Effect of egg cholesterol and dietary fats on plasma lipids, lipoproteins, and apoproteins of normal women consuming natural diets

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Abstract Nine normal women, 22 to 37 years old, consumed controlled quantities of natural foods to test their responses to dietary cholesterol and saturated fat. All diets contained, as percentage of calories, 14% protein, 31% fat, and 55% carbohydrate. The main sources of polyunsaturated and saturated fats were corn oil and lard, respectively, and egg yolk was used for cholesterol supplementation. All subjects participated in four diet protocols of 15 days duration, and each diet period was separated by 3 weeks without diet control. The first diet (corn) was based on corn oil, had a polyunsaturated to saturated fat ratio (P/S) of 2.14, and contained 130 mg of cholesterol. The second diet (corn+) was identical to the first but contained a total of 875 mg of cholesterol. The third diet (lard) was based on lard, had a P/S ratio of 0.64, and contained 130 mg of cholesterol. The fourth diet (lard+) was identical to the third, but contained 875 mg of cholesterol per day. Changes of the plasma lipid, lipoprotein and apoprotein parameters relative to the corn diet were as follows: *a*) the corn+ diet significantly increased total plasma cholesterol, HDL-cholesterol, LDL-cholesterol, and apoB levels; *b*) the lard diet significantly increased total cholesterol, HDL-cholesterol, and apoB; and *c*) the lard+ diet significantly increased the total cholesterol, HDL-cholesterol, LDL-cholesterol, and apoA-I and apoB levels. There were no significant variations in VLDL-cholesterol, triglyceride, or apoE levels with these diets. The diets affected both the number of lipoprotein particles as well as the composition of LDL and HDL. Compared to the corn diet, cholesterol and saturated fat each increased the number of LDL particles by 17% and 9%, respectively, and the cholesterol per particle by 9%. The combination of saturated fat and cholesterol increased particle number by 18% and particle size by 24%. Switching from lard+ to lard, corn+, or corn diets reduced LDL-cholesterol of the group by 18%, 11%, and 28%, respectively, while a large inter-individual variability was noted. In summary, dietary fat and cholesterol affect lipid and lipoprotein levels as well as the particle number and chemical composition of both LDL and HDL. There is, however, considerable inter-individual heterogeneity in response to diet. — **Zanni, E. E., V. I. Zannis, C. B. Blum, P. N. Herbert, and J. L. Breslow.** Effect of egg cholesterol and dietary fats on plasma lipids, lipoproteins, and apoproteins of normal women consuming natural diets. *J. Lipid Res.* 1987. **28:** 518–527.

Supplementary key words saturated fat • unsaturated fat • egg consumption • cholesterol • apoE • apoA-I • apoB • lipoproteins

The effect of dietary cholesterol and saturated fat in raising plasma cholesterol levels was defined by two different research groups in the mid 1960s (1, 2) and has been verified in numerous metabolic ward as well as outpatient diet studies (3–19). Some (20–22) but not all (3) cross section population studies have also established a correlation between dietary cholesterol and plasma cholesterol levels in humans. Earlier studies (1, 2) suggested that the P/S ratio did not affect the changes in plasma cholesterol in response to dietary cholesterol. However, most studies agree that the fat composition of the diet influences the plasma cholesterol changes in response to cholesterol consumption (5, 8, 19, 23–27). It has also been reported that polyunsaturated fats decrease (12, 13, 25–28), saturated fats increase (11), and cholesterol has no effect (6, 17) on plasma triglyceride levels.

Recent studies have focused on the effect of dietary lipids on plasma lipoproteins and apoproteins (14, 16, 19, 29–34). These studies have generally shown that dietary cholesterol increases LDL-cholesterol but either is without effect (35, 36) or increases (14, 19) HDL-cholesterol. Most investigators have observed a large inter-individual variability in response to cholesterol- and saturated fat-containing diets (4, 6, 18, 28, 31, 37–39).

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein.

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A few human experiments (25, 40–42) have shown that diets high in saturated fats and cholesterol cause accumulation of certain plasma lipoproteins (HDL_c, VLDL, IDL) which are rich in cholesteryl ester and apoE.

The egg contributes 46% of the daily cholesterol intake of the American diet (3) and several previous studies have used eggs as cholesterol source in human nutritional experiments (15, 19, 43–45). Two outpatient studies failed to demonstrate significant changes in plasma cholesterol when eggs were added or removed from the diets of the subjects (15, 43). Two other studies showed a small decrease in plasma cholesterol when the subjects eliminated eggs from their diets (14, 32, 44). Egg consumption was also associated with increases in LDL-cholesterol and apoB, and with small or negligible changes in HDL-cholesterol, apoA-I, and apoA-II (14, 32).

The present study was undertaken to evaluate the effect of commonly used dietary fats alone or in combination with egg yolk cholesterol on plasma lipids, lipoproteins, and apoproteins of human subjects consuming controlled natural diets.

METHODS

Selection of subjects

Nine healthy women, aged 23–37 years, volunteered to participate in this 60-day study. All of them were either Harvard Medical School students or laboratory technicians. The subjects were ambulatory and continued their normal activities for the duration of the experiment. They were advised not to change their daily routine or to alter their exercise habits. Drugs including birth control pills and alcohol use as well as smoking were not permitted. The subjects were menstruating and remained healthy during the entire study.

Diets

We used four dietary periods of 15 days each, separated from each other by 3-week ad lib periods. The sources of fat and the cholesterol content of the four dietary periods were: period 1: corn oil, 31% of calories, 130 mg of cholesterol per day, P/S = 2.14; period 2: corn oil, 31% of calories, 875 mg of cholesterol per day, P/S = 2.14; period 3: lard, 31% of calories, 130 mg of cholesterol per day, P/S = 0.64; period 4: lard, 31% of calories, 875 mg of cholesterol per day, P/S = 0.64.

The basic food items were the same in all dietary periods for all the subjects. Thus, in all four dietary periods the subjects consumed two teaspoons of jelly, five teaspoons of margarine,[1] 0.5 cup of orange juice, one cup of lettuce, 0.3 cup of celery, 0.5 cup of low fat yogurt, one apple, one banana, one cup of rice, 0.5 cup of frozen mixed vegetables, two slices of whole wheat bread, and either four ounces of chicken breast or three whole eggs depending on the period. In all periods the subjects consumed four to eight muffins especially made for each subject. The muffins were made from flour, sugar, egg whites, corn oil or lard, and egg yolks (only during the egg feeding periods). The ingredients were calculated in each period to maintain the required P/S ratios and the total amount of cholesterol. Thus, in order to adjust for the fat content of the three egg yolks during the egg-feeding period we decreased by an equal amount the fat content of the muffins given to the subjects. In this regard, the muffins served as a buffer which allowed for adjustment of the caloric content as well as the fat content of each diet. The food items that changed were the corn oil or the lard and the egg yolks as specified by each of the dietary periods. The composition and the caloric content of the foods were calculated with the aid of a computer program (HVH-CWRU Nutrient Data Base, Revision 5, April 1980, Case Western Reserve University).

Caloric requirements were determined by analysis of 3-day diet records kept by the subjects while on their ad lib diets and were adjusted early in the diet periods so that body weights remained constant. Vitamins were provided as "One a Day Plus Minerals" capsules (Miles Laboratories). Vitamin E was supplemented to 30 IU during the corn oil period using capsules (U.S.V. Laboratories).

Plasma lipid, lipoprotein, and apoprotein analyses

Fasting plasma samples were taken the first 2 days preceding each dietary period, day 10, and the last 2 days of each 15-day dietary period.

Subjects, resting in the sitting position for 5 min, underwent venipuncture and blood was obtained without tourniquet constriction. Specimens were collected in tubes containing EDTA (1 mg/ml blood) and placed on ice. Plasma was obtained by centrifugation at 2500 rpm for 20 min in a refrigerated centrifuge at 4°C. The plasma was used for simultaneous determination of total cholesterol and triglycerides by the Technicon AutoAnalyzer II method as specified by the Laboratory Manual of the Lipid Research Clinics (46, 47), and standardized by the Lipid Standardization Program of the Center for Disease Control in Atlanta. Another aliquot of plasma was used for lipoprotein fractionation by ultracentrifugation. During this procedure, 5 ml of plasma was overlayed with 1 ml of normal saline and centrifuged in a Beckman L5-65 ultracentrifuge for 18 hr at 120,000 *g* in a Beckman 40.3 rotor. After ultracentrifugation, the supernatant VLDL was harvested, dialyzed overnight against 4 l of water at 4°C, lyophilized, and used directly for two-dimensional gel electrophoretic analysis to determine apoE phenotypes, as

[1] An equivalent amount of corn oil substituted for the five teaspoons of margarine during the corn+ dietary period. This substitution was necessary to maintain the P/S ratio of 2.14 during this period.

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previously described (48). The volume of the infranatant solution was adjusted to 5 ml by addition of normal saline. An aliquot of the infranatant solution was treated with heparin and $MnCl_2$ to precipitate LDL; HDL remained in solution. Cholesterol and triglyceride concentrations were measured in the d < 1.006 g/ml infranatant fraction both before and after treatment with heparin and $MnCl_2$. VLDL lipids were estimated as the difference between values for total plasma and d < 1.006 g/ml infranatant fraction; LDL lipids were estimated as the difference between values for the infranatant solution before and after heparin-manganese precipitation, and HDL lipids were estimated as those of the infranatant solution after heparin-manganese precipitation.

ApoB radioimmunoassay was performed as described (49), using narrow density range LDL as the apoB standard. Apolipoproteins A-I and E were measured by double antibody radioimmunoassay with polyclonal antisera as previously described in detail (50, 51). Standards or unknowns were preincubated overnight in 50 mM sodium decyl sulfate, pH 7.4. The assays were performed in the presence of a final concentration of 5 mM sodium decyl sulfate. The within-assay coefficient of variation was 9% for the apoE radioimmunoassay and 10.6% for the apoA-I radioimmunoassay. The coefficients of variation for systematic between-assay variability were 3% for the apoE assay and 9% for the apoA-I assay. Direct measurement of the B protein in lipoprotein fractions was not carried out at the time these studies were performed. However, because the study subjects were normolipidemic, VLDL-apoB accounted for a very small portion of total plasma apoB — only about 8%. Thus, even large fractional errors in the estimation of VLDL-apoB would result in very small errors in the calculated value of LDL apoB (total plasma apoB minus VLDL-apoB). As a hypothetical example, using values similar to those found in the subjects of these studies, for a VLDL-cholesterol concentration of 20 mg/dl, we calculate VLDL protein as 110 μg/ml (55% of VLDL-cholesterol) and VLDL-apoB as 55 μg/ml (50% of VLDL protein). With total plasma apoB = 700 μg/ml, LDL-apoB is calculated as 700 − 55 = 645 μg/ml. If there had been an error in the estimate of VLDL-apoB as large as 30%, VLDL-apoB might have been 39 μg/ml and LDL-apoB would have been 661 μg/ml, only 2% greater than the first estimate, which was based on a 30% error in the estimation of VLDL-apoB. Estimates of LDL particle number were made assuming that all LDL particles have an equal content of apoB.

Statistical analyses were done using Statistical Analysis System (SAS) Programs.

RESULTS

Characteristics of study subjects

The age, weight, height, apoE phenotype, ad lib plasma cholesterol and triglyceride levels, and the caloric intake of each subject are shown in **Table 1.** All subjects were within 20% of ideal body weight except subject number 1 who was 20% overweight for her height. Initially caloric intakes were based on dietary records from 3 consecutive days several weeks before beginning the experimental diets. After the first 3 to 4 days, changes in caloric intakes were applied so that body weights remained constant. Compared to initial values, subjects lost a mean of 0.3 to 0.8 kg during the four diet periods with an average weight loss of 0.65 kg. The largest change, −0.8 kg, was observed during the corn and lard periods without cholesterol supplementation.

Lipids, lipoproteins, and apolipoprotein levels

The biochemical variables measured were total plasma-cholesterol, VLDL-cholesterol, LDL-cholesterol and HDL-cholesterol, total plasma triglycerides, apoE, apoA-I, and apoB. The ratios LDL-cholesterol/LDL-apoB, HDL-cholesterol/apoA-I, LDL-cholesterol/HDL-cholesterol, HDL-cholesterol/total cholesterol, and apoB/apoA-I were calculated. Lipid parameters of the group measured at day 10 of the diet were not significantly different from those measured on day 15. The data were analyzed by compar-

TABLE 1. Characteristics of study subjects

Subject	Age	Height	Weight	ApoE Phenotype	Caloric Intake	Ad Lib Cholesterol	Ad Lib Triglycerides
	yr	*cm*	*kg*		*kCal*	*mg/dl*	*mg/dl*
001	37	164.0	89.0	E4/3	2800	219	64
002	37	165.0	64.5	E3/2	1800	165	42
003	25	166.0	65.0	E3/2	1800	139	67
004	24	169.0	56.5	E3/3	1500	210	85
005	27	174.0	62.0	E4/4	1500	175	57
006	23	149.0	49.0	E3/3	1500	185	70
007	25	162.0	51.5	E3/3	1500	149	46
008	24	176.0	73.0	E3/2	2000	142	59
009	24	162.0	53.0	E3/2	1500	141	44
	27 ± 6	165 ± 8	62.6 ± 12.5		1767 ± 430	169 ± 30	59 ± 14

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ing the experimental diets both to the ad lib diets and to the corn oil diet (**Table 2**).

When compared to the ad lib diets: *a*) the corn diet significantly reduced ($P < 0.05$) total cholesterol, HDL-cholesterol and LDL-cholesterol, total apoA-I, total apoB, and LDL-apoB levels, as well as the ratios LDL-cholesterol/LDL-apoB and HDL-cholesterol/apoA-I. *b*) The corn plus cholesterol diet significantly reduced total cholesterol and LDL-cholesterol, and the ratios LDL-cholesterol/LDL-apoB and LDL-cholesterol/HDL-cholesterol. The ratios HDL-cholesterol/apoA-I and apoB/apoA-I were significantly increased. *c*) The lard diet significantly decreased total cholesterol and LDL-cholesterol, total apoA-I, total apoB, and LDL-apoB levels, and significantly increased total triglyceride levels. The ratios LDL-cholesterol/LDL-apoB and LDL-cholesterol/HDL-cholesterol were significantly decreased while HDL-cholesterol/apoA-I and HDL-cholesterol/total cholesterol were significantly increased. *d*) The lard plus cholesterol diet did not produce any significant changes relative to the ad lib diet.

When compared to the corn oil diet: *a*) the corn plus cholesterol diet significantly increased total cholesterol, HDL-cholesterol and LDL-cholesterol and total apoB and LDL-apoB levels. The ratios LDL-cholesterol/LDL-apoB, HDL-cholesterol/total cholesterol, HDL-cholesterol/apoA-I, and apoB/apoA-I were significantly increased. *b*) The lard diet significantly increased total cholesterol and HDL-cholesterol, apoB, and HDL-cholesterol/apoA-I. *c*) The lard plus cholesterol diet significantly increased total cholesterol, HDL-cholesterol and LDL-cholesterol, total apoA-I, total apoB, and LDL-apoB levels, and the ratios LDL-cholesterol/LDL-apoB and HDL-cholesterol/apoA-I.

Diet-induced changes in LDL and HDL

We also analyzed the effect of dietary lipids on the composition of LDL and HDL (**Table 3**). Recent determinations of the molecular weight of apoB-100 show that there is one apoB molecule per LDL particle (52), thus changes in LDL-apoB are equivalent to changes in the number of LDL particles. The analysis of the effect of the diet on the composition of LDL showed that addition of cholesterol to the corn oil diet increased the number of LDL particles by 17% and the amount of cholesterol per LDL particle by 9%, while addition of cholesterol to the lard diet increased the number of LDL particles by 9% and the amount of cholesterol per LDL particle by 13%. When cholesterol and saturated fat were consumed together, the effects were independent and additive with 18% increase in LDL particles and a 24% increase in cholesterol.

Similarly, addition of cholesterol to the corn oil diet resulted in a population of HDL particles relatively rich in cholesterol compared to the amount of apoA-I present. There was a 20% increase in the ratio of HDL-cholesterol/apoA-I. Saturated fat consumption alone also produced a population of HDL particles relatively enriched in cholesterol as the ratio of HDL-cholesterol/apoA-I increased by 14%.

Saturated fat and cholesterol consumption together resulted in a 11% increase in the apoA-I concentration with a 10% increase in the HDL-cholesterol/apoA-I ratio. The ad lib diet lipid, lipoprotein, and apolipoprotein levels for the nine subjects were not significantly different from the lard plus cholesterol dietary period. Although individuals vary in their response to dietary cholesterol and saturated fat, the behavior of the group can be predicted by empirical equations (53).

TABLE 2. Lipoprotein parameters during ad lib and control diet periods

	Ad Lib	Corn	Corn +	Lard	Lard +
Total chol (mg/dl)	169.4 ± 30.2	136.2 ± 25.3*	158.5 ± 24.6*,**	154.1 ± 31.9*,**	169.0 ± 30.2**
HDL chol (mg/dl)	48.5 ± 5.6	39.4 ± 7.0*	50.3 ± 8.8**	47.4 ± 7.5**	47.5 ± 6.3**
LDL chol (mg/dl)	102.3 ± 31.6	72.4 ± 27.2*	88.6 ± 26.1*,**	83.7 ± 30.0*	101.4 ± 32.9**
VLDL chol (mg/dl)	18.6 ± 4.8	24.3 ± 14.7	19.7 ± 6.1	23.0 ± 6.9	20.0 ± 2.3
Total triglycerides (mg/dl)	59.3 ± 14.0	62.7 ± 11.5	61.2 ± 15.7	70.5 ± 18.0*	67.3 ± 15.7
ApoE (mg/dl)	3.5 ± 0.7	3.3 ± 0.7	3.4 ± 0.7	3.5 ± 0.7	3.7 ± 0.9
ApoA-I (mg/dl)	127.3 ± 14.4	110.9 ± 12.3*	118.9 ± 17.7	118.2 ± 14.8*	122.2 ± 10.2**
ApoB (mg/dl)	71.0 ± 20.0	62.0 ± 19.0*	70.0 ± 21.0**	67.0 ± 22.0*,**	73.0 ± 22.0**
LDLapoB (mg/dl)	66.2 ± 19.6	57.3 ± 19.7*	65.3 ± 19.5**	61.0 ± 19.3*	66.4 ± 21.7**
LDL chol/LDL-apoB	1.54 ± 0.07**	1.26 ± 0.19*	1.36 ± 0.13*,**	1.36 ± 0.14*	1.53 ± 0.14**
HDLc/apoA-I	0.38 ± 0.03	0.36 ± 0.05*	0.42 ± 0.05*,**	0.40 ± 0.07*,**	0.39 ± 0.04**
LDL chol/HDL chol	2.17 ± 0.86	1.94 ± 1.01	1.87 ± 0.88*	1.82 ± 0.83*	2.23 ± 1.0
ApoB/apoA-I	0.57 ± 0.19	0.58 ± 0.20	0.61 ± 0.23*,**	0.58 ± 0.22	0.60 ± 0.24
HDL/total chol	0.30 ± 0.06	0.30 ± 0.08	0.33 ± 0.08**	0.32 ± 0.08*	0.29 ± 0.07

*Ad lib versus dietary period values significant at $P < 0.05$.
**Corn versus dietary period values significant at $P < 0.05$.

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TABLE 3. Effects of dietary lipids on the composition of LDL and HDL[a]

	Corn to Corn +	Lard to Lard +	Corn to Lard	Corn to Lard +
LDL-cholesterol	29 ± 32*	23 ± 14*	20 ± 33	46 ± 36*
LDL-apoB	17 ± 18*	9 ± 12	9 ± 19	18 ± 18*
LDL-cholesterol/LDL-apoB	9 ± 14	13 ± 10*	9 ± 17	24 ± 25*
HDL-cholesterol	28 ± 16*	2 ± 16	22 ± 21*	22 ± 15*
ApoA-I	8 ± 13	4 ± 10	7 ± 9	11 ± 11*
HDL-cholesterol/apoA-I	20 ± 7*	−2 ± 16	14 ± 17*	10 ± 11*

[a]The changes are expressed as % increases of the tabulated parameters.
*Significant at $P < 0.05$.

Individual variability in response to diet

As shown in **Table 4,** the mean plasma cholesterol change of the group in response to diet is in good agreement with the values predicted by the Keys (53) equation. The only discrepancy occurred when subjects switched from lard+ to lard where the observed change was substantially less than predicted.

The individual response to diet was evaluated and the results were informative. Using as reference the lipid, lipoprotein, and apoprotein parameters of the lard and cholesterol dietary period, we were able to assess the individual dietary response to cholesterol reduction, saturated fat reduction, or both. This analysis, presented in **Table 5** and **Figs. 1A and 1B,** shows a considerable individual variability in response to saturated fat- and/or cholesterol-containing diets. Thus a switch from the saturated fat, high cholesterol diet to the saturated fat, low cholesterol diet caused an 18% decrease of LDL-cholesterol for the group with individual changes ranging from −3% to −30%. Similarly, a switch from the saturated fat, high cholesterol diet to the polyunsaturated fat, high cholesterol diet caused an 11% decrease in LDL-cholesterol for the group with individual changes ranging from +6% to −23%. Finally, a switch from the saturated fat, high cholesterol diet to a polyunsaturated fat, low cholesterol diet caused a 28% decrease of LDL-cholesterol for the group with individual changes ranging from −6% to −50%.

Similar individual variability in response to diet was observed for total plasma cholesterol, apoB, apoE, and apoA-I. Thus, a switch from a saturated fat, high cholesterol diet to a saturated fat, low cholesterol diet decreased total plasma cholesterol by 9% for the group with individual changes ranging from −2% to −17%; apoB decreased 6% for the group with individual changes ranging from +7% to −17%; apoE decreased by 4% with individual changes ranging from +19% to −17%, and apoA-I decreased by 3% with individual changes ranging from +16% to −14%.

TABLE 4. Observed and calculated changes in plasma cholesterol concentration in response to diet

Dietary Modification	Observed	Expected Keys Equation (53)
	mg/dl	
Lard + to lard	14.9	22.5
Lard + to corn +	10.4	12.5
Lard + to corn	32.4	36.9
Corn to corn +	22.3	23.0
Corn to lard	18.9	19.8

The equation used to calculate changes in serum cholesterol is:

$$\text{chol} = 1.3(2\Delta S - \Delta P) + 1.5(z_1 - z_2)$$

where chol = serum cholesterol; S and P are the changes in saturated and polyunsaturated dietary fat, respectively, expressed in % of calories; and z_1 and z_2 = square roots of the dietary cholesterol (mg/day per 1000 kcal) in dietary periods 1 and 2, respectively.

ApoE phenotype and the ad lib lipid and lipoprotein values cannot predict the dietary response

Four of the nine women studied had the apoE 3/2 phenotype, three the E 3/3 phenotype, one had the E 4/3 phenotype, and another the E 4/4 phenotype. In this sample of nine women studied, the apoE phenotypes had no apparent effect on any of the variables measured. When we tried to correlate the ad lib lipid and lipoprotein and apoprotein parameters with the dietary response, we found that, although several ad lib parameters correlated with one or another change, none of them, including HDL-cholesterol, could predict uniformly the dietary responses of the subject (analysis is not shown).

DISCUSSION

In a recent study we evaluated the effects of corn oil, coconut oil, and cholesterol on plasma lipids, lipoproteins, and apoE in human subjects consuming formula diets (54). We found that corn oil and coconut oil had the only significant effects on lipids, lipoproteins, and apoE, but that the addition of 1 g per day of cholesterol did not have any effect. In the present study, we tested the validity of

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TABLE 5. Individual variability in HDL- and LDL-cholesterol in response to diet

Subject	Dietary Change: Lard + to Lard		Lard + to Corn +		Lard + to Corn	
	% Change in LDL Cholesterol	% Change in HDL Cholesterol	% Change in LDL Cholesterol	% Change in HDL Cholesterol	% Change in LDL Cholesterol	% Change in HDL Cholesterol
1	−11	−2	−12	0	−30	−25
2	−21	0	−23	+6	−24	−4
3	−30	−22	−21	−2	−23	−16
4	−14	+22	−22	+4	−50	−17
5	−14	+6	−1	+26	−6	−4
6	−21	+3	−16	−3	−17	−16
7	−16	−21	+5	−16	−12	−28
8	−3	+11	+6	+33	−43	−11
9	−30	+8	−19	+6	−49	−31
Mean ± SD	−18 ± 8	−1 ± 14	−11 ± 12	+6 ± 15	−28 ± 16	−17 ± 10

these findings using natural diets with fat and cholesterol content similar to the liquid formula diets.

The current protocols differed in three important aspects from the liquid formula protocol. First, the dietary periods were increased from 9 to 15 days. In this regard, previous studies (2, 6, 9, 17, 18, 55) have noted that most dietary effects on serum lipids are achieved within 2 weeks. Second, lard containing long chain saturated fatty acids was the source of saturated fat, whereas coconut oil containing medium chain fatty acids was used previously. Corn oil was the unsaturated fat used in both studies. Finally, crystalline cholesterol was added to the liquid formula diets whereas egg yolks provided additional cholesterol in the studies reported here.

Eggs have been used previously as the source of cholesterol in human nutritional experiments (15, 19, 43–45). An important improvement in the present study was that the diets were highly controlled.

In the present study, egg yolk cholesterol increased LDL-cholesterol levels in the presence of either saturated or polyunsaturated fat diets (Table 3). Egg yolk cholesterol when added to the corn oil-based diet significantly increased HDL-cholesterol levels (+11 mg/dl), but when cholesterol was added to the lard diet, this had no effect on HDL. The ratio of HDL-cholesterol to total cholesterol was in fact higher on corn and cholesterol (0.33) than corn alone (0.30). This finding is of theoretical interest and will require attention in future studies.

The mean group changes in plasma cholesterol in this study are in good agreement with theoretical values predicted by the Keys equation (53). The only discrepancy is in the switch from lard to lard+ where the observed change is appreciably lower than predicted.

As shown in Table 2, all values for the cholesterol-supplemented lard diet were almost identical to those for the ad lib diet. The cholesterol-supplemented corn oil diet and the cholesterol-poor lard diet provide another very interesting comparison. The ratio HDL-cholesterol/total cholesterol was 0.33 for corn+ and 0.32 for lard. The ratio of LDL-cholesterol/HDL-cholesterol was 1.87 and 1.82 for corn+ and lard, respectively. These ratios as well as the levels of all apolipoproteins were virtually identical on both these diets.

Few previous studies have examined the response of several plasma apolipoproteins to dietary perturbations. Increasing dietary cholesterol from 97 to 418 mg per day in free-living lactovegetarians did not change apoA-I and apoA-II levels but increased apoB by 6 mg/dl (32). In the extensive dietary experiments of Schonfeld et al. (19), only apolipoproteins A-I and B demonstrated dietary responsiveness, whereas apoproteins A-II, C-II, C-III, and E did not vary with dietary cholesterol or P/S ratio. These workers found that apoA-I levels were highest when the P/S ratio was 0.8 and the diet was supplemented with three to six eggs per day. Diets with comparable cholesterol enrichment and both higher and lower P/S ratios, however, resulted in lower apoA-I levels. In our studies, while apoA-I levels were higher in the lard+ diet than in any of the other experimental diets, the ratio of apoA-I/LDL-cholesterol was lowest in this diet. ApoB levels varied in a consistent fashion, rising and falling with the LDL-cholesterol concentration; but the percentage change in LDL-cholesterol was greater than that in apoB. Other studies (19, 33) have documented a similar relationship of apoB and LDL-cholesterol changes. Finally, the concentration of VLDL and plasma triglycerides and apoE varied over a relatively narrow range in this and in our previous study (54).

The present study suggests that saturated fat and cholesterol both increase both the number of LDL particles and the cholesterol content of the LDL particles. Mechanisms consistent with these changes have been proposed. A recent study suggested that the increase in LDL-cholesterol that results from a high cholesterol diet cor-

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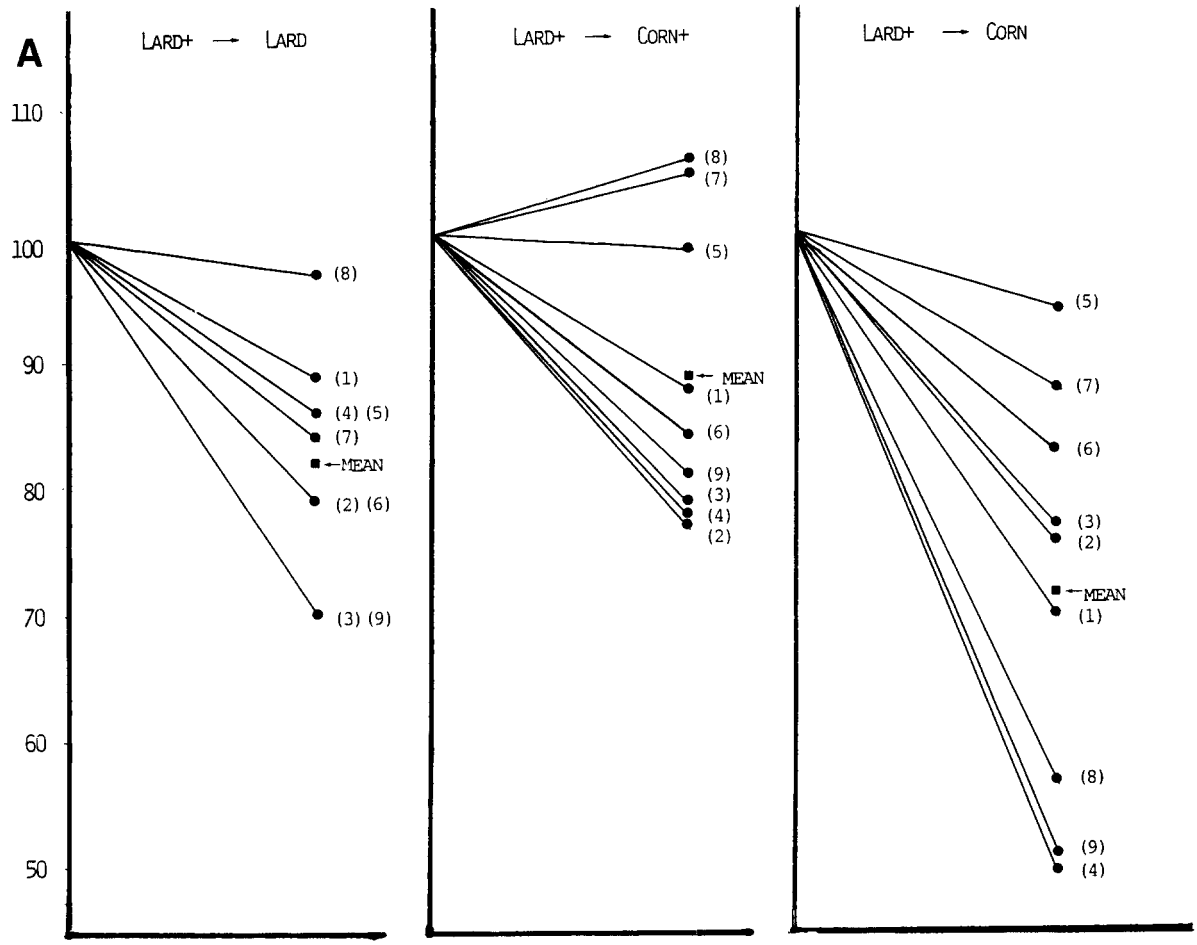

Fig. 1. Schematic representation of individual variability in LDL-cholesterol (panel A) and HDL-cholesterol (panel B) in response to diet. The individual percent changes of LDL-cholesterol and HDL-cholesterol for the three dietary periods (lard, corn+, corn) are connected by solid lines with the lard+ diet designated as 100%. Numbers in parentheses designate the individual subjects of Table 1. The mean percent change of LDL-cholesterol and HDL-cholesterol is indicated in panels A and B, respectively.

relates with a reduction of the LDL receptor activity of the circulating mononuclear cells (34). A decrease in the catabolic rate of LDL by a cholesterol-containing diet coupled with an increase in the rate of lipoprotein synthesis has also been described (56). The corn+ and the lard diets produced a relatively cholesterol-rich HDL with increased HDL-cholesterol/apoA-I ratios. Similar changes in LDL-cholesterol/LDL-apoB ratio (9) and HDL/apoA-I ratio (13, 26, 57) have also been reported by others.

A common observation of several previous studies is the large inter-individual variability of humans in response to cholesterol- and saturated fat-containing diets (4, 6, 18, 28, 31, 37–39). Since lipoprotein parameters of the lard+ diet closely matched those during the ad lib periods, we assessed inter-individual variation in the present study by comparing the various parameters obtained on the lard+ diets to those obtained during the other three nutritional periods. A switch from lard+ to lard, corn+, or corn is equivalent to the removal of cholesterol, saturated fat, or both from the diet. Saturated fat withdrawal, of course, necessitated substitution of monounsaturated and polyunsaturated fats. Such comparison shows that removal of cholesterol from the diet reduced the LDL-cholesterol of the group by 18% with individual decreases varying from −3% to −30%. Removal of saturated fats reduced the LDL-cholesterol by 11% with individual changes ranging from −23% to +6%. Removal of both the saturated fat and the cholesterol from the diet decreased LDL-cholesterol by 28% for the group with individual decreases ranging from −6% to −50%. Similar individual variability in response to diet was observed for total plasma cholesterol apoB, apoE, and apoA-I.

The current study confirms many others demonstrating that plasma cholesterol levels can be decreased by reducing the cholesterol content and increasing the polyunsaturated fat content of the diet. Ideally, reductions in LDL-cholesterol should be associated with stable or higher levels of HDL-cholesterol. This and other studies

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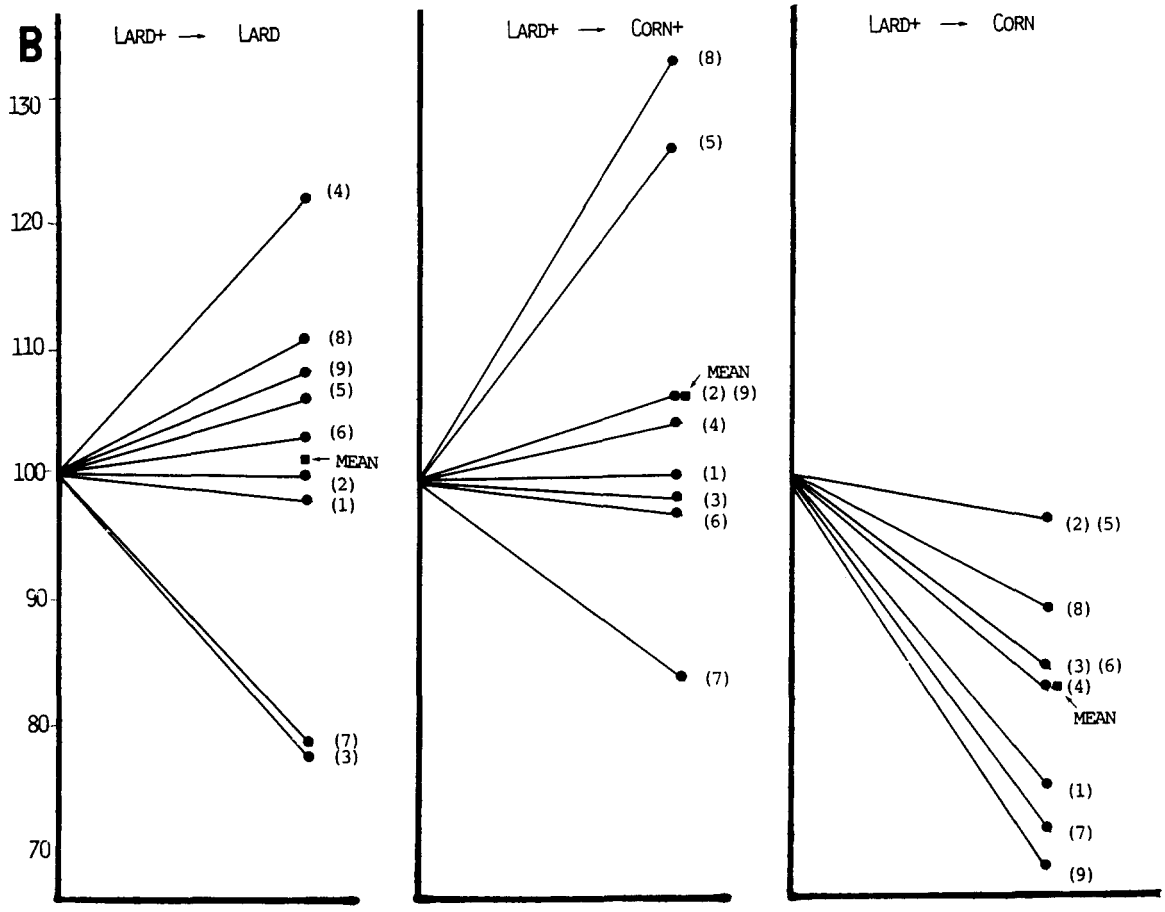

(19, 54) suggest that while corn oil and vegetable oils alone have a tendency to reduce total cholesterol and HDL-cholesterol, monosaturated fats also reduce plasma- and LDL-cholesterol in hypertriglyceridemic patients without affecting HDL-cholesterol (58). Modest reductions in total cholesterol and LDL-cholesterol without reduction in HDL-cholesterol have also been achieved in normal, and some hypertriglyceridemic, subjects with diets in which the fat source was in the form of either fish oil or vegetable oil (27, 59). However, since cholesterol contents of the fish and vegetable oils are different, these later studies cannot separate the effects of dietary fat and cholesterol on plasma lipid and lipoprotein levels.

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