Evaluating Serum α-Tocopherol (Vitamin E) in Terms of a Lipid Ratio*

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ABSTRACT

It is well known that accurate assessment of serum α-tocopherol requires knowledge of the serum lipids also present. Patient specimens (n = 307) within the standard reference limit of 5–18 mg/L were used to determine the following 95 percent reference interval for a lipid ratio of α-tocopherol to the sum of cholesterol and triglycerides [E/(C + T)]: 1.4 (1.20–1.56) – 5.7 (5.51–6.91) mg/g (90 percent confidence interval). In terms of α-tocopherol status, patients with low results (<5 mg/L) were normal on reevaluation with the lipid ratio in 47 percent of those examined (28 of 59), and elevated results (>18 mg/L) were normal or low in 58 percent (26 of 45). Elevated triglycerides developed from non-fasting specimens were one common reason for misleading results when lipids were not considered. When measuring α-tocopherol in a patient population, evaluation of the lipid content is needed for accurate assessment in a significant number of cases.

Introduction

Vitamin E is fat-soluble vitamin and an essential nutrient for humans.1 α-Tocopherol is the most biologically active form of vitamin E and the most relevant for clinical evaluation. Vitamin E and α-tocopherol are often used interchangeably,2 as they are here. It is well known that accurate assessment of serum vitamin E requires consideration of the lipid content of the specimen.3,4,5 However, textbooks of clinical chemistry typically list reference intervals only in terms of the simple serum concentration,2,6 and most clinical laboratories continue to report vitamin E in the same manner. The clinical reference laboratory at this institution analyzes about 500 serum specimens per month for vitamin E and, like most clinical laboratories, reports values in terms of the concentration. The purpose of the present study is to define an adult reference interval for the lipid ratio of α-tocopherol to the sum of cholesterol and triglycerides [E/(C + T)], and to illustrate the limitations of reporting α-tocopherol as a simple concentration in a patient population.

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There is a variety of reasons why the determination of a lipid ratio is not routine, the foremost being that it requires additional tests, specimen volume and expense. In addition, there is an assumption that the simple concentration is sufficiently accurate, at least in most cases. Other problems with a lipid ratio include questions about which lipids to use for the calculation, and the fact that a lipid ratio developed in one setting is often difficult to apply to other circumstances. Finally, some references suggest that serum is in adequate for the evaluation of vitamin E even after adjusting for the lipid content, and that other specimen types should be used.

Tissue concentration is probably the single best way to assess vitamin E, although tissue is not an acceptable specimen for clinical practice. Plasma and serum are used most often for clinical assessment, although the correlation with tissue is poor. Since blood contains about 1 percent of the total body stores of vitamin E, this small amount can be altered by many circumstances without significantly affecting the amounts in tissues and without any change in dietary intake. Other specimens used for assessment include platelets, leukocytes and erythrocytes. Functional tests such as the resistance of the erythrocyte against hydrogen peroxide-induced hemolysis are useful in certain circumstances, although many other factors in addition to vitamin E affect the results of such functional tests. The ideal method with which to assess vitamin E remains in dispute. No single test is completely adequate to evaluate vitamin E status, and a variety of testing may be needed, depending on clinical circumstances. However, it is clear that when using serum or plasma, a lipid ratio gives a more adequate assessment of vitamin E than does the simple serum concentration.

**Methods**

**α-Tocopherol assay.** α-Tocopherol was measured with a reverse-phase HPLC assay utilizing UV detection. (Retinol, retinyl palmitate and δ-/γ-tocopherol fraction were also seen on the chromatograms). Briefly, 100 μL ethanol (containing a retinyl acetate internal standard) was added to 100 μL patient serum to precipitate proteins. Hexane (1.0 mL) was then added to extract the fat-soluble components. The mixture was centrifuged at 5000 g for 3 minutes, and a portion of the hexane layer removed for drying at 40°C in a stream of nitrogen. The residue was resuspended in 50 μL ethanol and transferred to instrument vials. Instrumentation consisted of an HP1090 HPLC (Hewlett-Packard, Wilmington, DE) equipped with an HP 1050 diode array detector. The prepared specimen (30 μL) was injected onto a 5 μm Prodigy 250 mm × 4.6 mm column (Phenomenex, Torrance, CA) using gradient elution consisting of 90 percent methanol and 10 percent water, and 90 percent ethyl acetate and 10 percent isopropanol. The signal was monitored at 305 and 292 nm. Routine controls showed a CV of 8.3 percent at 3.6 and 10.8 mg/L. The laboratory is enrolled in the National Institute of Standards and Technology/National Cancer Institute (NIST/NCI) Fat-Soluble Vitamins Quality Assurance Program.

**Cholesterol and triglycerides assays.** Cholesterol was determined with a commercially available enzymatic assay based on cholesterol esterase and cholesterol oxidase on an automated instrument (BMC/Roche Hitachi 917, San Jose, CA). Likewise, triglycerides were determined with an enzymatic method based on lipase, glycerol kinase and glycerol phosphate oxidase on the same instrument. Routine controls showed a CV of 2 percent at 125 and 264 mg/dL for cholesterol, and 3 percent at 101 mg/dL and 2 percent at 265 mg/dL for triglycerides.

**Study specimens.** Study specimens were selected from patient specimens received for vitamin E analysis between April 7 and June 30, 1998. Specimens which fit the following criteria were used: 1) Testing complete, 2) age known, 3) age > 17 years old and 4) at least 0.25 mL serum remaining for determination of cholesterol and triglycerides. The lipid ratio in units of mg/g was calculated by dividing α-tocopherol (E, mg/L) by the sum of cholesterol (C, g/L) and triglycerides (T, g/L): E/(C + T).
Results

A total of 412 patient specimens received for serum α-tocopherol testing were collected and also tested for cholesterol and triglycerides. One specimen with an α-tocopherol of 308 mg/L was excluded as an outlier (a 24-year-old female with a cholesterol of 408 mg/dL and triglycerides of 2670 mg/dL); the next highest α-tocopherol was 48.5 mg/L. The remaining 411 specimens were defined as the study population. Of these, 307 were normal in terms of α-tocopherol (reference interval 5-18 mg/L), 2,6 59 were low (<5 mg/L) and 45 were elevated (>18 mg/L). Comparisons between simple concentrations and lipid ratios \( \frac{E}{C + T} \) are shown graphically in figure 1 (normal α-tocopherol), figure 2 (low α-tocopherol) and figure 3 (elevated α-tocopherol).

To calculate the reference interval for the lipid ratio, all specimens within the normal α-tocopherol reference interval of 5–18 mg/L were selected \( (n = 307) \). Using the nonparametric rank numbering method described by Solberg,10 the study specimens were ordered by increasing lipid ratio. The lower 2.5 percent limit corresponded to 1.42 mg/g (rank number 8), and the upper 97.5 percent to 5.71 mg/g (rank number 300). Using table 14–5 in Solberg,10 the lower 90 percent confidence interval was determined to be 1.20–1.56 mg/g (rank numbers 3–13), and the upper 90 percent confidence interval to be 5.51–6.91 mg/g (rank numbers 295–305). To facilitate comparison with another study, the 5–95 percent interval was also determined to be 1.6–5.3 mg/g.

Of the 307 specimens in the normal α-tocopherol interval of 5–18 mg/L (figure 1), the average lipid ratio was 3.1 mg/g (SD 1.0, range 1.0–8.6). The average age was 51 years old (SD 18, range 18–96), and the average cholesterol was 184 mg/dL (SD 52, range 67–339). The cholesterol distribution showed 12 < 100 mg/dL, 36 > 250 mg/dL and 3 > 300 mg/dL. The average triglycerides were 155 mg/dL (SD 97, range 35–714), and the distribution showed 69 > 200 mg/dL, 23 > 300 mg/dL and 8 > 400 mg/dL.

Of the 59 specimens with α-tocopherol below the reference limit of 5 mg/L (figure 2),
28 had a normal lipid ratio. The average age was 40 years old (SD 18, range 17-78), and the average cholesterol was 120 mg/dL (SD 38, range 41-258). The cholesterol distribution showed 15 < 100 mg/dL and 1 > 250 mg/dL. The average triglycerides were 104 mg/dL (SD 55, range 47-278), and the distribution showed 4 > 200 mg/dL. The average lipid ratio was 1.47 (SD 0.68, range 0.17–3.36).

Of the 45 specimens with \( \alpha \)-tocopherol above the reference limit of 18 mg/L (figure 3), 25 had a normal lipid ratio and one had a low lipid ratio. The average age was 61 years old (SD 15, range 18–82), and the average cholesterol was 243 mg/dL (SD 51, range 139–393). The cholesterol distribution showed 20 > 250 mg/dL and 4 > 300 mg/dL. The average triglycerides were 316 mg/dL (SD 301, range 73–1747), and the distribution showed 26 > 200 mg/dL, 15 > 300 mg/dL and 3 > 600 mg/dL. The average lipid ratio was 5.55 (SD 2.47, range 1.04–14.1).

Discussion

One problem with reporting vitamin E as a lipid ratio is the fact that a variety of lipids is present in the serum, and different studies use different lipids. In 1970, Horwitt and others advocated employing an assay for total lipids, but such assays are now seldom used. More suitable are the automated assays for cholesterol and triglycerides, which enjoy wide popularity and have excellent quality control. Phospholipids would also be included under ideal circumstances; however, the assays for phospholipids are not generally available and are not as well developed in terms of automation and quality control. The sum of cholesterol and triglycerides has been shown to give good correlation with the sum of cholesterol, triglycerides and phospholipids. In contrast, using either cholesterol or triglycerides alone does not give as good a correlation as the sum of the two.

Reference limits. The present study found a lower reference limit (90 percent confidence interval) for the lipid ratio of 1.42 mg/g (1.20–1.56), and an upper limit of 5.71 (5.51–6.91). It should be emphasized that the group used to estimate these limits comprised patients being evaluated specifically for vitamin E for diverse reasons unknown to the laboratory. The group
Comparing lipid ratios. To compare studies which used different lipids to calculate the lipid ratio, the following assumptions were made: The average cholesterol (C) was taken as 1.8 g/L (180 mg/dL), triglycerides (T) as 1.6 g/L and phospholipids (P) as 2 g/L. These values were substituted into the following equations: \( R_C = E/C \), \( R_{CT} = E/(C + T) \) and \( R_{CTP} = E/(C + T + P) \). The following relationships are grossly oversimplified but provide a straightforward method of comparison: \( R_{CT} = 0.53 \ R_{C} \) and \( R_{CT} = 1.6 \ R_{CTP} \).

Sokol and others\(^4\) found a lower reference limit for adults of 0.8 mg/g (0.6 mg/g for children). This study employed an older fluorometric assay which determined total vitamin E, and calculated a lipid ratio by dividing vitamin E by the sum of cholesterol, triglycerides and phospholipid \( E/(C + T + P) \). Compensating for the presence of phospholipid gives an adjusted lower limit of 1.3 mg/g, which compares to 1.4 (1.20–1.56) mg/g in the present study. The clinical status of patients in the present study was unknown, and results below the reference limit of 1.4 mg/g are likely to identify those at risk for deficiency. Overtly symptomatic deficiency is more likely to occur below the limit defined at 1.3 mg/g.

Winklhofer-Roob and others\(^11\) calculated a lipid ratio reference interval of 4.7–7.5 mg/g (converted from mmol/mol) based on cholesterol alone. The population examined was a healthy group of Swiss citizens, and the reference interval was based on an interval of 5–95 percent as opposed to 2.5–97.5%. Compensating for the lack of triglycerides gives an adjusted reference interval of 2.5–4.0 mg/g, compared to 1.6–5.3 mg/g (5–95%) in the present study. This is a result of the differences in the groups studied, with specimens collected from healthy individuals showing less variation than those collected from a patient population.
population. It is worth emphasizing that a reference interval based on healthy individuals is likely to identify a large percentage of patient-based populations as abnormal.

**Upper reference limit.** The present study found an upper reference limit of 5.7 mg/g, and values above this are presumably a consequence of excess oral supplementation. When following the principle that a balanced nutritional state is the most desirable, individuals with values above the upper limit should be counseled to decrease their level of supplementation. Excess vitamin E is well tolerated in adults and is generally regarded as nontoxic. However, excess vitamin E is associated with a variety of subtle changes, including depressed leukocyte function, interference with vitamin K activity, elevated serum triglycerides and reduction in serum thyroid hormones. Some authors attribute excess supplementation with causing hypertension, thrombophelebitis, pulmonary embolism and a number of other conditions of some patients. Christen and others suggest that excess \( \alpha \)-tocopherol may displace other vitamin E isomers, such as \( \gamma \)-tocopherol, which may be more active in specific circumstances, as in the detoxification of nitrogen radicals. Enstrom and Pauling propose that excess vitamin E supplementation is associated with increased mortality compared to more modest supplementation. In brief, it seems prudent to set an upper limit on a desirable lipid ratio.

**Case studies.** Some of the advantages to using a lipid ratio can be illustrated by examining specific cases. For example, specimen 24 (figure 1) was from a 34-year-old male and showed an \( \alpha \)-tocopherol of 7.6 mg/L, a normal serum cholesterol of 156 mg/dL and a significantly elevated triglycerides of 458 mg/dL (reference limit <200 mg/dL). In all likelihood, this represents a non-fasting specimen, and the lipid ratio of 1.24 mg/g demonstrates that this patient is actually vitamin E deficient. Among the specimens illustrated in figure 1, 69 of 307 (22 percent) showed elevated triglycerides. The majority of these probably represent specimens collected from non-fasting patients, although a small number are also from patients with hyperlipidemias. Two points are worth emphasizing: 1) an \( \alpha \)-tocopherol concentration within the standard reference interval of 5–18 mg/L does not ensure an adequate vitamin E status, and 2) common problems such as collection of non-fasting specimens can easily produce misleading results unless the lipid status is also known.

Specimen 151 (figure 1) is from a 22-year-old male and showed an \( \alpha \)-tocopherol of 11.9 mg/L. In this case, cholesterol and triglycerides were a modest 112 mg/dL and 57 mg/dL, respectively, and the lipid ratio of 7.04 mg/g is consistent with excess vitamin E supplementation.

**Low vitamin E.** Figure 2 compares \( \alpha \)-tocopherol concentrations <5 mg/L with the lipid ratio, which demonstrates that 28 of these 59 results (47 percent) are actually normal in terms of vitamin E status. For example, specimen 328 (figure 2) was from a 72-year-old female patient. The \( \alpha \)-tocopherol was low at 2.7 mg/L, but the associated cholesterol and triglycerides were also low at 57 and 50 mg/dL, respectively. The lipid ratio of 2.8 mg/g is consistent with adequate vitamin E status. The low cholesterol is concerning for other reasons, but this patient is not deficient in terms of \( \alpha \)-tocopherol.

**High vitamin E.** Figure 3 compares \( \alpha \)-tocopherol concentration > 18 mg/L with the lipid ratio, which demonstrates that 25 of these 45 results (56 percent) are actually normal in terms of vitamin E status and that one is actually deficient. Specimen 286 (figure 3) was from a 44-year-old female who had an elevated \( \alpha \)-tocopherol of 21.3 mg/L, an elevated cholesterol of 301 mg/dL and a massively elevated triglycerides of 1750 mg/dL. (It was not known if the patient was suffering from pancreatitis.) The lipid ratio of 1.04 mg/g correctly identifies this patient as vitamin E deficient.

Although elevated \( \alpha \)-tocopherol concentrations are often associated with hyperlipidemias, this is not always the case. For example, specimen 315 (figure 3) was from a 70-year-old male who had an \( \alpha \)-tocopherol of 33 mg/L, a cholesterol of 73 mg/dL and a triglycerides of 234 mg/dL. The lipid ratio is elevated at 14.1...
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mg/g and is consistent with excess supplementation. To distinguish between hyperlipidemia and excessive supplementation requires consideration of the lipid content.

\section*{Conclusions}

Calculation of a lipid ratio is considerably more complicated than the determination of a simple $\alpha$-tocopherol concentration, but also gives a more accurate assessment of vitamin E status. The reference interval determined in the present study was 1.4–5.7 mg/g for the ratio of $\alpha$-tocopherol to the sum of cholesterol plus triglycerides [$E/(C+T)$]. Individuals below the reference interval are at risk for marginal $\alpha$-tocopherol status, although other studies suggest symptomatic deficiency is not likely to develop until <1.3 mg/g. Values above the reference interval are presumably associated with excess supplementation but do not represent overt toxicity. When the goal is to achieve a balanced nutritional state, individuals above the upper limit should consider reducing their level of supplementation. Patient results below the standard concentration-based reference limit of 5 mg/L were normal in terms of a lipid ratio in 47 percent of those evaluated (28 of 59), and patient results above the upper reference limit of 18 mg/L were normal in 56 percent of those evaluated (25 of 45). Elevated triglycerides developed from specimens are a common reason for misleading results when the lipid status was unknown. When measuring serum $\alpha$-tocopherol in a patient population, knowledge of the associated lipid content is needed for accurate assessment in a significant number of cases.

\section*{References}