Heterogeneity of Cholesterol Homeostasis in Man

Response to Changes in Dietary Fat Quality and Cholesterol Quantity

Donald J. McNamara, Rachael Kolb, Thomas S. Parker, Hedda Batwin, Paul Samuel, Clinton D. Brown, and E. H. Ahrens, Jr.
The Rockefeller University, New York, New York 10021

Abstract

Studies were carried out to examine the effects of dietary fat and cholesterol on cholesterol homeostasis in man. 75 12-wk studies were carried out during intake of 35% of calories as either saturated or polyunsaturated fat, first low and then high in dietary cholesterol. Dietary fat and cholesterol intakes, plasma lipid and lipoprotein levels, cholesterol absorption and sterol synthesis in isolated blood mononuclear leukocytes were measured during each diet period. In 69% of the studies the subjects compensated for the increased cholesterol intake by decreasing cholesterol fractional absorption and/or endogenous cholesterol synthesis. When an increase in plasma cholesterol levels was observed there was a failure to suppress endogenous cholesterol synthesis. Plasma cholesterol levels were more sensitive to dietary fat quality than to cholesterol quantity.

The results demonstrate that the responses to dietary cholesterol and fat are highly individualized and that most individuals have effective feedback control mechanisms.

Introduction

Numerous studies, either under steady state conditions on a metabolic ward or in free-living outpatients, have demonstrated a large patient-to-patient variability in response to a dietary cholesterol challenge and to a shift in dietary fat quality (1–40). However, only in a limited number of studies have metabolic responses other than changes in plasma lipid levels been investigated: studies utilizing sterol balance techniques (1–20) have demonstrated that the major metabolic changes in response to a dietary cholesterol challenge are suppression of endogenous cholesterol synthesis and/or increased re-excretion of absorbed dietary cholesterol as fecal neutral steroids (2–10). Some patients are able to decrease the fractional absorption of dietary cholesterol (2), or to increase bile acid synthesis and excretion (8, 9), while others may experience an expansion of tissue stores of cholesterol (2, 5, 9). Changes in dietary fat quality have been reported to exert no effect on whole body cholesterol metabolism (11, 15, 18, 20) or, in hypertriglyceridemic patients, to increase endogenous fecal neutral steroid excretion (17). Shifting the quality of the dietary fat has been reported to alter low density lipoprotein (LDL) synthesis and/or its fractional catabolic rate (20, 36, 37). The degree of precision in regulatory responses to a dietary cholesterol challenge in the general population remains unknown. Numerous studies in free-living outpatients suggest that a majority have relatively precise feedback control mechanisms, since an increased dietary cholesterol intake fails to significantly increase plasma cholesterol levels in the majority of subjects (3, 4, 10, 24–31). In sharp contrast to the relatively minor effect of dietary cholesterol on plasma cholesterol levels, the quality of dietary fat has been shown to have a more consistent influence on plasma lipid levels: a shift from a saturated to a mono- or polyunsaturated fat (PUFA)[1] diet will lower plasma cholesterol levels in the majority of subjects (11–20, 32–40). However, the extent of cholesterol lowering varies greatly from patient to patient (41–44).

Over the past five years technical developments have made it possible to study cholesterol homeostasis in free-living outpatients by a combination of three procedures: (*a*) refinement in measurement of dietary cholesterol intake using dietary records (45); (*b*) quantitation of the absorption of dietary cholesterol by the isotope ratio method (46–48); and (*c*) analysis of changes in the rate of endogenous cholesterol synthesis by assay of sterol synthesis rates in freshly isolated peripheral blood mononuclear leukocytes (MNL) (49–51).

The present report describes the first application of these methods in a study designed to evaluate the effects of dietary fat quality and cholesterol quantity on both plasma lipid and lipoprotein levels and on cholesterol absorption and synthesis in a succession of 6-wk dietary periods in a population of male outpatient volunteers. A total of 75 studies were carried out in 50 individuals, comparing a low cholesterol (~ 250 mg/d) versus a high cholesterol (~ 800 mg/d) diet during intake of 35% of calories as either a PUFA (polyunsaturated to saturated fat ratio [P/S] ~ 1.5) or saturated fat (SFA) (P/S ~ 0.3) diet. In 25 of these volunteers, all four dietary periods were investigated. We observed a range of regulatory responses: in the majority of studies the feedback control mechanisms were sufficiently precise to compensate for a large increase in absorbed dietary cholesterol without increasing plasma cholesterol levels. In those studies where the patients exhibited a significant increase in plasma cholesterol levels upon intake of a high cholesterol diet, there was a lack of sufficient feedback suppression of endogenous cholesterol synthesis and thus they were unable to maintain cho-

Address reprint requests to Dr. McNamara at his present address, Dept. of Nutrition and Food Science, 309 Shantz Bldg., U. of Arizona, Tucson, AZ 85721. Dr. Parker's present address is Dept. of Biochemistry, Cornell U. Medical Center, NY, NY 10021; Dr. Batwin's present address is Beth Israel Medical Center, NY, NY 10003; Dr. Samuel's present address is Dept. of Medicine, Albert Einstein College of Medicine, Bronx, NY 10461; and Dr. Brown's present address is Dept. of Medicine, Downstate Medical Center, Brooklyn, NY 11203.

Received for publication 21 February 1986 and in revised form 22 January 1987.

J. Clin. Invest.

0021-9738/87/06/1729/11 $1.00
Volume 79, June 1987, 1729–1739

1. *Abbreviations used in this paper:* MNL, mononuclear leukocytes; P/S, polyunsaturated to saturated fat ratio; PUFA, polyunsaturated fat; SFA, saturated fat.

lesterol homeostasis. The results indicate that the primary determinants of a given subject's plasma cholesterol level are (*a*) sensitivity to dietary fat quality, and (*b*) the degree of precision of the feedback control responses to dietary cholesterol. These responses were highly variable from patient to patient.

Methods

Volunteers. 50 male volunteers were selected from our outpatient study population. All were asymptomatic for cardiovascular diseases and free of secondary causes of hyperlipidemia. Outside of the dietary interventions described below, no attempts were made to alter life-style patterns, and patients were encouraged to maintain their usual habits regarding smoking, exercise, and mealtime- and work-schedules. Some key characteristics of this study group are given in Table I.

Study design. The overall study design is presented schematically in Fig. 1. The study was described to each volunteer and informed consents were obtained after appropriate review and approval of the study by the Rockefeller University Hospital Institutional Review Board. Potential candidates were initially screened by routine physical examination which included blood and urine chemistries and hematological tests. Suitable individuals were randomized into one of the two dietary groups and instructed in the dietary guidelines (see below) on two clinic visits 1 wk apart. 4 wk after the last dietary instruction, patients attended the outpatient clinic weekly for 3 wk for analysis of cholesterol absorption, collection of dietary records, and blood sampling. At the end of week 6 each patient added three large eggs per day to his diet, and the studies were repeated on weeks 10, 11, and 12.

Those patients involved in the dietary fat exchange cross-over study (n = 25) were instructed in exchanging the quality of their dietary fat, and the entire study protocol was then repeated. No undesirable side effects or symptoms were reported throughout these studies.

Dietary instruction and evaluation. Each patient was instructed by a registered dietitian (H. Batwin) in maintaining a low cholesterol diet in which 35% of calories were to be derived from fat, either from SFA (P/S ~ 0.3, n = 36), which was derived primarily from dairy products, or from PUFA (P/S ~ 1.5, n = 39), which was derived from vegetable oils and margarines. Eggs and organ meats were excluded from the diets during the low cholesterol periods. After 6 wk on the low cholesterol diet each patient was instructed to add three large eggs per day to the original diet, which would reduce calories derived from meat/fish/poultry to compensate for those added by eggs. Each patient was trained in portion-size assessment using Nasco food models and instructed in maintaining dietary records as described previously (45). 9 d of dietary records were collected for each dietary period during weeks 4 to 6 and 10 to 12; these were evaluated in consultation with the nutritionist. Portion sizes were converted to standard units; P/S and daily cholesterol intakes were calculated using a computer program containing the fat and cholesterol contents of commonly eaten foodstuffs obtained from Tables of Food Composition (52). Previous studies (45) have demonstrated that, under conditions of proper training in dietary recordkeeping and portion-size assessment, with adherence to a low cholesterol diet, and with collection of at least 9 d of dietary records, a quantitatively reliable estimate of daily cholesterol intake (accurate to ±15%) can be obtained in free-living outpatients. By increasing daily dietary cholesterol intake through addition of three large eggs per day (~ 750 mg cholesterol), total dietary cholesterol intakes can be estimated by 9 d of dietary records with an even greater degree of reliability than during the low cholesterol period. Preliminary studies suggest a similar degree of reliability in estimating dietary fat quality (unpublished observations).

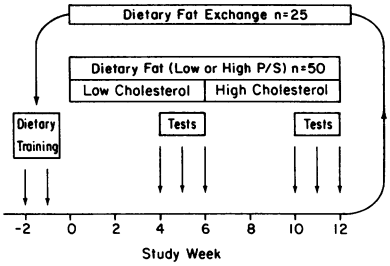

Figure 1. Study design. The overall study design entailed patient recruitment and training in dietary guidelines before the initial 6-wk dietary period on a low cholesterol diet. During weeks 4 to 6 the patients were tested to determine dietary cholesterol intake from 9-d dietary records, measurement of plasma lipid and lipoprotein levels in triplicate, quantitation of dietary cholesterol absorption by the isotope-ratio method, and collection of blood samples for assay of MNL sterol synthesis rates. The patients were then placed on a high cholesterol intake for 6 wk and the tests repeated during weeks 10 to 12. 25 patients exchanged their dietary fats and repeated the study as indicated by the box labeled dietary fat exchange, n = 25.

*Table I. Characteristics of the Volunteers during Their Intake of Either of Two Types of Dietary Fat at 35% of Total Calories**

	Dietary fat	
	PUFA (n = 39)	SFA (n = 36)
Age (*yr*)	47.3±10.1 (44.0–50.6)	46.9±10.2 (43.4–50.3)
Weight (*kg*)	75.8±10.6 (72.3–79.2)	79.0±11.3 (75.2–82.8)
Height (*cm*)	171.7±6.5 (169.6–173.8)	173.1±6.8 (170.7–175.3)
BMI	25.6±2.7 (24.8–26.5)	26.3±2.9 (25.3–27.2)

BMI, Body mass index (kilograms per meter squared).
* Data presented as means±1 SD with the 95% confidence interval given in parentheses. n, Number of volunteers tested on the stated regimen.

Plasma lipids and lipoproteins. Fasting plasma samples were obtained from each volunteer on weeks 4–6 and 10–12 of the study and assayed for total cholesterol and triglyceride by enzymatic analysis (53, 54) using commercial kits (Boehringer-Mannheim Biochemicals, Indianapolis, IN). Plasma LDL and high density lipoproteins (HDL) were isolated by centrifugation in a Beckman airfuge (55) and the HDL fraction obtained after phosphotungstic acid-$MgCl_2$ precipitation (56); the cholesterol concentrations of each fraction were measured enzymatically. The mean coefficient of variation for plasma total cholesterol levels measured three times during each dietary period was 5.4% (3.1% SD, n = 150). Since the coefficient of analytical variation for a plasma cholesterol standard was 2.1% (n = 90), the physiological variability of plasma cholesterol levels in a given patient was 2.5-fold greater than the analytical variation (57); we considered both sources of variation to be acceptably small.

Materials. Radiolabeled compounds ([2-^{14}C]acetate, 4-^{14}C-cholesterol, and 1,2-^{3}H-cholesterol) were purchased from New England Nuclear, Boston, MA. Radiolabeled cholesterols were subjected to thin-layer chromatography on silica gel G in benzene/ethyl ether, 4:1 vol/vol, and the cholesterol fractions were eluted with absolute ethanol. Radioisotopic materials were prepared and infused as previously described (46). The radiochemical reliability of each batch of 1,2-^{3}H-cholesterol used in these studies was tested as described by Davidson et al. (58), and appropriate correction factors were used to determine the doses of 1,2-^{3}H-cholesterol administered.

Cholesterol absorption measurements. Exogenous cholesterol absorption was measured during weeks 5 and 11 of the study by the plasma isotope-ratio method originally described by Zilversmit and Hughes (59) and modified by Samuel et al. (46, 47). All doses of radiolabeled cholesterol were administered in the morning after a 14-h fasting period. Previously reported studies have demonstrated that the coefficient of

variation for the isotope-ratio measurement of exogenous cholesterol absorption in outpatients is ±6% (48). Mass absorption of cholesterol (milligrams per day) during each dietary period was calculated in each volunteer from the known mean daily dietary cholesterol intakes obtained from the dietary records (45) and the fractional absorption of cholesterol obtained from the plasma isotope-ratio measurements.

MNL sterol synthesis. Peripheral blood MNL were isolated according to the method of Boyum (60) and assayed for sterol synthesis rates by analysis of [2-^{14}C]acetate incorporation into sterols as previously described (49, 50). The results are presented as picomoles [2-^{14}C]acetate incorporated per hour per 10^6 cells.

Statistical analysis. Statistical analysis of differences between paired means were carried out using Student's *t* test (61). Statistical significance was determined using a two-tailed statistical model. All data are presented below as means±1 SD with the 95% confidence interval presented in parentheses. The data are presented in two sections: the overall results for the 75 studies in 50 volunteers studied in two dietary periods, then the results for those 25 individuals who were studied during all four dietary periods.

Results

Effect of dietary cholesterol on plasma lipids and lipoprotein levels

Dietary fat and cholesterol intakes. We achieved the dietary goals of this study in that there were significant differences in P/S of the diets of the two dietary fat groups, and significant differences in dietary cholesterol intakes between the low and high cholesterol periods in the two groups (Table II). The data indicate that both the PUFA and SFA groups exhibited a small decrease in the P/S of the diets when shifted from a low to a high cholesterol intake; this decrease was significant only in the PUFA group. The decrease in P/S in the PUFA group arose in part from the addition of egg fat to the diet and in part from a reduction in PUFA intake. Both diet groups increased their dietary cholesterol intake from an average of 240 mg/d to > 800 mg/d when three large eggs were added to the baseline diet. Dietary cholesterol intakes during the low cholesterol period were lowest in the PUFA group, which reflected their reduced intake of animal products. Even in the SFA group, daily cholesterol intakes were < 300 mg/d on average.

The data presented in Table II demonstrate that during the low cholesterol period the mean exogenous cholesterol absorption was 61%, and decreased to 55% during the dietary cholesterol challenge. In both groups (PUFA and SFA), these reductions in the fractional absorption of cholesterol were significant ($P < 0.01$). Nevertheless, in both groups the increased intakes of cholesterol resulted in significant increases in the total amount (mass) of cholesterol absorbed: a fourfold increase in the PUFA group and a 2.6-fold increase in the SFA group.

Plasma lipid and lipoprotein changes. The increased dietary cholesterol intakes failed to cause significant increases in mean levels of plasma total, LDL, or HDL cholesterol in either the PUFA or SFA groups; mean plasma triglyceride levels also remained constant throughout (Table III). However, individual patients exhibited substantial heterogeneity in response to an increased cholesterol intake in both groups (Fig. 2), with statistically significant increases ($P < 0.05$) in total plasma cholesterol observed in 8 of the 75 patients (five in the PUFA group and three in the SFA group), and significant decreases in 3 volunteers (one in PUFA and two in SFA). We found no relationship between the baseline plasma cholesterol level and sensitivity to a dietary cholesterol challenge, regardless of the quality of dietary fat (Fig. 2).

Since the plasma cholesterol response might be expected to relate to the increment in the amount of dietary cholesterol absorbed (milligrams per kilogram per day) upon moving from the low to high dietary cholesterol intake (62), we have related the percent change in plasma cholesterol to the difference in absorbed cholesterol between the low and high intake periods. Table IV demonstrates that the majority of patients, irrespective of the actual difference in absorbed cholesterol, were able to maintain a constant plasma cholesterol level within ±5%, the established total variability (see Methods). Of the 75 studies carried out, only 23 patients exhibited an increase in plasma cholesterol that was > 5%, and in only 8 was the change significant; in contrast, 8 patients actually reduced their total cholesterol levels on the higher intakes, with significant decreases occurring in 3 volunteers.

Effect of dietary cholesterol on in vivo cholesterol metabolism

Increases in absorbed dietary cholesterol were accompanied by significant reductions in MNL sterol synthesis rates (Table V).

*Table II. Dietary Intakes and Cholesterol Absorption in Volunteers**‡*

	PUFA (n = 39)		SFA (n = 36)	
Cholesterol intake	Low	High	Low	High
P/S of dietary fat	1.90±0.90§ (1.61–2.19)	1.45±0.50§ (1.29–1.61)	0.32±0.18 (0.26–0.38)	0.27±0.15 (0.22–0.32)
Cholesterol Intake (*mg/d*)	192±60§ (172–211)	820±102§ (787–853)	288±64‖ (267–310)	863±161‖ (809–918)
Absorption (%)	60.5±12.6¶ (56.4–64.6)	57.2±13.5¶ (52.8–61.5)	61.5±12.6§ (57.2–65.8)	53.5±12.6§ (49.2–57.7)
Absorbed (*mg/d*)	116±44§ (101–130)	469±131§ (426–511)	176±51‖ (159–194)	464±153‖ (412–515)

* Data presented as mean±1 SD with the 95% confidence interval given in parentheses. ‡ Values in the same row with common superscripts are significantly different. § $P < 0.001$. ‖ $P < 0.001$. ¶ $P < 0.01$.

*Table III. Plasma Lipid and Lipoprotein Cholesterol Levels in Volunteers**

Cholesterol intake	PUFA (n = 39)		SFA (n = 36)	
	Low	High	Low	High
Plasma lipids	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*
Cholesterol				
Total	218±49 (202–234)	224±46 (209–239)	243±50 (226–260)	248±51 (231–265)
LDL	131±41 (117–144)	138±39 (126–151)	144±38 (131–157)	150±42 (135–164)
HDL	42±10 (39–45)	44±9 (41–47)	45±10 (41–48)	46±12 (42–50)
Triglyceride	201±139 (156–246)	189±131 (147–232)	223±149 (173–274)	226±183 (164–287)

* Data presented as mean±1 SD with the 95% confidence interval given in parentheses.

Table IV. Changes in Absorbed Dietary Cholesterol vs. Percent Change in Plasma Total Cholesterol Levels

Change in absorbed dietary cholesterol	% Change in plasma cholesterol					
	> −5	±5	+6 to +10	+11 to +15	> +15	Total
*mg/kg per d**						
>6.5	0	6	2	1	2	11
4.0–6.4	2	19	5	5	3	34
1.5–3.9	5	18	4	0	0	27
<1.4	1	1	0	1	0	3
Total no. tested	8	44	11	7	5	75
Individuals with significant changes‡	3	0	1	2	5	11

* Milligrams of absorbed cholesterol per day are expressed per kilogram of total body weight.
‡ Significant difference in plasma cholesterol levels in the two diet periods, $P < 0.05$.

In both the PUFA and SFA groups the increased intake of cholesterol caused a 21% or greater reduction in the incorporation of radiolabeled acetate into sterols in freshly isolated MNL. When individual data are considered rather than means of group data, the degree of suppression of MNL sterol synthesis was found to be linearly related to the mass of cholesterol (milligrams per kilogram per day) actually absorbed by the volunteers (Fig. 3). We have classified our volunteers as either compensators (those who maintained a constant plasma cholesterol level when dietary cholesterol intake was increased, n = 52) or noncompensators (those exhibiting a 5% or greater increase, mean = 12%, in plasma cholesterol levels when given a dietary cholesterol challenge, n = 23). As shown in Table VI, the plasma cholesterol levels were unchanged in the compensators upon shifting from a low to high cholesterol intake, and these compensators exhibited a significant 26% reduction in MNL sterol synthesis. In contrast, the noncompensators had significant increases in plasma cholesterol levels and a nonsignificant 12% reduction in the rate of acetate incorporation into sterols in their MNL. The patient groups classified as compensators and noncompensators had similar baseline values for body weight, body mass index, percent cholesterol absorption, and MNL sterol synthesis rates.

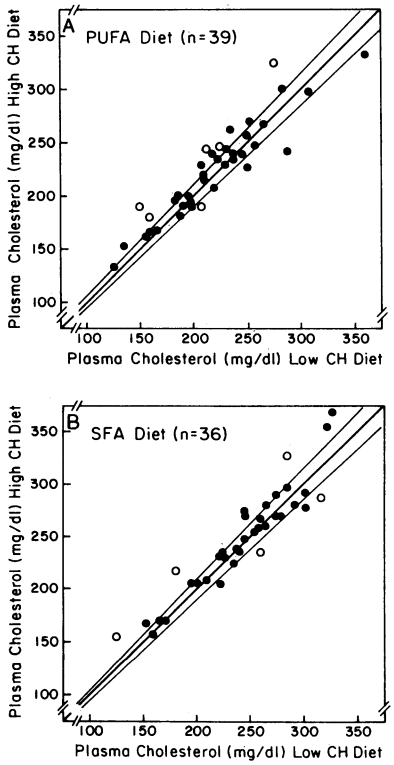

Figure 2. Individual plasma cholesterol responses to a dietary cholesterol challenge. Plasma total cholesterol levels (milligrams per deciliter) are plotted against the line of identity (±5%) for each patient during the low versus high dietary cholesterol intakes for the PUFA group (*A*) and the SFA group (*B*). Open symbols represent significant changes ($P < 0.05$).

These results recall those of Nestel and Poyser (3) and Maranhao and Quintao (9): patients who demonstrated the most effective feedback suppression of endogenous cholesterol synthesis by dietary cholesterol (as measured by sterol balance techniques) were able to maintain a constant plasma cholesterol level on a high cholesterol diet. The data presented here are consistent with the hypothesis that most individuals possess precise feedback

Table V. Cholesterol Synthesis Rates as a Function of Cholesterol Intake in Volunteers‡*

Cholesterol intake	PUFA (n = 39)		SFA (n = 36)	
	Low	High	Low	High
Mononuclear cell sterol synthesis (*pmol/h per 10^6 cells*)	9.7±2.9§ (8.8–10.7)	7.7±2.1§ (7.0–8.4)	9.2±2.6‖ (8.3–10.0)	7.0±1.9‖ (6.3–7.7)
% Reduction	−21		−24	

* Data presented as mean±1 SD with the 95% confidence interval given in parentheses.
‡ Values in the same row with common superscripts are significantly different.
§ $P < 0.001$.
‖ $P < 0.001$.

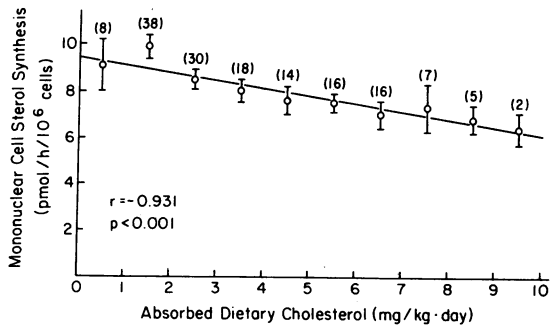

Figure 3. Relationship between absorbed dietary cholesterol and the rate of sterol synthesis in freshly isolated blood MNL. The rates of incorporation of [2-^{14}C]acetate into sterols in freshly isolated blood MNL (picomoles per hour per 10^6 cells) plotted against the mass of absorbed dietary cholesterol (milligrams per kilogram per day).

control mechanisms and are able to maintain a relatively constant plasma cholesterol level when given a moderate dietary cholesterol challenge.

There were also differences between compensators and noncompensators in terms of overall metabolic responses to the high cholesterol diet. Within the group classified as compensators, 29% had a reduction of MNL sterol synthesis rates of 15% or more, 17% had a 10% or greater reduction in the fractional absorption of cholesterol, and 35% decreased both cholesterol fractional absorption and sterol synthesis. In contrast, the noncompensators exhibited a smaller percentage of regulatory responses, with 35% decreasing cholesterol fractional absorption, 35% decreasing synthesis, and 22% expressing reductions in both synthesis and fractional absorption.

Effect of dietary fat quality on plasma lipid and lipoprotein levels

25 of the volunteers completed four dietary study periods of low and high cholesterol intakes on low and high P/S fat diets. This allowed us to evaluate the interactions of dietary fat quality and cholesterol quantity on plasma lipids and lipoproteins and on cholesterol metabolism in the same patients. The characteristics of this subset of patients are presented in Table VII along with the results of their diet analyses. As shown in Table VIII the addition of three large eggs per day to an otherwise low cholesterol diet did not result in an elevation in plasma cholesterol levels in either the PUFA or SFA groups. In contrast, the shift from a PUFA to a SFA diet caused significant ($P < 0.05$) increases in plasma total and LDL cholesterol.

The values for the individual patient responses to the shift in P/S of the diet during the low cholesterol intake periods are shown in Fig. 4. (Similar correlations were seen for plasma cholesterol concentrations during the high cholesterol intake periods.) Analysis of the individual patient data resulting from the shift in dietary fat quality demonstrated that 7 of the 25 volunteers had a significant increase in plasma cholesterol on the SFA low cholesterol regimen ($P < 0.05$). A similar plot (not shown) of the individual data on the high cholesterol intake showed that 6 of 25 had a significant increase in plasma cholesterol on the SFA high cholesterol diet as compared with the PUFA high cholesterol diet. There was no effect on plasma HDL cholesterol or triglyceride levels of the shift in dietary fat quality.

Effect of dietary fat quality on in vivo cholesterol metabolism

The data presented in Table IX demonstrate that the quality of dietary fat did not alter the precision of the regulatory responses to the dietary cholesterol challenge; dietary cholesterol absorption and endogenous cholesterol synthesis were reduced to a similar extent in both the SFA and PUFA trials. During both the PUFA and SFA feeding periods the rate of sterol synthesis in MNL expressed a negative correlation with absorbed dietary cholesterol, and both groups had similar intercepts and slopes relating absorbed dietary cholesterol (milligrams per kilogram per day) and MNL sterol synthesis rates (Table IX). These facts demonstrate that changes in MNL sterol synthesis in response to the dietary cholesterol challenge are independent of the type of fat fed.

Discussion

The role of diet in hyperlipidemia and cardiovascular disease is less than clear (43). Conflicting opinions (63, 64) arise from the contrasting data obtained in controlled metabolic ward studies versus those in free-living, outpatient populations (65); and, in part, from the use of nonphysiological dietary cholesterol intakes or P/S fats during challenge periods. In most studies plasma cholesterol levels are used as the study end points, while less is known about the metabolic consequences of dietary factors on the regulation of cholesterol homeostasis: intake and absorption, endogenous synthesis, catabolism to bile acids, tissue flux, and fecal excretion. Metabolic ward studies in small numbers of patients have demonstrated that these regulatory factors are affected by dietary fat quality and cholesterol quantity, even when plasma cholesterol levels remain unchanged. The literature displays ample evidence of significant patient-to-patient variation in the

*Table VI. Relationship between Changes in Plasma Cholesterol Levels and Sterol Synthesis Rates**

	Compensators (n = 52)			Noncompensators (n = 23)		
Cholesterol intake	Low	High	P	Low	High	P
Plasma cholesterol (*mg/dl*)	237±45	234±41	NS	214±58	240±63	<0.001
% Increase	0			12		
MNL sterol synthesis (*pmol/h per 10^6 cells*)	9.6±2.7	7.1±2.1	<0.001	9.1±2.7	8.0±1.7	NS
% Reduction	−26			−12		

* Data presented as mean±1 SD.

*Table VII. Cross-over Study in 25 Volunteers**‡*

	PUFA (n = 25)		SFA (n = 25)	
Cholesterol intake	Low	High	Low	High
Characteristics				
Age (*yr*)	48.9±10.3	—	—	—
	(44.6–53.1)	—	—	—
Weight (*kg*)	77.5±11.2	77.5±11.4	78.2±11.7	78.5±11.8
	(72.8–82.1)	(72.9–82.3)	(73.3–83.0)	(73.6–83.4)
Height (*cm*)	171.9±6.4	—	—	—
	(169.2–174.5)			
BMI (kg/m^2)	26.1±2.9	26.1±3.0	26.3±3.0	26.5±3.1
	(24.9–27.3)	(24.9–27.4)	(25.1–27.5)	(25.2–27.7)
Dietary intakes				
P/S of dietary fat	1.98±0.93§	1.43±0.47§	0.34±0.19	0.26±0.15
	(1.60–2.37)	(1.24–1.62)	(0.26–0.42)	(0.20–0.33)
Cholesterol (*mg/d*)	183±59‖	815±101‖	272±58¶	836±167¶
	(159–208)	(773–857)	(248–296)	(767–905)

BMI, body mass index. * Data presented as mean±1 SD with the 95% confidence interval given in parentheses. ‡ Values in the same row with common superscripts are significantly different. § $P < 0.01$. ‖ $P < 0.001$. ¶ $P < 0.001$.

precision of these compensatory responses to dietary factors (24–31, 37–39, 44, 66–70).

In the present study we tested a range of intakes of dietary cholesterol from less than to slightly more than that consumed in the average American diet (71), and variations in fat quality from levels less than that in the average American diet (P/S of 0.3 vs. 0.45) to slightly higher than that recommended (1.5 vs. 1.0). The study results bracket what might be expected, in terms of plasma cholesterol levels and cholesterol homeostasis, within the dietary regimens characteristic of the general population.

*Table VIII. Plasma Lipid and Lipoprotein Cholesterol Levels in 25 Volunteers in Cross-over Study**‡*

	PUFA (n = 25)		SFA (n = 25)	
	Low	High	Low	High
Plasma lipids	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*
Cholesterol				
Total	227±50§	230±44‖	240±49§	245±52‖
	(207–248)	(212–248)	(220–260)	(224–267)
LDL	136±41¶	142±38§	144±42¶	154±46§
	(120–153)	(126–157)	(127–162)	(135–173)
HDL	43±10	45±8	44±10	44±10
	(39–47)	(42–49)	(40–48)	(40–48)
Triglyceride	196±121	180±128	210±134	205±142
	(146–246)	(128–233)	(155–265)	(146–263)

* Data presented as mean±1 SD with the 95% confidence interval given in parentheses.
‡ Values in the same row with common superscripts are significantly different.
§ $P < 0.01$.
‖ $P < 0.01$.
¶ $P < 0.05$.

In this study we have applied a series of new techniques which allow the determination of the effects of dietary fat and cholesterol on plasma lipid and lipoprotein levels, and on cholesterol absorption and endogenous synthesis, and thus are able to relate the changes in plasma lipids to metabolic responses and regulatory mechanisms.

Effects of cholesterol quantity and fat quality on plasma cholesterol levels. The results of the 75 studies carried out in 50 patients indicate that the addition of dietary cholesterol (given as eggs) has a marginal influence on plasma lipid levels. Changes in dietary fat quality, on the other hand, had a consistent but small effect on plasma cholesterol levels: ~ 20% of the patients

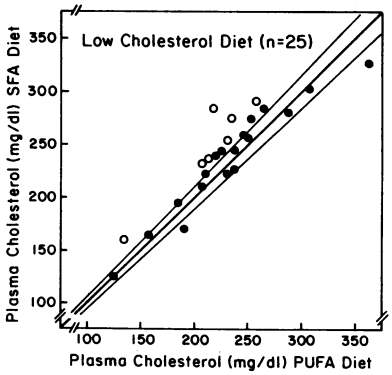

Figure 4. Individual plasma cholesterol responses to a change in dietary fat quality. Plasma total cholesterol levels (milligrams per deciliter) are plotted against the line of identity (±5%) for each patient during the SFA and PUFA regimens on a low dietary cholesterol intake. Open symbols represent significant changes ($P < 0.05$). Changes in plasma cholesterol levels were significant in 7 of 25 volunteers. A similar plot for the high cholesterol diet comparison showed that plasma levels were significantly higher on the SFA diet in 6 of 25 patients.

Table IX. Regulatory Responses in Cross-over Group[*‡§]*

	PUFA (n = 25)		SFA (n = 25)	
Cholesterol intake	Low	High	Low	High
Cholesterol				
Absorption (%)	59.0±13.8 (53.3–64.6)	56.7±13.6 (51.1–62.3)	59.6±11.0[‖] (55.0–64.1)	54.3±14.3[‖] (48.4–60.2)
Absorption (*mg/d*)	108±45[‖¶] (89–126)	461±127[¶] (408–513)	160±35[‖**] (145–175)	458±173** (386–529)
MNL sterol synthesis (*pmol/h per 10^6 cells*)	9.8±2.8[¶] (8.6–10.9)	7.5±2.2[¶] (6.6–8.4)	9.0±2.6** (7.9–10.1)	7.1±2.0** (6.3–7.9)

* Data presented as mean±1 SD with the 95% confidence interval given in parentheses. ‡ Values in the same row with common superscripts are significantly different. § Correlations between absorbed dietary cholesterol (micrograms per kilogram per day) and MNL sterol synthesis rates for the two dietary fat-fed groups were: PUFA group, intercept 10.05 pmol/h per 10^6 cells; slope −0.363 pmol/h per 10^6 cells per milligram per kilogram absorbed cholesterol; $r = -0.363$, $P < 0.01$; and SFA group, intercept 9.46 pmol/h per 10^6 cells; slope −0.376 pmol/h per 10^6 cells per milligram per kilogram absorbed cholesterol; $r = -0.376$, $P < 0.01$. ‖ $P < 0.05$. ¶ $P < 0.005$. ** $P < 0.005$.

exhibited a decrease in plasma cholesterol on a high P/S fat diet, regardless of cholesterol intake.

Sensitivity to dietary cholesterol varied between patients and was independent of the baseline plasma cholesterol level. Those patients exhibiting the greatest increase in plasma cholesterol levels were predominantly those who absorbed the largest amount of dietary cholesterol. The majority of patients were able to compensate effectively by maintaining unchanged plasma cholesterol levels. We did not observe a difference in sensitivity to dietary cholesterol in the PUFA- and SFA-fed groups, in contrast to a previous report (30). Similar results have been reported by others (38, 72) and support the thesis that dietary fat quality and dietary cholesterol are independent in terms of changes in plasma cholesterol levels, and that fat quality has no effect on the compensatory responses to a cholesterol challenge. Neither dietary cholesterol nor the P/S of the diet altered plasma HDL cholesterol levels, presumably due to the moderate ranges of cholesterol and fat P/S used in this study.

The lack of effect of modest increases in dietary cholesterol on plasma cholesterol concentrations has been reported by a number of other investigators (24–27, 66, 68); in contrast, higher intakes can result in increases in plasma cholesterol levels (21, 28, 73). The addition of 500 mg/d of dietary cholesterol to an ordinary American diet usually has little effect on plasma cholesterol levels (24–27, 30, 68, 69); only two studies report significant increases (29, 70).

Any evaluation of studies relating changes in dietary cholesterol to changes in plasma cholesterol must consider the following variables: (*a*) duration of the dietary challenge, since transient effects can occur (69); (*b*) degree of patient-to-patient variability in response to the challenge, since mean values do not indicate the large variability of responses (44, 74); (*c*) number of plasma samples obtained before and during the study, since physiological variations can be large (57); and (*d*) type of diets (formula versus solid food) fed to the study subjects. In the range of cholesterol intakes similar to the American diet, the majority of the data indicate that dietary cholesterol has a minor effect on plasma cholesterol levels in most people; however, subsets of the population exhibit sensitivity to changes in cholesterol intake (74).

Large changes in fat P/S have consistently resulted in lowered plasma cholesterol levels (references 12–20 and many earlier reports). The modest changes tested here resulted in smaller effects: shifting from a dietary fat P/S of 0.3 to 1.8 resulted in a 5.5% mean reduction in total plasma cholesterol levels; a significant reduction in plasma cholesterol levels occurred in 7 of the 25 patients studied. Similar variability in plasma cholesterol responses to modest shifts in fat quality have been reported (39, 67, 75–79). A recent report indicated that dietary beef fat (P/S 0.05) did not elevate plasma cholesterol levels as much as coconut oil (P/S 0.05), and was comparable with plasma levels on safflower oil (P/S 10.2) (79). Recent studies have also shown that intake of monounsaturated fatty acids can also be an effective cholesterol-lowering modality (40).

The present study measured a dietary shift in P/S slightly more than that recommended to the public (64): since the mean reduction in plasma cholesterol was only 5.5%, we would anticipate a smaller change upon moving the population's diet from P/S 0.45 to 1.0 (64).

Effects in individual patient of cholesterol quantity and fat quality on cholesterol metabolism. The lack of a significant dietary cholesterol effect on plasma cholesterol levels in this population is due in part to two regulatory responses found in the majority of patients: (*i*) a small but significant reduction in the fractional absorption of exogenous cholesterol; and (*ii*) the feedback suppression of endogenous cholesterol synthesis. There was no relationship between the fractional absorption of dietary cholesterol and plasma LDL cholesterol levels (Fig. 5) during the low dietary cholesterol phase of the study, or during the high intake period (data not shown), as had previously been reported (80).

Others have shown that intake of a high cholesterol diet can significantly reduce MNL 3-hydroxy-3-methylglutaryl coenzyme A reductase activity (28) and cellular LDL receptor activity (28, 81). In the present study a high cholesterol diet reduced the rate of acetate incorporation into sterols in blood MNL, and the sensitivity of the feedback response was related to the total amount of dietary cholesterol absorbed. Since MNL sterol synthesis rates reflect whole body synthesis rates (49–51), we would predict that the average increment of absorbed dietary cholesterol

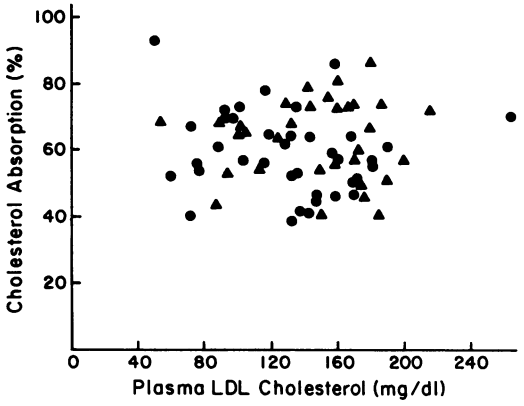

Figure 5. Relationship between percent cholesterol absorption and plasma LDL cholesterol levels during the low cholesterol intake period. Plasma LDL cholesterol levels (milligrams per deciliter) for the subjects on the PUFA diet (●) and SFA diet (▲) plotted against percent cholesterol absorption during the low dietary cholesterol intakes.

of 4.3 mg/kg per d between the two dietary cholesterol periods would reduce endogenous synthesis by an equivalent amount. Whole body endogenous cholesterol synthesis averages 11 mg/kg per d (82), and a decrease of 4.3 mg/kg per d would represent a 39% reduction in synthesis; we observed a 22% reduction in mononuclear cell sterol synthesis. Since other regulatory responses exist that were not quantitated (increased biliary reexcretion and/or increased bile acid synthesis) (2–10), it can be assumed that these responses also contributed to maintenance of plasma cholesterol levels. The differences in synthesis rates observed between compensators and noncompensators are similar to other reports using sterol balance methods (3, 9); patients with precise feedback control resist a dietary cholesterol-induced increase in plasma cholesterol levels, while patients who fail to suppress endogenous synthesis experience increases in plasma cholesterol upon intake of high cholesterol diets. Studies by Oh et al. (74) suggest that classification of patients as compensators and noncompensators may depend on the amount of cholesterol fed, and that at extremely high dietary cholesterol intakes a number of subjects still exhibit effective compensatory responses. The results demonstrate that the degree of saturation of dietary fat has no effect on MNL sterol synthesis nor on the feedback response to dietary cholesterol.

Individualized patient responses to a dietary intervention were reported by us as early as 1971 (2), and have since been recognized in numerous studies (5, 9, 17, 28, 39). The importance of this metabolic heterogeneity has been discussed (41–44, 83, 84) in terms of its importance in evaluating the effects of any dietary intervention on plasma cholesterol levels and whole body cholesterol metabolism. In this study we observed significant heterogeneity in our study population both in terms of dietary fat and cholesterol sensitivity and the precision of regulatory responses. Most patients effectively compensated for a dietary cholesterol challenge by feedback suppression of endogenous cholesterol synthesis coupled with a reduction in dietary cholesterol fractional absorption, while other patients maintained a constant plasma cholesterol level by compensatory mechanisms we are not yet able to measure in outpatients, such as increased biliary reexcretion of cholesterol. For the patients classified as noncompensators the failure of the regulatory mechanisms resulted in an increase in plasma cholesterol levels when dietary cholesterol was increased.

Implications of the results. We found no evidence to support the hypothesis that reducing dietary cholesterol intake from the current level of 450 mg/d to < 300 mg/d will significantly reduce plasma cholesterol levels in the general population. Our data do suggest that reductions in dietary cholesterol may benefit a percentage of people who lack precise feedback control of endogenous cholesterol synthesis; in these terms 31% were noncompensators.

In terms of mean changes, dietary fat quality has a consistent impact on plasma cholesterol levels, but the changes were small in moving from P/S 0.3 to 1.5. In volunteers studied during all four dietary periods the shift from a diet with a P/S of 0.26 and 836 mg/d of cholesterol to a diet of P/S 1.98 and 183 mg/d of cholesterol produced a mean reduction of 8% in total plasma cholesterol. By comparing the mean changes between the four dietary periods, we estimate that the change due to the shift in P/S was 6% and to dietary cholesterol 2%. Thus, dietary fat quality has a larger and more consistent effect on plasma cholesterol than does dietary cholesterol.

The data suggest that the efficacy of changes in dietary fat and cholesterol cannot be predicted for individual patients due to the large patient-to-patient variations observed. Furthermore, our study does not provide criteria on which to separate compensators from noncompensators, short of carrying out dietary trials in individual patients. The results illustrate the importance of modifying the dietary intervention to meet the needs of the patient and that patient's responsiveness to dietary factors. As previously discussed (84), there is little evidence to justify the use of one diet to treat all hypercholesterolemic patients, and the use of other dietary alternatives, such as a high P/S diet, a monounsaturated fat diet, or even the inclusion of fish oils in the diet may be beneficial in reducing plasma cholesterol levels in some hypercholesterolemic patients. Only by detailed follow-up to determine the plasma cholesterol lowering efficacy of any dietary change can the most appropriate intervention for an individual patient be determined by his physician.

The long-term significance of differences between compensators and noncompensators remains to be defined. Studies by Clarkson et al. (85) on the regression of atherosclerosis in rhesus monkeys demonstrated that animals maintained at the same plasma cholesterol levels differed in their rates of progression or regression of atherosclerosis, depending upon whether they were compensators ("hyporesponders," in their terminology) or noncompensators ("hyperresponders"). If similar differences in lesion progression or regression exist in human beings then it becomes important to identify and intervene in hypercholesterolemic individuals who are noncompensators: these may be the individuals at highest risk for atherosclerosis and the most responsive to dietary intervention. Further studies are needed to determine the genetics of noncompensation and whether dietary interventions can effectively reduce heart disease risk in this group as compared with the relatively diet-insensitive compensators.

Acknowledgments

The authors wish to express their gratitude to the volunteers who participated in this study for their involvement and cooperation. The ex-

cellent technical assistance of Mary Lee, Florence Chu, Eleanor Mathusek, Ludmilla Mallikin, and Margaret Rudolph, and the services of the Rockefeller University Hospital Out-Patient Clinic are gratefully acknowledged.

This work was supported in part by U. S. Public Health Service grants (HL24190 and HL28727 from the National, Heart, Lung and Blood Institute, FR-00102 from the General Clinical Research Center Branch, Division of Research Resources, and CA29502 from the National Cancer Institute), and by grants from the American Egg Board, the National Dairy Council, the Herman Goldman Foundation, and the Jean and Louis Dreyfus Foundation.

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