The hypolipidemic action of probucol: a study of its effects on high and low density lipoproteins

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Abstract This study examines the effects of probucol (1 g/day) on the plasma concentration, composition, and metabolism of low and high density lipoproteins (LDL and HDL) in eleven hyperlipidemic subjects, (seven Type II and four Type IV). The drug lowered plasma cholesterol in the Type II patients by 11% ($P < 0.02$) without affecting triglyceride. Both LDL and HDL cholesterol levels fell by 6% and 26%, respectively. The small reduction in the former was not associated with a change in the composition of the lipoprotein nor with a measurable alteration in the level of circulating apoLDL. Kinetic studies revealed that probucol had no consistent effect on either the synthesis or catabolism of apoLDL. However, probucol did exert a potent influence on HDL, lowering the level of this lipoprotein in both the Type II and Type IV patients despite the fact that total plasma cholesterol in the latter group was unchanged by treatment. The fall in HDL mass largely affected the HDL_3 subfraction; HDL_2, which was initially low in our subjects, did not show a consistent response to therapy. Not all of the constituents in HDL were equally affected by the drug. Specifically, the fall in total plasma apoA levels (which derived from significant reductions in the rates of synthesis of apoproteins A-I and A-II) was less than that of HDL cholesterol. Direct measurement of the composition of the lipoprotein confirmed that during therapy it carried less cholesterol per unit protein. The significance of these observations in relation to the prophylaxis of ischemic heart disease is not yet clear, but it seems prudent at present to use probucol selectively in subjects who show a substantial hypocholesterolemic response that derives primarily from a reduction in circulating LDL.—**Atmeh, R. F., J. M. Stewart, D. E. Boag, C. J. Packard, A. R. Lorimer, and J. Shepherd.** The hypolipidemic action of probucol: a study of its effects on high and low density lipoproteins. *J. Lipid Res.* 1983. **24:** 588–595.

Supplementary key words HDL_2 • HDL_3 • fractional clearance rate • apoA-I • apoA-II • apoLDL

In 1970 Barnhart, Sefranka, and McIntosh (1) first described the effect of a sulfur-containing bis-phenol (4,4′-(isopropylidenedithio) bis (2,6-di-t-butylphenol)) on the plasma lipids of a number of animal species. The drug significantly lowered plasma cholesterol in mice, rats, dogs, and monkeys, and a similar effect was also observed in man (2). The chemical structure of this compound, now known as probucol (Dow Chemical Co., Indianapolis, IN), differs completely from that of other lipid-lowering agents and consequently one might expect that its mechanism of action would also be unique. The drug is normally administered at a dose of 1 g/day and on this regimen plasma and tissue levels rise to plateau values within 3 to 4 months (3, 4). The compound is only sparingly water soluble, resulting in limited absorption from the gut, minimal urinary excretion, and substantial retention in body fat stores (3, 4). It is relatively free from side effects and is tolerated well by patients over the long term (4).

Probucol effectively lowers plasma cholesterol in Type II hyperlipoproteinemic subjects without changing triglyceride levels (5). Its action (5, 6) appears to be directed towards lowering cholesterol in both the low and high density lipoprotein fractions (LDL and HDL) of the plasma which, in a recent extensive study (6), fell by approximately 8.4% and 26%, respectively, giving an overall decrement of 10.7% in total plasma cholesterol. The mechanisms whereby these effects are achieved are not yet known although a preliminary animal study (7) has invoked suppression of lipoprotein synthesis. Nestel and Billington (8) suggested a similar action with regard to the influence of the drug on HDL apoA-I production in man. Since this drug is so radically different from other hypolipidemic agents and significantly reduces the level of HDL cholesterol, a "negative risk factor" for ischemic heart disease, it is important that we come to an understanding of its influence on lipoprotein metabolism as a whole. Consequently, we have examined a number of metabolic parameters in a group of Type II and Type IV hyperlipoproteinemic subjects before and during drug treatment. In this paper we report our findings.

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; HDL_2, HDL subfraction 2, d 1.063–1.125 g/ml; HDL_3, HDL subfraction 3, d 1.125–1.210 g/ml; CHD, 1,2 cyclohexanedione; apo, apolipoprotein.

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MATERIALS AND METHODS

This study was approved by the Ethical Committee of Glasgow Royal Infirmary.

Subjects

Eleven subjects gave their informed consent to the study. Seven were Type II hyperlipoproteinemic (3 Type IIa, 4 Type IIb) and four were Type IV according to the criteria of Fredrickson, Levy, and Lees (9). Family studies were not performed and so genotypic classification was not possible. However, secondary causes of their hyperlipoproteinemia were excluded by appropriate biochemical tests of hepatic, renal, endocrine, and hematologic function. All subjects were examined on an out-patient basis and ate their regular diet which, from a 7-day intake record, contained approximately 15% of calories as protein, 40% as carbohydrate, and 45% as fat, with a polyunsaturated/saturated ratio of 0.15. The patients were advised to maintain a constant dietary intake; frequent measurements of their body weight during each phase of the study showed that this parameter remained constant (**Table 1**). Prior to the study they had received no drug therapy (including the contraceptive pill) for at least 1 month. For 3 days before and throughout the study they were given 60 mg of KI twice daily to prevent uptake of radioiodide by the thyroid gland.

Turnover study protocols

The subjects, whose lipid and lipoprotein profiles were presented in Table 1 were divided into two groups. The first group, comprising three Type IIa and three Type IIb patients, was used to examine the effects of probucol on LDL metabolism, while analysis of HDL kinetics was performed in the second. Both groups were studied twice, first during a control phase and then after 3 months of probucol therapy (0.5 g twice daily). Measured plasma probucol levels indicated that compliance to therapy was excellent.

LDL study

The design of this study has been described in previous publications (10, 11). Briefly, LDL (d 1.030–1.050 g/ml) was prepared from the plasma of each subject by rate zonal ultracentrifugation (12) and divided into two aliquots. One was labeled with ^{125}I and the other with ^{131}I (13). The latter was modified (14) with 1,2 cyclohexanedione to give a product (CHD/LDL) which has been characterized elsewhere (15). Twenty-five μCi of each labeled tracer (approximately 0.2–0.5 mg of protein) was sterilized by filtration through 0.22 μm filters (Millipore Corp. Bedford, MA) and injected in rapid succession into the bloodstream of the donor via an indwelling catheter. Blood samples were withdrawn at 10 min and then daily, after an overnight fast, for the next 14 days. The radioactivity present in a 2.0-ml aliquot of plasma was measured in a twin channel gamma spectrometer (Packard Instruments, Downers Grove, IL) and the results were used to construct decay curves for each isotope. The curves were analyzed by the method of Matthews (16) to obtain the fractional catabolic rates of the native and modified lipoproteins. The difference between these was a measure of receptor-mediated LDL catabolism. The plasma LDL pool size was determined according to Langer, Strober, and Levy (17).

HDL study

The metabolism of apolipoproteins (apo) A-I and A-II was examined in HDL as follows. The lipoprotein (d 1.063–1.210 g/ml) was isolated from 10 ml of plasma from a fasting subject, washed once at d 1.210 g/ml, dialyzed against 0.15 M NaCl, 0.01% Na_2 EDTA, 0.01 M Tris buffer, pH 7.0, and labeled with ^{125}I (18). Fifty μCi of the sterilized labeled tracer (approximately 1.0 mg of protein) was administered to the donors by intravenous injection. Plasma samples were then withdrawn at 10 min and daily thereafter (following an overnight fast) for 14 days. HDL was re-isolated from 10 ml of plasma, obtained at each time point, by the ultracentrifugation procedure described above, delipidated by addition of an equal volume of tetramethylurea, and the solubilized apoHDL was applied to 1-cm diameter by 8-cm long cylindrical preparative urea/PAGE gels (19) to separate apolipoproteins A-I and A-II. These were located by immersing the gels in a solution of 0.01% magnesium 1-anilino-naphthalene-8-sulfonate (20) and comparing the resulting pattern with purified apoA-I and apoA-II standards run on identical gels. The appropriate fluorescent bands were excised and the proteins were eluted (21) by electrophoresis into Spectrapor (Spectrum Medical Industries Inc, Los Angeles, CA) dialysis bags. More than 90% of the radioactivity in each band was recovered by this procedure. The apoA-I and apoA-II specific activities, determined by measuring the protein content (22) and ^{125}I radioactivity in the fractions, were used to construct decay curves from which fractional catabolic rates were obtained by Matthews' procedure (16). Plasma apoA-I and A-II levels were determined by electroimmunoassay (23).

HDL subfraction measurements

Following both phases of each turnover study, 50 ml of plasma from each participant (fasting) was subjected to rate zonal ultracentrifugation to separate HDL_2 and HDL_3. The plasma concentrations of these subfractions were calculated from the rate zonal elution profiles and

TABLE 1. Effects of probucol on plasma lipids and lipoproteins in Type II and Type IV hyperlipoproteinemia

Subject (Sex)	Hyperlipoproteinemic Phenotype	Age	Body Weight (n = 15)		Plasma Triglyceride (n = 15)		Plasma Cholesterol (n = 15)		Cholesterol (n = 5) in VLDL		in LDL		in HDL	
			Control	Drug	Control	Drug	Control	Drug	Control	Drug	Control	Drug	Control	Drug
		yr	*kg*		*mg/dl*		*mg/dl*				*mg/dl*			
JR (M)	IIa	42	72.6[a] ± 0.7	72.0 ± 0.3	131.9 ± 32.7	141.6 ± 7.1	315.4 ± 4.3	281.0 ± 3.9	23.6 ± 10.1	23.2 ± 5.8	230.7 ± 12.9	217.1 ± 3.9	61.1 ± 6.9	40.2 ± 2.7
AA (F)	IIa	43	64.5 ± 0.5	63.7 ± 0.6	163.7 ± 41.6	138.9 ± 20.6	284.4 ± 10.4	250.8 ± 6.6	15.5 ± 12.8	13.5 ± 5.4	207.0 ± 17.8	193.5 ± 3.5	61.9 ± 3.5	43.7 ± 3.5
CL (F)	IIa	54	57.0 ± 0.2	56.4 ± 0.3	172.6 ± 12.4	215.1 ± 37.2	317.0 ± 5.8	284.4 ± 17.0	33.3 ± 17.0	29.0 ± 8.5	210.9 ± 10.4	206.3 ± 12.0	72.4 ± 6.6	49.5 ± 4.6
JB (M)	IIb	60	75.5 ± 0.6	76.1 ± 0.4	229.2 ± 46.9	347.8 ± 132.8	281.7 ± 19.7	264.7 ± 14.3	26.3 ± 5.0	43.7 ± 14.7	199.7 ± 16.6	186.5 ± 13.0	55.7 ± 7.4	34.4 ± 3.5
RO'N (M)	IIb	39	63.2 ± 0.5	63.5 ± 0.4	220.4 ± 27.4	181.4 ± 43.4	344.8 ± 17.0	315.8 ± 18.9	39.0 ± 8.5	28.6 ± 9.7	252.7 ± 12.0	246.1 ± 22.8	53.4 ± 3.9	41.0 ± 10.4
RW (M)	IIb	48	96.8 ± 0.5	95.1 ± 0.6	374.4 ± 26.6	292.9 ± 32.7	350.2 ± 18.9	348.3 ± 22.1	49.9 ± 10.1	46.4 ± 8.7	235.7 ± 13.2	234.1 ± 17.4	74.7 ± 8.1	70.0 ± 5.8
DC (M)	IIb	49	82.3 ± 0.4	81.3 ± 0.2	235.4 ± 39.8	230.0 ± 38.9	323.1 ± 15.1	240.3 ± 10.1	50.3 ± 36.8	23.2 ± 15.5	218.7 ± 33.7	175.3 ± 10.1	54.2 ± 2.7	42.6 ± 2.3
JMcD (M)	IV	58	61.2 ± 0.1	65.3 ± 0.3	290.3 ± 121.2	289.4 ± 40.7	236.1 ± 23.2	253.5 ± 8.5	38.7 ± 17.8	53.4 ± 36.4	135.5 ± 5.8	152.9 ± 1.5	61.9 ± 3.5	47.2 ± 2.0
AK (M)	IV	61	78.8 ± 0.1	81.9 ± 0.2	577.1 ± 117.7	332.7 ± 50.4	224.5 ± 10.8	195.4 ± 22.4	96.8 ± 24.4	44.9 ± 12.0	85.1 ± 22.4	108.7 ± 16.3	45.3 ± 18.6	41.8 ± 2.3
MC (M)	IV	52	65.3 ± 0.4	67.0 ± 0.4	462.9 ± 108.0	874.4 ± 102.7	263.2 ± 15.9	256.6 ± 23.2	81.3 ± 16.6	137.4 ± 19.4	143.2 ± 19.4	102.1 ± 6.9	42.6 ± 4.3	17.0 ± 3.5
JF (M)	IV	57	84.7 ± 0.6	85.0 ± 0.7	294.7 ± 17.7	270.8 ± 72.6	260.5 ± 13.5	233.7 ± 15.9	107.2 ± 7.0	113.8 ± 24.8	110.3 ± 1.4	84.8 ± 5.4	43.7 ± 1.2	35.2 ± 3.9
Paired *t* test vs. control (11 subjects)			NS		NS		$P < 0.01$		NS		NS		$P < 0.001$	
Paired *t* test vs. control (Type II subjects)			NS		NS		$P < 0.02$		NS		$P < 0.01$		$P < 0.001$	

[a] Mean ± SD.

the subfraction compositions were determined as outlined elsewhere (24).

RESULTS

Probucol at a dose of 1 g/day lowered plasma cholesterol in our Type II subjects by 11% ($P < 0.02$) but had no significant effect on the level of this lipid in the Type IV patients (Table 1). Plasma triglyceride and very low density lipoprotein (VLDL) cholesterol remained the same before and during treatment. The drug consistently decreased HDL cholesterol (by 26%, $P < 0.01$) in all subjects, but its influence on LDL was phenotype-dependent, occurring only in the Type II individuals; even within this group the reduction that was achieved averaged only 6% ($P < 0.01$) and varied between 1% and 20%. The metabolism of the protein moiety of LDL was examined by injecting ^{125}I-LDL and ^{131}I-CHD/LDL. The latter does not interact with the high affinity LDL receptor and therefore provides a measure of receptor-independent apoLDL catabolism. Consequently, the difference between its plasma fractional clearance rate and that of native LDL is an index of the activity of the receptor pathway. This approach showed that the raised plasma apoLDL concentration in the Type II hyperlipoproteinemic subjects (**Table 2**) was associated with decreased fractional clearance of the lipoprotein through the receptor pathway. The fractional clearance rate of CHD/LDL was similar to that previously recorded in normals (10) and did not change during probucol treatment. Likewise, on average, the activity of the receptor pathway remained unaffected by the drug, both in terms of the fractional and absolute catabolism of LDL. Nevertheless, there was substantial inter-individual variation both in the control value for receptor-mediated catabolism and in its response to therapy. Those patients with higher receptor-mediated LDL clearance showed no change, while in the others (e.g., JR, RW) the low basal receptor activity tended to increase during drug treatment.

The influence of probucol on the concentration and metabolism of HDL in the plasma was more dramatic. **Table 3** documents the response of the HDL apoprotein and subfraction levels to treatment. Plasma apoA-I and apoA-II fell in all subjects, reaching levels of statistical significance ($P < 0.01$ and $P < 0.001$, respectively) for the group as a whole. Independent analysis of the data from the Type II subjects confirmed that the reductions were also significant in this group. Although the trend in the Type IV patients followed the same pattern, significance levels were not achieved here largely because the apoprotein decrements in one subject (MC) were extreme (apoA-I fell 51% and A-II, 20%), almost match-

TABLE 2. Effects of probucol on LDL apoprotein catabolism

Subject	Plasma ApoLDL Concentration		ApoLDL Fractional Catabolic Rate				ApoLDL Absolute Catabolic Rate					
			Receptor-Mediated		Receptor-Independent		Receptor-Mediated		Receptor-Independent		Total	
	Control	Drug	Control	Drug	Control	Drug	Control	Drug	Control	Drug	Control	Drug
	mg/dl		*pools/day*				*mg/kg per day*					
JR	182	147	0.042	0.099	0.175	0.158	2.8	5.2	11.5	8.3	14.3	13.5
AA	159	142	0.112	0.087	0.190	0.196	5.8	3.4	9.8	7.8	15.6	11.2
CL	175	187	0.110	0.125	0.152	0.156	5.6	7.7	7.7	9.6	13.3	17.3
JB	164	166	0.094	0.067	0.244	0.260	4.9	4.7	12.8	13.0	17.7	17.7
RO'N	233	274	0.034	0.041	0.205	0.250	2.9	3.5	17.4	21.3	20.3	24.9
RW	168	172	0.005	0.023	0.211	0.200	0.3	1.3	11.6	11.3	11.9	12.6
Mean ± SD	180 ± 27	173 ± 30	0.066 ± 0.045	0.074 ± 0.038	0.196 ± 0.032	0.203 ± 0.044	3.7 ± 2.1	4.3 ± 2.1	11.8 ± 3.3	11.9 ± 5.0	15.5 ± 3.1	16.2 ± 5.0
Paired *t* test vs. control	NS		NS		NS		NS		NS		NS	

TABLE 3. Effects of probucol on HDL apoprotein and subfraction concentrations

Subject	Plasma ApoA-I (n = 8)		Plasma ApoA-II (n = 8)		Plasma HDL_2		Plasma HDL_3	
	Control	Drug	Control	Drug	Control	Drug	Control	Drug
				mg/dl				
JR	136 ± 16	115 ± 10	42 ± 3	32 ± 2	42.5	12.1	419	193
AA	143 ± 12	134 ± 10	39 ± 4	34 ± 3	37.0	6.4	285	200
CL	154 ± 13	149 ± 6	48 ± 2	46 ± 3	25.0	8.5	234[a]	200
JB	155 ± 3	137 ± 5	34 ± 1	30 ± 2	15.9	30.5	318	311
RO'N	135 ± 4	126 ± 6	39 ± 7	33 ± 4	8.7	11.2	331	321
RW	148 ± 12	135 ± 7	57 ± 1	55 ± 2	80.0	34.9	412	409
DC	129 ± 14	105 ± 9	33 ± 5	28 ± 5	13.4	10.6	348	190
JMcD	127 ± 11	114 ± 11	45 ± 2	40 ± 3	14.5	12.5	416	257
AK	132 ± 4	114 ± 10	34 ± 3	33 ± 2	15.0	21.6	352	268
MC	141 ± 4	69 ± 9	49 ± 3	39 ± 3	8.0	16.0	309	99
JF	132 ± 14	117 ± 12	40 ± 4	37 ± 4	16.9	10.0	259	247
Mean ± SD	139 ± 10	120 ± 21	42 ± 7	37 ± 8	25.2 ± 21.2	15.8 ± 9.3	335 ± 63	245 ± 83
Paired *t* test vs. control	$P < 0.01$		$P < 0.001$		NS		$P < 0.01$	

[a] HDL_3 on the zonal elution profile was poorly resolved from residual plasma proteins.

Correlation between HDL cholesterol and total HDL mass: *1*) control phase (omitting CL) $r = 0.77$, $P < 0.01$; *2*) drug phase $r = 0.67$, $P < 0.02$.

ing the 60% fall in his HDL cholesterol. The changes in the plasma HDL subfraction concentrations were more difficult to interpret. Examination of the data as a whole showed that there was no significant change in HDL_2, while HDL_3 fell by 27% ($P < 0.01$). However, the effect of the drug varied according to the initial HDL subfraction distribution. In those hyperlipoproteinemic subjects whose plasma HDL_2 concentration on zonal centrifugation is low (24, 25), any drug-induced change is more likely to affect HDL_3. However, where the initial levels of HDL_2 were substantial (e.g., in subjects JR, AA, CL, and RW) they did fall in response to treatment, in agreement with unpublished observations from this laboratory[2] that probucol consistently reduces this subfraction in normolipemic subjects.

The compositions of LDL, HDL_2, and HDL_3 determined before and during drug treatment are shown in **Table 4.** The medication had no effect on LDL composition in the Type II group. However, as could be predicted from the relative reductions in plasma HDL cholesterol (26%) and apoprotein levels (13%), the compositions of the HDL subfractions were altered. Specifically, their protein contents rose and there was a significant decrease ($P < 0.01$) in the cholesterol/protein ratio in HDL_3. This trend, significant in the group as a whole, was also apparent when the patients were segregated according to phenotype.

The reductions in apoA-I and apoA-II produced by probucol were associated with lower rates of synthesis of these proteins. Examination of the data from all five hypertriglyceridemic subjects showed that probucol suppressed production of apolipoproteins A-I and A-II by 39% ($P < 0.01$) and 37% ($P < 0.05$), respectively. This effect was also apparent in the results from the four Type IV hyperlipoproteinemic patients, although here only the fall in apoA-I synthesis was statistically significant. The fractional clearance rates of both apoproteins did not change consistently (**Table 5**).

DISCUSSION

Probucol has been approved for clinical use in the United States since 1977, and now reports are beginning to appear that detail its mechanism of action. It is generally agreed that the drug is effective in lowering plasma cholesterol. Several clinical trials (5, 6, 26–28) have reported decrements of between 10% and 15%, but most concede that there is significant inter-patient variability in response, some showing a substantial reduction while in others the cholesterol level does not change. Our own findings reflect this situation. We observed a mean fall of 11% in our hypercholesterolemic subjects, with a range of 26% to 1%. Although both LDL and HDL contributed to the cholesterol reduction, its variability was attributable to the former since the drop in HDL in response to the drug was consistent in all of our subjects.

The metabolism of LDL was examined in a group of six hypercholesterolemic patients in an attempt to define the mechanism whereby probucol lowers the cho-

[2] Atmeh, R. F., C. J. Packard, and J. Shepherd. Unpublished observations.

TABLE 4. Effects of probucol on lipoprotein compositions

Subjects	Lipoprotein	Composition: Free Cholesterol Control	Free Cholesterol Drug	Esterified Cholesterol Control	Esterified Cholesterol Drug	Triglyceride Control	Triglyceride Drug	Phospholipid Control	Phospholipid Drug	Protein Control	Protein Drug	Cholesterol/Protein Ratio Control	Cholesterol/Protein Ratio Drug
		g/100 g											
Type II	LDL (n = 6)	9.9 ± 3.7[a]	7.6 ± 3.1	40.0 ± 5.9	40.1 ± 6.5	3.2 ± 2.3	3.1 ± 1.1	24.9 ± 4.9	23.6 ± 4.4	25.5 ± 2.4	26.9 ± 2.3	1.32 ± 0.11	1.17 ± 0.15
Type IIa	HDL_2 (n = 3)	4.8 ± 0.3	4.1 ± 1.2	20.5 ± 2.8	15.1 ± 5.6	3.1 ± 1.3	2.7 ± 1.9	31.2 ± 2.3	29.8 ± 3.5	42.5 ± 2.8	47.8 ± 1.8	0.40 ± 0.02	0.28 ± 0.10
Type IIb	HDL_2 (n = 4)	5.1 ± 2.6	4.3 ± 2.2	14.6 ± 3.5	14.3 ± 5.3	5.2 ± 1.8	2.4 ± 1.6	31.6 ± 4.9	31.0 ± 1.3	43.5 ± 1.8	47.6 ± 4.9	0.32 ± 0.09	0.28 ± 0.12
Type IV	HDL_2 (n = 4)	4.9 ± 3.2	3.9 ± 1.9	17.4 ± 5.4	14.5 ± 2.5	7.7 ± 1.7	4.2 ± 1.2	28.1 ± 6.3	27.0 ± 2.7	41.9 ± 4.0	49.6 ± 3.8	0.38 ± 0.20	0.27 ± 0.06
Type IIa	HDL_3 (n = 3)	2.4 ± 0.6	1.8 ± 0.6	13.9 ± 0.9	11.5 ± 3.9	2.3 ± 0.4	4.5 ± 5.1	26.3 ± 4.6	24.7 ± 2.1	55.0 ± 5.7	57.5 ± 0.6	0.20 ± 0.03	0.15 ± 0.05
Type IIb	HDL_3 (n = 4)	1.9 ± 0.2	1.4 ± 0.7	13.8 ± 1.8	9.9 ± 1.5	4.5 ± 3.7	5.8 ± 6.3	27.6 ± 6.6	27.0 ± 3.2	52.4 ± 3.1	56.0 ± 2.9	0.19 ± 0.02	0.13 ± 0.02
Type IV	HDL_3 (n = 4)	2.0 ± 0.3	1.8 ± 1.1	12.8 ± 2.6	9.5 ± 2.9	3.7 ± 2.3	2.4 ± 0.7	27.7 ± 4.0	25.5 ± 3.1	52.0 ± 2.3	61.0 ± 1.1	0.18 ± 0.04	0.12 ± 0.02
All	HDL_2 (n = 11)	5.0 ± 2.3	4.4 ± 2.1	17.2 ± 4.5	14.6 ± 4.1	5.5 ± 2.4	3.1 ± 1.6	30.2 ± 4.8	29.2 ± 2.9	42.6 ± 2.8	48.3 ± 3.6[b]	0.36 ± 0.07	0.25 ± 0.08
All	HDL_3 (n = 11)	2.1 ± 0.4	1.6 ± 0.8	13.5 ± 1.9	10.2 ± 2.7	3.6 ± 2.5	4.2 ± 4.5	27.3 ± 4.7	25.8 ± 2.8	53.7 ± 3.9	58.2 ± 2.9[b]	0.19 ± 0.03	0.13 ± 0.03[b]

[a] Mean ± SD.
[b] $P < 0.01$, paired t test vs. control.
LDL compositions were measured in the six Type II subjects whose LDL metabolism was examined.

TABLE 5. Effects of probucol on high density lipoprotein metabolism

	ApolipoproteinA-I Kinetics				Apolipoprotein A-II Kinetics			
	Fractional Clearance Rate		Absolute Clearance Rate		Fractional Clearance Rate		Absolute Clearance Rate	
Subject	Control	Drug	Control	Drug	Control	Drug	Control	Drug
	pools/day		*mg/kg per day*		*pools/day*		*mg/kg per day*	
DC	0.156	0.095	5.6	3.2	0.395	0.145	3.63	1.30
JMcD	0.255	0.205	10.9	7.3	0.221	0.138	3.35	1.72
AK	0.280	0.240	11.1	8.6	0.284	0.251	2.90	2.60
MC	0.264	0.306	15.9	9.0	0.150	0.149	3.14	2.48
JF	0.268	0.142	10.9	5.4	0.155	0.114	1.91	1.37
Mean ± SD	0.236 ± 0.049	0.198 ± 0.082	10.9 ± 3.6	6.7 ± 2.4	0.241 ± 0.102	0.159 ± 0.052	2.99 ± 0.66	1.89 ± 0.61
Paired *t* test vs. control	NS		$P < 0.01$		NS		$P < 0.05$	

lesterol level in this fraction. All subjects responded to the drug as expected but the average reduction in LDL cholesterol was small (6%, $P < 0.01$), and in four we could detect no significant change in apoLDL concentration. Consequently, it was impossible to ascribe the observed reduction in LDL cholesterol to either a fall in apoLDL synthesis or an increase in its catabolism. Similar results have been reported recently by Nestel and Billington (8) who found no significant change in LDL pool size or catabolism during probucol administration to five hypercholesterolemic subjects.

The influence of the drug on HDL metabolism is more clearcut. Treatment lowered both HDL cholesterol and apoprotein A-I and A-II levels, the latter as a result of suppressed synthesis. These significant findings establish the earlier proposal of Nestel and Billington (8) that the drug suppresses apoA-I synthesis. In a group of four Type II subjects, they were able to show a consistent (but not significant) reduction in this parameter. Therefore, both Type II and Type IV subjects seem to make the same response to the drug, at least in terms of apoA-I kinetics. The above changes were accompanied by a fall primarily in the level of HDL_3, the major apoA-containing particle in the plasma (24, 29), particularly in our hyperlipoproteinemic subjects whose initial HDL_2 levels were low (Table 3). But it should be noted that this response, though applicable to the group as a whole, may not apply to the Type IIb individuals who showed little change in HDL subfraction levels during treatment. Since the percentage fall in plasma HDL cholesterol (26%) was double that of total apo-A (13%), it could be inferred that probucol treatment lowered the cholesterol/protein ratio in the HDL_3 fraction. This, in fact, was confirmed by direct compositional analysis (Table 4). Thus, probucol seems to have effects on HDL composition and metabolism that are independent of the phenotype of the subject. The levels of both apoA-I and apoA-II fall in response to a reduction in their synthesis and the total mass of HDL in the plasma is reduced. Moreover, HDL cholesterol changes disproportionately so that there is a decrement in the content of cholesterol in both HDL_2 and HDL_3. Despite the fact that HDL is regarded as a negative risk factor for coronary heart disease (30), the multiplicity of effects of probucol on this parameter makes it difficult to assess how the drug will influence the apparent antiatherogenic function of the lipoprotein. Indeed, preliminary evidence (31) suggests that such therapy may reduce cardiovascular risk. Nevertheless, since the efficacy of the drug is variable from patient to patient, it is prudent to match probucol treatment with those subjects whose response in terms of LDL-lowering is good.

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