Suggested lower cutoffs of serum zinc concentrations for assessing zinc status: reanalysis of the second National Health and Nutrition Examination Survey data (1976–1980)[1–3]

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ABSTRACT

Background: The risk of zinc deficiency in populations can be estimated by comparing serum zinc data with statistically defined lower cutoffs derived from a presumably healthy population. Serum zinc data are available from a large sample of the US population assessed during the second National Health and Nutrition Examination Survey (NHANES II). Although the original analysis of these data considered fasting status and the time of day of blood sampling, it did not account for potentially confounding variables that may affect the serum zinc concentration, such as age, sex, and health status.

Objective: The objective was to describe variations in serum zinc concentration by age, sex, and other characteristics and to recommend lower cutoffs for presumably healthy persons.

Design: Serum zinc data from NHANES II were analyzed by using analysis of variance and covariance models to identify and describe variables significantly associated with serum zinc concentration; 2.5th percentile curves were produced and used to establish age- and sex-based lower cutoffs.

Results: Age and sex were significant confounders of serum zinc concentration, so separate lower cutoffs were derived for children and adolescent and adult males and females. Other minor confounding variables were identified. Tentative lower cutoffs for pregnancy and oral contraceptive use were also derived.

Conclusions: The interpretation of population serum zinc data with the use of lower cutoffs should account for the age and sex of the subjects, pregnancy and oral contraceptive use, and fasting status and time of day of blood collection. *Am J Clin Nutr* 2003;78:756–64.

KEY WORDS Serum zinc, zinc deficiency, second National Health and Nutrition Examination Survey, NHANES II

INTRODUCTION

In recent decades, a large amount of information has accumulated about the possible widespread occurrence of zinc deficiency (1) and the various associated health consequences, which include growth faltering (2); an increased prevalence of infections (3) and impaired neurobehavioral function in children (4, 5); poor pregnancy outcomes, such as impaired fetal development (6, 7) and infant health (8); and reduced immunocompetence in the elderly (9). This information indicates an urgent need to assess the prevalence of zinc deficiency in representative samples of at-risk populations with the use of direct indicators of zinc status.

Currently, the serum or plasma zinc concentration is the most widely used biochemical indicator of zinc status and is the only biochemical indicator of zinc status for which adequate reference data are available. Although serum or plasma zinc is not considered to be a reliable indicator of zinc status in individual persons, a growing body of evidence suggests that it may be a useful indicator of a population's zinc status (6, 10, 11). The second National Health and Nutrition Examination Survey (NHANES II) of the United States (1976–1980) included serum zinc concentration in its biochemical assessments. The analysis of these data led to the derivation of cutoffs for low serum zinc concentrations (12), which have been used since to assess zinc status. Separate cutoffs were derived on the basis of the fasting state and the time of day of sample collection. It is apparent, however, that the serum zinc concentration also varies with age and sex (12) and health status (13), so the use of a single cutoff for all age and sex groups may not be appropriate.

Thus, the objectives of the current study were to reanalyze the NHANES II data to *1*) describe the variation in serum zinc concentration according to age, sex, and other characteristics (eg, health and physiologic status) in addition to the time of day of sample collection and the fasting status of the subjects; *2*) assess the appropriateness of the currently used cutoffs for serum zinc concentration, and *3*) present new recommendations for appropriate cutoffs, as necessary.

SUBJECTS AND METHODS

NHANES II was a nationwide survey of 27 801 people between 6 mo and 74 y of age that was conducted in the United

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States between 1976 and 1980. Information was collected on current and past health status, health-related behaviors, presence of medical conditions, use of medications, dietary intakes, stature, and a variety of biochemical indicators. Details of the survey design, methodology, data collected, and procedures for blood preparation and laboratory analyses were reported previously (14, 15). Questionnaires used in the survey, survey data, and variable codes were downloaded from the Internet (http://www.cdc.gov/nchs/about/major/nhanes/nhanesii.htm; October, 2000).

Blood samples were successfully collected from 17 797 of 18 549 participants aged ≥ 3 y; serum zinc concentrations for 14 770 participants were available for the analysis. Certain groups were deliberately oversampled in the survey; thus, it was necessary to apply sample weighting factors to reflect the actual means and prevalences for the US population subgroups. Serum zinc data were log transformed before the analysis to yield a more symmetrical distribution.

In the first phase of analysis, major variables with significant main or interaction effects on log serum zinc were identified with the use of analysis of variance models. The major variables included in the model were age (in y), sex, time of the day of blood collection in a fasting or nonfasting state ("time/fasting status"), and their interaction terms. The variable "time/fasting status" was used to categorize samples as morning fasting, morning nonfasting, afternoon, or evening. "Morning samples" were those collected before 1200, "afternoon samples" were those collected between 1200 and 1800, and "evening samples" were those collected after 1800. A subset of 5903 adult participants aged ≥ 20 y was requested to fast before the morning blood collection for the assessment of blood triacylglycerol. Of the subjects in this subset, those who reported that their last meal was eaten ≥ 8 h before the blood collection were considered to be in a fasting state; these samples are referred to as "morning fasting" samples. Samples from subjects who had blood drawn in the morning but who were not requested to fast are referred to as "morning nonfasting" samples; subjects who were not asked to fast were not questioned about the time of their last meal, and it is possible that some of the subjects arrived in a fasting state of their own accord.

To achieve a parsimonious model, interaction terms were removed in a stepwise fashion, as long as no higher-order interaction involving the same terms remained in the model. For each of the 3 major variables, log serum zinc was predicted from the resulting model, and the antilogarithm was calculated. Smoothed curves for the 50th percentile of serum zinc concentration by age were created for each of the significant major variables by entering age in years as a 4th-order polynomial function. The same procedures described above were carried out for the SD of log serum zinc to examine potential differences in the variability of data among the 3 major variables.

For the second phase of analysis, all other relevant variables derived from the survey were reviewed to identify factors known or suspected to affect serum zinc concentration, independent of the zinc status of the subjects (ie, present or recent pregnancy or lactation; use of oral contraceptives, steroids, or other hormones; low serum albumin concentration; elevated or low white blood cell counts; diabetes; diarrhea; anemia; and cigarette smoking). Those variables with a significant association with log serum zinc concentration were identified by using analysis of covariance, with the major confounding variables identified in the first phase of analysis (ie, sex, age, and time/fasting status) being controlled for. Data for subjects with factors having a significant relation with serum zinc concentration were removed before further analysis.

The sample 2.5th percentile was calculated numerically at each age (in y), borrowing data from the 2 surrounding ages to avoid bias due to small sample size; data were smoothed following the same procedures as used for the 50th percentile curves. Age groups were then formed in 5-y intervals, from 0–4 to 70–74 y. Because serum zinc data were available only for children aged ≥ 3 y, the first age group represents data for children 3–4 y of age only. The midpoint of the 2.5th percentile for each age group was determined, and curves were developed for each sex and time/fasting status group.

The need to establish separate cutoffs based on the major variables and by age group was then assessed. It was reasoned that the absolute difference in serum zinc concentration occurring among age groups beyond which a separate cutoff should be established should exceed a moderate level of analytic measurement error for serum zinc concentration. Flame atomic absorption spectrophotometry is a common analytic method for measuring zinc in biological samples. With this analytic method, a CV < 1% is possibly achievable, a CV < 5% is reasonably achievable, and a CV < 10% is expected to be achieved (16). On the basis of the overall geometric mean serum zinc concentration for the NHANES data set (86 μg/dL), a CV of 5% would allow differences of 4.3 μg Zn/dL to be measured with confidence that the difference does not occur because of measurement error. This value was thus used to define meaningful differences in serum zinc concentration between the various groups.

RESULTS

Of the 14 770 subjects for whom serum zinc data were available, data for 1307 were excluded from further analysis, largely because of inadequate information on the duration of fasting in the subset of 5903 subjects who were requested to fast before their blood was drawn. The reasons for these exclusions and the number of subjects in each category are summarized in **Figure 1**. The overall mean and the effect of the 3 major variables on serum zinc concentrations are summarized in **Table 1**.

Effect of major confounding variables on serum zinc concentrations

Sex

Serum zinc concentrations differed significantly between males (88.4 ± 0.2 μg/dL) and females (83.3 ± 0.2 μg/dL; $P < 0.0001$). The data points and smoothed 50th percentile curves of serum zinc by sex and year of age are shown in **Figure 2**. During childhood, males had lower serum zinc concentrations than did females, but this reversed in late childhood (≈10 y of age) when the concentrations in males began to exceed those of females. Throughout late adulthood (≈40 y of age and older), the magnitude of sex differences decreased with age and all but disappeared after ≈60 y of age. Differences between males and females were more pronounced in the morning samples than in the afternoon or evening samples.

Age

Age was significantly associated with serum zinc concentration ($P < 0.0001$) (Figure 2). Serum zinc concentrations were lowest in young children, increased steadily with age, peaked between 18

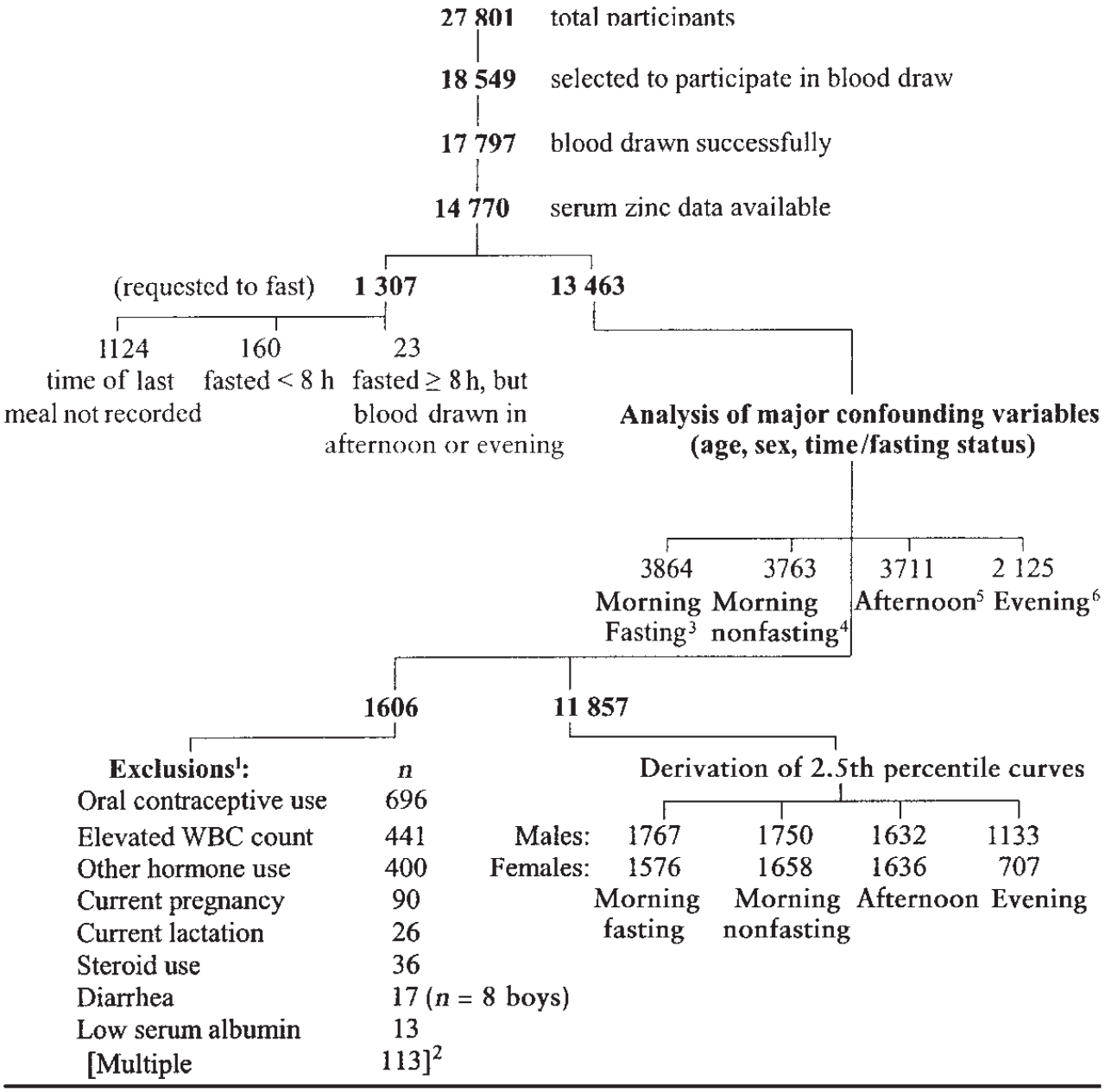

FIGURE 1. Summary of the number of subjects included and excluded from the analysis of serum zinc concentration, derived from an analysis of data from the second US National Health and Nutrition Examination Survey, 1976–1980. [1]Refer to the text for specific criteria. [2]113 subjects were excluded on the basis of multiple criteria. [3]Samples were collected before 1200 in subjects aged ≥ 20 y who had fasted for ≥ 8 h. [4]Samples were collected before 1200 from subjects who were not requested to fast and who were assumed to have eaten before the collection. [5]Samples were collected in the afternoon between 1200 and 1800. [6]Samples were collected in the evening after 1800. WBC, white blood cell.

and 25 y of age, decreased slowly during adulthood, and dropped off after 65–70 y of age.

Time of day

The time of day that blood samples were drawn had a significant effect on serum zinc concentrations ($P < 0.0001$), such that serum zinc was higher in the morning samples than in the afternoon or evening samples. This trend was less pronounced in subjects ≈60 y of age and older, ie, serum zinc in the morning samples decreased to meet the afternoon and evening concentrations. The evening and afternoon samples were similar except for those from adults ≈40 y of age and older, after which time serum zinc in the evening samples began to exceed the values in the afternoon samples. Data points and smoothed curves for the 50th percentile of serum zinc concentration by time of day of blood collection and year of age are shown in **Figure 3**.

Morning fasting compared with morning nonfasting samples

Fasting samples were derived for a subset of 5904 adult subjects (> 20 y of age) only, and most of the fasting samples were collected in the morning; therefore, only these samples were included (Figure 3). The serum zinc concentration was greater in

TABLE 1
Adjusted serum zinc concentration in subgroups of participants with factors known to affect serum zinc concentration independently of zinc status

Variable and data included in analysis	Mean serum zinc[1]	Mean serum zinc[2]	Variables controlled for	*P*
	μg/dL	*μg/dL*		
Overall	85.6 ± 0.1		Sex, age, time/fasting status	
Major variables				
Sex				
Males	88.4 ± 0.2		Time/fasting status, age	<0.0001
Females	83.3 ± 0.2			
Time/fasting status[3]				
Morning fasting	93.4 ± 0.2		Sex, age	<0.0001
Morning nonfasting	89.0 ± 0.2			
Afternoon	80.1 ± 0.2			
Evening	79.0 ± 0.3			
Age				
3–9 y	82.5 ± 0.3		Sex, time/fasting status	<0.0001
≥10 y	86.4 ± 0.1			
Minor variables[4]				
Oral contraceptive use				
Women aged ≥13 y	81.2 ± 0.4	83.0 ± 0.2	Time/fasting status, age	<0.0001
Elevated WBC count, $>11.5 \times 10^9$/L[5]	82.7 ± 0.6	85.3 ± 0.1	Sex, time/fasting status, age	<0.0001
Hormone use, age ≥17 y	81.4 ± 0.6	83.0 ± 0.1	Sex, time/fasting status, age	<0.02
Pregnant women				
Age 14–42 y	69.8 ± 1.1	83.2 ± 0.3	Time/fasting status, age	<0.0001
Lactating women				
Age 14–42 y	78.3 ± 2.1	82.6 ± 0.3	Time/fasting status, age	<0.05
Steroid use, age ≥14 y	81.3 ± 2.1	85.5 ± 0.1	Sex, time/fasting status, age	<0.05
Diarrhea, age <10 y				
Boys	68.9 ± 3.4	79.1 ± 0.4	Time/fasting status, age	<0.05
Girls	88.0 ± 5.2	78.4 ± 0.4	Time/fasting status, age	NS
Low serum albumin, <3.5 g/dL	71.2 ± 3.6	85.4 ± 0.1	Sex, age, time/fasting status,	<0.001
All exclusions	82.9 ± 0.3	85.6 ± 0.1	Sex, age time/fasting status	<0.0001

[1] Adjusted geometric $\bar{x}$ ± SE.
[2] Adjusted geometric $\bar{x}$ ± SE of comparison group with equivalent sex and age as indicated in variable description.
[3] *See* Subjects and Methods for details.
[4] All variables represent a condition that was current at the time of the interview.
[5] WBC, white blood cell.

the morning fasting samples than in the morning nonfasting samples, although the magnitude of this difference varied with age. For young adults (≈20–30 y of age), there was little difference in serum zinc concentrations between the morning fasting and the morning nonfasting samples, but this difference increased steadily with age until ≈60–65 y of age.

The CV for serum zinc concentration is used as a measure of dispersion of this variable in the present analysis. Because serum zinc is approximately log-normally distributed, the SD of the log-transformed variable is related to the CV of the untransformed variable. The CV for serum zinc concentration differed significantly by age ($P = 0.0001$), sex ($P = 0.0002$), and time/fasting status ($P = 0.015$). The CV for serum zinc was greater in children (14.5% at 7 y of age, ie, the median age of those <10 y of age) than in adolescents and adults (13.1% at 34 y, ie, the median age of those ≥10 y) and was slightly greater in men (15.4%) than in women (14.3%). The CVs for time/fasting status were 14.5% for morning fasting, 14.6% for morning nonfasting, 15.8% for afternoon, and 14.2% for evening.

Effect of other potentially confounding variables on serum zinc concentrations

After identification of the abovementioned significant variables, additional characteristics found to be associated with serum zinc concentration but likely to be independent of the subject's zinc status were as follows: low serum albumin (<3.5 g/dL); high white blood cell count ($>11.5 \times 10^9$/L); current pregnancy or lactation (females aged 14–42 y only); current use of oral contraceptives (females aged ≥13 y), steroids (≥14 y), or other hormones (≥17 y); and current diarrhea (3–9 y). Current diarrhea had a significant interaction with sex, such that only boys had a significantly lower serum zinc concentration. The numbers of subjects excluded from further analyses for each of these variables are summarized in Figure 1. The adjusted mean serum zinc concentrations for each of the potentially confounding variables and the resultant *P* values, tested in an analysis of covariance model, are summarized in Table 1. Other variables tested but found not to have a significant effect ($P > 0.05$) on serum zinc concentration were as follows: low white blood cell count ($<3.4 \times 10^9$/L), presence of anemia (self-reported), recent pregnancy or lactation (past 12 mo, but not current), self-reported diabetes, and cigarette smoking (≥12 y only).

An assessment of the smoothed 2.5th percentile data for the 11 857 remaining subjects, by 5-y age groups, indicated that meaningful differences (ie, >4.3 μg/dL) in serum zinc concentration existed between sexes, between morning fasting and morning nonfasting samples, and between afternoon and morning nonfasting samples but not between afternoon and evening samples. Although the magnitude of sex differences in the 2.5th percentile

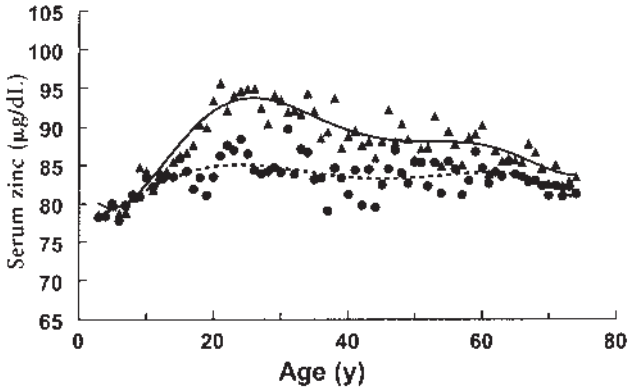

FIGURE 2. Percentiles (50th) of serum zinc concentration by age and sex (▲, males; ●, females) derived from an analysis of data from the second US National Health and Nutrition Examination Survey, 1976–1980.

data varied with age, these differences exceeded 4.3 μg/dL through most of adulthood (≈30–49 y of age). Therefore, it is reasonable to consider separate reference cutoffs for males and females. For both males and females, morning fasting and morning nonfasting samples differed by > 4.3 μg/dL from ≥ 40–45 y of age, whereas differences between morning nonfasting and afternoon samples differed substantially for all age groups. Because differences between afternoon and evening samples were not substantial for any age group, these data were combined. On the basis of these observations, separate reference curves for the 2.5th percentiles were developed for each sex and, within each sex, for morning fasting, morning nonfasting, and combined afternoon and evening samples (**Figure 4**, A and B).

Finally, for each of these reference curves, the differences between age groups were assessed to determine where it was justified to establish separate cutoff values. Starting at the tail ends of each curve, where the cumulative difference in serum zinc concentrations between midpoints of adjacent age groups exceeded

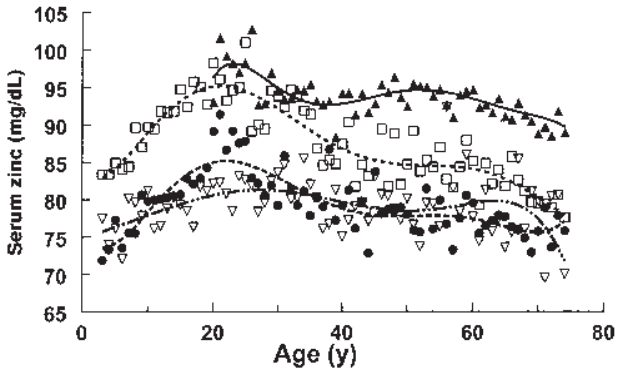

FIGURE 3. Percentiles (50th) of serum zinc concentration by age and time of day of blood sample collection and fasting status, derived from an analysis of data from the second US National Health and Nutrition Examination Survey, 1976–1980. Data for each time of day and fasting status group are shown as follows: ▲, morning fasting (≥ 8 h); □, morning nonfasting; ●, afternoon nonfasting; ▽, evening nonfasting. *See* Subjects and Methods for details.

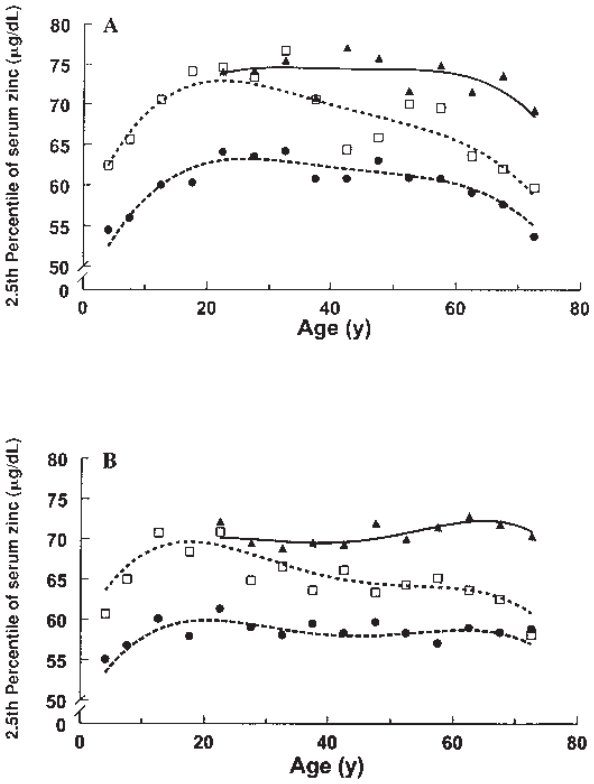

FIGURE 4. Percentiles (2.5th) of serum zinc concentration for males (A) and females (B) aged 3–74 y, by age and time of day of blood sample collection and fasting status, derived from the second US National Health and Nutrition Examination Survey, 1976–1980. Symbols represent the midpoint for 5-y age intervals. Curves were fitted by using a fourth-order polynomial function for age in years. Data for each time of day and fasting status group are shown as follows: ▲, morning fasting (≥ 8 h); □, morning nonfasting; ●, afternoon and evening combined. *See* Subjects and Methods for details.

4.3 μg/dL, a separate cutoff was derived. The suggested lower cutoffs for serum zinc concentration by age group, sex, and time/fasting status are given in **Table 2**.

It was of interest to examine serum zinc data separately for pregnant women. The NHANES II data set provided results for 99 women during pregnancy; however, only 61 valid data points remained after the exclusion of subjects who also had other characteristics found to affect serum zinc concentration. Time/fasting status was not found to significantly affect mean serum zinc concentrations in pregnant women, and all groups were combined to attain sufficient sample size for further analyses. The 50th percentile of serum zinc concentrations, by month of pregnancy, is given in **Figure 5**. Mean serum zinc decreased steadily throughout pregnancy from 72 ± 2.7 μg/dL during the first month to 61 ± 1.7 μg/dL during the ninth month. Although this sample size was not adequate to derive a 2.5th percentile with reliability for each month of pregnancy, an analysis of variance of log serum zinc by trimester indicated that the 2.5th percentile for the first trimester was 56 μg/dL; the 2.5th percentile for the second and third trimesters did not differ significantly, and the pooled value was 50 μg/dL (**Table 3**).

Given the relatively large number of women in the survey who were currently using oral contraceptive agents, it also seemed

TABLE 2
Percentiles (2.5th) of serum zinc concentration based on age group, sex, and fasting status and time of day of blood collection (time/fasting status)[1]

	2.5th Percentile of serum zinc concentration			
Sex and time/fasting status[2]	3–9 y	10–64 y	≥65 y	10 to ≥70 y
		μg/dL		
Male				
Morning fasting[3]	NA	74 ± 0.5	72 ± 0.8	—
Morning nonfasting	65 ± 0.7	70 ± 0.6	61 ± 1.0	—
Afternoon	56 ± 0.7	61 ± 0.4	56 ± 1.0	—
Female				
Morning fasting[3]	NA[3]	—	—	70 ± 0.4
Morning nonfasting	64 ± 0.8	—	—	66 ± 0.4
Afternoon	57 ± 0.7	—	—	59 ± 0.4

[1] Percentiles ± SE. Data derived from the second National Health and Nutrition Examination Survey. NA, not available. For conversion to μmol/L, divide by 6.54.
[2] *See* Subjects and Methods for details.
[3] Based on data from subjects aged ≥20 y.

appropriate to assess the 2.5th percentiles for this group. Time/fasting status was a significant factor, and the 2.5th percentiles were derived for each of these groups following the same method as described above for determining age- and sex-group cutoffs for the included subjects. The results for oral contraceptive users are presented in Table 3. Because the 2.5th percentiles differed by >4.3 μg/dL between the afternoon and evening blood samples, separate lower cutoffs were derived for each of these time groups. Although data were available for women >45 y of age, 2.5th percentiles are not given for these women. Except for the morning nonfasting group, cumulative differences in serum zinc concentration between the 5-y age groups were >4.3 μg/dL after 40–44 y of age, which, according to our method, indicates that a separate lower cutoff should be set. However, the number of older women who used oral contraceptives was small (n = 10), and the data did not follow the expected pattern for time/fasting status. Therefore, a single 2.5th percentile for the evening samples is presented, which includes data from oral contraceptive users aged 15–39 y.

DISCUSSION

We reanalyzed the NHANES II data on serum zinc concentration to develop reference cutoffs for population assessment based on the 2.5th percentile, after we controlled for factors that affect serum zinc deficiency independent of zinc status. Specific cutoffs were developed based on sex, age, and time/fasting status. Tentative lower cutoffs were also proposed for pregnant women and for women using oral contraceptives.

The difference in serum zinc concentrations between males and females was equivalent to 5.6% of the overall mean serum zinc concentration for all participants, and the difference in serum zinc concentrations between nonfasting children (3–9 y of age) and nonfasting adolescents and adults (≥10 y of age) was 4.3%. In comparison, differences introduced by fasting state (morning fasting compared with morning nonfasting) and time of day (morning compared with afternoon) were 7.3% and 9.5%, respectively. Although the differences introduced by age and sex were of a somewhat lesser magnitude than were those introduced by time/fasting status, they were significant and substantial; therefore, all 4 variables should be considered when setting lower cutoffs for interpreting serum zinc status.

The diurnal variation in circulating zinc concentrations is largely a result of metabolic changes after meal consumption, although some variation may occur as a result of normal circadian variations in metabolism (17, 18). Meal consumption results in a decrease in serum zinc concentrations, which is cumulative with repeated meals (17, 19), whereas overnight and daytime fasting result in increased circulating zinc concentrations (17). Differences in serum zinc concentration by sex (20–22) and by age (20, 23) were noted previously. Possible reasons for these age- and sex-based differences include differences in serum albumin concentrations and in lean body mass. Concentrations of albumin and zinc in serum were strongly correlated, because ≈80% of zinc in the circulation is bound to albumin. In the present analysis, changes in serum albumin concentration with age explained most of the variability in serum zinc concentrations in adults aged ≥20 y and ≈50% of the variability among those aged <20 y. Differences in serum albumin concentrations explained nearly 50% of the difference in serum zinc concentrations between sexes. Estrogen and progesterone are associated with lower serum zinc concentrations in women when these hormones are at their highest concentrations during the ovulatory and luteal phases of the menstrual cycle (24). Data from Pinna et al (25) suggest that the size of the rapidly

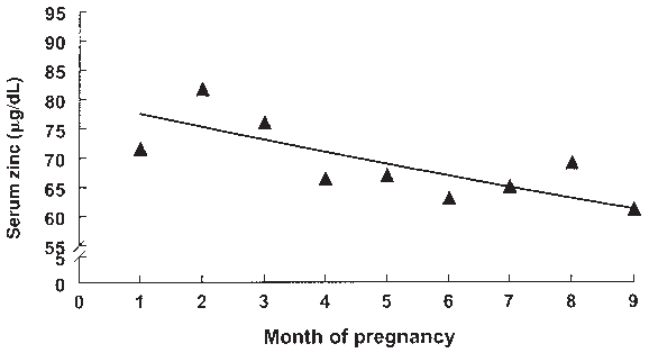

FIGURE 5. Median serum zinc concentration by month of pregnancy, derived from an analysis of data from the second US National Health and Nutrition Examination Survey, 1976–1980.

TABLE 3
Tentatively proposed lower cutoffs (2.5th percentile) for the assessment of serum zinc concentration in pregnant women or women using oral contraceptive agents (15–44 y of age)[1]

	Lower cutoffs of serum zinc concentration
	μg/dL
Pregnancy	
First trimester	56
Second and third trimesters	50
Oral contraceptive use[2]	
Morning fasting samples[3]	65
Morning nonfasting samples	61
Afternoon samples	57
Evening samples[4]	53

[1] Data derived from the second National Health and Nutrition Examination Survey.
[2] *See* Subjects and Methods for details.
[3] Data derived from women aged 20–44 y only.
[4] Data derived from women aged 15–39 y only.

exchangeable zinc pool, of which serum zinc is a part, is directly associated with lean body mass ($r = 0.91$, $P < 0.01$), which may partly explain the lower serum zinc concentrations found in women and the elderly than in men.

Data were removed for subjects with conditions that were known to affect serum zinc concentration but that cannot necessarily be attributed to a change in zinc status. As in the previous analyses of NHANES II survey results, data from subjects with low serum albumin concentrations, subjects with elevated white blood cell counts, and current users of oral contraceptives were eliminated from the analysis because of significantly lower serum zinc concentrations (Table 1). Severe decreases in serum albumin occur with conditions such as cirrhosis and protein-energy malnutrition (13). With concurrent infections, as indicated by high white blood cell counts, the noted decrease in serum zinc concentrations (26) probably occurs because of the release of cytokines, which stimulates hepatic metallothionein synthesis and leads to hepatic sequestration of circulating zinc (27, 28). C-reactive protein, an acute phase response factor, was not measured in NHANES II, but it may also be used to identify subjects with concurrent infections and to control for this confounding variable in the analysis of serum zinc results (29). Use of oral contraceptive agents has been documented in other studies to affect serum zinc concentration (30, 31).

Unlike the previous analysis, we removed data for subjects who were using steroids or other hormones because of the observed effects of physiologic or pharmacologically induced changes in hormone or steroid concentrations on serum zinc concentration (24, 30–32). Finally, subjects with a current episode of diarrhea were found to have significantly lower serum zinc concentrations than were other participants and, therefore, were also removed. Acute diarrhea may result in large losses of endogenous zinc through the intestine (33); therefore, serum zinc concentrations during diarrhea may reflect acute changes in zinc metabolism and not necessarily the true zinc status. These conditions should be considered as possible confounding factors in the analysis of serum zinc status in populations, and associated data may be excluded or controlled for during analysis.

In the current analysis, we found no significant differences in serum zinc concentration among the women who had been pregnant or lactating within 1 y before the survey was conducted ($P > 0.05$; analysis of covariance); therefore, unlike in the previous analysis of these data (12), we did not eliminate these data from consideration. Data for women who were currently pregnant or lactating were, however, analyzed separately. Serum zinc concentrations decreased during pregnancy as a consequence of blood volume expansion and possibly because of hormonal changes. In the current analysis, the decrease in serum zinc concentration appears to have been largely due to hemodilution, because the ratio of serum zinc to albumin, as a marker of change in blood volume, was relatively constant by month of pregnancy and did not differ significantly between pregnant and nonpregnant women ($P = 0.23$). A longitudinal study of changes in plasma zinc concentrations during pregnancy among apparently healthy US women consuming adequate amounts of zinc, reported mean plasma zinc concentrations in the third (71 μg/dL; $n = 9$) and ninth (57 μg/dL; $n = 16$) months of pregnancy that were comparable with the respective median serum zinc concentrations from NHANES II (76 and 63 μg/dL, respectively) (34). The 90% confidence limits in the longitudinal study for the second and ninth months of gestation (54 and 40 μg/dL, respectively) were also similar to the 2.5th percentiles derived in the current analysis for women during the first and second and third trimesters combined (55 and 46 μg/dL, respectively); the latter values can be used tentatively as lower cutoffs for pregnancy until further reference data are available.

Unfortunately, the number of lactating women for whom serum zinc data were available and were not excluded for other reasons was small ($n = 23$); therefore, it was not possible to derive reliable estimates of the 2.5th percentiles for this group. Until further reference data become available for lactation, the lower cutoffs for nonpregnant women may be used with the recognition that the proportion of lactating women with low serum zinc concentrations may be overestimated.

Because women of childbearing age are generally at high risk of nutritional deficiencies, they are often oversampled in representative surveys, and many of them use oral contraceptive agents. It may not be desirable to exclude from assessments the serum zinc data from oral contraceptive users or appropriate to compare their results with lower cutoffs for nonusers of oral contraceptives. Therefore, tentative lower cutoffs for serum zinc for oral contraceptive users are presented, albeit with the precaution that the effects of oral contraceptives on serum zinc may vary as a result of different formulations of these hormones.

Serum zinc concentrations were not measured in children < 3 y of age in NHANES II. Two smaller studies collected serum zinc data with the intent of establishing pediatric reference values, including this age group (23, 35); however, only one of the studies (23) disaggregated the data for children < 3 y of age and did not control for time/fasting status (35). The 2.5th percentile reported for the 9–23-mo-old Australian children was 59 μg/dL ($n = 132$), and that for the 3–5-y-old children was 52 μg/dL ($n = 226$). These values are intermediate to those found in the NHANES II survey for blood samples collected in the morning and afternoon from children 0–5 y of age (Table 3). Thus, until further reference data are available for children < 3 y of age, it appears appropriate to use the lower cutoffs for the 0–5-y age group presented in Table 3.

Serum zinc concentration is not considered to be a reliable indicator to diagnose mild or moderate zinc deficiency in individual persons. Serum zinc is fairly well maintained within a normal range during short-term zinc depletion because of efficient homeostatic mechanisms and, therefore, may show measurable changes only when zinc depletion is prolonged or severe (36). Nonetheless, measurable differences in serum zinc concentration have been observed to occur in groups or populations in response to changes in dietary zinc intakes and clinical conditions associated with zinc deficiency. For example, serum zinc concentrations vary

TABLE 4
Suggested lower cutoffs (2.5th percentile) for the assessment of serum zinc concentration in population studies[1]

Time/fasting status[2]	Lower cutoffs of serum zinc concentration			
	Children aged <10 y	Females aged ≥10 y	Males aged ≥10 y	Pilch and Senti (12)[3]
		μg/dL		*μg/dL*
Morning fasting[4]	—	70	74	70
Morning nonfasting	65	66	70	65
Afternoon	57	59	61	60

[1] Data derived from the second National Health and Nutrition Examination Survey (NHANES II).
[2] Time of day of blood sample collection and fasting status; *see* Subjects and Methods for details.
[3] From a previous analysis of NHANES II serum zinc data for all age and sex groups combined.
[4] Based on data from subjects aged ≥20 y only.

on the basis of differences in total dietary intakes or changes in the likely absorption of the forms of dietary zinc among groups of subjects (37–39). Median serum zinc concentrations in women during pregnancy were found to predict infant birth weight in response to zinc supplementation (6). A low serum zinc concentration (<54.9 μg/dL) was shown to predict an increased risk of diarrhea among children in India (10). Also, in a previous meta-analysis of the effect of zinc supplementation on growth in children, the initial mean serum zinc concentration was negatively correlated with the magnitude of the growth response (11). Although this relation was not observed in an updated meta-analysis (2), it was noted that studies of severely malnourished children included in the previous meta-analysis were omitted in the updated version, so it is possible that the relation was masked because of a smaller range of mean serum zinc values available among the studies included in the updated analysis. Therefore, it appears that serum zinc is a useful indicator of population zinc status, such that a high proportion of individual persons with low serum zinc concentrations suggests an elevated risk of zinc deficiency within the population.

The 2.5th percentiles for serum zinc concentration presented in Table 1 may be simplified for convenient use as lower cutoffs in survey assessments, as summarized in **Table 4**. Although the lower cutoffs derived in the present study for children and nonpregnant women by time/fasting status do not differ markedly from those suggested previously, cutoffs derived for men were substantially higher in the morning samples. If the previously suggested lower cutoffs were applied to the assessment of zinc status in men, the prevalence of zinc deficiency in men would be somewhat underestimated.

In conclusion, we have presented suggested lower cutoffs for the assessment of risk of zinc deficiency in human populations based on serum zinc concentration, taking into account 4 major confounding variables: age, sex, time of day of blood sampling, and fasting state of the subjects. As population-based health and nutrition surveys in at-risk areas begin to include biochemical assessments of zinc status, each of the abovementioned variables should be considered in the design of the survey and during data interpretation. Several other factors associated with significantly lower serum zinc concentrations and presumably independent of zinc status were also identified, and these should be treated as confounding variables when they occur.

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