The Repeatability of Serum Carotenoid, Retinoid, and Tocopherol Concentrations in Specimens of Blood Collected 15 Years Apart[1]

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Abstract

Community-wide programs to collect blood for a research serum bank were carried out in Washington County, Maryland in 1974 and 1989. Of the 8395 persons who participated in both programs, 64 were controls in a nested case-control study of the association of antioxidant micronutrients with subsequent breast cancer, and 30 and 166 were controls in similar studies of lung and prostate cancer. Assay results for five carotenoids, two retinoids, and two tocopherols in samples of blood collected 15 years apart were thus available for comparisons of micronutrient concentrations. The mean Spearman rank order correlation coefficient for all comparisons was 0.44, with two coefficients greater than 0.60 and two less than 0.30. Blood pressure readings at the two blood collections had a mean rank order correlation coefficient of 0.46. Because blood pressure readings in 1974 were shown to be significantly predictive of atherosclerosis 15–18 years later, the present results suggest that ranked concentrations of antioxidant micronutrients from a single sample are sufficiently representative to be used as predictors of subsequent concentrations and are thus suitable for assessment as risk factors for subsequent illnesses.

Introduction

In an extensive review of the known and potential causes of cancer, Doll and Peto (1) included the possibility that cellular damage by oxidative free radicals might be prevented by dietary antioxidants delivered to the cells by the blood and that β-carotene was a reasonable candidate for such a protective substance. This idea became the primary stimulus for numerous subsequent studies of the association of cancer with dietary and serum[3] concentrations of a variety of antioxidants, notably the carotenoids, vitamin E, vitamin C, and selenium (a marker for glutathione peroxidase).

Most of these studies have relied on histories of dietary intake or biochemical assays of serum. Dietary histories have the advantage of indicating average consumption over relatively long periods of time, but their validity suffers from the frailties of human memory and the uncertain pertinence of intake to the micronutrient concentrations in the fluids to which cells are exposed. Serum concentrations are much more likely to reflect cellular exposures, but they suffer from the fact that they are often measured at only one time and may not be representative of usual concentrations.

There are relatively few reports of intraindividual variability of serum levels of micronutrient antioxidants such as carotenoids, retinol, or tocopherols. Most are based on small numbers of subjects and have relatively short intervals between assays. All are concerned with absolute concentrations. Their findings are summarized in Table 1.

Among studies for which coefficients of variation were reported or could be calculated, these indices were 0.10 or less for shorter periods [1 day or one menstrual cycle (2, 3, 6, 7)] but ranged from 0.11–0.47 when specimens were taken a year or more apart (3, 10, 11). Correlation coefficients ranged from 0.68–0.95 for comparisons over short periods but fell to a range of 0.22–0.81 for longer intervals (8, 9, 11).

Whereas it would be ideal if individual serum concentrations remained essentially the same for long periods of time, this is unlikely. Variability can arise from changes in dietary intake, metabolic changes associated with age, and diurnal and seasonal variations. In addition, if recent and past specimens are assayed at the same time, changes due to long-term storage are possible. Even if assays are performed at the time of blood collection, it is unlikely that all assay conditions will remain constant if years separate the repeat assays. However, the significance of serum micronutrients as biomarkers for future disease can still be estimated if persons who have high concentrations at one time tend to have relatively high concentrations at a later time, and if persons with low concentrations also tend to rank low in the future. It is possible that rank order correlations will be high enough for rank, grouped or individual, to be used in analyses, even if other measures of agreement are poor. Unfortunately, only in the study among Belize children could a rank order correlation be calculated: it was 0.87 for retinol (5).

Three nested case-control studies of breast, lung, and prostate cancer in Washington County, Maryland performed repeated assays of carotenoids, retinoids, and tocopherols on

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[3] Unless otherwise specified, "serum" will include both plasma and serum.

Table 1 Summary of previous findings regarding within-individual variation of serum concentrations of carotenoids, retinol, α-tocopherol, and ascorbic acid

	Ref. no. of the study										
	2	3	4	5	6	7	8	9	10	3	11
Period of study	Day	Day	2 wk	2 wk	Menstrual cycle	Menstrual cycle	Season 6 mo	Season 5–9 mo	Season 12 mo	1 yr	22 yrs
Index of variation	CV[a]	CV	CCC	CCC	CV	CV	ICC	Difference	CV	CV	PMC
No. of subjects	15	33	23	23	30	12	92	66	18	29	442
α-Carotene		0.09	0.95		0.09	0.01			0.47	0.31	
β-Carotene	0.06	0.08	0.92		0.05	0.04	0.81[b]	50%	0.36	0.20	0.43
Cryptoxanthin		0.08	0.91		0.06				0.37	0.20	
Lutein/zeaxanthin		0.09	0.59		0.10	0.05			0.29	0.18	
Lycopene			0.89		0.06	0.05			0.43	0.18	
Total carotenoids			0.68			0.04					
Retinol	0.04			0.81		0.05	0.70		0.14		
α-Tocopherol	0.05						0.57[c]	No difference	0.11		0.47
Ascorbic acid							0.29				0.22

[a] CV, coefficient of variation; CCC, concordance correlation coefficient; ICC, intraclass correlation coefficient; PMC, product-moment correlation coefficient.
[b] α- and β-carotene.
[c] α- and γ-tocopherol.

serum from 260 individuals selected as normal controls (12).[4] These individuals had donated blood for a serum bank in 1974 and again in 1989. This study compares the assay results for both time periods by rank order correlation and also compares these coefficients with those for blood pressure determinations done at the times of blood donation.

Materials and Methods

From August through November, 1974 and again from May through November, 1989, community-wide programs were conducted in Washington County, Maryland to collect blood for a serum bank and subsequent cancer research. Mobile trailers were moved to many different parts of the county to allow all segments of the adult population an opportunity to participate. Approximately one-third took part in the 1974 program (CLUE I) and a similar proportion participated in the 1989 program (CLUE II).

In 1974, a brief history was first taken. After the several minutes required for this procedure, blood pressure was measured by trained nurses using a mercury sphygmomanometer with the subject seated. Diastolic pressures were defined as the point of disappearance of the Korotkov sounds. The lowest of three determinations was recorded. Finally, blood was drawn into a 15-ml vacutainer (Becton Dickinson, Rutherford, NJ), allowed to clot at room temperature for 30 min, and then refrigerated at 4°C until the serum was separated, usually within 3–4 h. The serum was kept at −70°C until aliquots for assays were prepared by thawing in ice water under dim yellow light. The assay specimens were shipped to the laboratory under dry ice and kept there at −70°C until assayed.

In 1989, the procedures were similar. The only major difference was that blood was collected in 20-ml vacutainers containing heparin (Becton Dickinson) and kept at 4°C until the plasma was separated 2–6 h later. The plasma was kept frozen at −70°C until aliquoted, shipped to the laboratory, and assayed. For the present study, specimens from CLUE I and CLUE II were aliquoted, shipped, and assayed similarly at the same time.

To study repeatability over time, assay results from previous studies were used from controls matched to the breast, lung, or prostate cancer cases who had donated blood in 1974 and again in 1989. Serum and plasma from breast and prostate cancer controls were handled similarly and assayed for carotenoids, retinoids, and tocopherols by high-performance liquid chromatography (13, 14). For lung cancer controls, the high-performance liquid chromatographic procedure differed slightly (15). For the breast and prostate cancer controls, assays were performed by the Department of Biomedical Research, Our Lady of Mercy Medical Center (Bronx, NY) in 1995 and 1998, respectively; assays of specimens from lung cancer controls were performed at the Department of Epidemiology, School of Public Health, University of Minnesota (Minneapolis, MN) in 1995.

It was not appropriate to compare only the absolute concentrations of the various micronutrients in the 1974 and 1989 paired specimens. The 1974 specimens were sera, whereas the 1989 specimens were plasma; storage times were markedly different for serum and plasma; and the subjects were 15 years older in 1989 with concomitant changes in lifestyle. Because assays for the breast, lung, and prostate cancer controls were done at different times and in different laboratories, results for participants in these three studies were analyzed separately by Spearman's rank order correlation coefficients (16). Systolic and diastolic blood pressure values were treated similarly to obtain their rank order correlation coefficients.

Results

Paired results of serum and plasma assays were available for 64 breast cancer controls, 30 lung cancer controls, and 166 prostate cancer controls (Table 2). As expected from the types of cancer to which controls were matched, most subjects for the present study were males. Breast cancer controls were younger than the other two groups, of whom two-thirds were more than 65 years of age in 1989. Most of the controls were still married. Lung cancer controls were somewhat better educated than the breast or prostate cancer controls. Only 13.0% of the total group had changed smoking habits between 1974 and 1989 (they had quit smoking during that period).

The mean concentrations of the various analytes, which are shown in Table 3, differed among the three groups of controls, presumably because of differences in the populations, storage times, and assay locations. There was also considerable variation in the percentage differences in the mean values between the 1974 and 1989 specimens. The mean difference for all analytes was −2.9% (SD, 15.4). Disregarding sign, the mean percentage difference was 11.7% (SD, 10.1).

Mean systolic blood pressures were remarkably similar at the two times. Mean diastolic pressures varied somewhat more, decreasing by 5.8%, 5.9%, and 7.7% among breast, lung, and prostate controls, respectively.

Spearman rank order correlations are shown in Table 4. For the various analytes, the mean value was 0.445 with a SD

[4] K. J. Helzlsouer, personal communication.

Table 2 Percentage distribution of controls for cases of cancer of the breast, lung, and prostate, by status in 1989

Characteristics	Matched controls for cases of cancer of		
	Breast (n = 64)	Lung (n = 30)	Prostate (n = 166)
Sex			
Male	0	46.7	100.0
Female	100.0	53.3	0
Age (yrs)			
<65	53.1	33.3	33.1
65–74	35.9	46.7	54.2
75+	10.9	20.0	12.7
Marital status			
Married	65.6	60.0	89.8
Not married[a]	34.4	40.0	10.2
Years of school completed			
<12	35.9	20.0	39.8
12	50.0	53.3	40.4
13+	14.1	27.7	19.9
Smoking history[b] (1974 and 1989)			
Never (1974 & 1989)	53.1	46.7	27.7
Former (1974 & 1989)	14.1	26.7	51.8
Smoker (1974, former 1989)	12.4	20.0	12.0
Smoker (1974 & 1989)	20.4	6.7	8.4

[a] Single, separated, divorced, widowed.
[b] Smoking includes cigarettes, pipes, and cigars.

of 0.106. Two coefficients were less than 0.30: (*a*) α-carotene among lung cancer controls; and (*b*) retinyl palmitate among prostate cancer controls. Two coefficients were greater than 0.60: (*a*) cryptoxanthin among lung cancer controls; and (*b*) α-tocopherol among prostate cancer controls. For only two coefficients, α-carotene and lycopene among lung cancer controls, did the 95% confidence limits include 0. For blood pressure determinations, the mean rank order correlation coefficient was 0.46. None of the confidence limits included 0.

To see whether seasonal variations affected the rank order correlations, four pairs of blood collection months were selected: (*a*) September 1974 and spring (May and June) 1989; (*b*) October and November 1974 and spring 1989; (*c*) October and November 1974 and summer (July and August) 1989; and (*d*) September through November in both years. Rank order correlation coefficients were calculated for each of the micronutrients and for each of the seasonal period pairs. No clear cut differences in the rank order coefficients by season were observed, although there was a suggestion that they were least for carotenoid concentrations in serum collected in September 1974 and again in spring 1989. Repeatability might also have been affected because marital or smoking status changes between 1974 and 1989 might have been associated with dietary changes. However, such status changes were too few to allow meaningful comparisons to be made.

The failure of mean blood pressure levels to increase with the aging of the study population stimulated a closer look at the data. Of the 260 participants in this study, 67 who were not taking blood pressure medication in 1974 were doing so in 1989. Their systolic and diastolic blood pressures decreased by an average of 9.4 and 9.5 mm Hg, respectively. Regression to the mean also played a part. Among persons not taking blood pressure medication at either time, those with systolic pressures over 150 mm Hg in 1974 showed an average decrease in systolic pressure of 15.4 mm Hg, whereas those with systolic pressures less than 130 mm Hg increased by only 7.2 mm. Diastolic pressures showed a similar phenomenon: readings more than 95 mm decreased by an average of 14.3 mm, whereas 1974 readings of less than 80 mm Hg were 1.6 mm higher in 1989. It is also possible that apprehension about participating in a blood donation program was less on the second experience than it had been initially and that this might have affected blood pressure levels.

Discussion

It should be obvious that the population for this study is a selected one. First, to have donated blood in 1974 and 1989 required not only a survival of 15 years but also the ability to be ambulatory at each time. To have volunteered to give blood on two occasions for scientific study also sets these participants apart from the two-thirds who did not donate blood. Comparisons with the 1975 private census and the 1990 official Census showed that the participants tended to be female, in the 50–70-year age group, better educated, and nonsmokers. To the extent that the participants were more likely to maintain the same lifestyle and dietary habits from 1974 to 1989 than nonparticipants, the results in this study may overemphasize repeatability. We know of no feasible way to check this possibility. It should also be remembered that giving blood was not entirely an act of volunteering in support of a good cause. In 1974, many persons avowedly participated to have their blood pressures checked, and in 1989, an even higher proportion came to have a free cholesterol assay.

For a number of reasons, the micronutrient concentrations in specimens collected in 1974 and 1989 could not be expected to be the same in absolute terms. Serum was collected in 1974, and plasma was collected in 1989. However, many persons with high concentrations at one time were still in the group with high concentrations later on. For this reason, it seems reasonable to assess serum micronutrient concentrations as risk factors for disease in terms of their relative concentrations assessed by rank order correlation coefficients of assay results of specimens from the same individuals done at different times.

Although the rank order correlation coefficients for the micronutrients assayed in specimens taken in 1974 and 1989 are only moderately high (mean, 0.445; SD, 0.106), their magnitude is similar to those for the blood pressure readings at the same times (mean, 0.457; SD, 0.089). Blood pressure determinations from the 1974 project have been shown to be significantly predictive of subsequent coronary disease and increased atherosclerotic changes in the carotid arteries (17). This study involved the 1702 Washington County residents who took part in the 1974 blood collection program and who, as participants in the Atherosclerosis Risk in Communities study, were given a thorough cardiovascular examination in the period 1987 through 1989 (18). That examination included a history of previous cardiovascular disease and an ultrasound examination of both carotid arteries. Persons were classified as hypertensive in 1974 if their systolic blood pressures were 140 mm Hg or higher or if their diastolic blood pressures were 90 mm Hg or higher. The relative risk of having had a heart attack or coronary artery surgery between 1974 and 1989 associated with this classification of hypertension was 2.2 (95% confidence interval, 1.3–3.5). Similarly, among persons with no history of coronary disease or stroke before 1989, the hypertensives in 1974 had a relative risk of 2.0 (95% confidence interval, 1.4–2.8) of having increased subclinical atherosclerosis of the carotid arteries in 1989–1992.

These findings and the similarity of rank order correlation coefficients between micronutrients and blood pressure suggest that measurements of various micronutrients from a single sample can provide useful risk factors for disease processes in which they are suspected of playing a part. To confirm this possibility, future studies should report within-person variability in terms of rank order in addition to absolute values.

Table 3 Mean serum concentrations in 1974 and percentage differences of 1989 values from those of 1974 for specified micronutrients and for blood pressures among matched controls for breast, lung, and prostate cancer cases, Washington County, Maryland

	Breast (n = 64)			Lung (n = 30)			Prostate (n = 166[a])		
	1974	% Difference[b]	P	1974	% Difference	P	1974	% Difference	P
Micronutrients (units)									
Total carotenoids (μg/dl)	105.4	−4.6	0.36				63.3	−2.4	0.47
α-Carotene (μg/dl)	4.5	−21.9	0.26	4.2	7.4	0.54	3.1	18.8	0.01
β-Carotene (μg/dl)	19.8	−25.5	<0.01	22.4	−2.4	0.84	11.4	−5.3	0.51
Cryptoxanthin (μg/dl)	13.2	−1.4	0.89	9.4	−11.1	0.22	7.8	5.1	0.48
Lutein/zeaxanthin (μg/dl)	27.5	−7.0	0.27	22.1	−19.0	<0.01	14.8	−12.2	<0.01
Lycopene (μg/dl)	40.8	−14.8	0.14	41.9	−9.1	0.33	40.1	17.2	<0.01
α-Tocopherol (mg/dl)	1.31	7.3	0.18	0.78	9.0	0.17	1.34	8.3	0.02
γ-Tocopherol (mg/dl)	0.25	−4.3	0.38				0.26	20.6	<0.01
Retinol (μg/dl)	59.0	9.8	<0.01				67.9	4.0	0.01
Retinyl palmitate (μg/dl)	12.8	−49.1	<0.01				11.5	7.2	0.39
Blood pressure									
Systolic (mm Hg)	131.4	−0.8	0.67	139.2	0.1	0.95	140.5	−2.5	0.02
Diastolic (mm Hg)	82.6	−5.8	<0.01	84.9	−5.9	<0.01	88.1	−7.7	<0.01

[a] n for α-carotene is 143, and n for retinyl palmitate is 140.
[b] % Difference of 1989 mean concentration from that of 1974.

Table 4 Spearman rank order correlation coefficients (rho) between 1974 and 1989 values for selected micronutrients and for blood pressures among matched controls for breast, lung, and prostate cancer cases, Washington County, Maryland

	Breast		Lung		Prostate	
	rho	95% CI[a]	rho	95% CI[a]	rho	95% CI[a]
Micronutrients (units)						
Total carotenoids (μg/dl)	0.48	0.25–0.65			0.46	0.32–0.57
α-Carotene (μg/dl)	0.48	0.26–0.65	0.25	−0.13–0.57	0.37	0.22–0.51
β-Carotene (μg/dl)	0.46	0.24–0.64	0.54	0.21–0.76	0.52	0.40–0.63
Cryptoxanthin (μg/dl)	0.51	0.30–0.68	0.66	0.39–0.83	0.46	0.32–0.57
Lutein/zeaxanthin (μg/dl)	0.46	0.24–0.64	0.46	0.10–0.71	0.44	0.30–0.56
Lycopene (μg/dl)	0.32	0.07–0.53	0.35	−0.02–0.64	0.35	0.20–0.48
α-Tocopherol (mg/dl)	0.46	0.23–0.64	0.46	0.11–0.71	0.61	0.50–0.70
γ-Tocopherol (mg/dl)	0.53	0.32–0.69			0.48	0.34–0.59
Retinol (μg/dl)	0.35	0.10–0.55			0.58	0.46–0.67
Retinyl palmitate (μg/dl)	0.32	0.08–0.53			0.21	0.04–0.37
Blood pressure						
Systolic (mm Hg)	0.47	0.22–0.63	0.62	0.32–0.80	0.39	0.24–0.51
Diastolic (mm Hg)	0.38	0.14–0.57	0.47	0.12–0.71	0.41	0.28–0.54

[a] CI, confidence interval.

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