erably higher than those reported by other methods. Results for samples from our study group measured simultaneously with the RA-1000 were approximately 30% lower than the Ektachem-700 range. Despite the differences in reference intervals measured with the two analyzers, both sets of data showed an obvious decreasing trend with age.

The reference interval for amylase in children ages one to 19 years was 30–100 U/L (n = 470), similar to the adult normal range. No sex-related difference was noted. Although Aggett and Taylor reported a different range, using a Phadebas blue starch method, our study supports their finding that adult amylase concentrations may be reached by one year of age (*12*).

The reference interval for lipase was 15–120 U/L for boys and girls between ages four and 19 years. Data from younger children were not available.

Age- and sex-specific reference intervals were determined in a pediatric population of multiple ethnic origins, for analytes measured with the Ektachem-700 multilayer film analyzer. Except for LDH and GGT, these reference intervals were not strikingly instrument dependent. The *absolute values* for age- and sex-specific upper and lower reference limits for an analyte may differ from data obtained by using a different method, thus making it incumbent on each laboratory to determine the direct transferability of these data to its own specific method and equipment. However, the *trend* of reference limits to increase or decrease with increasing age, and the sex-related effect on reference limits for some variables in postpubertal children, were method independent. This study provides a framework for comparison of reference data obtained by other methods.

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Age- and Sex-Specific Pediatric Reference Intervals and Correlations for Zinc, Copper, Selenium, Iron, Vitamins A and E, and Related Proteins

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Age- and sex-specific reference intervals based on the 0.025 and 0.975 fractiles of data derived from a healthy pediatric population are presented for zinc, copper, selenium, iron, ferritin, retinol, α-tocopherol, and related analytes in serum. Age was an important covariate for copper, selenium, retinol, and tocopherol, and ferritin in boys. Strong correlations were found between retinol and retinol-binding protein, prealbumin (transthyretin), α-tocopherol, and selenium. Tocopherol was highly correlated with both cholesterol and triglycerides. We found no relationship between serum zinc and either retinol or retinol-binding protein. Despite exclusion of children in whom anemia, microcytosis, or variant hemoglobins were found, the 0.025 fractile for iron in several age groups was even less than the concentration considered to indicate poor iron nutritional status.

Additional Keyphrases: *tocopherol · retinol · retinol-binding protein · transthyretin · cholesterol · triglycerides*

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We have defined age- and sex-specific reference intervals for serum zinc, copper, selenium, retinol, α-tocopherol, iron, transferrin saturation, and ferritin in a healthy pediatric population.

Materials and Methods

The study population and the general and statistical methods are described elsewhere (*1*). Specific precautions were observed throughout this study to minimize sample contamination for the trace-element analyses and prevent

photodestruction of vitamin A. Blood was collected by venipuncture into siliconized Vacutainer Tubes (Becton-Dickinson, Mississauga, Ontario, L5J 2M8, Canada), containing no anticoagulant, for trace-element analysis. The second tube collected from each child was used for the trace-element studies.

Samples were covered in foil and kept upright to clot, to prevent contact with the stopper. After centrifugation (1300 × *g*, 10 min), aliquots of serum were removed for the trace-element studies and recentrifuged to ensure that no erythrocytes were present. Hemolyzed samples were excluded from the study. Aliquots were stored at −70 °C until analysis. Samples for trace-element studies in neonates were specifically collected for this purpose at the time of other blood sampling. Because of sample constraints and expense, trace elements were measured for only a small subgroup of the healthy term newborns.

Zinc was measured by atomic absorption spectrometry with deuterium background correction, with a microsampling technique, in the Varian AA-1475 instrument (Varian Canada Inc., Georgetown, Ontario, Canada). Copper and selenium were measured by electrothermal atomic absorption spectroscopy in a Varian GTA-95 instrument with deuterium background correction (*2*). Iron was measured by a Ferrozine method (*3*), with Sigma Chemical Co. reagents, in the RA-1000 random-access analyzer (Technicon Instruments, Tarrytown, NY). Transferrin was measured by nephelometry (Behring LN kit; Behringwerke, Marburg F.R.G.), and transferrin-binding capacity and saturation were calculated (*4, 5*). Ferritin was measured by immunoassay with "Ferrizyme" reagents (Abbott Laboratories, Ltd., Diagnostics Division, Mississauga, Ontario, L5N 3R7 Canada) in the Abbott Quantum II analyzer. Retinol and α-tocopherol were simultaneously quantified by "high-performance" liquid chromatography (HPLC), with modifications of reported methods (*6, 7*). HPLC equipment and the C_{18} guard column were from Waters Chromatography Division, Milford, MA 01757. The analytical column (250 × 4.6 mm, steel, packed with 10-μm RSIL C_{18} HL) was from Alltech Associates, Inc., Deerfield, IL 60015. Because not every analyte was measured for each child, sample numbers vary for different analytes.

Results and Discussion

Nineteen children were found to have unsuspected anemia, thalassemia syndromes, or variant hemoglobins (confirmed by electrophoresis). As part of an associated study, we had derived age-related reference ranges for erythrocyte mean cell volume. Microcytosis was defined as a mean cell volume below the 0.025 fractile for the given age interval.

Table 1. Age- and Sex-Specific Reference Intervals (0.025–0.975 Fractiles) and Quality-Control Data for Zinc, Copper, and Selenium in Serum

		Zinc	Copper	Zinc:copper		Selenium
Age group, y	n	μmol/L		ratio	n	μmol/L
(0–5 d)	27	9.9–21.4	1.4– 7.2	0.5–1.1	20	0.72–1.20
1–5	77	10.3–18.1	12.6–23.6	0.5–1.1	30	1.22–1.82
6–9	44	11.8–16.4	13.2–21.4	0.6–1.1	30	1.29–2.05
10–14					30	1.31–2.35
15–19					27	1.31–2.35
10–14	36♂	11.6–15.4	12.6–19.0	0.4–1.1		
15–19	55♂	9.8–17.9	10.1–18.4	0.8–1.4		
10–14	23♀	12.1–18.0	12.9–18.9	0.6–1.3		
15–19	31♀	9.2–15.4	11.3–25.2	0.5–1.2		
		Mean CV, %	Mean CV, %			Mean CV, %
Quality-control data						
Level 1		21.1 3.9	30.2 2.9			2.98 4.6
Level 2		11.6 5.0	15.3 3.9			1.88 3.4
Level 3		5.7 7.3	7.1 4.9			1.28 5.7

Table 2. Age- and Sex-Specific Reference Intervals (0.025–0.975 Fractiles) and Quality-Control Data for Iron and Related Variables in Serum

		Iron	CTIBC[a]		Ferritin,
Age group, y	n	μmol/L		TRF-SAT[a]	μg/L
1–5	44	4–25	48–79	0.07–0.44	6–24
6–9	50	7–25	43–91	0.17–0.42	10–55
Males					
10–14	31	5–24	54–91	0.11–0.36	23–70
14–19	65	6–29	52–102	0.06–0.33	23–70
Females					
10–14	40	8–26	57–103	0.02–0.40	6–40
15–19	110	5–33	52–101	0.06–0.33	6–40
		Mean CV, %			Mean CV, %
Quality-control data					
Level 1		40 1.8			388 4.6
Level 2		17 2.5			81 6.3

[a] Calculated transferrin iron-binding capacity (CTIBC) and transferrin saturation (TRF-SAT) calculated as suggested by Tsung et al. (*4*).
CTIBC (μmol/L) = transferrin (g/L) × 23.1.
Transferrin saturation = iron (μmol/L)/CTIBC.

Data from children with abnormal hemoglobins, anemia, or microcytosis were excluded from consideration in the studies of iron and related parameters.

Reference intervals are shown for serum analytes zinc, copper, and selenium in Table 1; for iron, transferrin, transferrin saturation, and ferritin in Table 2; and for retinol, α-tocopherol, and tocopherol:lipid ratios in Table 3.

Age- and Sex-Related Effects on Concentrations in Serum

Serum concentrations of zinc declined slightly with increasing age in girls ($r = -0.28, P < 0.001$, n = 150) but not in boys ($r = -0.09, P = 0.28$, n = 116). There was a marked sex-related difference in the decrease of copper in serum with age. The decrease in copper in boys was significant ($r = -0.59, P < 0.001$, n = 116), the negative correlation being less for girls ($r = -0.2, P = 0.02$, n = 147). Boys older than 10 years showed a significant decrease in serum copper, whereas values were high for a subgroup of older girls. The increase in some of the postpubertal girls was probably ascribable to higher concentrations of ceruloplasmin, as has been noted elsewhere (*1*); it may also be due to a pubertal estrogen effect or to use of oral contraceptives (*8, 9*).

Selenium increased steadily in both sexes from the neonatal period until around 10 years, when adult values were reached. For both selenium and copper, values in the term neonate are about 50% of adult values, whereas the reference interval for zinc in serum from the neonate is similar to that of the adult.

Iron in serum did not change with age, but transferrin increased in both sexes, such that a slight, but not significant, decrease of transferrin saturation occurred with increasing age. Ferritin increased with age in boys ($r = 0.49, P < 0.001$, n = 42) but not in girls ($r = 0.1, P = 0.42$, n = 70). Despite the exclusion of data from children with anemia, microcytosis, or thalassemia syndromes, our lower reference intervals for serum iron and transferrin saturation, as defined by the 0.025 fractile for this healthy pediatric population, are below the limits used to define "less than acceptable" for nutritional health in other studies (*10*).

Concentrations of retinol ($r = 0.6, P < 0.001$, n = 180) and α-tocopherol ($r = 0.3, P < 0.001$, n = 162) also increased with age. As recommended by Horwitt et al. (*11*), we expressed the tocopherol concentration in terms of tocopherol:lipid ratio. The ratios of tocopherol to cholesterol and tocopherol to lipid (cholesterol + triglyceride) were constant between ages one and 19 years. Lower limits of tocopherol:cholesterol were similar to those previously reported for children (*11, 12*). Unlike the strong correlation of tocopherol with cholesterol ($r = 0.6, P < 0.001$, n = 148), the relationship for retinol was weaker ($r = 0.23, P = 0.003$, n = 161).

Relationships among Concentrations of Micronutrients in Serum

Vitamins A and E. Strong correlations were found between the concentration of retinol in serum and the concentration of α-tocopherol [$r = 0.3$, n = 159, $P < 0.001$, vitamin A = 0.8 + (0.02 × vitamin E)] and selenium [$r = 0.5$, n = 58, $P < 0.001$, vitamin A = 0.07 + (0.8 × selenium)]. Retinol correlated highly with retinol-binding protein [$r = 0.6$, n = 116, $P < 0.001$, vitamin A = 0.8 + (0.01 × retinol-binding protein)] and prealbumin, as would be expected, owing to the tight binding of retinol to its binding protein and the association of that complex with prealbumin in the circulation (*13*).

Although serum zinc may be involved in the mobilization of vitamin A from the liver (*13–15*), we found no correlation between serum zinc and retinol, retinol-binding protein, or prealbumin (Figure 1). This differs from one previous report (*14*), but is consistent with other previous findings (*16*). In several disease states, zinc status clearly alters retinol and

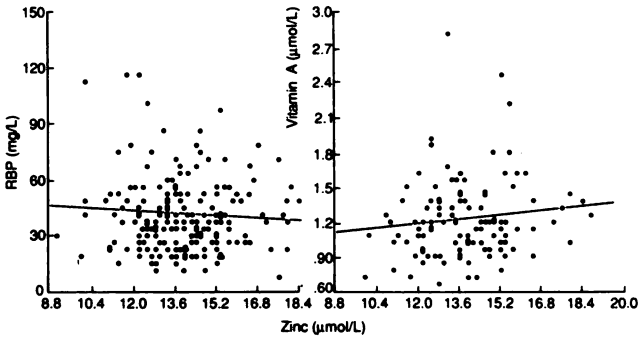

Fig. 1. Relationship of zinc concentration in serum to (*left*) retinol-binding protein [$r = -0.04$, n = 176, $P = 0.6$, RBP = 48 − (0.47 × zinc)] and (*right*) vitamin A [$r = 0.1$, n = 109, $P = 0.14$, vitamin A = 0.8 + (0.03 × zinc)]

Table 3. Age- and Sex-Specific Reference Intervals (0.025–0.975 Fractiles) and Quality-Control Data for Retinol, Tocopherol, and for Tocopherol:Cholesterol (T:C), Tocopherol:Triglyceride (T:T), and Tocopherol:Lipid (T:L) Ratios

		Retinol		Tocopherol				
Age group, y	n	μmol/L				T:C	T:T	T:L
1–6	62	0.7–1.5		7–21		3–6	8–33	3–5
7–12	23	0.9–1.7		10–21		3–6	10–53	2–5
13–19	24	0.9–2.5		13–24		3–5	11–32	2–4
		Mean	CV, %	Mean	CV, %			
Quality-control data								
Patients' pooled sera		1.63	2.6	26	3.5			

Table 4. Correlations between Trace-Element Concentrations in Serum

	Copper			Selenium			Iron		
	n	r	P	n	r	P	n	r	P
Zinc	261	0.06	0.32	23	−0.11	0.59	150	−0.09	0.28
Copper				24	−0.13	0.54	146	−0.07	0.36
Selenium							53	0.17	0.20

retinol-binding protein concentrations (*15*) but there does not appear to be such a relationship in the healthy child.

The independent correlation between retinol and α-tocopherol is interesting. This has been reported previously in vitamin-supplemented individuals but was not seen in normal children (*17*). Retinol and α-tocopherol are absorbed (*18*) and transported in the circulation (*13, 19*) by different mechanisms, so neither of these factors explains the relationship between these concentrations. Tocopherol is associated with circulating lipoproteins, predominantly low-density lipoprotein, in normal individuals (*19*), which accounts for the strong positive correlation of tocopherol with total cholesterol and with lipid (cholesterol + triglyceride), and the weaker correlation with triglyceride. Expressing tocopherol in terms of cholesterol eliminates the age dependence of tocopherol concentrations in children (Table 3). There was no independent correlation of retinol or tocopherol with iron, copper, or zinc.

Zinc, copper, iron, selenium. No correlation was found between any pairs of the four elements studied (Table 4). The reference interval for the zinc:copper ratio also remained fairly constant over all ages, ranging from a low of 0.4 to a high of 1.3. Although Klevay (*20*) has claimed that an increase in the dietary zinc:copper ratio increases cholesterol and promotes atherosclerosis, we found only a weak association of the serum zinc:copper ratio with serum cholesterol concentration in our study ($r = 0.18, P = 0.08$, n = 94).

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