Seasonal Variations in Plasma Micronutrients and Antioxidants

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Abstract
Plasma samples were collected at monthly intervals for a period of 1 year from a group of healthy nonsmoking men and women (n = 21) living in Honolulu, HI. Analysis of plasma cholesterol and triglyceride levels showed marked seasonal variations, with higher mean levels in winter months and lower values in the summer. Cholesterol and triglycerides were highly and inversely correlated with plasma levels of the provitamin A carotenoids. Mean β- and α-carotene levels were highest in late summer and fall. Plasma retinol levels were significantly lower in the summer and higher in the winter. Variations (either between individuals or seasonally) in plasma retinol were unrelated to plasma provitamin A carotenoid levels. Plasma levels of α-tocopherol, γ-tocopherol, β-cryptoxanthin, and lutein were also higher in the winter and lower in the summer. Significant seasonal correlations, both positive and negative, with environmental variables, such as temperature, solar UV radiation, and rainfall, are noted for many of these plasma micronutrients. The number of samples required to accurately characterize long-term plasma levels for an individual generally ranged from 1 to 4. However, plasma retinol levels exhibited the highest ratio of intra- to interindividual variability, suggesting the need for multiple sampling (>8 samples) for this micronutrient. Some of this variability for retinol was associated with seasonal changes. Assessment by a diet history of food and supplement intake of micronutrients and phytochemicals for 1 year showed good agreement with 1-year mean plasma levels for most carotenoids, vitamin C, and α-tocopherol. Retinol, γ-tocopherol, cholesterol, and triglyceride levels in plasma were unrelated to estimates of dietary intake. It may be important to consider seasonal variation in the design and analysis of future epidemiological and prevention studies.

Introduction
Numerous plasma micronutrients and antioxidants have been associated with cancer risk in epidemiological studies (1–3). Many of these substances are thought to be either risk enhancing factors or preventive agents in aging-related diseases, such as cholesterol in the development of ischemic cardiovascular disease (4), and vitamin E in the prevention of heart disease (5, 6). Retinoids, carotenoids, tocopherols, and other antioxidants have been shown to prevent cancer in experimental cell and animal studies (6–10), and supporting data for their role in preventing human cancers are found in numerous epidemiological studies, measuring consumption of and/or plasma levels of dietary antioxidants (7, 11–14). Most epidemiological studies rely on single measurements of analytes without clear evidence that a single plasma measurement is adequate to characterize an individual with respect to the particular group being studied. In some cases, intra-individual variation may be sufficiently high relative to inter-individual variability to cause misclassification of a substantial number of individuals. Indeed, several investigators have suggested that multiple samples are required for many serum compounds of interest (15–18). Tangney et al. (17) reported that as many as 3 samples must be analyzed for β-carotene to identify the true serum concentration at the 90% confidence level, while 4 samples were required for retinol and triglycerides.

High individual variability in plasma levels of naturally occurring substances over repeated measurements may be the result of variability in dietary intake, excretion, metabolism, physiology, and/or analytical method. By minimizing interassay variability and identifying factors that influence circulating levels, the number of samples required for analysis can be minimized. Recent improvements in analytical methodology and use of appropriate internal standards have minimized errors and variability in the analytical process, leaving intra-individual variation as the primary source for misclassification. Although changes in diet may affect levels of some nutrients, there is evidence that some lipids vary even on controlled diets, suggesting that other factors may regulate plasma levels (19). Some investigators have reported seasonal variability for serum cholesterol (20–22) and β-carotene (23–28), while others found no effect (16, 18) or disputed the conclusions of earlier studies (29). Seasonal variation has also been reported for bilirubin (30), urea (30), vitamin D (31), mortality (32), and blood pressure (33). In most cases, these studies have been performed in temperate zones where considerable seasonal changes in diet, temperature, and sunlight exposure occur. This study describes the magnitude of seasonal changes for a number of plasma micronutrients and antioxidants associated with aging-related diseases in a semitropical environment. Estimates of the number of samples required to characterize a usual plasma level of an individual and correlations of dietary nutrient intake with plasma levels are also presented.
Subjects and Methods

Subjects. Twenty-seven individuals who were not on any medications and were willing to donate 7 ml of blood at monthly intervals over the 1-year period, May 1992–April 1993, were recruited from the University of Hawaii Cancer Research Center. The participants were healthy nonsmoking men (n = 13) and women (n = 14). Twenty-one (78%) of the participants completed the study with 2 or fewer missing samples (241 of a possible 252 samples were obtained in this subgroup), and values from these individuals (10 men and 11 women) were used in the analyses reported. No dietary instructions were given to participants.

The subgroup was composed of individuals of Caucasian (76%), Asian (14%), and mixed (10%) ancestry, ranging in age from 31 to 63 years (mean, 41 years for men and 43 years for women). The majority of study participants did not take vitamin supplements on a routine basis. Values for the 8 individuals who reported occasional use of a multivitamin and/or ascorbic acid were included in the analysis, as their exclusion did not materially affect the results.

Blood samples were collected in the morning after an overnight fast at approximately 1-month intervals, using 7-ml green-top vacutainer tubes containing 100 United States Pharmacopeia units of sodium heparin. In general, one-half of the study group was sampled at the beginning of the month, and the other half was sampled during mid-month. Samples were immediately put on ice and protected from light during subsequent work-up. After centrifugation (850 × g) for 30 min at 4°C, the plasma supernatant was placed in cryovials and stored at −70°C for later analysis. Fresh plasma (2 ml) was mixed with 2 ml of 10% metaphosphoric acid, stored at −70°C, and analyzed within 1 month for vitamin C levels.

Analytical Methods. Plasma lipid-phase micronutrients and antioxidants were measured as described previously (34) with the use of synthesized retinol laurate (35) as an internal standard for retinol quantitation instead of the retinol palmitate used in earlier studies. Ascorbate was assayed spectrophotometrically with the use of dichlorophenolindophenol (36). Plasma cholesterol and triglyceride levels were determined spectrophotometrically with the use of enzymatic kits (352-50 and 339-50, respectively) from Sigma Chemical Co. (St. Louis, MO). Values reported for α- and β-carotene, β-cryptoxanthin, and lutein/zeaxanthin are for the trans-isomers.

Laboratory accuracy and precision in the measurement of plasma micronutrients and antioxidants were maintained and assessed by participation in the National Institute of Standards round robin. This involved the measurement of blind plasma samples sent from the National Institute of Standards to approximately 33 participating laboratories across the United States every 4 months. During the 12 months of this study our average bias from the accepted values, as determined by averaging values from various “core laboratories,” was 6.0, 7.0, and 7.1% for retinol, α-tocopherol, and β-carotene, respectively (n = 19). Precision, as assessed by the coefficient of variation for 14 duplicate samples, was observed to be 4.3, 2.4, and 3.2%, respectively.

Diet Histories. Following the final blood sample, trained interviewers administered a diet history questionnaire to estimate the usual intake of approximately 250 food items during a typical month (or months when particular fruits were in season) in the previous year. Both frequencies and quantities consumed were estimated. Participants selected usual amounts from color photographs showing three portion sizes, or referred to common household serving units. The diet history was developed from food records of a representative sample of the major ethnic groups in Hawaii and has been tested for validity (37) and reproducibility (38, 39).

The average daily dietary intakes were computed from a comprehensive food composition database derived from data provided by the United States Department of Agriculture (40), McCance and Widdowson's The Composition of Foods (41), Science and Technology Agency of Japan (42), and other published and analyzed data. The data set has been expanded to include the specific tocopherols (43) and the five major carotenoids (44) found in serum. Normal temperature and rainfall data for the Honolulu, HI area were obtained from the Hawaii State Data Book (45).

Statistical Analysis. In the analysis of the data, some analytes were log transformed, as log (x + 1), in order for the distributions to approximate normality. The monthly values for each analyte were modeled with a one-way random effects ANOVA (46) to obtain estimates of the intra- and intersubject variability. Since no measurements were made on consecutive days, we can assume the monthly values are independent.

The number of days of measurement (D) necessary to rank individuals correctly according to their long-term analyte levels was computed using the formula proposed by Nelson et al. (47):

$$D = \left( \frac{r^2}{1-r^2} \right) \frac{s^2}{s^2 + s^2_i}$$

where $r$ is the unobservable correlation between the measured and the true underlying values for an individual for a day, and $s^2_w$ and $s^2_i$ are the observed within and between subject variances computed by ANOVA. A value of $r = 0.90$ was chosen, since at least 80% of subjects in the extreme thirds of the distribution would be correctly classified, and <1% would be grossly misclassified in the incorrect extreme thirds. The number of days required increases with the precision desired on the measurement, that is, $D$ increases with $r$. Ninety-five% confidence intervals were computed about $D$ as:

$$\frac{r^2}{1-r^2} \left( r^2 k s^2 + s^2_i \right) \frac{F_{0.05,u,v}}{F_{0.95,u,v}}$$

where $F_{*,u,v}$ is the critical value of the $F$ distribution; $u = n - 1$, where $n$ is the number of subjects; $v = \Sigma m_i - n$, where $m_i$ is the number of days of observation for subject $i$, and

$$k = \frac{\Sigma m_i - \Sigma m_i^2}{n-1}$$

Correlations between analytes were computed by Pearson’s correlation coefficient, $r$. The mean analyte level

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3. M. Buzzard, personal communication.
within-subject variance ratios are given along with the estimated number of measurements required to characterize an individual with respect to the group. For most analytes, intrasubject variation was smaller than that between subjects, and two or three samples appeared adequate to characterize an individual. Retinol was a notable exception, requiring 8 samples to achieve the indicated level of accuracy.

Correlations between analytes, based on individual means, are given in Table 2. A number of micronutrients were found to be correlated, possibly indicating common dietary sources or related metabolic effects. The tocopherols (γ-tocopherol particularly) were positively correlated with triglycerides, consistent with their common dietary source from oils (48). Plasma cholesterol levels, which are influenced by saturated fat consumption (49), showed a significant positive correlation with both the triacylglycerides and tocopherols. Mean plasma retinol (vitamin A) levels, which are tightly regulated physiologically by retinol-binding protein (50), varied over a relatively narrow range (525–940 ng/ml) among the subjects and were not correlated with plasma provitamin A carotenoid levels. Retinol was significantly correlated with triglyceride levels (in agreement with Herbeth et al. (51)), and somewhat surprisingly, with plasma lutein/zeaxanthin levels ($r = 0.5$), although these carotenoids are not converted into retinol.

Table 3 shows correlations over time for the 6 bimonthly mean levels for each analyte. Positive correlations over time were observed between retinol and lutein/zeaxanthin ($r = 0.64$) and between retinol and the provitamin A carotenoid, ß-cryptoxanthin ($r = 0.62$). In contrast, there was no significant correlation between seasonal changes in plasma retinol and the pro-vitamin A carotenoids, α- and ß-carotene. Both changes in lutein/zeaxanthin and retinol were correlated positively with normal seasonal changes in rainfall and negatively with temperature (Table 3). In the Hawaiian Islands, mean temperature and rainfall are highly correlated, reflecting increased rainfall during the cooler winter months. Solar radiation is also significantly decreased (by ~50%) in the winter (52). As a result, positive correlations with rainfall also represent inverse correlations with solar UV light exposure.

To visualize the seasonal variations suggested in Table 3 for plasma levels of micronutrients or antioxidants, bimonthly mean levels, or the mean deviation from yearly averages of individuals, were plotted as a function of time of year. In general, both methods showed similar effects in those instances where a significant seasonal variation was observed. Fig. 1 shows an approximate 12% change in mean cholesterol levels over the year, varying inversely with mean temperature. There appeared to be a 1-month lag between temperature and cholesterol levels ($r = -0.831; P = 0.04$ when correlated with temperature for the month preceding sample collection). Triglycerides showed a similar pattern (Fig. 2) and, like cholesterol, were strongly inversely correlated with changes in concentrations of the main plasma provitamin A carotenoids (α-carotene + ß-carotene + ß-cryptoxanthin; Fig. 1) and with temperature (Fig. 2). α-Carotene exhibited an approximate 50% change between high and low mean seasonal values (Fig. 2). ß-Carotene also showed a significant seasonal variability of approximately 25% (Fig. 3), with the highest levels in the late summer and early fall. Although α- and ß-carotene were positively correlated with temperature ($r = 0.56$), changes in
plasma levels appeared to lag behind temperature changes by approximately 1 month. When the correlational analysis was carried out by comparing plasma levels of \( \beta \)-carotene with mean temperature for the month preceding sample collection (e.g., the mean bimonthly temperature for April and May was compared with the mean bimonthly \( \beta \)-carotene level for samples obtained in May and June), the correlation coefficient was 0.91 \((P = 0.01)\). The tocopherols (Fig. 4), \( \beta \)-cryptoxanthin, and lutein/zeaxanthin (Fig. 5) were found at higher levels in plasma during the winter months and their changes were inversely correlated with changes in \( \beta \)-carotene (Table 3). The graph for tocopherols displays monthly values because it more clearly showed the seasonal effect. Plasma lycopene levels were also slightly elevated in the winter but not significantly \((P = 0.25)\).

Mean plasma retinol for the group was found to vary throughout the year from a high of 819 ng/ml in early winter to a low of 676 ng/ml in the summer (Fig. 6). This is particularly significant in light of the low interindividual variation for retinol (see Table 1). The seasonal change in retinol was strongly and positively correlated with normal mean monthly rainfall levels for Honolulu (Fig. 6). The seasonal correlation did not change with exclusion of those persons consuming vitamin A supplements.

\begin{table}
\centering
\caption{Pearson correlations between individuals for 1-year means of plasma analytes \((n = 21)\)}
\begin{tabular}{lcccccccc}
\hline
 & \( \alpha \)-Carotene & \( \beta \)-Carotene & \( \beta \)-Cryptoxanthin & Lycopene & Total carotenoids & Cholesterol & Triglycerides & \( \alpha \)-Tocopherol & \( \gamma \)-Tocopherol & Retinol & Vitamin C \\
\hline
Lutein/zeaxanthin* & 0.22 & -0.18 & 0.49 & 0.09 & 0.21 & -0.10 & 0.18 & -0.22 & 0.06 & 0.50 & 0.11 \\
\( \alpha \)-Carotene* & 0.75 & 0.47 & 0.18 & 0.84 & 0.24 & -0.05 & 0.58 & -0.36 & 0.15 & 0.68 & 0.79 \\
\( \beta \)-Carotene* & & & 0.45 & -0.06 & 0.84 & 0.0 & -0.40 & 0.61 & 0.52 & -0.26 & -0.01 \\
\( \beta \)-Cryptoxanthin* & & & & 0.31 & 0.75 & 0.0 & -0.01 & 0.32 & -0.32 & 0.32 & 0.31 \\
Lycopene & & & & & 0.35 & 0.32 & 0.44 & 0.10 & 0.36 & 0.34 & -0.10 \\
Total carotenoids* & & & & & & 0.07 & -0.14 & 0.55 & -0.38 & 0.07 & 0.68 \\
Cholesterol & & & & & & & 0.61 & 0.55 & 0.52 & 0.38 & 0.07 \\
Triglycerides & & & & & & & & 0.24 & 0.58 & 0.55 & -0.18 \\
\( \alpha \)-Tocopherol* & & & & & & & & & -0.10 & 0.24 & 0.60 \\
\( \gamma \)-Tocopherol & & & & & & & & & & 0.19 & -0.40 \\
Retinol* & & & & & & & & & & & -0.12 \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Correlations over time for micronutrients, antioxidants, and environmental variables}
\begin{tabular}{lcccccccccccc}
\hline
 & Lutein/ & Zeaxanthin* & \( \alpha \)-Carotene & Carotene & \( \beta \)-Cryptoxanthin & Lycopene & Total carotenoids & Retinol & Cholesterol & Triglycerides & \( \alpha \)-Tocopherol & \( \gamma \)-Tocopherol & Vitamin C & Temperature & Rainfall \\
\hline
Lutein/ & -0.71 & -0.52 & 0.39 & 0.33 & 0.64 & 0.69 & 0.33 & 0.52 & 0.49 & 0.38 & -0.89 & 0.74 & & & \\
Zeaxanthin* & & 0.80 & -0.31 & 0.35 & -0.18 & -0.80 & -0.83 & -0.56 & -0.51 & -0.14 & 0.54 & -0.17 & & & \\
\( \alpha \)-Carotene* & & & -0.76 & 0.37 & -0.42 & -0.61 & -0.64 & -0.89 & -0.83 & -0.35 & 0.56 & -0.29 & & & & \\
\( \beta \)-Carotene* & & & & 0.16 & 0.62 & 0.15 & 0.19 & 0.93 & 0.94 & -0.63 & -0.63 & 0.59 & & & & \\
\( \beta \)-Cryptoxanthin* & & & & & & 0.36 & -0.36 & -0.43 & -0.01 & 0.09 & -0.09 & 0.42 & 0.66 & & & \\
Total carotenoids* & & & & & & & 0.36 & -0.36 & -0.43 & -0.01 & 0.09 & -0.09 & 0.42 & 0.66 & & & \\
Retinol* & & & & & & & & 0.42 & -0.32 & 0.62 & 0.46 & 0.14 & -0.69 & 0.80 & & & \\
Cholesterol & & & & & & & & & 0.39 & 0.33 & 0.30 & 0.46 & -0.58 & 0.33 & & & & \\
Triglycerides* & & & & & & & & & & 0.39 & 0.44 & -0.20 & -0.21 & -0.18 & & & & \\
\( \alpha \)-Tocopherol* & & & & & & & & & & & 0.88 & -0.46 & -0.60 & 0.47 & & & & & \\
\( \gamma \)-Tocopherol & & & & & & & & & & & & -0.39 & -0.73 & 0.58 & & & & & \\
Vitamin C & & & & & & & & & & & & -0.07 & 0.08 & & & & & & \\
Temperature & & & & & & & & & & & & & -0.91 & & & & & & \\
\hline
\end{tabular}
\end{table}
vitamin C, proportionally more intra- than interindividual temperature for the bimonthly period produced similar re-
tene and retinol (P < 0.01). Models substituting rainfall and temperature for the bimonthly period produced similar results. Since seasonality can affect both within and between person variability, we also investigated how season affected these two variance components. For all analytes except vitamin C, proportionally more intr- than interindividual variability was explained by seasonality (data not shown).

However, the $s_w^2:s_g^2$ ratios were not changed substantially, and the number of days of measurement was reduced by 1 day only for $\alpha$-carotene and retinol.

Average yearly serum values for micronutrients and antioxidants were compared to estimates of the dietary intake for the year, as assessed by a diet history questionnaire. Good correlations were found between dietary intake and plasma level for lutein/zeaxanthin ($r = 0.61; P < 0.01$) and $\beta$-cryptoxanthin ($r = 0.68; P < 0.01$). Acceptable correlations were found for $\alpha$-carotene ($r = 0.26$), $\beta$-carotene ($r = 0.21$), lycopene ($r = 0.39$), vitamin C ($r = 0.25$), and $\alpha$-tocopherol ($r = 0.24$). Inclusion of both food and supplement intake increased the degree of correlation markedly.

Table 4 shows the $r^2$, the amount of variability in plasma nutrients accounted for, as calculated by different models. Model 1 is the base one-way random effects ANOVA model. Model 2 measures the effect of seasonality on variability by adding bimonthly dummy variables as fixed effects. $r^2$ values increased substantially for $\alpha$-caro-
tene and retinol ($P < 0.001$). Models substituting rainfall and temperature for the bimonthly period produced similar results. Since seasonality can affect both within and between person variability, we also investigated how season affected these two variance components. For all analytes except vitamin C, proportionally more intr- than interindividual variability was explained by seasonality (data not shown).

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Variations in Plasma Micronutrients supplements had no effect on the observed seasonal variation. 

Comparison (45). Exclusion of data from one individual who took vitamin A level for 21 individuals for the indicated 2-month period; Fig. 6. 

0.01

P values for F-test comparing Model 1 and Model 2 were all 0.01, except for the vitamin C model where \( P = 0.06 \). The direct photolytic degradation of retinol is unlikely, however, as previous research has shown no effect of UV exposure on plasma retinol in humans, although significant reductions in plasma carotenoids were observed (56). A seasonal and latitudinal association between light exposure and plasma vitamin D levels is well established (31, 57, 58) and is inverse to the association we observed for retinol. Recent observations concerning cross-recognition of and synergistic interaction with nuclear receptors by lipophilic vitamins and steroid hormones, including vitamins A, D, and thyroid hormones, suggest the possibility that vitamin A levels might rise to compensate for deficiencies in vitamin D resulting from decreased solar UV in the winter (59–61). Others have reported increased carotenoid and retinoid levels in various disease states, such as anorexia nervosa and diabetes, resulting from decreased thyroid hormone levels (62, 63). The mean retinol levels for men and women reported here are very similar to those observed by Ito et al. (64) (785 and 681 ng/ml for men and women, respectively) who found no seasonal change for retinol concentrations, although they found higher \( \beta \)-carotene levels, and intake of vitamin A and its precursors did not correlate with plasma retinol levels. Mean daily caloric intake, as estimated from the diet histories, was 1536 ± 473 for women and 2250 ± 638 for men. 

Table 4 Variability explained by different models, as measured by \( r^2 \)

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Model 1 ( ^a )</th>
<th>Model 2 ( ^b,c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lutein/zeaxanthinin ^f</td>
<td>0.61</td>
<td>0.65</td>
</tr>
<tr>
<td>( \beta )-Cryptoxanthinin ^f</td>
<td>0.76</td>
<td>0.72</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.59</td>
<td>0.62</td>
</tr>
<tr>
<td>( \alpha )-Carotene ^d</td>
<td>0.58</td>
<td>0.68</td>
</tr>
<tr>
<td>( \beta )-Carotene ^d</td>
<td>0.88</td>
<td>0.89</td>
</tr>
<tr>
<td>Total carotenoids ^ f</td>
<td>0.83</td>
<td>0.84</td>
</tr>
<tr>
<td>Retinol ^d</td>
<td>0.39</td>
<td>0.48</td>
</tr>
<tr>
<td>( \gamma )-Tocopherol</td>
<td>0.65</td>
<td>0.67</td>
</tr>
<tr>
<td>( \alpha )-Tocopherol ^d</td>
<td>0.82</td>
<td>0.83</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.74</td>
<td>0.76</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.72</td>
<td>0.76</td>
</tr>
<tr>
<td>Triglycerides ^d</td>
<td>0.77</td>
<td>0.78</td>
</tr>
</tbody>
</table>

\( ^a \) Model 1, unique identifier as random effect. 
\( ^b \) Model 2, unique identifier as random effect, dummy variables for bi-monthly period as fixed effect. 
\( ^c \) \( P \) values for F-test comparing Model 1 and Model 2 were all ≤0.01, except for the vitamin C model where \( P = 0.06 \). 
\( ^d \) Log-transformed value used in calculation. 

for \( \beta \)-carotene \( (r = 0.83; \ P < 0.01) \), ascorbate \( (r = 0.83; \ P < 0.01) \), and \( \alpha \)-tocopherol \( (r = 0.64; \ P < 0.01) \). Fat consumption from food was not related to fasting plasma triglyceride levels, and intake of vitamin A and its precursors did not correlate with plasma retinol levels. Mean daily caloric intake, as estimated from the diet histories, was 1536 ± 473 for women and 2250 ± 638 for men. 

Discussion The demonstration of a significant association between a particular plasma constituent and disease incidence requires that either a sufficient number of repeat samples from each individual be obtained such that an accurate characterization can be made of the usual level of that individual, or that the number of subjects in the study be sufficiently large enough to compensate for the reduction in statistical power resulting from the intraindividual variability in the exposure variable. The latter option is difficult due to a restriction in the number of cases, whereas the additional cost from multiple samples, whether from the same individual or through the recruitment of additional subjects, may be prohibitive. Furthermore, as intervention trials are initiated to test the efficacy of diets to prevent disease, objective and cost-effective assessments of compliance, such as those provided by analysis of blood components, are needed. Clearly, any means of reducing intraindividual variability or accounting for some of the observed variability will improve the design of epidemiological studies and intervention trials. Improvements in analytical methodology in recent years, along with the introduction of relevant internal standards and quality control programs, have significantly reduced interassay variability as a source of intraindividual variation. However, the results reported here suggest that multiple samples are still required for the accurate measurement of many micronutrients and antioxidants. Our data would suggest that the numbers needed for carotenoids are somewhat less than determined in previous reports (15–17) and are somewhat greater for retinol (53). This may be the result of differences in the study populations or seasonal effects. 

Seasonal changes in plasma constituents contribute to observed variability. In the case of retinol, seasonal variation in the means is almost one-half as large as the intraindividual variation suggesting that adjustment for seasonal effects could reduce the number of samples required. As shown in Table 4, compensation for seasonal changes resulted in reduced variability, as measured by the \( r^2 \) and in one fewer measurement required for retinol and \( \alpha \)-carotene. Although in the absence of vitamin A deficiency, serum retinol levels are physiologically regulated (50), little is known concerning possible seasonal changes or other factors affecting normal circulating levels (50, 54). The level of retinol-binding protein appears to regulate the plasma concentration of this micronutrient (55) and supplementation with retinoids or provitamin A carotenoids does not affect serum levels of retinol except in cases of deficiency (25, 54). The positive correlation of plasma retinol with normal monthly rainfall and negative correlations with seasonal changes in mean temperature and solar UV radiation suggest that environmental conditions may affect circulating levels of retinol. The direct photolytic degradation of retinol is unlikely, however, as previous research has shown no effect of UV exposure on plasma retinol in humans, although significant reductions in plasma carotenoids were observed (56).
tene levels in the winter. Nierenberg et al. (23) also reported significant sex differences in plasma retinol and the lack of an association between retinol and β-carotene levels (22). The positive seasonal association between retinol and lutein/zeaxanthin is noteworthy but presumably not causally related because lutein is not converted to vitamin A.

Many studies have observed a seasonal variation for serum cholesterol levels (20–22), while others have questioned the validity of the data based on statistical considerations (29). In the present study, both cholesterol and triglycerides, determined by two independent analytic methods, showed similar strong seasonal variations. For cholesterol, the seasonal amplitude of 20 mg/dl (12%) is >50% of the SD observed for the study group (179 ± 37 mg/dl) and compares favorably with previous studies (20–22). Other sources of variation may be associated with illness, the menstrual cycle in women (65), or changes in fat consumption (49).

The strong seasonal variation observed for α- and β-carotene may be the result of dietary changes, as have been postulated in other studies reporting a similar seasonal effect for β-carotene (24, 66, 67). Mangoes represent one potentially significant seasonal source of α- and β-carotenes in Hawaii and may account for the large rise in plasma α-carotene in July at the peak of mango season. Seasonality explained more of the variability (particularly intraindividual) in α-carotene than in β-carotene levels. In general, most other foods are available on a year-round basis and no significant changes in consumption based on availability would be expected. However, cost and quality of fresh fruits and vegetables vary on a seasonal basis and may affect consumption significantly. The strong correlation between some plasma carotenoids and mean temperature during the month immediately preceding sample collection (α-carotene and β-carotene positively; lutein/zeaxanthin negatively) suggests the need for additional research to determine if there is a causal relationship between environmental factors, such as temperature and plasma carotenoid levels. The lowest plasma β-carotene levels occurred in the March/April period (289 ng/ml) and highest levels in September/October (368 ng/ml). Nierenberg et al. (23), looking at 1750 patients with nonmelanoma skin cancer at various United States sites, observed a similar seasonal effect, reporting levels of 154 ng/ml in the spring and 202 ng/ml in the summer. Although the absolute values were less than in the current study, the magnitude of seasonal change was similar. Lux and Naidoo (27) reported a 2-fold increase in plasma β-carotene levels from July to December for 12 healthy volunteers in Australia, where temperature and solar UV exposure are inverse to those in Hawaii and those reported in the study by Nierenberg et al. (23). Lutein levels, in our study, were highest in January/February (247 ± 22 ng/ml) and lowest in July/August (195 ± 10 ng/ml) and showed a significant sex difference. In contrast, Olmedilla et al. (28) recently reported no significant sex differences for lutein/zeaxanthin mean values and observed higher values for women in the spring and for men in the summer in Spain. They did, however, observe increased β-cryptoxanthin levels in the winter and increased α-carotene and β-carotene in the summer in agreement with our data. Lycopene and vitamin C showed no substantial seasonal variability in our study, although plasma lycopene levels appeared to be slightly higher in winter.

Although the objective of this study was not the validation of the diet history questionnaire, the very satisfactory correlations with mean plasma concentrations, particularly for total intakes of vitamin C, β-carotene, α-tocopherol, and food intakes of β-cryptoxanthin and lutein, indicate that the diet history developed for the Hawaii population is a satisfactory instrument for assessing the average dietary intakes for this diverse group of subjects. Similar to Willett et al. (68), our data reveal that dietary intake is a poor indicator of plasma retinol and therefore unlikely to be the source of the seasonal changes observed. Plasma lycopene concentrations were poorly correlated with consumption, consistent with a previous intervention study in which significant increases in dietary lycopene had only marginal effects on plasma levels (69). Previous studies (70) have reported that β-carotene consumption can depress serum and tissue α-tocopherol levels. The strong inverse seasonal correlation (r = −0.89) observed between plasma α-tocopherol and β-carotene levels also suggests an interactive effect for these two dietary antioxidants.

The seasonal effects observed in this study are intriguing in light of the relatively modest seasonal changes that occur in the Hawaiian environment. Although the monthly mean temperature varies <10°F throughout the year, the absence of insulation, heating, and air conditioning in homes means that individuals in Hawaii may be exposed to ambient conditions to a larger extent than someone living in a more extreme climate, where indoor climate is better regulated. As a consequence, the extent to which the observed seasonal changes are related to changes in temperature may be significant. Indeed, effects of temperature, if they are causally related, may be masked or inverted in areas in which winter heating and summer air conditioning are used extensively. Differences in sunlight exposure are more modest in Hawaii (2-fold increase in UV radiation between winter and summer) (52) relative to those experienced at higher latitudes, and except in the case of a household effect, one would predict a greater magnitude of change for a similar study at a higher latitude if this was a determining factor. Bates et al. (25) reported significantly lower plasma retinol levels in the Gambian population relative to those observed in more Northern climates, even after supplementation with vitamin A. Although this may have been due to ethnic differences, increased sunlight exposure cannot be ruled out as a possible explanation for their observation.

The variations observed in the present study, while moderate and of unknown physiological impact, could represent significant contributions to both intra- and interindividual variability and their effects on epidemiological and prevention studies need to be considered. For example, short dietary intervention and epidemiological studies might be targeted for a fixed season of the year. In case-control studies, it may be important to collect serum from matched pairs during the same season. Conversely, if long-term analyte levels for individuals are needed, the study may need to include a blood sample from each of several seasons. In addition, seasonal changes in cholesterol of the magnitude seen here could affect clinical assessment of risk, as well as treatment. Ultimately, with increased knowledge of the factors regulating plasma micronutrients, we may better understand their function in human health and nutrition.

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