

The Tocopherol Pattern in Human Serum Is Markedly Influenced by Intake of Vitamin E Drugs—Results of the German National Health Surveys

H.-U. Melchert* and E. Pabel

Robert Koch-Institute, RKI 623 (Bereich Tempelhof), D-12101 Berlin, Germany

ABSTRACT: During national and regional health surveys, done from 1984–1995 in Germany, consumption data for all drugs used by the participants—approximately 18,000 persons—in the last 7 d before the examination were monitored with a detailed drug-usage questionnaire. The groups examined are representative for the national and regional German inhabitant population aged 25–69 yr. In serum samples of subsamples of the study participants, all tocopherols were measured by isocratic high-performance liquid chromatography (Si 60 column, fluorescence detection). Consumption data for tocopherol-containing drugs showed that up to 5% of females and up to 3% of males of the study population used those drugs. During the study period, the serum content of α -tocopherol (mean values \pm SD) rose from 7.5 ± 2.6 mg/L serum to 11.8 ± 2.8 mg/L serum for nonusers and from 11.9 ± 4.3 mg/L serum to 15.3 ± 4.9 mg/L serum in tocopherol-drug users. Throughout all studies, it could be shown that β - and γ -tocopherol were heavily reduced in those persons taking daily doses ≥ 50 mg α -tocopherol. The reduction of the two tocopherols is dose-dependent and especially pronounced in females using high-dose α -tocopherol drugs. Owing to the emerging evidence of the physiological importance concerning the balance of the different tocopherols in biological systems, the possible benefits of using natural tocopherol mixtures from plant origin instead of pure *RRR*- α -tocopherol, gained from permethylation procedures, as vitamin supplements in human nutrition should be considered.

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KEY WORDS: Fluorescence detection, German health surveys, HPLC, human serum, α -, β -, γ - and δ -tocopherol, vitamin E drugs.

Despite the fact that vitamin E drugs have been very popular for some years and are sold as OTC preparations (OTC = over-the-counter, prescription-free drugs), relatively little is known about the true consumption data and the serum concentrations of the different tocopherols in representative population groups of users and nonusers of α -tocopherol-containing drugs. The widespread use of tocopherols in the food and

pharmaceutical industry as antioxidants has led to unintended exposure of consumers to α -tocopherol. Many kinds of food and beverages and some pharmaceutical products contain α -tocopherol in relatively small amounts, but owing to regular consumption of those products, exposure has reached a considerable extent. Because information concerning the amount of OTC drugs consumed is sparse, we decided to install nested pharmacoepidemiological studies in the three waves of the German cardiovascular prevention study (1) and in following studies in the region of the former German Democratic Republic (GDR) and in Berlin. Those studies give reliable information concerning all types of drugs used in the inhabitant population of Germany (2). The aim of our studies was to look for time trends of serum concentrations of the different tocopherols in serum samples of study participants who use α -tocopherol-containing drugs in comparison to persons taking no medication at all.

EXPERIMENTAL PROCEDURES

The study participants of the four national surveys (approx. 18,000 persons) examined are representative for the German inhabitant population aged 25–69 yr for the western part and for the former GDR. Their drug-consumption pattern was recorded with a detailed standardized questionnaire. The questionnaire covered drug consumption over the 7 d preceding the examination. Additionally, a complete set of sociodemographic and socioeconomic data of the participants was collected. Clinical chemistry, hematological or other physiological data were determined from the blood and serum samples of all persons. Further study details were published earlier (3). The method for determining the different tocopherols has been published (4). Briefly, the method uses first an Extrelut[®] (Merck, Darmstadt, Germany) extraction on minicolumns prepared with Pasteur pipettes. Serum (100 μ L) is applied on the Extrelut column, and after waiting 5 min, 200 μ L methanol is added to break the lipid/protein bonds. Hexane (5 mL) as eluant then yields the total lipid extract. This total lipid extract is evaporated to dryness, dissolved in 100 μ L hexane, and purified from possibly interfering lipid components by chromatography on silica gel (Kieselgel, Art. Nr. 10757.1000; Merck, Darmstadt, Germany). This step too

*To whom correspondence should be addressed at Robert Koch-Institute, Fg.: RKI 623, Bereich Tempelhof, General-Pape-Str. 62-66, D-12101 Berlin, Germany.

E-mail: melcherth@rki.de.

is done with silica gel minicolumns made from Pasteur pipettes by applying all of the last extract onto the column and using 4 mL hexane/diethyl ether (5:1, vol/vol) as eluant. This last extract is evaporated to dryness, and the remainder is dissolved in 100 μ L of high-performance liquid chromatography (HPLC) mobile phase for straight-phase HPLC determination of the different tocopherols. Experimental conditions are: HPLC-System, HP 1090 (Hewlett-Packard, Waldbronn, Germany); Lichrosorb Si 60 (Merck) column 7 μ m (250 mm \times 4.6 mm); mobile phase, isooctane/isopropanol (95.5:0.5, vol/vol); eluant flow, 1.25 mL/min; fluorescence detector, HP 1046A (Hewlett-Packard), $\lambda_{\text{ex}} = 295$ nm, $\lambda_{\text{em}} = 330$ nm; injection volume, 20 μ L. The rate of recovery for all tocopherols measured by this method is 98%, and the day-to-day precision is $\pm 5\%$ for amounts of ≥ 1 mg tocopherol/L serum. The entire method was validated by gas chromatography–mass spectrometry measurements by using off-line control of the different HPLC tocopherol peaks with MAT 44 S-equipment (Varian MAT, Bremen, Germany) after derivatization to trimethylsilyl derivatives.

RESULTS

Table 1 shows the numbers of participants in the different German health surveys and the years in which the surveys were done. In all surveys, the number of vitamin product users of all kinds was between 19 (survey T1) and 25% (T2) (data not shown). For α -tocopherol-containing products, the number of users was between 5 (T0) and 2% (survey in the former GDR) of the study population. In general, in all surveys, females consumed α -tocopherol-containing products twice as frequently as males.

Table 2 gives the mean values for α -, β -, and γ -tocopherol concentrations in the serum for all those study participants who did use α -tocopherol-containing products in the last 7 d before the examination, and Table 3 shows the data for randomly assigned control persons, matched by age and sex, who had used no medication at all in the 7 d preceding examination. An analysis of all data, taking into consideration the amount of α -tocopherol used daily by the study participants, showed a clear dose–response relationship for the α -tocopherol concentration in the serum sample and an inverse relationship with the amount of β - and γ -tocopherol in the serum sample. Figure 1 shows the results for females of survey T2

TABLE 1
Study Participants of the German National and Regional Health Surveys

Study years (survey codes)	Persons examined		
	Males	Females	Total
1984–1986 (T0)	2392	2354	4746
1987–1989 (T1)	2650	2685	5335
1990–1991 (T2)	2622	2684	5306
1991–1992 (GDR) ^a	1273	1120	2393
1995 (Berlin)	171	207	378

^aGerman Democratic Republic.

TABLE 2
Concentration of Different Tocopherols in Serum Samples of Tocopherol-Drug Users^a

Survey	α -Tocopherol (mg/L)	β -Tocopherol (mg/L)	γ -Tocopherol (mg/L)
	mean \pm SD	mean \pm SD	mean \pm SD
T0 ($n = 178$)	11.92 \pm 4.28	0.08 \pm 0.10	0.44 \pm 0.37
T1 ($n = 59$)	12.72 \pm 4.19	0.09 \pm 0.10	0.64 \pm 0.54
T2 ($n = 84$)	15.34 \pm 4.92	0.13 \pm 0.11	0.36 \pm 0.31
GDR ($n = 44$)	8.22 \pm 3.43	0.14 \pm 0.10	0.82 \pm 0.48
Berlin ($n = 30$)	15.83 \pm 4.88	0.09 \pm 0.09	0.63 \pm 0.47

^aSee Table 1 for abbreviations.

TABLE 3
Concentration of Different Tocopherols in Serum Samples of No-Drug Users^a

Survey	α -Tocopherol (mg/L)	β -Tocopherol (mg/L)	γ -Tocopherol (mg/L)
	mean \pm SD	mean \pm SD	mean \pm SD
T0 ($n = 133$)	7.50 \pm 2.59	0.16 \pm 0.10	0.69 \pm 0.43
T1 ($n = 122$)	9.84 \pm 2.86	0.20 \pm 0.11	0.88 \pm 0.68
T2 ($n = 159$)	11.80 \pm 2.78	0.24 \pm 0.10	0.64 \pm 0.29
GDR ($n = 108$)	7.57 \pm 3.20	0.18 \pm 0.10	1.01 \pm 0.48
Berlin ($n = 64$)	12.54 \pm 3.24	0.19 \pm 0.08	1.13 \pm 0.57

^aSee Table 1 for abbreviations.

who consumed <50 mg α -tocopherol daily in comparison to the female control group with no medication. Figure 2 shows the results for those females of survey T2 who used >50 mg α -tocopherol daily compared with the control group with no medication in the preceding 7 d.

DISCUSSION

The results of the five surveys, done between 1984–1995 in Germany, revealed that vitamin supplements are regularly used by approximately 20% of the German inhabitant population aged 25–69 yr. More than 50% of the users are women. This holds true for usage in the old and new “Bundesländer” of Germany. These data are based on self-assessment of the study participants.

A detailed drug questionnaire showed that the use of α -tocopherol-containing drugs was subject to consumption changes in the time from 1984 to 1995. Consumption dropped from nearly 5 to less than 2% of female users from 1984 to 1989 and then rose again to over 4% of female users in 1995. Males consumed α -tocopherol-containing drugs less than females in all surveys. These results could be confirmed by determination of tocopherols in serum samples of the study participants. The serum analyses showed a steadily growing exposure to α -tocopherol in all persons. So, the serum content of α -tocopherol rose from a mean value of 7.50 \pm 2.59 mg/L serum in 1984 to 12.54 \pm 3.24 mg/L serum in the 1995 survey in no-drug users. The region of the former GDR showed

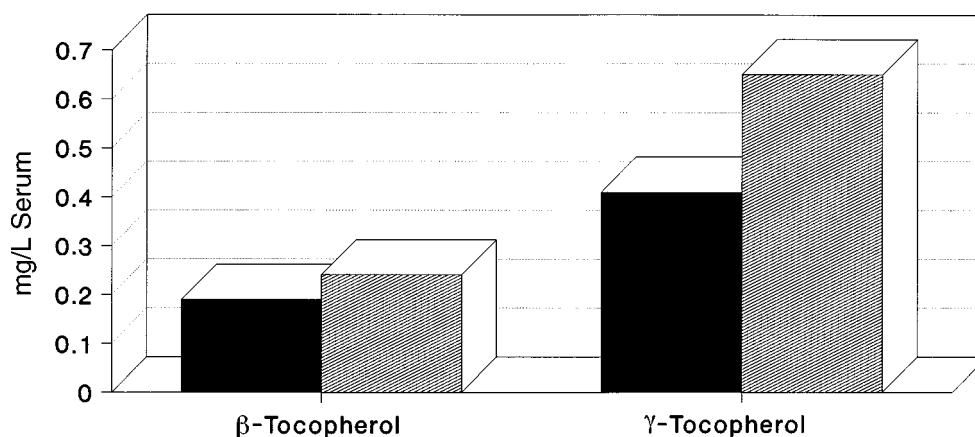


FIG. 1. β - and γ -Tocopherol concentration, in sera of female tocopherol-drug users with consumption of <50 mg α -tocopherol/day from survey T2. Users, $n = 33$; nonusers, $n = 104$. Solid bars: use of <50 mg tocopherol/day; cross-hatched bars, no-drug users.

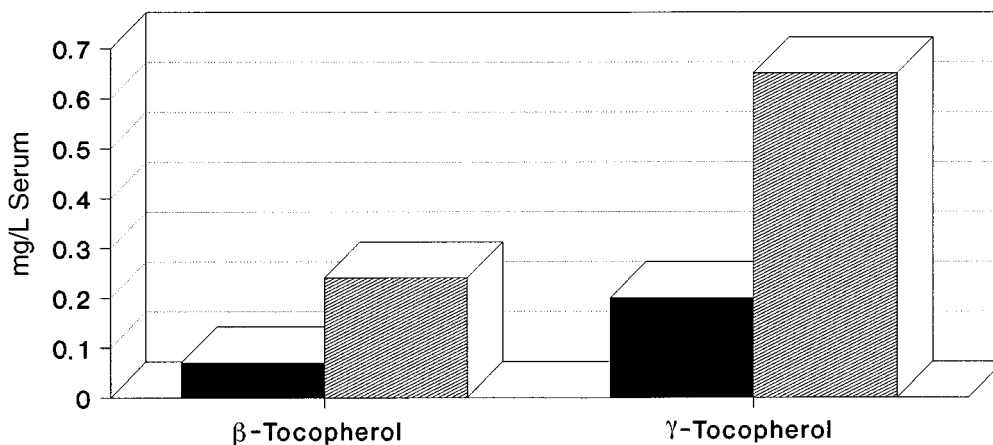


FIG. 2. β - and γ -Tocopherol concentrations in sera of female tocopherol-drug users with consumption of >50 mg α -tocopherol/day from survey T2. Users, $n = 27$; nonusers, $n = 104$. Solid bars: use of >50 mg tocopherol/day; cross-hatched bars, no-drug users.

in the 1992 survey mean values of 7.57 ± 3.20 mg α -tocopherol/L serum.

These surveys show that use of α -tocopherol-containing drugs leads to pronounced changes in the pattern of the different serum tocopherols. β - and γ -Tocopherols are clearly diminished in persons who use α -tocopherol-containing drugs. This takes place in a dose-dependent manner and is seen clearly in those study participants who use >50 mg α -tocopherol/single dose daily. δ -Tocopherol could be found in amounts from 0.01 mg/L serum to 0.5 mg/L serum (data not shown) and seems also to be diminished by consumption of α -tocopherol.

Owing to the emerging evidence of the potential physiological importance of a balanced presence in human sera (5–8) of all the different tocopherols, use of natural tocopherol mixtures as vitamin supplements should be considered when such medication seems necessary.

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