



Erythrocyte tocopherol isomers in the investigation of vitamin E deficiency

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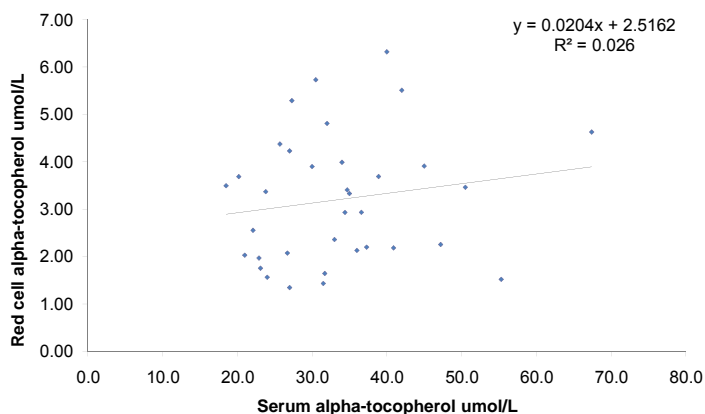
ABSTRACT

We have established a method for erythrocyte α - and γ -tocopherol using reverse-phase HPLC, as an adjunct to plasma measurement in diagnostically difficult situations. The extraction procedure for erythrocytes is more challenging than for plasma, since it requires the complete disruption of red cell membranes prior to extraction into heptane. The derived reference interval for erythrocyte α -tocopherol was 1.3-5.7 $\mu\text{mol/l}$ and for plasma 25 - 60 $\mu\text{mol/l}$. According to our results for vitamin E-replete subjects, the mean ratio of erythrocyte to plasma α -tocopherol concentration is 0.10 (range 0.03-0.19). This figure is somewhat lower than previous reports, which have suggested mean erythrocyte /plasma ratios ranging from 0.15 to 0.21.

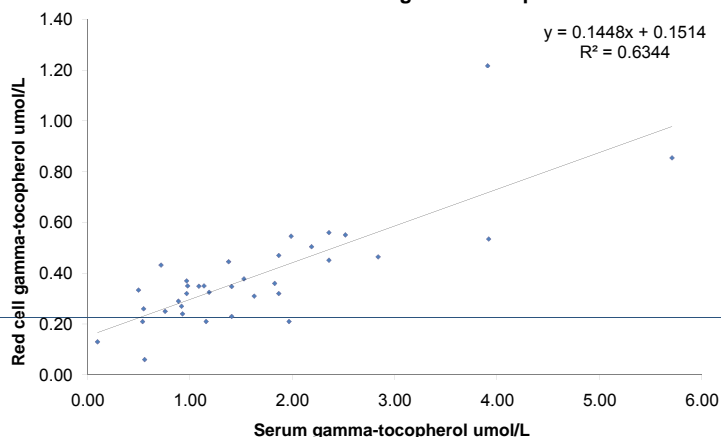
METHODS

To prepare red cells for analysis, heparinised whole blood was centrifuged for 10 minutes, the plasma removed from the cells, and then the cells washed three times in phosphate-buffered saline, pH 7.40, before freezing overnight to disrupt lipid membranes. The samples were thawed and 250 μL of red cell lysate was mixed with 1.0 mL of methanol:triton X-100 (9:1) containing 20 mM sodium dodecyl sulphate (SDS) and 148 μM tocopherol acetate (internal standard). The extracts were vortexed for 5 minutes, before the addition of 1.0 mL of heptane with 0.5 gm/L butylated hydroxytoluene (BHT) and further vortex mixing for 30 minutes. Serum samples were also extracted into heptane and BHT. 600 μL of heptane supernatant was then taken into a fresh glass tube for vacuum evaporation at 40°C, before re-constitution in HPLC mobile phase. HPLC conditions for serum samples were as described by Thurnam [1]. HPLC conditions for red cell extracts were: mobile phase – methanol, 1 ml/minute, injection volume 10 μl , and fluorescence detection with excitation at 294 nm and emission at 336 nm.

Red cell and serum alpha-tocopherol



Red cell and serum gamma-tocopherol



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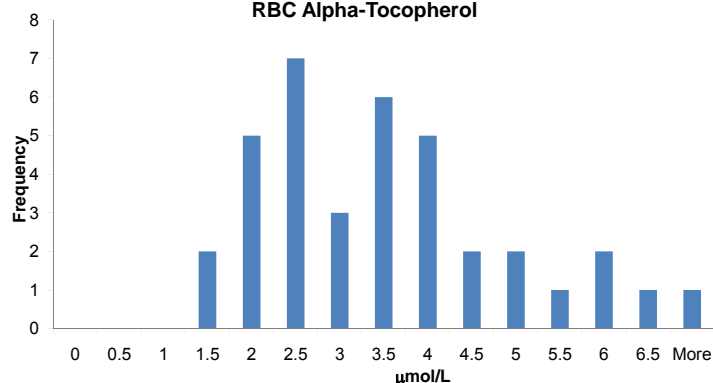
WHY MEASURE RED CELL TOCOPHEROLS?

Serum α - and γ -tocopherol concentrations are commonly used as markers of vitamin E status, but in certain conditions may have a limited diagnostic value. For example, the serum level in patients suffering from ataxia with vitamin E deficiency (AVED syndrome) can fluctuate rapidly during supplementation, rather than achieving a steady state. The erythrocyte tocopherol content is less susceptible to fluctuations after supplementation and is a possible marker of tissue vitamin E status.

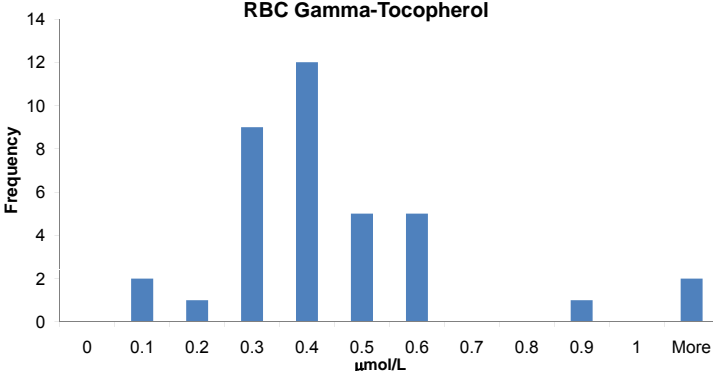
The maintenance of serum concentrations of α -tocopherol normally depends on the action of α -tocopherol transfer protein, secreted by hepatocytes, to preferentially take up α -tocopherol from the portal blood, while other forms of vitamin E are more rapidly metabolized and excreted [2,3]. As a result, blood and cellular concentrations of other vitamin E isomers are lower than those of α -tocopherol and have been the subjects of less research [3,4].

The initial aim of this study was to establish the vitamin E status of a subject with AVED syndrome, in which there is an abnormality of α -tocopherol transfer protein. In such a case, the erythrocyte tocopherol content is a possible marker of tissue vitamin E status. The subject monitored had developed a sensory neuropathy due to axonal nerve damage (as judged by electrophysiological studies) and then took 1200 IU of vitamin E per day (a high dose) for 8 months. Plasma α -tocopherol was measured as 23.0 $\mu\text{mol/l}$, which is below the reference interval, but the erythrocyte α -tocopherol was 1.97 $\mu\text{mol/l}$ (normal), with an erythrocyte /plasma ratio of 0.09, which is near the median value for this ratio. These results suggest that the intensive supplementation programme undertaken had resulted in vitamin E accumulation in the tissues, even though the plasma level remained low.

RBC Alpha-Tocopherol



RBC Gamma-Tocopherol



CONCLUSIONS

Erythrocyte tocopherol measurement thus has a possible role in the investigation of difficult cases of vitamin E deficiency. As can be seen from the figures opposite, there is a good correlation between between serum and erythrocyte γ -tocopherol, but not between serum and erythrocyte α -tocopherol.

Chow² measured the distribution of tocopherols in serum and red blood cells, reporting a range for serum α -tocopherol of 15.3-34.8 $\mu\text{mol/l}$ and for erythrocyte α -tocopherol of 2.1-4.2 $\mu\text{mol/L}$, with a mean erythrocyte /plasma ratio of 0.15. Mino et al.³ measured serum and red cell tocopherols in a Japanese population and recorded a serum α -tocopherol range of 12.4-36.9 $\mu\text{mol/l}$, an erythrocyte range of 2.3-5.6 $\mu\text{mol/l}$, with a mean erythrocyte/plasma ratio of 0.18. The influence of supplementation dosages on serum and erythrocyte tocopherols was researched by Lehmann et al⁴, who reported mean values for α -tocopherol in serum of 23.9 $\mu\text{mol/l}$ and in erythrocytes of 5.1 $\mu\text{mol/l}$, with a mean erythrocyte /plasma ratio of 0.21.

Our results for vitamin E-replete subjects suggest a range for the erythrocyte /plasma α -tocopherol ratio of 0.03-0.19 (mean value 0.10), which is lower than that of previous investigators. Our results also suggest a range for the erythrocyte /plasma γ -tocopherol ratio of 0.11-0.67 (mean value 0.28), which underlines the different physiological mechanisms for the tissue handling of tocopherol isomers.

We have found no previous reports in the literature of the erythrocyte /plasma γ -tocopherol ratio.

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