



Zinc supplementation of malnourished schoolboys in Iran: increased growth and other effects^{1, 2, 3}

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ABSTRACT The effects of zinc, 40 mg daily, given as zinc carbonate, in combination with a supplement of egg-white protein (10 g daily), corn oil, minerals, and vitamins to fulfill many requirements, were evaluated in 13-year-old prepubertal village schoolboys in southern Iran. Thirty-five of the 49 boys participating had subnormal plasma zinc concentrations. The boys continued their usual diets in which unleavened wholemeal wheat bread rich in phytate was the main staple. A similar group serving as controls received the same supplements without zinc. A second control group received only the protein supplement. Observations were continued for 18 months.

Significantly increased heights, weights, and bone ages occurred in those receiving the supplementary zinc, despite zinc concentrations in plasma that remained subnormal throughout the study in most of the boys. These gains occurred mainly during the final 12 months of supplementation.

Serum total protein and albumin concentrations were moderately decreased initially in approximately 75% of the boys. Concentrations of total protein became normal during the first 3 months of treatment.

The results demonstrate a clearly defined stimulus to growth brought about by supplementation with zinc when adequate amounts are given. Contrary to findings in a previous study, no statistically significant stimulation of gonadal development was detected in the zinc-supplemented group compared with the controls. However, a tendency toward accelerated sexual development in the zinc supplemented boys was evident. *Am. J. Clin. Nutr.* 27: 112-121, 1974.

A syndrome of hypogonadal dwarfism was described by Prasad et al. (1) in 1961 in Iran. Two years later, Prasad et al. (2) demonstrated its relationship to zinc deficiency in Egypt. The role of zinc was confirmed subsequently by Halsted et al. in Iran (3). Prepubertal growth ceases in those affected and maturation of gonads and other changes associated with puberty fail to occur. Sandstead (4) has pointed out that prepubertal development increases the need for zinc. Consequently, in the absence of an increased intake, symptoms of deficiency may develop. The belief that lack of zinc is crucial receives further support from the low concentrations of zinc in plasma that are characteristic of the syndrome and by its

successful treatment by means of zinc supplementation in conjunction with a nutritious diet (3).

However, a number of trials in which zinc

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was administered to children in populations believed to be zinc deficient have failed to bring about the stimulation of growth that would be anticipated by provision of an essential dietary component that had been in short supply. This was true of the 6- to 12-year-olds (mainly boys) tested by Mahloudji et al. (5) in the village of Kherak in the Shiraz Region. Similarly, Ronaghy et al. (6) found no effect on growth of prepubertal schoolboys in an earlier stage of the present study carried out with boys at a school in the town of Marvdasht. They did, however, observe an increased maturation of genitalia. Carter et al. (7) previously had failed to demonstrate effects on growth when zinc supplementation was tested in Egyptian boys as subjects in a village locale. Because of these negative results, skepticism has persisted concerning the importance of zinc in human nutrition and of the existence of marginal human zinc deficiency in populations in which dwarfism has developed.

The purpose of the present study was to learn whether these failures could have been in part the result of administration of insufficient quantities of zinc as a dietary supplement. Although the amount used in the unsuccessful trials was double or treble the amounts provided in Western diets and hence considered to be adequate, it has become clear that much of the zinc in the cereal-rich diets of rural Iran is unavailable for absorption from the intestine. Another possible explanation of the ineffectiveness of zinc in the earlier studies is that the time periods during which supplementation was continued were insufficient for a response to be manifested. For these reasons, a second trial of zinc supplementation at Marvdasht was carried out with a daily supplement of 40 mg zinc as zinc carbonate instead of the 28 mg previously given as zinc sulfate. Supplementation was

continued during 2 school years (October to May).

To further insure that the diet was nutritionally complete, supplements of protein, corn oil, minerals, and vitamins were given. Care was taken also to select boys of uniform bone age and at the ages (13 to 14 years) when the events leading to puberty would normally undergo acceleration.

Methods

Fifty schoolboys, aged 13 years, who were enrolled in two schools in Marvdasht agreed to participate. Marvdasht is a town of approximately 10,000 persons located midway between Shiraz and Persepolis. However, nearly all of the participants were from nearby villages. The boys were divided into two groups (B and C) of 20 boys each and one of 10. The boys comprising the latter group (A), who served as controls, attended a different school, but like the others, they came from nearby villages. Bone ages were the same in the three groups. Only one boy failed to complete the study but some were unwilling to submit to venipuncture at all times.

Although the purpose of the supplements given in the current study was the same as before (6), the form was different (Table 1). In this experiment, a high protein-vitamin liquid supplement was given to groups B and C instead of the cookies used previously. The protein was supplied by dried egg white, the vitamins were crystalline, and the minerals were reagent grade when available. The oil was mixed with the dry components. When added to water this gave a smooth suspension that was acceptable to the boys. Group A received a non-nutrient supplement (except for carbohydrate) that was identical in appearance to the supplement for groups B and C. The mineral supplements were mixed in the laboratory and assayed for zinc prior to the capsule-filling, which was done mechanically. The levels of minerals were the same as before, except that magnesium was omitted in this study and zinc (when present) was at a higher level.

The supplements were given each day at 11 AM when school was in session (ordinarily 6 days a week) and under the supervision of the schoolmasters, beginning on November 4, 1969. Zinc and other mineral capsules were administered to boys in group C at the same time as the other liquid supplements. The boys of group A were given lactose-containing placebo capsules. The capsules for group B were the same as for group C except that zinc was omitted. All capsules were similar in appearance. The boys continued their usual diets and ways of life during the 20-month period of the study. No supplements were given during the summer holiday, which extended from the end of May to the beginning of October. Both boys and schoolmasters were motivated to cooperate by the opportunity which the study provided to contribute information that had potential benefit for the people of Iran. However, unannounced visits were made on different days once or twice each week by a physician

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TABLE 1
Composition of dietary supplements

Constituent	Group A, amount/ day	Groups B and C, amount/ day
Liquid supplement		
Dried egg white, g	0	10
Corn oil, g	0.7	6
Sucrose, g	20	20
Vitamin mix, g ^a	0	1
Carrageenan, g	0.2	0.2
Citric acid, g	0.2	0.2
Food color, g ^b	0.0025	0
Glucose, g	10	0
Corn starch, g	0.2	0
Total dry weight, g	31.3025	37.4
Water, ml	135	135
Calories	177	127
Zinc (contaminant) μ g	30	30
Capsules, 2^c		
	Lactose	Minerals

^a The following vitamins were dispersed in 0.67 g glucose: vitamin A palmitate (dry, water-dispersible), 6,000 IU; vitamin D₃, 500 IU; DL-alpha-tocopheryl acetate (dry, water-dispersible form), 50 IU; thiamin-HCl, 2 mg; riboflavin, 2.5 mg; niacin, 25 mg; D-calcium pantothenate, 20 mg; pteroylglutamic acid, 0.1 mg; D-biotin, 0.3 mg; pyridoxine-HCl, 4 mg; vitamin B₁₂, 10 μ g; and ascorbic acid, 90 mg.

^b Yellow food coloring to match that of the vitamins in the supplement for groups B and C.

^c The capsules for groups B and C supplied the following milligrams of mineral elements (form in parentheses) per day: Ca, 100 (CaCO₃); Fe, 100 (ferrous fumarate); Mn, 5 (MnSO₄·H₂O); Co, 0.1 (CoSO₄·7H₂O); Cu, 1.1 (CuSO₄); Cr, 0.15 (CrCl₃·6H₂O); Mo, 2 (Na₂MoO₄·2H₂O); Ni, 0.5 (NiCl₂·6H₂O); Se, 0.1 (Na₂SeO₃); I, 0.15 (KIO₃); and F, 1 (NaF). Group C also received Zn, 40 mg (zinc carbonate).

participating in the study to make certain that the supplements were being administered as instructed.

Bread of the type consumed in their homes was obtained from 15 of the boys. Ten regularly ate tanok, the unleavened wholemeal wheat village bread that has been shown to be rich in phytate (8). Phytate concentrations of these samples averaged 675 mg/100 g of dried bread. The remainder varied tanok with sangak, a leavened bread of lower but still appreciable phytate content. The latter averaged 450 mg/100 g of dried bread. A recent survey of food consumption by Maleki (9) included some of the boys participating in the present experiment. This showed that bread supplied at least 50% of the daily caloric intake. As a result, the phytate intakes were high. The supplements added 177 kcal daily, which helped somewhat to overcome the caloric deficiency characteristic of village diets (9).

Blood and random samples of urine were collected from the boys at the beginning and at four subsequent intervals. The final blood collection was on May 11, 1971.

Zinc and calcium concentrations were measured in plasma obtained by using 20% sodium citrate trihydrate (w/v) as anticoagulant, 0.04 ml being used for 5 ml blood. Zinc concentrations were determined by aspiration of 1:3 (10) dilutions of plasma with water into a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer. Calcium was similarly measured in 1:5 dilutions that included 1 volume of 5% lanthanum chloride. Standard solutions of these metals (Hartman-Leddon Co., Philadelphia, Pa.) were used for calibration and Versatol Control Serum was used as an analytical control standard. Operation conditions were those recommended for air-acetylene fuel mixtures by the manufacturer (11). Well-nourished healthy adults who were examined at intervals during the study were found to have plasma zinc concentrations of $95 \pm 12.3 \mu\text{g}/100 \text{ ml}$ (mean and SD). Zinc concentrations in erythrocytes were measured after diluting 100 μ l packed cells to 10 ml with deionized water. A small amount of protein precipitate was removed by centrifugation.

Sulfonated bathophenanthroline was used for measurement of serum iron concentrations (12) and transferrin-bound iron was measured after removal of unbound iron by magnesium carbonate as described by Ramsay (13). Serum proteins were separated by electrophoresis on cellulose acetate using the Gelman apparatus and procedures (14). Total serum protein concentrations were obtained with the aid of the biuret method (15). Inorganic phosphorus measurements in plasma were made by reduction of phosphomolybdate with ascorbic acid in trichloroacetic acid filtrate. *p*-Nitrophenylphosphate was used as substrate (16) for alkaline phosphatase activity measurements.

Randomly collected specimens of urine were analyzed for creatine and creatinine periodically (17). However, no significant differences in creatine excretion could be detected in the outputs of the three groups and the results are omitted from the tables. This was true also of hemoglobin concentrations in blood which initially averaged 12.8, 13.3, and 12.8 g/100 ml in groups A, B, and C, respectively. They underwent no significant changes subsequently.

Sexual development was evaluated by a scoring system described in a previous report (6).

The bone ages of the groups A, B, and C were compared with the corresponding averages of Greulich and Pyle (18). Special attention was paid to a) the radial epiphysis and epiphyses of the second to fifth metacarpals, b) the ossification centers of the sesamoid in the tendon of the adductor pollicis, c) the epiphyses of the proximal phalanges of the second to fifth finger, d) the epiphysis of middle phalanx of the fifth finger, and e) the tips of the epiphyses of the distal phalanges of the second to fifth finger.

It should be noted that there was often a marked discrepancy between the bone age of the carpals as compared with that of the distal epiphysis of radius and ulna, as well as the epiphyses of the phalanges, the carpals being almost invariably the more retarded.

Results

Growth

Table 2 shows that the zinc-treated boys (group C) made gains in height and weight that exceeded those of the boys who received identical supplementation minus zinc (group B) during the 20-month treatment period. This response occurred mainly during the 2nd year. After the first 7 months of treatment, the gains in height exceeded those of the other groups by only a moderate and statistically insignificant margin, whereas gains in weight were less than those of the other groups.

Bone age

Bone age changes were small during the first 6 months and there were no significant differences among the groups. However, during the 2nd year, bone development of the zinc-supplemented group surpassed that of the other groups by a substantial and statistically significant margin. (Table 3).

Sexual development

Table 4 shows the appraisal of the development of genitalia at the beginning and end of

the supplementation program. Although the boys in the group who had received zinc showed a higher proportion of developed genitalia at the end of the study, the difference was not sufficient to attain statistical significance when evaluated by the chi-square method.

Biochemical findings

Subnormal concentrations of plasma zinc in 35 of 49 boys and moderately decreased serum albumin concentrations in 36 of 47 are the more noteworthy deviations from normal composition of the initial blood samples (Table 5). Other abnormalities included lowered serum total protein (23 of 47), elevated gamma globulin concentrations (26 of 46), and elevated serum alkaline phosphatase activities (7 of 42).

After 3 months of supplementation, plasma zinc concentrations had declined in groups B and C. Low zinc concentrations found in erythrocyte hemolysates at this time supported the results of plasma analyses. No significant improvement had occurred in May, and, indeed,

TABLE 2
Relationship of growth increments to treatment with zinc

	Days	Group A ^a		Group B ^b		Group C ^c	
		No.	Mean ± SD	No.	Mean ± SD	No.	Mean ± SD
Height, cm							
1969 height		10	134.0 ± 3.1	19	134.1 ± 2.9	20	133.1 ± 3.97
Nov. 1969–May 1970 increment	182	9	5.0 ± 3.36	16	5.68 ± 2.06	14	6.10 ± 1.22
Significance of difference (1969–1970) ^d			NS		<i>P</i> < 0.10 but > 0.05		
May 1970–May 1971 increment	536	10	8.25 ± 4.82	19	8.90 ± 2.60	20	10.49 ± 2.38
Significance of difference (1970–1971)			NS		<i>P</i> < 0.001		
Weight, kg							
1969 initial weight		8	31.3 ± 2.49	19	31.5 ± 2.97	19	30.6 ± 3.05
Nov. 1969–May 1970 increment		7	2.09 ± 1.76	14	1.79 ± 0.96	14	1.66 ± 1.35
May 1970–May 1971 increment		8	2.56 ± 1.54	15	2.63 ± 1.57	13	3.68 ± 1.80
Significance of difference (1970–1971)			NS		<i>P</i> < 0.001		

Significance of mean difference in growth increments 1969 to 1971 by *t* test. NS = not significant at 5% level or less.

^a Placebo supplement only. ^b Complete supplement without zinc. ^c Complete supplement including zinc. ^d Differences between A vs B and B vs C evaluated by *t* test.

the lowest average for the group as a whole was found the following October. The experiment ended with subnormal plasma zinc concentrations persisting in two-thirds of the boys. Those of group C who received zinc did not differ from the others in this respect nor in erythrocyte zinc concentrations.

Within 3 months, serum albumin and total protein concentrations had become normal in most of the boys. At the end all were normal with only two exceptions. Group B made more rapid progress in this respect than did the other two groups. Iron concentrations in serum rose gradually toward normal. The rise in the two iron-supplemented groups, B and C, did not exceed that of group A which received no additional iron. There was a significant rise in iron-binding capacity during the final 8-month period by all groups.

Serum inorganic phosphorus concentrations tended to be in the low normal range during most of the study. However, calcium concentrations in plasma were maintained within normal limits with a few exceptions. The 400 units of vitamin D included in the supplements of groups B and C may have been partly responsible.

Discussion

Stimulation of growth of the prepubertal schoolboys who received zinc in this trial of zinc supplementation is clearly demonstrated. Gains in height and weight were significantly greater than those in the two control groups. The significant increases in bone age of the zinc-treated group are in agreement with these observations. Although it is curious that the superiority of zinc supplementation did not become clear until the 2nd year of the supplementation program, the low initial bone ages, 9 to 10 years, undoubtedly contributed to the delay. Zinc supplementation may have gradually accelerated prepubertal changes and so made possible the initiation of the first stages of a prepubertal growth spurt in the later stages of the study. It is also worth noting that the acceleration of growth in this group occurred despite plasma zinc concentrations that remained subnormal. However, it is possible that the concentrations remained subnormal in most of the boys receiving zinc supplementation because of increased demands by the tissues for zinc associated with the higher rates of growth in this group.

TABLE 3
Effect of zinc supplements on bone age

	Group A ^a		Group B ^b		Group C ^c	
	No.	Mean ± SD	No.	Mean ± SD	No.	Mean ± SD
Bone age, years						
October, 1969	6	9.45 ± 0.53 ^d	11	9.66 ± 0.54	11	9.55 ± 0.69
Increment, months						
October 1969 to May 1971	6	17.3 ± 65 ^e	11	13.1 ± 2.3	11	22.9 ± 2.5
Significance ^f						
B vs C		<i>P</i> = <0.001				
A vs C		<i>P</i> = 0.02				

^a Placebo supplement only. ^b Complete supplement without zinc. ^c Complete supplement including zinc. ^d Mean ± SD. ^e Mean ± SE. ^f Differences between means evaluated by Student's *t* test.

TABLE 4
Development of genitalia (numbers of subjects)^a

	Group A ^b		Group B ^c		Group C ^d	
	Infantile	Adult	Infantile	Adult	Infantile	Adult
October, 1969	7	1	13	0	12	1
May, 1971	5	3	9	4	5	8

^a Scores based upon maturational changes. See (6) for scoring method. ^b Placebo supplement only. ^c Complete supplement without zinc. ^d Complete supplement including zinc.


In the previous study at Marvdasht (6), zinc concentrations in plasma were found to be normal, although no effect on growth was demonstrated. Different techniques for measurement of zinc concentrations were used in the two studies, that of Hackley et al. (19) in the first and that of Reinhold et al. (10) in the second. A more important factor, probably, was the shipment of plasma samples from Shiraz to Washington for examination during the first study, whereas in the second, measurements could be made in Shiraz. A comparison of zinc analyses in the two locations showed that concentrations of samples transported to Washington rose by a mean of 23 $\mu\text{g}/100$ ml over values found in Shiraz. The difference is attributed to uptake of zinc from the containers, although these had been leached with 15% HCl for several days before use. The two laboratories did not differ in accuracy when locally collected plasmas were examined.

Human zinc deficiency as it occurs in Iran is believed to be the result of interference with zinc absorption by the large amount of phytate present in the unleavened wholemeal wheat bread, tanok, which is the major staple of the village diet. Although the village child may ingest much larger amounts of zinc than does a child consuming a Western diet, the zinc in the diet of the former is relatively unavailable because it is complexed by phytate. By increasing the amount of zinc supplement provided in the current experiment, the additional zinc may have been less firmly bound and thus available for absorption from the intestine. The inclusion of calcium and iron in the supplement also may have been helpful because each element is capable of combining with phytate and decreasing the reactivity of the phosphate groups of the latter with zinc. However, this action may be partially offset by the formation of firm complexes of zinc with calcium phytate. The delayed response to zinc suggests the further possibility that the 40 mg zinc given daily was marginal.

The appropriate level of supplementation to reverse the adverse changes in a deficient organism is an important consideration for which there are, as yet, no clear answers. The nutrient requirements of experimental animals typically follow a log dose response (20). This means that large amounts of a nutrient are required to produce a relatively small response

just below the requirement level. This is true for zinc in chicks and rats (21). The boys in this study fall in this range of moderate rather than severe deficiency. Therefore, it is reasonable that a larger supplement of zinc would have caused marked increases in growth and sexual development, possibly during the 1st school year, as well as increases in plasma zinc level.

The high phytate intakes of Iranian villagers affect the metabolism of calcium (22, 23), iron (24), and possibly phosphorus as well as of zinc. As a result, the zinc deficiency that occurs is undoubtedly associated with states of depletion, if not deficiency of these elements. The supplement for groups B and C supplied approximately five times the recommended dietary allowance for iron but only a fraction of that for calcium (25). The response to zinc may have been modified particularly as a result of low calcium intake.

An improvement in plasma protein concentrations in the course of the experiment was shared by all groups. Caughey (28) believes that a lack of protein rather than of zinc is primarily responsible for retardation of growth and of pubertal development of adolescents in Iranian villages. The existence of protein deficiency of moderate degree in village boys is confirmed by the present study. However, repletion of protein in group B was not followed by a stimulus to growth comparable to that produced when extra zinc was provided. The results support the belief that zinc is a principal limiting factor in the nutrition of children when the intake of unleavened wholemeal bread is high. 

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TABLE 5
Biochemical studies
Group A (placebo supplement only)

	Serum										
	Zinc, $\mu\text{g}/100\text{ ml}$		Ca., mg/100 ml		Total protein, g/100 ml	Albumin, g/100 ml	Gamma globulin, g/100 ml	Alkaline phosphatase, units	P, mg/100	Iron, $\mu\text{g}/100\text{ ml}$	Transferrin concentration, $\mu\text{g}/100\text{ ml}$
	Plasma	RBC	Plasma	RBC							
1969											
Nov. 4											
Mean	64		9.7		6.5	3.0	1.9	5.9			
SE	3.4		0.13		0.24	0.08	0.16	1.13			
Abnormal	9 ^a		0		3 ^a	10 ^a	8 ^b	0			
Normal	1		10		7	0	2	7			
1970											
Feb. 10											
Mean	77	8.6	9.5		7.0	3.4	1.7	7.9	4.3		
SE	3.4	1.47	0.09		0.10	0.17	0.09	0.45	0.13		
Abnormal	4 ^a	1 ^a	0		0	6 ^a	9 ^b	3 ^b	0		
Normal	3	2	4		10	4	1	7	10		
May 22											
Mean	70		9.7		6.8	3.4	1.7	6.9	5.7	91	
SE	4.7		0.17		0.12	0.14	0.08	0.53	0.21	6.6	
Abnormal	5 ^a		0		0	3 ^a	7 ^b	1 ^b	0	2 ^a	
Normal	2		7		8	5	1	7	8	8	
Oct. 11											
Mean	58	9.4	9.4		6.8	4.0	1.4	7.2	4.5	74	264
SE	3.8	0.67	0.13		0.12	0.22	0.13	0.41	0.15	5.9	15
Abnormal	8 ^a	3 ^a	1 ^a		0	1 ^a	5 ^b	0	0	4 ^a	4 ^a
Normal	1	6	8		9	8	5	9	9	5	5
1971											
May 11											
Mean	74	9.1	9.3		6.7	4.2	1.26	8.0	4.2	105	316
SE	5.3	0.46	0.23		0.25	0.17	0.09	1.6	0.17	9.6	18
Abnormal	4 ^a	3 ^a	2 ^a		1 ^a	0	0	7 ^b	0	0	1 ^a
Normal	4	4	6		7	8	7	0	8	7	5

Group B (complete supplement without zinc)

TABLE 5 - Group C Continued

	Serum										
	Zinc, µg/100 ml		Ca, mg/100 ml		Total protein, g/100 ml	Albumin, g/100 ml	Gamma globulin, g/100 ml	Alkaline phosphatase, units	P, mg/100	Iron, µg/100 ml	Transferrin concentration, µg/100 ml
	Plasma	RBC	Plasma	RBC							
1970											
Feb. 10											
Mean	54	6.7	9.2	6.7	6.7	3.4	1.7	8.0	4.7		
SE	1.7	0.27	0.10	0.08	0.08	0.06	0.06	0.56	0.15		
Abnormal	19 ^a	9 ^a	2 ^a	0	0	6 ^a	15 ^b	6 ^b	0		
Normal	0	0	16	19	19	11	4	1	17		
May 22											
Mean	64	9.7	9.7	6.7	6.7	3.3	1.8	6.0	5.0	83	
SE	3.3	0.10	0.10	0.12	0.12	0.08	0.09	0.28	0.24	3.8	
Abnormal	9 ^a	0	0	1 ^a	1 ^a	7 ^a	13 ^b	2 ^b	0	4 ^a	
Normal	3	11	11	15	15	9	3	14	16	9	
Oct. 11											
Mean	65	8.3	9.5	6.7	6.7	3.8	1.5	7.1	4.7	93	273
SE	3.7	0.36	0.07	0.20	0.20	0.14	0.10	0.49	0.13	12	14
Abnormal	12 ^a	9 ^a	0	2 ^a	2 ^a	1 ^a	7 ^b	1 ^b	1 ^a	6 ^a	6 ^a
Normal	2	5	14	12	12	13	7	13	13	8	8
1971											
May 11											
Mean	67	7.8	9.6	6.5	6.5	4.3	1.5	8.7	4.3	113	382
SE	3.5	0.39	0.08	0.54	0.54	0.12	0.043	0.70	0.11	7.2	18
Abnormal	9 ^a	6 ^a	0	0	0	0	6 ^b	4 ^b	0	0	2 ^b
Normal	3	5	12	12	12	12	6	9	13	13	11
Expected values found in well-nourished 13-year-old boys ^c											
Minimum	75	8.5	8.8	6.0	6.0	3.3	0.8	3.4 ^d	3.7 ^e	75	250
Mean	95	11.0	9.5	6.9	6.9	4.1	1.1	9.0	5.5	113	350
Maximum	115	14.0	10.2	7.8	7.8	4.9	1.4	9.0	7.0	175	450

^a Below lower limit found in healthy prepubertal boys. ^b Above upper limit found in healthy prepubertal boys. ^c Based on findings in young adults, well-nourished and in good health. Other than for alkaline phosphatase activity and phosphorus concentrations in serum, no difference due to age is to be expected. ^d From (26). ^e From (27).

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