Dropped out
368	12/11/57	9/3/97	-----	-----	-----	-----	-----	Not eligible
470	3/8/66	-----	-----	-----	-----	-----	-----	Dropped out
456	7/21/75	-----	-----	-----	-----	-----	-----	Dropped out
428	7/17/75	-----	-----	-----	-----	-----	-----	Dropped out
204	2/11/57	2/18/97	-----	-----	-----	-----	-----	Dropped out
285	9/11/68	-----	-----	-----	-----	-----	-----	Dropped out
342	1/7/73	8/20/97	-----	-----	-----	-----	-----	Not eligible
313	9/26/58	-----	-----	-----	-----	-----	-----	Dropped out
242	4/8/74	4/1/97	-----	-----	-----	-----	-----	Dropped out
126	2/1/63	11/6/96	Normal	12/13/96	-----	-----	-----	Dropped out
055	5/31/68	4/5/96	-----	-----	-----	-----	-----	Dropped out
321	3/29/74	-----	-----	-----	-----	-----	-----	Dropped out

114	12/14/60	8/29/96	Low	10/4/96	10/28/96	-----	-----	Dropped out
369	9/9/67	NE	-----	-----	-----	-----	-----	Not eligible
212	11/5/75	NE	-----	-----	-----	-----	-----	Not eligible
290	3/10/67	5/6/97	Normal	5/24/97	-----	-----	-----	Pregnant
037	8/16/71	2/23/96	-----	-----	-----	-----	-----	Dropped out
171	11/25/70	NE	-----	-----	-----	-----	-----	Not eligible
081	1/29/65	NE	-----	-----	-----	-----	-----	Not eligible
353	4/13/68	8/15/97	-----	-----	-----	-----	-----	Not eligible
537	11/8/69	NE	-----	-----	-----	-----	-----	Not eligible
283	8/17/61	-----	-----	-----	-----	-----	-----	Dropped out
273	10/25/60	4/22/97	-----	-----	-----	-----	-----	Not eligible
109	8/8/67	8/16/96	Normal	8/26/96	-----	-----	-----	Dropped out
522	12/21/67	5/6/98	-----	-----	-----	-----	-----	Not eligible
476	1/26/64	1/14/98	Normal	1/29/98	2/4/98	4/3/98	-----	Moved away
280	10/12/56	6/16/98	-----	-----	-----	-----	-----	Dropped out
543	3/12/58							Dropped out
437	6/12/64	11/14/97	-----	-----	-----	-----	-----	Not eligible
134	12/24/66	NE	-----	-----	-----	-----	-----	Not eligible
184	6/18/74	NE	-----	-----	-----	-----	-----	Not eligible
102	4/18/68	NE	-----	-----	-----	-----	-----	Not eligible
347	1/3/58	8/26/97	-----	-----	-----	-----	-----	Not eligible
484	5/28/57	-----	-----	-----	-----	-----	-----	Dropped out
344	10/22/64	8/22/97	Low	9/12/97	-----	-----	-----	Dropped out
048	3/12/68	-----	-----	-----	-----	-----	-----	Not eligible
392	2/10/68	9/26/97	Low					Dropped out
396	7/29/76	-----	-----	-----	-----	-----	-----	Dropped out
089	12/14/67	NE	-----	-----	-----	-----	-----	Not eligible
178	2/7/59	NE	-----	-----	-----	-----	-----	Not eligible
180	11/1/64	-----	-----	-----	-----	-----	-----	Dropped out
097	8/3/56	10/2/96						Dropped out
146	5/20/68	10/9/96	Low	11/22/96	-----	-----	-----	Dropped out
100	6/24/69	8/5/96	-----	-----	-----	-----	-----	Dropped out
252	11/2/56	NE	-----	-----	-----	-----	-----	Not eligible
020	1/7/96	2/19/96	-----	-----	-----	-----	-----	Dropped out
550	10/14/68	NE	-----	-----	-----	-----	-----	Not eligible
108	11/26/56	-----	-----	-----	-----	-----	-----	Dropped out
548	5/29/69							Dropped out
415	8/16/77	NE	-----	-----	-----	-----	-----	Not eligible
402	9/25/74	9/18/97	-----	-----	-----	-----	-----	Not eligible
488	12/14/58	-----	-----	-----	-----	-----	-----	Dropped out
112	3/15/58	8/12/96	-----	-----	-----	-----	-----	Not eligible
362	11/10/58	NE	-----	-----	-----	-----	-----	Not eligible
042	3/10/61	3/12/96	-----	-----	-----	-----	-----	Dropped out
400	8/16/66	NE	-----	-----	-----	-----	-----	Not eligible
205	2/24/59	4/11/97	Normal	5/7/97	6/16/97	8/5/97	9/29/97	Completed
061	8/7/74	NE	-----	-----	-----	-----	-----	Not eligible
104	5/21/68	8/5/96	Normal	8/23/96	9/14/96	-----	-----	Dropped out
417	3/23/62	10/17/97	-----	-----	-----	-----	-----	Not eligible

078	12/10/63	NE	-----	-----	-----	-----	-----	Not eligible
070	5/11/65	11/4/96	-----	-----	-----	-----	-----	Dropped out
259	3/31/74	-----	-----	-----	-----	-----	-----	Dropped out
529	2/27/73							Dropped out
265	11/13/60	4/2/97	Low	6/3/97	7/17/97	10/8/97	11/24/97	Completed
192	8/16/64	2/14/97	-----	-----	-----	-----	-----	Not eligible
542	5/11/71							Dropped out
302	5/16/69	5/22/97	-----	-----	-----	-----	-----	Not eligible
187	6/30/65	NE	-----	-----	-----	-----	-----	Not eligible
182	5/6/63	1/9/97	-----	-----	-----	-----	-----	Not eligible
080	12/11/56	NE	-----	-----	-----	-----	-----	Not eligible
053	1/15/69	3/25/96	-----	-----	-----	-----	-----	Dropped out
256	8/15/58	-----	-----	-----	-----	-----	-----	Dropped out
492	8/19/58	-----	-----	-----	-----	-----	-----	Dropped out
399	1/5/57	NE	-----	-----	-----	-----	-----	Not eligible
058	5/6/72	NE	-----	-----	-----	-----	-----	Not eligible
395	2/27/62	9/26/97	Normal	6/19/98	7/8/98			Dropped out
036	11/7/63	-----	-----	-----	-----	-----	-----	Dropped out
152	10/27/58	-----	-----	-----	-----	-----	-----	Dropped out
093	4/24/77	NE	-----	-----	----	----	----	Not eligible
254	2/21/76	NE	-----	-----	-----	-----	-----	Not eligible
245	5/28/71	------	-----	-----	-----	-----	-----	Dropped out
412	10/14/66	10/28/97	-----	-----	-----	-----	-----	Not eligible
533	1/28/66							Dropped out
405	2/20/58	6/30/98						Dropped out
056	11/5/67	NE	-----	-----	-----	-----	-----	Not eligible
447	2/1/66	-----	-----	-----	-----	-----	-----	Pregnant
309	3/17/75	NE	-----	-----	-----	-----	-----	Not eligible
298	3/3/75	-----	-----	-----	-----	-----	-----	Dropped out
328	11/5/60	-----	-----	-----	-----	-----	-----	Dropped out
167	10/11/71	3/10/97	Normal	5/3/97	-----	-----	-----	Dropped out
122	3/2/55	NE	-----	-----	-----	-----	-----	Not eligible
221	2/11/69	2/28/97	Low	4/10/97	5/8/97	6/30/97	8/27/97	Completed
531	6/8/66	-----	-----	-----	-----	-----	-----	Dropped out
318	5/29/75	-----	-----	-----	-----	-----	-----	Dropped out
363	11/17/68	-----	-----	-----	-----	-----	-----	Dropped out
377	12/20/69	-----	-----	-----	-----	-----	-----	Dropped out
443	6/26/70	12/22/97	-----	-----	-----	-----	-----	Not eligible
168	3/16/67	11/20/96	-----	-----	-----	-----	-----	Not eligible
229	12/9/57	6/17/97	Low	8/9/97	9/16/97	11/19/97	2/12/98	Completed
094	10/20/71	NE	-----	-----	----	----	----	Not eligible
367	5/24/66	9/8/97	-----	-----	-----	-----	-----	Not eligible
077	7/13/71	6/13/96	Low	6/27/96	10/9/96	-----	-----	Dropped out
536	3/6/79	6/9/98	Low	7/1/98				Dropped out
464	8/26/97	3/9/98	-----	-----	-----	-----	-----	Dropped out
222	7/22/75	2/26/97	-----	-----	-----	-----	-----	Not eligible
491	11/28/75	3/18/98	-----	-----	-----	-----	-----	Not eligible
257	5/17/60	4/2/97	Low	6/10/97	-----	-----	-----	Dropped out

183	11/12/77	1/10/97	-----	-----	-----	-----	-----	Dropped out
288	9/9/74	-----	-----	-----	-----	-----	-----	Dropped out
416	12/11/72	NE	-----	-----	-----	-----	-----	Not eligible
381	11/28/59	9/11/97	-----	-----	-----	-----	-----	Not eligible
287	3/20/57	NE	-----	-----	-----	-----	-----	Not eligible
390	4/6/60	-----	-----	-----	-----	-----	-----	Dropped out
258	6/9/69	4/14/97	Low	5/15/97	5/29/97	7/28/97	9/22/97	Completed
333	5/26/63	8/14/97	-----	-----	-----	-----	-----	Not eligible
494	6/30/67	3/9/98	Low	3/24/98	4/15/98	6/4/98		Dropped out
173	5/3/72	NE	-----	-----	-----	-----	-----	Not eligible
451	3/2/73	12/16/97	Normal	1/23/98	2/10/98	4/30/98	6/25/98	Completed
216	2/4/68	------	-----	-----	-----	-----	-----	Dropped out
544	11/16/66	6/12/98	-----	-----	-----	-----	-----	Not eligible
035	3/8/61	NE	-----	-----	-----	-----	-----	Not eligible
502	7/22/61							Dropped out
228	9/18/77	2/28/97	Low	4/3/97	-----	-----	-----	Dropped out
553	7/23/66							Dropped out
332	5/21/61	-----	-----	-----	-----	-----	-----	Dropped out
086	8/27/66	NE	-----	-----	-----	-----	-----	Not eligible
454	1/30/71	-----	-----	-----	-----	-----	-----	Dropped out
281	10/12/74	5/11/97	-----	-----	-----	-----	-----	Dropped out
111	11/24/58	------	-----	-----	-----	-----	-----	Dropped out
262	1/5/57	-----	-----	-----	-----	-----	-----	Dropped out
133	3/16/58	NE	-----	-----	-----	-----	-----	Not eligible
284	10/16/63	NE	-----	-----	-----	-----	-----	Not eligible
172	7/1/73	NE	-----	-----	-----	-----	-----	Not eligible
038	5/12/72	-----	-----	-----	-----	-----	-----	Dropped out
511	8/23/58	-----	-----	-----	-----	-----	-----	Dropped out
495	11/10/72	3/9/98						Not eligible
468	8/20/59	-----	-----	-----	-----	-----	-----	Dropped out
307	10/15/77	5/27/97	Low	7/3/97	8/6/97	10/1/97	11/18/97	Completed
510	4/9/63	4/24/98	Low					Dropped out
217	4/17/75	NE	-----	-----	-----	-----	-----	Not eligible
452	9/27/62	NE	-----	-----	-----	-----	-----	Not eligible
518	1/21/73	5/6/98	-----	-----	-----	-----	-----	Not eligible
062	11/8/60	NE	-----	-----	-----	-----	-----	Not eligible
166	8/28/70	NE	-----	-----	-----	-----	-----	Not eligible
478	7/3/94	-----	-----	-----	-----	-----	-----	Dropped out
208	7/21/69	2/13/97	-----	-----	-----	-----	-----	Moved away
232	2/13/97	NE	-----	-----	-----	-----	-----	Not eligible
243	6/8/73	4/1/97	-----	-----	-----	-----	-----	Not eligible

Appendix 2. Results of Kinetic Studies

Table 1.
Characteristics of Subjects (n = 50)

	Mean	SD	CV	Median	Min	Max
Age, years	29.1	5.4	0.186	28.5	19	39
Body weight, kg	63.5	13.5	0.212	60.0	45.8	108.4
Body height, m	1.631	0.071	0.044	1.625	1.490	1.780
Body mass index, kg/m^2	24.0	5.3	0.220	22.2	16.9	38.4
Fat free mass[#], kg	44.5	6.0	0.135	43.6	34.0	65.2
Plasma zinc, ng/mL	718	97	0.135	717	492	939
Serum ferritin, ng/mL	23.5	16.8	0.712	18.0	6	78
Serum iron[&], µg/dL	77.0	35.3	0.459	68.0	29.0	183.0
Erythrocyte protoporphrin[$], µg/dL	54.4	24.7	0.453	48.0	20.0	131.0
Hemoglobin, g/dL	13.1	0.8	0.062	13.1	11.4	15.5

[#] n = 48
[$] n = 46
[&] n = 49

Table 2.
Zn Kinetic Parameters (n = 48)

	Mean	SD	CV	Median	Min	Max
Coefficients in exponentials (n = 48)						
H_1	0.955	0.274	0.286	0.921	0.527	1.815
g_1, day^{-1}	163	32	0.194	164	111	287
H_2	0.0603	0.0252	0.418	0.0610	0.0234	0.1272
g_2, day^{-1}	12.01	4.47	0.372	12.87	3.53	20.76
H_3	0.01574	0.00387	0.246	0.01583	0.00880	0.02635
Transfer rate constants (n = 48)						
k_{11}, day^{-1}	152.2	30.9	0.203	151.2	102.0	274.4
k_{12}, day^{-1}	21.94	6.18	0.282	23.06	7.77	35.10
k_{21}, day^{-1}	68.6	12.4	0.181	68.7	44.5	100.8
k_{13}, day^{-1}	1.329	0.264	0.198	1.315	0.706	1.901
k_{31}, day^{-1}	83.6	30.3	0.362	86.1	175.2	31.4
Zn pool sizes (n = 48)						
Q_1, mg	2.18	0.59	0.272	2.09	1.10	3.47
Q_2, mg	7.79	4.61	0.591	6.17	2.62	20.12
Q_3, mg	130.4	33.7	0.258	123.0	74.7	233.1
EZP, mg	140.4	34.5	0.246	131.5	79.7	238.6

Zn fluxes between pools (n = 48)						
F_{21}, mg/day	148.8	48.4	0.325	147.3	65.4	278.1
F_{31}, mg/day	173.2	60.1	0.347	158.1	79.4	320.2
Turnover rate, mg/day	323.7	78.0	0.241	309.3	187.6	538.5
Zn pool sizes per body weight (n = 48)						
Q_1/BW, µg/kg	35.5	10.4	0.292	32.0	17.2	60.8
Q_2/BW, µg/kg	129.8	85.8	0.661	101.3	43.6	386.5
Q_3/BW, mg/kg	2.08	0.37	0.180	2.15	1.25	2.80
EZP/BW, mg/kg	2.25	0.41	0.181	2.30	1.33	3.09
Zn fluxes per body weight (n = 48)						
F_{21}/BW, mg/kg/day	2.44	0.90	0.369	2.30	1.07	5.00
F_{31}/BW, mg/kg/day	2.72	0.60	0.222	2.84	1.32	4.20
Turnover rate/BW, mg/kg/day	5.19	1.00	0.192	5.06	3.28	7.69
Zn pool sizes per fat-free mass (n = 45)						
Q_1/FFM, µg/kg	48.5	12.1	0.250	49.7	24.9	73.4
Q_2/FFM, µg/kg	166.1	94.5	0.569	137.4	63.9	447.5
Q_3/FFM, mg/kg	2.95	0.54	0.184	2.95	1.84	4.24
EZP/FFM, mg/kg	3.16	0.57	0.180	3.08	1.95	4.45
Zn fluxes per fat free mass (n = 45)						
F_{21}/FFM, mg/kg/day	3.25	1.00	0.308	3.20	1.57	5.74
F_{31}/FFM, mg/kg/day	3.89	1.04	0.268	3.84	1.82	6.73
Turnover rate/FFM, mg/kg/day	7.19	1.40	0.194	7.13	4.57	11.31

EZP: Rapidly exchangeable zinc pool, a sum of Q_1, Q_2 and Q_3
FFM: Fat-free mass

Table 3.
Correlation Coefficients Between Pool Sizes & Body Weight, Fat-Free Mass or Plasma Zn Concentration

	Body weight n = 48		Fat free mass n = 46		Plasma Zn n = 48	
	r	p	r	p	r	p
Q_1	0.267	0.067	0.335	0.023	0.503	0.0003
Q_2	-0.130	0.378	-0.045	0.765	0.644	0.0001
Q_3	0.685	0.001	0.684	0.001	0.112	0.448
EZP	0.657	0.001	0.668	0.001	0.204	0.164
F_{21}	0.079	0.595	0.105	0.488	0.536	0.0001
F_{31}	0.795	0.001	0.698	0.001	-0.086	0.563
Turnover rate	0.656	0.001	0.601	0.001	0.252	0.084

Table 4.
Comparison of Plasma Zn & Characteristics of Subjects Among Quartiles of Plasma Zn

	n	Plasma Zn	Age	Weight	Height	Body mass index	Fat free mass	Ethnicity
		ng/mL	years	kg	m	kg/m^2	kg	W vs Minority
Quartile I (Low)	12	602±46[2]	29.5±6.8	62.8±17.6	1.63±0.08	23.7±6.3	44.1±7.9	7/5
Quartile II	13	684±22[2]	30.2±4.5	63.2±12.5	1.63±0.07	23.7±4.1	44.0±5.0	7/6
Quartile III	13	739±17[2]	29.2±6.7	64.9±13.1	1.61±0.06	25.3±5.9	44.6±5.5	9/3
Quartile IV (High)	12	851±58	27.3±2.8	63.2±12.0	1.66±0.07	23.0±4.8	45.4±6.1[$]	10/2

[1]Values are mean ± SD.
[2]Significantly different from Quartile IV (High), $P < 0.001$ (Dunnett's simultaneous multiple comparison test).
[$]$n = 10$.

Table 5.
Comparison of Coefficients in the Truncated Triexponential Function & Zn Kinetic Parameters in the Closed Mammillary Model Among Quartiles of Plasma Zn

Parameter	Quartile I Low	Quartile II	Quartile III	Quartile IV High
n	12	13	11	12
Coefficients in exponentials				
H_1	0.962±0.180[2]	1.126±0.294[2]	1.039±0.234[2]	0.685±0.146
g_1, day^{-1}	164±15[4]	185±39[2]	166±32[4]	139±19
H_2	0.0722±0.0223[2]	0.0671±0.0246[3]	0.0641±0.0248[3]	0.0375±0.0142
g_2, day^{-1}	14.55±2.99[2]	13.41±3.46[2]	12.40±4.92[3]	7.59±3.23
H_3	0.0170±0.0043[4]	0.0164±0.0034	0.0162±0.0047	0.0134±0.0023
Transfer rate constants				
k_{11}, day^{-1}	152±15	171±38[2]	155±32[4]	129±17
k_{12}, day^{-1}	25.9±4.7[2]	23.8±4.6[2]	22.0±5.2[3]	15.9±5.8
k_{21}, day^{-1}	64.0±10.9	73.1±14.8	66.6±11.1	69.9±11.9
k_{13}, day^{-1}	1.47±0.22[4]	1.37±0.28	1.26±0.25	1.20±0.24
k_{31}, day^{-1}	87.5±15.7[4]	98.3±32.6[3]	88.4±36.8[4]	59.5±18.8
Zinc pool sizes				
Q_1, mg	2.07±0.45[2]	1.87±0.52[2]	1.97±0.47[2]	2.82±0.44
Q_2, mg	5.30±1.83[2]	5.81±2.41[2]	6.68±3.61[2]	13.45±4.64
Q_3, mg	125±37	128±29	132±43	137±28
EZP, mg	132±38	136±31	140±43	153±26
Zinc fluxes between pools				
F_{21}, mg/day	133±38[2]	133±34[2]	132±41[2]	197±49
F_{31}, mg/day	184±66	174±55	167±69	167±58
Turnover rate, mg/day	317±93	313±66	300±69	364±75
Zinc pool sizes per body weight				

Q_1/BW, μg/kg	33.9±6.7[2]	30.3±8.9[2]	32.5±11.0[3]	45.4±8.1
Q_2/BW, μg/kg	86.7±27.5[2]	94.5±422[2]	113.5±72.9[2]	226.1±100.1
Q_3/BW, mg/kg	2.02±0.41	2.06±0.44	2.07±0.42	2.17±0.21
EZP/BW, mg/kg	2.14±0.42	2.19±0.46	2.22±0.45	2.44±0.23
Zinc fluxes per body weight				
F_{21}/BW, mg/kg/day	2.19±0.66[2]	2.17±0.72[2]	2.21±0.94[2]	3.20±0.91
F_{31}/BW, mg/kg/day	2.92±0.59	2.75±0.66	2.57±0.57	2.61±0.59
Turnover rate/BW, mg/kg/day	5.12±1.07	5.02±0.96	4.78±0.81[4]	5.81±0.93
Zinc pool sizes per fat-free mass[$]				
Q_1/FFM, μg/kg	47.5±9.1[3]	42.7±12.3[2]	44.9±12.1[3]	60.5±6.8
Q_2/FFM, μg/kg	122±41[2]	130±58[2]	154±89[2]	276±104
Q_3/FFM, mg/kg	2.82±0.55	2.99±0.61	2.91±0.58	3.10±0.43
EZP/FFM, mg/kg	2.99±0.56	3.16±0.64	3.11±0.59	3.44±0.42
Zinc fluxes per fat-free mass[$]				
F_{21}/FFM, mg/kg/day	3.07±0.91[3]	2.95±0.85[3]	3.04±1.08[3]	4.07±0.85
F_{31}/FFM, mg/kg/day	4.13±1.04	3.98±1.11	3.66±1.05	3.76±1.04
Turnover rate/FFM, mg/kg/day	7.20±1.69	7.08±1.43	6.70±1.09[4]	7.84±1.20

[1]Values are mean ± SD (n).
[2]Significantly different from Quartile IV (High), $P < 0.001$ (Dunnett's simultaneous multiple comparison test)
[3]Significantly different from Quartile IV (High), $P < 0.01$ (Dunnett's simultaneous multiple comparison test)
[4]Significantly different from Quartile IV (High), $P < 0.05$ (Dunnett's simultaneous multiple comparison test)
[$]Number of subjects in Quartile II and Quartile IV are 12 and 10, respectively.
EZP: Rapidly exchangeable zinc pool, a sum of Q_1, Q_2 and Q_3
FFM: Fat-free mass

Table 6.
Comparison Serum Electrolytes Among Quartiles of Plasma Zn

	n	Ca	Na	K	Chloride	Bicarbonate	Anion gap
		mg/dL	mmol/L	mmol/L	mmol/L	mmol/L	mmol/L
Quartile I (Low)	12	8.94±0.26	140.0±1.3	4.08±0.26	102.8±1.5	24.2±1.6[4]	13.0±1.5[4]
Quartile II	13	8.98±0.29	141.1±1.9[4]	3.95±0.24	102.9±1.6	25.7±1.8	12.5±2.3
Quartile III	13	8.78±0.20[4]	139.5±1.6	3.80±0.23[4]	102.8±1.8	25.1±2.4	11.5±2.3
Quartile IV (High)	12	9.03±0.28	139.5±1.9	4.03±0.29	102.4±1.4	26.1±1.1	11.0±1.4

[1]Values are mean ± SD.
[2]Significantly different from Quartile IV (High), $P < 0.001$ (Dunnett's simultaneous multiple comparison test)
[3]Significantly different from Quartile IV (High), $P < 0.01$ (Dunnett's simultaneous multiple comparison test)
[4]Significantly different from Quartile IV (High), $P < 0.05$ (Dunnett's simultaneous multiple comparison test)

Table 7.
Comparison of Urinary Zn Excretion Rate (ZnExR) Among Quartiles of Plasma Zn

	n	ZnExR	ZnExR/BW	ZnExR/FFM	ZnExR constant
		μg/day	μg/kg/day	μg/kg/day	day^{-1}
Quartile I (Low)	7	184±78(7)[3]	3.44±1.61(7)[3]	4.56±1.92(7)[3]	0.099±0.047(7)
Quartile II	11	456±309(11)[4]	7.01±4.42(11)[4]	10.17±6.65(11)[4]	0.307±0.253(10)
Quartile III	10	604±416(10)	8.93±5.57(10)	13.02±8.29(10)	0.329±0.243(9)
Quartile IV (High)	5	907±580(5)	13.88±8.20(5)	19.46±11.54(5)	0.300±0.162(5)

[1]Values are mean ± SD (n).
[2]Significantly different from Quartile IV (High), $P < 0.001$ (Dunnett's simultaneous multiple comparison test)
[3]Significantly different from Quartile IV (High), $P < 0.01$ (Dunnett's simultaneous multiple comparison test)
[4]Significantly different from Quartile IV (High), $P < 0.05$ (Dunnett's simultaneous multiple comparison test)
[5]Zn excretion rate constant for Quartile I was significantly lower than that for the combination of Quartiles II, III and IV (0.314±0.224, mean ± SD, $P = 0.0002$).

Table 8.
Comparison of Urinary Zn excretion and Rate Constant Between Subjects With or Without a History of Urinary Tract Infections (UTI)

	No report of UTI	Past of UTI	P
N	20	13	
Urinary zinc excretion, μg/day	615±479	353±232	0.045
Urinary zinc excretion per body weight, μg/kg/day	9.30±6.66	5.68±3.52	0.051
Urinary zinc excretion per fat free mass, μg/kg/day	13.4±9.7	8.02±4.98	0.047
Urinary zinc excretion rate constant, day^{-1}	0.313±0.251	0.168±0.100	0.029

[1]Values are mean ± SD.
Difference was statistically analyzed by Welch's t-test.

Table 9.
Comparison of Fe Status Indices of Subjects with Plasma Zn ≥ 700 ng/mL and Plasma Zn < 700 ng/mL

	plasma Zn ≥ 700 ng/mL	plasma Zn < 700 ng/mL	p
Plasma Zn, ng/mL	778±72 (30)	629±49 (20)	0.0001
Serum ferritin, ng/mL	27.3±19.5(30)	17.9±9.3 (20)	0.026
Serum Fe, μg/dL	87.0±36.8(27)	62.8±28.3(19)	0.016
Saturation of Fe binding capacity, %	29.6±15.7(27)	20.6±10.0(19)	0.022
Hemoglobin, g/dL	13.1±0.8 (30)	13.0±0.8 (20)	0.897

[1]Values are mean ± SD (n).
Difference was statistically analyzed by Welch's t-test.

Table 10.
Fe Status Indices by Plasma Zn Quartile

	Serum ferritin	Serum Fe	Saturation of Fe binding capacity	Erythrocyte protoporphyrin	Hemoglobin
	ng/mL	µg/dL	%	µg/dL	g/dL
Quartile I (Low)	19.0±11.1(12)	63.9±29.6(12)	21.0±11.4(12)[4]	56.7±19.3(12)	12.9±0.8(12)
Quartile II	21.8±13.7(13)	66.5±26.2(11)[4]	21.2± 6.9(11)	66.0±34.3(12)[4]	13.1±0.7(13)
Quartile III	23.1±19.9(13)	83.5±36.2(12)	27.3±11.7(13)	52.8±23.7(13)	13.1±1.0(13)
Quartile IV (High)	30.4±20.4(12)	94.9±42.4(11)	34.5±20.8(11)	43.3±13.5(12)	13.1±0.7(12)

[1]Values are mean ± SD (n).
[2]Significantly different from Quartile IV (High), $P < 0.001$ (Dunnett's simultaneous multiple comparison test)
[3]Significantly different from Quartile IV (High), $P < 0.01$ (Dunnett's simultaneous multiple comparison test)
[4]Significantly different from Quartile IV (High), $P < 0.05$ (Dunnett's simultaneous multiple comparison test)

Table 11.
Frequency of Consumption of Foods: Percentage of Users & Mean Frequency[1]

	Percentage users	Frequency
	%	times/wk[2]
Vitamin-C fortified drinks	24	1.3±2.3
Coffee	79	8.8±7.0
Tea	71	3.0±4.9
Lettuce	97	3.4±3.3
Beans	94	1.7±1.7
Bran breakfast cereals	32	1.8±2.1
Beef	82	2.4±2.0
Chicken	82	2.3±1.3
Egg	85	1.9±1.9
Yogurt	68	1.7±1.7
Milk	79	3.7±5.9
Orange juice	88	4.0±5.3

[1]Foods used for multiple regression analyses
[2]Mean±SD.

Table 12.
Associations of Serum Ferritin (ng/mL) with Food Frequency and Gynecological History[1, 2]

Variable	Regression coefficient	SE	p	Partial correlation coefficient
Constant	16.5	2.9	0.001	-
Beef	3.84	1.12	0.002	0.542
Bleeding through menstrual pads	-15.1	6.1	0.021	-0.421
Bran breakfast cereals	-2.52	1.47	0.097	-0.309

[1]Multiple regression analysis
[2]$n = 32$, $R^2 = 0.326$, SEE = 11.5, $p = 0.010$

Table 13.
Associations of Erythrocyte Protoporphyrin (μg/dL) with Food Frequency & Gynecological History[1,2]

Variable	Regression coefficient	SE	p	Partial correlation coefficient
Constant	45.5	6.60	0.001	-
Milk	2.55	0.82	0.004	0.508
Bleeding through menstrual pads	33.0	11.2	0.006	0.487
Egg	-6.09	2.60	0.027	-0.404
Beans	5.93	2.92	0.052	0.359

[1]Multiple Regression Analysis
[2]$n = 32$, $R^2 = 0.396$, SEE = 22.5, $p = 0.006$

Table 14.
Associations of Hemoglobin (g/dL) with Food Frequency & Gynecological History[1,2]

Variable	Regression coefficient	SE	p	Partial correlation coefficient
Constant	12.3	0.2000	0.001	-
Beef	0.190	0.0590	0.003	0.506
Coffee	0.0361	0.0156	0.028	0.389
Orange juice	0.0425	0.0234	0.080	0.314

[1]Multiple Regression Analysis
[2]$n = 34$, $R^2 = 0.515$, SEE = 0.6, $p = 0.00006$

Table 15.
Associations of Hematocrit (%) with Food Frequency & Gynecological History[1,2]

Variable	Regression coefficient	SE	p	Partial correlation coefficient
Constant	36.2	0.500	0.001	-
Orange juice	0.199	0.050	0.001	0.612
Beef	0.463	0.135	0.002	0.557
Lettuce	0.292	0.086	0.002	0.554
Yogurt	-0.410	0.178	0.030	-0.411
Bran breakfast cereals	0.326	0.178	0.079	0.338

[1]Multiple Regression Analysis
[2]$n = 32$, $R^2 = 0.697$, SEE = 1.3, $p = 0.00001$

Table 16.
Associations of Red Blood Cell Count (millions/μL) with Food Frequency & Gynecological History[1,2]

Variable	Regression coefficient	SE	p	Partial correlation coefficient
Constant	4.37	0.0600	0.001	-
Orange juice	0.0177	0.0078	0.030	0.384
Yogurt	-0.0493	0.0025	0.054	-0.344

[1]Multiple Regression Analysis
[2]$n = 33$, $R^2 = 0.239$, SEE = 0.23, $p = 0.017$

Table 17.
Associations of Coffee Consumption (times/wk) with Red Blood Cell Indices[1], Serum Fe Concentration & Percentage Saturation of Fe Binding Capacity (SIBC)

Item	n	Constant	Regression coefficient	SE	P value	Correlation coefficient
MCV, fL	34	85.9	0.332	0.117	0.008	0.448
MCH, pg	34	29.0	0.154	0.049	0.003	0.487
MCHC, %	34	33.7	0.0469	0.0160	0.006	0.460
Fe, µg/dL	33	53.8	2.14	0.71	0.005	0.476
SIBC, %	33	18.0	0.770	0.265	0.007	0.462

[1]Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC).

Table 18.
Associations of the central Zn pool size Q_1 per fat-free mass (µg/kg) with Food Frequencies & Gynecological History[1, 2]

Variable	Regression coefficient	SE	p	Partial correlation coefficient
Constant	44.4	2.3	0.001	-
Bleeding through menstrual pads	-13.0	4.6	0.009	-0.482
Bran breakfast cereals	-2.65	1.11	0.024	-0.425
Beef	1.84	0.86	0.042	0.387

[1]Multiple regression analysis
[2]$n = 30$, $R^2 = 0.317$, SEE = 8.6, $p = 0.018$

Table 19.
Associations of the lesser peripheral Zn pool size Q_2 (mg) with Food Frequencies & Gynecological History[1, 2]

Variable	Regression coefficient	SE	p	Partial correlation coefficient
Constant	5.07	1.07	0.001	-
Yogurt	1.30	0.40	0.003	0.537
Bran breakfast cereals	-1.14	0.46	0.020	-0.438
Beef	0.760	0.356	0.042	0.386
Orange juice	-0.225	0.130	0.095	-0.322

[1]Multiple regression analysis
[2]$n = 31$, $R^2 = 0.372$, SEE = 3.49, $p = 0.013$

Table 20.
Associations of the Greater Peripheral Zn Pool Size Q_3 per Fat-Free Mass (mg/kg) with Food Frequencies & Gynecological History[1, 2]

Variable	Regression coefficient	SE	p	Partial correlation coefficient
Constant	2.44	0.14	0.001	-
Bleeding through menstrual pads	-0.932	0.230	0.001	-0.629
Beef	0.142	0.038	0.001	0.603
Lettuce	0.124	0.037	0.003	0.547
Yogurt	-0.137	0.061	0.035	-0.408
Tea	0.036	0.017	0.049	0.382

[1]Multiple regression analysis
[2]$n = 31$, $R^2 = 0.538$, SEE = 0.39, $p = 0.001$

Table 21.
Associations of the Rapidly Exchangeable Zn Pool Size (EZP, i.e., sum of Q_1, Q_2 & Q_3) per Fat-Free Mass (mg/kg) with Food Frequencies & Gynecological History[1,2]

Variable	Regression coefficient	SE	p	Partial correlation coefficient
Constant	2.89	0.12	0.001	-
Beef	0.146	0.045	0.003	0.520
Bleeding through menstrual pads	-0.680	0.247	0.010	-0.462

[1]Multiple regression analysis
[2]$n = 31$, $R^2 = 0.322$, SEE = 0.47, $p = 0.004$

Table 22.
Associations of the Zn turnover rate[1] per Fat-Free Mass (mg/day/kg) with Food Frequencies & Gynecological History[2,3]

Variable	Regression coefficient	SE	p	Partial correlation coefficient
Constant	6.61	0.35	0.001	-
Beef	0.369	0.129	0.008	0.490
Bleeding through menstrual pads	-1.53	0.69	0.036	-0.397
Bran breakfast cereals	-0.360	0.165	0.039	-0.392

[1] sum of fluxes from the central Zn compartment
[2]Multiple regression analysis
[3]$n = 30$, $R^2 = 0.308$, SEE = 1.29, $p = 0.008$

Table 23.
Comparisons of Subjects Taking or Not Taking Oral Contraceptives[1]

	No oral contraceptives	Taking oral contraceptives	p-value[2]
Age, years	30.2±5.5 (35)	26.6±4.3 (15)	0.019
BMI[3], kg/m^2	24.9±5.7 (35)	21.8±3.2 (15)	0.018
Q_1/FFM, µg/kg	45.9±10.9 (32)	54.6±13.0 (13)	0.041
F_{21}/FFM, mg/d/kg	2.94±0.86 (32)	4.01±0.95 (13)	0.002
k_{21}, day^{-1}	64.6±10.8 (33)	77.3±11.6 (15)	0.001
TIBC, µg/dL	302±48 (34)	337±48 (12)	0.042
Alkaline phosphatase, U/L	61.2±17.3 (35)	47.4±14.0 (15)	0.006
APTT[4], sec	29.8±3.3 (32)	27.4±2.7 (13)	0.017

[1]Data are mean ± SD (number of subjects).
[2]p-values are based on Welch's t test.
[3]BMI: Body mass index
[4]APTT: Activated partial thromboplastin time

Table 24.
Comparison of electric taste thresholds between normozincemic (plasma Zn ≥ 700 ng/mL) and hypozincemic subjects (plasma Zn < 700 ng/mL)

	Normozincemia	Hypozincemia	P
	dB	dB	
Chorda tympani, left side	4 (2, 8); 17	-4 (-6, 3.5); 18	0.047
Chorda tympani, right side	4 (2, 6); 16	-4 (-6, 3); 16	0.028
Glossopharyngeal nerve, left side	12 (8,18); 16	4 (-2, 10); 18	0.034
Glossopharyngeal nerve, right side	15 (8,21); 17	1 (-3, 10); 18	0.036

[1]Values are median (25 percentile, 75 percentile); n.
Mann-Whitney U-test statistically analyzed difference.

Table 25.
Number of Low Q_2 Classified by Plasma Zn Concentration (n = 48)[1, 2]

	Q_2 < 7.3 mg	Q_2 ≥ 7.3 mg
Plasma Zn < 750 ng/mL	27	6
Plasma Zn ≥ 750 ng/mL	3	12

[1]Fisher's exact probability (two-tail): p = 0.0001
[2]Odds ratio: 18.0; 95% confidence range: 3.8 to 84.3

Table 26.
Number of Low Q_2 Classified by Serum Ferritin Concentration Among Subjects Not Taking Oral Contraceptives (n = 35)[1, 2]

	Q_2 < 7.3 mg	Q_2 ≥ 7.3 mg
Serum ferritin < 23 ng/mL	20	3
Serum ferritin ≥ 23 ng/mL	4	8

[1]Fisher's exact probability (two-tail): p = 0.0022
[2]Odds ratio: 13.3; 95% confidence range: 2.4 to 73.5

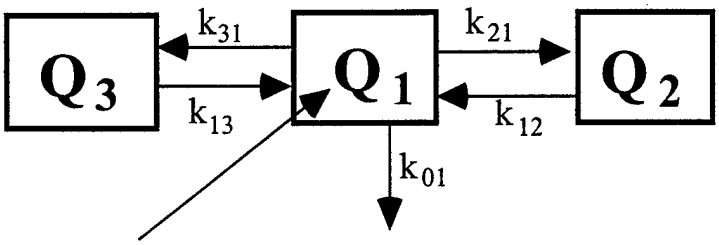

Figure 1. The diagrammatic representation of the mammillary model for human zinc kinetics

Legend to Figure 1.

Q_1 denotes a size of the central Zn pool (Pool 1).
Q_2 denotes a size of the first peripheral Zn pool (Pool 2), which may have a regulatory role in Zn metabolism and may settle in liver and another active metabolic organs.
Q_3 denotes a size of the second peripheral Zn pool (Pool 3), which may exert a biologically important function because the pool size Q_3 is kept unchanged in hypozincemia found in the subjects. The rate constant k_{01} can be neglected within 24 hours after intravenous injection of Zn isotope. The plasma zinc disappearance is described as bi-exponential function with a constant term in this situation "closed mammillary model". Urinary Zn excretion rate constant that contributes to k_{01} is calculated as urinary Zn excretion divided by Q_1.

The Zn fluxes from Pool 1 to Pool 2 (F_{21}) and from Pool 2 to Pool 1 (F_{12}) are equivalent because of the steady state assumption, which is general in the stationary kinetic model. The Zn fluxes from Pool 1 to Pool 3 (F_{31}) and from Pool 3 to Pool 1 (F_{13}) are equivalent.

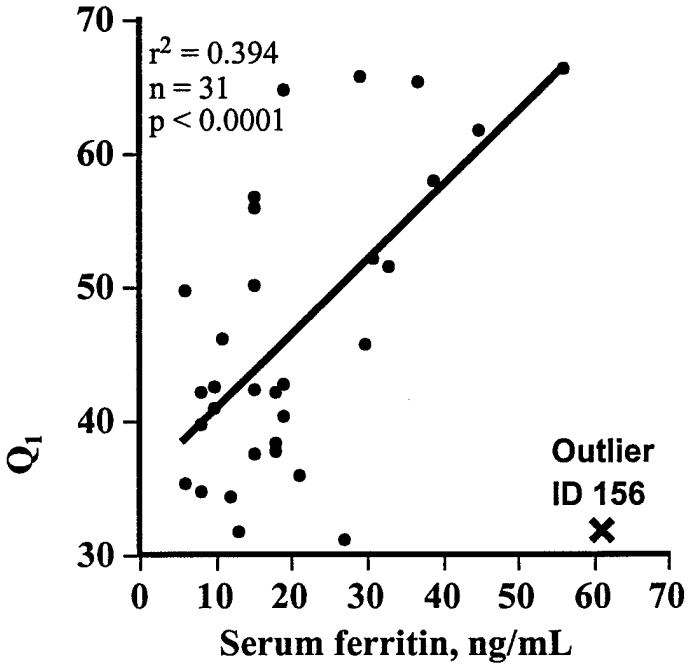

Figure 2. Linear relationship between serum ferritin concentrations and the central Zn pool size Q_1 per fat-free mass in the subjects not taking oral contraceptives.

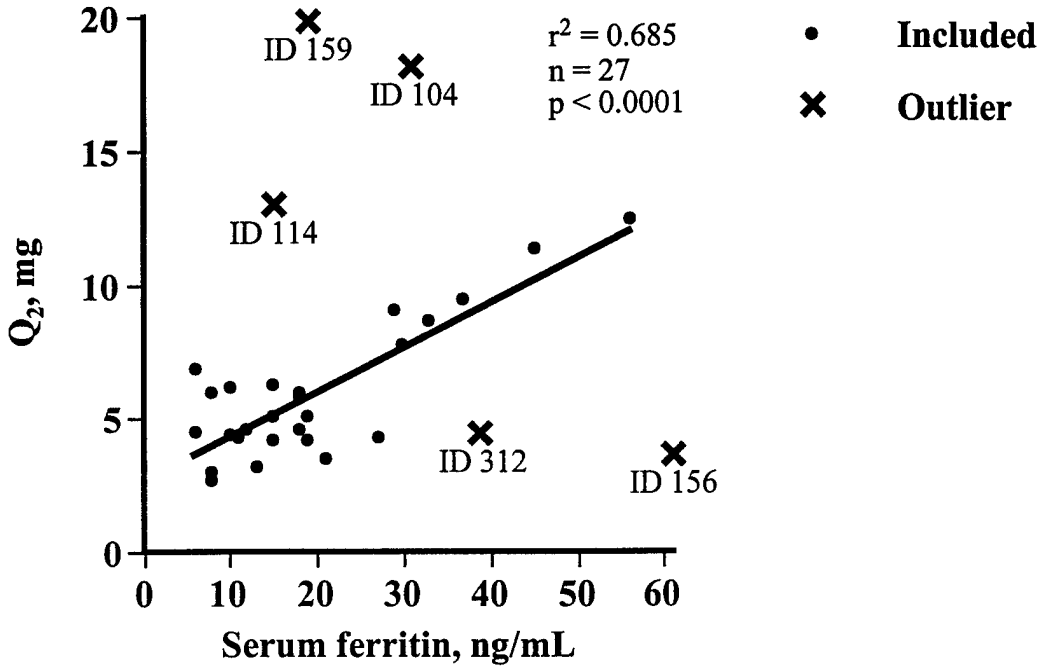

Figure 3. Linear relationship between serum ferritin concentration and the lesser peripheral Zn pool size Q_2 in the subjects not taking oral contraceptives. Crosses indicate outliers that were not included in the analysis.

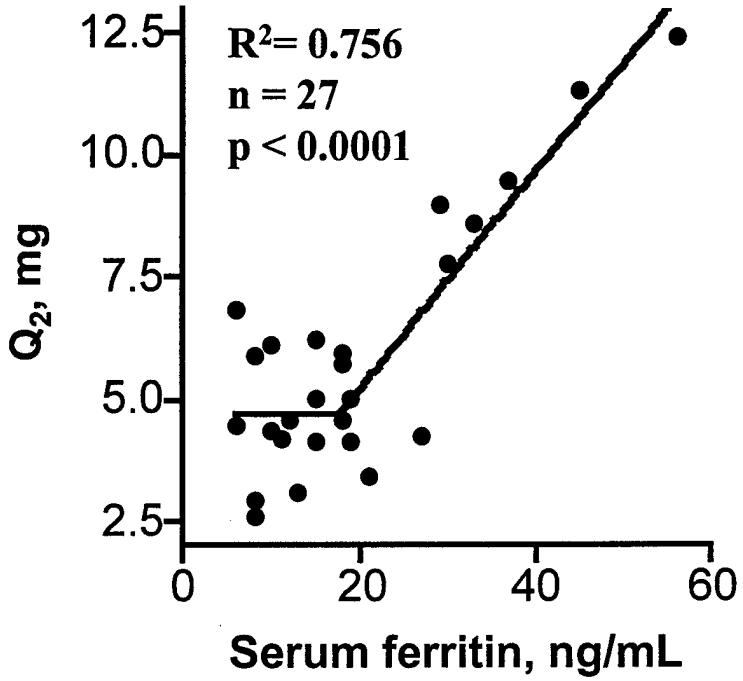

Figure 4. Nonlinear relationship between serum ferritin and the lesser peripheral Zn pool size Q_2 in the subjects not taking oral contraceptives. Statistical outliers were removed from the regression. The function employed for curve fitting was the broken-line equation.

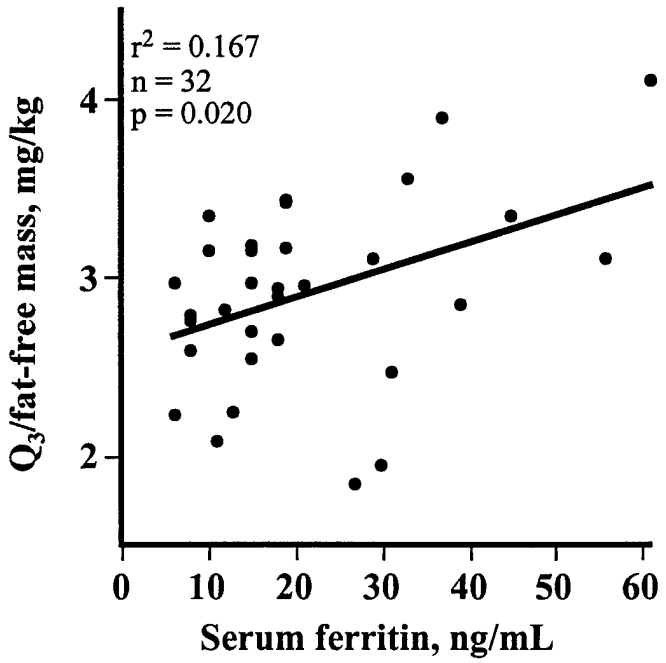

Figure 5. Linear relationship between serum ferritin concentration and the greater peripheral Zn pool size Q_3 per fat-free mass in the subjects not taking oral contraceptives.

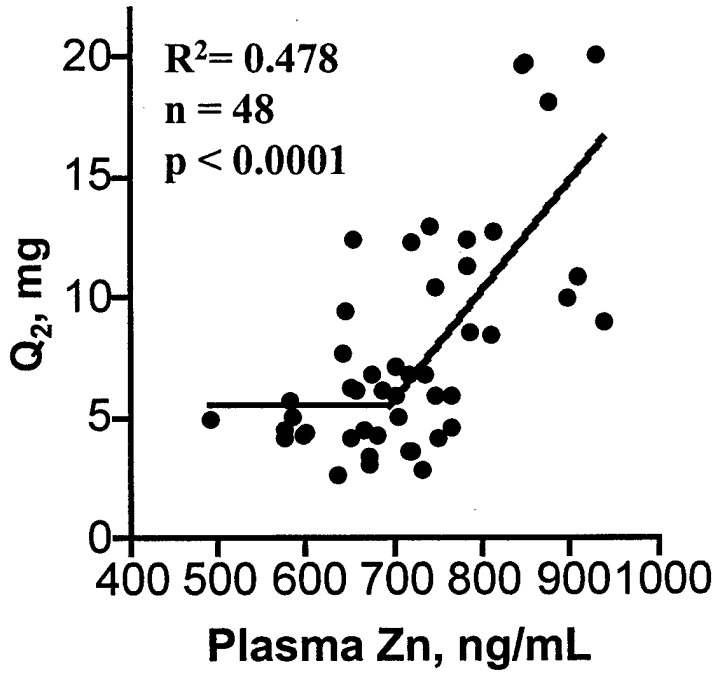

Figure 6. Broken-line equation applied to plasma Zn vs Q_2 plot of 48 subjects

Appendix 3
JOURNAL ARTICLE.

Sadagopa Ramanujam, VM, Yokoi, K, Egger, NG, Dayal, HH, Alcock, NW, Sandstead, HH. Polyatomics in zinc isotope ratio analysis of plasma samples by inductively coupled plasma-mass spectrometry and applicability of nonextracted samples for zinc kinetics. Biological Trace Element Research 1999; 68: 143-58.

PRESENTATIONS

1. Yokoi, K, Sadagopa Ramanujam, VM, Egger, NG, Dayal, HH, Alcock, NW, Sandstead, HH. Comparison of triexponential and truncated models in plasma zinc kinetics. Poster,1996 annual meeting of the Central Society for Clinical Research, Chicago, IL, September 20-22, 1996.

2. Yokoi, K, Egger, NG, Sadagopa Ramanujam, VM, Alcock, NW, Sandstead, HH. A convenient approach to determine the rapidly exchangeable zinc pool in humans. Poster, 37th annual meeting of the American College of Nutrition, San Francisco, CA, October 11-13, 1996.

3. Sadagopa Ramanujam, VM, Yokoi, K, Egger, NG, Dayal, HH, Alcock, NW, Sandstead, HH. Simplified pretreatment method for plasma samples applicable to zinc kinetic studies. Poster (#2354), annual meeting of the FASEB: Experimental Biology 97, New Orleans, LA, April 6-9, 1997.

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