Effects of long-term intake of retinol on selected clinical and laboratory indexes[1–3]

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ABSTRACT

Background: Chemopreventive agents developed to be used in a moderate-risk but otherwise healthy population need to be both efficacious and to have minimal adverse effects.

Objective: The objective of this study was to evaluate the adverse effects of long-term retinol intake in a skin cancer chemoprevention trial in a large population at moderate risk for skin cancer.

Design: Participants (n = 2297) were randomly assigned to receive retinol [7576 retinol equivalents (RE), or 25 000 IU] or a placebo daily. The adverse effects of retinol intake were studied by monitoring 14 clinical symptoms and laboratory indexes. The median follow-up time was 3.8 y.

Results: No adverse effects concerning the 14 symptoms were observed. Significant differences in alkaline phosphatase ($P < 0.0001$), triacylglycerol ($P < 0.0001$), cholesterol ($P = 0.04$), and HDL ($P = 0.01$) were observed over time between the 2 groups. After 49 mo of follow-up, alkaline phosphatase was 7% higher, triacylglycerol was 11% higher, cholesterol was 3% higher, and HDL was 1% lower in the retinol group than in the placebo group.

Conclusions: Because a 1% increase in cholesterol concentrations has been reported to be associated with a 2% increase in coronary artery disease risk, long-term ingestion of 7576 RE vitamin A/d should be considered with caution. However, further studies are needed to confirm this finding. *Am J Clin Nutr* 1999;69:937–43.

KEY WORDS Retinol, skin cancer, prevention, actinic keratoses, humans, long-term effects, Arizona, adults

INTRODUCTION

One of the primary goals of every chemoprevention trial is to establish the safety of the putative chemopreventive agents under investigation. The target population for a chemopreventive agent is commonly at high risk for a specific cancer, but otherwise healthy; therefore, any intervention to reduce cancer incidence must have negligible adverse effects.

Results from studies in both animals (1) and humans (2–4) suggest that retinoids may be beneficial in preventing cancer. Retinoids other than retinol have been most commonly used in human chemoprevention trials (2, 3, 5, 6). A disadvantage of one of the most commonly tested synthetic retinoids, isotretinoin, is that it causes adverse effects such as increases in triacylglycerol concentrations and skeletal changes even at relatively low doses (5, 7).

The adverse effects of high doses of retinol are well documented (8). However, there is limited information available on the effects of low doses of retinol in humans [7576 retinol equivalents (RE)/d, or 25 000 IU/d]. Goodman et al (9) reported observing no adverse effects of retinol (7576 RE/d) when given for a median of 1.5 y in a double-blind, placebo-controlled trial. In a large lung cancer chemoprevention trial, Omenn et al (10) observed an increase in lung cancer incidence and in the risk of death from lung cancer and cardiovascular disease when a combination of 7576 RE retinyl palmitate/d and 30 mg β-carotene/d was given for ≤10 y. In the pilot cohorts of the Carotene and Retinol Efficacy Trial (CARET), Omenn et al (11) reported a significant increase in triacylglycerol in those receiving retinol or retinyl palmitate alone or in combination with β-carotene, which the authors stated was not clinically significant. However, no significant differences were reported between the retinol and placebo groups in the other 13 monitored symptoms, in liver function test results, or in 33 newly diagnosed conditions (10). Vitamin A supplementation of up to 14 242 RE/d (47 000 IU/d) for 5 y was reported to have no toxic effects in 116 healthy elderly persons (12). In contrast, several case reports of liver damage after ingestion of 7576 RE vitamin A/d for 2, 7, or 10 y were described by Herbert (13). In a study of 41 consecutive patients with vitamin A hepatotoxicity, the mean (±SEM) daily dose of vitamin A assessed in 29 of these patients was 29 032 ± 5215 RE (95 806 ± 17 209 IU) and the mean duration of use was 7.17 ± 1.21 y (14); however, the minimum continuous dose reported was 7576 RE/d over 6 y.

Alcohol intake has been shown to both deplete vitamin A in the liver and exacerbate the hepatotoxic effect of vitamin A (15). In animal experiments, relatively low doses of vitamin A (5 times normal intakes) alone resulted in no detectable adverse affects; however, liver damage occurred when vitamin A was combined with alcohol (15).

It is not clear at what level of exposure vitamin A may be ter-

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atogenic because few epidemiologic data are available (16). However, in a large study, Rothman et al (17) reported the ratio of the prevalence of defects associated with cranial-neural-crest tissue to be 4.8 for mothers who had consumed ≥3030 RE vitamin A/d (10000 IU/d) compared with those who had consumed ≤1515 RE vitamin A/d (5000 IU/d). They further suggested a threshold risk near an intake of 3030 RE vitamin A/d.

In the present study, the long-term effects of retinol (7576 RE/d) on clinical symptoms as well as on hematologic and biochemical indexes were evaluated in a double-blind, placebo-controlled chemoprevention trial in a population at moderate risk for skin cancer.

SUBJECTS AND METHODS

Subjects

Two thousand, two hundred ninety-seven men and women residing in Arizona who had ≥10 clinically diagnosed actinic keratoses were recruited for the study and were randomly assigned to receive either retinol or a placebo. Subjects had a median age of 63 y and 70% were male; 81% reported having no prior skin cancers. Demographic and baseline characteristics did not differ significantly between the 2 groups. Forty-four percent of the participants were classified as having skin that always or usually burns on exposure to the sun and 59% reported having >10 h of sun exposure per week. The study, conducted at the Arizona Cancer Center, was approved by the University of Arizona Human Investigations Committee and informed, written consent was obtained from all participants.

Study design

Of the 2297 participants, 1157 (50.4%) were randomly assigned to receive retinol (7576 RE/d) and 1140 (49.6%) to receive a placebo that contained vegetable oil. Further details of the trial design are described elsewhere (18). Briefly, the study results showed that daily supplementation with 7576 RE/d was effective in preventing squamous cell carcinoma of the skin, but did not prevent basal cell carcinoma of the skin (19). Monitored indexes were selected based on reports in the literature regarding the putative adverse effects of retinol or other retinoids. Participants were followed for a median of 3.8 y. Data from blood analyses or symptom assessments conducted after a subject discontinued taking the capsules were excluded from the analyses.

Assessment of clinical symptoms

Symptoms were assessed routinely in person by a trained interviewer 1 mo after randomization and every 6 mo thereafter while subjects were active in the study (ie, taking the study capsules). In addition, symptoms were assessed when a participant called to report a symptom or reported a symptom on the self-administered questionnaire, which was completed by participants between regular visits and returned by mail. Symptoms reported at the routine visits were not dependent on the participant's initiation of the symptom assessment and so are considered the most consistent assessment by the investigators. Hence, only symptoms reported at the routine visits are reported here.

At each visit, participants were specifically asked if they had experienced dry skin, cheilitis, headaches, fatigue, or any other health problems since their last visit. In addition to the 4 symptoms listed above, 10 other symptoms were classified as possibly related to retinol ingestion: alopecia, conjunctivitis, dysuria, epistaxis, exanthema, menstrual changes, musculoskeletal stiffness and pain, nausea or vomiting, peeling palms or soles, and skin infections.

On the basis of the subjects' self-reports, the interviewer categorized the severity of each reported symptom with a score of 1–4, 1 being the least severe. If subjects reported a symptom with a score that was classified by the interviewer as ≥2, subjects were instructed to decrease their dose to one capsule every other day for ≥1 mo. If the symptom had disappeared or improved after this time period, or if the subjects attributed the symptom to something other than the capsules, the subjects were instructed to resume the full dose. If the symptom persisted or worsened over a 3-mo period at the reduced dose, the subjects were asked to stop taking the capsules; however, they remained in the study for the collection of end point data.

Assessment of laboratory effects

Blood indexes were assessed at enrollment (3 mo before randomization), 1 mo after randomization, and annually thereafter while subjects were active in the study. At enrollment, participants were instructed to fast for 12 h before blood was drawn; for subsequent blood collections, fasting was optional. Participants who had abnormal baseline blood concentrations of the following indexes at enrollment were ineligible for the study: serum aspartate aminotransferase >0.83 μkat/L, serum alanine aminotransferase >0.92 μkat/L, cholesterol >7.11 mmol/L, platelet count $<100 \times 10^9$/L, white blood cell count $<4 \times 10^9$/L, and hemoglobin <140 g/L (men) or <120 g/L (women). Assessment of these indexes was mandatory during active participation in the trial; however, because the additional laboratory costs were minimal, most participants had a full blood profile conducted at each laboratory assessment.

Doses were modified, following a set protocol, if any of the criteria shown in **Table 1** were met. These criteria are conservative and, in normal medical practice, such criteria being met would not necessarily require action to be taken. Initially, if outside the acceptable range, the blood index was remeasured. If the index being measured was still outside the acceptable range, the capsule dose was reduced by half for 3 mo. If the blood index remained outside the acceptable range for this 3-mo period, capsule administration was discontinued. If the blood value returned to within the acceptable range, capsule administration resumed and the index was remeasured 1 mo after reinitiating the intervention. An exception to this rule was made in 1987; participants who had hemoglobin concentrations lower than the acceptable range maintained the half dose (1 capsule taken every other day) provided their hemoglobin concentrations were stable and did not continue to fall. Ninety-three percent of participants were ≥75% compliant 1 mo postrandomization; this value dropped to 86% at 55 mo postrandomization. Compliance with supplementation did not differ significantly between the 2 groups.

Most of the blood analyses were conducted by a commercial laboratory; however, if a participant was unable to attend one of the study sites or have blood drawn at a facility run by the commercial laboratory, they were encouraged to have the analyses conducted at a physician's office or laboratory of their choice. Although monitoring of alkaline phosphatase and triacylglycerols was not part of the study protocol, these indexes were included in the following analysis because they have been reported to change in response to retinol intake. Measurements

TABLE 1
Symptoms and blood indexes: criteria for dose modification

Symptom or blood index	Criteria for dose modification
Aspartate aminotransferase	> 0.83 μkat/L
Alanine aminotransferase	> 0.92 μkat/L
Cholesterol	> 7.76 mmol/L
Platelet count	$< 100 \times 10^9$/L
White blood cell count	$< 4 \times 10^9$/L
Hemoglobin	
Men	< 140 g/L
Women	< 120 g/L
Alopecia	25–50% hair loss; clinically apparent
Cheilitis	Poorly controlled with emollients
Conjunctivitis	Poorly controlled with artificial tears
Dry skin	Partially controlled with emollients
Dysuria	≥4 episodes/wk
Epistaxis	≥3 episodes/wk or blood loss > 10 mL
Exanthema	Severe and lasting > 1 d
Fatigue, chronic	Difficulty performing normal functions
Headache	3–4 episodes/wk above preactive intervention frequency; analgesics control symptom effectively
Menstrual changes	Amenorrhea
Musculoskeletal stiffness or pain	Analgesics control symptom
Nausea and vomiting	Moderate or > 1 emesis/d for ≥3 d
Peeling palms or soles	Poorly controlled with emollients
Skin infections	≥2/mo

of HDL and LDL cholesterol were completed in 89% of subjects.

Data analyses

In the analysis of trends for laboratory indexes, only data for participants who had completed laboratory tests for each assessment through month 49 are included in the analysis. A natural log transformation was conducted to minimize the right skewing of the laboratory data. Student's *t* test, repeated-measures analysis of variance, and the chi-square test were used to determine possible differences in hematologic and biochemical indexes between intervention groups. The chi-square test was used to determine differences in clinical symptoms between the 2 groups. Dunnett's test was used to compare biochemical and hematologic indexes at each time point with baseline values within each treatment group. Bonferroni correction was applied when making multiple comparisons between treatment groups for each biochemical and hematologic index. SAS/STAT (version 6; SAS Institute Inc, Cary, NC) was used for the statistical analyses.

RESULTS

Seventy percent of the study population completed 49 mo of assessments.

Clinical symptoms

The clinical symptoms reported by participants during the trial are shown by group in **Table 2**. More than one symptom may have been reported by each patient. The most commonly reported symptoms were headaches, dry skin, and fatigue. There were no significant differences between groups in the frequency of symptoms categorized as ≥2.

Hematologic and biochemical assessments

TABLE 2
Clinical symptoms with a score ≥2 by group and time postrandomization[1]

Symptom and group	Time postrandomization					
	1 mo	13 mo	25 mo	37 mo	49 mo	61 mo
Alopecia						
Retinol	1	3	0	1	2	0
Placebo	2	0	0	0	1	0
Conjunctivitis						
Retinol	1	0	1	0	0	0
Placebo	0	0	0	1	1	0
Epistaxis						
Retinol	1	1	0	0	0	0
Placebo	0	0	0	0	1	0
Cheilitis						
Retinol	2	1	4	0	1	2
Placebo	1	2	3	2	2	0
Dry skin						
Retinol	5	6	4	4	0	2
Placebo	3	2	1	0	2	1
Exanthema						
Retinol	3	1	1	2	2	1
Placebo	3	2	1	0	2	1
Peeling palms or soles						
Retinol	0	0	0	3	0	0
Placebo	0	0	0	0	0	0
Skin infection						
Retinol	0	0	0	0	0	0
Placebo	0	0	0	0	0	0
Headaches						
Retinol	7	5	1	2	3	1
Placebo	12	9	3	5	2	0
Fatigue						
Retinol	3	5	1	2	0	0
Placebo	5	2	3	1	1	0
Stiffness						
Retinol	1	1	1	0	0	0
Placebo	3	1	1	0	0	0
Dysuria						
Retinol	1	0	0	1	0	0
Placebo	0	0	0	0	0	0
Menstrual changes						
Retinol	0	0	0	0	0	0
Placebo	0	0	0	0	0	0
Nausea or vomiting						
Retinol	0	0	1	1	0	0
Placebo	0	0	0	0	0	0
Number of subjects						
Retinol	1124	1012	924	843	667	378
Placebo	1140	1008	945	852	677	375

[1] There were no significant differences between groups at any time point (chi-square test).

The distribution of abnormal hematologic and biochemical indexes over the duration of the trial, by group, is shown in **Table 3**; some subjects had more than one abnormal blood index during the trial. There were no significant differences between groups in the frequency of abnormal blood indexes. Geometric means for the hematologic and biochemical indexes assessed at each visit, by group, are shown in **Table 4**. Significant differences between groups were observed over time for alkaline phosphatase, triacylglycerols, cholesterol, and HDL.

A significantly greater increase in alkaline phosphatase was

TABLE 3
Number of abnormal hematologic results by group and postrandomization[1]

Index and group	Time postrandomization					
	1 mo	13 mo	25 mo	37 mo	49 mo	61 mo
Cholesterol						
Retinol	19 (1.7)	14 (1.4)	11 (1.2)	12 (1.4)	6 (0.9)	3 (0.8)
Placebo	31 (2.7)	22 (2.2)	17 (1.8)	14 (1.6)	11 (1.6)	2 (0.5)
Liver enzymes						
Retinol	28 (2.5)	19 (1.9)	31 (3.4)	34 (4.0)	14 (2.1)	8 (2.1)
Placebo	24 (2.1)	34 (3.4)	34 (3.6)	45 (5.3)	32 (4.7)	17 (4.5)
White blood cell count						
Retinol	19 (1.7)	29 (2.8)	16 (1.7)	10 (1.2)	13 (1.9)	5 (1.3)
Placebo	34 (3.0)	15 (1.5)	22 (2.3)	23 (2.7)	18 (2.7)	10 (2.7)
Hemoglobin						
Retinol	37 (3.3)	48 (4.7)	47 (5.1)	67 (7.9)	43 (6.4)	25 (6.6)
Placebo	31 (2.7)	35 (3.5)	60 (6.3)	78 (9.2)	59 (8.7)	35 (9.3)
Platelet count						
Retinol	2 (0.2)	1 (0.1)	2 (0.2)	1 (0.1)	0	1 (0.3)
Placebo	2 (0.2)	0	0	0	2 (0.3)	3 (0.8)
Number of subjects						
Retinol	1124	1012	924	843	667	378
Placebo	1140	1008	945	852	677	375

[1] Percentages in parentheses. There were no significant differences between groups at any time point (chi-square test).

observed over time in the retinol group than in the placebo group. The difference in the geometric mean for alkaline phosphatase was small (retinol group: 1.16 μkat/L; placebo group: 1.11 μkat/L) at 13 mo but was greater by 49 mo (retinol group: 1.42 μkat/L; placebo group: 1.32 μkat/L).

Over time, triacylglycerol concentrations were significantly higher in the retinol group than in the placebo group, beginning 1 mo after randomization and continuing for the remainder of the trial. For both groups, the greatest increase in triacylglycerols between time points was the increase between baseline and 1 mo (retinol group: 1.42–1.69 mmol/L; placebo group: 1.38–1.52 mmol/L), most likely because participants were required to fast before the initial blood sample was taken but were not required to fast before subsequent blood sample collections.

Cholesterol concentrations decreased during the trial in the placebo group, but remained relatively stable in the retinol group, resulting in a significant difference in cholesterol concentrations over time between the 2 groups. HDL decreased over time in both groups, but the decline was significantly greater in the retinol group. LDL did not differ significantly between the 2 groups. LDL:HDL differed significantly between the retinol and placebo groups over time (time × treatment interaction: $P < 0.001$), increasing from a mean (±SD) of 3.0 ± 1.08 to 3.3 ± 1.18 in the retinol group and from 3.1 ± 1.17 to 3.2 ± 1.14 in the placebo group. There were no significant time × treatment interactions between the retinol and placebo groups in the other blood indexes measured; however, there were significant changes over time in all blood indexes.

DISCUSSION

In this long-term intervention trial, retinol supplementation was not associated with any of 14 monitored clinical symptoms. This finding agrees with that of Goodman et al (9), who reported no adverse effects for 10 routinely assessed clinical symptoms after oral intake of 7576 RE vitamin A/d for a median of 1.5 y. Even though no significant differences in reported symptoms were observed between the retinol and placebo groups, some symptoms were frequently reported by participants in both groups. The most commonly reported symptoms were headaches, dry skin, and fatigue, most likely because these were 3 of the 4 symptoms that the participants were asked about specifically by the interviewer. Goodman et al (9) reported "anxiety and depression" to be the most common symptom observed in their study; this symptom was not assessed in the present study. However, in Goodman et al's (9) study, skin redness and dryness was the second most common symptom reported, followed by fatigue, bone pain, and headaches, which is similar to the results reported here. The frequent reporting of these symptoms in the placebo group reinforces the need for double-blind, placebo-controlled trials when assessing any intervention for potentially adverse effects that are common in the population.

Pastorino et al (20) noted that at any one time, 50–60% of participants taking 90 909 RE (300 000 IU) retinyl palmitate/d reported desquamation and mucosal dryness. However, he did not report the frequency of these symptoms in the placebo group; hence, it is impossible to assess whether a considerably higher dose of retinol than used in our study might cause mucocutaneous adverse affects or whether these effects were simply more common in the population participating in this study.

No significant associations were observed between the number of abnormalities in hematologic or biochemical indexes and retinol intake. Such overt changes are those often monitored by the safety monitoring committee of a trial. An unfavorable association of overt changes in blood indexes can bring about the early closure of a trial. A somewhat higher number of abnormalities in blood indexes was observed in the present study than in other trials. For example, an average of 3% of participants in the present study experienced an elevation in either alanine or aspartate aminotransferase at at least one time point during the study compared with 1.8% of participants in the placebo group of Tangrea et al (5) and 0.84% (in aspartate aminotransferase) of the placebo group of Goodman et al (9).

Compliance with the regimen for capsule ingestion did not

TABLE 4
Geometric mean blood indexes by group and time postrandomization[1]

Index and group	Baseline	Time postrandomization					Main effects and interactions		
		1 mo	13 mo	25 mo	37 mo	49 mo	Retinol (treatment) effect	Time effect	Treatment × time interaction
Alkaline phosphatase (μkat/L)							0.006	0.0001	0.0001
Retinol (n = 581)									
Mean	1.12	1.13	1.16	1.23[2,3]	1.33[3,4]	1.42[3,5]	—	—	—
UCI	1.14	1.15	1.19	1.26	1.36	1.45	—	—	—
LCI	1.09	1.10	1.14	1.20	1.30	1.38	—	—	—
Placebo (n = 562)									
Mean	1.11	1.11	1.11	1.16	1.24[3]	1.32[3]	—	—	—
UCI	1.14	1.13	1.14	1.19	1.27	1.35	—	—	—
LCI	1.08	1.08	1.09	1.13	1.20	1.28	—	—	—
Triacylglycerol (mmol/L)							0.0002	0.0001	0.0001
Retinol (n = 580)									
Mean	1.42	1.69[3,4]	1.80[3,4]	1.81[3,5]	1.79[3,5]	1.77[3,4]	—	—	—
UCI	1.48	1.78	1.89	1.88	1.87	1.85	—	—	—
LCI	1.35	1.61	1.72	1.73	1.72	1.69	—	—	—
Placebo (n = 567)									
Mean	1.38	1.52[6]	1.60[3]	1.57[3]	1.59[3]	1.59[3]	—	—	—
UCI	1.32	1.45	1.53	1.50	1.53	1.52	—	—	—
LCI	1.45	1.59	1.67	1.64	1.66	1.66	—	—	—
Platelet count (× 10^9/L)							0.119	0.0001	0.13
Retinol (n = 562)									
Mean	223	226	228	231	229	225	—	—	—
UCI	227	230	233	235	234	230	—	—	—
LCI	218	222	224	226	225	221	—	—	—
Placebo (n = 548)									
Mean	229	225	234	236	234	230	—	—	—
UCI	234	230	238	240	239	234	—	—	—
LCI	225	221	229	231	230	225	—	—	—
Aspartate aminotransferose (μkat/L)							0.07	0.0018	0.13
Retinol (n = 593)									
Mean	0.36	0.37	0.37	0.36	0.35	0.36	—	—	—
UCI	0.37	0.37	0.37	0.37	0.37	0.37	—	—	—
LCI	0.36	0.36	0.36	0.35	0.34	0.35	—	—	—
Placebo (n = 572)									
Mean	0.36	0.36	0.36	0.35	0.35	0.34[6]	—	—	—
UCI	0.37	0.37	0.36	0.36	0.36	0.35	—	—	—
LCI	0.35	0.35	0.35	0.34	0.34	0.33	—	—	—
Alanine aminotransferase (μkat/L)							0.01	0.0001	0.15
Retinol (n = 592)									
Mean	0.37	0.36	0.36	0.38	0.37	0.34[6]	—	—	—
UCI	0.38	0.37	0.38	0.40	0.38	0.36	—	—	—
LCI	0.36	0.34	0.35	0.37	0.35	0.33	—	—	—
Placebo (n = 570)									
Mean	0.35	0.35	0.35	0.36	0.35	0.32[7]	—	—	—
UCI	0.36	0.37	0.36	0.37	0.37	0.33	—	—	—
LCI	0.33	0.34	0.34	0.35	0.34	0.30	—	—	—
Hemoglobin (g/L)							0.08	0.0001	0.38
Retinol (n = 572)									
Mean	155	153[6]	152[7]	150[3]	149[3]	148[3]	—	—	—
UCI	156	154	153	151	150	149	—	—	—
LCI	154	152	151	149	148	147	—	—	—
Placebo (n = 569)									
Mean	154	152	151[7]	149[3]	147[3]	147[3]	—	—	—
UCI	155	153	152	150	148	148	—	—	—
LCI	153	151	150	148	146	145	—	—	—
White blood cell count (× 10^6/L)							0.79	0.0001	0.61
Retinol (n = 574)									
Mean	6.18	6.04	5.99	6.03	5.98	5.96	—	—	—
UCI	6.31	6.17	6.11	6.15	6.11	6.10	—	—	—
LCI	6.05	5.91	5.87	5.90	5.85	5.83	—	—	—
Placebo (n = 569)									
Mean	6.16	6.11	6.03	5.99	6.03	5.98	—	—	—
UCI	6.29	6.23	6.14	6.11	6.15	6.10	—	—	—
LCI	6.04	5.99	5.92	5.87	5.91	5.86	—	—	—

(Continued)

TABLE 4 *(Continued)*

Index and group	Baseline	Time postrandomization					Main effects and interactions		
		1 mo	13 mo	25 mo	37 mo	49 mo	Retinol (treatment) effect	Time effect	Treatment × time interaction
Cholesterol (mmol/L)							0.02	0.0001	0.04
Retinol (n = 599)									
Mean	5.64	5.64	5.67	5.67[2]	5.64	5.57[2]	—	—	—
UCI	5.72	5.72	5.75	5.76	5.72	5.66	—	—	—
LCI	5.55	5.55	5.58	5.59	5.55	5.49	—	—	—
Placebo (n = 577)									
Mean	5.59	5.52	5.56	5.50	5.49	5.42[6]	—	—	—
UCI	5.68	5.61	5.65	5.58	5.57	5.50	—	—	—
LCI	5.50	5.44	5.48	5.42	5.41	5.34	—	—	—
HDL cholesterol (mmol/L)							0.37	0.0001	0.01
Retinol (n = 466)									
Mean	1.26	1.21	1.17[7]	1.14[3]	1.14[3]	1.16[3]	—	—	—
UCI	1.29	1.24	1.21	1.17	1.17	1.19	—	—	—
LCI	1.22	1.18	1.14	1.11	1.11	1.13	—	—	—
Placebo (n = 500)									
Mean	1.24	1.22	1.21	1.16[7]	1.17[7]	1.17[7]	—	—	—
UCI	1.28	1.26	1.24	1.19	1.20	1.20	—	—	—
LCI	1.21	1.19	1.18	1.14	1.14	1.14	—	—	—
LDL cholesterol (mmol/L)							0.21	0.0001	0.12
Retinol (n = 466)									
Mean	3.55	3.47	3.52	3.57	3.58	3.61	—	—	—
UCI	3.63	3.56	3.60	3.66	3.66	3.70	—	—	—
LCI	3.47	3.39	3.43	3.48	3.49	3.53	—	—	—
Placebo (n = 500)									
Mean	3.55	3.44	3.44	3.48	3.50	3.49	—	—	—
UCI	3.63	3.52	3.53	3.56	3.58	3.57	—	—	—
LCI	3.47	3.36	3.36	3.40	3.42	3.42	—	—	—

[1] LCI, 95% lower confidence interval; UCI, 95% upper confidence interval.
[2,4,5] Significantly different from placebo (Student's t test with Bonferroni correction): [2] $P < 0.05$, [4] $P < 0.01$, [5] $P < 0.001$.
[3,6,7] Significantly different from baseline (Dunnett's test): [3] $P < 0.001$, [6] $P < 0.05$, [7] $P < 0.01$.

differ significantly between the 2 groups, indicating that dose modification initiated by study personnel or the participants did not affect the likelihood of observing significant differences in adverse effects of treatment between the 2 groups.

Significant changes over time were observed in all blood indexes monitored. These changes may have been due to the increasing age of the study population or to regression to the mean, a phenomenon that is observed when multiple laboratory measurements are made over time and when entry to a trial is restricted to participants who fulfill certain laboratory criteria (21). However, some of the changes in mean values over time may have been due to changes in the laboratory methods used during the study. Even though the same commercial laboratories conducted the blood chemistry analyses throughout the trial, different equipment and reagents were used that affected the analysis of alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase. The laboratory provided conversion factors to use for these 3 indexes to adjust for major changes; however, minor changes may have occurred for which the laboratory felt the calculation of a conversion factor was not justified. However, any changes in laboratory practices should not have affected comparisons between the 2 groups.

No significant differences were observed in alanine aminotransferase and aspartate aminotransferase between the 2 groups, suggesting that continuous ingestion of 7576 RE vitamin A/d for 49 mo did not cause liver damage. The smallest continuous dose of vitamin A leading to cirrhosis reported by Geubel et al (14) was 7576 RE/d for 6 y.

The difference in alkaline phosphatase concentrations between groups continued to increase with time. A similar difference in alkaline phosphatase between the 2 groups was reported by Omenn et al (22) during the pilot phase of CARET. After intervention with β-carotene (15 mg/d) and retinyl palmitate (7576 RE/d) for 24 mo in a group of asbestos-exposed workers, the alkaline phosphatase concentration increased from 82 ± 21 to 94 ± 26 IU/L. However, Goodman et al (9) reported no significant difference in alkaline phosphatase between groups in a 2 × 2 factorial study in which vitamin A (7576 RE/d) and β-carotene were given; the median follow-up time was 1.5 y. Pastorino et al (20) reported a nonsignificant increase in alkaline phosphatase in participants treated with 90 909 RE (300 000 IU) retinyl palmitate/d for 24 mo. He reported that mean concentrations rose from 2.58 to 3.53 μkat/L (155 to 212 IU/dL) in the intervention group, whereas no change was seen in the placebo group. Elevations in alkaline phosphatase have been observed in animals given retinoids (23). It has been suggested that the elevation is due to the production of an alkaline phosphatase isoenzyme in the bone; however, there is no direct evidence of this. Low doses of isotretinoin have been shown to cause bone changes (7); however, we are unaware of similar effects of retinol.

In other chemoprevention studies that tested the effects of retinoids, triacylglycerols are the one biochemical index that has consistently been elevated (3–5, 11). Our results are consistent with these findings; however, the role of plasma triacylglycerols as an independent risk factor for cardiovascular disease is still unclear.

The results of a meta-analysis of 17 prospective studies suggest that plasma triacylglycerol is a risk factor for cardiovascular disease in both men and women (24). The statistically significant adjusted relative risks for a 1-mmol/L increase in triacylglycerol was reported to be 1.14 for men and 1.37 for women. The maximum difference between the 2 groups in this study was 0.23 mmol/L.

A significant difference in cholesterol concentrations between the retinol and placebo groups was observed in the present study. Cholesterol concentrations in the retinol group were relatively stable throughout the study, in contrast with a steady decline in concentrations in the placebo group, resulting in a 2–3% difference in cholesterol concentrations between the retinol and placebo groups. The decline in the placebo group may have been due in part to a temporal effect. Johnson et al (25) reported a continuing decline in cholesterol concentrations in both males and females of all age groups between 1976 and 1980 and 1988 and 1991. In contrast with the findings of our study, Pastorino et al (20) observed an increase in cholesterol concentrations over the 24-mo duration of their trial. The authors suggested that this increase may have been due to a change in diet after lung resection and after the subjects stopped smoking. Pastorino et al (20) reported that no significant differences were observed between the intervention groups. Omenn et al (11) noted that no significant differences in cholesterol were observed between treatment groups in the pilot group of CARET. Synthetic retinoids have been reported to cause an increase in cholesterol with a concomitant decrease in HDL (26–28).

We have no explanation for the differences in cholesterol concentrations seen between the retinol and placebo groups other than retinol intervention. Even though these differences were small (2–3%), if replicated, they could prove important because it has been suggested by cholesterol-lowering clinical trials that a 1% reduction in serum cholesterol can reduce coronary artery disease risk by 2% (29). The significantly greater decline in HDL over time in the group receiving retinol, which in part resulted in the significant difference between groups over time in the ratio of LDL to HDL, would also confer an increased risk to those receiving retinol. This finding should be investigated further, especially in light of the increased deaths from cardiovascular disease seen in the intervention arm of CARET (10). If replicated, this finding would warrant an investigation into the threshold level of such an effect so guidance could be given to the public regarding vitamin A supplementation.

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