Serum Retinol Levels Throughout 2 Years of Cholesterol-Lowering Therapy

M. Muggeo, M.G. Zenti, D. Travia, A. Sartori, S. Trimeloni, L. Grigolini, M.S. Graziani, and M. Cigolini

Some studies have reported an inverse correlation between serum cholesterol level and risk of cancer. This correlation might be due to a decrease in serum retinol, a lipid-soluble vitamin that controls cell proliferation and differentiation. We evaluated the influence of cholesterol-lowering therapy on serum retinol in 102 subjects (mean ± SE: aged 47.1 ± 4.1 years; body mass index, 23.8 ± 0.6 kg/m^2) with primary hypercholesterolemia treated for 2 years with different therapeutic protocols. Twenty-two subjects had been treated with diet alone, 35 with diet and fibrates, 37 with diet and hepatic hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase inhibitors (statins), and eight with diet and cholestyramine. Postabsorptive serum retinol, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride levels were determined at baseline and every 3 months. Baseline TC and LDL-C were significantly lower in the diet-treated group than in other groups. No intergroup differences were found in pretreatment levels of triglycerides and serum retinol. After 2 years of treatment, TC and LDL-C serum levels were not significantly decreased in the diet-alone group, whereas they were decreased by 20% and 24%, respectively, in the gemfibrozil group, 28% and 34% in the statins group; and 21% and 27% in the cholestyramine group. In the entire population (N = 102), serum retinol was 3.46 ± 0.08 μmol/L before therapy and 3.76 ± 0.07 after 2 years of therapy ($P < .001$). Serum retinol increased in diet- and statin-treated groups, but not in fibrate- and resin-treated groups. Serum retinol-binding protein ([RBP] n = 37 subjects) was unchanged after the 2-year cholesterol-lowering therapy (50.6 ± 3.2 mg/L before and 50.9 ± 2.8 after, P = NS). In the pooled data, changes in serum cholesterol and retinol were unrelated to each other ($r = .053$, $P = .60$). Our data suggest that (1) long-term hypocholesterolemic therapy does not decrease serum retinol levels; (2) prolonged dietary treatment of hypercholesterolemia increases serum retinol levels; and (3) this diet-dependent effect is counteracted by combined fibrate or resin, but not statin, treatment.

RETINOIDS MAY EXERT an antineoplastic effect by inhibition of both the transformation and promotional stage of the neoplastic process.[1,2] Several epidemiologic studies have shown an inverse relationship between vitamin A intake (and serum retinol concentrations) and cancer.[3-8] Furthermore, some reports suggested an association between low serum cholesterol and increased mortality rates for noncardiovascular diseases, as well as an increased risk of cancer.[9-15] In a recent study, plasma vitamin A levels were higher in subjects with hypercholesterolemia and decreased in response to cholesterol-lowering interventions.[16] We hypothesized that cholesterol-lowering therapy may have the potentially adverse effect of decreasing vitamin A and that this in turn may lead to increased cancer risk, even though a vitamin A–deficient status was never demonstrated in hypocholesterolemic neoplastic patients.[7,17-19] To test the premise of this hypothesis, we studied 102 subjects who were being treated with diet either alone or in combination with the most common cholesterol-lowering drugs. We have found that long-term successful cholesterol-lowering therapy does not decrease serum retinol levels, independently of the type of treatment.

From the Cattedra di Malattie del Ricambio, Istituto di Chimica Clinica, and Istituto di Clinica Medica, Università di Verona; and Laboratorio Analisi Chimico Cliniche, Ospedale Maggiore, Verona, Italy.

Submitted March 9, 1994; accepted June 11, 1994.

Supported in part by the Consiglio Nazionale delle Ricerche (84.00693.44, 85.02265.44, 86.00498.44, 87.01405.44, and 88.00770.44) and the Ministero dell'Università e della Ricerca Scientifica e Tecnologica 1988-1992.

Address reprint requests to Professor M. Muggeo, Cattedra di Malattie del Ricambio, Università di Verona, Ospedale Civile Maggiore, I-37126 Verona, Italia.

0026-0495/95/4403-0019$03.00/0

SUBJECTS AND METHODS

One hundred two subjects (53 men, 49 women) diagnosed with primary hypercholesterolemia (n = 72 for phenotype IIa and n = 30 for phenotype IIb, according to Fredrickson's classification[20]) participated in the study. All patients were on an isocaloric standardized diet that consisted of 55% carbohydrate, 30% fat (10% polyunsaturated, 10% monounsaturated, and 10% saturated), and 15% protein. Daily cholesterol and alcohol intakes were less than 300 mg and less than 50 g, respectively. Dietary treatment increased fruit and vegetable intake in these patients in comparison to their previous diet. Patients were allocated to four different cholesterol-lowering treatment groups according to our clinical experience, in accordance with the guidelines of the National Cholesterol Education Program.[21] Patients with low-density lipoprotein-cholesterol (LDL-C) serum levels greater than 190 mg/dL (4.9 mmol/L) or greater than 160 mg/dL (4.1 mmol/L) in combination with definite coronary heart disease or two other risk factors were treated with fibrates, hepatic hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase inhibitors, or cholestyramine. Patients with LDL-C less than 160 mg/dL or between 160 and 190 mg/dL but without coronary heart disease, were treated with diet alone. Since cholesterol-lowering therapy was always associated with diet treatment, subjects on diet alone were used as controls to determine the net effect of each hypolipidemic drug on serum retinol levels. We deemed it unethical to treat a subgroup of patients that fulfilled clinical criteria for combined diet + drug treatment with diet alone. Thus, 22 subjects (16 IIa, six IIb) were treated with diet alone, 35 (24 IIa, 11 IIb) with diet and gemfibrozil (600 mg twice per day), 37 (26 IIa, 11 IIb) with diet and HMG CoA reductase inhibitors (pravastatin or simvastatin 10 to 40 mg/d), and eight (6 IIa, 2 IIb) with diet and cholestyramine (8 to 12 g/d). Treatment with statins was initiated with simvastatin 10 mg or pravastatin 20 mg; doses were increased up to 40 mg/d if LDL-C was still above the target level.[21] Gemfibrozil and cholestyramine doses were kept constant throughout the study.

The four groups of subjects were similar with respect to sex distribution, age, and body mass index (Table 1).

Medical visits were scheduled every 3 months from the beginning of therapy. On each visit, a complete medical history (which included specific questions on possible untoward effects of therapy)

Table 1. Baseline Characteristics of 102 Subjects With Primary Hypercholesterolemia (mean ± SEM) Allocated to Four Treatment Groups

	Diet Alone (n = 22)	Diet + Fibrates (n = 35)	Diet + Statins (n = 37)	Diet + Resins (n = 8)	*P*
Phenotype IIa/IIb	16/6	24/11	26/11	6/2	—
Sex (M/F)	11/11	19/16	19/18	4/4	—
Age (yr)	47.7 ± 3.2	55.0 ± 2.1	51.6 ± 1.9	40.6 ± 6.6	NS
BMI (kg/m²)	23.7 ± 0.8	24.7 ± 0.5	24.5 ± 0.6	23.0 ± 0.7	NS
TC (mmol/L)	6.2 ± 0.19	7.9 ± 0.28	8.9 ± 0.27	7.4 ± 0.36	<.0001
HDL-C (mmol/L)	1.27 ± 0.06	1.31 ± 0.04	1.34 ± 0.07	1.34 ± 0.29	NS
LDL-C (mmol/L)	4.3 ± 0.18	5.8 ± 0.27	6.7 ± 0.24	5.2 ± 0.30	<.0001
TG (mmol/L)	1.54 ± 0.19	1.69 ± 0.12	1.71 ± 0.17	1.75 ± 0.60	NS
Retinol (μmol/L)	3.63 ± 0.18	3.57 ± 0.12	3.29 ± 0.11	3.20 ± 0.66	NS

NOTE. *P* refers to ANOVA among treatment groups.
Abbreviations: BMI, body mass index; TG, triglycerides.

and physical examination were obtained, and patient compliance with the diet was assessed and dietary instructions were reinforced. At each visit, the following laboratory tests were performed by standard routine methods: complete blood count (hemoglobin, hematocrit, total and fractional WBC, and platelets), prothrombin time, fibrinogen, total protein, albumin, protein electrophoresis, urea nitrogen, creatinine, uric acid, conjugated and unconjugated bilirubin, alkaline phosphatase, γ-glutamyltransferase, aspartate aminotransferase, alanine aminotransferase, glucose, electrolytes (Ca, Na, and K), creatine phosphokinase, and standard urine analysis. At the beginning of the study and at 3-month intervals, blood was also withdrawn after a 12-hour fast for determination of serum lipid and retinol concentrations. Blood samples for retinol determination were taken with a light-protected system (ie, syringes and tubes were wrapped in aluminum foil). Serum was separated by low-speed centrifugation (100 × *g* for 10 minutes) after clotting. Total cholesterol (TC) and triglyceride levels were determined in whole serum by manual enzymatic methods.[22,23] High-density lipoprotein cholesterol (HDL-C) level was measured by an enzymatic assay after dextran-sulfate precipitation.[24] LDL-C levels were calculated according to the formula reported by Friedewald et al.[25] Serum aliquots for retinol analysis were protected from light and stored at −20°C until analyzed. Retinol level was measured by a high-performance liquid chromatography method.[26] In 37 subjects, serum retinol-binding protein (RBP) was also assayed by a nephelometric method[27] at the beginning of the study and after 1 and 2 years of treatment. RBP level was also measured in 18 normocholesterolemic healthy volunteers (nine men, nine women) who participated as controls for RBP values. At baseline, serum cholesterol and serum retinol values were also assessed in a sample of 70 normocholesterolemic subjects (35 men, 35 women) aged 40 ± 1.3 years who were studied as a control group for retinol values.

In six subjects, three normocholesterolemic and three hypercholesterolemic, cholesterol and retinol levels were measured at baseline and after cholesterol-lowering therapy in plasma lipoprotein fractions (very-low-density lipoprotein, LDL, and HDL) obtained by sequential preparative ultracentrifugation.[28]

All variables are presented as the mean ± SE. Differences in variables among the four treatment groups were evaluated by ANOVA. Differences in serum lipid and retinol values between the basal state and different therapy periods were analyzed by Student's *t* test for paired data. Pearson's *r* correlation coefficients between changes in serum cholesterol and those in serum retinol after 12 and 24 months of treatment were also calculated.

RESULTS

Table 1 lists baseline characteristics of subjects with primary hypercholesterolemia allocated to different therapeutic interventions. In the diet-alone group, TC and LDL-C serum values were significantly less than those in other groups. No differences were found among the four groups in pretreatment levels of triglycerides and retinol.

After 2 years, TC and LDL-C serum levels did not significantly change from baseline in the diet-alone group, whereas they decreased by 20% and 24% in the gemfibrozil group ($P < .01$), 28% and 34% in the statin group ($P < .01$), and 21% and 27% in the cholestyramine group ($P < .05$) (Fig 1). After a 2-year lipid-lowering treatment, triglyceride and HDL-C serum values were not statistically different from baseline in any group.

Tables 2 through 5 list lipid serum values (TC, LDL-C, HDL-C, and triglycerides) before therapy and at different intervals during the therapy period.

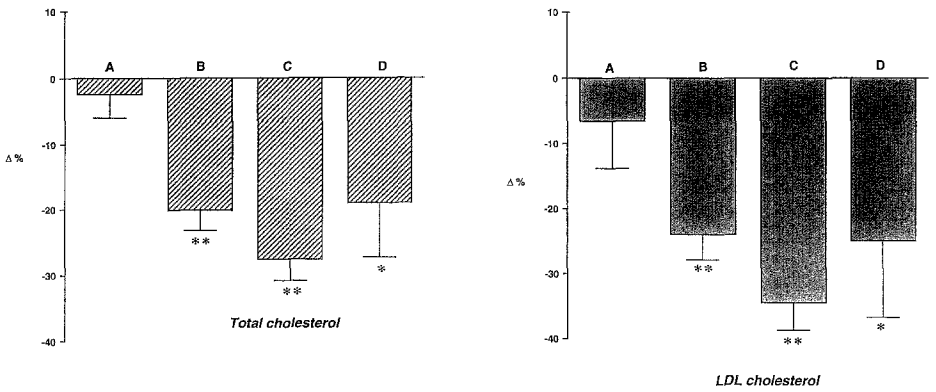

Fig 1. Percent change (Δ%) in serum lipids after 2 years of lipid-lowering treatment in the 4 intervention groups (A, diet alone; B, diet + fibrates; C, diet + HMG CoA reductase inhibitors; D, diet + anion-exchange resins). *$P < .05$, **$P < .01$: in comparison to respective initial values.

Table 2. TC Serum Values (mmol/L) at Beginning of Study and During Lipid-Lowering Treatment (mean ± SEM)

	Diet Alone (n = 22)	Diet + Fibrates (n = 35)	Diet + Statins (n = 37)	Diet + Resins (n = 8)
Basal	6.2 ± 0.19	7.9 ± 0.28	8.9 ± 0.27	7.4 ± 0.36
3 mo	6.0 ± 0.19	7.1 ± 0.18†	7.3 ± 0.28†	6.5 ± 0.57
6 mo	6.1 ± 0.20	6.8 ± 0.17†	6.9 ± 0.24†	5.7 ± 0.41*
9 mo	5.9 ± 0.30	6.9 ± 0.26†	6.9 ± 0.25†	6.1 ± 0.57
12 mo	6.1 ± 0.20	6.7 ± 0.19†	7.0 ± 0.22†	6.2 ± 0.45*
15 mo	6.0 ± 0.25	6.6 ± 0.23†	6.7 ± 0.26†	5.9 ± 0.37*
18 mo	5.9 ± 0.27	6.8 ± 0.33†	6.7 ± 0.28†	6.3 ± 0.64
21 mo	5.7 ± 0.15	6.4 ± 0.21†	6.4 ± 0.24†	5.5 ± 0.37*
24 mo	6.0 ± 0.18	6.3 ± 0.14†	6.4 ± 0.24†	5.8 ± 0.19*

*$P < .05$.
†$P < .01$ *v* basal.

At baseline, hypercholesterolemic subjects showed no difference in serum retinol values as compared with 70 normocholesterolemic subjects (total cholesterol, 7.8 ± 0.17 *v* 5.09 ± 0.07 mmol/L, $P < .0001$; retinol, 3.46 ± 0.08 *v* 3.32 ± 0.09 μmol/L, P = NS). After a 2-year treatment, serum retinol increased from 3.63 ± 0.18 to 3.79 ± 0.16 μmol/L in the diet-alone group (P = NS), from 3.57 ± 0.12 to 3.73 ± 0.10 in the gemfibrozil group (P = NS), from 3.29 ± 0.11 to 3.71 ± 0.13 in the statins group ($P < .05$), and from 3.20 ± 0.66 to 3.99 ± 0.73 in the cholestyramine group (P = NS). Figure 2 shows serum retinol and TC serum profiles during treatment. Subjects on diet alone showed a transient increase in serum retinol, which attained statistical significance after 9, 12, and 21 months of treatment. In the statin-treated group, the 24-month serum retinol profile resembled that of diet-treated subjects, but showed an even more pronounced increase over baseline. In contrast, both fibrate- and resin-treated patients showed no significant changes in serum retinol from baseline throughout the treatment.

Serum RBP, which was measured in 37 subjects (eight of diet-treated group, 13 of gemfibrozil-treated group, and 16 of HMG CoA reductase inhibitors–treated group), showed no change after either 1 or 2 years of therapy (50.6 ± 3.2 mg/L before and 50.9 ± 2.8 after 2-year treatment, P = NS; Fig 3). Serum RBP values in 37 hypercholesterolemic subjects were not different from those found in 18 normocholesterolemic subjects (TC, 7.08 ± 0.17 *v* 4.94 ± 0.15 mmol/L, $P < .0001$; RBP, 50.6 ± 3.2 *v* 45.7 ± 2.6 mg/L, P = NS).

Table 3. LDL-C Serum Values (mmol/L) at Beginning of Study and During Lipid-Lowering Treatment (mean ± SEM)

	Diet Alone (n = 22)	Diet + Fibrates (n = 35)	Diet + Statins (n = 37)	Diet + Resins (n = 8)
Basal	4.3 ± 0.18	5.8 ± 0.27	6.7 ± 0.24	5.2 ± 0.30
3 mo	4.3 ± 0.19	5.1 ± 0.18†	5.1 ± 0.26†	4.5 ± 0.54
6 mo	4.2 ± 0.19	4.9 ± 0.17†	5.0 ± 0.23†	3.7 ± 0.36*
9 mo	3.9 ± 0.21	4.8 ± 0.23†	4.9 ± 0.24†	4.0 ± 0.53
12 mo	4.2 ± 0.21	4.7 ± 0.19†	5.1 ± 0.24†	4.2 ± 0.39*
15 mo	4.2 ± 0.23	4.5 ± 0.24†	4.7 ± 0.25†	3.8 ± 0.52*
18 mo	4.1 ± 0.26	4.8 ± 0.33†	4.7 ± 0.26†	4.2 ± 0.64*
21 mo	4.0 ± 0.16	4.5 ± 0.23†	4.5 ± 0.22†	3.5 ± 0.58*
24 mo	4.0 ± 0.20	4.4 ± 0.16†	4.4 ± 0.21†	3.8 ± 0.41*

*$P < .05$.
†$P < .01$ *v* basal.

Table 4. HDL-C Serum Values (mmol/L) at Beginning of Study and During Lipid-Lowering Treatment (mean ± SEM)

	Diet Alone (n = 22)	Diet + Fibrates (n = 35)	Diet + Statins (n = 37)	Diet + Resins (n = 8)
Basal	1.27 ± 0.06	1.31 ± 0.04	1.34 ± 0.07	1.34 ± 0.29
3 mo	1.21 ± 0.06	1.31 ± 0.04	1.29 ± 0.05	1.47 ± 0.38
6 mo	1.29 ± 0.08	1.37 ± 0.05	1.34 ± 0.05	1.52 ± 0.41
9 mo	1.27 ± 0.06	1.37 ± 0.06*	1.45 ± 0.07*	1.34 ± 0.24
12 mo	1.31 ± 0.07	1.42 ± 0.05*	1.37 ± 0.07	1.37 ± 0.04
15 mo	1.29 ± 0.06	1.37 ± 0.06	1.37 ± 0.06	1.50 ± 0.32
18 mo	1.29 ± 0.06	1.39 ± 0.04*	1.34 ± 0.06	1.37 ± 0.28
21 mo	1.24 ± 0.06	1.34 ± 0.06	1.29 ± 0.07	1.24 ± 0.17
24 mo	1.18 ± 0.06	1.32 ± 0.05	1.29 ± 0.05	1.34 ± 0.26

*$P < .05$.

No significant correlation was found between percent changes in serum cholesterol and in serum retinol after either 1 or 2 years of treatment (Fig 4).

To verify whether cholesterol-lowering therapy could shift retinol among different lipoprotein fractions, we measured retinol concentrations in lipoprotein (very–low-density lipoprotein, LDL, and HDL) fractions in three normocholesterolemic and three hypercholesterolemic subjects (at baseline and after treatment). No differences in lipoprotein retinol values were found either between normocholesterolemic and hypercholesterolemic subjects or within hypercholesterolemic subjects before and after treatment (data not shown).

DISCUSSION

The main finding of this study is that prolonged (2-year) cholesterol-lowering therapy caused no reductions of serum retinol levels in patients with primary hypercholesterolemia. These results are different from those reported by Smith et al,[16] who found a concomitant plasma vitamin A decrease and serum cholesterol reduction in 16 hypercholesterolemic subjects on four different treatments (three subjects were treated only with diet, four with fenofibrate, two with cholestyramine, and seven with lovastatin). However, several differences between the two studies should be highlighted. First, our results were obtained on a much larger cohort of patients (102 *v* 16) who were checked on a

Table 5. Triglyceride Serum Values (mmol/L) at Beginning of Study and During Lipid-Lowering Treatment (mean ± SEM)

	Diet Alone (n = 22)	Diet + Fibrates (n = 35)	Diet + Statins (n = 37)	Diet + Resins (n = 8)
Basal	1.54 ± 0.19	1.69 ± 0.12	1.71 ± 0.17	1.75 ± 0.60
3 mo	1.31 ± 0.17	1.39 ± 0.23	1.52 ± 0.14	1.34 ± 0.27
6 mo	1.46 ± 0.16	1.22 ± 0.11†	1.37 ± 0.11†	1.18 ± 0.48
9 mo	1.37 ± 0.18	1.37 ± 0.14†	1.23 ± 0.09†	1.14 ± 0.26
12 mo	1.43 ± 0.21	1.23 ± 0.13†	1.30 ± 0.08†	1.15 ± 0.11
15 mo	1.17 ± 0.20	1.23 ± 0.12†	1.44 ± 0.13	0.96 ± 0.22*
18 mo	1.17 ± 0.15	1.32 ± 0.11†	1.48 ± 0.14	1.60 ± 0.41
21 mo	1.66 ± 0.32	1.37 ± 0.12†	1.47 ± 0.13	1.77 ± 0.55
24 mo	1.56 ± 0.22	1.42 ± 0.15	1.61 ± 0.16	1.45 ± 0.45

*$P < .05$.
†$P < .01$ *v* basal.

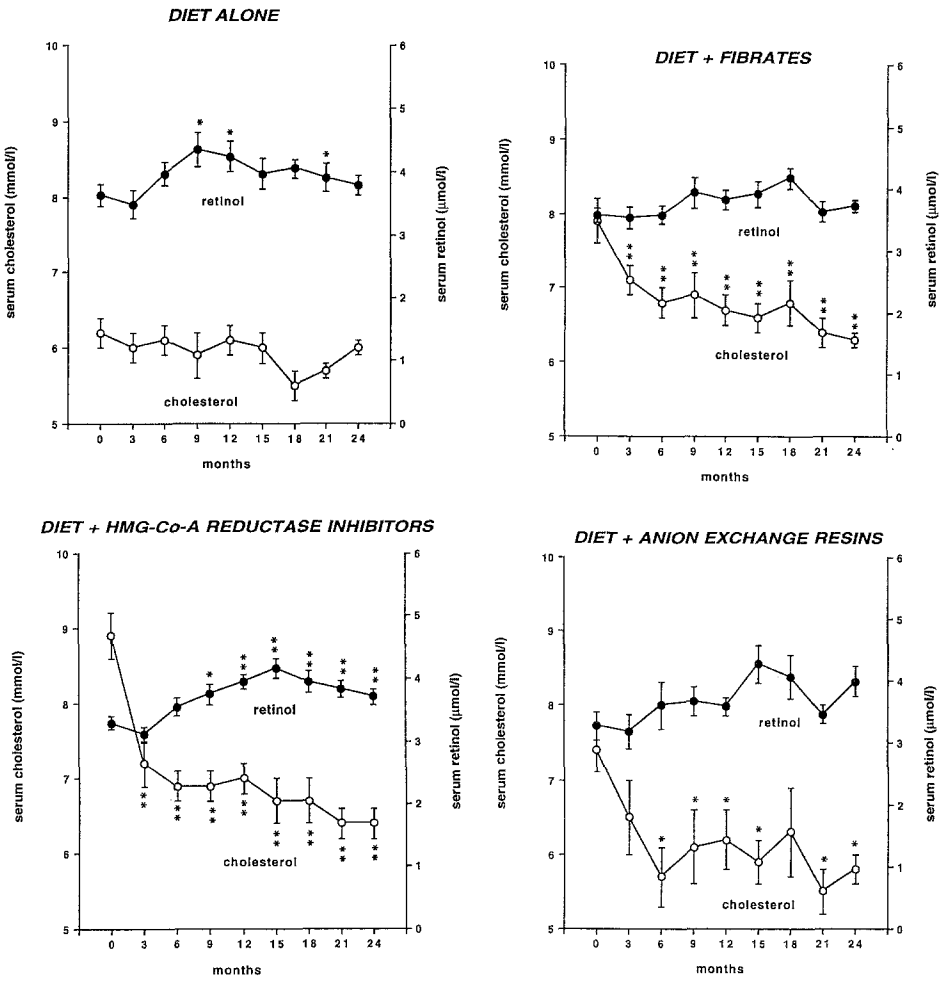

Fig 2. Serum retinol and TC levels during 2-year lipid-lowering treatment in the 4 intervention groups. *P < .05, †P < .01: *v* basal.

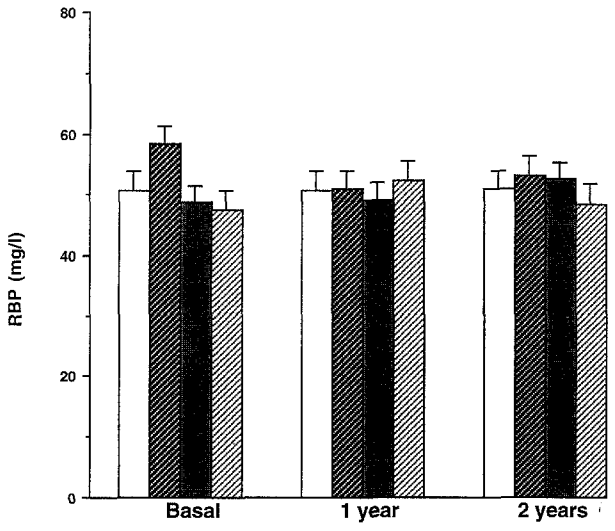

Fig 3. Serum RBP (mg/L) in 37 hypercholesterolemic subjects at baseline and after 1 and 2 years of lipid-lowering treatment. No significant intergroup or time-related differences were found. (□) All; (▨) A, diet alone (n = 8); (●) B, diet + fibrates (n = 13); (▨) C, diet + HMG CoA reductase inhibitors (n =16).

3-month basis throughout 2 years (duration of treatment was not specified in the report by Smith et al). Second, in our patients serum retinol levels were comparable to control values, whereas Smith et al detected higher-than-control retinol concentrations in their patients. Thus, two different subsets of patients might have been included in the two studies. Third, in our study retinol serum levels were determined by high-performance liquid chromatography, whereas Smith et al evaluated total vitamin A concentrations using a spectrofluorimetric method. Fourth, serum retinol levels of normocholesterolemic subjects in this study are somewhat higher than values found in North European normal subjects,[29] although our subjects were on a regular diet. This discrepancy may be partially explained by the fact that the average Italian diet is richer in fruit and vegetables. High intakes of fruit and/or vegetables might be associated with an increase in serum retinol levels, as suggested by our findings in patients treated with diet alone (see below).

In this study, not only did we not document any decrease in serum retinol levels during long-term successful cholesterol-reduction treatment, but we also observed somewhat increased retinol levels. Patients on diet alone actually showed an increase in serum retinol during the 2 years of study, despite no concomitant changes in serum cholesterol levels. This finding could be partially explained by a higher

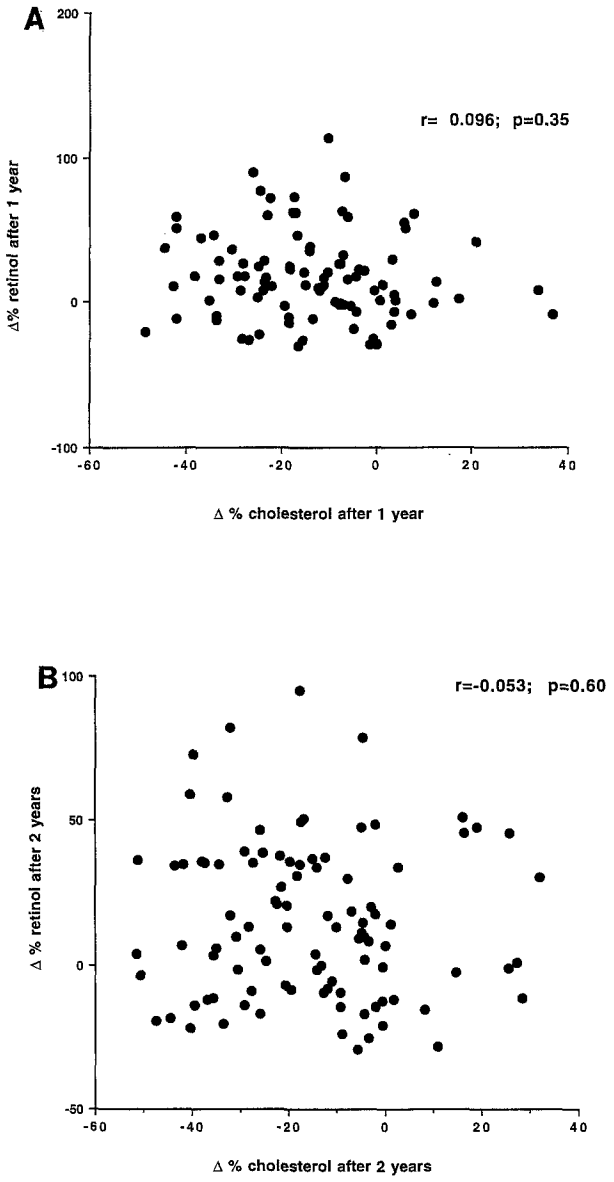

Fig 4. Scattergram of the % changes in cholesterol and in retinol serum values after 1 (A) and 2 (B) years of cholesterol-lowering therapy in hypercholesterolemic subjects.

vitamin A intake during the study, because the diet we recommended had a higher fruit and vegetable intake than the standard Italian diet.

Drug treatment significantly interfered with the diet-induced increase in serum retinol concentration. In fact, only in the statin-treated group was the increase in serum retinol levels maintained and possibly increased. In contrast, resin and fibrate regimens were associated with no changes in serum retinol levels throughout 2 years. Therefore, these data suggest that cholesterol-lowering drugs can differently affect serum retinol levels. One could speculate that despite similar cholesterol-lowering effects, statin treatment does not affect the diet-induced retinol increase, whereas fibrate and resin treatments blunt it. Triglyceride metabolism is affected by both fibrates (increased chylomicron clearance) and resins (reduction in chylomicron synthesis), but not by statins, and triglyceride and retinol metabolism are strictly interrelated.[30] Fibrates and resins might therefore affect retinol metabolism through effects on triglyceride metabolism. Our findings suggest that patients who receive treatment with resins or fibrates might be advised to supply their diet with carotenoid-rich foods.

It should be emphasized that serum retinol levels do not necessarily reflect levels of retinoids in liver and other tissues.

Serum retinol levels are indeed maintained within a normal range despite wide variations in dietary and liver vitamin A levels.[29] Thus, normal serum retinol levels are compatible with low tissue retinol content. The latter is more sensitive to dietary retinol intake and might be the key parameter in determining the relationship, if one indeed exists, between retinol and cancer. Since in our study no assessment of tissue retinol content was made, the effects of cholesterol-lowering therapy on tissue vitamin A metabolism remain undefined.

In the present study, no relationship between serum RBP and cholesterol levels was found. In fact, hypercholesterolemic subjects had serum RBP levels similar to those of normocholesterolemic control subjects. Furthermore, no cholesterol-lowering treatment caused significant changes in serum RBP levels after 1 or 2 years of treatment.

Finally, it should be emphasized that these results were obtained with the standard therapeutic protocols used in a lipid clinic, ie, dietary treatment was often associated with drug therapy and the latter was chosen according to patients' clinical features. In conclusion, results of our study are not consistent with the hypothesis that cholesterol-lowering therapies reduce serum retinol concentrations and thereby enhance the risk of cancer.

REFERENCES

1. Sporn MB, Dunlop NM, Newton DL, et al: Prevention of chemical carcinogenesis by vitamin A and its synthetic analogues (retinoids). Fed Proc 35:1322-1338, 1976

2. Sporn MB, Newton DL: Chemoprevention of cancer with retinoids. Fed Proc 38:2528-2534, 1979

3. Hirayama T: Diet and cancer. Nutr Cancer 1:67-81, 1979

4. Mettlin C, Graham S, Swenson M: Vitamin A and lung cancer. J Natl Cancer Inst 62:1435-1438, 1979

5. Mettlin C, Graham S: Dietary risk factors in human bladder cancer. Am J Epidemiol 110:255-263, 1979

6. Peto R, Doll R, Buckley JD, et al: Can dietary β-carotene materially reduce human cancer rates? Nature 290:201-207, 1981

7. Kark JD, Smith AH, Hames CG: Serum retinol and the inverse relationship between serum cholesterol and cancer. Br Med J 284:152-154, 1982

8. Marenah CB, Lewis B, Hassal D, et al: Hypocholesterolaemia and non-cardiovascular disease: Metabolic studies on subjects with low plasma cholesterol concentrations. Br Med J 286:1603-1606, 1983

9. Wysowski DK, Gross TP: Deaths due to accidents and violence in two recent trials of cholesterol-lowering drugs. Arch Intern Med 150:2169-2172, 1990

10. Strandberg TE, Salomaa VV, Naukkarinen VA, et al: Long-term mortality after 5 year multifactorial primary prevention

of cardiovascular diseases in middle-aged men. JAMA 266:1225-1229, 1991

11. Rose G, Blackburn H, Keys HL, et al: Colon cancer and blood cholesterol. Lancet 1:181-183, 1974

12. Kark JD, Smith AH, Hames CG: The relationship of serum cholesterol to the incidence of cancer in Evans County. J Chronic Dis 33:311-322, 1980

13. Beaglehole R, Foulkes MA, Prior IAM, et al: Cholesterol and mortality in New Zealand Maoris. Br Med J 280:285-287, 1980

14. National Institutes of Health: Cholesterol and non cardiovascular mortality. JAMA 244:25, 1980

15. Williams RR, Sorlie PD, Feinleib M, et al: Cancer incidence by levels of cholesterol. JAMA 245:247-252, 1981

16. Smith DK, Greene JM, Leonard SB, et al: Vitamin A in hypercholesterolemia. Am J Med Sci 304:20-24, 1992

17. Wald N, Idle M, Boreham J, et al: Low serum vitamin A and subsequent risk of cancer. Lancet 2:813-815, 1980

18. Smith AH, Hoggard BM: Retinol, carotene and the cancer/cholesterol associations. Lancet 1:1371-1372, 1981

19. Stahelin HB, Bues E, Rosel F, et al: Vitamin A, cardiovascular risk factor, and mortality. Lancet 1:394-395, 1982

20. Fredrickson DS, Levy RI, Lees RS (eds): Fat Transport in Lipoproteins: An Integrated Approach to Mechanisms and Disorders. N Engl J Med 276:34-44, 94-103, 148-156, 215-224, 273-281, 1967

21. The expert panel: Report of the national cholesterol education program expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. Arch Intern Med 148:36-69, 1988

22. Siedel J, Hagele EO, Ziegenhorn J, et al: Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. Clin Chem 29:1075-1080, 1983

23. Wahlefeld AW: Triglycerides: Determination after enzymatic hydrolysis, in Bergmeyer HU (ed): Methods of Enzymatic Analysis. New York, NY, Academic, 1974, pp 1831-1836

24. Warnick GR, Benderson J, Albers JJ: Dextran sulfate–Mg^{2+} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem 28:1379-1388, 1982

25. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low density lipoprotein cholesterol in plasma without the preparative ultracentrifuge. Clin Chem 18:499-552, 1972

26. Miller KW, Lorr NA, Yang CS: Simultaneous determination of plasma retinol, tocopherol, lycopene, and carotene by high performance liquid chromatography. Anal Biochem 138:340-345, 1984

27. Anderson RJ, Stemberg JC: A rate nephelometer for immunoprecipitation measurement of specific serum proteins, in Ritchie RF (ed): Automated Immunoanalysis, vol 2. New York, NY, Dekker, 1978, pp 410-469

28. Lindgren FT: Preparative ultracentrifugal laboratory procedures and suggestions for lipoprotein analysis, in Perkins EG (ed): Analysis of Lipids and Lipoproteins. Champaign, IL, American Oil Chemist's Society, 1975, pp 204-224

29. Underwood BA: Vitamin A in animal and human nutrition, in Sporn MB, Roberts AB, Goodman DS (eds): The Retinoids. Academic, 1984, New York, NY, pp 281-392

30. Goodman DS: Vitamin A and retinoids in health and disease. N Engl J Med 310:1023-1031, 1984