Reference values for plasma concentrations of vitamin E and A and carotenoids in a Swiss population from infancy to adulthood, adjusted for seasonal influences

Brigitte M. Winklhofer-Roob, Martin A. van’t Hof, and David H. Shmerling

In a cross-sectional survey, plasma concentrations of α- and γ-tocopherol, α- and β-carotene (cis and trans isomers), lycopene, and retinol were determined by reversed-phase HPLC, and ratios of plasma α-tocopherol to cholesterol were calculated in 208 Swiss individuals ages 0.4–38.7 years. The influence of age, sex, and season of sampling was studied. Age was a significant predictor of all plasma concentrations except α-carotene. No sex-related differences were observed. Season of sampling affected α-tocopherol and retinol (higher in winter) and γ-tocopherol and cholesterol concentrations (higher in winter and spring than in the other seasons). After correction for seasonal influences, age differences were 0.24 μmol/L per year for α-tocopherol, 0.04 μmol/L per year for retinol, and 0.04 μmol/L per year for cholesterol concentrations; ratios of plasma α-tocopherol to cholesterol were not affected by age. We constructed age-specific reference intervals from the regression line and a multiple of the standard deviation. Separate regression equations are presented for seasons with low and high values.

INDEXING TERMS: tocopherol • lycopene • retinol • carotene • cholesterol • age-related effects • pediatric chemistry • variation, source of • epidemiology

Both in health and disease, increasing interest is being directed to the significance of optimal vitamin and antioxidant status. Evidence has accumulated from epidemiological studies that serum α-tocopherol concentrations and mortality from ischemic heart disease are inversely related [1–3], as are serum carotenoid concentrations and the incidence of several types of cancer [4]. Additional significance regarding several aspects of immune function has also been recognized [5, 6].

Antioxidant status evidently varies considerably among populations [1]. Moreover, changes over time in dietary habits affect not only the intake of fruits and vegetables but also the consumption of polyunsaturated fatty acids, all of which influence the oxidant–antioxidant balance.

Impaired antioxidant status has been identified in several disorders, such as cholestatic liver disease [7], exocrine pancreatic insufficiency [8], nutritional deprivation due to protein calorie malnutrition [9], and acquired immunodeficiency syndrome (AIDS) [10]. Intervention studies for correction of these deficiencies have been conducted or are still in progress [11–13]. In these situations, definition of threshold values for initiation of intervention and successful treatment is critical. Preferably, reference values obtained from the general healthy population living in the same area should be used to define this threshold.

Optimal nutrition in childhood and adolescence is becoming increasingly a general concern because development of certain diseases may be determined very early in life. Autopsy studies, for instance, confirm the presence of atherosclerotic lesions in the aortas and coronary arteries of teenagers and young adults [14]. In general, vitamin status is believed to differ between children and adults, but data comparing concentrations of different antioxidant vitamins and micronutrients between childhood and adulthood in the same population are limited to French [15] and Japanese individuals [16]. Additional data have focused only on α-tocopherol [17, 18] and retinol concentrations [19] in North American [17] and Hispanic individuals [18, 19].
The purpose of this study was to determine cross-sectional age trends and reference intervals for plasma concentrations of tocopherols, carotenoids, and retinol in a Swiss pediatric and adult population living in the Zurich area.

Materials and Methods

STUDY POPULATION
The study population comprised 208 Swiss individuals—92 males and 116 females, median age 12.6 years (range, 0.4–38.7 years)—who lived in the Zurich area. Participants in the pediatric age range were patients attending the hospital either for orthopedic problems and follow-up visits after orthopedic or orthognathic surgery, ear–nose–throat problems, minor surgery, or for constitutional short stature. None had gastrointestinal or metabolic disorders likely to affect their nutritional status. All had venipunctures for the medical problem leading to their hospital visit, and an additional 2 mL of blood was drawn for this study. Adults volunteering for this investigation were clinically healthy nonsmoking staff members from the same hospital. None was taking vitamin supplements. A greater number of pediatric participants than adults were enrolled because greater variability was expected in children and adolescents than in young and middle-aged adults. Plasma samples were collected between June 1991 and September 1993; 52 samples were obtained in spring (March–May), 31 in summer (June–August), 30 in fall (September–November), and 95 in winter (December–February). Age was recorded in all cases to the completed month, in decimals, each month being 0.08 years. The study was approved by the Ethics Committee of the Department of Pediatrics, University of Zurich.

ANALYTICAL METHODS
Blood was drawn into EDTA (1.6 g/L)-containing tubes (Sarstedt Monovette; Nürmbrecht, Germany) from fasted individuals, protected from light with aluminum foil, and centrifuged without delay at 2000g for 8 min at room temperature. Plasma was separated and kept at −20 °C for no more than 4 days before analysis. Plasma α- and γ-tocopherol, α- and β-carotene, lycopene, and retinol concentrations were determined by reversed-phase HPLC according to the method of Hess et al. [20] in the laboratory of the Vitamin Research Department of F. Hoffmann-La Roche, Basel, Switzerland. Within-day reproducibility (CV) was 2.3% for α-tocopherol, 2.4% for γ-tocopherol, 9.4% for α-carotene, 4.3% for β-carotene, 2.1% for lycopene, and 2.0% for retinol as described [20]. Plasma cholesterol concentrations were determined in the same laboratory with a COBAS-BIO instrument (F. Hoffmann-La Roche) and a diagnostic kit (Merckotest 14350; Merck, Darmstadt, Germany) for which the CV was 4%. Plasma α-tocopherol:cholesterol ratios were calculated as the index of choice for vitamin E status [21].

STATISTICAL ANALYSIS
To obtain approximately gaussian distributions, we had to log-transform the measurement data for plasma γ-tocopherol, β-carotene, and lycopene concentrations and α-tocopherol:cholesterol ratios. Analysis of covariance was used to study the effects of age, age2, sex, and season of sampling on plasma concentrations of the antioxidant vitamins and micronutrients, assuming an absence of interaction. The influence of age2 was considered so we could study deviations from linearity with age. For all variables that were dependent on age, the relationship with age was best fitted by a linear regression line. The data obtained in this study were not sufficient to determine whether the standard deviation (SD) would change with age; therefore, we assumed a constant SD over the whole age range. Because no sex-related differences were evident, we combined the data for males and females. The raw data for variables that showed significant seasonal influences were corrected accordingly. For those variables for which age was an influential factor, we constructed age-specific reference intervals from the regression lines plus or minus 1.645 SD to obtain the central 90% prediction limits (corresponding to the 5th through 95th percentiles). For analytes that did not show an age-dependency, the 5th and 95th percentiles were calculated for the results over the whole age range. To construct the age-specific reference intervals, we used the methodology described recently by Royston [22] as far as was applicable. Partial correlations were used to study the effects of age and cholesterol concentrations on the different variables adjusted for seasonal influences. SPSS-X release 4.1 (SPSS, Chicago, IL) and Statgraphics Plus for Windows (Manugistics, Rockville, MD) were used for statistical procedures. P <0.05 was considered significant.

Results

SOURCES OF VARIATION
Analysis of covariance showed a significant effect of age on the plasma concentrations of all of the analytes except on α-carotene, but no effect of sex. Therefore, age-specific but not sex-specific reference intervals were calculated.

Influences of season of sampling. Plasma α-tocopherol and retinol concentrations were highest in winter, and γ-tocopherol and cholesterol concentrations were higher in winter and spring than in the other seasons; the other variables did not show significant seasonal influences. The data points presented in Fig. 1 are corrected for these seasonal influences and represent the values from the seasons with the lower values. The plasma α-tocopherol:cholesterol ratios were higher in summer and in winter than in the other seasons; however, the numerator and the denominator of this ratio each has its own pattern of seasonal changes, making any differences in the combined data difficult to interpret. We therefore decided not to correct for seasonal influences the raw data for the ratio.
Fig. 1. Plasma concentrations of antioxidant vitamins and micronutrients in 208 randomly selected Swiss individuals plotted against age.

Reference values for plasma vitamin E and A and carotenoids

Influence of age on plasma concentrations of antioxidant vitamins and micronutrients. Plasma vitamin A concentrations increased by 1.5% per year, and plasma carotenoid concentrations increased by 1.6% per year. Reference intervals for the two variables that did not show significant age differences were calculated for the whole age range (Table 2).
Table 1. Linear regression analysis on age dependency of the different antioxidants and micronutrients determined in 208 Swiss individuals.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Season*</th>
<th>Regression equation</th>
<th>% residuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontransformed data</td>
<td>Intercept</td>
<td>Slope</td>
<td>r</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>Nonwinter</td>
<td>18.6</td>
<td>0.240</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>21.1</td>
<td></td>
</tr>
<tr>
<td>Retinol</td>
<td>Nonwinter</td>
<td>0.81</td>
<td>0.037</td>
</tr>
<tr>
<td></td>
<td>Winter</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Summer + fall</td>
<td>3.49</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>Winter + spring</td>
<td>3.80</td>
<td></td>
</tr>
<tr>
<td>Loge-transformed data</td>
<td>Intercept</td>
<td>Slope</td>
<td>r</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>Summer + fall</td>
<td>-0.30</td>
<td>0.0156</td>
</tr>
<tr>
<td></td>
<td>Winter + spring</td>
<td>-0.07</td>
<td></td>
</tr>
<tr>
<td>β-Carotene</td>
<td>All</td>
<td>-0.56</td>
<td>0.0129</td>
</tr>
<tr>
<td>cis-β-Carotene</td>
<td>All</td>
<td>-1.45</td>
<td>0.0117</td>
</tr>
<tr>
<td>trans-β-Carotene</td>
<td>All</td>
<td>-0.60</td>
<td>0.0131</td>
</tr>
<tr>
<td>Lycopene</td>
<td>All</td>
<td>-1.00</td>
<td>0.0160</td>
</tr>
<tr>
<td>No age dependency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loge α-tocopherol:cholesterol ratio</td>
<td>All</td>
<td>1.67</td>
<td>0.0018</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>All</td>
<td>0.41</td>
<td>-0.0025</td>
</tr>
</tbody>
</table>

* For analytes with significant seasonal influences, different intercepts for seasons with low and high values are shown.

The mean value for the reference population at a given age can be calculated according to \( y = \text{intercept} + \text{slope} \times \text{age} \). To obtain the 90% reference range (5th to 95th percentiles), subtract from and add to the mean value for the reference population a multiple of the SD \((1.645 \times \text{SD})\).

Table 1 provides the information necessary for calculating the reference intervals for these analytes in subjects of a given age from the regression equation \( y = \text{intercept} + \text{slope} \times \text{age} \) and the residual SDs. Where indicated, separate intercepts for seasons with low and high values are presented. These data can further be used to calculate z-scores for comparing individual measurement data obtained in clinical and research settings with the reference values presented here. An example for the calculations is shown in the Appendix.

**Correlation with Cholesterol**

As a constitutional component of the lipoproteins, cholesterol exerts a carrier function for the lipophilic antioxidants in blood. We therefore wondered whether the increase in cholesterol concentrations might have determined the increase of these analytes with age. Partial correlations between age, cholesterol, and plasma concentrations of the various antioxidants and vitamins (corrected for seasonal influences) are compared with Pearson correlations in Table 3, which shows that all analytes except α-carotene are correlated with cholesterol concentrations. Correlations with cholesterol were stronger than the correlations with age for α-tocopherol and β-carotene but not for retinol.

**Discussion**

The data presented provide information about season-adjusted current plasma concentrations and cross-sectional age trends from infancy to adulthood for toco-
Table 3. Correlation coefficients for age and plasma cholesterol concentrations with concentrations of antioxidant vitamins and micronutrients in 208 individuals.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age</th>
<th>Cholesterol*</th>
<th>Partial correlation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Tocopherol*</td>
<td>0.50</td>
<td>0.76</td>
<td>0.70</td>
</tr>
<tr>
<td>γ-Tocopherol (loga)</td>
<td>0.33</td>
<td>0.37</td>
<td>0.27</td>
</tr>
<tr>
<td>β-Carotene (loga)</td>
<td>0.22</td>
<td>0.36</td>
<td>0.31</td>
</tr>
<tr>
<td>cis-β-Carotene (loga)</td>
<td>0.19</td>
<td>0.23</td>
<td>0.16</td>
</tr>
<tr>
<td>trans-β-Carotene (loga)</td>
<td>0.23</td>
<td>0.35</td>
<td>0.29</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>-0.09</td>
<td>-0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Lycopene (loga)</td>
<td>0.31</td>
<td>0.42</td>
<td>0.33</td>
</tr>
<tr>
<td>Retinol*</td>
<td>0.70</td>
<td>0.52</td>
<td>0.34</td>
</tr>
</tbody>
</table>

* Adjusted for seasonal influences.

a Partial correlation with cholesterol (adjusted for seasonal influences), controlled for age.

Specific reference intervals. Depending on the reference values being considered, more or less complex and sophisticated curve-fitting methodologies have been proposed. To ensure that the linear model was the one that best fit the data, we checked several characteristics: e.g., the effect of age, the gaussian distribution of the sample, and the validity of the calculated 90% reference intervals.

Sex-related differences were not observed for any of the antioxidant vitamins and micronutrients under investigation—which agrees well with some other studies [18, 25, 26] but contrasts with those that reported higher plasma retinol concentrations in males and higher α- and β-carotene concentrations in females [15, 16, 23, 24, 27]. Whether this reflects a lack of differences in dietary habits between men and women in the Swiss population, in contrast to the case in other populations, remains to be investigated.

Season of sampling had a significant effect on plasma concentrations of α- and γ-tocopherol and retinol but not on carotenoids. For all three, the highest concentrations were found in winter, and γ-tocopherol was also high in spring. The effects of season (winter vs nonwinter) were estimated to be 2.48 µmol/L for α-tocopherol (16% of mean α-tocopherol concentrations) and 0.23 µmol/L (21%) for retinol; the effects for γ-tocopherol and cholesterol (winter vs spring and summer and fall) were 25% of mean values for γ-tocopherol and 9% for cholesterol concentrations. Further interpretation of these seasonal trends is limited by the cross-sectional nature of this study. Only a longitudinal study, combined with dietary intake data, will be able to elucidate possible determinants of these trends. The influence of season on α-tocopherol concentrations found in this study contrasts to Finnish [28] and Spanish [27] observations of stable α-tocopherol concentrations. That season of sampling did not influence plasma carotenoid concentrations is in agreement with a North American study [29] but contrasts with a Spanish investigation showing highest α- and β-carotene concentrations in summer [27] and a Finnish study with the highest values in fall [28].

Cholesterol concentrations increased from childhood to adulthood as shown before [30]. In a longitudinal study a decrease in cholesterol concentrations during the early teenage years and a subsequent increase have been reported, with these changes occurring ~2 years earlier in girls than in boys [31]. A recent analysis of data from adults (ages 27 to 62) showed an increase not only in the mean values but also in the spread (SD) of cholesterol concentrations, particularly after the age of 40 [22]. Except for French children with serum cholesterol concentrations as high as 4.5 mmol/L [26], values in other populations, e.g., Italian [25], English [25], and North American adults [24, 32], were comparable to those found in this study.

Plasma α-tocopherol concentrations were strongly correlated with cholesterol concentrations, confirming the findings of other studies [11, 17, 21]. Partial correlations, controlling for the age effect, showed correlations be-
tween cholesterol and all variables except a-carotene. The correlations for a-tocopherol and β-carotene were clearly stronger than their correlations with age, whereas the opposite was true for retinol concentrations. These results suggest that the increase of almost all variables with age can at least in part be attributed to the age-related increase in cholesterol concentrations.

Because of the close correlation between plasma a-tocopherol and lipid concentrations, both plasma a-tocopherol:cholesterol and a-tocopherol:total lipids ratios have been proposed as desirable indexes of vitamin E status [21]. Using the former ratio alleviated the changes with age observed in both numerator and denominator; the ratio was practically constant over the whole age range. This finding was unexpected because (a) supplementation studies have shown that lipoproteins can be loaded quite extensively with vitamin E, resulting in plasma a-tocopherol:cholesterol ratios that exceeded 10 mmol/mol, with only minor indications of a plateau effect [11]; (b) a close relation of the intake of a-tocopherol and cholesterol cannot be expected; and (c) the mechanisms for regulation of blood concentrations of cholesterol are not the same as those for a-tocopherol. As for all other vitamins and micronutrients included in this study, we did not attempt to investigate any possible determinants of their plasma concentrations except for correlations with cholesterol concentrations.

Comparison with other populations was restricted to plasma a-tocopherol concentrations because plasma a-tocopherol:cholesterol ratios were not presented in most studies. Swedish [33] and Japanese children [16] had clearly lower plasma a-tocopherol concentrations than did French [15, 26] and Swiss children of this study. Spanish [27] and Southern Italian adults [25] showed higher concentrations than our Swiss adult population and than English [25], Austrian [34], and Finnish adults [35]. Thus, a European north–south gradient was evident, with the Swiss population showing intermediate concentrations. Data from North American adults showed relatively low concentrations, even though 18% took vitamin E supplements [23]. Similar concentrations were found for nonusers in another study, whereas those for users of vitamin E supplements were substantially higher [24].

Plasma γ-tocopherol accounts for only a minor proportion of vitamin E in the blood, most likely because of preferential incorporation of α-tocopherol into lipoproteins [36]. In the Swiss population we studied, γ-tocopherol concentrations increased with age, but to a lesser extent than did α-tocopherol. Appropriate data for comparison of plasma γ-tocopherol concentrations in other populations were not available.

Among all vitamins and micronutrients investigated, retinol concentrations showed the strongest dependency on age: a positive slope of 0.04 μmol/L per year. This increase is in agreement with other studies [15, 16, 26]. Malvy et al. [26] showed that postpubertal values tend to be slightly higher than prepubertal values and that fluctuations with age are more pronounced in males than in females. They also showed that concentrations of the main carrier for retinol in plasma, retinol-binding protein, increase throughout childhood. In contrast to studies conducted in Finland [28], Spain [27], France [15, 26], and Japan [16], which showed generally higher plasma retinol concentrations in males, no sex-related difference was observed in our study population. Values in the Swiss pediatric age group of this study were lower than those in Swedish [33] children and one group of French children [26], but were comparable with those in another group of French children [15]. Those in adults of this study were lower than in Finnish [35] and Japanese individuals [16] but comparable with those in Spanish adults [27]. A survey in a North American population showed plasma retinol concentrations of 2.18 μmol/L for men and 1.84 μmol/L for women; roughly a third of these individuals were taking vitamin A supplements [23].

A recent study compared English and Southern Italian adults and found exceptionally high plasma concentrations of β-carotene in both groups [25]. In contrast, Austrian [34], Spanish [27], and North American adults [23], Japanese individuals over the whole age range [16], French children [26] and adults [15], and the Swiss population of this study showed considerably lower values—which raises the question of analytical differences.

A few investigations have shown that all-trans-β-carotene is predominant in human plasma [37–40]. Cis isomers are present in various fruits and vegetables, and it is not clear whether isomerization reactions, including potential isomer discrimination at the uptake or transport level, might explain why 9- and 13-cis-isomer concentrations are generally very small in plasma compared with trans-β-carotene but are present in higher concentrations in tissues [37]. This study, which comprises the most comprehensive data currently available on total cis and trans isomers of β-carotene in human plasma, shows a 5% contribution of the cis form to total plasma β-carotene. Of the studies that differentiated between 9- and 13-cis isomers of β-carotene, some identified 9-cis (10% of all-trans-β-carotene) [38, 39] and some 13-cis (5% of total β-carotene) [37, 40] as the main cis isomer of β-carotene in plasma.

Another carotenoid, lycopene, exerts only one-twelfth the vitamin A activity of β-carotene but is a more efficient quencher of singlet oxygen than is β-carotene [41]. Comparison with the few data available showed that the Swiss study population had higher plasma concentrations of lycopene than did Spanish [27], Japanese [16] and Austrian individuals [34], whereas the concentrations in North American adults were even slightly higher [23]. Plasma α-carotene concentrations in the Swiss population were comparable with those in Japanese [16], about twice as high as in Spanish [27], and three times those in North American adults [23].
In summary, season-adjusted actual reference intervals for ages 0–40 years were established in a Swiss population living in the German-speaking part of Switzerland. These values may be useful for comparison of plasma concentrations both in healthy subjects and in patients with various disorders who are at risk for developing deficiencies in these vitamins and micronutrients.

The authors thank Willy Schüep and Richard Salkeld, Vitamin Research Department of F. Hoffmann-La Roche Ltd., Basel, Switzerland, for the determinations of plasma antioxidants and micronutrients, and Dorothea Stössel, Christa Wächter, and the nurses of the Department of Pediatric Surgery, University of Zurich, for their help in enrolling the study participants. This work was supported by grants from the Austrian Science Foundation (Charlotte Bühler Research Grant, H062), the International Foundation for the Promotion of Nutrition Research and Nutrition Education (ISFE), Switzerland, and Milupa Ltd., Puch, Austria.

References

Appendix: Comparison of Individual Measurement Data with the Reference intervals by Using z-Scores; Example for Log-Transformed Data
To obtain the z-score for a given individual measurement, e.g., 0.20 μmol/L lycopene in a 9-year-old subject, (a) calculate the log of the mean value for the reference population at age 9 years, according to \[
y = \text{intercept} + \text{slope} \times \text{age (shown in Table 1): } y = -1.00 + 0.016 \times 9 = -0.856.\]
(b) Take the log of the individual measurement value: log 0.20 = −1.609. (c) Calculate the z-score: z-score = (log of individual measurement value − log of the mean value for the reference population)/residual SD (shown in Table 1); that is, (−1.609 + 0.856)/0.51 = −1.476. Thus the individual measurement value is 1.48 SD below the mean value for the reference population, or z-score = −1.48.