Influence of short term dietary cholesterol and fat on human plasma Lp[a] and LDL levels

Spencer A. Brown,* Joel Morrisett,* Josef R. Patsch,*† Rebecca Reeves,* Antonio M. Gotto, Jr.,* and Wolfgang Patsch*

Department of Medicine,* Baylor College of Medicine and The Methodist Hospital, Houston, TX 77030, and Department of Medicine,† University of Innsbruck, Innsbruck, Austria

Abstract The relationship between plasma levels of Lp[a] and LDL was examined using dietary regimens. In 81 normolipidemic male outpatients, dietary cholesterol was increased by consuming six eggs per day from a mean (SD) level of 311 (162) to 1430 (198) mg per day. Mean (SD) LDL-cholesterol levels increased from 102 (26) mg/dl to 120 (33) mg/dl ($P < 0.001$), while mean (SD) Lp[a] levels were 5.5 (6.1) mg/dl on the basal diet and 5.6 (6.4) mg/dl on the cholesterol-rich diet. No significant correlation was observed between increases in either LDL-cholesterol or apolipoprotein B to Lp[a], nor was there any relationship between individual baseline levels of Lp[a] and dietary-induced changes of Lp[a]. Fourteen of the 81 participants were reexamined under strict nutritional control. Four diets with 40% of calories as fat, but differing in the type of fat and the amount of cholesterol, were administered sequentially to all subjects. As expected, mean (SD) LDL-cholesterol and apolipoprotein B levels were highest on the saturated fat, high cholesterol diet (112 (32) mg/dl and 79 (22) mg/dl) and lowest on the polyunsaturated fat, low cholesterol diet (77 (27) mg/dl and 53 (18) mg/dl). In contrast, mean Lp[a] levels did not significantly change among the four diets (range 4.2–4.9 mg/dl). No correlation of Lp[a] responses with changes in plasma lipids, apolipoproteins, or lipoproteins was observed on any diet. These data suggest that determinants of plasma Lp[a] levels are distinctly different from the determinants of plasma LDL levels in normolipidemic males. —**Brown, S. A., J. Morrisett, J. R. Patsch, R. Reeves, A. M. Gotto, Jr., and W. Patsch.** Influence of short term dietary cholesterol and fat on human plasma Lp[a] and LDL levels. *J. Lipid Res.* 1991. **32:** 1281–1289.

Supplementary key words lipoproteins · Lp[a] · apolipoprotein B · diet · atherosclerosis

Lipoprotein[a] (Lp[a]) comprising a unique class of plasma lipoproteins has been identified as an independent risk factor for coronary heart disease (1–5, see reviews 6–8). While genetic factors may play the predominant role in determining plasma concentrations of Lp[a] (9, 10), metabolic factors may also modulate Lp[a] plasma levels (11–14). Of the antihyperlipidemic drugs, niacin used with neomycin has been reported to decrease plasma levels of Lp[a] (11). Reports vary on the effects of drugs that inhibit HMG-CoA reductase in that increases (12) or no changes of Lp[a] plasma levels (13) were observed. Anabolic steroid therapy in postmenopausal women resulted in substantial reduction of plasma Lp[a] (14). Finally, Lp[a] levels are reported to decrease in patients after heart transplants (15). The mechanisms whereby these perturbations alter plasma Lp[a] levels are not known.

Effects of diet on Lp[a] levels have also been studied (16–19). A dietary challenge in a single subject did not influence the Lp[a] level, while the apolipoprotein B (apoB) level was dramatically elevated (16). In a later study, 2 subjects were fed excess cholesterol for 28 days which resulted in a dramatic increase of apoB, but no change in Lp[a] (17). In 36 subjects fed a lacto-ovo vegetarian diet, mean low density lipoprotein (LDL)-cholesterol levels decreased 23% while no change was observed in mean Lp[a] levels (18). In contrast, 22 or 32 subjects fed a diet rich in fish oil showed reductions of 25% in mean Lp[a] levels and 11.4% in mean apoB levels. In the remaining 10 subjects, there was no change in Lp[a] levels (19).

Recent studies by Utermann et al. (20) revealed a multiplicative interaction between LDL receptor status in subjects with familial hypercholesterolemia and Lp[a] plasma levels. However, reports on the role of the LDL receptor in plasma Lp[a] catabolism are less clear in normolipidemic subjects (21–24). To gain further insight of the determinants of plasma Lp[a] in normolipidemic subjects, we measured Lp[a] levels subsequent to dietary manipulations known to affect LDL receptor expression (25–27).

Two different dietary protocols were used. The first was an outpatient study on a large number of normolipidemic subjects who first consumed a prescribed basal diet of low cholesterol followed by a diet supplemented with eggs. In the second protocol, 14 subjects from the first study were

Abbreviations: Lp[a], lipoprotein[a]; LDL, low density lipoprotein; HDL, high density lipoprotein; Chol, cholesterol; TG, triglyceride.

fed four different diets under strict nutritional control. These inpatient diets contained the same amount of fat but differed in type of fatty acids as well as the amount of cholesterol. We report here that both the outpatient and the inpatient protocols produced pronounced effects on plasma LDL-chol and apoB levels, but had no or very little effect on plasma Lp[a] levels.

METHODS

Subjects

Subjects were recruited locally. Inclusion criteria were: healthy males between the ages of 20–50 years; within 30% of ideal body weight, as determined by the Metropolitan Life Insurance tables (28); and less than 240 mg/dl for fasting total plasma cholesterol, 200 mg/dl for fasting total plasma triglycerides, and 175 mg/dl for LDL-chol through initial screening procedures. None of the subjects had a history or signs of liver, heart, kidney, or thyroid disease. In the first dietary protocol, 91 men started the protocol. Results were complete for 81 subjects with 1 subject lacking an apoB measurement. The mean (SD) age of the study subjects was 29.6 (5.8) years and the mean (SD) body mass index was 23.4 (2.3). In the second dietary protocol, 14 of the 81 outpatient subjects participated.

Study design

Outpatient. Two diets were administered during the 6-week outpatient diet (**Fig. 1**). For the first 3 weeks (weeks 1–3), the dietary goals were: 45% calories from carbohydrate, 15% from protein, and 40% from fat with an emphasis on saturated fats. Participants were asked to limit ethanol intake to 3% of total calories. Dietary cholesterol was restricted to 300 mg per day. In the next 3 weeks (weeks 4–6) dietary cholesterol intake was increased by consuming six eggs per day. This corresponds to approximately 1300 mg cholesterol per day using the revised 213 mg cholesterol content per egg as stated in 1989 by the U.S. Department of Agriculture. The same approximate distribution of fat, protein, and carbohydrate was prescribed during this dietary phase and subjects were not restricted in their use of cholesterol-containing foods. Throughout the study, registered dietitians provided group instruction to adjust sources of total fat, saturated fat, and dietary cholesterol. Lists of appropriate and inappropriate food choices as well as sample menus incorporating the proper foods into the recommended dietary pattern were distributed. In the egg-supplemented phase, multiple recipes were used for incorporating the eggs into the overall diet. All subjects kept 4-day food records (2 weekdays and 2 weekend days) that were documented by the dietitian during weekly visits to the clinic. Food records were first analyzed for all 81 individuals by a nutrient database from Computrition, Inc., Chatsworth, CA. However, the analysis of percent contribution of individual dietary fatty acids required a more sophisticated database. Complete food records from a random sample of 20 individuals were reanalyzed and nutrient calculation was performed using the Minnesota Nutrition Data System (NDS) software, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN (Food Database version 4A; Nutrient Database version 19). The data derived from NDS software did not differ significantly from the Computrition database in terms of percent contribution of carbohydrate, protein, and fat to total calories. Dietary compliance was evaluated through multiple interviews and phone calls. Body weight was relatively stable throughout the study protocol. Mean (SD) body weights at the begin-

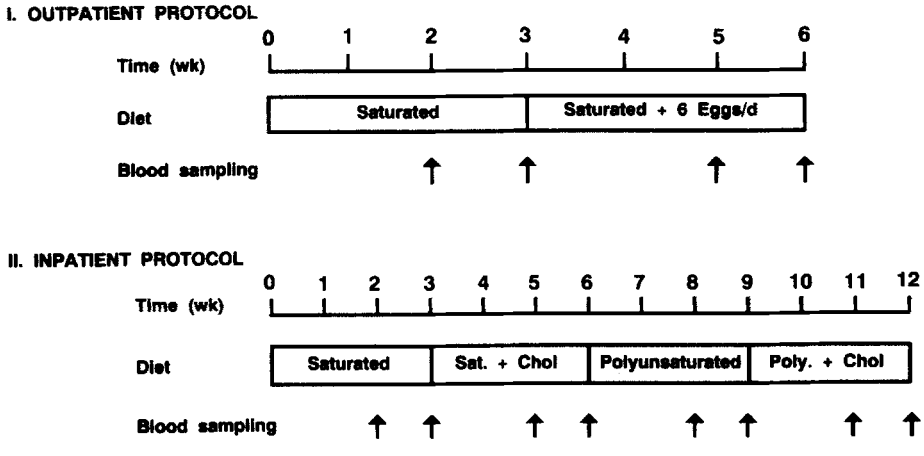

Fig. 1. Study design of outpatient and inpatient protocols.

ning of the study, after the saturated diet (week 3), and after the diet supplemented with six eggs per day (week 6) were 164.0 (19.9) lbs, 164.5 (20.1) lbs, and 165.5 (19.6) lbs, respectively. Fasting blood sampling was performed at the end of the second, third, fifth, and sixth week of this protocol (Fig. 1). Subjects were instructed to maintain their usual lifestyles during the study period.

Inpatient. Four different diets, each for 3 weeks, were administered sequentially during a 12-week inpatient study (Fig. 1). All subjects completed the full protocol. The dietary goals of the inpatient diets, in terms of total calories, were: 40% as fat, 45% as carbohydrate, and 15% as protein. The diets differed in their content of monounsaturated, polyunsaturated, and saturated fatty acids and in the amount of cholesterol. The diets were administered in the following sequence: *1*) saturated fatty acid-rich diet with <300 mg dietary cholesterol/day; *2*) saturated fatty acid-rich diet with approximately 1500 mg dietary cholesterol/day; *3*) polyunsaturated fatty acid-rich diet with <300 mg dietary cholesterol/day; and *4*) polyunsaturated fatty acid-rich diet with approximately 1500 mg dietary cholesterol/day. The saturated fatty acid-rich diets contained fat sources primarily from butter and animal fat, while the polyunsaturated fatty acid-rich diets used mainly safflower oil and safflower margarine. Dietary cholesterol was increased by subjects consuming six eggs/day as in the outpatient protocol. Subjects were asked to limit ethanol intake to an equivalent of two beers per day.

All diets were planned at a 2700 calorie level with adjustments for weight fluctuations for each individual. Loss of body weight resulted in the addition of food items to the diets which did not alter the daily dietary protocol, particularly in terms of the type of fat. All diets were nutritionally complete according to the requirements for essential nutrients by the Food and Nutrition Board of the National Research Council, National Academy of Sciences. One 7-day menu cycle was developed for each diet period by the research dietitians and the food production dietitian. The meals were prepared by the Metabolic Kitchen of The Methodist Hospital. Meal preparation was supervised by a registered dietitian who was responsible not only for food production but also for menu implementation. Accuracy in portion sizes and fat content for each food was obtained by weighing and measuring both individual food components and actual foods.

Lunch and dinner meals were served Monday–Friday in a private dining area close to the kitchen facility. The following day's breakfast meal was given to the subjects at dinner. Weekend meals were boxed and carried home. Subjects were required to consume all foods served. Each subject was given a copy of the diet for each dietary phase with an additional sheet for tracking exercise, illness, medication use, alcohol intake, and foods eaten that were not designated on the diet. Subjects were sampled for blood at the end of the second and third week of each of the four diets. Mean and SD levels of each dietary component were determined from an analysis on all foods for 1 week in each dietary period by the NDS software package.

Laboratory measurements

Total plasma cholesterol (29) and triglycerides (30) were measured enzymatically on a Cobas-Bio centrifugal analyzer (Roche Diagnostics, Montclair, NJ) with the respective enzymatic kits (Cat. Nos. 236691 and 701912; Boehringer Mannheim Diagnostics, Indianapolis, IN). High density lipoprotein (HDL)-cholesterol was determined by measuring cholesterol in the supernatant liquid after precipitation of the plasma with $MgCl_2$ and dextran sulfate (31). LDL-chol was calculated according to Friedewald, Levy, and Fredrickson (32). Apolipoprotein A-I (apoA-I) (33) and apoB levels (34) were determined by RIA methods. The coefficients of laboratory variation were calculated from both CDC serum pools and internal laboratory quality control plasma pools at multiple analyte levels. They were: 2.5% for cholesterol, 2.7% for triglycerides, 3.7% for HDL-chol, 5.2% for LDL-chol, 7.0% for apoA-I, and 9.0% for apoB measurements.

The levels of Lp[a] were determined by an enzyme-linked immunoassay (ELISA) using a method previously described (35). Rabbits were immunized with Lp[a] and an antiserum that was nonspecific for Lp[a] was prepared. The rabbit anti-Lp[a] serum was absorbed with human LDL and the resultant LDL-antiapoB precipitate was removed. The supernatant yielded a single precipitin line with Lp[a], but no reaction with LDL Ouchterlouny gels. Patient samples were stored at 4°C and analyzed within 48 h. Purified Lp[a] was isolated from the plasma of individuals with high levels of this lipoprotein. After measurement of its protein content and immunoreactivity, it was used as a primary standard in all assays. The standard dilution curve was linear with a correlation coefficient of 0.90. Thus, Lp[a] levels reported represent the total protein moiety of Lp[a]. Intra- and inter-assay coefficients of variation were 4% and 9.0%, respectively. For normal Lp[a] protein levels in the range of 1 to 10 mg/dl, the contributions of plasminogen at physiologic concentrations (200 mg/dl) was negligible (36).

Statistical methods

Statistical analysis for the comparison of two dietary phases in the first protocol was performed using a paired *t*-test. Comparison for statistical significance among the four diets used ANOVA. For nonparametric statistics, the Kendall rank correlation was performed to test comparison of analytes among the four diets in the second dietary protocol (37). A P value of <0.05 was considered significant. Each analyte value represents the mean of two separate blood samples during the respective dietary phase. Lp[a] and plasma triglyceride data were sig-

TABLE 1. Summary of dietary intake

Diet	Percent Total Calories			Chol	Percent of Total Fatty Acid			
	CHO	Protein	Fat		16:0	18:0	18:1	18:2
				mg/day				
Outpatient protocol[a]								
Saturated	46.8 (9.0)	15.0 (3.8)	37.6 (7.8)	311 (162)	20 (3)	10 (2)	34 (5)	15 (6)
Sat. + Chol	44.0 (8.2)	16.2 (3.5)	41.3 (6.8)	1430 (198)	21 (2)	10 (1)	34 (4)	14 (4)
Inpatient protocol[b]								
Saturated	40.4 (1.5)	16.7 (0.8)	44.0 (1.1)	279 (27)	17 (2)	7 (2)	44 (4)	15 (5)
Sat. + Chol	40.7 (2.2)	17.2 (1.6)	43.1 (3.0)	1434 (47)	17 (2)	7 (1)	45 (4)	16 (4)
Polyunsat.	39.5 (0.7)	17.1 (1.2)	44.4 (1.8)	220 (48)	12 (1)	5 (1)	23 (2)	48 (2)
Poly. + Chol	39.2 (2.1)	16.2 (2.2)	45.1 (4.1)	1368 (30)	13 (1)	5 (1)	22 (1)	47 (2)

[a]All foods in each diet were assessed for 7 days during the dietary regimen. Data are expressed as mean (SD) in a random subset of 20 of the 81 subjects.
[b]All foods in each diet were assessed for 7 days during the dietary regimen. Data are expressed as mean (SD) in 14 subjects.

nificantly skewed. These data were examined either in non-log or log-transformed data sets.

RESULTS

Outpatient studies

The actual dietary intake as assessed by food records is given in **Table 1.** While the study objective was achieved in terms of dietary cholesterol, the increase in dietary cholesterol was associated with increases in dietary fat at the expense of carbohydrate. Mean baseline total plasma cholesterol levels increased significantly by 12% ($P < 0.02$), while mean plasma triglyceride and apoA-I levels remained unaltered on the egg-supplemented diet (**Table 2**). Elevation of total cholesterol levels on the cholesterol-rich diet was reflected in significant increases of LDL-chol (17%, $P < 0.001$), HDL-chol (5.4%, $P < 0.001$) and apoB (15%, $P < 0.001$) levels. In contrast to LDL-chol and apoB elevations, mean baseline Lp[a] levels remained unchanged on the cholesterol-rich diet ($P < 0.911$).

The relationship between Lp[a] and LDL-chol was examined in each diet; there was no correlation between LDL-chol and Lp[a] levels. There were significant, although weak, correlations between log Lp[a] and LDL-chol levels both on the baseline diet ($r = 0.365$, $P = 0.008$) and on the cholesterol-rich diet, ($r = 0.275$, $P = 0.013$). When the changes in log Lp[a] from the cholesterol-rich diet were plotted against the changes of LDL-chol, no correlation was observed either when the changes were expressed in absolute values (**Fig. 2**) or as a percentage (data not shown).

Similarly, there was a significant correlation of log Lp[a] levels with apoB levels on the baseline diet ($r = 0.335$, $P = 0.002$). This correlation was not maintained on the cholesterol-rich diet ($r = 0.207$, $P = 0.065$). No correlation was found between log Lp[a] and apoB changes that resulted from the addition of six eggs to the diet ($r = 0.002$, $P = 0.986$, **Fig. 3**). As expected, strong significant correlations between LDL-chol and apoB were observed on the baseline ($r = 0.635$) and the cholesterol-rich diets ($r = 0.623$). No correlation of Lp[a] or log Lp[a] was observed under any of these conditions to apoA-I or HDL-chol.

We examined whether the baseline concentration of either Lp[a] or LDL-chol affected the response of either analyte to the cholesterol-rich diet. Therefore, all subjects were ranked in quartiles, by ascending order, according to their baseline Lp[a] or LDL-chol levels (**Fig. 4**). The mean percent response of LDL-chol on the cholesterol-rich diet did not differ among the quartiles derived from LDL-chol (panel A) or Lp[a] (panel B). Variability, expressed as standard deviation, of the LDL-chol response tended to be higher at the lowest levels of baseline LDL-

TABLE 2. Effects of dietary cholesterol on plasma lipid, lipoprotein, and apolipoprotein levels in 81 outpatients

Diet	Chol	TG	HDL-Chol	LDL-Chol	ApoA-I	ApoB	Lp[a]
Sat.	171 (27)	82 (32)	52 (12)	102 (26)	128 (24)	76 (24)	5.5 (6.1)
Sat. + six eggs/day	192 (35)[a]	85 (38)	55 (13)[b]	120 (33)[b]	130 (23)	88 (29)[b]	5.6 (6.4)

Results are given as mean (SD) of two separate blood draws in mg/dl.
[a]$P < 0.02$ and [b]$P < 0.001$ (paired t-test).

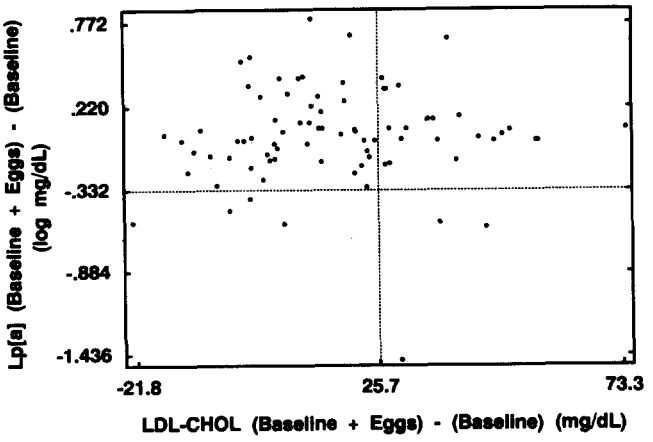

Fig. 2. Correlation between changes in plasma log Lp[a] and LDL-chol levels observed in 81 males upon increasing dietary cholesterol.

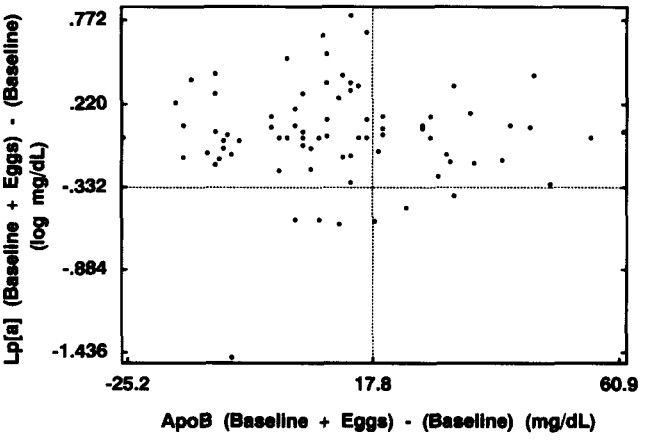

Fig. 3. Correlation between changes in plasma log Lp[a] and apoB levels observed in 80 males upon increasing dietary cholesterol.

chol and Lp[a], but the differences in variability among the quartiles were not significant. The mean percent response of Lp[a] levels on the cholesterol-rich diet tended to be higher in the lower baseline quartiles of either LDL-chol (panel C) or Lp[a] (panel D), but these trends were not statistically significant. Lp[a] variability fluctuated and lower variability tended to be associated with higher baseline Lp[a] levels. These trends were not statistically significant and may have resulted from analytical variability at low Lp[a] plasma concentrations.

Inpatient study

Fourteen subjects from the outpatient phase were studied under strict metabolic control on four diets

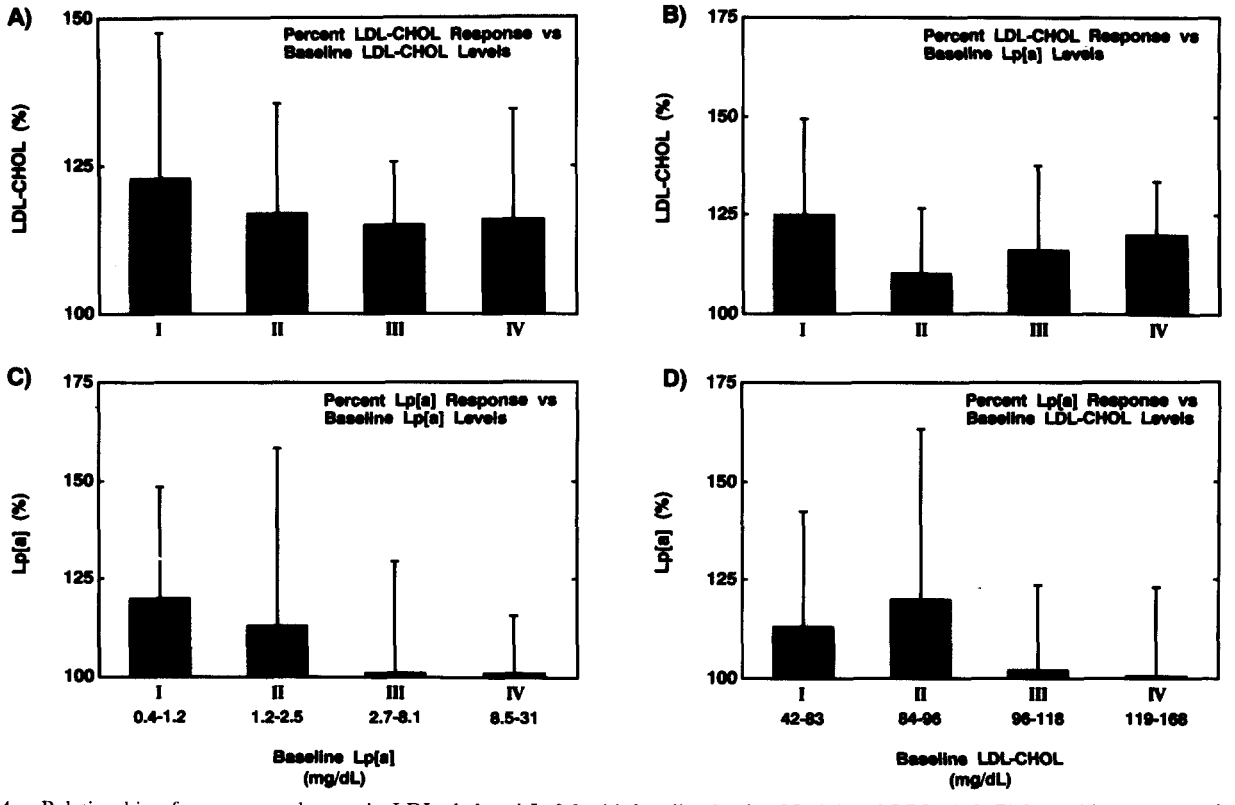

Fig. 4. Relationship of percentage changes in LDL-chol and Lp[a] with baseline levels of Lp[a] and LDL-chol. Eighty subjects were ranked by quartiles either by baseline Lp[a] levels (panels A and C) or baseline LDL-chol levels (panels B and D). The responses to a cholesterol-rich diet, expressed as a percent of baselines levels, are shown for LDL-chol (panels A and B) and Lp[a] (panels C and D). The enclosed boxes and bars refer to changes in the mean and SD, respectively.

TABLE 3. Dietary effects on plasma lipid, lipoprotein, and apolipoprotein levels in 14 inpatients

Diet	Chol	TG	HDL-Chol	LDL-Chol	ApoA-I	ApoB	Lp[a]
Sat.	152 (30)	64 (32)	51 (11)	88 (28)[c]	137 (19)	66 (16)	4.4 (4.6)
Sat. + eggs	178 (28)[a]	64 (22)	54 (12)	112 (32)[c,d]	137 (13)	79 (21)[e,f]	4.9 (5.1)
Poly.	137 (23)[a,b]	58 (14)	48 (7)	77 (26)[d]	140 (46)	53 (17)[e]	4.3 (4.3)
Poly. + eggs	160 (34)[b]	61 (18)	53 (11)	96 (33)	157 (40)	63 (21)[f]	4.2 (4.1)

Data are expressed as mean (SD) in mg/dl. These diets are described in Methods.
[a-f]pairs differing at $P < 0.05$ by analysis of variance.

(Table 1). In these subjects, all the dietary goals of the inpatient phase were achieved. The proportions of carbohydrate, fat, and protein were similar in all diets and the dietary cholesterol levels changed as designed. Subjects fed the cholesterol-rich saturated fat diet had elevations of 27% ($P < 0.05$) in LDL-chol and 19% in apoB levels above the saturated fat diet levels, while no significant increases in HDL-chol or apoA-I levels were observed (**Table 3**). Subjects progressing from the cholesterol-rich saturated fat diet to the diet high in polyunsaturated fat showed decreases in LDL-chol of 31% ($P < 0.05$) and in apoB levels of 33% ($P < 0.05$), but HDL-chol and apoA-I levels did not change. Subjects were then switched from the low cholesterol, polyunsaturated fat diet to a similar diet containing excess cholesterol. As a result, elevations of LDL-chol (25%), apoB (19%), HDL-chol (10%), and apoA-I (12%) were observed. Mean changes in Lp[a] were 11.3%, 12.3%, and 0% in progressing through the four diets. These tendencies in Lp[a] changes did not achieve statistical significance and were less than half of those observed for LDL-chol. Individual LDL-chol and Lp[a] responses to the four diets are shown in **Fig. 5** and **Fig. 6**, respectively. No significant correlations were present between changes of log Lp[a] and either LDL-chol or apoB on any of the inpatient diets.

DISCUSSION

The main objective of this study was to determine the effect of diet on plasma levels of Lp[a]. To maximize dietary-induced effects on lipoprotein concentrations, rather extreme diets known to induce significant changes in LDL-chol were chosen. Our study diets did affect plasma lipid and lipoprotein levels as expected from previous studies. Changes in LDL-chol and HDL-chol were nearly identical to those described by us nearly a decade ago in a different population (38). The new finding of this study is that Lp[a] plasma levels showed only minimal response to these dietary perturbations both in the outpatient study in 81 subjects and in the rigorously controlled inpatient study in 14 subjects. Our study further establishes the insensitivity of plasma Lp[a] levels to diets in humans that was previously suggested by earlier obser-

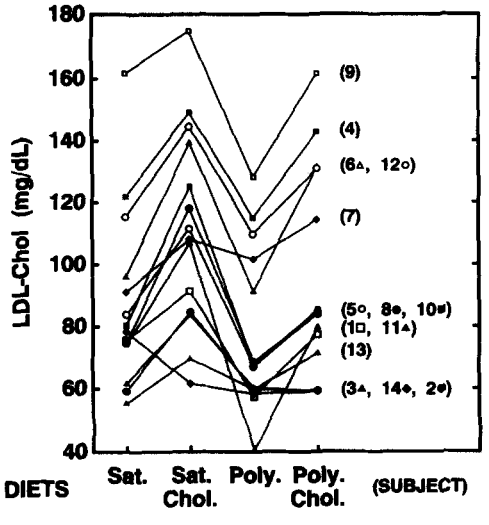

Fig. 5. Dietary effects on LDL-cholesterol levels in 14 inpatient subjects. LDL-chol was measured on each of the four inpatient diets. Individual subjects are identified by a designated number and symbol.

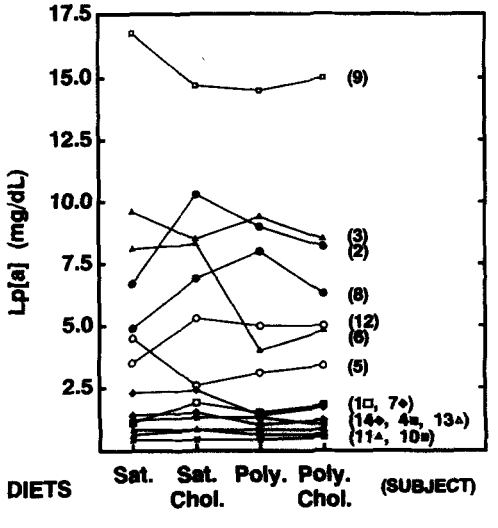

Fig. 6. Effects of diets on Lp[a] levels in 14 inpatient subjects. Lp[a] was determined on each of the four inpatient diets. Individual subjects are identified by a designated number and symbol.

vations (16–18). Furthermore, our study is consistent with results obtained in baboons showing no or very little effect of atherogenic diets on plasma Lp[a] levels (39, 40).

Because of the large number of subjects studied, a number of interesting findings emerged. Unexpectedly, the magnitude and variability of LDL-chol or apoB response to cholesterol-rich diets did not depend on basal levels of these analytes. Furthermore, each of the quartiles, ranked by baseline LDL-chol, contained individuals who did or did not increase LDL-chol as a result of excess dietary cholesterol. These findings indicate that the mechanism(s) controlling an individual's response to dietary cholesterol are operative at and insensitive to a wide range of LDL-chol levels. Similarly, Lp[a] response to dietary cholesterol was independent of either baseline LDL-chol, apoB, or Lp[a] concentrations. Lp[a] exists in multiple phenotypic forms that are inversely associated with Lp[a] plasma levels (41, 42). Although we did not analyze Lp[a] phenotypes in our study subjects, our results suggest that Lp[a] phenotypes play no major role in the response of plasma LDL and Lp[a] levels to the diets used in this study.

The lack of Lp[a] response to our diets is not too surprising since genetic factors are thought to be more important than environmental factors in determining Lp[a] levels in plasma (9, 10). Recently, Utermann et al. (20) showed that the LDL receptor gene locus can affect plasma levels of Lp[a]. This interaction appeared multiplicative as Lp[a] concentrations corrected for phenotype effects appeared to be threefold higher in subjects carrying a functionally defective allele for the LDL receptor locus. The biochemical mechanism explaining this interaction has not been clarified. If the elevation of Lp[a] observed in FH patients was solely due to reduced catabolism of Lp[a] via a reduced number of LDL receptors, we should have observed significant increases of Lp[a] along with LDL-chol in our study subjects, as previous studies have demonstrated a down-regulation of LDL-receptor expression by excess dietary cholesterol (25, 26, 42). Conversely, enhanced expression of the LDL receptor pathway by the polyunsaturated, low cholesterol diet should have resulted in lowering of both LDL and Lp[a] (43). Since the affinity of Lp[a] to the LDL receptor may be lower than that of LDL (24), a lower response of Lp[a] may be expected. Indeed, mean levels of Lp[a] showed a tendency to decrease as a result of the polyunsaturated, low cholesterol diet, but this difference was not statistically significant. Thus, it is possible that small changes in Lp[a] that might have occurred were not detected due to intra-individual and analytical variability. However, inspection of data in Figs. 5 and 6 shows no consistent trend of Lp[a] to any diet, but reveals that the small differences in mean Lp[a] levels in going from the saturated fat, high cholesterol diet to the polyunsaturated fat, low cholesterol diet was primarily due to subject 6.

Results on the importance of the LDL receptor in the removal of Lp[a] from the circulation are at variance (21–24, 44) and synthesis of Lp[a] may indeed be a more important determinant of Lp[a] plasma levels (45). Excess dietary cholesterol reduces endogenous cholesterol synthesis (46, 47). In heterozygotes for familial hypercholesterolemia though, endogenous cholesterol synthesis may either be similar or increased compared to nonaffected individuals (48, 49). The results in the dietary and genetic models may thus result from differences in one or more regulatory steps determining the metabolism of Lp[a] and point to an interaction between hepatic cholesterol and Lp[a] synthesis that may determine plasma levels of Lp[a] attained.

Our data were derived from subjects who were normocholesterolemic and normotriglyceridemic but had a wide range of Lp[a] levels. Thus, as our study population consisted of healthy, normolipidemic males, our conclusions may not necessarily be extrapolated to subjects with dyslipoproteinemic disorders. Furthermore, long term dietary effects might differ from those observed in these short term studies.

Elevated levels of Lp[a] are associated with increased risk of coronary heart disease and the risk is reported to be potentiated by high levels of LDL (50, 51). Although diet does not alter Lp[a] levels, an LDL-lowering diet would seem prudent not only for decreasing LDL levels but to decrease the interactive risk resulting from high Lp[a] levels in the presence of elevated LDL-chol levels.

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REFERENCES

1. Dahlen, G., K. Berg, T. Gillnas, and C. Ericson. 1975. Lp[a]: pre-beta-lipoprotein in Swedish middle-aged males and in patients with coronary heart disease. *Clin. Chem.* **7:** 334–341.
2. Kostner, G., P. Avogaro, G., Cazzolato, E. Marth, G. Bittolo-Bon, and G. B. Quinci. 1981. Lipoprotein Lp[a] and the risk for myocardial infarction. *Atherosclerosis.* **38:** 51–61.
3. Koltringer, P., and G. Jurgens. 1985. A dominant role of lipoprotein[a] in the investigation and evaluation of parameters indicating the development of cervical atherosclerosis. *Atherosclerosis.* **58:** 187–198.
4. Dahlen, G., J. R. Guyton, M. Arrar, J. A. Farmer, J. A. Kautz, and A. M. Gotto, Jr. 1986. Association of levels of lipoprotein Lp[a], plasma lipids and other lipoproteins with coronary heart disease documented by angiography. *Circulation.* **74:** 758–765.
5. Rhoads, G. G., G. Dahlen, K. Berg, N. E. Morton, and A. L. Dannenberg. 1986. Lp[a] lipoprotein as a risk factor

for myocardial infarction. *J. Am. Med. Assoc.* **256:** 2540–2544.
6. Morrisett, J. D., J. R. Guyton, J. W. Gaubatz, and A. M. Gotto, Jr. 1987. Lipoprotein[a]: structure, metabolism and epidemilogy. *In* Plasma Lipoproteins. A. M. Gotto, Jr., editor. Elsevier Science Publishers B.V., New York. 129–152.
7. Utermann, G. 1990. The mysteries of lipoprotein[a]. *Science.* **246:** 904–910.
8. Loscalzo, J. 1990. Lipoprotein[a], a unique risk factor for atherothrombotic disease. *Arteriosclerosis.* **10:** 672–679.
9. Sing, C., J. S. Schultz, and D. C. Schreffler. 1974. The genetics of the Lp antigen. II. A family study and proposed models of genetic control. *Ann. Hum. Genet. (London)* **38:** 47–56.
10. Albers, J. J., P. Wahl, and W. R. Hazzard. 1974. Quantitative genetic studies of the human plasma Lp[a] lipoprotein. *Biochem. Genet.* **11:** 475–486.
11. Gurakar, A., J. M. Hoeg, G. M. Kostner, N. M. Papadoulous, and H. B. Brewer. 1985. Levels of lipoprotein Lp[a] decline with neomycin and niacin treatment. *Atherosclerosis.* **57:** 293–301.
12. Kostner, G. M., D. Gavish, B. Leopold, K. Bolzano, M. S. Weintraub, and J. L. Breslow. 1989. HMG-CoA reductase inhibitors lower LDL-cholesterol without reducing Lp[a] levels. *Circulation.* **80:** 1313–1319.
13. Thiery, J., V. W. Armstrong, J. Schleef, C. Creutzfeldt, W. Creutzfeldt, and D. Seidel. 1988. Serum lipoprotein Lp[a] concentrations are not influenced by an HMG-CoA reductase inhibitor. *Klin. Wochenschr.* **66:** 462–463.
14. Albers, J. J., H. M. Taggart, D. Applebaum-Bowden, S. Haffner, C. H. Chestnut III, and W. R. Hazzard. 1984. Reduction of lecithin:cholesterol acyltransferase, apolipoprotein D and the Lp[a] lipoprotein with the anabolic steroid Stanozolol. *Biochim. Biophys. Acta.* **795:** 293–296.
15. Farmer, J. A., C. M. Ballantyne, W. Patsch, J. D. Morrisett, C. Payton-Ross, O. H. Frazier, A. M. Gotto Jr., and J. B. Young. 1990. Lp[a] and apolipoprotein changes after heart transplant. *Arteriosclerosis.* **10:** 844a.
16. Albers, J. J., V. G. Cabana, G. R. Warnick, and W. R. Hazzard. 1975. Lp[a]: relationship to sinking pre-b lipoprotein, hypercholesterolemia, and apolipoprotein B. *Metabolism.* **24:** 1047–1054.
17. Albers, J. J., J. L. Adolphson, and W. R. Hazzard. 1977. Radioimmunoassay of human plasma Lp[a] lipoprotein. *J. Lipid Res.* **18:** 331–338.
18. Masarei, J. R. L., I. L. Rouse, W. J. Lynch, K. Robertson, R. Vandongen, and L. J. Beilin. 1984. Effects of a lacto-ovo vegetarian diet on serum concentration of cholesterol, triglyceride, HDL-C, HDL^2-C, HDL^3-C and Lp[a]. *Am. J. Clin. Nutr.* **40:** 468–479.
19. Hermann, W., J. Biermann, H. G. Lindhof, and G. Kostner. 1989. Modification of the atherogenic risk factor Lp[a] by supplementary fish oil administration in patients with moderate physical training. *Med. Klin.* **84:** 429–433.
20. Utermann, G., F. Hopplichler, H. Dieplinger, M. Seed, G. Thompson, and E. Boerwinkle. 1989. Defects in the low density lipoprotein receptor gene affect lipoprotein[a] levels: multiplicative interaction of two gene loci associated with premature atherosclerosis. *Proc. Natl. Acad. Sci. USA.* **86:** 4171–4174.
21. Hofmann, S. A., D. L. Eaton, M. S. Brown, W. J. McConathy, J. L. Goldstein, and R. L. Hammer. 1990. Overexpression of human low density lipoprotein receptors leads to accelerated catabolism of Lp[a] lipoprotein in transgeneic mice. *J. Clin. Invest.* **85:** 1542–1547.
22. Maartmann-Moe, K., and K. Berg. 1981. Lp[a] lipoprotein enters cultured fibroblasts independently of the plasma membrane low density lipoprotein receptor. *Clin. Genet.* **20:** 352–362.
23. Armstrong, V. W., A. K. Walli, and D. Seidel. 1985. Isolation, characterization, and uptake in human fibroblasts of an apo[a]-free lipoprotein obtained on reduction of lipoprotein[a]. *J. Lipid Res.* **26:** 1314–1323.
24. Armstrong, V. W., B. Harrach, H. Robenek, M. Helmhold, A. K. Walli, and D. Seidel. 1990. Heterogeneity of human lipoprotein Lp[a]: cytochemical and biochemical studies on the interaction of two Lp[a] species with the LDL receptor. *J. Lipid Res.* **31:** 429–441.
25. Packard, C. J., L. McKinney, K. Karr, and J. Shepherd. 1983. Cholesterol feeding increases low density lipoprotein synthesis. *J. Clin. Invest.* **72:** 45–51.
26. Mistry, F. N., N. E. Miller, M. Laker, W. B. Hazzard, and B. Lewis. 1981. Individual variation in the effects of dietary cholesterol on plasma lipoproteins and cellular cholesterol hemostasis in man. *J. Clin. Invest.* **67:** 493–502.
27. Spady, D. K., and J. M. Dietschy. 1988. Interaction of dietary cholesterol and triglycerides in the regulation of hepatic low density lipoprotein transport in the hamster. *J. Clin. Invest.* **81:** 300–309.
28. 1979 Build Study. 1983. Society of Actuaries and Association of Life Insurance Medical Directors of America. Metropolitan Life Insurance Company.
29. Siedel, J., E. Hagele, J. Ziegenhorn, and A. W. Wahlenfeld. 1983. Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. *Clin. Chem.* **29:** 1075–1080.
30. Nagele, U., E. Hagele, G. Sauer, E. Wiedermann, P. Lehmann, A. W. Wahlefeld, and W. Gruber. 1984. Reagent for the enzymatic determination of serum total triglyceride with improved lipolytic efficiency. *J. Clin. Chem. Clin. Biochem.* **22:** 165–174.
31. Warnick, G. R., J. Benderson, and J. J. Albers. 1982. Dextran sulfate-Mg^{2+} precipitation procedure for quantification of high-density-lipoprotein cholesterol. *Clin. Chem.* **28:** 1379–1388.
32. Friedewald, W. T., R. I. Levy, and D. S. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18:** 499–502.
33. Brown, S. A., C. E. Rhodes, K. Dunn, A. M. Gotto, Jr., and W. Patsch. 1988. Effect of blood collection and processing on radioimmunoassay results for apolipoprotein A-I in plasma. *Clin. Chem.* **34:** 920–924.
34. Brown, S. A., D. F. Epps, J. K. Dunn, A. R. Sharrett, J. R. Patsch, A. M. Gotto, Jr., and W. Patsch. 1990. Effect of blood collection and processing on radioimmunoassay results for apolipoprotein B in plasma. *Clin. Chem.* **36:** 1662–1666.
35. Gaubatz, J. W., G. L. Cushing, and J. D. Morrisett. 1986. Quantitation, isolation, and characterization of human lipoprotein[a]. *Methods Enzymol.* **129:** 167–186.
36. Gaubatz, J. W., K. I. Ghanem, J. Guevara, M. L. Nava, W. Patsch, and J. D. Morrisett. 1990. Polymorphic forms of human apolipoprotein[a]: Inheritance and relationship of their molecular weights to plasma levels of lipoprotein[a]. *J. Lipid Res.* **31:** 603–613.
37. Snedecor, G. W., and W. G. Cochran. 1980. Statistical Methods. 7th ed. Iowa State University Press, Ames, IA. 192–193.
38. Schonfeld, G., W. Patsch, L. L. Rudel, C. Nelson, M. Ep-

stein, and R. E. Olson. 1982. Effects of dietary cholesterol and fatty acids on plasma lipoproteins. *J. Clin. Invest.* **69:** 1072–1080.
39. Rainwater, D. L., G. S. Manis, and J. L. VandeBerg. 1989. Hereditary and dietary effects of apolipoprotein[a] isoforms and Lp[a] in baboons. *J. Lipid Res.* **30:** 549–558.
40. Neven, L., A. Khalil, D. Pfaffinger, G. M. Fless, E. Jackson, and A. M. Scanu. 1990. Rhesus monkey model of familial hypercholesterolemia: relation between plasma Lp[a] levels, apo[a] isoforms, and LDL-receptor function. *J. Lipid Res.* **31:** 633–643.
41. Utermann, G., H. G. Kraft, H. J. Menzel, T. Hopferwieser, and C. Seitz. 1988. Genetics of the quantitative Lp[a] lipoprotein trait. I. Relation of Lp[a] glycoprotein phenotypes to Lp[a] lipoprotein concentrations in plasma. *Hum. Genet.* **78:** 41–46.
42. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science.* **232:** 34–41
43. Ventura, M. A., L. A. Woollett, and D. K. Spady. 1989. Dietary fish oil stimulates hepatic low density lipoprotein transport in the rat. *J. Clin. Invest.* **84:** 528–537.
44. Krempler, F., G. M. Kostner, A. Roscher, F. Haslauer, K. Bolzano, and F. Sandhofer. 1983. Studies on the role of specific cell surface receptors in the removal of lipoprotein[a] in man. *J. Clin. Invest.* **71:** 1431–1441.
45. Krempler, F., G. M. Kostner, K. Bolzano, and F. Sandhofer. 1980. Turnover of lipoprotein[a] in man. *J. Clin. Invest.* **65:** 1483–1490.
46. Quintao, E., S. M. Grundy, and E. H. Ahrens, Jr. 1971. Effect of dietary cholesterol on the regulation of total body cholesterol in man. *J. Lipid Res.* **12:** 233–247.
47. McNamara, D. J., R. Kolb, T. S. Parker, H. Batwin, P. Samuel, C. D. Brown, and E. H. Ahrens, Jr. 1987. Heterogeneity of cholesterol homeostasis in man. Response to changes in dietary fat quality and cholesterol quantity. *J. Clin. Invest.* **79:** 1729–1739.
48. Schwarz, K. B., J. Witzum, G. Schonfeld, S. M. Grundy, and W. E. Conner. 1979. Elevated cholesterol and bile acid synthesis in a young patient with homozygous familial hypercholesterolemia. *J. Clin. Invest.* **64:** 756–760.
49. Bilheimer, D. W., J. L. Goldstein, S. M. Grundy, and M. S. Brown. 1975. Reduction in cholesterol and low density lipoprotein synthesis after portacaval shunt surgery in a patient with homozygous familial hypercholesterolemia. *J. Clin. Invest.* **56:** 1420–1430.
50. Armstrong, V. M., P. Cremer, E. Eberle, A. Manke, F. Schulze, H. Wieland, H. Krezer, and D. Seidel. 1986. The association between serum Lp[a] concentrations and angiographically assessed coronary atherosclerosis. Dependence on serum LDL levels. *Atherosclerosis.* **62:** 249–257.
51. Seed, M., F. Hoppichler, D. Reavely, S. McCarthy, G. R. Thompson, E. Boerwinkle, and G. Utermann. 1990. Relation of serum lipoprotein[a] concentration and apolipoprotein[a] phenotype to coronary heart disease in patients with familial hypercholesterolemia. *N. Engl. J. Med.* **332:** 1494–1499.