Effects of a low fat diet with and without intermittent saturated fat and cholesterol ingestion on plasma lipid, lipoprotein, and apolipoprotein levels in normal volunteers

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Abstract Diets low in saturated fat and cholesterol are recommended to the American public for improving plasma lipoprotein patterns and reducing the risk of heart disease. However, since dietary intake cannot always be controlled, the effects of different degrees of dietary saturated fat lowering and occasional high saturated fat and cholesterol meals on the expected lipoprotein pattern improvement of these diets needs to be defined. In the current study, we compared lipid, lipoprotein, and apolipoprotein levels in 14 young normal volunteers on a metabolic ward when they were consuming a high saturated fat diet (42% fat), an AHA Phase II diet (25% fat), and a third diet which approximated the AHA Phase I diet (30% fat). The latter actually consisted of intermittent ingestion of meals high in saturated fat and cholesterol on the background of an AHA Phase II diet (Intermittent Saturated Fat diet). When compared to the high saturated fat diet, the AHA Phase II diet significantly reduced total, low density lipoprotein (LDL), and high density lipoprotein (HDL) cholesterol, apoB, and apoA-I levels, and improved the LDL/HDL cholesterol ratio, whereas the intermittent saturated fat diet lowered total and LDL cholesterol and apoB levels, and also improved the LDL/HDL cholesterol ratio. When compared to the AHA Phase II diet, the intermittent saturated fat diet raised total and HDL cholesterol levels. Thus, in these normal volunteers, intermittent saturated fat ingestion, in the context of an overall 30% fat diet and a 25% fat diet, did not differ with respect to the effect on improving the LDL/HDL cholesterol ratio. — **Denke, M. A., and J. L. Breslow.** Effects of a low fat diet with and without intermittent saturated fat and cholesterol ingestion on plasma lipid, lipoprotein, and apolipoprotein levels in normal volunteers. *J. Lipid Res.* 1988. **29:** 963–969.

Supplementary key words diet responsiveness • saturated fat • cholesterol • lipoproteins • apolipoproteins • AHA Phase II diet • LDL cholesterol • HDL cholesterol • apoA-I • apoB

Prompted by the results of The Lipid Research Clinics Coronary Primary Prevention Trial (1), demonstrating that decreasing total and LDL cholesterol levels reduces cardiovascular events, an NIH consensus panel (2) recently recommended that all Americans above 2 years of age adopt a low fat diet to improve their lipid profile. Specific diet recommendations include reducing dietary fat from 40 to 30% of calories, reducing saturated fat to 10% of calories, increasing polyunsaturated fat to no more than 10% of calories, and limiting cholesterol intake to 300 mg/day. This has also been recommended by the American Heart Association (AHA) and called a Phase I diet (3). For individuals on this diet whose cholesterol levels remain greater than the 75th percentile (> 240 mg/dl), the NIH consensus panel recommended an even more stringent lowering of dietary fat to 25% of calories with only 8% from saturated fat and limiting cholesterol intake to 200 mg/day. This is the equivalent of the AHA Phase II diet.

These dietary recommendations appear to be sensible in light of the ample data in the literature showing that changing from an average American diet to one lower in saturated fat and cholesterol reduces total and LDL cholesterol levels (4–10). However, the benefits of progressing from a 30% to a 25% fat diet cannot be regarded as proven. There are few studies examining the effects of different degrees of dietary fat lowering in the same individuals (11), so we cannot be sure of how much additional LDL cholesterol lowering will result. Moreover, recent evidence indicates that low fat diets may actually reduce HDL cholesterol levels (11–14) and this might adversely affect cardiovascular disease risk. These considerations make it urgent that we expand our knowledge of the effects on lipoprotein levels of progressing from an AHA Phase I to a Phase II diet.

Abbreviations: LDL, low density lipoprotein; HDL, high density lipoprotein; ELISA, enzyme-linked immunosorbent assay.

Another important issue when counselling a 25% fat diet is the difficulty of strict adherence and the consequences of dietary indiscretions. One can, with effort, establish a pattern of eating an AHA Phase II diet at home. However, in our complex society, many of our meals are taken outside the home and it is almost impossible to avoid occasional meals that are high in saturated fat and cholesterol. It is not known whether such meals have a major or a minor impact on undoing the beneficial effect of a low fat diet on the plasma lipoprotein pattern. This practical dilemma arises for physicians and patients almost daily and additional knowledge on this subject is required for proper dietary counselling.

In the current study, we have tried to address these issues by comparing lipid, lipoprotein, and apolipoprotein levels in normolipidemic subjects consuming a high saturated fat diet, an AHA Phase II diet, and a third diet that approximates an AHA Phase I diet overall. The latter actually consists of intermittently ingesting meals high in saturated fat and cholesterol while mainly consuming an AHA Phase II diet. Although many real life dietary indiscretions are also higher in calories, to avoid confounding weight gain with diet change, our study examined an isocaloric substitution of a high saturated fat meal for a low one. It was found that, compared to the high saturated fat diet, the AHA Phase II diet lowered total, LDL, and HDL cholesterol, apoB, and A-I levels, with an improvement in the LDL/HDL cholesterol ratio. The addition of a meal high in saturated fat and cholesterol to the AHA Phase II diet every other day, while significantly elevating total and HDL cholesterol, did not raise the LDL/HDL cholesterol or apoB/apoA-I ratios.

METHODS

Study subjects

Fourteen normal volunteers between the ages of 19 and 21 years were hospitalized in the Rockefeller University Hospital General Clinical Research Center for a 77-day metabolic diet study. Subject characteristics including age, sex, apoE phenotype, height, weight, quetelet index, and ad lib lipid and lipoprotein levels are listed in **Table 1.** During the study, subjects were not on medications and none of them were participating in strenuous physical exercise programs.

Dietary manipulations

There were three 24-day diet periods separated by 2–3 days of ad lib feeding. The caloric requirement for each subject was initially estimated using the Harris-Benedict equation (15) and adjustments were made, when necessary, to maintain a steady weight during the entire period of study. Subjects were asked to eat all of the food served to them and maintain their physical activity at a constant rate.

Diets consisted of natural foods, whose composition had been determined by the USDA and listed in the Handbook 8 food tables (16). For variety, 2-day menus were developed and rotated through each diet period. For each day, half of the calories were allocated to breakfast and lunch, and the other half to dinner and a snack. Each of these half-day periods were matched for their fat, protein, and carbohydrate content, including the type of fat and amount of cholesterol (**Fig. 1**).

The three diets were: *a*) AHA Phase II diet. This diet consisted of 25% of calories from fat with 6% saturated,

TABLE 1. Subject characteristics

Subject	Sex	Age	ApoE Phenotype	Height	Weight	Quetelet Index[a]	Lipid/Lipoprotein Levels[b]				
							Chol	TG	VLDL	LDL	HDL
		yr		*cm*	*kg*				*mg/dl*		
1	F	20	4/3	170	60	2.08	167	63	16	106	46
2	F	19	3/3	165	59	2.17	174	69	14	111	49
3	F	20	3/3	161	69	2.66	200	130	22	116	62
4	F	19	3/2	162	64	2.44	191	63	16	114	61
5	F	20	3/3	160	66	2.58	184	71	14	115	55
6	F	19	3/2	160	55	2.15	129	68	11	66	53
7	F	20	3/3	160	63	2.46	110	33	6	29	74
8	M	21	3/3	182	99	2.99	160	104	24	87	49
9	M	20	3/3	180	76	2.35	159	86	24	91	44
10	M	21	4/3	184	74	2.19	206	90	19	140	46
11	M	21	3/3	175	76	2.48	178	73	13	119	46
12	M	21	4/4	170	63	2.18	204	58	10	117	77
13	M	20	3/3	177	70	2.23	151	74	13	94	44
14	M	20	3/3	182	74	2.23	153	90	17	101	36

[a] Quetelet Index is weight (kg)/[height (cm)]^2 × 1000.

[b] Ad lib plasma lipid and lipoprotein levels (mean of two determination) for each subject prior to entry into the study; VLDL, LDL, and HDL are expressed in terms of their cholesterol concentration.

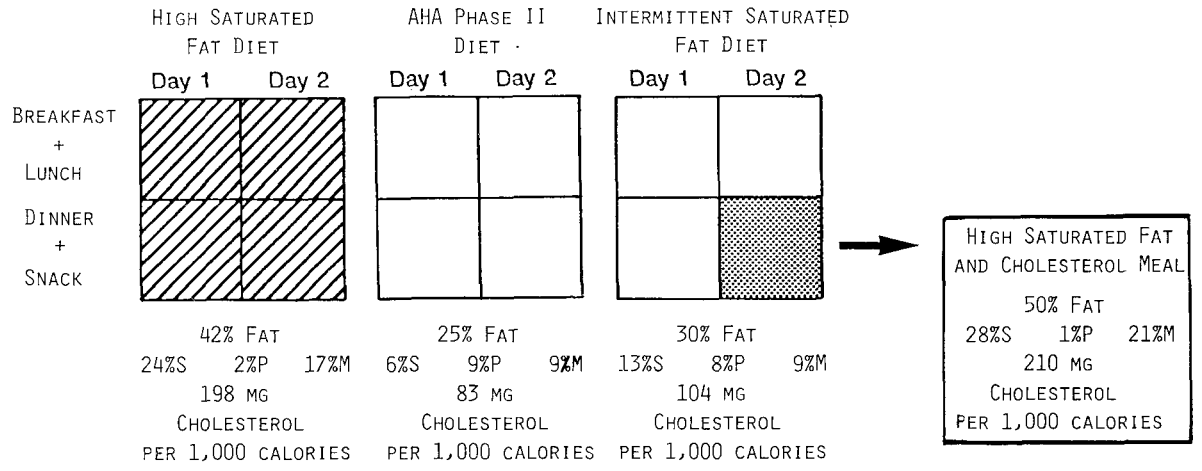

Fig. 1. Design of high saturated fat, AHA Phase II, and Intermittent Saturated Fat diets. Two-day menus were rotated through each diet period. All diets were calculated so that 50% of the calories were consumed during breakfast and lunch and 50% during dinner and an evening snack. These half-day diets were all matched in composition including grams of saturated and polyunsaturated fat and cholesterol. On day 2 of the 2-day rotating diet, the intermittent saturated fat diet substituted an isocaloric meal high in saturated fat and cholesterol for the dinner and evening snack. S, saturated; P, polyunsaturated; M, monounsaturated.

9% polyunsaturated, and 9% monounsaturated; 60% of calories from carbohydrate; and 15% of calories from protein. The ratio of polyunsaturated to saturated fats was 1.5, and the diet contained 83 mg of cholesterol per 1,000 calories.

b) High Saturated Fat diet. This diet consisted of 42% of calories from fat with 24% saturated, 2% polyunsaturated, and 17% monounsaturated; 43% of calories from carbohydrate; and 15% of calories from protein. The ratio of polyunsaturated to saturated fats was 0.08 and the diet contained 198 mg of cholesterol per 1,000 calories.

c) Intermittent Saturated Fat diet. This diet consisted principally of the AHA Phase II diet, but on each day-2 of this diet, a high saturated fat and cholesterol meal was substituted for the dinner and snack. Hence, for the 2-day period, 75% of calories came from the AHA Phase II diet and 25% of calories came from a meal high in saturated fat and cholesterol. The average dietary intake over the entire 2-day period was 30% of calories from fat with 13% saturated, 8% polyunsaturated, and 9% monounsaturated; 55% of calories from carbohydrate; and 15% from protein. The ratio of polyunsaturated to saturated fats was 0.6 and the diet contained 104 mg of cholesterol per 1,000 calories.

To adjust for possible diet order effects between the AHA Phase II and the intermittent saturated fat diet periods, the high saturated fat diet was assigned to the middle diet period and the first and third diet periods were randomized between the AHA Phase II diet and the intermittent saturated fat diet.

Lipid, lipoprotein and apolipoprotein analyses

A fasting blood specimen was drawn in EDTA for analysis at baseline and daily during the last 10 days of each of the three 24-day diet periods. Plasma was obtained and lipid and lipoprotein measurements were done on fresh unfrozen specimens. Aliquots of plasma were immediately frozen at −70°C for apolipoprotein determinations at a later date. Total cholesterol and triglycerides were determined enzymatically using Boehringer-Mannheim kits. HDL cholesterol was determined after apoB-containing lipoproteins were precipitated by the dextran sulfate-Mg^{2+} method (17). LDL plus HDL cholesterol was determined on the infranatant after removing VLDL cholesterol by airfuge ultracentrifugation (Beckman Instruments). LDL cholesterol was determined by subtracting the HDL cholesterol value from the cholesterol in the intranatant. VLDL cholesterol was determined by subtracting the cholesterol in the infranatant from the total cholesterol. Lipoprotein analyses were performed on each of the 10 specimens for each subject for each dietary period. Cholesterol and HDL cholesterol determinations were standardized by the Lipid Standardization Program of the Center for Disease Control in Atlanta, Georgia.

Frozen aliquots of plasma obtained on days 14, 15, 17, 20, and 22 of each diet period were assayed for apoA-I and apoB levels by sandwich ELISA. The assay plates were coated with antibody, either a monospecific polyclonal goat antibody to apoA-I (generously supplied by Dr. Peter Herbert) or a rabbit antibody to apoB. After overnight incubation and washing, the plates were treated with ELISA-grade BSA to block nonspecific binding sites. Multiple dilutions of antigen were then applied and incubated for 2 hr at 37°C. After rewashing, the plates were incubated for 2 hr with alkaline phosphatase-conjugated antibody. The plates were then rewashed, incubated with phosphatase substrate, and color development was read at 410 nm in a Dynatech plate reader (Dynatech, Alexandria,

VA). Assays were standardized with a reference serum pool supplied by the Center for Disease Control and two frozen control sera, whose apolipoprotein values were determined by three independent laboratories: Brown University/Miriam Hospital; the Lipid Metabolism Laboratory, USDA Human Nutrition Center on Aging; and the Northwest Lipid Research Clinic. Assays were rejected when the control sera values were more than 15% above or below their target value. To avoid potential bias from plate to plate variations, each assay plate contained one specimen from each of the three diets for the same subject.

Apolipoprotein E phenotyping

Five ml of plasma was added to each of two Quick-seal ultracentrifuge tubes, ½ × 2½ in. The tubes were filled to the top with saline, sealed, and spun at 39,000 rpm for 20 hr at 4°C using a 40.3 Beckman rotor. The floating VLDL layer was removed with a syringe and transferred to a glass tube for delipidation with ethanol–acetone 1:1 at a temperature of −20°C. The tubes were vortexed, kept at −20°C for 1 hr, spun, and the supernatant was discarded. The pellet was washed twice with cold ether, and dissolved in 0.2 to 0.3 ml of a buffer containing 6 M urea, 0.01 M Tris HCl, 0.01 M dithiothreitol, pH 8.6. Protein concentration was determined by a Bio-Rad assay using bovine serum albumin as a reference. A one-dimensional isoelectric focusing gel was prepared in a 6 M urea buffer containing 7.5% acrylamide, 0.2% N,N'-methylene-bis-acrylamide (BIS) and 2% ampholines of pH 4–6.5. The gel was prefocused for 1 hr at 45°C at 110 V, and then 50 μg of VLDL protein was applied to each gel lane. Focusing was carried out for 17 hr at 4°C at 250 V. After focusing, the gels were fixed in trichloroacetic acid–sulfosalicylic acid, stained with Coomassie Blue (0.1%) in methanol–water–acetic 4.5:4.5:1.0 and destained. Specimens from individuals with known apoE phenotypes were run along with new samples to aid in the interpretation of the results.

Statistical analysis

Mean lipid and lipoprotein levels were determined for each subject for the last 10 days of each diet period. Mean apolipoprotein levels were determined from 5 of the last 10 days of each diet period. To test for differences among the dietary periods, a multivariate analysis of variance for repeated measures was performed utilizing the BMDP statistical software package (18). Statistically significant differences among the dietary regimens were tested at the $P < 0.05$ level by the Wilks' Lambda and calculated overall F statistic. Under the protection of this significant multivariate F value, a univariate analysis of variance was performed for each dependent variable. Significant univariate test results were further analyzed by the post hoc procedure (Tukey pairwise comparison test) to isolate differences among the diets (19).

RESULTS

Group diet responses

Mean lipid, lipoprotein, and apolipoprotein values for the study subjects on each diet are listed in **Table 2.** Compared to the high saturated fat diet, the AHA Phase II diet significantly ($P < 0.01$) lowered the levels of total cholesterol 17%, LDL cholesterol 21%, HDL cholesterol 10%, apoB 13%, and apoA-I 8%. The LDL/HDL cholesterol ratio significantly improved from 2.00 to 1.77. Compared to the AHA Phase II diet, the intermittent saturated fat diet significantly increased the levels of total cholesterol 5% and HDL cholesterol 6% and the LDL/HDL and apoB/apoA-I ratios did not change significantly. Compared to the high saturated fat diet, the intermittent saturated fat diet significantly lowered the levels of total cholesterol 12%, LDL cholesterol 16%, and apoB 10%. The LDL/HDL cholesterol ratio significantly improved from 2.00 to 1.74. The apoB/apoA-I ratio did not significantly differ amongst all three diets.

TABLE 2. Lipid, lipoprotein, and apolipoprotein levels for each diet period

Fraction	High Saturated Fat Diet	AHA Phase II Diet	Intermittent Saturated Fat Diet
		mg/dl ± SD	
Chol	169 ± 28	142 ± 24[a]	149 ± 24[a,b]
TG	76 ± 23	75 ± 18	70 ± 16
VLDL	16 ± 5	14 ± 3	14 ± 3
LDL	100 ± 27	80 ± 22[a]	84 ± 22[a]
HDL	53 ± 12	48 ± 13[a]	51 ± 11[b]
ApoB	68 ± 16	59 ± 16[a]	61 ± 14[a]
ApoA-I	124 ± 26	114 ± 24[a]	119 ± 22
LDL/HDL	2.00 ± 0.67	1.77 ± 0.15[a]	1.74 ± 0.53[a]
ApoB/apoA-I	0.57 ± 0.18	0.54 ± 0.17	0.53 ± 0.16

[a] Significantly different from the high saturated fat diet by Tukey post hoc comparison test, $P < 0.01$.
[b] Significantly different from the AHA Phase II diet by Tukey post hoc comparison test, $P < 0.05$.

Individual diet responses

Mean total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, apoB, and apoA-I values for each of the 14 subjects on the three diets are plotted in **Fig. 2.** Even in this group of normal volunteers, absolute levels and responses to dietary change can be seen to vary greatly. An analysis was performed to see whether height, weight, quetelet index, or lipoprotein and apolipoprotein levels on the ad lib diet correlated with the magnitude of an individual's LDL or HDL cholesterol change when going from the high saturated fat to the AHA Phase II diet. The only significant correlations were between ad lib cholesterol and LDL cholesterol and the change in LDL cholesterol levels ($r = 0.563$ and $P = 0.035$, and $r = 0.675$ and $P = 0.008$, respectively).

Individual day-to-day variation

Even in the context of a metabolic ward study, the subjects exhibited significant day-to-day variation in lipid, lipoprotein, and apolipoprotein levels. **Table 3** shows the interassay coefficient of variation for the measurements made in this study and compares them to the mean of each individual's day-to-day variation. The interassay variations account for from 10 to 60% of the day-to-day variations. The day-to-day variations are smallest for the total, LDL and HDL cholesterol determinations, larger for the apoB and apoA-I assays, and largest for the triglyceride and VLDL cholesterol measurements. Surprisingly, despite the change in daily dietary composition on the intermittent saturated fat diet, the day-to-day variation was no greater than on the other two diets (data not shown). In addition, there was no obvious change in the lipoprotein pattern on the mornings after the high saturated fat and cholesterol meal.

DISCUSSION

The contribution of diet to the development of atherosclerosis has been well documented. The studies of Ancel Keyes (20) and others have shown that in Westernized societies the incidence of coronary heart disease is proportional to the percent of calories from saturated fat and the amount of cholesterol in the diet. These dietary constituents have been shown to raise the levels of total and LDL cholesterol (21), which are both strong risk factors for coronary heart disease (22). Diminishing dietary saturated fat and cholesterol has been proposed as a way to reduce total and LDL cholesterol (3). Such recommendations have been made more urgently by various groups, since the Lipid Research Clinic CPPT showed that lowering LDL cholesterol reduced coronary heart disease incidence in man (1). However, the best diet to achieve these ends continues to be debated (11).

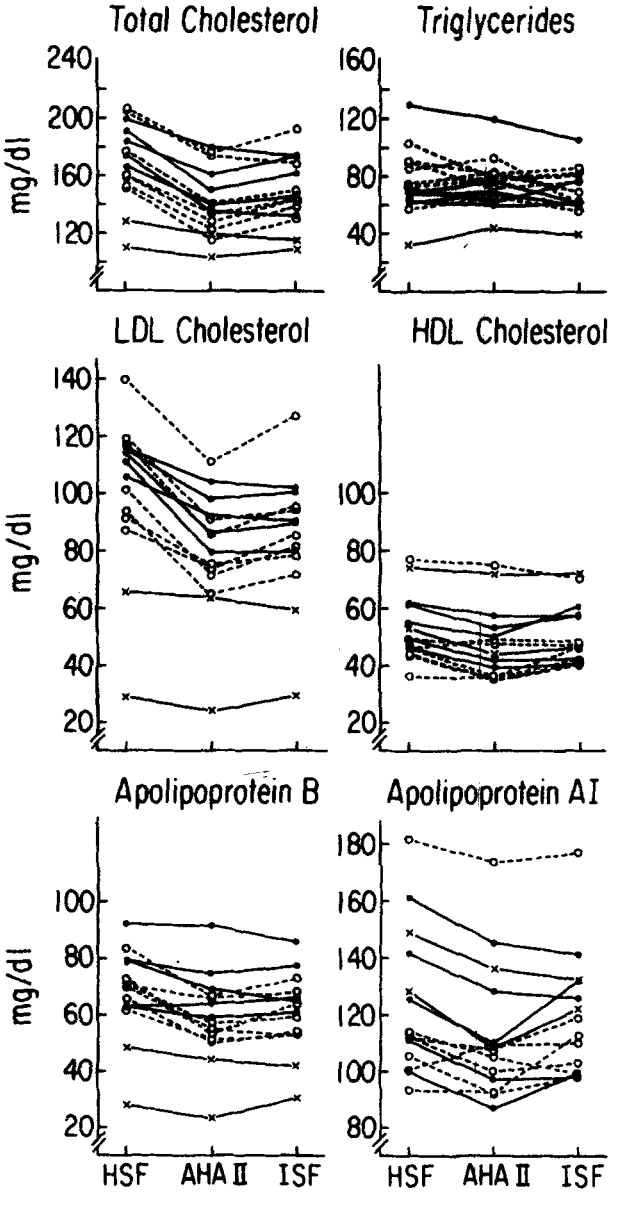

Fig. 2. Individual lipid, lipoprotein, and apolipoprotein responses to diets. Men are depicted with open circles and dashed lines; women with solid circles and solid lines. Two women whose LDL cholesterol was less than 65 mg/dl on the high saturated fat diet are depicted with x and solid lines.

The current study was designed to determine the effect of two important dietary perturbations in normal humans on plasma lipid, lipoprotein, and apolipoprotein patterns. First, the effects of 30% and 25% of calories as fat diets, or the AHA Phase I and II diets, were compared. With a baseline of a 42% fat diet, both low fat diets lowered LDL cholesterol and apoB levels. However, only the 25% fat diet significantly lowered HDL cholesterol and apoA-I levels. The 30% fat diet achieved a significantly higher HDL cholesterol than the 25% fat diet. The 30% and 25% fat diets did not differ significantly with respect to

TABLE 3. Coefficient of variation by assay type

Assay Type	Coefficient of Variation	
	Assay[a]	Individual Day-to-Day[b]
	%	
Total cholesterol	1.0	5.5
Total triglyceride	2.7	20.3
VLDL cholesterol	3.8	38.6
LDL cholesterol	3.8	7.9
HDL cholesterol	2.4	7.4
Apolipoproteins B	5.2	15.6
Apolipoprotein A-I	5.2	8.6

[a] Assay: coefficient of variation for same sample run on 5 different assay days.

[b] Individual, day-to-day: mean coefficient of variation for each of the 14 subjects' ten lipid and lipoprotein measurements and five apolipoprotein measurements on each of the three diet periods.

the LDL/HDL cholesterol ratio. Considerable epidemiological evidence exists indicating that LDL cholesterol and HDL cholesterol are independent risk factors for coronary heart disease and that the ratio of these two levels is a better predictor of disease incidence than either one alone (23, 24). On this basis, one might conclude that in normolipidemic subjects the 30% and 25% fat diets confer equal protection against coronary heart disease compared to a high saturated fat diet.

In terms of the effects of low fat, low cholesterol diets on total and LDL cholesterol levels, our results are comparable to others in the literature. Diets equivalent to the AHA Phase I 30% fat diet have lowered LDL cholesterol from 9 to 32% (8, 10, 14) and diets equivalent to the AHA Phase II 25% fat diet have lowered LDL cholesterol from 18 to 26% (4, 9, 25). In the current metabolic ward study, where compliance was maximal, the LDL lowering was 16 and 21% on the two diets, respectively.

There are several studies in the literature documenting the HDL cholesterol lowering effect of low fat diets (4–6, 10, 11, 13, 26). In one study, a 20% fat formula diet lowered HDL cholesterol levels 30% compared to a 40% fat formula diet (26). In another study, it was found that 40% and 30% fat solid food diets produced comparable HDL cholesterol levels, whereas a 20% fat diet lowered these levels 15% (11). In the current study, compared to the high saturated fat diet (42% fat), the 30% fat diet lowered HDL cholesterol 4% and the 25% fat diet lowered it 10%. Thus, because of their effect on HDL cholesterol levels, diets with less than 30% of calories as fat do not appear to result in a further improvement in the LDL/HDL cholesterol ratio. In the current study, we were also able to show the effects of low fat diets on plasma levels of apoB and apoA-I. Compared to the average American diet, the 25% fat diet significantly lowered apoB and apoA-I, 13% and 8%, respectively, whereas the 30% fat diet only significantly lowered apoB levels 10%.

The second dietary perturbation examined in this study was the effect of the intermittent ingestion of a high saturated fat and cholesterol meal on a background of a low saturated fat, low cholesterol diet. This is an important practical question unaddressed in the literature. Prior to this study, we had no idea whether a meal high in saturated fat and cholesterol every other day for dinner would completely abolish the benefits of an AHA Phase II diet. Our results suggest that this did not occur. This has several implications for dietary counselling. It suggests that, when an improved LDL/HDL cholesterol ratio is the goal, an AHA Phase I diet is sufficient and a more stringent diet is not required. Alternatively, it suggests that where diet is controllable, such as in the home, one should strive for an AHA Phase II diet, but that ingestion of the occasional high saturated fat, cholesterol meal, under circumstances encountered in the real world that are difficult to control, is acceptable. We favor the latter interpretation.

It is well known that individuals respond differently to diet (27, 28). In the change from the high saturated fat diet to the AHA Phase II diet, all subjects reduced their LDL cholesterol level, but the range of responses was −3 to −30%. For HDL cholesterol, 12 of 14 subjects showed a reduction and the range of responses was +7 to −22%. Determinants of individual variation in diet response are constitutional and probably of both an acquired and genetic nature. These are yet to be defined in humans. In the current study, the magnitudes of the LDL and HDL responses were not correlated. In terms of the LDL cholesterol decrease when changing from a high to lower fat diet, this correlated with ad lib total cholesterol and LDL cholesterol levels. This suggests that people with higher levels are more diet responsive.

In summary, it appears that for a group of young normal volunteers, LDL cholesterol levels are related to the amount of dietary saturated fat and cholesterol. In addition, dietary indiscretion on the background of a low fat diet does not have a deleterious effect on the LDL/HDL cholesterol ratio, because of the dietary effect on HDL. This study suggests that in free living populations, one should aim for a low fat diet, but one may be able to accept the inevitable occasional dietary indiscretions without anticipating that the LDL/HDL cholesterol ratio will be impaired. Similar studies must be accomplished in additional normal individuals as well as obese and/or hyperlipidemic patients before such dietary prescriptions can be generally recommended.

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