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PLASMA CONCENTRATIONS OF RETINOL, TOCOPHEROLS, AND CAROTENOIDS AND PLATELET FATTY ACIDS IN A GROUP OF FEMALE IOWA CENTENARIANS

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Abstract: Objective: Dietary and biochemical assessment of nutritional status of centenarians in assisted care/nursing home settings. *Methods:* Twenty-four centenarian rural and small-town Midwestern, Caucasian women (98.4 ± 3.4 y, mean ± SD, range 95 to 108 y) were studied for dietary intake, plasma concentrations of retinol, retinol-binding protein (RBP), tocopherols, carotenoids, and platelet fatty acid (FAs) concentrations. *Results:* Diet provided a daily intake of 1710 kcal, 71 g protein, 58 g fat, and 234 g carbohydrate. Plasma retinol concentration was $1.95 \pm 0.52 \,\mu\text{M}$, RBP concentration was $2.48 \pm 0.66 \,\mu\text{M}$, and the percent saturation of RBP was 78%. Concentrations of α- and γ-tocopherol were 27.8 ± 11.0 and $5.23 \pm 2.12 \,\mu\text{M}$, respectively. The major plasma carotenoids were β-carotene ($0.44 \pm 0.21 \,\mu\text{M}$), lycopene ($0.43 \pm 0.20 \,\mu\text{M}$), lutein + zeaxanthin ($0.32 \pm 0.17 \,\mu\text{M}$), and α-carotene ($0.15 \pm 0.06 \,\mu\text{M}$). Plasma retinol and RBP concentrations were highly correlated (r = 0.982). There were significant positive correlations between plasma total cholesterol and retinol, RBP, γ-tocopherol, lutein, α-carotene, β-carotene, and platelet 18:2 (μ g in 2x10s cells/mL). There were significant positive correlations between dietary 20:4 and platelet 18:0 FAs, dietary 20:4 and serum LDL cholesterol, platelet 18:1 (%FA) and serum HDL-cholesterol. *Conclusions:* Biochemical values were generally within normal/acceptable ranges, confirming that adequate nutritional status could be maintained in a long-term care setting among centenarians who did not have major chronic, wasting diseases.

Key words: β-carotene, blood platelets, centenarian, nutritional status, vitamin A, vitamin E, aged, 80 and over.

Introduction

The elderly are a growing component of the world's population and may have specific nutritional needs. A number of studies have explored the nutritional status of the elderly; by necessity, these studies have measured plasma or serum concentrations of nutrients to assess status. Pilch presented data from NHANES I and II and HHANES studies showing fewer incidences of low serum vitamin A values in the elderly (45 to 74-y group) than in other groups (1). Woo et al. found a non-significant trend toward slightly lower total plasma vitamin A values and retinol-binding protein (RBP) in an elderly group (>75 y) in China, with no discernable effect of age on plasma vitamin E concentrations (2). Plasma RBP concentrations were found to be normal, although females >76 y had the lowest RBP concentrations

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reported in a study of elderly subjects by Munro et al. (3). Serum vitamin E concentrations in elderly females (aged 65-92 y) were higher than those measured in young females (aged 20-49 y), but the plasma vitamin E:triacylglycerol ratios were the same in both groups (4). In other studies of institutionalized (5) and free-living elderly (6), plasma retinol and RBP concentrations were within normal ranges, with few subjects showing plasma retinol <1 μM (29 $\mu g/dl$).

Many studies have found an association between dietary intake and platelet phospholipid fatty acids; increased mono- and polyunsaturated fatty acid (MUFA and PUFA) intake was reflected by increased platelet MUFA and PUFA concentrations (7-14). In both the Georgian Centenarian and the New England Centenarian Studies, the impact or lack of diseases and how that contributes to the health status and survivorship of centenarians was examined (15-16). Goodwin (17) investigated the nutritional status of institutionalized elderly individuals and concluded that their nutritional status was not adequate. However, few of the subjects in these studies were centenarians, and none of the preceding studies examined plasma retinol and carotenoids together. We have examined the relationship

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between dietary intake and plasma concentrations of retinol, RBP, tocopherols, individual carotenoids, and platelet fatty acids in an exceptional group of female centenarians from rural and small-town Midwest US, and we report those results here.

Materials and methods

Subjects

Female Caucasian subjects (N = 24), ages 95 to 108 y $(98.4 \pm 3.4 \text{ y, mean} + \text{SD})$ participated in this study in 1990 (18). All were natives of central Iowa, with 22 subjects living in long-term care facilities and two living in associated residential units. All were in good physical and mental health at recruitment. Most (85%) lived on a farm during the majority of their lives. Sixty-five percent had mothers that lived >80 y (3 lived to 100 y) and 46% had fathers that lived >80 y (maximum 97 y). Education levels were: 61% elementary school, 19% high school, and 19% college. This study was approved by the Iowa State University Institutional Review Board. Informed consent was obtained from each subject, their physicians, and their next of kin. Under the direction of the consulting dietitian, the dietary staff kept dietary intake records for each resident for 3 days (two week days and one weekend day). Many of the subjects chose their own meals from a menu and food preferences were known by dietary staff in planning meals for the other subjects.

Subjects were interviewed to obtain demographic and weight history. To increase and verify the information obtained from the subject, a relative or support person was also interviewed. Anthropometric measures of weight and knee height were performed by the same investigator. Blood samples were obtained after an overnight fast for routine hematological and clinical analysis. Additional samples were centrifuged to isolate platelets and plasma as described (19). Plasma samples were frozen for later analysis of retinol, tocopherols, and carotenoids. Platelets were resuspended in 0.5 mL physiological saline to give a concentration of 2 x 108 cells/mL and frozen for later analysis of fatty acids. Dietary intake was coded for analysis by the USDA Computerized Nutrient Data Base for Standard Reference (update January 1990). The data analysis for this paper was generated by PROC GLM and PROC CORR using SAS software, Release 6.06 of the SAS System (SAS Institute Inc., Cary NC © 1989).

Plasma retinol, tocopherols, and carotenoids

Thawed plasma was extracted with ethanol:hexane, and retinyl hexanoate was added as internal standard. The extract of each sample was analyzed by gradient reversed-phase high-pressure liquid chromatography

(20). Absorbance was monitored at 450 nm (for carotenoids) and at 300 nm (for retinol and tocopherols). Retinol, tocopherols, and carotenoids were identified by comparison of retention times with those of authentic standards, and were quantified by peak area. This HPLC method did not separate zeaxanthin from lutein. Retinol, $\alpha\text{-}$ and $\gamma\text{-}tocopherol$, lutein, lycopene, and $\alpha\text{-}$ and $\beta\text{-}carotene$ standards were purchased commercially (Sigma Chemical Co., St. Louis, MO) and were purified by reversed-phase HPLC when necessary. All standards were quantified by absorbance spectroscopy; purity was confirmed by absorbance spectra and HPLC.

Immunodiffusion assay of plasma retinolbinding protein

Aliquots (20 µl) of thawed plasma were analyzed for RBP by radial immunodiffusion (LC-Partigen Retinol-binding protein Kit, Behring Diagnostics, Somerville, NJ), and were quantified by comparison of the area of each precipitin ring with those of standards.

Platelet fatty acids

Internal standard, heptadecanoic acid, was added to all samples and the fatty acids were extracted three times with hexane. Fatty acid methyl esters (FAMEs) were prepared as detailed (21). FAMEs were analyzed by isothermal gas chromatography on a packed stainless steel column (100/120 mesh Gas Chrom Q II with 10% Silar 10C coating; Alltech Associates, Deerfield, IL) with flame ionization detection and identified using a standard mix (Matreya, Inc, Pleasant Gap, PA).

Results

Body weight was $57.4 \pm 8.6 \text{ kg}$ (range 39.7-78.1 kg), knee height was 49.1 ± 2.1 cm (range 44.6-53.3 cm); calculated height was 1.52 ± 0.04 m (range 1.44-1.59 m) [75.00 + (1.91 x knee height [cm])-(0.17 x age)] (22); and body mass index was $25.0 \pm 3.5 \text{ kg/m}^2$ (range 17.9 to 33.8) kg/m²). Daily dietary intake of these subjects was $1710 \pm$ 217 kcal (range 1170 to 2041 kcal). Dietary protein, carbohydrate, and fat intakes were 16.8% (range 13.3 to 21.2%), 55% (45.7 to 62.8%), and 30.5% (range 22.2 to 37.8%), respectively. The polyunsaturated to saturated fat ratio was 0.41 (range 0.22 to 0.87) (Table 1). The centenarians had adequate intakes of the macronutrients, as well as linoleic acid (14.5%), α -linolenic (1.3%), and cholesterol (283 mg). Total fat consisted of 44.6% saturated fat, 38.8% MUFA, and 16.6% PUFA. The platelet membrane fatty acid profile was comprised of 42.1% saturated fat, 27.8% MUFA, and 30.8 % PUFA (Table 2). Their average intakes of the following vitamins and minerals were also adequate: vitamin A (1399 RE),



ascorbic acid (131 mg), thiamin (1.5 mg), riboflavin (2.45 mg), niacin (17.8 mg), vitamin B6 (1.81 mg), vitamin B12 (7.27 μg), iron (15.0 mg), phosphorus (1363 mg), sodium (2519 mg), zinc (14.7 mg), and copper (1110 μ g). The centenarians had inadequate dietary intakes of vitamin E (5.95 mg), pantothenic acid (4.57 mg), folate (205 μ g), calcium (1123 mg), magnesium (254 mg), and potassium (3133 mg) (Table 1). (Note: This study was performed before food folic acid fortification, which is reflected in the folate intake values.)

Table 1 Mean 3-day dietary intake of Iowa centenarians (N=24)

Nutrient	Mean (SD)	Range	Guidelines ¹
Macronutrients			
Energy (kcal)	1710 (217)	1170 - 2040	1600
Protein (g)	71 (10)	51 – 91	46
Protein (%)	16.8 (2.0)	13.34 - 21.21	10 – 35
Carbohydrate (g)	234 (34)	182 – 308.5	130
Carbohydrate (%)	55.0 (4.48)	45.68 – 62.83	45 – 65
Fat (g)	58 (11)	29 – 80	45 - 05
Fat (%)	30.5 (3.84)	22.15 – 37.79	20 - 35
Saturated fat, total (g)	21.8 (5.60)	10.80 - 30.20	(<10%)
Monounsaturated fat, total(g)	19.0 (4.63)	8.30 – 30.10	(-20/0)
Polyunsaturated fat, total (g)	8.11 (1.77)	4.93 – 11.6	
Poly/saturated fat ratio	0.41 (0.15)	0.22 - 0.87	
Linoleic acid (g)	8.40 (1.67)	4.60 - 11.60	(5-10%)
Alpha – linolenic acid (g)	0.77 (0.14)	0.50 - 0.99	(0.6 - 1.2%)
Cholesterol (mg)	283 (97.9)	146 – 464	<300
Vitamins	()		
Vitamin A (IU)	7369 (2788)	2903 - 13026	
Vitamin A (RE)	1399 (621)	608.9 - 2674	700 μg RAE
Vitamin E (mg)	5.95 (2.08)	2.06 - 10.50	15
Vitamin α tocopherol (mg)	0.94 (0.43)	0.34 - 1.71	
Ascorbic acid (mg)	131.2 (37.0)	59.4 - 205.9	<i>7</i> 5
Thiamin (mg)	1.53 (0.52)	0.90 - 3.20	1.1
Riboflavin (mg)	2.45 (0.73)	1.60 - 4.90	1.1
Niacin (mg)	17.8 (6.45)	10.8 - 37.6	14
Pantothenic acid (mg)	4.57 (0.91)	3.2 - 6.3	5
Vitamin B6 (mg)	1.81 (0.59)	1.00 - 3.40	1.5
Vitamin B12 (µg)	7.27 (6.36)	2.50 - 25.40	2.4
Folate (µg)	205 (75.3)	116.4 - 494.5	400
Minerals			
Calcium (mg)	1123 (344)	567.6 - 2102.3	1,200
Iron (mg)	15.0 (4.87)	7.9 - 29.0	8
Magnesium (mg)	253.8 (49.6)	166.7 - 362.8	320
Phosphorus (mg)	1363 (271)	893.3 - 2054	700
Potassium (mg)	3133 (457)	2394 - 4082	4,700
Sodium (mg)	2519 (471)	1606 - 3542	<2,300
Zinc (mg)	14.7 (16.7)	6.90 - 78.5	8
Copper (µg)	1108 (845)	600 - 4400	900

1. U.S. Department of Agriculture and U.S. Department of Health and Human Services. Dietary Guidelines for Americans, 2010, Appendixes 5 & 6. 7th Edition, Washington, DC: U.S. Government Printing Office, December 2010.

Most hematologic and serum biochemical values were within the normal range indicating adequate levels of bone marrow, renal and hepatic function and no detectable levels of infection (Table 3). However, osmolarity values were 305.2 ± 5.1 mOsm/kg (range 292.2 to 314.6), which indicated some dehydration (normal range 281-297 mOsm/kg) after the overnight fast. Blood glucose was 95.2 mg/dL including two subjects who had diabetes. The mean (range) for the 22 non-diabetics was 91.1 (77-105 mg/dL) and 139 and 141 mg/dL for the diabetics. 50% had low hemoglobin values between 9.6 and 11.2 g/dL.

Plasma retinol concentration was $1.95 \pm 0.52 \mu M$ (Mean \pm SD) and RBP was 2.48 \pm 0.66 μM , and these were highly $_{25}$

correlated (r = 0.982). Plasma retinol concentrations (and hence RBP concentrations) were weakly correlated with cholesterol and bilirubin (Table 4). Retinol (and RBP) were weakly correlated with serum albumin and albumin/globulin ratios (r = 0.51, 0.47). Mean α tocopherol concentration was $27.8 \pm 11 \mu M$; y-tocopherol concentration was $5.23 \pm 2.12 \mu M$. The major plasma carotenoids were β -carotene (0.44 \pm 0.21 μ M), lycopene $(0.43 \pm 0.20 \mu M)$, lutein and zeaxanthin $(0.32 \pm 0.17 \mu M)$, and α -carotene (0.15 \pm 0.06 μ M) (Table 5). Lutein and α and β -carotene were correlated with total cholesterol (p<0.04, 0.05, 0.002, respectively) but not with triacylglycerols. Lutein concentrations were correlated with plasma lycopene concentrations (p<0.01) and weakly with β -carotene (p<0.1); lycopene was also correlated with α -carotene (p<0.004) and β -carotene (p<0.002), and α -carotene was correlated with β -carotene (p<0.0001).

Significant positive correlations existed between plasma total cholesterol and retinol (r=0.420), RBP (r=0.411), γ -tocopherol (r=0.626), lutein (r=0.412), α carotene (r=0.411), β -carotene (r=0.593), the sum of carotenoids (r=0.548), and platelet 18:2 (µg in 2x108cells/ mL) (r=0.437). There were also significant positive correlations between platelet 18:1 (%FA) and serum HDL-cholesterol (r=0.426), α -tocopherol triacylglycerols (r=0.754), and platelet 20:4 (%FA) and total bilirubin (r=0.450). Retinol and RBP were negatively correlated with total bilirubin (r=-0.418 and -0.410, respectively) (Table 4). Dietary 20:4 was positively correlated with platelet 18:0 FAs and serum LDLcholesterol (r=0.440 and 0.521, respectively).

Table 2 Dietary fatty acids (g) and platelet fatty acids (µg in 2x108 cells/mL) from Iowa centenarians (N=24)

	Dietary Fatty (%) Acids (g)		Platelet Fatty Acids (μg)		(%)
	Mean (SD)		Mean	SD SD	
4:0 Butyric	0.49 (0.26)				
6:0 Caproic	0.27 (0.16)				
8:0 Caprylic	0.17 (0.09)				
10:0 Capric	0.40 (0.20)				
12:0 Lauric	0.47 (0.22)				
14:0 Myristic	1.97 (0.82)				
16:0 Palmitic	10.6 (2.77)		6.20	2.13	19.8
18:0 Stearic	4.79 (1.38)		7.21	2.33	22.3
20:0 Arachidic (n=15)	0.003 (0.005)				
22:0 Behenic (n=15)	0.005 (0.005)				
14:1 Myristoleic (n=15)	0.002 (0.004)				
16:1 Palmitoleic	1.16 (0.40)				
18:1 Oleic	20.55 (4.29)		8.99	3.53	27.8
20:1 Eicosenoic	0.09 (0.04)				
22:1 Erucic	0.003 (0.005)				
18:2 Linoleic	8.40 (1.67)		2.52	1.04	7.9
18:3 Alpha Linolenic	0.77 (0.14)				
18:3 Gamma Linolenic	0.013 (0.022)				
20:4 Arachidonic	0.11 (0.05)		7.38	2.91	22.9
20:5 Eicosapentaenoic	0.065 (0.104)				
22:5 Docosapentaenoic	0.010 (0.011)				
22:6 Docosaĥexaenoic	0.092 (0.102)				
Total Saturated	21.8 (5.60)	44.6			42.1
Total Monounsaturated	19.0 (4.63)	38.8			27.8
Total Polyunsaturated	8.11 (1.77)	16.6			30.8

Table 3
Serum biochemical and hematologic parameters in Iowa centenarians

Parameter	N	Mean (SD)	Range	Normal Range
Total cholesterol (mg/dL)	24	189.6 (33.6)	127 – 250	0 – 199
HDL-cholesterol (mg/dL)	23	46.5 (14.3)	23 – 89	35 – 85
VLDL-cholesterol (mg/dL)	16	23.9 (13.4)	6 – 55	5 – 40
LDL-cholesterol (mg/dL)	16	118.8 (34.3)	68 – 191	0 – 129
Triglycerides (mg/dL)	24	151.8 (162.6)	34 - 870	10 - 250
Blood urea nitrogen (mg/dL)	24	24.6 (6.5)	13 - 42	7 – 26
Serum creatinine (mg/dL)	24	1.26 (0.34)	0.5 - 2.1	0.5 - 1.5
BUN / creatinine ratio	24	19.9 (4.0)	12 - 29	6 – 22
Total protein (g/dL)	24	6.6 (0.6)	5.7 - 7.4	6.0 - 8.5
Total globulin (g/dL)	24	3.0 (0.6)	2.1 - 4.1	1.5 - 4.5
Serum albumin (g/dL)	24	3.6 (0.3)	3.1 - 4.3	3.5 - 5.5
Albumin / globulin ratio	24	1.19 (0.27)	0.8 - 2.0	1.1 - 2.5
Lactate dehydrogenase (IU/L)	24	165.8 (33.1)	103 - 254	100 - 250
SGOT (AST) (IU/L)	24	21.2 (9.06)	8 – 51	0 - 50
Total bilirubin (mg/dL)	24	0.5 (0.3)	0.2 - 1.1	0.1 - 1.2
Gamma-glutamyl transferase (IU/L)	17	31.4 (55.7)	3 – 227	0 - 45
Alanine aminotransferase SGPT (ALT) (IU/L)	24	14.0 (12.8)	2 - 49	0 - 50
Alkaline phosphatase (IU/L)	24	93.6 (26.3)	58 – 178	40 - 150
Calcium (mg/dL)	24	9.1 (0.4)	8.4 - 10.0	8.5 - 10.6
Phosphorous (mg/dL)	17	3.9 (0.3)	3.3 - 4.6	2.5 - 4.5
Sodium (meg/L)	17	141.2 (2.6)	135 - 145	135 - 148
Potassium (meg/L)	17	4.4 (0.3)	3.8 - 4.9	3.5 - 5.5
Chloride (meq/L)	17	103.4 (2.0)	100 - 107	94 - 109
Uric acid (mg/dL)	24	6.76 (1.87)	3.5 - 9.6	2.2 - 7.7
Glucose (mg/dL)	24	95.2 (15.4)	77 - 141	60 - 115
Total iron (mcg/dL)	24	68.0 (12.6)	42 – 92	40 - 180
White blood cells (cells/uL)	24	5904 (1521)	2800 - 9400	4000 - 16000
Red blood cells (M/uL)	24	4.05 (0.59)	3.04 - 5.57	4.2 - 5.5
Hemoglobin (g/dL)	24	12.0 (1.9)	9.6 - 17.6	12 - 16
Hematocrit (%)	24	35.8 (5.1)	29.6 - 52.0	37 - 47
Mean corpuscular volume MCV (fL)	24	88.5 (4.2)	80 - 97	84 - 94
Mean corpuscular hemoglobin MCH (pg)	24	29.7 (1.7)	26 – 33	26 - 32
Mean corp. hemoglobin conc MCMC (%)	24	33.3 (1.13)	31.0 - 36.0	32 - 36
T4-Thyroxine, Total (ug/dL)	7	8.9 (1.0)	7.6 - 10.5	4.5 - 12
Osmolarity (mOsm/kg) calculated	24	305.2 (5.1)	292 – 314	281 – 297

Table 4
Significant correlations among plasma carotenoids, platelet fatty acids and plasma parameters, (CI) and statistical significance

Parameters		r (CI)	P value
Retinol (µM)	Total bilirubin (mg/dL)	-0.418 (0.042)	0.05
(,,,,,	Total cholesterol (mg/dL)	0.420 (0.041)	0.05
	RBP (μM)	0.982 (0.0001)	0.0001
RBP (µM)	Total bilirubin (mg/dL)	-0.410 (0.047)	0.05
. ,	Total cholesterol (mg/dL)	0.411 (0.046)	0.05
α-Tocopherol (μM)	Triacylglycerol (mg/dL)	0.754 (0.0001)	0.0001
γ-Tocopherol (μM)	Total cholesterol (mg/dL)	0.626 (0.001)	0.005
	RBC (M/μL)	-0.436 (0.033)	0.05
	Hemoglobin (g/dL)	-0.465 (0.022)	0.05
	Hematocrit (%)	-0.432 (0.039)	0.05
	Lutein (µM)	0.9999 (0.0001)	0.0001
Lutein + zeaxanthin (μ M)	Total cholesterol (mg/dL)	0.412 (0.046)	0.05
	Lycopene (µM)	0.494 (0.014)	0.05
Lycopene (µM)	β-carotene (μM)	0.606 (0.002)	0.005
	α-carotene (μM)	0.560 (0.005)	0.005
α-Carotene (μM)	Total cholesterol (mg/dL)	0.411 (0.046)	0.05
	β-carotene (μM)	0.870 (0.0001)	0.0001
β-Carotene (μM)	Total cholesterol (mg/dL)	0.593 (0.002)	0.005
Total carotenoids	Total cholesterol (mg/dL)	0.548 (0.006)	0.01
	β-carotene (μM)	0.853 (0.0001)	0.0001
Platelet FA 18:2 (µg)	Total cholesterol (mg/dL)	0.437 (0.033)	0.05
Platelet %FA 18:1	HDL cholesterol (mg/dL)	0.426 (0.043)	0.05
Platelet %FA 20:4	Total bilirubin (mg/dL)	0.450 (0.0275)	0.05

 $\begin{array}{c} \textbf{Table 5} \\ \textbf{Plasma retinol, tocopherols, and carotenoids in Iowa} \\ \textbf{centenarians } (N=24) \end{array}$

	Mean (SD)	range
D-4:	1.05 (0.52)	1.08 – 3.29
Retinol (µM)	1.95 (0.52)	
Retinol-binding protein (µM)	2.48 (0.66)	1.51 - 4.09
% Saturation	78.4 (4.24)	67.7 - 85.5
α-Tocopherol (μM)	27.8 (11.0)	14.6 - 67.3
γ-Tocopherol (μM)	5.23 (2.12)	1.1 - 8.4
Lutein + Zeaxanthin (µM)	0.32 (0.17)	0.09 - 0.79
Lycopene (µM)	0.43 (0.20)	0.07 - 0.88
α-Carotene (μM)	0.15 (0.06)	0.08 - 0.34
β-Carotene (μM)	0.44 (0.21)	0.17 - 1.06
α-Tocopherol μM/total lipid	0.086 (0.025)	

Discussion

The standard serum biochemical and hematologic parameters of these subjects generally fell within normal ranges confirming good health. Osmolarity values indicated 92% of the subjects were dehydrated (23). Of the centenarians taking a diuretic (66%), all exhibited a mOsm/kg concentration >297 mOsm/kg. Several subjects had elevated plasma concentrations of one or more indicators, further suggesting mild dehydration;

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however, no subject displayed consistently high values for multiple indicators of dehydration.

Plasma retinol values were normal and retinyl ester concentrations were low to nondetectable, as is usual in fasting plasma. Various other surveys have reported plasma retinol means of 1.6 to 3.4 µM (24) and the 50th percentile values in the US are 1.71 µM for all females and 2.10 μ M for females >71 y (25). Bates et al. (26) found serum retinol concentrations of 2.15 µM in British females aged >80 y. Retinol-binding protein was 2.48 µM, which fits a published range of 1.9 to 2.4 µM (27). The percent saturation (molar ratio of retinol to RBP) was 78%; values of 82 to 91% have been reported (27). The lack of relationship to plasma tocopherols or carotenoids is not surprising, because retinol and RBP concentrations are homeostatically regulated (27), whereas plasma tocopherol and carotenoids concentrations tend to reflect recent dietary intake (28). Basile et al. (29) have also reported normal serum concentrations of retinol and αtocopherol in centenarians.

Although plasma concentrations of retinol or RBP are not quantitative indicators of vitamin A status, the combined observations that: a) retinol and RBP concentrations were well within normal ranges; b) RBP saturation with retinol was normal; c) plasma carotenoid concentrations were normal; and d) dietary intake was adequate, all argue that these subjects were in good vitamin A nutriture (30).

The major plasma carotenoids were β -carotene, lycopene, lutein, and α -carotene. β -Carotene and lycopene each consitituted one-third of the total plasma carotenoids. Lesser amounts of other carotenoids, such as cryptoxanthin, were detectable but were not quantified. Previous surveys have reported mean plasma β -carotene values of 0.21 to 1.15 µM (24). We found higher concentrations of lycopene, α -carotene, and β -carotene than those reported by Mecocci et al. in a European population of centenarians (31); presumably these differences are due to diet. Total plasma carotenoid concentrations, and ratios of specific carotenoids, reflect recent dietary intake (32), so the diversity of carotenoid concentrations and ratios observed in this study is expected. Of the carotenoid interrelationships, only α carotene/β-carotene showed a regression slope greater than 0.8. This suggests that a diversity of dietary carotenoid intakes was reflected in the plasma carotenoid profiles (32). It has previously been reported that total serum carotenoids in the elderly are weakly correlated with dietary carotene intake, but less so than with serum cholesterol concentrations (33).

 α -and γ -Tocopherol were the only vitamin E forms detectable, and α -tocopherol concentrations fit with published ranges (i.e., 16 to 36 μ M) (24, 34). There was no correlation between α - and γ -tocopherol concentrations (29). Plasma α -tocopherol concentrations were highly

correlated with plasma triacylglycerol concentrations (p<0.0001), and γ -tocopherol concentrations were strongly correlated with total plasma cholesterol (p<0.001). These observations are consistent with the view that these two forms of vitamin E are metabolized differently in the liver (35). Dietary vitamins A and E were not significantly correlated with plasma vitamin A and E measures or the carotenoids.

In this study, the total saturated fatty acid pattern in the platelets mirrored that of the dietary total saturated fatty acids. The dietary percent of MUFA and PUFA concentrations were not mirrored in the platelet fatty acid profile; the concentrations were the inverse of what we expected based on the three-day food intake records just before the blood draws. Platelets circulate for 8-12 days; therefore, the platelet fatty acid concentrations are reflective of their long-term diet intake (36).

This study complements other reports (1-6, 31) of vitamin A, carotenoids, and tocopherol status in the elderly and uniquely presents data for plasma retinol, RBP, tocopherols, and individual carotenoids in the same subjects who were unusually elderly, but in good health. There is good agreement for analyte values among these studies, and values fall within normal ranges. These normal values indicate that there was no effect of ageing on nutrient status (tocopherols and carotenoids) or nutrient mobilization (retinol) in this extremely elderly population. However, nutrient intakes of the subjects, who lived in long-term care facilities and did not prepare their own meals, are not typical of all elderly individuals; caloric intake of some rural elderly who live alone fall as low as 600 kcal/d (M. J. Oakland, unpublished observations, 1990). Hence these values reflect the nutritional status that may be obtained and maintained in the elderly, not the nutritional status of all elderly individuals.

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References

- Pilch SM (1987) Analysis of vitamin A data from the Health and Nutrition Examination Surveys. J Nutr 117:636-640.
- Woo J, Ho S, Mak YT, Shek CC, Swaminathan R (1988) Vitamin A and E status in healthy elderly Chinese in Hong Kong. Eur J Clin Nutr 42:689-695.
- Munro HN, McGandy RB, Hartz SC, Russell RM, Jacob RA, Otradovec CL (1987) Protein nutriture of a group of free-living elderly. Am J Clin Nutr 46:586-592.
- Sadowski JA, Hood SJ, Dallal GE, Garry PJ (1989) Phylloquinone in plasma from elderly and young adults: factors influencing its concentration. Am J Clin Nutr 50:100-108.
- Sahyoun NR, Otradovec CL, Harz SC, Jacob RA, Peters H, Russell RM, McGandy RB (1988) Dietary intakes and biochemical indicators of nutritional



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- status in an elderly, institutionalized population. Am J Clin Nutr 47:524-533.

 6. Garry PJ, Hunt WC, Bandrofchak JL, VanderJagt D, Goodwin JS (1987)

 Vitamin A intake and plasma retinol levels in healthy elderly men and
- women. Am J Clin Nutr 46:989-994. 7. Li D, Sinclair A, Mann N, Turner A, Ball M, Kelly F, Abedin L, Wilson A
- (1999) The association of diet and thrombotic risk factors in healthy male vegetarians and meat-eaters. Eur J Clin Nutr 53:612-9.
 Nelson GJ, Schmidt PC, Bartolini G, Kelley DS, Kyle D (1997). The effect of
- dietary arachidonic acid on platelet function, platelet fatty acid composition, and blood coagulation in humans. Lipids 32:421-425.
- Christensen JH, Christensen MS, Dyerberg J, Schmidt EB (1999) Heart rate variability and fatty acid content of blood cell membranes: a dose-response study with n-3 fatty acids. Am J Clin Nutr 70:331-337.
- Vicario IM, Malkova D, Lund EK, Johnson IT (1998) Olive oil supplementation in healthy adults: effects in cell membrane fatty acid composition and platelet function. Ann Nutr Metab 42:160-169.
- Caprari P, Scuteri A, Salvati AM, Bauco C, Cantafora A, Masella R, Modesti D, Tarzia A, Marigliano V (1999) Aging and red blood cell membrane: a study of centenarians. Exp Gerontol 34:47-57.
- Guillot N, Caillet E, Laville M, Calzada C, Lagarde M, Vericel E (2009) Increasing intakes of the long-chain ω-3 docosahexaenoic acid: effects on platelet functions and redox status in healthy men. FASEB J 23:2909-2916.
 Wood DA, Butler S, Riemersma RA, Thomson M, Oliver MF (1984) Adipose
- Wood DA, Butler S, Riemersma RA, Thomson M, Oliver MF (1984) Adipose tissue and platelet fatty acids and coronary heart disease in Scottish men. Lancet 2(8395):117-121.
- Fokkema MR, Brouwer DA, Hasperhoven MB, Hettema Y, Bemelmans WJE, Muskiet FAJ (2000) Polyunsaturated fatty acid status of Dutch vegans and omnivores. Prostaglandins Leukot Essent Fatty Acids 63:279-285.
- Arnold J, Dai J, Nahapetyan L, Arte A, Johnson MA, Hausman D, Rodgers WL, Hensley R, Martin P, MacDonald M, Davey A, Siegler IC, Jazwinski SM, Poon LW (2010) Predicting successful aging in a population-based sample of Georgia centenarians. Curr Gerontol Geriartr Res. doi:10.1155/2010/989315.
- Evert J, Lawler E, Bogan H, Perls T (2003) Morbidity profiles of centenarians: survivors, delayers, and escapers. J Gerontolol A Biol Sci Med Sci 58:232-237
- Goodwin JS (1989) Social, psychological and physical factors affecting the nutritional status of elderly subjects: separating cause and effect. Am J Clin Nutr 50:1201-1209.
- Oakland MJ, Speer MG (1993) Dietetic research in long-term care facilities: nutrient intake and nutritional status indicators in the oldest old. Top Clin Nutr 8:26-31.
- Stewart JW, Kaplan ML, Beitz DC (2001) Pork with a high content of polyunsaturated fatty acids lowers LDL cholesterol in women. Am J Clin Nutr 74:179-187.
- Tanumihardjo SA, Koellner PG, Olson JA (1990) The Modified Relative Dose Response (MRDR) assay as an indicator of vitamin A status in a population of well-nourished American children. Am J Clin Nutr 52:1064-1067.
- 21. Morrison WR, Smith LM (1964) Preparation of fatty acid methyl esters and

- dimethylacetals from lipids with boron fluoride-methanol. J Lipid Res 5:600-
- Pini R, Tonon E, Cavallini MC, Bencini F, Di Bari M, Masotti G, Marchionni N (2001) Accuracy of equations for predicting stature from knee height, and assessment of statural loss in an older Italian population. J Gerontol A Biol Sci Med Sci J 56A:B3-B7.
- Thomas DR, Tariq SH, Makhdomm S, Haddad R, Moinuddin A (2004) Physician misdiagnosis of dehydration in older adults. J Am Med Dir Assoc 5:S30-S34.
- DeLeenheer AP, Nelis HJ, Lambert WE, Bauwens RM (1988) Chromatography of fat-soluble vitamins in clinical chemistry. J Chromatogr 429:3-58
- Ballew C, Bowman BA, Sowell AL, Gillespie C. (2001) Serum retinol distributions in residents of the United States: third National Health and Nutrition Examination Survey, 1988-1994. Am J Clin Nutr 73:586-593.
- Bates CJ, Prentice A, Finch S (1999) Gender differences in food and nutrient intakes and status indices from the National Diet and Nutrition Survey of people aged 65 years and over. Eur J Clin Nutr 53:694-699.
- Goodman DS (1984) Plasma retinol-binding protein. In: Sporn MB, Roberts AB, Goodman DS (eds), The Retinoids, vol. 2. Academic Press, New York, pp 41-88.
- Brady WE, Mares-Perlman JA, Bowen P, Stacewicz-Sapuntzakis M (1996)
 Human serum carotenoid concentrations are related to physiologic and lifestyle factors. J Nutr 126:129-137.
- Basile G, Gangemi S, Lo BC, Mento A, Nicita-Mauro C, Crisafulli G, Merendino R A, Ientile R, Nicita-Mauro V (2003) Correlation between serum retinol and alpha-tocopherol levels in Italian centenarians. J Nutr Sci Vitaminol (Tokyo) 49:287-288.
- Olson JA (1987) Recommended Dietary Intakes (RDI) of vitamin A in humans. Am J Clin Nutr 45:704-716.
- Mecocci P, Polidori MC, Troiano L, Cherubini A, Cecchetti R, Pini G, Straatman M, Monti D, Stahl W, Sies H, Franceschi C, Senin U. (2000) Plasma antioxidants and longevity: a study on healthy centenarians. Free Radic Biol Med. 28: 1243-1248; erratum in Free Radic Biol Med (2000) 29(5):486.
- 32. Parker RS (1989) Carotenoids in human blood and tissues. J Nutr 119:101-104.
- Kergoat M-J, Leclerc BS, PetitClerc C, Imbach A (1986) Determinants of total serum carotene concentrations in institutionalized elderly. J Am Geriatr Soc 36:430-436
- Talwar DK, Azharuddin MK, Williamson C, Teoh YP, McMillan DC, St. J O'Reilly D, (2005) Biological variation of vitamins in blood of healthy individuals. Clin Chem 51: 2145-2150.
- Traber MG, Kayden HJ (1989) Preferential incorporation of alpha-tocopherol versus gamma-tocopherol in human lipoproteins. Am J Clin Nutr 49:517-526.
- Hillyer CD, Silberstein LE, Ness PM, Anderson KC, Roback JD (2007) Blood Banking and Transfusion Medicine: Basic Principles and Practice, 2nd edition. Churchill Livingstone (Elsevier), Philadelphia, p 458.



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