Postprandial Plasma Retinyl Ester Response Is Greater in Older Subjects Compared with Younger Subjects
Evidence for Delayed Plasma Clearance of Intestinal Lipoproteins

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Abstract

Postprandial vitamin A and intestinal lipoprotein metabolism was studied in 86 healthy men and women, aged 19–76 yr. Three independent experiments were carried out. In the first experiment, a supplement dose of vitamin A (3,000 retinol equivalents [RE]) was given without a meal to 59 subjects, aged 22–76 yr. In the second experiment, 20 RE/kg body wt was given with a fat-rich meal (1 g fat/kg body wt) to seven younger subjects (aged < 50 yr) and seven older subjects (aged ≥ 50 yr). In both experiments, postprandial plasma retinyl ester response increased significantly with advancing age ($P < 0.05$). In the third experiment, retinyl ester-rich plasma was infused intravenously into nine young adult subjects (aged 18–30 yr) and nine elderly subjects (aged ≥ 60 yr), and the rate of retinyl ester disappearance from plasma during the subsequent 3 h was determined. Mean (±SE) plasma retinyl ester residence time was 31±4 min in the young adult subjects vs. 57±8 min in the elderly subjects ($P < 0.05$). These data are consistent with the concept that increased postprandial plasma retinyl ester concentrations in older subjects are due to delayed plasma clearance of retinyl esters in triglyceride-rich lipoproteins of intestinal origin. (*J. Clin. Invest.* 1990. 85:883–892.) retinyl ester • retinol • vitamin A • triglyceride-rich lipoprotein • chylomicron • atherosclerosis

Introduction

Epidemiological studies have implicated nutritional factors in the development or prevention of various diseases associated with aging. However, information regarding the metabolism of such nutrients in older humans is limited. For example, intake of vitamin A has been identified with the prevention of certain types of cancer (1, 2), but few studies have addressed the metabolism of this nutrient in older individuals.

Evidence in the literature suggests that older humans have greater endogenous vitamin A reserves than younger humans, which, in turn, could influence vitamin A metabolism. In national surveys, such as the Health and Nutrition Examination Surveys of 1971–1980 (HANES I and II), vitamin A concentration in fasting plasma was found to increase with advancing age (3). In a survey in Boston, long-term daily use of vitamin A supplements was linked to evidence of excessive hepatic vitamin A stores in older humans, but not in young adults (4). In rats receiving standard rat feed containing the vitamin A requirement, liver vitamin A was found to be greater in older vs. younger animals (5). Taken together, these data suggest that aging is associated with greater endogenous vitamin A storage pools, and, therefore, a lower margin of safety for vitamin A intake. Since excessive vitamin A intake has known adverse effects (6–11), and since intake of the vitamin may increase among the aged because of the reported anticancer properties of the vitamin (1, 2), a better understanding of vitamin A nutriture and metabolism in the aged is required.

Vitamin A occurs as preformed vitamin A esters (retinyl esters) in dietary animal sources and also in vitamin supplements. During digestion, retinyl esters are hydrolyzed to vitamin A alcohol (retinol) and taken up by enterocytes. The vitamin is then reesterified and incorporated into the core of triglyceride-rich chylomicrons as retinyl esters (12, 13). Chylomicrons are released first into the lymph and then into the circulation, where lipoprotein lipase on vascular endothelium hydrolyzes chylomicron-triglyceride, and chylomicron remnants are formed (14, 15). Chylomicron remnants are rapidly removed from plasma by the liver via receptor-mediated endocytosis (16–18). Retinyl esters entering the liver are primarily reesterified and stored, but some may be hydrolyzed to retinol and released to plasma bound to retinol-binding protein (19). By virtue of their nearly complete association with chylomicrons and chylomicron remnants (20, 21), and lack of release by the liver (22), retinyl esters in plasma after vitamin A administration serve not only as the predominant postprandial plasma metabolites of ingested vitamin A, but also as useful postprandial indicators of intestinally derived, triglyceride-rich lipoproteins (TRL)[1] (23, 24).

Cohn et al. (25–27) have recently characterized postprandial lipoprotein metabolism in healthy humans, and have found that older subjects have greater postprandial triglyceridemia after a fat-rich meal than do younger subjects (25). This finding suggests that intestinal TRL remain in the plasma compartment longer for older vs. younger individuals. Since intestinal TRL may play a role in the development of atherosclerosis (28), and since risk of atherosclerosis and coronary artery disease increases with advancing age (29), a better understanding of postprandial intestinal lipoprotein metabolism in the aged is required.

The aim of the present study was to evaluate the relationship between aging and postprandial vitamin A and intestinal

1. *Abbreviations used in this paper:* RE, retinol equivalent; SAAM, simulation, analysis, and modeling (program); TRL, triglyceride-rich lipoprotein.

lipoprotein metabolism by measuring retinyl esters in postabsorptive plasma. We found that aging results in greater rises in postprandial plasma retinyl ester concentrations, and provide evidence which is consistent with the concept that increased postprandial plasma retinyl ester concentrations observed in older subjects are due to delayed plasma clearance of intestinal TRL.

Methods

Human subjects. Volunteers living in Boston, MA were recruited for three independent experiments by posting announcements in the local media and at area schools and businesses. Interested volunteers underwent a screening examination which included a medical history, physical examination, and a routine clinical blood chemistry profile. Volunteers with any history or biochemical evidence of liver, kidney or pancreatic disease, anemia, diabetes, hyperlipidemia, alcoholism, active ulcer, or malabsorption were excluded. Women who were pregnant or lactating, or who were taking oral contraceptives were also excluded. Subjects were not taking mineral oil, medications that interfered with fat-soluble vitamin absorption (cholestyramine or neomycin), or large doses of vitamin A (at least 10 times the recommended dietary allowance) within the past year. Subjects were asked not to take any nutrient supplement for at least 2 wk before admission into the studies. A combined total of 86 individuals (42 men, 44 women; aged 19–76 yr) were accepted into the three experiments. All studies were conducted in the Metabolic Research Unit at the U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University. Informed written consent was obtained from all volunteers under the guidelines established by the Human Investigation Review Committee of Tufts University and the New England Medical Center.

Analysis of plasma samples. Venous blood was collected in 0.1% EDTA tubes and immediately protected from light. Plasma was separated from red blood cells by centrifugation at 1,000 g for 15 min at 4°C, and stored at −70°C until analyzed. Plasma cholesterol and triglyceride concentrations were measured by an enzymatic assay using a bichromatic analyzer (ABA-200, Abbott Laboratories, Irving, TX) (30). Plasma retinol and retinyl ester concentrations were measured under red lights by normal-phase high-performance liquid chromatography as described by Bankson et al. (31). In this method, all the major plasma retinyl esters (retinyl palmitate, stearate, and oleate) coeluted as a single peak in the chromatogram.

Experiment 1: vitamin A tolerance in subjects of different ages. The relationship between age and postprandial vitamin A metabolism was investigated by carrying out vitamin A tolerance tests in 59 subjects of different ages (28 men, 31 women; aged 22–76 yr). Efforts were directed toward recruiting an approximately equal number of subjects in each age-decade group, so as to obtain a continuous data set with respect to age. During the 4-d study period, subjects were fed a diet containing 100 g of fat per day. After a 24-h equilibration period on the steady fat diet, stools were collected for the remaining 3 d and analyzed for fat content by the method of Van de Kamer et al. (32). All subjects were found to have a fecal fat excretion of < 9 g/d; mean±SE was 3.0±0.3 g/d.

On the second day of the study, 3,000 retinol equivalents (RE) of vitamin A (three times the recommended dietary allowance) were given after a 14-h overnight fast. The vitamin was given as retinyl palmitate in corn oil (P1M0, a gift from Hoffman LaRoche Inc., Nutley, NJ). Subjects did not receive breakfast, but received a lunch 5 h after the oral vitamin A dose. The lunch consisted of a turkey breast sandwich on white bread, cranberry juice, almond butter, and carbonated soda, and contained no preformed vitamin A and < 1 RE of β-carotene. The meal and oral vitamin A preparations were not equilibrated to body weight. Blood samples (5 ml) were collected in the fasting state ($t = 0$), and 2, 3, 4, 5, 6, 7, and 8 h after the 3,000 RE of vitamin A were given.

Tolerance curves were constructed by plotting the plasma retinyl ester concentration vs. time. The maximum rise in the postprandial plasma retinyl ester concentration was calculated by subtracting the fasting ($t = 0$) value from the highest postprandial value. As a composite index of the rate of entry and the rate of exit of retinyl esters in plasma, the total increase in postprandial plasma retinyl ester was calculated by the trapezoidal method (after the fasting value was subtracted) as the area under the retinyl ester response curve.

The relationships of maximum rise and total increase in postprandial plasma retinyl ester to age, sex, body weight, fecal fat excretion, and fasting plasma cholesterol, triglyceride, retinol, and retinyl ester concentrations were determined statistically by correlation and regression analysis. Subjects were separated at age 50 yr into two approximately equally sized age groups for further analysis. The *t* test was used to statistically compare mean postprandial plasma retinyl ester concentrations between younger subjects (age < 50 yr, n = 29) and older subjects (age ≥ 50 yr, n = 30).

Experiment 2: vitamin A, fat-feeding in subjects of different ages. To investigate the relationship between age and postprandial plasma retinyl ester concentrations for vitamin A given physiologically (i.e., with a meal), 14 subjects were prospectively recruited for a younger (age < 50 yr) and an older (age ≥ 50 yr) age group. Efforts were directed toward recruiting an equal number of men and women for each group. After a 14-h overnight fast, seven younger subjects (three men, four women; aged 22–48 yr) and seven older subjects (three men, four women; aged 50–67 yr) were fed vitamin A in a fat-rich meal. The fat-rich meal (1.0 g soybean oil/cream [1:1]/kg body wt) contained 20 RE of vitamin A/kg body wt. The vitamin A in the meal was ~ 1.5 times the recommended dietary allowance. 20 ml of blood was collected for plasma retinyl ester analysis in the fasting state ($t = 0$), and 3, 6, 9, and 12 h after the meal. Except for water and/or carbonated soda, subjects fasted for the 12-h duration of the study. TRL fractions ($d < 1.006$ g/ml) were collected by density ultracentrifugation (L8-80, Beckman Instruments, Inc., Fullerton, CA) at 100,000 g at 4°C for 18 h. For both whole plasma and TRL, the maximum rise and total increase (area under the curve) in postprandial plasma retinyl ester were determined from 0 to 12 h as described for the vitamin A tolerance experiment (experiment 1). The *t* test was used to statistically compare the data between the two age groups.

Experiment 3: plasma retinyl ester clearance in subjects of different ages. Postprandial plasma retinyl ester metabolism was evaluated further by carrying out a retinyl ester clearance experiment. The nature of this experiment warranted additional exclusion guidelines beyond those of the previous experiments. Subjects with a history of congestive heart failure, shortness of breath, angina, or other evidence of pulmonary or cardiovascular disease were excluded. Subjects who were less than or greater than 20% of their reference weight as determined from the 1983 Metropolitan Life Insurance reference weights (33) were excluded. Subjects with elevated fasting plasma triglyceride or serum glucose concentrations were also excluded. Serum glucose concentration was measured by an enzymatic assay using a centrifugal analyzer (Cobas Fara, Roche Diagnostic Systems, Monclair, NJ) (34). Apo E phenotype was determined in all subjects by minigel isoelectric focusing of TRL protein (35).

Given the invasive nature of the experiment and the known variability of the data (20), we maximized the possibility of observing an age difference by recruiting subjects to fill widely differing age groups, i.e., age 18–30 yr and age ≥ 60 yr. Only those subjects with the common apo E 3/3 phenotype were accepted for study. Nine young adult subjects (five men, four women; aged 19–28 yr) and nine elderly subjects (four men, five women; aged 62–73 yr) were studied. Three middle-aged men (aged 32–45 yr), who did not fulfill the strict recruitment criteria with respect to age, body weight, and apo E phenotype, were also studied. Finally, two subjects (both men, aged 20 and 28 yr), each with an apo E 2/2 phenotype, which is known to be associated with impaired plasma clearance of chylomicron remnants (36–42), were studied.

Retinyl ester clearance experiments were carried out as described by Berr and Kern (20). After a 14-h overnight fast, each volunteer was given a fat-rich meal containing 60,000 RE of vitamin A and 40 g of soybean oil/cream (1:1) per square meter of body surface area. Body surface area was estimated from height and weight (43). 5 h after ingestion of the meal, 2 U of plasma was collected by plasmapheresis and stored at room temperature in a light-shielded container. 42 h later, the plasma was returned to the donor by rapid intravenous infusion into a forearm vein. 2 ml of blood was collected for plasma retinyl ester analysis before infusion (preinfusion, $t = 0$), at 10, 15, 20, 25, and 30 min subsequent to infusion, and every 10 min thereafter for 3 h. To ensure that retinyl ester in infused plasma was primarily in TRL, an additional 20 ml of blood was collected at 0 and 10 min. For these samples, TRL fractions ($d < 1.006$ g/ml) were separated by density ultracentrifugation as described in the vitamin A, fat-feeding experiment (experiment 2) and the concentration of retinyl ester was determined.

To construct decay curves, the postinfusion retinyl ester concentration was subtracted by the preinfusion value, and then expressed as a percentage of the 10-min retinyl ester value. Compartmental analysis using simulation, analysis, and modeling (SAAM) programs (44, 45) were applied to fit the decay curves to a one-, two-, or three-exponential decay function. The best fit of the data (determined by the lowest fractional standard deviation of the slopes and y-intercepts) was used to generate apparent decay constants and residence times (the residence time is equal to the average weighted $t_{1/2}$). For all subjects studied, a monoexponential decay function provided the best fit of the data. An apparent first-order decay constant, k_d, was calculated from SAAM, and the apparent volume of distribution, V_d, for retinyl ester was estimated from age, sex, and body weight (46). The rate of plasma retinyl ester clearance was calculated as the apparent decay constant, k_d, multiplied by the apparent volume of distribution, V_d.

Mean weight and fasting concentrations of serum glucose and plasma cholesterol, triglyceride, retinol, and retinyl ester were compared between the two age groups by the t test. For the kinectic decay data, significantly different variance between the two age groups, as determined by the F test for equality of variance, dictated the use of nonparametric procedures (47). Hence, the Wilcoxon rank test was used to statistically compare the kinetic decay data between the two age groups (47). To obtain a continuous data set with respect to age, the three middle-aged subjects were included with the original 18 study subjects and relationships among the data were determined statistically by correlation analysis.

Results

Mean age, body weight, and fasting plasma cholesterol, triglyceride, retinol, and retinyl ester concentrations for the 86 subjects who participated in the experiments are shown in Table I. Age was positively correlated with fasting plasma cholesterol ($r = 0.42$, $P < 0.001$), triglyceride ($r = 0.24$, $P < 0.02$), retinol ($r = 0.37$, $P < 0.001$), and retinyl ester ($r = 0.26$, $P < 0.05$) concentrations. Body weight was positively correlated with fasting plasma triglyceride concentration ($r = 0.26$, $P < 0.02$). Cholesterol was positively correlated with fasting plasma triglyceride ($r = 0.55$, $P < 0.001$), retinol ($r = 0.37$, $P < 0.01$), and retinyl ester ($r = 0.22$, $P < 0.05$) concentrations. As shown in Table I, older subjects (age $\geq$ 50 yr) had significantly greater mean fasting plasma concentrations of cholesterol ($P < 0.001$), triglyceride ($P < 0.01$), retinol ($P < 0.05$), and retinyl ester ($P < 0.05$) than younger subjects (age < 50 yr).

In the vitamin A tolerance experiment (experiment 1), postprandial plasma retinyl ester response after administration of 3,000 RE of vitamin A without a meal was measured as (a) the maximum postprandial rise from fasting retinyl ester concentrations and (b) the total postprandial retinyl ester increase as determined by area under the postprandial plasma retinyl ester response curve. The relationships of age to maximum rise and total increase in postprandial plasma retinyl ester are shown in Fig. 1. Age was positively correlated with both maximum rise (Fig. 1 A) and total increase (Fig. 1 B) in postprandial plasma retinyl ester. Fasting plasma triglyceride concentration was also positively correlated with both maximum rise ($r = 0.46$, $P < 0.001$) and total increase ($r = 0.46$, $P < 0.001$) in postprandial plasma retinyl ester. Fasting plasma retinol concentration was also positively correlated with both maximum rise ($r = 0.34$, $P < 0.01$) and total increase ($r = 0.33$, $P < 0.01$) in postprandial plasma retinyl ester. The maximum rise and total increase in postprandial plasma retinyl ester were not significantly correlated with body weight, fecal fat, or fasting plasma cholesterol or retinyl ester concentrations.

Multiple regression analysis was undertaken to determine which parameters could best predict the postprandial plasma retinyl ester response (Table II). The fasting plasma concentration of triglyceride ($P < 0.002$) and retinol ($P < 0.05$) were both significant positive predictors of both maximum rise and total increase in postprandial plasma retinyl ester. Age was also an independent positive predictor of total increase in postprandial plasma retinyl ester ($P < 0.05$).

To graphically display the postprandial plasma retinyl ester response curves, subjects were separated at age 50 yr into two approximately equally sized groups. Fig. 2 shows the

Table I. Characteristics of Subjects

Subject group	Age	Body weight	Fasting plasma concentration			
			Cholesterol	Triglyceride	Retinol	Retinyl ester
	yr	*kg*	*mmol/liter*	*mmol/liter*	*μmol/liter*	*nmol/liter*
All subjects	49±2	71±2	4.94±0.10	1.02±0.05	1.71±0.05	48±5
Younger (age < 50 yr)	32±1	72±3	4.53±0.11	0.89±0.05	1.57±0.05	38±5
Older (age ≥ 50 yr)	65±1	70±2	5.30±0.14*	1.15±0.07‡	1.85±0.07§	58±8§

Values are mean±SE. Plasma was taken from subjects who fasted for 14 h overnight. 43 younger subjects (24 men, 19 women; aged 22–48 yr) and 43 older subjects (18 men, 25 women; aged 50–67 yr) were studied. One subject from the vitamin A, fat-feeding experiment (experiment 2) and seven subjects from the retinyl ester clearance experiment (experiment 3) were also in the vitamin A tolerance experiment (experiment 1). In this table, only data from the vitamin A tolerance experiment for these subjects were used. Significantly different from younger subjects: *$P < 0.001$, ‡ $P < 0.01$, § $P < 0.05$.
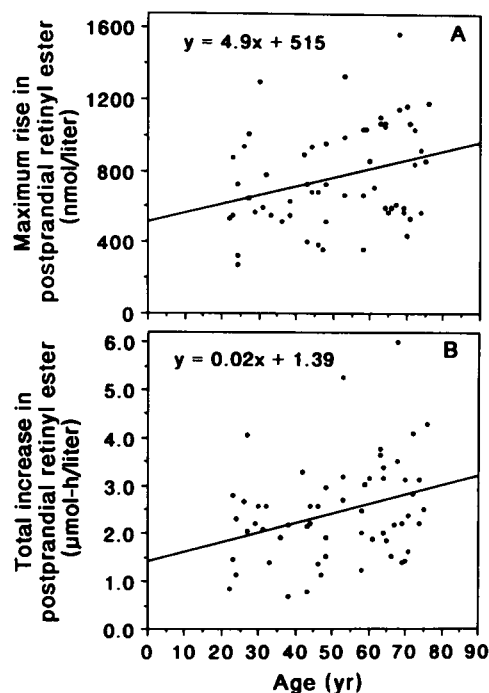
Figure 1. Postprandial plasma retinyl ester response in human subjects given 3,000 RE of vitamin A (as retinyl palmitate) without a meal. Each symbol represents the result from a single individual. The solid line represents a least squares fit to the data. (A) Correlation between age and maximum rise from fasting retinyl ester concentrations; $r = 0.30$, $P < 0.02$. (B) Correlation between age and total increase in postprandial plasma retinyl ester as measured by area under the retinyl ester response curve; $r = 0.33$, $P < 0.01$.
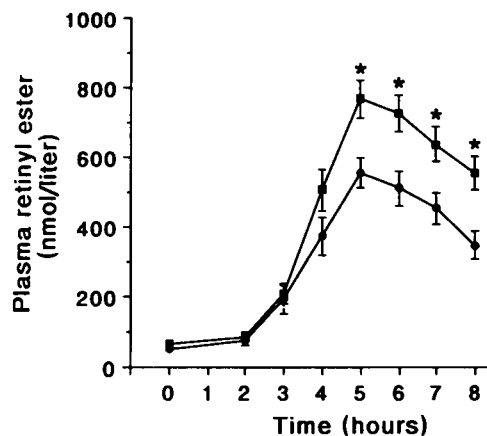

Figure 2. Postprandial plasma retinyl ester concentrations in human subjects given 3,000 RE of vitamin A (as retinyl palmitate) without a meal. Data are presented as mean±SE for 28 younger subjects (17 men, 11 women) and 31 older subjects (11 men, 20 women): age < 50 yr (•) and age ≥ 50 yr (■). *Significantly different from younger subjects: $P < 0.02$.

Table II. Predictors of Postprandial Plasma Retinyl Ester Response in 59 Subjects Given 3,000 RE of Vitamin A as Retinyl Palmitate

Multiple regression variable	Maximum rise in postprandial retinyl ester* (nmol/liter) Coefficient (SE)	Total increase in postprandial retinyl ester‡ (μmol·h/liter) Coefficient (SE)
Intercept	349	1.29
Age (yr)	3 (2)	0.02 (0.01)‖
Sex (male = 1, female = 2)	−29 (75)	−0.15 (0.28)
Body weight (kg)	−1 (2)	−0.01 (0.01)
Fecal fat (g/day)	7 (15)	0.03 (0.06)
Cholesterol (mmol/liter)§	−29 (47)	−0.24 (0.17)
Triglyceride (mmol/liter)§	289 (88)¶	1.27 (0.32)¶
Retinol (μmol/liter)§	151 (73)‖	0.61 (0.27)‖
Retinyl ester (nmol/liter)§	−1 (1)	−0.01 (0.02)
	Multiple $R^2 = 0.39$	Multiple $R^2 = 0.36$

* The maximum rise in postprandial retinyl ester concentration was calculated by subtracting the fasting value from the highest postprandial value.
‡ Total increase in postprandial retinyl ester was calculated by the trapezoidal method (after the fasting value was subtracted) as the area under the postprandial plasma retinyl ester response curve.
§ Taken from 14-h overnight fasting plasma.
‖ $P < 0.002$.
¶ $P < 0.05$.

mean±SE postprandial plasma retinyl ester concentration for 28 younger subjects and 31 older subjects. The older subjects had significantly greater postprandial plasma retinyl ester concentrations than the younger subjects at 5–8 h after the vitamin A dose ($P < 0.02$).

We extended the findings of the vitamin A tolerance experiment by carrying out a second experiment in which vitamin A was given in a physiological amount in association with a fat-rich meal to seven younger and seven older subjects. As shown in Table III, retinyl ester was measured in whole plasma and in the TRL fraction of plasma ($d < 1.006$ g/ml). At all time points, the retinyl ester concentration in whole plasma and in the TRL fraction was higher in older subjects as compared with younger subjects, and was significantly different ($P < 0.05$) at 6 h for whole plasma and at 9 h for TRL. The maximum rise and total increase (area under the curve) in postprandial plasma retinyl ester were both significantly greater for older subjects than for younger subjects ($P < 0.05$). The total postprandial increase in TRL-retinyl ester was also significantly greater for older subjects than for younger subjects ($P < 0.05$).

To determine if the greater postprandial plasma retinyl ester concentrations observed in older subjects were due to decreased plasma retinyl ester removal, plasma retinyl ester clearance tests were carried out in nine young adult subjects (aged 18–30 yr) and nine elderly subjects (aged ≥ 60 yr). The rate of plasma retinyl ester clearance after a rapid infusion of retinyl ester-rich plasma for the two age groups is shown in Fig. 3. To construct decay curves, postinfusion retinyl ester concentrations ($t = 0$) were subtracted by the preinfusion values, and the differences were expressed as a percentage of the 10-min retinyl ester values, as described by Berr and Kern (20). Using SAAM compartmental modeling programs (44, 45), retinyl ester clearance was found to be monoexponential for all subjects. The fractional standard deviations for the apparent first-order decay constants and y-intercepts were < 0.05 for all subjects. For monoexponential decay, the residence time is equivalent to the $t_{1/2}$.

| Subject group | Retinyl ester concentration | | | | | Maximum rise in postprandial retinyl ester* | Total increase in postprandial retinyl ester‡ |
| | *h* | | | | | | |
	0	3	6	9	12		
	nmol/liter					*nmol/liter*	*μmol · h/liter*
Whole plasma							
Younger (aged < 50 yr)	39±6	140±10	103±8	92±12	69±8	82±10	0.62±0.06
Older (aged ≥ 50 yr)	64±13	142±18	180±16§	151±16	99±18	130±18§	0.92±0.13§
Triglyceride-rich lipoproteins							
Younger (aged < 50 yr)	14±3	68±7	68±15	42±6	21±7	68±12	0.44±0.05
Older (aged ≥ 50 yr)	19±7	93±13	108±19	85±18§	32±14	100±12	0.75±0.11§

Values are mean±SE. After a 14-h overnight fast, subjects received a fat-rich meal (1 g fat/kg body wt) containing 20 RE of vitamin A (as retinyl palmitate)/kg body wt. Subjects remained fasting for the 12-h duration of the study. Seven younger subjects (three men, four women; aged 22–48 yr) and seven older subjects (three men, four women; aged 50–67 yr) were studied. * The maximum rise in postprandial retinyl ester concentration was calculated by subtracting the fasting value from the highest postprandial value. ‡ Total increase in postprandial retinyl ester was calculated by the trapezoidal method (after the baseline value was subtracted) as the area under the postprandial retinyl ester response curve. § Significantly different from younger subjects: $P < 0.05$.

Age, body weight, apo E phenotype, and fasting serum glucose and plasma cholesterol, triglyceride, retinol, and retinyl ester concentrations, as well as the kinetic decay data for all subjects studied in the clearance experiment (experiment 3), are shown in Table IV. The mean apparent first-order decay constant and rate of clearance for elderly subjects were both approximately twofold lower than for the young adult subjects ($P < 0.05$). The mean residence time for the elderly subjects was almost twofold greater than for the young adult subjects ($P < 0.05$). For correlation analysis, the three middle-aged men were included with the original 18 study subjects so as to obtain a continuous data set with respect to age. A statistically significant negative correlation between age and the

retinyl ester decay constant ($r = -0.46$, $P < 0.05$) was found. Furthermore, a statistically significant positive correlation between age and residence time ($r = 0.52$, $P < 0.02$) was found. None of the kinetic decay parameters were correlated with fasting serum glucose or plasma cholesterol, triglyceride, retinol, or retinyl ester concentrations, or body weight. The two subjects with apo E 2/2 phenotypes (both men, aged 20 and 28 yr) had fivefold lower decay constants, over fourfold greater residence times, and over fourfold lower clearance rates than their young adult counterparts (Table IV).

We found that the increase in the concentration of retinyl ester from preinfusion to 10-min postinfusion plasma was due primarily to an increase in retinyl ester in the TRL fraction. The increase in retinyl ester from preinfusion to 10-min post-infusion TRL ($d < 1.006$ g/ml) accounted for 97±3% (mean±SE) of the overall increase in retinyl ester in whole plasma for all subjects.

Discussion

In the vitamin A tolerance experiment (experiment 1), we administered a dose of the vitamin similar to that in a multivitamin preparation, and found a significant positive correlation between age and the maximum rise and total increase (area under the curve) in postprandial plasma retinyl ester. To investigate this finding further, we administered vitamin A (dose dependent on body weight) in association with a fat-rich meal to a selected group of younger and older subjects. In agreement with the vitamin A tolerance experiment, we observed that older subjects achieved greater maximum rises and total increases in postprandial plasma retinyl ester than younger subjects. Further, we noted that the postprandial rise in plasma retinyl ester concentration was mainly due to a rise in retinyl ester in the TRL fraction of plasma ($d < 1.006$ g/ml). Finally, to investigate the process of postprandial plasma retinyl ester clearance, we infused retinyl ester-rich plasma to a selected group of young adult and elderly subjects and determined the subsequent rate of plasma retinyl ester decay. These
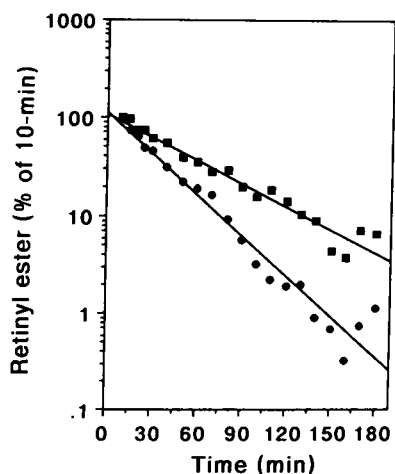

Figure 3. Rate of plasma retinyl ester clearance after intravenous infusion of retinyl ester-rich plasma. 5 h after receiving 60,000 RE and 40 g of fat (soybean oil/cream = 1:1) per square meter body surface area, plasma was collected from each subject by plasmapheresis. 42 h later, the plasma was returned to the donor by a rapid intravenous infusion, and the disappearance in retinyl esters from plasma was monitored for the next 3 h. To construct decay curves, the postinfusion retinyl ester concentration was subtracted by the preinfusion value, and then expressed as a percentage of the 10-min retinyl ester value. Data are presented as mean values from nine young adult subjects (five men, four women) and nine elderly subjects (four men, five women): age 18–30 yr (●), age ≥ 60 yr (■).

Table IV. Characteristics of Subjects in the Retinyl Ester Clearance Experiment (Experiment 3)

Subject	Age	Body weight	Fasting plasma concentration*					Kinetic decay data[‡]				Apo E phenotype
			Glucose	Cholesterol	Triglyceride	Retinol	Retinyl ester	V_d	k_d	Residence time	Clearance	
	yr	kg	mmol/liter	mmol/liter	mmol/liter	μmol/liter	nmol/liter	liter	min^{-1}	min	ml/min	
Young adult (aged < 30 yr)												
Women												
1	19	59.2	4.6	4.29	0.71	1.18	29	4.3	0.038	26	163	3/3
2	22	59.6	4.4	4.11	0.43	1.32	28	4.2	0.021	48	88	3/3
3	27	58.0	4.4	4.34	0.35	1.00	21	4.1	0.023	43	94	3/3
4	27	51.4	4.4	4.34	0.76	1.45	43	3.4	0.054	18	184	3/3
Men												
5	20	60.5	4.2	4.11	0.81	1.28	40	4.2	0.039	26	168	3/3
6	23	70.5	4.9	3.93	0.65	1.38	75	5.4	0.106	9	572	3/3
7	27	71.0	4.9	4.58	0.90	0.99	36	5.4	0.027	27	146	3/3
8	28	77.8	4.5	4.78	1.10	1.57	48	5.5	0.028	36	140	3/3
9	29	72.2	4.7	4.63	0.88	1.43	34	5.4	0.021	47	113	3/3
Mean	25	64.5	4.6	4.16	0.73	1.32	39	4.6	0.040	31	186	
(SE)	(1)	(2.9)	(0.1)	(0.18)	(0.08)	(0.07)	(5)	(0.2)	(0.012)	(4)	(50)	
Median	27	60.5	4.5	4.29	0.76	1.38	36	4.4	0.028	27	146	
Elderly (aged ≥ 60 yr)												
Women												
10	63	60.1	4.9	5.40	1.13	1.57	44	4.2	0.018	56	76	3/3
11	63	60.2	4.6	4.45	0.87	1.72	31	3.9	0.030	34	117	3/3
12	70	75.5	3.9	5.35	1.10	1.38	41	4.4	0.020	51	88	3/3
13	73	55.4	5.4	5.15	1.04	1.24	40	3.5	0.020	50	70	3/3
14	73	67.5	4.8	5.90	1.72	1.48	39	4.1	0.010	96	41	3/3
Men												
15	62	67.8	3.9	4.01	0.79	1.18	60	4.7	0.019	52	89	3/3
16	63	84.6	3.9	4.99	0.70	1.69	41	5.5	0.009	109	50	3/3
17	67	85.5	4.8	4.63	1.05	2.09	143	5.4	0.032	31	173	3/3
18	71	77.5	5.0	5.07	0.56	1.73	75	5.0	0.033	31	165	3/3
Mean	67	70.4	4.6	4.99[§]	0.99	1.57	57	4.5	0.021[§]	57[§]	96[§]	
(SE)	(2)	(3.6)	(0.2)	(0.19)	(0.11)	(0.09)	(12)	(0.2)	(0.003)	(9)	(15)	
Median	67	67.8	4.8	5.07	1.04	1.57	41	4.4	0.020	51	88	
Middle-aged (aged 30–60 yr)												
19	32	63.0	4.4	5.09	0.79	1.49	48	4.8	0.045	22	216	4/3
20	45	129.6	5.2	6.59	1.16	2.01	70	7.1	0.039	26	277	3/3
21	45	61.0	4.5	4.58	1.04	1.52	43	4.1	0.046	23	189	4/3
Apo E 2/2												
22	20	77.5	3.9	3.41	1.20	1.34	115	5.8	0.008	130	46	2/2
23	28	91.7	3.6	4.47	0.95	1.62	461	6.4	0.008	132	52	2/2

* Taken from 14-h overnight fasting blood.   [‡] Decay curves were constructed as described in Fig. 2. Plasma volume of distribution (V_d) of retinyl ester was determined from standardized tables (46). The apparent first-order decay constant (k_d) and residence time were determined by compartmental analysis (44, 45). The rate of plasma retinyl ester clearance = $V_d \times k_d$.   [§] Significantly different from young adult subjects: $P < 0.05$.

experiments revealed that elderly subjects had a slower rate of retinyl ester removal from plasma than young adult subjects indicating that plasma clearance of intestinal TRL is delayed in the aged.

The absorption of vitamin A in oral dose or feeding studies, such as in the vitamin A tolerance and fat-feeding experiments, depend on such factors as gastric emptying rate, intestinal motility, and pancreatic and gall bladder secretions. Al-though several investigations have found an age-related decrease in gastric emptying of both liquid (48–50) and solid foods (49, 50), intestinal motility and mouth-to-cecum transit time was unaltered by increasing age (50–52). Stimulated pancreatic secretions and pancreatic enzyme activities in duodenal contents of rats and humans appear to decrease slightly with advancing age (53–56). However, Arora et al. (57) have recently shown in 114 healthy subjects that fecal fat excretion,

and, thus, fat absorption, changes little as one ages, suggesting that the decrease in pancreatic enzyme secretions is not great enough to impair normal digestion. Little information is available regarding changes in biliary secretions with increasing age. Decreased gastric emptying rate, intestinal motility, or biliary or pancreatic secretions among the aged would lead, if anything, to a lower absorption of vitamin A, and consequently, a lower postprandial plasma retinyl ester response, rather than a greater response as observed in the present investigation.

The absorption of vitamin A in oral dose or feeding studies also depends on the resistance of the diffusion barrier at the intestinal cell surface. Hollander and Morgan (58) have shown in rats that the rate of uptake of radiolabeled vitamin A by perfused intestinal segments increases with advancing age, and have suggested that this finding is due to an age-related decrease in the resistance of the diffusion barrier at the intestinal cell surface. However, Fleming and Barrows (59) have shown that, in rats given tritiated vitamin A, the amount of radioactivity recovered in stools over 18 h did not differ significantly by age group, suggesting no effect of age on vitamin A uptake. In view of the experiments of Hollander and Morgan (58), however, one must consider the possibility that vitamin A uptake is greater in older vs. younger humans.

To circumvent the difficulties of investigating postprandial vitamin A metabolism by oral dose studies, we have used the retinyl ester infusion technique described by Berr and Kern (20) to isolate the process of postprandial retinyl ester clearance from that of vitamin A absorption. This technique is more accurate than oral dose experiments for studying retinyl ester clearance because it avoids the assumptions required regarding the absorption process, such as constant input over time. In the retinyl ester infusion experiments, retinyl ester-rich plasma is collected by plasmapheresis and reinfused 42 h later, and the rate of plasma retinyl ester decay is used to estimate the rate of plasma chylomicron remnant clearance. For this experiment to be valid, retinyl esters in plasmaphered plasma must remain with chylomicrons and their remnants during isolation and storage, and after infusion. We have found that 97% of the rise in the retinyl ester concentration from preinfusion plasma to 10-min postinfusion plasma was due to a rise in the retinyl ester concentration in TRL ($d < 1.006$ g/ml), suggesting that retinyl esters remain with chylomicrons and their remnants during the 42-h storage period, and 10 min after infusion. Retinyl esters must also be quantitatively removed by the liver with minimal resecretion into plasma. Although animal studies have suggested that hepatic secretion of retinyl ester is possible, the amount released is minimal relative to the amount of retinyl ester in postprandial plasma (22, 60). The validity of this method, as carried out in our laboratory, is supported by the similarity of our results to those of Berr and Kern (20). The mean (±SE) residence time ($t_{1/2}$) for retinyl ester decay was 31±4 min for our nine young adult subjects (aged 19–28 yr) and 29±6 min for the eight young adults (aged 20–35 yr) in their study (20). The validity of the present method is also supported by the diminished rate of clearance in two subjects with apo E 2/2 phenotypes (Table IV), who would be expected to have a defect in plasma chylomicron remnant clearance (36–42).

Delayed clearance of postprandial plasma retinyl esters in older individuals could be due to diminished catabolism of chylomicrons. Catabolism of chylomicrons occurs via the action of lipoprotein lipase on vascular endothelium, which hy-

drolyzes chylomicron-triglyceride resulting in delipidation of the particles and the subsequent formation of chylomicron remnants (14, 15). Since hepatic receptors recognize chylomicron remnants rather than chylomicrons (16), hydrolysis of chylomicron-triglyceride by lipoprotein lipase is potentially a rate-limiting step in the removal of intestinal TRL from plasma. Several studies have shown that in humans, heparin-induced lipoprotein lipase activity decreases significantly with increasing age (61–65). For example, Huttunen et al. (64), using an immunochemical method for separating postheparin plasma activities of lipoprotein lipase from hepatic lipase, demonstrated a statistically significant negative correlation between age and activity of postheparin plasma lipoprotein lipase in 47 healthy men and 35 healthy women. Similarly, Weintraub et al. (65) have shown that increased postprandial plasma retinyl ester concentrations in older subjects was associated with decreased lipoprotein lipase activity. Thus, a decrease in lipoprotein lipase activity with advancing age is one possible explanation for delayed chylomicron remnant clearance.

Delayed clearance of postprandial plasma retinyl esters in older individuals could also be due to diminished hepatic chylomicron remnant receptor activity. In dogs, binding of ^{125}I-labeled lipoproteins to hepatic receptors decreased dramatically with increasing age for the LDL receptor, but only slightly for the chylomicron remnant receptor (as measured by ^{125}I-apo E HDL binding) (66). However, the dogs ranged in age from 7 wk to 21 mo which represents a progression from immature to adult animals, but does not include aged animals. The relationship between old age and hepatic chylomicron remnant receptor activity for five swine (different breeds) and three humans (one man, two women) was inconclusive (66). Thus, the effect of aging on hepatic chylomicron remnant receptor activity requires further study.

In the vitamin A tolerance experiment, we have shown that fasting plasma triglyceride concentration is highly predictive of postprandial plasma retinyl ester response (Table II). Consonant with this finding is that fasting plasma triglyceride concentration is highly predictive of postprandial triglyceridemic response (25, 67–69). Taken together, these data suggest that the preexisting TRL pool is metabolically related to the postprandial plasma response in intestinal lipoprotein. Nestel (67) has postulated that since both chylomicron-triglyceride and triglyceride of endogenous origin share many common metabolic pathways, particularly in liver, adipose tissue and plasma, the rate at which chylomicron-triglyceride is hydrolyzed and removed from plasma is influenced by the magnitude of the preexisting liver, adipose tissue, and plasma pools of triglyceride. Preexisting endogenous triglyceride could compete with chylomicron-triglyceride for lipolytic sites on lipoprotein lipase, or removal sites on adipose tissue. Further, endogenous triglyceride-rich VLDL could compete with chylomicron remnants for removal sites on hepatocytes. Known differences between younger and older humans in fat mass and endogenous plasma triglyceride concentration may therefore play a role in the age-related differences observed in the clearance of intestinal TRL from plasma.

Measurement of fasting plasma retinol and retinyl ester concentrations are accepted tools for determining the vitamin A nutritional status of humans (4, 70). In the present study, we have confirmed the finding that fasting plasma retinol concentrations increase with advancing age (71), and demonstrate

that fasting plasma retinyl ester concentrations also increase with advancing age (Table I). The increased fasting plasma vitamin A concentrations, in which delayed clearance of retinyl esters in chylomicron remnants could play a role, may be circulating in plasma unbound to retinol-binding protein (72–75). Unbound vitamin A has potential adverse effects on cell membrane stability, and is thought to be related to some of the clinical manifestations of vitamin A toxicity (76, 77). Although greater fasting plasma vitamin A concentrations in the aged is consistent with the hypothesis that older humans have greater endogenous vitamin A reserves than younger humans, further research is required to determine whether these findings represent a normal response to aging.

Delayed plasma clearance of intestinal lipoproteins among older subjects has significant implications for the pathogenesis of coronary artery disease. Since chylomicrons are the principal carriers of dietary cholesterol, Zilversmit (28) has postulated that their conversion to chylomicron remnants on vascular endothelium is an atherogenic event. The process involves the binding of chylomicrons to the arterial surface, the hydrolysis of triglyceride by lipoprotein lipase, and the subsequent internalization of the cholesterol-rich chylomicron remnant by the arterial smooth muscle. In support of this hypothesis, Floren et al. (78) have demonstrated that uptake of chylomicron remnants results in cholesterol accumulation in cultured human arterial smooth muscle cells. Further support is given by clinical and angiographic studies, which have implicated postprandial lipoproteins in the development of coronary artery disease (79–83). The findings of the present study suggest that older individuals have a decrease in the rate of intestinal lipoprotein clearance and therefore may be more susceptible to the development of atherosclerosis.

Acknowledgments

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