U-shape relationship between change in dietary cholesterol absorption and plasma lipoprotein responsiveness and evidence for extreme interindividual variation in dietary cholesterol absorption in humans

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Abstract A possible relationship between change in dietary cholesterol absorption and plasma lipoprotein responsiveness was examined in 18 normal subjects fed low fat low cholesterol, high fat low cholesterol, and high fat high cholesterol diets. For the group, neither dietary cholesterol nor dietary fat affected the percentage dietary cholesterol absorption, whereas dietary cholesterol intake raised total and LDL-C and dietary fat raised total, LDL, and HDL-C. On a fixed diet there was approximately a 2-fold variation among subjects in percentage dietary cholesterol absorption. Subjects also varied in response to dietary cholesterol and fat with regard to dietary cholesterol absorption and plasma lipoprotein responsiveness. There was a U-shaped parabolic relationship between dietary cholesterol-induced percent change in LDL-C and the change in percentage dietary cholesterol absorption ($R^2 = 0.62$, $P = 0.005$). A similar but weaker relationship characterized the responsiveness of HDL-C ($R^2 = 0.38$, $P = 0.05$). For the group, increased cholesterol intake raised dietary cholesterol mass absorption from 1.6 to 4.6 mg/kg per day, but the range of increase was from 1 to 4.7 mg/kg per day. Increased fat intake also affected dietary cholesterol mass absorption with most subjects displaying a strong inverse relationship between fat intake and mass absorption ($r = -0.77$, $P < 0.003$). In summary: *i*) the percentage change in dietary cholesterol absorption in response to dietary cholesterol does appear to regulate diet responsiveness of LDL and HDL-C, and *ii*) the large variability in percent absorption and changes in percentage and mass absorption in response to dietary cholesterol suggest the presence of genetically determined differences among individuals in the regulation of dietary cholesterol absorption.—Sehayek, E., C. Nath, T. Heinemann, M. McGee, C. E. Seidman, P. Samuel, and J. L. Breslow. **U-shape relationship between change in dietary cholesterol absorption and plasma lipoprotein responsiveness and evidence for extreme interindividual variation in dietary cholesterol absorption in humans. *J. Lipid Res.* 1998.** 39: **2415–2422.**

Supplementary key words dietary fat • LDL • HDL

Atherosclerotic cardiovascular disease is the number one public health problem in the United States, and by the year 2020 it is predicted this will be true world wide. This disease is a complex genetic disease with many genes involved and important gene–environment interactions. Epidemiological, clinical, and animal studies have clearly established an important role for dietary cholesterol and saturated fat in atherosclerosis susceptibility. Numerous studies have shown that increased consumption of cholesterol and saturated fat are associated with increased plasma levels of LDL cholesterol and increased risk of cardiovascular diseases, whereas low dietary cholesterol and low saturated fat have the opposite effect (1). However, it has been repeatedly observed that there is great interindividual variation in plasma lipoprotein responsiveness to dietary cholesterol and saturated fat. This variation is presumably genetic but the specific genes involved are largely unknown.

It has been previously shown in humans that for each 100 mg/day increase in dietary cholesterol the mean plasma cholesterol level rises 7 mg/dl, but some individuals are unresponsive and have even decreasing cholesterol levels, while others show an exaggerated response, with increases of more than 2-fold the mean (2–6). In human studies, increasing saturated fat intake also increases cholesterol levels with similar interindividual variability (7, 8). In human studies, it has been suggested that the ability to down-regulate endogenous cholesterol synthesis in response to a dietary challenge limits an individual's plasma lipoprotein responsiveness (9, 10). In animal studies, evidence has been provided that species fed a low cholesterol

Abbreviations: HFHC, high fat high cholesterol; HFLC, high fat low cholesterol; LFLC, low fat low cholesterol.

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diet and have high hepatic cholesterol synthesis, which can down-regulate synthesis in response to cholesterol feeding, are less responsive to dietary challenge than species in which hepatic cholesterol synthesis is low (11). Thus metabolic processes, and the genes that control them, that regulate individual differences in endogenous cholesterol synthesis may be fundamental to understanding plasma lipoprotein responsiveness to dietary challenge.

Another metabolic process that could influence diet responsiveness is the absorption of dietary cholesterol from the intestine, as this represents the first obligatory step that allows dietary cholesterol to exert its metabolic effects. After absorption, dietary cholesterol is transported by chylomicrons and their remnants mainly to the liver where it can directly influence lipoprotein production and removal pathways (7, 11). Individual differences in absorption could influence lipoprotein responsiveness in this manner. Differences in dietary cholesterol absorption could also account for differences in the endogenous cholesterol synthesis proposed in human and animal studies to account for plasma lipoprotein responsiveness.

The current study was undertaken to determine the relationship, if any, between dietary cholesterol absorption and plasma lipoprotein responsiveness. Eighteen normal subjects were fed low fat low cholesterol, high fat low cholesterol, and high fat high cholesterol diets and the percentage dietary cholesterol absorption and plasma lipoprotein responsiveness were measured. The percentage change in dietary cholesterol absorption in response to dietary cholesterol was found to be highly correlated with the percent change in LDL and HDL cholesterol levels and the relationships were best described by U-shaped parabolic curves. Documentation was also provided for large individual differences in *i*) dietary cholesterol absorption and *ii*) the dietary cholesterol affect on percentage dietary cholesterol absorption and dietary cholesterol mass absorption. These differences suggest genetic variation among humans in the regulation of dietary cholesterol absorption.

METHODS

Subjects

Eighteen normal volunteers were recruited through advertisements posted at The Rockefeller University and neighboring institutions or through college undergraduate work–study programs. There were no exclusions based on gender, race, or ethnic background. Subjects had normal thyroid, renal, and liver function tests and no systemic diseases by history or physical examination. None of the subjects were smokers or on any medication, including birth control pills. There were 10 males and 8 females varying in age from 19 to 60 years (mean ± SD of 30.3 ± 13.3) with body mass indices (BMI) from 17.0 to 27.3 (mean ± SD of 23.2 ± 2.9). The distribution of apoE phenotypes was E3/3, 44.4%; E4/3, 22.2%; E3/2, 22.2%; E4/4, 5.6%; and E2/2, 5.6%. All subjects were normolipidemic upon initial screening with lipid and lipoprotein levels between 10th and 90th percentile for age and sex based on Lipid Research Clinic data (12).

Experimental protocol

The subjects were studied on the inpatient unit of The Rockefeller University Hospital and encouraged to continue their usual physical activity. The study design was a randomized crossover trial of three isocaloric, natural food diets that differed in dietary fat and/or cholesterol. The diets, as described below, were: low fat low cholesterol (LFLC), high fat low cholesterol (HFLC), and high fat high cholesterol (HFHC). Each metabolic diet was consumed for 3 weeks. The diets were randomly assigned to each subject with every set of three subjects in Latin squares balanced for sequence, so that each diet followed the others twice, with an equal number of subjects on each diet. All subjects completed the study with no dropouts. Fasting lipoprotein profiles were obtained four times in the third week of each diet period. We have previously shown that under metabolic ward conditions, similar to the ones in this study, changes in dietary fat and cholesterol result in a new steady state in plasma lipoprotein levels by day 15 with no drift between days 15 and 25 (13). The study protocol was approved by the Institutional Review Board of The Rockefeller University, and each subject signed an informed consent prior to the study.

Diets

Meals were prepared by the nutrition staff of The Rockefeller University Hospital Clinical Research Center. The diets consisted of common ingredients of known composition listed in the USDA Handbook 8 (14), and the foods were weighed to the nearest 0.1 gram. A 2-day rotating menu was provided throughout each study period. Breakfast and dinner were routinely consumed at the Clinical Research Center and lunches were usually packed for convenience. Subjects were instructed not to eat any foods other than the metabolic diets provided and all foods served had to be consumed by 8 pm on the same day. The physicians and research nutritionists communicated with each subject daily to encourage compliance. The initial caloric requirement for each subject was estimated according to the Harris-Benedict equation with an adjustment for physical activity (15). The caloric requirements ranged from 2300 to 3500 kcal, (mean ± SD of 2789 ± 362). Subjects were kept in a metabolic steady state with no significant changes in weight or physical activity during the study. The composition of the diets is shown in **Table 1**. The LFLC diet conformed to the American Heart Association Phase II Diet and contained 60% carbohydrate, 15% protein, 25% fat (26% saturated, 40% monounsaturated and 34% polyunsaturated fatty acids), and 80 mg cholesterol/ 1000 kcal/day, the equivalent of 0.04% weight/weight (w/w) dietary cholesterol. The HFLC diet was characterized by 42% carbohydrates, 15% protein, 43% fat (44% saturated, 40% monounsaturated and 16% polyunsaturated fatty acids), and 80 mg cholesterol/ 1000 kcal/day (0.04% w/w dietary cholesterol). The HFHC diet was identical to the HFLC diet except for cholesterol content of 200

TABLE 1. Diet composition

	LFLC	HFLC	HFHC
Carbohydrates (%)	60	42	42
Protein (%)	15	15	15
Fat (%)	25	43	43
Fatty acids composition			
Saturated (%)	26	44	44
Monounsaturated (%)	40	40	40
Polyunsaturated (%)	34	16	16
P/S ratio	1.5	0.35	0.35
Cholesterol (mg/1000 kcal/d)	80	80	200

mg/1000 kcal per day that is equivalent to 0.1% w/w dietary cholesterol. The compositions of the diets were verified by chemical analysis of composites of each day of the three metabolic diets by Hazelton Laboratories (Madison, WI).

Measurements of lipid, lipoproteins, and apolipoprotein E genotyping

In the third week of each diet period (days 16, 17, 19, and 20) four fasting plasma samples anticoagulated with EDTA were obtained after a 12-h overnight fast for lipid and lipoprotein measurements. The values used for analysis were the average of these four measurements for each subject on each diet. Total cholesterol and triglycerides were determined by enzymatic methods using Boehringer Mannheim reagents. HDL-cholesterol was determined after precipitation of non-HDL-C by dextran sulfate (16). LDL-C plus HDL-C was determined on the infranatant after airfuge ultracentrifugation (Beckman Instruments). LDL-C was the difference between the infranatant and HDL-C value. VLDL-C was the difference between total and the infranatant cholesterol. Total and HDL-cholesterol values were standardized by the Lipid Standardization Program of the Centers for Disease Control and Prevention supported by the National Heart, Lung, and Blood Institute (17). Apolipoprotein E genotyping was determined according to Hixson and Vernier (18).

Cholesterol absorption

Cholesterol absorption was determined by the isotope ratio method as described by Zilversmit and Hughes (19) and modified for human studies by Samuel, Crouse, and Ahrens (20). Briefly, on the third week of each dietary period (day 16) subjects were fasted overnight and radiolabeled cholesterol was administered intravenously and orally between 8 and 10 am. For intravenous administration, [1,2-^{3}H]cholesterol dissolved in 1 ml of ethanol was suspended in 150 ml of saline and immediately infused. Residual radioactivity in the infusion set was measured after toluene extraction and subtracted from the total radioactivity to calculate the administered dose. For oral administration, [4-^{14}C] cholesterol dissolved in 1 ml of ethanol was mixed with 5 ml of milk in a glass beaker and immediately ingested. Subsequently, another 5 ml of milk was added to the beaker and this too was ingested. Residual radioactivity remaining in the beaker was measured by ethanol extraction and the net amount administered was determined. Dosage of radioactivity varied from 1 to 2 μCi of [1,2-^{3}H]- and [4-^{14}C]cholesterol per assay. Radioactivity was measured in a Beckman LS 5000TD model scintillation counter (Beckman Instruments Co., Fullerton, CA) with automatic quench compensation after Compton spectrum measurement. Plasma ^{3}H and ^{14}C labels were determined on morning blood samples drawn on days 18, 19, 20, and 21 of each diet. Percent cholesterol absorption was calculated using the equation:

$$\%\ \text{absorption} = {}^{14}\text{C}/{}^{3}\text{H ratio} \times \frac{\text{intravenous } {}^{3}\text{H dose (dpm)}}{\text{oral } {}^{14}\text{C dose (dpm)}} \times 100$$

Mass absorption of dietary cholesterol was calculated by multiplying the daily cholesterol intake by the percent cholesterol absorption and expressed as mg cholesterol/kg body weight per day.

Statistical analysis

The study was designed as a three-treatment, three-period crossover trial with no wash-out periods between treatments. Sample size was calculated during the design phase of the trial, using a power of 80% and a significance level of 0.05. During sample size calculations, it was assumed that there would be no carryover effects from one diet to the next. Data analysis was performed using a computer model in Excel 7.0 (Microsoft®, 1996), which used formulas for the analysis of crossover studies found in Fleiss (21). Data were examined for the amount of variation attributable to subjects, periods, treatment effects, carryover effects, and residual effects for each variable of interest under study (TC, TG, VLDL-C, LDL-C, HDL-C, and percentage dietary cholesterol absorption). There were no significant carryover effects for any of the variables except HDL-C. For HDL-C, the variation due to crossover effects resulted in an F-ratio of 11.58 with 2 and 30 degrees of freedom. This corresponds to a P value less than 0.001 indicating evidence for the presence of some carryover effects for HDL-C. For variables in which there was evidence for a treatment effect but no evidence of a carryover effect, pairwise differences between treatments were examined using Tukey's HSD.

The relationship between cholesterol absorption and plasma lipoprotein responsiveness was investigated using a polynomial regression model. The multiple coefficient of determination (R^2) was used as a measure of the goodness of fit of each model. Pairwise dot plots were also used to examine changes in important parameters between treatments. All data analyses were done in SPSS 7.5 on a Gateway 2000 E-3000 computer running the Windows 95 operating system.

RESULTS

The percentage absorption of dietary cholesterol for each subject on the different diets is shown in **Fig. 1**. For the group, neither dietary cholesterol nor dietary fat sig-

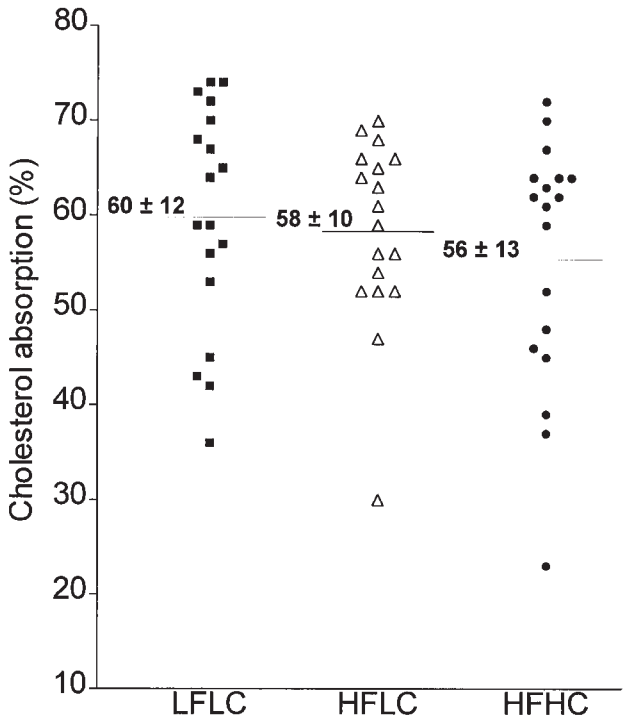

Fig. 1. Variability in dietary cholesterol absorption rates according to diets. Each subject consumed LFLC (■), HFLC (△), and HFHC (●) diet. Cholesterol absorption rates were measured during the third week of each dietary period. Displayed figures are the group mean ± SD values of cholesterol absorption rates on each diet.

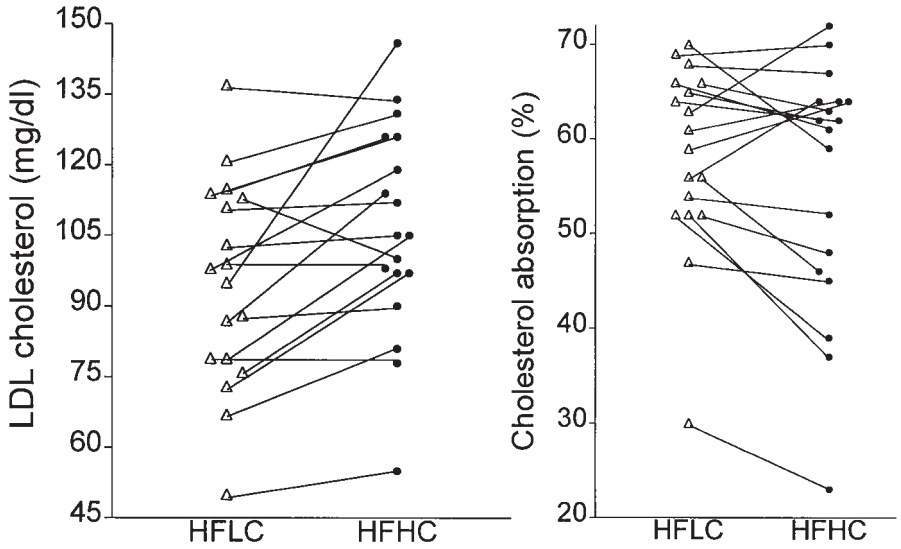

Fig. 2. Variability in LDL-C and cholesterol absorption responsiveness to dietary cholesterol. Displayed are the subjects' LDL-C levels and cholesterol absorption rates on HFLC (△) and HFHC (●) diets.

nificantly altered the percentage dietary cholesterol absorption. It is important to note, however, that regardless of diet type, the individuals within the group differed markedly in the percentage dietary cholesterol absorption. For example, on the LFLC diet percentage dietary cholesterol absorption varied from 36 to 74%. No significant relationships were found between apoE genotype and cholesterol absorption rates.

Individual changes in percentage dietary cholesterol absorption in response to dietary cholesterol are shown in **Fig. 2**. The responsiveness of percentage dietary cholesterol absorption varied markedly among individuals with some subjects increasing, some maintaining, and others decreasing their values in response to dietary cholesterol. Similar variability characterized the responsiveness of percentage dietary cholesterol absorption to dietary fat (data not shown).

For the group as a whole, the plasma lipid and lipoprotein levels on each diet and the isolated effects of increasing dietary cholesterol (HFHC–HFLC) and dietary fat (HFLC–LFLC) are shown in **Table 2**. Dietary cholesterol increased LDL-C levels by 11.6 ± 14.9 mg/dl, whereas dietary fat increased LDL-C 6.8 ± 7.3 mg/dl. There was no significant effect of dietary cholesterol on HDL-C levels, but increased dietary fat increased HDL-C 5.2 ± 5.3 mg/dl. Finally, dietary cholesterol and dietary fat did not significantly change triglyceride or VLDL-C levels. Thus dietary cholesterol and dietary fat were shown to have different effects on the plasma lipoprotein pattern.

Individual changes in LDL-C levels in response to dietary cholesterol are shown in Fig. 2. Like dietary cholesterol absorption, the LDL-C responsiveness varied markedly among individuals with some subjects increasing, some maintaining, and others decreasing their LDL-C in response to dietary cholesterol. Similar variability characterized the responsiveness of LDL-C to dietary fat (data not shown).

Next, possible relationships were sought between percentage dietary cholesterol absorption and LDL-C levels. During none of the diet periods was there a relationship between these variables (data not shown). However, there are many factors that might influence LDL-C levels aside from dietary cholesterol absorption and these might obscure a possible relationship. Therefore, to minimize the

TABLE 2. Effect of diets and isolated effects of dietary fat and dietary cholesterol on plasma lipids and lipoprotein levels

	LFLC	HFLC	HFHC	HFLC-LFLC (Fat)	HFHC-HFLC (Cholesterol)
			mg/dl		
TC	153.6 ± 19.2	166.6 ± 24.1	177.7 ± 25.6[b]	12.9 ± 9.1	11.7 ± 18.7
TG	80.2 ± 26.8	84.0 ± 27.8	77.5 ± 23.2		
VLDL-C	19.3 ± 7.4	19.3 ± 8.7	18.7 ± 5.9		
LDL-C	87.9 ± 18.3	94.7 ± 21.9	106.1 ± 23.2[a,b]	6.8 ± 7.3	11.6 ± 14.9
HDL-C	46.9 ± 11.9	53.2 ± 13.0	52.1 ± 13.5	5.2 ± 5.3	1.2 ± 4.4

Value are given as mean ± SD. (HFLC-LFLC) and (HFHC-HFLC) are the changes in lipid/lipoprotein levels in response to dietary fat and dietary cholesterol, respectively.

[a] $P < 0.05$ versus HFLC.

[b] $P < 0.01$ versus LFLC.

effect of other variables, a relationship was sought between dietary cholesterol-induced changes in percentage dietary cholesterol absorption and percent change in LDL-C levels. To accomplish this, for each subject the value of LDL-C on the HFLC diet was subtracted from the values on the HFHC diet and the difference was expressed as percent change after normalization to the value on the HFLC diet. Individual changes in cholesterol absorption were calculated by subtracting the percentage absorption on HFLC from the percentage absorption on HFHC diet. As shown in **Fig. 3**, for the 18 subjects in the study, the plot of dietary cholesterol-induced change in percentage dietary cholesterol absorption versus percent change in LDL-C level reveals a non-linear relationship. A U-shaped parabolic curve, which best describes the relationship, indicates a high coefficient of multiple determination between the variables ($R^2 = 0.62$, $P = 0.005$). This suggests that almost two-thirds of the variation in the dietary cholesterol-induced change in LDL-C is explained by the dietary cholesterol-induced change in percentage dietary cholesterol absorption. Further analysis of this relationship suggests two populations of individuals. Analyzing the data for the top and bottom half of the dietary cholesterol-induced changes in percentage dietary cholesterol absorption, as shown in the Fig. 3 insets, revealed significant linear correlations in the top and bottom groups, $r = 0.71$; $P < 0.04$ and $r = -0.82$; $P < 0.007$, respectively. To determine whether the U-shaped curve is influenced by outliers, we removed the subject with a 50% change in LDL-C and the subject that reduced his LDL-C by over 10%. The new analysis resulted in R^2 value of 0.46 and $P = 0.019$, suggesting that the U-shape relationship is not heavily influenced by these outliers. Analysis of dietary cholesterol-induced change in percentage dietary cholesterol absorption versus percent change in HDL-C levels also revealed a parabolic relationship, but this was weaker with an $R^2 = 0.38$, $P = 0.05$ explaining a smaller proportion of the variation in HDL-C responsiveness (**Fig. 4**). In addition, the dietary cholesterol-induced changes in LDL-C and HDL-C were correlated ($r = 0.48$, $P < 0.04$, data not shown). Finally, no significant relationships were found between dietary fat-induced changes in percentage dietary cholesterol absorption and dietary fat-induced changes in LDL-C or HDL-C levels.

Differences among individuals in the mass of dietary cholesterol absorbed (mg dietary cholesterol/kg body weight per day) under similar dietary constraints were also examined. This was assessed by multiplying the cholesterol in the diet (mg/kcal per day) by the daily kcal ingested by the percentage dietary cholesterol absorption. As shown in **Fig. 5A**, on the HFLC diet an average of 1.7 ± 0.4 mg cholesterol/kg per day was absorbed with a range from 1 to 2.6 mg cholesterol/kg per day. On the HFHC diet, an average of 4.6 ± 1.2 mg dietary cholesterol/kg per day was absorbed with a range from 2 to 6.6 mg dietary cholesterol/kg per day. In switching from the HFLC to the HFHC diet in different individuals, the range of increase in cholesterol absorption was from 1 to 4.7 mg dietary cholesterol/kg per day. Dietary fat also affected the mass absorption of dietary cholesterol. As shown in Fig. 5B, most individuals decreased but some maintained and others increased their dietary cholesterol mass absorption. Moreover, the subgroup of 13 subjects that responded to dietary fat by decreased dietary cholesterol mass absorption was characterized by a strong and inverse relationship between fat intake (gm/day) and the decrease in dietary cholesterol absorbed per kg body weight per day ($r = -0.77$, $P < 0.003$; **Fig. 6**). These results suggest independent effects of dietary cholesterol and dietary fat on dietary cholesterol mass absorption and remarkable

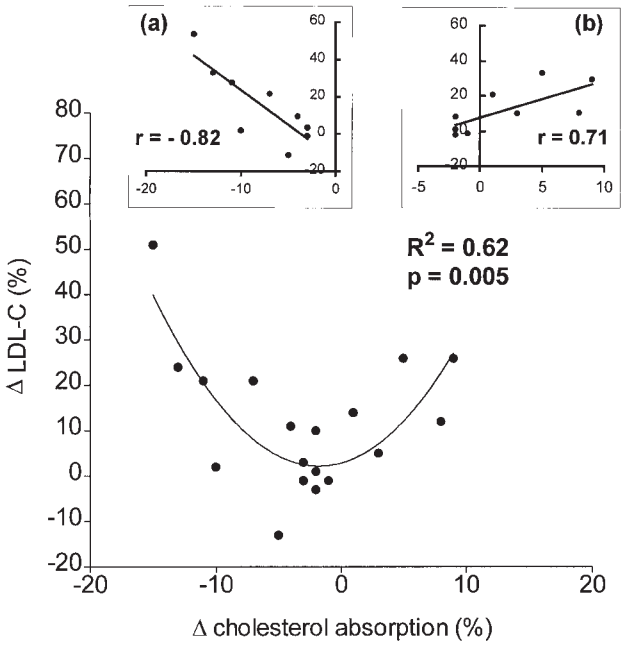

Fig. 3. Relationships of changes in LDL-C and cholesterol absorption rates in response to dietary cholesterol. Subjects LDL-C and cholesterol absorption responsiveness (shown in Fig. 2) are displayed as % change in response to dietary cholesterol. The U-shaped parabolic relationship was characterized by $R^2 = 0.62$, $P = 0.005$. Insets show the relationship of LDL-C responsiveness to cholesterol absorption in the lower (inset a; $r = -0.82$) and upper (inset b; $r = 0.71$) fiftieth percentiles of subjects cholesterol absorption response to dietary cholesterol.

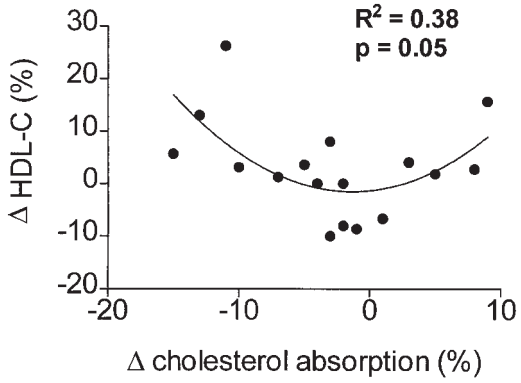

Fig. 4. Relationships of changes in HDL-C and cholesterol absorption rates in response to dietary cholesterol. The parabolic relationship was characterized by $R^2 = 0.38$ and $P = 0.05$.

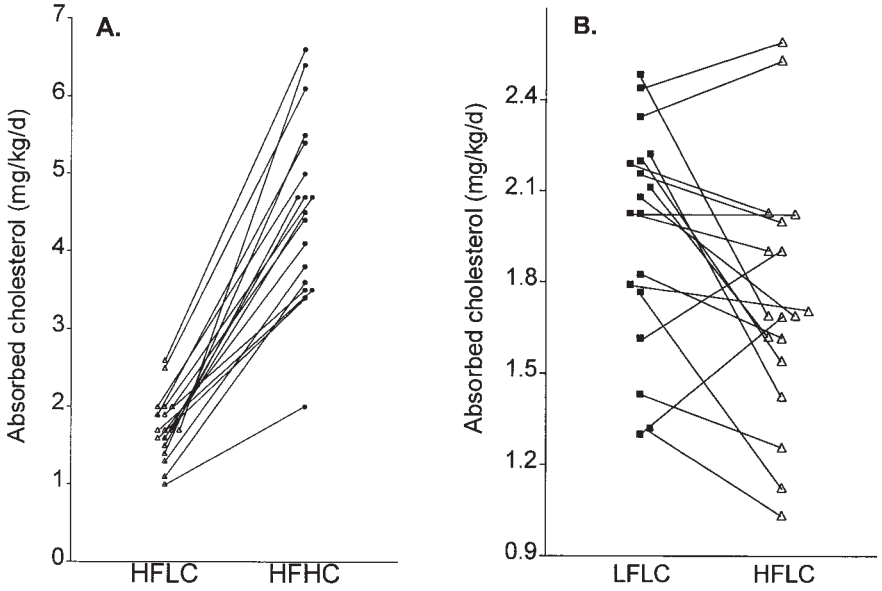

Fig. 5. Subjects variability in dietary cholesterol mass absorption in response to dietary cholesterol (A) and dietary fat (B). Dietary cholesterol mass absorption was calculated by multiplying the individual daily cholesterol intake by cholesterol absorption rates. Displayed are the individuals' mass absorption of dietary cholesterol on LFLC (■), HFLC (△), and HFHC (●) diets.

interindividual variation in dietary cholesterol mass absorption in response to dietary fat and dietary cholesterol.

DISCUSSION

In the current study we have shown: *i*) marked interindividual variability in the effect of dietary cholesterol on percentage dietary cholesterol absorption and LDL-C levels; *ii*) a relationship between dietary cholesterol-induced changes in the percentage of dietary cholesterol absorbed and changes in LDL-C and HDL-C levels, best characterized by U-shaped parabolic curves; *iii*) dietary cholesterol and dietary fat independently affect dietary cholesterol mass absorption; *iv*) in subjects that decreased their dietary cholesterol mass absorption in response to dietary fat, the decrease was strongly related to their fat intake; and *v*) the increase in dietary cholesterol mass absorption in response to dietary cholesterol was characterized by marked interindividual variability.

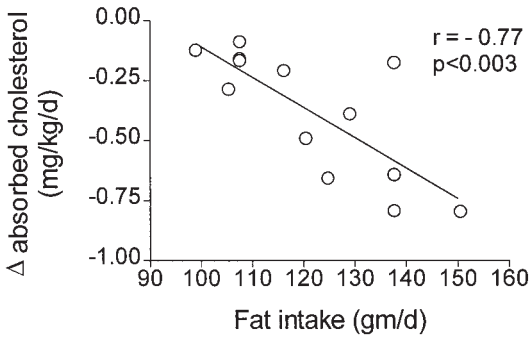

Fig. 6. Relationships of dietary cholesterol mass absorption to fat intake. Displayed are the values in a subset of 13 subjects that responded to dietary cholesterol by decreased dietary cholesterol mass absorption ($r = -0.77$, $P < 0.003$).

Numerous studies in animals and humans have shown large variability in plasma lipoprotein responsiveness to dietary cholesterol. Strain differences in plasma cholesterol responsiveness have been shown in mice, rats, and rabbits (2, 22, 23), and variability in responsiveness among individual animals from the same species was reported in rabbits and monkeys (2, 24). Careful studies by Katan and Beynen (3) were able to show the presence of large variability in plasma cholesterol responsiveness in humans. These investigators reported that whereas some individuals respond to dietary cholesterol by a large increase in plasma cholesterol, some maintain and others even decrease their plasma cholesterol levels (3). Furthermore, by repeated trials in individuals with different levels of responsiveness, the same group has shown that the pattern of response is reproducible and displays a characteristic feature of individual subjects (4–6). The large variability in LDL-C response to dietary cholesterol in our study (Table 2 and Fig. 2) agrees with these findings. Moreover, our results, derived under metabolic ward conditions that minimized confounding environmental effects, suggest that the observed interindividual differences in LDL-C response may be due to genetic factors that control responsiveness to dietary cholesterol.

As compared to LDL-C responsiveness, the interindividual variability in changes in percentage dietary cholesterol absorption in response to dietary cholesterol and fat has received much less attention. Animal and human studies support the presence of considerable intra-species vari-

ability in percentage dietary cholesterol absorption. Strain differences in percentage dietary cholesterol absorption have been reported in mice (25), and differences among individual animals from the same species were reported in monkeys (26, 27). In humans, a number of clinical studies showed that individuals differ in percentage dietary cholesterol absorption (28, 29). Our results support these observations (Fig. 1). However, in the present study we also examined for each subject the change in the percentage dietary cholesterol absorption in response to changes in dietary cholesterol and dietary fat and we found marked interindividual differences in the responsiveness of percentage dietary cholesterol absorption to these nutrients (Fig. 2).

The main objective of the current study was to search for a relationship between dietary cholesterol absorption and plasma lipoprotein levels. Unfortunately, during none of the diet periods was there a correlation between percentage dietary cholesterol absorption and lipoprotein levels. However, this may not have been unexpected as the regulation of lipoprotein levels is complex. To concentrate on the effects of dietary cholesterol and fat, we instead looked for diet-induced changes in percentage dietary cholesterol absorption and lipoprotein levels. This revealed that the dietary cholesterol-induced percent change in the plasma level of LDL-C, and less so of HDL-C, is significantly and strongly related to the induced change in the percentage dietary cholesterol absorption (Figs. 3 and 4). The nature of these relationships is best represented by U-shaped parabolic curves. Analysis of these relationships suggests that compared to the values displayed by the curves' nadir, every decrease or increase in percentage dietary cholesterol absorption resulted in a similar response to dietary cholesterol, e.g., an increase in plasma levels of LDL-C and HDL-C. Although cautious analysis of our data precludes outlier effects, it is important to note that the U-shaped relationships in our study derive from data in 18 healthy individuals on strictly controlled dietary conditions and this calls for studies that will address these relationships in additional populations under different dietary conditions.

How do opposite effects of dietary cholesterol on the percentage dietary cholesterol absorbed result in a similar effect on plasma lipoprotein levels? Although the current study was not designed to address this important question, we have carried out recent studies in the mouse that might be relevant to our findings in humans. In these studies we found that in C57Bl6 mice the percentage dietary cholesterol absorption was strongly and inversely related to biliary cholesterol concentration (30). In other mouse strains we found that other biliary constituents also influenced percentage dietary cholesterol absorption, leading to the general hypothesis that percentage dietary cholesterol absorption is regulated by the capacity of the bile to solubilize cholesterol which is a function of the relative amounts of biliary cholesterol, phospholipids and bile acids (E. Sehayek and J. L. Breslow, unpublished data). Recent data from Dr. D. W. Russell's laboratory (31) in studies with 7α-hydroxylase-deficient mice also suggest that the quality of the bile acids is important. Schwarz et al. (31, 32) found that 7α-hydroxylase-deficient mice have altered bile acid composition and strongly suppressed dietary cholesterol absorption. Moreover, studies in African green monkeys and rabbits with low plasma cholesterol responsiveness to dietary cholesterol reported that cholesterol feeding resulted in suppression and stimulation of 7α-hydroxylase in the non-responsive monkeys and rabbits, respectively. In each case, these contrasting effects on 7α-hydroxylase were used to explain the resistance of plasma cholesterol responsiveness to dietary cholesterol in these animals (33, 34). We speculate that in humans, opposing changes in the percentage dietary cholesterol absorption may result from genetic variability in biliary composition response to dietary cholesterol and that these changes are coupled with, as yet to be defined, metabolic events that increase the concentrations of plasma LDL and HDL-C levels.

The role of dietary fat in determining the percentage dietary cholesterol absorbed is well established. Human studies have shown that very low fat intake dramatically decreases dietary cholesterol absorption, probably because intraluminal lipolysis of dietary fat promotes absorption (35, 36). In the present study we showed that the increase in dietary fat intake was associated with a remarkable interindividual variability in dietary cholesterol mass absorption (Fig. 5), and that there was a strong and inverse relationship between fat intake and the decrease in dietary cholesterol mass absorption in the subgroup of individuals that suppressed their absorption in response to dietary fat. These results strongly suggest that excessive fat intake may interfere, at least in some subjects, with the absorption of dietary cholesterol from the intestine.

In the present study we also showed remarkable interindividual differences in the increase of dietary cholesterol mass absorption in response to dietary cholesterol (Fig. 5). In the aforementioned recent studies in C57Bl6 mice we showed that dietary cholesterol absorption appears to be a saturable process characterized by a K_m of 0.4% w/w dietary cholesterol and a V_{max} of 0.65 mg cholesterol/gm body weight per day (30). In the present study individual subjects fed diets containing 0.04% and 0.1% w/w dietary cholesterol (HFLC and HFHC diets, respectively) had up to a 4.7-fold difference in the increase in mass of dietary cholesterol absorbed (Fig. 5). These results suggest that, in humans, individual subjects may differ markedly in K_m and/or V_{max} values within the range of normal human dietary cholesterol consumption. This marked interindividual variability in dietary cholesterol mass absorption in response to dietary cholesterol, under conditions that minimize environmental confounding effects, strongly supports the assumption that in humans genetic factors may regulate the kinetics of dietary cholesterol absorption.

In summary, the results of the present study emphasize the variability in dietary cholesterol absorption and plasma lipoprotein response to dietary cholesterol and dietary fat in humans, and show that dietary cholesterol and dietary fat independently affect the absorption of dietary

cholesterol from the intestine. Finally, the relationship of plasma lipoprotein responsiveness to dietary cholesterol is complex and demands further studies that will clarify the details of the interactions between the mechanisms that govern the absorption of dietary cholesterol and plasma lipoprotein metabolism.

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