Determination of tocotrienol and tocopherol isomers at physiological concentration by HPLC in Caucasian and Japanese women

Che Sam Lo, Mark L Wahlqvist, Yoshimitsu Horie*, Kazuyo Horie** and Naiyana Wattanapenpaiboon

*Department of Medicine, Monash Medical Centre, Monash University, Melbourne, Australia, *Nagoya Municipal Women's Junior College, Nagoya, Japan, and **Aichi Gakusen University, Okazaki, Aichi, Japan.*

A sensitive, specific and simple method for simultaneous evaluation of tocopherol and tocotrienol isomers in human serum by normal phase HPLC with a fluorescence detector has been developed. Tocopherol and tocotrienol isomers are measurable in physiological concentration in human serum by this method. There is no significant difference in serum alpha- and beta-tocopherols and alpha-, gamma-, and delta-tocotrienols between Caucasian and Japanese subjects. However, serum gamma- and delta-tocopherol concentrations in Japanese women are significantly higher than in Caucasian women.

Introduction

Most interest in vitamin E centres around cardiovascular disease and cancer because of is natural antioxidant capabilities[1-3]. Little is known about tocotrienol and tocopherol isomer status in health or disease or how such status varies with food culture or ethnicity.

In the present study we sought: (1) to develop an HPLC method for the simultaneous assessment of serum tocotrienol and tocopherol isomer status, applicable to clinical and population-based studies; and (2) to assess the status of these compounds in those with disparate food cultures, Caucasian and Japanese.

Subjects and methods

The study involved 14 apparently healthy Caucasian women from Melbourne, Australia and 21 age-matched healthy Japanese women from Nagoya, Japan (Table 1).

Each subject was asked not to eat or drink anything other than water after 8.00 pm until after the blood test the following morning. A fasting blood sample was collected at 8.30–9.00 am for tocotrienol and tocopherol isomer estimation.

The serum samples were prepared as follows. (1) Storage at –70°C. (2) Extraction and measurement were conducted in a darkroom with a red filtered light. (3) 300µl of serum were precipitated with 300µl of methanol and the tocotrienol and tocopherol isomers extracted with 1.2ml of hexane. (4) Samples were then vortexed and centrifuged and 0.8ml of supernatant pipetted into a 3ml brown bottle. (5) The sample was dried under nitrogen and redissolved in 50µl of hexane. (6) Duplicate 20µl samples were applied through the U6K injector to the HPLC column.

The HPLC column was µ-Porosil, 10µm (30 cm x 0.46 cm ID); the mobile phase was hexane/isopropanol (99.5/0.5 by volume); the flow rate was 2.2 ml/min and detection was with an F-1050 Hitachi fluorescence detector at EX 298 nm, EM 325 nm with attenuation 8; ambient temperature was 20–25°C. Tocopheryl acetate

Table 1. The age, stature, body weight and body mass index (BMI) of Caucasian and Japanese women (Mean ± SEM).

Subjects	n	Age (years)	Stature (cm)	Body weight (kg)	BMI (kg/m²)
Caucasian	14	49±2 (40–62)	163±1 (154–170)	63±3 (49–94)	23.8±1.4 (19.5–35.3)
Japanese	21	50±1 (41–59)	155±1 (143–161)	54±1 (43–64)	22.5±0.6 (16.8–28.1)

Correspondence: Dr C.S. Lo, Department of Medicine, Monash Medical Centre, Monash University, Clayton Road, Clayton, Melbourne, Victoria 3168, Australia.

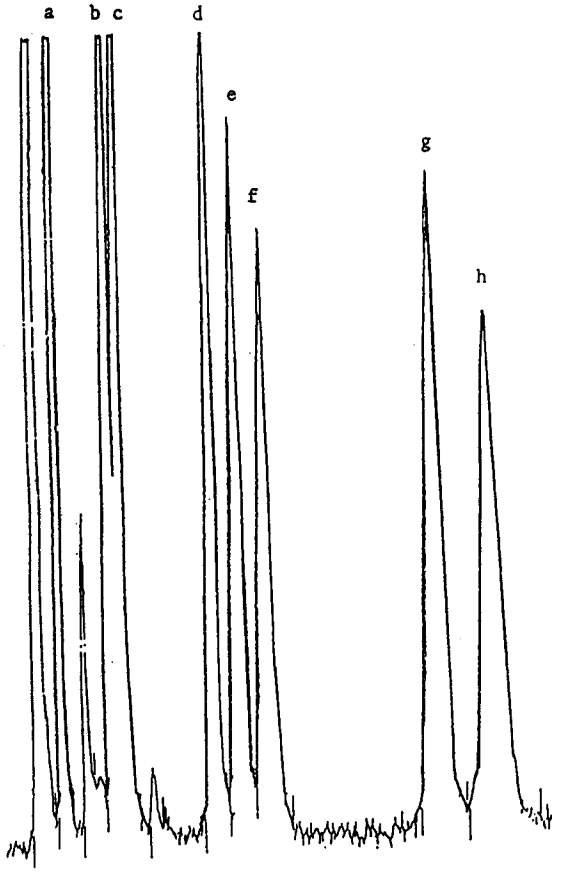


Figure 1. Chromatogram of a standard solution. a: tocopheryl acetate as internal standard (RT 1.99); b: alpha-tocopherol (RT 3.13); c: alpha-tocotrienol (RT 3.39); d: beta-tocopherol (RT 5.39); e: gamma-tocopherol (RT 5.97); f: gamma-tocotrienol (RT 6.58); g: delta-tocopherol (RT 10.35); h: delta-tocotrienol (RT 11.53).

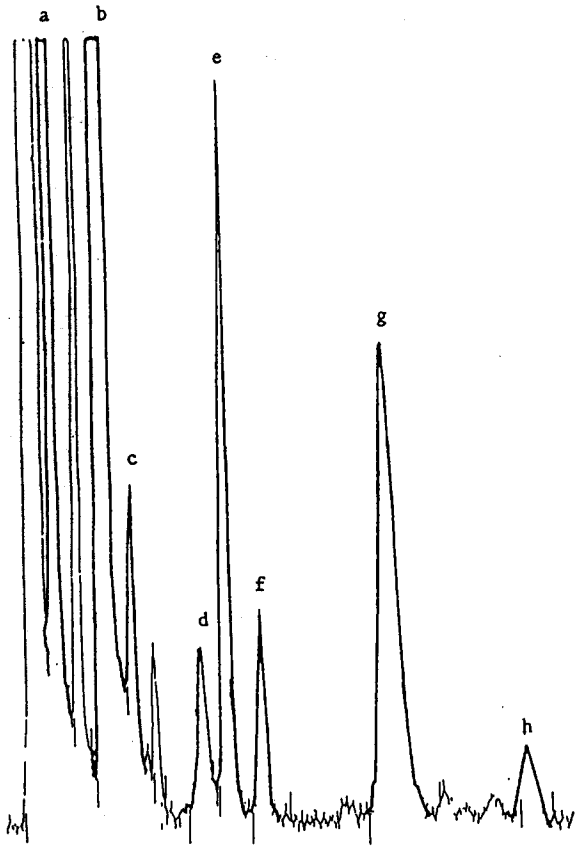


Figure 2. Chromatogram of a serum extract. a: tocopheryl acetate as internal standard (RT 1.97); b: alpha-tocopherol (RT 3.07); c: alpha-tocotrienol (RT 3.73); d: beta-tocopherol (RT 5.29); e: gamma-tocopherol (RT 5.80); f: gamma-tocotrienol (RT 6.57); g: delta-tocopherol (RT 9.54); h: delta-tocotrienol (RT 12.58).

was used as an internal standard. Figures 1 and 2 are typical chromatograms for standard tocotrienols and tocopherols and for serum from a Caucasian women. Reproducibility and recovery of tocotrienol and tocopherol isomers are shown in Table 2.

Results

Tocotrienol and tocopherol isomer levels in women's serum are shown in Table 3. There are no significant differences in serum alpha-tocopherol, alpha-tocotrienol, beta-tocopherol, gamma-tocotrienol and delta-tocotrienol between Caucasian and Japanese women. However, Japanese women had significantly higher gamma-tocopherol and delta-tocopherol concentrations than did

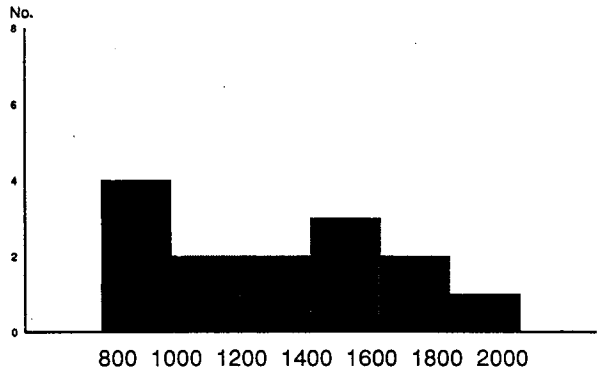
Table 2. Reproducibility and recovery of tocotrienol and tocopherol isomers.

Vitamin E isomers	Reproducibility (%CV, n=8)	Recovery (%, n=8)
alpha-tocopherol	2.3	101.9
alpha-tocotrienol	5.0	100.9
beta-tocopherol	7.8	98.5
gamma-tocopherol	5.2	96.0
gamma-tocotrienol	3.2	92.2
delta-tocopherol	5.8	96.6
delta-tocotrienol	8.2	95.5

Table 3. Tocotrienol and tocopherol isomer levels in women's sera, (μg/100 ml serum, mean±SEM).

	Japanese (n=21)	Caucasian (n=14)
alpha-tocopherol	1134 ± 67	1205 ± 98
alpha-tocotrienol	37.8 ± 11.9	27.8 ± 6.2
beta-tocopherol	21.4 ± 1.3	20.0 ± 1.7
gamma-tocopherol	109.0 ± 9.0**	43.3 ± 6.5
gamma-tocotrienol	22.8 ± 1.5	31.5 ± 11.0
delta-tocopherol	23.2 ± 3.7*	8.1 ± 0.08
delta-tocotrienol	12.4 ± 1.8	8.6 ± 1.1
Total tocopherol	1287.2 ± 74.0	1276.1 ± 102.0
Total tocotrienol	73.0 ± 13.74	68.4 ± 13.3

n is the number of subjects. The mean±SEM are shown. The significant differences between Caucasian and Japanese women are indicated by * $P<0.005$, ** $P<0.001$.



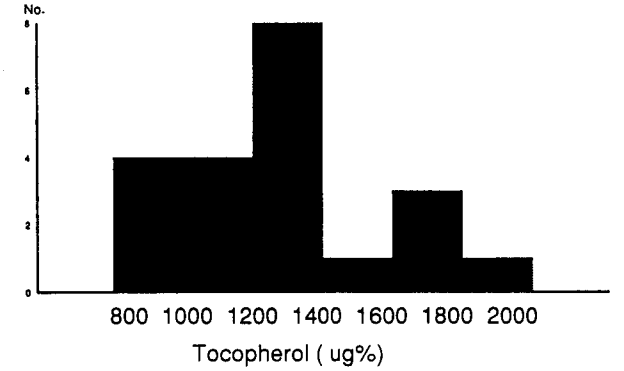
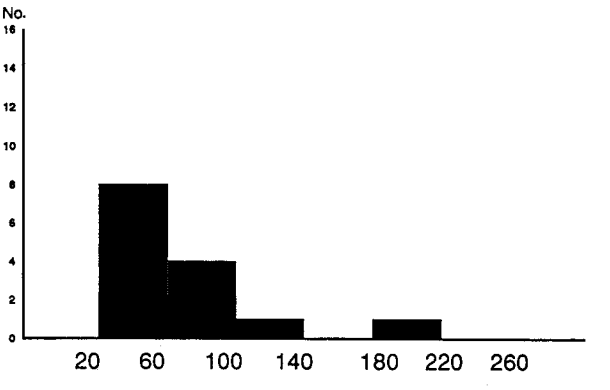


Figure 3. Distribution of serum tocopherol of 14 Caucasian women (top) and 21 Japanese women (bottom).



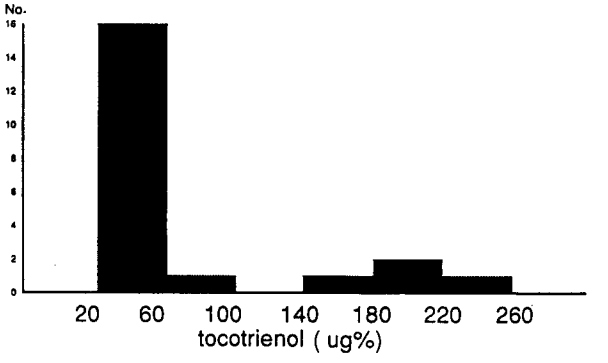


Figure 4. Distribution of serum tocotrienol of 14 Caucasian women (top) and 21 Japanese women (bottom).

Table 4. Tocopherol and tocotrienol content of food.[4,5]

Food	Tocopherol (mg%)				Tocotrienol (mg%)			
	Alpha	Beta	Gamma	Delta	Alpha	Beta	Gamma	Delta
Coconut oil	0.5	—	0	0.6	0.5	—	—	—
Cottonseed oil	38.9	—	38.7	0	0	—	—	—
Olive oil	5.1	—	Tr	0	0	—	—	—
Palm oil	25.6	—	31.6	7.0	14.3	3.2	—	28.6
Peanut oil	18.9	—	21.4	2.1	0	—	—	—
Rapeseed oil	23.6	—	38.0	1.2	0	—	—	—
Safflower oil	39.6	—	17.4	24.0	0	—	—	—
Soybean oil	7.9	—	59.3	26.4	0	—	—	—
Sunflower oil	48.7	—	5.1	0.8	0	—	—	—
Wheatgerm oil	119.4	71.0	26.0	27.1	2.7	18.1	—	—
Maize oil	11.2	5.0	60.2	1.8	0	—	—	—
Barley pearl, raw	0.2	0.3	—	—	1.2	—	—	—
Bran wheat	1.6	1.0	—	—	1.1	—	—	—
Flour wholemeal (100%)	1.0	0.7	—	—	0.4	—	—	—
Oatmeal raw	0.8	—	0.1	0	1.0	—	—	—
Rice, polished, raw	0.3	—	0.3	—	—	—	—	—
Rye flour (100%)	0.8	0.4	0.3	—	1.5	—	—	—
All-bran	2.0	—	1.6	—	—	—	—	—
Grapenuts	1.6	—	0.6	—	—	—	—	—
Puffed wheat	1.7	—	2.5	—	—	—	—	—
Whole yellow corn	1.5	—	5.1	—	0.5	—	—	—
Shredded wheat	1.0	—	2.0	—	—	—	—	—
Weetabix	1.8	—	2.2	—	—	—	—	—
Peach	1.3	—	—	—	—	—	—	—
Strawberry	1.2	—	—	—	—	—	—	—
Apple	0.3	—	—	—	—	—	—	—
Asparagus	1.6	—	—	—	—	—	—	—
Broccoli	2.0	—	—	—	—	—	—	—
Spinach	2.5	—	—	—	—	—	—	—
Carrot	0.4	—	—	—	—	—	—	—

Table 5. Correlation (r) between serum tocopherol, tocotrienol and BMI.

		Tocopherol					Tocotrienol			
Subject	n	Alpha	Beta	Gamma	Delta	Total	Alpha	Gamma	Delta	Total
Caucasian women	14	–0.05	0.03	–0.14	0.37	–0.05	–0.11	–0.37	–0.07	–0.39
Japanese women	20	0.13	0.15	0.22	–0.11	0.16	0.07	0.32	0.25	0.14
Combined	34	0.06	0.07	–0.11	–0.14	0.03	–0.01	0.04	0.06	–0.11

No correlation was significant at $P<0.05$.

Table 6. Tocopherol and tocotrienol in pre- and post-menopausal women's sera (μg/100 ml serum, mean±SEM).

		Pre-menopause	Post-menopause	*P*
Tocopherol	Alpha	1056.6±66.5	1268.7±85.3	0.059
	Beta	18.6±1.6	23.0±1.7	0.07
	Gamma	75.1±10.7	89.6±13.6	0.41
	Delta	13.4±1.7	20.9±5.1	0.16
	Total	1187.4±75.5	1402.2±80.6	0.06
Tocotrienol	Alpha	28.6±8.4	32.2±11.4	0.80
	Gamma	29.8±8.7	22.5±2.3	0.42
	Delta	9.9±1.4	11.5±2.2	0.54
	Total	68.2±12.5	66.6±13.9	0.93

Caucasian women (Table 3). Distribution of tocopherol and tocotrienol in women's serum are shown in Figures 3 and 4.

Discussion

A sensitive, specific and simple method of simultaneous evaluation of alpha-, beta-, gamma-, delta-tocopherols and alpha-, gamma-, delta-tocotrienols in human serum by normal phase HPLC with a spectrofluorometer has been developed. Tocotrienol and tocopherol isomers are measurable in physiological concentration in humen serum by this method.

There is no significant difference in serum levels of alpha- and beta-tocopherols and alpha-, gamma-, and delta-tocotrienols between Caucasian and Japanese subjects. However, serum gamma- and delta-tocopherol concentrations in Japanese women are significantly higher than in Caucasian women.

The reasons for these differences are not clear. One possibility is that Caucasian and Japanese women have different intake of the isomers on account of the cultural food differences. For example, soybean oil is peculiarly high in gamma- and delta-tocopherols which is consumed more by people in Japan than by people in Australia. There are, however, few data on tocopherol and tocotrienol isomers composition of food (Table 4[4,5]). Other possibilities are that there is interconversion of isomers or distribution in body compartments, that is different in the two groups of women. That serum tocotrienol level is usually less than tocopherol level would be consistent with conversions of tocotrienol to tocopherol, although this may simply reflect intake. However, either the absorption, the catabolism or the excretion of tocopherol and tocotrienol may also be different between Caucasian and Japanese women.

To compare the possibility that the level of body fatness may be a contributory factor to tocopherol and tocotrienol status, we related these serum measurements to body mass index (BMI), but there was no significant difference in our study (Table 5). In addition, there is also no significant difference of tocopherol and tocotrienol status between pre- and post-menopausal women in our study (Table 6).

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摘　要

本文介紹了一個同時測定人體血清中生育酚和生育三烯酚的簡單，特異和敏銳的方法。這個高壓液層析法是用螢光檢測器進行的。它可以測出血清中生育酚和生育三烯酚的生理濃度。在高加索(白人)婦女與日本婦女之間沒有發現血清中的α-, β-生育酚和α-, γ-, δ-生育三烯酚有明顯的差異，但是，日本婦女血清中的γ-和δ-生育酚卻明顯地高於高加索婦女。

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要約

トコフェロールおよびトコトリエノール類を同時に測定し、ヒト血清のそれらを生理的濃度で評価できる高感度で特異的かつ簡便な方法を、蛍光検出器付順相高速液体クロマトグラフィーを用いて開発した。この方法を用いて、在豪コーカサス女性および日本在住日本女性の血清のそれらを測定すると、α－，β－トコフェロールおよびα－，γ－，δ－トコトリエノールは両者に有意差がなかったが、γ－，δ－トコフェロールは日本女性のほうがコーカサス女性よりも有意に高いことがわかった。