Lipid standardization of serum fat-soluble antioxidant concentrations: the YALTA study1–3

Myron Gross, Xinhua Yu, Peter Hannan, Christian Prouty, and David R Jacobs Jr

ABSTRACT

Background: Blood lipids can influence fat-soluble antioxidant concentrations and confound their interpretation as indicators of antioxidant intake status and disease risk.

Objectives: The objectives were to identify lipoproteins that can confound the interpretation of serum fat-soluble antioxidants, to evaluate the amount of the confounding, and to recommend a method for standardizing blood concentrations of fat-soluble antioxidants.

Design: Several methods of lipid standardization of fat-soluble antioxidants were evaluated in a large cohort of young adults with the use of both cross-sectional and longitudinal data analysis.

Results: Tocopherol and carotenoid concentrations were associated with plasma total cholesterol and its components, LDL, HDL, and VLDL cholesterol (estimated as plasma total triacylglycerols/5), some of which were independent predictors for all of the fat-soluble antioxidants. Among supplement nonusers, the most amphiphatic (polar) of the antioxidants (α-tocopherol, γ-tocopherol, and zeaxanthin plus lutein) and lycopene were associated strongly with these lipid fractions ($R^2 = 0.09, 0.40$). Consistent with a causal association in which blood antioxidant concentrations change as blood lipid concentrations change, similar relations were found for changes in blood antioxidant and lipid concentrations over a 7-y period. Concentrations of the remaining carotenoids (β-cryptoxanthin, α-carotene, and β-carotene) had a weaker association with plasma lipoproteins ($R^2 < 0.06$). Similar relations were found for supplement users.

Conclusions: The simultaneous adjustment of the concentrations of tocopherols, zeaxanthin plus lutein, and lycopene for VLDL, HDL, and LDL cholesterol is recommended. This method is practical and can provide a basis for the standardization of carotenoid and tocopherol concentrations.


KEY WORDS Serum antioxidant concentration, blood lipids, lipid standardization, lipoproteins, total cholesterol, fat-soluble vitamins, young adults

INTRODUCTION

Several carotenoids and tocopherols act in the maintenance of essential cellular functions and are classified as vitamins or precursors of vitamins. In addition to these well-established functions (1, 2), the fat-soluble compounds have antioxidant activities and have been associated with the prevention of lipid peroxidation and chronic diseases (3–9), but they have had little effect in most clinical trials to date (10–13). A critical aspect of an evaluation of these activities is the assessment of the carotenoid and tocopherol status in human subjects. Generally, status has been assessed by a measurement of carotenoid and tocopherol concentrations in blood, without or with an adjustment for a limited set (1 or 2) of blood lipids. This approach provides an indication of status, but it has a significant limitation, in that antioxidant concentrations in blood are influenced by the concentrations of most blood lipids rather than of a single component (14, 15). Differences in blood lipid concentrations can confound the interpretation of antioxidant concentrations (16, 17). Even partial adjustment for lipids has been shown to improve estimates of antioxidant intake (15, 18, 19), status (14, 16, 17), and associations with disease (20, 21), and lipid adjustments have been incorporated into many studies (22–25). Our goal is identification of a more complete set of plasma lipids and lipoproteins, which contribute to the variation in antioxidant concentrations in human populations and define adjustments for the standardization of antioxidant concentrations.

Tocopherol and carotenoid concentrations have been associated with concentrations of total cholesterol, phospholipid, triacylglycerol, lipoprotein, and apolipoprotein in blood (14, 20, 21, 26–28). Several methods have been used to reduce the confounding of antioxidants with blood lipid concentrations (21, 29, 30). Each of these methods has significant limitations (31).

We hypothesize that each blood lipoprotein uniquely influences serum concentrations of fat-soluble antioxidants. Moreover, a lipid standardization of antioxidant concentrations that involves HDL, LDL, and VLDL (each represented by its cholesterol concentration) separately will be more indicative of the antioxidant status in human populations than will a cumulative measure of lipoproteins, such as total cholesterol.

With the use of data from the Young Adults Longitudinal Trends in Antioxidants (YALTA) study [an ancillary study of the

Received January 30, 2002.
Accepted for publication May 30, 2002.

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2 Supported by grant no. 1RO1-HL53560-01A1 from the National Heart, Lung, and Blood Institute.

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Coronary Artery Risk Development in Young Adult (CARDIA) study], analyses were performed to further define the influence of blood lipids on fat-soluble antioxidant concentrations. Specifically, we evaluated associations between 7 serum fat-soluble antioxidants and the fasting concentrations of total, LDL, HDL, and VLDL cholesterol; apolipoprotein (apo) B; and apo A-I in this population-based setting.

SUBJECTS AND METHODS

The YALTA study measured serum antioxidant concentrations in frozen samples as an ancillary study to the CARDIA study, which is a longitudinal cohort study that has tracked the evolution of cardiovascular disease risk factors in human subjects beginning at age 18–30 y. In 1985 and 1986, black and white men and women (n = 5115) were recruited in Birmingham, AL; Chicago; Minneapolis; and Oakland, CA (32). Recruitment was population-based, and subjects were recruited at random by telephone or door-to-door contact in the first 3 cities and at random from members of the large Kaiser Permanente Medical Care Plan in Oakland. The overall response rate was ≈50% (33). By design, the sample in each of the 4 cities was balanced by race (black or white), sex, age (18–24 y compared with 25–30 y), and educational level (high school or less compared with at least some college), and approximately equal numbers of subjects were recruited from each site (34). Participants were reexamined during follow-up at years 2, 5, 7, and 10. Most of the surviving cohort (78.5%) participated in the year 10 examination, between June 1995 and June 1996. Higher rates of participation occurred in years 2, 5, and 7 than in year 10 (35). All participants in the CARDIA baseline examination were included in this study, except 147 who were not fasting for at least 8 h and those with insufficient quantities of stored blood. The number of participants in each part of the analysis is specified in the tables.

Blood samples were drawn after an overnight fast by venipuncture into evacuated tubes. The plasma was separated by centrifugation, transferred into airtight vials, and stored at −70 °C until it was shipped on dry ice to the Northwest Lipid Research Laboratories, University of Washington, Seattle. Total cholesterol and triacylglycerols were measured enzymatically within 6 wk of collection (36). HDL cholesterol was measured after the precipitation of LDL containing lipoproteins with dextran sulfate and magnesium chloride (37). LDL cholesterol was calculated with the use of the equation of Friedewald et al (38). Radioimmunoassays were performed to measure apo A-I and apo B-100 (39). The technical error, reported previously (20), was 3.3% for total cholesterol, 3.9% for HDL cholesterol, 8.4% for triacylglycerols, 7.0% for apo A-I, and 8.6% for apo B.

In the YALTA study carried out between 1995 and 1998 at the Molecular Epidemiology and Biomarker Research Laboratory, University of Minnesota, serum antioxidant vitamins were measured. The sera for α- and γ-tocopherol analysis and analysis of the carotenoids α- and β-carotene, lycopene, zeaxanthin plus lutein, and β-cryptoxanthin were from CARDIA study years 0 and 7 and were stored at −70 °C. The tocopherols and carotenoids were measured with an HPLC-based assay. The assay was a modification of the method of Bieri et al (40) with calibration as described by Craft et al (41) and sample handling as described by Gross et al (42). The primary modification was the simultaneous detection of tocopherols and carotenoids. The α- and γ-tocopherols were detected by the use of a second absorbance channel set at 292 nm. The retention time of α- and γ-tocopherol was 5.0 and 4.5 min, respectively. Other modifications were the addition of disopropylethylamine (0.015%) in the HPLC solvent as an aid to analyte recovery and the use of tocopher acetate, as an internal standard for tocopherols. Calibration was performed with pure compounds (Hoffman-LaRoche, Basel, Switzerland; Sigma Chemical Co, St Louis). Quality-control procedures included routine analysis of plasma and serum control pools containing high and low concentrations of each analyte.

In addition, the laboratory routinely analyzed National Institutes of Standards and Technology reference sera and was a participant in the National Institutes of Standards and Technology Fat-Soluble Vitamin Quality Assurance Group. The CVs were <10% for all analytes and control pools. Previously, we showed that the intraclass correlation coefficients (ratio of between-person variance to between- plus within-person variance) were 0.93 for α-carotene, 0.98 for β-carotene, 0.73 for zeaxanthin plus lutein, 0.97 for β-cryptoxanthin, 0.73 for lycopene, and 0.93 for α-tocopherol (20).

Results are presented separately for nonusers and users of supplements. A supplement user was defined as any person consuming vitamins A, E, and C in excess of his or her intake from foods alone. The source of vitamin A in many of the supplements includes α- and β-carotene. In the analysis, all findings were similar after adjustment or stratification for race and sex (data not shown). Thus, unadjusted findings are presented.

Cross-sectional analyses used data only from the baseline (year 0) of the CARDIA study. To assess the general pattern of association of antioxidants with plasma lipids, the mean level of each antioxidant was plotted according to 20 categories of total cholesterol. After the exclusion of missing data, each one-twentieth contained ≈240 people. This procedure was repeated for one-twentieths of concentrations of LDL, HDL, and VLDL cholesterol; apo A-I; and apo B. The correlation coefficient (r) is calculated on individual data, and P values and coefficients are reported for each analysis. Because of our conceptual interest in lipoprotein cholesterol fractions, we estimated VLDL cholesterol as total triacylglycerols divided by 5, an estimate based on the equation of Friedewald et al (38). Thus, the results for triacylglycerols exactly parallel those for VLDL cholesterol.

With multiple linear regression, each serum antioxidant vitamin was analyzed as a dependent variable on lipoprotein characteristics by the use of a series of models. Model 1 contained only plasma total cholesterol as an independent variable. Model 2 had only plasma LDL cholesterol; model 3 had plasma total cholesterol and VLDL cholesterol; model 4 had plasma LDL, HDL, and VLDL cholesterol simultaneously. Note that because total cholesterol is the sum of LDL, HDL, and VLDL cholesterol, model 1 differs from model 4 only in that the 3 lipoprotein cholesterol fractions have a common coefficient in model 1, whereas they have different coefficients in model 4. Model 5 used regression to analyze the change in antioxidant concentrations (year 7 – year 0) on the corresponding changes in LDL, HDL, and VLDL cholesterol. This last model evaluated the within-person associations of changes in serum antioxidant concentrations with changes in lipoprotein concentrations. Two additional models included only apo A-I and apo B-100, and the 5 independent variables—LDL, HDL, and VLDL cholesterol; apo A-I; and apo B-100 (data not shown). In total, 49 regression analyses were run (7 antioxidants each with 7 combinations of independent variables). In the regression models, antioxidants are expressed in micromolar concen-
trations and lipoprotein cholesterols in millimolar concentrations. Cross-sectional models (1–4) were evaluated by the conditional F test that compares the $R^2$ in nested models. To evaluate the change in model 5 against the best cross-sectional model (model 4), we used repeated-measures data for years 0 and 7 of the study. We compared 2 additional models: the first model used separate coefficients for the cross-sectional and change components for each lipoprotein fraction, and the second model used these component coefficients pooled. We tested the difference between these two models with the log-likelihood ratio test based on 3 df. Statistical analyses were performed with SAS software, versions 6, 12, and beyond (SAS Institute, Inc, Cary, NC).

RESULTS

General description

Lipid and antioxidant measurements were obtained from > 92% of all CARDIA study participants. Approximately one-third (30%) of persons in the population were users of vitamin supplements. A multivitamin or other combination containing vitamins A, E, and C was taken by 63% of the supplement users. The use of a vitamin C (a nonfat-soluble antioxidant vitamin) supplement alone qualified a person as a supplement user because vitamin C actively regenerates vitamin E and may spare lipid-soluble antioxidants in general. As shown in Table 1, antioxidant concentrations were 7–49% higher in supplement users than in supplement nonusers, with the exceptions of γ-tocopherol and lycopene concentrations, which were, respectively, 14% and 5% lower. The difference in mean plasma lipid concentrations between supplement users and supplement nonusers was < 0.03879 mmol/L (< 3%) for total, VLDL, HDL, and LDL cholesterol and was statistically significant only for HDL cholesterol.

Linear association

The cholesterol and lipoproteins of plasma were major correlates of the fat-soluble antioxidants (Figure 1). For instance, serum tocopherol and carotenoid concentrations were higher in persons with high LDL cholesterol than in those with low LDL cholesterol. Each antioxidant has a linear relation with the LDL-cholesterol concentration on its natural scale, even in the lowest and highest 5% of the sample. The slopes of the relations were steepest for the antioxidants occurring at the highest concentrations, and lowest for those occurring at the lowest concentrations, with the modifications that the associations with HDL cholesterol were similar to the association between each antioxidant and LDL cholesterol, with the modification that the associations with HDL cholesterol were less pronounced than those with total and LDL cholesterol.
Each plasma antioxidant concentration was evaluated by regression on total cholesterol (model 1), on LDL cholesterol (model 2), on the combination of total and VLDL cholesterol (model 3), and on the combination of HDL, LDL, and VLDL cholesterol (model 4). Regression coefficients for models 3 and 4 are shown in Table 2 (models 1 and 2 are not shown). The combination of LDL, HDL, and VLDL cholesterol concentrations (model 4) accounted for significantly \( P \leq 0.03 \) more of the variability in antioxidant concentrations than did total cholesterol (model 1), LDL cholesterol alone (model 2), or the combination of total and VLDL cholesterol (model 3) for all antioxidants, with minor exceptions. The exceptions were \( \gamma \)-tocopherol, for which models 3 and 4 accounted for similar amounts of variation, and lycopene, for which models 1 and 4 accounted for similar amounts of variation. The combination of total and VLDL cholesterol concentrations (model 3) accounted for a large portion of the overall variation in antioxidant concentrations that are due to the lipoproteins and for more of the variability in antioxidant concentrations than did total cholesterol (model 1) and LDL cholesterol alone (model 2). The differences in \( R^2 \) values between models 2 and 3 were significant \( P < 0.001 \) in all comparisons except for lycopene. Model 1 explained more of the variance than did model 2, but less than did model 3. For comparison, the \( R^2 \) values for each antioxidant in model 1 in supplement nonusers and users, respectively, were as follows: \( \alpha \)-tocopherol, 0.330 and 0.187; \( \gamma \)-tocopherol, 0.073 and 0.074; zeaxanthin, 0.074 and 0.053; \( \beta \)-cryptoxanthin, 0.028 and 0.017; lycopene, 0.093 and 0.075; \( \alpha \)-carotene, 0.010 and 0.000; and \( \beta \)-carotene, 0.027 and 0.002. In model 3, these values were increased in supplement nonusers and users, respectively: \( \alpha \)-tocopherol, 0.050 and 0.033; \( \gamma \)-tocopherol, 0.037 and 0.006; zeaxanthin, 0.016 and 0.017; \( \beta \)-cryptoxanthin, 0.032 and 0.023; lycopene, −0.003 and −0.005; \( \alpha \)-carotene, 0.010 and 0.016; and \( \beta \)-carotene, 0.033 and 0.038.

The absolute \( R^2 \) values for model 4 tended to be lower in the supplement users than in the nonusers (Table 2). This is most apparent for \( \alpha \)-tocopherol, for which the \( R^2 \) values were 0.23 and 0.40 (model 4) in the supplement users and nonusers, respectively. The differences in \( R^2 \) values (model 4) between these groups for each antioxidant were highly significant \( P \leq 0.03 \) for all antioxidants except zeaxanthin plus lutein, \( \beta \)-cryptoxanthin, and lycopene.

The model 4 \( R^2 \) values differed widely between antioxidants in both the supplement user and nonuser groups. The most amphi-
thetic (polar) antioxidants (\( \alpha \)-tocopherol, \( \gamma \)-tocopherol, and zeaxanthin plus lutein) and lycopene had the highest \( R^2 \) values in both the supplement user and nonuser groups. The regression coefficients in the supplement nonusers (Table 2) ranged from a −0.087 \( \mu \text{mol/L} \) change to a 8.86 \( \mu \text{mol/L} \) change in antioxidant concentration, for a 1.0-mmol/L change in plasma lipid. In the supplement users (Table 2), coefficients ranged from a −0.178 \( \mu \text{mol/L} \) change to an 11.17 \( \mu \text{mol/L} \) change in antioxidant concentration, for a 1.0-mmol/L change in plasma lipid. The model 4 lipoprotein coefficients for supplement users and nonusers were generally highest for \( \alpha \)-tocopherol, \( \gamma \)-tocopherol, and lycopene.

For almost all antioxidants, each lipoprotein in model 4 had an independent association \( P \leq 0.02 \) with antioxidant concentra-
tions. There were 3 exceptions: 1) VLDL cholesterol was unrelated to zeaxanthin plus lutein and \( \alpha \)-carotene among supplement nonusers; 2) VLDL cholesterol was unrelated to zeaxanthin plus lutein among supplement users; and 3) LDL cholesterol was unrelated to \( \alpha \)-carotene among supplement users. The model 4 coefficients for the tocopherols were highest with VLDL cholesterol, and those for HDL and LDL cholesterol followed. Except for lycopene in the supplement nonusers, HDL cholesterol was more closely associated than was LDL cholesterol with each of the carotenoid concentrations. The association of VLDL cholesterol with carotenoids varied across antioxidants. VLDL cholesterol had a significant inverse association with \( \beta \)-cryptoxanthin and \( \alpha \)-carotene among supplement nonusers. Lycopene had a signifi-
cant positive association with VLDL cholesterol among both supplement users and nonusers. Among supplement users, \( \beta \)-cryptoxanthin and \( \alpha \)- and \( \beta \)-carotene had a significant inverse association with VLDL cholesterol. Thus, the specific lipoprotein moieties varied in their relations with the various antioxidants.

In predicting \( \alpha \)-tocopherol, regression coefficients were higher in the supplement nonusers than in the users (Table 2), eg, 2.99 and 2.86, respectively, for LDL in model 4.
TABLE 2
Associations between serum antioxidant vitamin concentrations and plasma cholesterol and lipoprotein concentrations in participants of the CARDIA study from year 0 (1985–1986, baseline) to year 7 (1992–1993)

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Total cholesterol</th>
<th>LDL</th>
<th>HDL</th>
<th>VLDL</th>
<th>R²</th>
<th>P</th>
<th>Chi-square versus change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intercept</td>
<td>β</td>
<td>SE</td>
<td>β</td>
<td>SE</td>
<td>β</td>
<td>SE</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>Nonusers</td>
<td>3</td>
<td>47.8</td>
<td>3.05</td>
<td>0.087</td>
<td>4.95</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>Users</td>
<td>4</td>
<td>2.58</td>
<td>2.86</td>
<td>0.089</td>
<td>4.81</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>1.95</td>
<td>2.89</td>
<td>0.15</td>
<td>4.31</td>
<td>0.35</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>Nonusers</td>
<td>3</td>
<td>1.91</td>
<td>0.52</td>
<td>0.043</td>
<td>1.73</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Users</td>
<td>4</td>
<td>1.68</td>
<td>0.50</td>
<td>0.044</td>
<td>0.71</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0.66</td>
<td>0.53</td>
<td>0.074</td>
<td>0.75</td>
<td>0.17</td>
</tr>
<tr>
<td>Zeaxanthin and lutein</td>
<td>Nonusers</td>
<td>3</td>
<td>0.10</td>
<td>0.053</td>
<td>0.0030</td>
<td>0.072</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>Users</td>
<td>4</td>
<td>0.0083</td>
<td>0.045</td>
<td>0.0030</td>
<td>0.13</td>
<td>0.0077</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0.011</td>
<td>0.043</td>
<td>0.0046</td>
<td>0.11</td>
<td>0.011</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>Nonusers</td>
<td>3</td>
<td>0.060</td>
<td>0.024</td>
<td>0.0019</td>
<td>0.069</td>
<td>0.0066</td>
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<tr>
<td></td>
<td>Users</td>
<td>4</td>
<td>0.035</td>
<td>0.022</td>
<td>0.0020</td>
<td>0.044</td>
<td>0.0050</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0.0021</td>
<td>0.018</td>
<td>0.0034</td>
<td>0.034</td>
<td>0.0080</td>
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<tr>
<td>Lycopene</td>
<td>Nonusers</td>
<td>3</td>
<td>0.123</td>
<td>0.10</td>
<td>0.0055</td>
<td>0.048</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Users</td>
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<td>0.125</td>
<td>0.10</td>
<td>0.0057</td>
<td>0.099</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>0.046</td>
<td>0.077</td>
<td>0.011</td>
<td>0.11</td>
<td>0.025</td>
</tr>
</tbody>
</table>

(Continued)
Serum antioxidant concentrations increased in proportion to an increase in plasma lipid levels in both supplement nonusers and supplement users (models 5, Table 2), which indicated an effect of lipids on antioxidant concentrations. Among supplement nonusers, each lipoprotein was a significant independent predictor, with minor exceptions. These exceptions included VLDL cholesterol for \( \beta \)-cryptoxanthin and lycopene, HDL cholesterol for \( \beta \)-carotene, and all lipoproteins for \( \alpha \)-carotene. Among the supplement users, the results indicated similar but less pronounced relations. These findings identified changes in lipoprotein concentrations as determinants of antioxidant concentrations over the course of a longitudinal epidemiologic study.

Application of adjustment formulas

In the comparison of models 1–4, model 4 generally accounted for significantly more of the variation in antioxidant concentrations than did the other models. This finding indicated that each of the lipoprotein moieties independently influenced antioxidant concentrations. Although the contribution of HDL and calculated LDL cholesterol concentrations may be trivial in small populations, the choice of model 3 rather than model 4 or 5 should be considered carefully. Two models, 4 and 5, contain each of the lipoprotein moieties independently influenced antioxidant concentrations. Among supplement nonusers, each lipoprotein was a significant independent predictor, with minor exceptions. These exceptions included VLDL cholesterol for \( \beta \)-cryptoxanthin and lycopene, HDL cholesterol for \( \beta \)-carotene, and all lipoproteins for \( \alpha \)-carotene. Among the supplement users, the results indicated similar but less pronounced relations. These findings identified changes in lipoprotein concentrations as determinants of antioxidant concentrations over the course of a longitudinal epidemiologic study.

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In the comparison of models 1–4, model 4 generally accounted for significantly more of the variation in antioxidant concentrations than did the other models. This finding indicated that each of the lipoprotein moieties independently influenced antioxidant concentrations. Although the contribution of HDL and calculated LDL cholesterol concentrations may be trivial in small populations, the choice of model 3 rather than model 4 or 5 should be considered carefully. Two models, 4 and 5, contain each of the lipoprotein moieties independently influenced antioxidant concentrations. Among supplement nonusers, each lipoprotein was a significant independent predictor, with minor exceptions. These exceptions included VLDL cholesterol for \( \beta \)-cryptoxanthin and lycopene, HDL cholesterol for \( \beta \)-carotene, and all lipoproteins for \( \alpha \)-carotene. Among the supplement users, the results indicated similar but less pronounced relations. These findings identified changes in lipoprotein concentrations as determinants of antioxidant concentrations over the course of a longitudinal epidemiologic study.
Several previous studies showed that blood lipids can confound the interpretation of blood antioxidant concentrations and that lipid-adjusted antioxidant concentrations are more sensitive indicators of antioxidant status, intake, and risk of disease than are unadjusted antioxidant concentrations. These studies used selective measures of blood lipids in their adjustments and thus represent an incomplete adjustment for the influence of blood lipids. A more comprehensive approach is taken in the current study, which considered all major lipids in plasma, as defined by the major circulating lipoproteins. The results have identified lipoproteins that influence blood antioxidant concentrations, described the extent of this influence in a human population, and compared several models of lipid adjustment.

Results of the current study show statistically significant linear relations between blood fat-soluble antioxidant and lipoprotein concentrations. These associations were found in cross-sectional observations between people, and they persisted through the evaluation of changes in antioxidant and lipoprotein concentrations with time, ie, when based on within-person longitudinal changes over a 7-y period, which strongly suggests that blood lipid influences antioxidant concentrations. In general, the participants who had increased blood lipid concentrations also had increased serum antioxidant levels and vice versa. In contrast, the ingestion of additional antioxidant vitamins in the form of supplements had little, if any, apparent influence on plasma total or lipoprotein cholesterol levels.

A model with independent adjustments for each major lipoprotein (LDL, HDL, and VLDL) accounted for as much or more variance (highest $R^2$) in antioxidant concentrations that was due to changes in blood lipid concentrations than did models with total cholesterol or subsets of the lipoproteins only. This finding indicates that lipoproteins individually provide unique contributions toward the concentrations of antioxidants. For some antioxidants, the additional contribution of HDL and LDL may be small relative to that in model 3 (cholesterol and VLDL only).

In almost all cases, each lipoprotein particle was related in a positive manner to antioxidant concentrations. Exceptions were described in a previous report (18). These inverse associations may be a result of dietary patterns associated with hypertriglyceridemia. Hypertriglyceridemia has been linked with high intakes of fat and simple carbohydrates (44) and low intakes of fruits and vegetables, the primary sources of carotenoids. This pattern of intake could yield low antioxidant and high VLDL concentrations in blood. In addition, the conversion of β-cryptoxanthin, α-carotene, and β-carotene—primary sources of vitamin A—to vitamin A in the presence of low intakes may further lower their concentrations and contribute toward an inverse association as

### TABLE 3
Changes in cholesterol and antioxidant vitamin concentrations from year 0 to year 7 in participants of the CARDIA study who consistently used or did not use vitamin supplements

<table>
<thead>
<tr>
<th></th>
<th>Supplement nonusers</th>
<th></th>
<th>Supplement users</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Year 7 − year 0$^a$</td>
<td></td>
<td>Year 0$^a$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Year 0$^a$</td>
<td></td>
<td>Year 7 − year 0$^a$</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.006 ± 0.016 [1869]</td>
<td>4.578 ± 0.861 [1869]</td>
<td>0.015 ± 0.031 [536]</td>
<td>4.580 ± 0.864 [536]</td>
</tr>
<tr>
<td>LDL</td>
<td>−0.035 ± 0.014 [1854]</td>
<td>2.845 ± 0.815 [1854]</td>
<td>−0.048 ± 0.027 [534]</td>
<td>2.817 ± 0.794 [534]</td>
</tr>
<tr>
<td>HDL</td>
<td>−0.031 ± 0.006$^b$ [1869]</td>
<td>1.355 ± 0.331 [1869]</td>
<td>−0.026 ± 0.012 [536]</td>
<td>1.402 ± 0.347 [536]</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.069 ± 0.008$^a$ [1868]</td>
<td>0.378 ± 0.246 [1868]</td>
<td>0.077 ± 0.012$^a$ [536]</td>
<td>0.362 ± 0.215 [536]</td>
</tr>
<tr>
<td>Antioxidants (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>2.160 ± 0.120$^a$</td>
<td>20.400 ± 5.28</td>
<td>2.880 ± 0.48$^a$</td>
<td>26.400 ± 8.640</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>0.696 ± 0.046$^a$</td>
<td>4.874 ± 2.089</td>
<td>0.232 ± 0.093$^a$</td>
<td>3.714 ± 1.620</td>
</tr>
<tr>
<td>Zeaxanthin plus lutein</td>
<td>0.009 ± 0.003$^a$</td>
<td>0.313 ± 0.147</td>
<td>0.013 ± 0.007</td>
<td>0.350 ± 0.172</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>0.001 ± 0.002</td>
<td>0.141 ± 0.090</td>
<td>−0.001 ± 0.005</td>
<td>0.184 ± 0.126</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.043 ± 0.007$^a$</td>
<td>0.569 ± 0.275</td>
<td>0.059 ± 0.012$^a$</td>
<td>0.532 ± 0.255</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>0.012 ± 0.001$^a$</td>
<td>0.043 ± 0.061</td>
<td>0.030 ± 0.004$^a$</td>
<td>0.074 ± 0.082</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.015 ± 0.004$^a$</td>
<td>0.255 ± 0.212</td>
<td>0.157 ± 0.020$^a$</td>
<td>0.357 ± 0.307</td>
</tr>
</tbody>
</table>

$^a$For cholesterol concentrations, $n$ in brackets. For antioxidant concentrations, $n = 1875$ supplement nonusers and 537 supplement users.

$^b$x ± SE.

$^c$x ± SD.

$^d$Slope significantly different from zero, $P < 0.001$. 

lipoprotein moieties and provide a basis for adjustment. It is important that model 4 is based on the measurement of between-subject change and that model 5 is based on the measurement of within-subject change. A comparison of the regression coefficients from model 4 and model 5 found a statistically significant within-subject change. A comparison of the regression coefficients can be developed for a given population with the inclusion of lipoprotein moieties and provide a basis for adjustment. It is important that model 4 is based on the measurement of between-subject change and that model 5 is based on the measurement of within-subject change. A comparison of the regression coefficients from model 4 and model 5 found a statistically significant within-subject change.
compared with other carotenoids. Unmeasured confounding is a possible limitation of all observational studies and could potentially influence coefficients of the equations. However, the general similarity of coefficients in the within-subject and between-subject equations (models 4 and 5), despite a statistically significant difference between the equations, suggests the absence of significant confounding for the coefficients in the models and the applicability of these findings to a larger population.

The pattern of association between lipoproteins and antioxidants varied for tocopherols and carotenoids. Tocopherols had high coefficients (an indicator of concentration in the particles) for VLDL, followed by HDL and then LDL. In contrast, all of the carotenoids involved a high HDL coefficient followed by LDL coefficients and a negative and/or statistically nonsignificant coefficient for VLDL particles. These unique patterns of distribution may be a result of antioxidant solubility and routes of absorption, of lipoprotein metabolism, and of the activity of transport proteins. Tocopherols are readily absorbed, transported via the lymph system into the circulation, and taken up by the liver. Hepatic tocopherols bind to the α-tocopherol transfer protein (45). This protein has a central role in the packaging of tocopherols in VLDL and regulation of blood tocopherol concentrations, because it preferentially identifies the α form of tocopherol and tends to exclude other forms of tocopherol from blood (46). The VLDL particles are metabolized to intermediate-density lipoprotein and then LDL particles with the retention of a small portion of their original α-tocopherol and carotenoids (47). HDL particles play a key role in the accumulation and exchange of α-tocopherol from other lipoproteins and tissues. These exchanges can occur through thermodynamic partitioning (diffusion) between particles (48, 49).

Overall, α-tocopherol is shuttled in a specific manner into lipoproteins. On the other hand, no transport proteins have been reported for the carotenoids. The carotenoids may be distributed primarily on the basis of thermodynamic partitioning rather than being directed into VLDL and specific transport protein routes. Some, but not all, reports have found the absorption and transport of carotenoids via the portal vein rather than via the lymph system as occurs with tocopherols (50), which indicates a less specific path of distribution.

Despite the high apparent concentration (reflected in the coefficients) of tocopherol in VLDL particles, these particles are not a large contributor to total serum tocopherol concentrations. A short half-life and low concentration limit the amount of total tocopherol in VLDL particles. Their contribution to total serum tocopherol concentration can be estimated by multiplying the mean VLDL cholesterol concentration (in mmol/L) by its coefficient and comparing this value to the mean tocopherol concentration. Similar computations performed with carotenoids are consistent with fractionation studies: most circulating carotenoids are distributed between LDL and HDL particles, with a small amount in VLDL particles (51). The polar carotenoids are generally more concentrated in HDL particles than in LDL particles, whereas hydrocarbon carotenoids are shifted further toward LDL particles than are the polar carotenoids (52).

Elevated concentrations of antioxidants among supplement users may reflect higher intakes of vitamin E and β-carotene and a sparing action on other antioxidants, thereby protecting them from oxidation. Although only 6 subjects explicitly reported β-carotene supplementation, more subjects probably were ingesting it, given that it is generally the source of vitamin A in vitamin A supplements. In addition, supplement users may be more health conscious; they report consuming more servings of fruits and vegetables than do supplement nonusers in some populations (53). The particular pattern of food intake by supplement users apparently would not include good sources of γ-tocopherol and lycopene, such as meat and pasta dishes (54).

Standardization is particularly useful for the most amphiphatic antioxidants (α-tocopherol, γ-tocopherol, and zeaxanthin plus lutein) and lycopene. However, lipid adjustment may be beneficial for some of the other antioxidants (β-cryptoxanthin and β-carotene) in certain situations. These include well-controlled feeding studies, cholesterol-lowering studies, and intervention studies wherein small changes of antioxidant concentrations may be important markers of status or wherein lipid-lowering interventions can mask the true changes in antioxidant concentrations.

The use of total cholesterol or total cholesterol and triacylglycerol for standardization can be a suitable second choice and is less costly than performing a lipoprotein profile. The use of LDL alone or apolipoproteins had marginal value in standardizing the fat-soluble antioxidant concentrations.

PH and XY performed the data and statistical analyses; CP performed the chemical analysis and data collection for all antioxidant measurements; DJ oversaw the statistical analysis and study design and aided in writing the manuscript; and MG provided the conceptual basis of the study, oversaw most aspects of the project, and wrote the final manuscript.

REFERENCES