Determinants of plasma cholesterol responsiveness to diet

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Plasma cholesterol change, or 'responsiveness', to dietary saturated fat modification has long been acknowledged. The present study sought to determine the specific, predicted response of each cholesterol subfraction to known dietary manipulations. Two metabolically controlled diets, one with a low polyunsaturated:saturated fat (low P:S) ratio, and one with a high P:S ratio were fed in a crossover design to sixty-seven normolipidaemic subjects pooled from six foregoing metabolic studies. A series of statistical analyses was performed to identify the lipids and subfractions independently affected by the diet crossover. Multivariate analysis of variance revealed that the changes in total cholesterol (ΔTC), low-density-lipoprotein-cholesterol (ΔLDL-C), and high-density-lipoprotein-cholesterol (ΔHDL-C) were the only statistically significant diet-specific 'responsive' lipids. Multiple regression was performed to identify the independent predictors of ΔTC, ΔLDL-C and ΔHDL-C. It was found that age (years), extent of change in dietary saturated fat, and baseline LDL-C (mg/l) levels determine LDL-C change, while extent of change in saturated and polyunsaturated fat, and baseline HDL-C (mg/l) levels can predict HDL-C change. A series of equations to predict lipoprotein responsiveness to diet are derived for potential use in clinical practice.

Plasma cholesterol: Dietary fat: Lipoproteins: Man

Numerous studies have focused on the factors that regulate total cholesterol (TC) levels and can exert a beneficial change in lipid concentrations (Castelli *et al.* 1986; Lerner & Kanner, 1986; Martin *et al.* 1986; Nutrition Committee, American Heart Association, 1988). By far the most intensely studied of these influences has been dietary, and more specifically the change in dietary saturated fatty acids (SFA). Several investigators have studied the extent of change, or 'responsiveness', as seen by a predictable change in total cholesterol (ΔTC), to a change in dietary fat (Ahrens *et al.* 1957; Keys *et al.* 1957, 1965*a*, *b*; Grande & Anderson, 1964; Hegsted *et al.* 1965; Keys & Parlin, 1965; Jacobs *et al.* 1983).

The earliest ground-breaking work in the area of cholesterol responsiveness to dietary change was done by Keys *et al.* (1957, 1965*a*, *b*). In 1965, following a study conducted under metabolic conditions, this group proposed an interrelationship between a change in dietary fatty acid composition and a subsequent ΔTC, which was later validated (Keys *et al.* 1965*a*, *b*). However, these researchers (Keys *et al.* 1965*a*; Jacobs *et al.* 1983) have only focused on the ΔTC. Although change in plasma TC manifests mainly in the low-density-lipoprotein subfraction (LDL-C), a change in dietary fatty acids seems to affect the high-density-lipoprotein (HDL-C) fraction as well (Ehnholm *et al.* 1984; Kay *et al.* 1984; Sacks *et al.* 1986). To date no attempts have been made to predict specific changes in these lipoprotein subfractions with diet.

In a study by Grundy & Vega (1988) subjects fed on metabolically-controlled formula diets with markedly contrasting polyunsaturated:saturated (P:S) values showed a wide variability in HDL-C response change (ΔHDL-C) with diet in subjects with higher baseline

levels, being more responsive to dietary change, i.e. there was a greater change in cholesterol for relatively lower change in dietary fat. Studies from the same laboratory (Grundy *et al.* 1986; Grundy, 1989) have shown that monounsaturated fatty acids (MUFA) are as effective as polyunsaturated fatty acids (PUFA) in lowering LDL-C and that the former do not appear to have the potential to lower HDL-C. Thus, both baseline HDL-C levels and the type of fatty acid replacement may be predictive of HDL-C lowering. We therefore pooled data from six similar studies (Fisher *et al.* 1983; Wissel *et al.* 1987; Zanni *et al.* 1987; Denke & Breslow, 1988; Weintraub *et al.* 1988; Brinton *et al.* 1990) to describe the differential responsiveness to strict, metabolically controlled diets of the major cholesterol variables, TC, LDL-C and HDL-C.

MATERIALS AND METHODS

The present analysis is a composite of six previous studies (Table 1) conducted at two research institutions, The Rockefeller University in New York City and Harvard Medical School, Boston, Massachusetts. Research for all six studies was linked under the direction of a single senior investigator, Dr Jan Breslow (Fisher *et al.* 1983; Wissel *et al.* 1987; Zanni *et al.* 1987; Denke & Breslow 1988; Weintraub *et al.* 1988; Brinton *et al.* 1990). The key common denominators of the six studies were metabolically controlled dietary change, identical biochemical methods, and similar study populations and research design permitting linkage for pooled analysis. For clarity of presentation, crossover is described as a change from a low P:S to high P:S regimen, as this is the regimen prescribed in practice.

Study subjects

Studies providing the database for this analysis spanned 9 years (1980–8). Before data analysis, predetermined selection criteria were developed: subjects had to be in excellent health and older than 18 years with lipoprotein profiles between the 25th and 75th percentile rank according to Lipid Research Clinics standards (Rifkind, 1989). Volunteers were recruited from staff and undergraduate work-study students from the two study institutions. Normocholesterolaemic patients who had entered the lipid clinic of each University for routine evaluation were also included.

The initial pooled subject group comprised seventy normolipidaemic participants; one subject was dropped from the analysis after developing severe hypertriglyceridaemia and two subjects (Weintraub *et al.* 1988) failed to complete the study diet protocol. Thus, a final pool of sixty-seven subjects participated in the six metabolic studies to completion and comprised the database for this analysis. Informed consent was obtained from all volunteers after review of the study protocols at each of the two locations.

Table 2 shows the selected clinical characteristics of age, body mass index (kg/m²; BMI), energy intakes, baseline (*ad lib.*) total lipids, cholesterol subfractions and ratios of all study subjects. This group comprised thirty-four men and thirty-three women with an average age of 25 years. The average BMI was 23 kg/m²; an average energy intake (kJ) was estimated for each subject to ensure weight maintenance. During the baseline period, females exhibited higher HDL-C than males ($P < 0.05$).

Diet protocols

A low P:S diet (P:S $\leqslant 0.6$) was designed to exaggerate a typical diet rich in SFA and cholesterol, while a high P:S diet (P:S > 1.0) was tailored to simulate a more therapeutic diet rich in PUFA, low in SFA and considerably lower in dietary cholesterol. At both institutions, two such diets were administered to all subjects with an intervening 2-week

Table 1. *Study design and dietary changes (low P:S diet composition – high P:S diet composition)**

(Mean values and standard deviations)

Dietary change... Group	n	Total fat (%)		SFA (%)		MUFA (%)		PUFA (%)		Cholesterol (mg/4200 kJ per d)		Protein (%)		Carbohydrate (%)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
All subjects	67	17	17	18	6	3	6	10	8	113	161	0		−9	14
Rockefeller University site															
1. Brinton et al. (1990)	11	32		22		12		0		60		0		−32	
2. Denke & Breslow (1988)	16	17		18		13		−7		116		0		−17	
3. Weintraub et al. (1988)	8	0		12		4		−14		−6		0		0	
4. Wissel et al. (1987)	4	0		22		3		−29		159		0		0	
Harvard University site															
1. Fisher et al. (1983)	19	0		22		6		−17		0		0		0	
2. Zanni et al. (1987)	9	0		7		0		5		496		0		0	

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

* A negative value indicates an increase in the dietary component.

Table 2. *Characteristics by sex of normolipidaemic subjects participating in Rockefeller University and Harvard University studies†*

(Mean values and standard deviations)

Group	n	Age (years)		BMI (kg/m²)		Energy intake (×4200 kJ/d)		TC (mg/l)		TG (mg/l)		VLDL-C (mg/l)		LDL-C (mg/l)		HDL-C (mg/l)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
All subjects	67	25	6	23	3	2·7	8	1680	300	740	380	210	130	970	270	510	110
Males	34	27**	7	24	3	3·3**	3	1710	270	800	470	220	170	1020	250	480*	110
Females	33	23	4	22	3	2·1	7	1650	330	680	250	200	80	920	280	540	110

TC, Total cholesterol; TG, triacylglycerols; VLDL-C, very-low-density-lipoprotein-cholesterol; LDL-C, low-density-lipoprotein-cholesterol; HDL-C, high-density-lipoprotein-cholesterol; BMI, body mass index (kg/m²).

Mean values were significantly different from those for female subjects: * $P < 0.05$, ** $P < 0.01$.

† For details of subjects and dietary changes, see Table 1 and pp. 272–276.

Table 3. *Comparison of dietary fat source and fatty acid composition (g/100 g total fatty acids) of low-and high-polyunsaturated:saturated fat (P:S) diets*

(Mean values and standard deviations)

Diet	Major dietary fat source	SFA				MUFA ($C_{18:1}$)		PUFA ($C_{18:2}$)	
		$C_{12:0}$–$C_{16:0}$		$C_{18:0}$					
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low P:S	Butterfat, lard or coconut oil	90	7	17	7	94	3	90	7
High P:S	Safflower oil, maize oil	74	7	16	7	96	2	99	1
Crossover (low P:S – high P:S; % change)		16		1		−2		−9	

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

A negative value indicates an increase in the dietary component.

washout in each study. One diet with a low P:S ratio or one with a high P:S ratio were fed to each patient in a randomized order. A washout period separated each metabolic diet, so that each subject was given either a high-P:S diet followed by washout followed by low-P:S diet, or used a low-P:S diet first followed by a high-P:S diet with an intervening washout. Subjects were required to consume all food at each meal and were instructed to maintain their usual levels of physical activity throughout all phases of the study. To adjust for differences in energy intake across studies, the percentage of energy derived from each nutrient was calculated, and percentage change (% Δ) calculated by difference.

The % Δ for diet composition for each study are shown in Table 1 and fatty acid compositions are presented in Table 3 and in the published studies (Fisher *et al.* 1983; Wissel *et al.* 1987; Zanni *et al.* 1987; Denke & Breslow, 1988; Weintraub *et al.* 1988; Brinton *et al.* 1990). Three of the six diet studies maintained the same total fat content at 310 g/kg (Fisher *et al.* 1983; Zanni *et al.* 1987) or 420 g/kg (Weintraub *et al.* 1988) following the diet crossover. The three remaining studies (Wissel *et al.* 1987; Denke & Breslow, 1988; Brinton *et al.* 1990) reduced the total fat content. All studies showed a reduction in energy intake derived from SFA and a mean percentage decrease in total energy intake of 18%. Except in the study carried out by Zanni *et al.* (1987), all dietary studies decreased the amount of MUFA by 3–12%, with a mean decline of 3%. All diet studies either maintained or increased the proportion of total energy derived from PUFA by 0–29%, with a mean increase of 10%. The diet crossover (Table 3) reduced $C_{12:0} - C_{16:0}$ chain lengths by 16% and stearate ($C_{18:0}$) by an additional 1%. Oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$) concentrations of the diets were increased by 2 and 9% respectively. Dietary cholesterol was reduced during the high-P:S phase compared with low-P:S diet levels. Two exceptions were the formula diet study of Fisher *et al.* (1983), where there was no added cholesterol, and the study by Weintraub *et al.* (1988), which approximated dietary cholesterol intakes across both diet periods.

Data were adjusted for differences in diet and clinical characteristics in the present study.

Analysis

A minimum of three blood samples were collected at the end of each diet period and averaged, at both the Rockefeller and Harvard sites, and plasma quantified for total lipids and cholesterol subfractions by the same methods and standardized at the Center for Disease Control, Atlanta, Georgia. TC and triacylglycerol (TG) levels were assayed enzymically utilizing reagents from Boehringer Mannheim Biochemicals, Indianapolis, IN, USA. The HDL-C level was quantified as previously described (Warnick *et al.* 1982). The HDL-C plus LDL-C concentrations were determined after ultra centrifugation to separate the VLDL-C subfraction. LDL-C and VLDL-C levels were determined by difference as reported previously by Denke & Breslow (1988).

As current guidelines recommend changes in absolute amount of lipids (mg/l), the change in lipid levels, calculated as the difference between the average lipid concentration measured during the low-P:S diet and the corresponding repeated lipid level quantified following the high-P:S diet, was derived individually for each subject. From these values dependent variables (i.e. 'diet-responsive' lipids) were calculated: ΔTC, ΔTG, ΔVLDL-C, ΔLDL-C, and ΔHDL-C. All statistical analyses were calculated using Biomedical Computer Programs statistical software on a VAX 780 mainframe computer (BMDP: 1d, 2r, 4v, 8d and 6r; BMDP Software, Carey, NC, USA).

To identify the specific diet-responsive lipids, a multivariate analysis of covariance (MANCOVA) was performed with age, baseline lipoprotein profiles, group differences in diet (i.e. total fat, fatty acid composition, and cholesterol), diet-type (formula *v.* solid-food

diets) and study site (Rockefeller *v.* Harvard University). Partial correlations and multivariate regression analyses (BMDP 6r; BMDP Software) were conducted to isolate the potential predictors of the identified diet-responsive variables (ΔTC, ΔLDL-C, ΔHDL-C). By univariate analysis, the independent clinical variables included the following: age (years), sex, BMI, energy intake (kJ/d), and diet variables expressed as % Δ following the diet crossover. The independent dietary composition variables including total nutrient compositions (% Δ total fat, % Δprotein, and % Δcarbohydrate), fatty acids compositions (% ΔSFA, % ΔPUFA and % ΔMUFA), and dietary cholesterol, expressed as square root of difference between the two metabolic diets and their ability to predict lipid changes, were studied. Baseline TC, LDL-C and HDL-C concentrations obtained before the metabolic studies were entered into the final model. All independent variables in the final regression model had coefficients significantly different from zero ($P < 0.05$). For ease of interpretation the initial equation will be modified in a beneficial direction for each variable, that is to say a drop in LDL-C from the low- to high-P:S diet is seen as an improvement. The correlation between the estimated ΔTC computed from the Minnesota equation, derived by Keys *et al.* (1957, 1959, 1965*a*, *b*), and using our derived model was calculated using BMDP2r (BMDP Software).

RESULTS

ΔTC, ΔLDL-C and ΔHDL-C pooled across all metabolic diet studies and by individual groups are presented in Table 4, with the MANCOVA results also shown . Values for ΔTG and ΔVLDL-C were not significant and, thus, are not shown. The effect of the diet crossover was statistically significant ($P < 0.001$) in predicting these diet-responsive lipids. Across and within the six study groups a wide range of lipid responsiveness was observed in the total lipid changes and cholesterol subfraction differences. For all subjects combined, crossover from a low- to a high-P:S diet resulted in an average reduction in TC of 380 mg/l with associated decreases in all subfractions. LDL-C and HDL-C subfractions showed decreases of 280 and 80 mg/l respectively following the dietary crossover. These changes were reflected in LDL-C ranging from 120 to 650 mg/l for the Brinton *et al.* (1990) and Wissel *et al.* (1987) groups respectively. Even more dramatic, the change in HDL-C showed a 12-fold difference across study groups. Weintraub *et al.* (1988) showed a 10 mg/l increase in HDL-C, whereas the Brinton *et al.* (1990) study subjects showed HDL-C decreased by an average of 120 mg/l. This variability was used to advantage in isolating the predictors of lipoprotein responsiveness.

A profile of independent variables (clinical, dietary and baseline lipids) was pooled across all studies and the univariate relationship was determined.

Change in TC and LDL-C

The univariate correlates for ΔTC and ΔLDL-C revealed that age (years) and the baseline TC and LDL-C levels were the significant univariate clinical and baseline lipid predictors of the changes. Among the metabolic diet variables, the decrease in SFA and increase in PUFA were correlated with both ΔTC and ΔLDL-C.

Change in HDL-C

The univariate correlates for ΔHDL-C were sex, baseline VLDL-C level, and HDL-C concentration (Table 5). All dietary change variables, except protein, were correlated with ΔHDL-C. Among the metabolic diet variables, a decrease in SFA and MUFA, or dietary cholesterol resulted in greater lowering of HDL-C. Conversely, an increase in dietary carbohydrate and PUFA promoted HDL-C lowering.

Table 4. *The effect of changing from a low-polyunsaturated:saturated fat (P:S) diet to a high-P:S diet on diet responsive lipids in normolipidaemic subjects participating in the Rockefeller University and Harvard University studies**

(Mean values and standard deviations)

Cholesterol and subfraction response (mg/l)…	ΔTC		ΔLDL-C		ΔHDL-C	
	Mean	SD	Mean	SD	Mean	SD
All subjects	380	120	280	110	80	50
Rockefeller University Study						
1. Brinton *et al.* (1990)	430	50	260	80	120	60
2. Denke & Breslow (1988)	320	50	230	70	80	20
3. Weintraub *et al.* (1988)	280	60	180	30	−10	30
4. Wissel *et al.* (1987)	730	90	450	160	110	70
Harvard University Study						
1. Fisher *et al.* (1983)	410	60	320	120	80	20
2. Zanni *et al.* (1987)	310	40	290	50	90	10

ΔTC, change in total cholesterol; ΔLDL-C, change in low-density-lipoprotein-cholesterol; ΔHDL-C, change in high-density-lipoprotein-cholesterol.

Mean values were analysed by multivariate analysis of covariance (MANCOVA): overall diet effect, $P < 0.0001$; ΔTC, $P < 0.03$; ΔLDL, $P < 0.05$; ΔHDL, $P < 0.0005$.

* For details of subjects and dietary changes, see Table 1 and pp. 272–276.

Change in TC

The best predictor of ΔTC was a two-variable model ($P < 0.001$):

$$\Delta TC \text{ (mg/l)} = 0.79(\text{age; years}) + 1.03(\% \Delta SFA) \qquad (1)$$
$$(\text{SE } 0.12) \qquad (\text{SE } 0.20)$$

accounting for 85% of the variance. Inspection of the standardized regression coefficient (0·47–0·48) showed a similar contribution of these two variables to prediction of the ΔTC. There was no improvement in the adjusted R^2 change with addition of a third variable to this model. The Minnesota equation (Keys *et al.* 1957, 1965*a*, *b*; Hegsted *et al.* 1965) when applied to our results explained 82% of the variance in ΔTC.

Change in LDL-C

The best model for ΔLDL-C was a three-variable model ($P < 0.001$):

$$\Delta LDL\text{-}C \text{ (mg/l)} =$$
$$0.71(\text{age; years}) + 1.03(\% \Delta SFA) + 0.22(\text{baseline LDL-C (mg/l)}) - 28.41 \qquad (2)$$
$$(\text{SE } 0.30) \qquad (\text{SE } 0.28) \qquad (\text{SE } 0.07) \qquad (\text{SE } 0.30)$$

This model accounted for 36% of the variance in ΔLDL-C, with similar contributions by all variables to model prediction.

Change in HDL-C

The best three-variable model ($P < 0.001$) included dietary SFA and PUFA, plus the baseline HDL-C level. The multiple-regression equation was:

$$\Delta HDL\text{-}C \text{ (mg/l)} = 0.31(\% \Delta SFA) - 0.40(\% \Delta PUFA) + 0.13(\text{baseline HDL-C (mg/l)}) \qquad (3)$$
$$(\text{SE } 0.12) \qquad (\text{SE } 0.10) \qquad (\text{SE } 0.04)$$

and accounted for a predictive variance of 74%. Sex was a significant predictor ($P < 0.01$),

Table 5. *Univariate determinants of dietary responsiveness to low-polyunsaturated: saturated fat (P:S) and high P:S diets in sixty-seven normolipidaemic subjects participating in the Rockefeller University and Harvard University studies*†

	Total lipids	Cholesterol subtractions	
	ΔTC	ΔLDL-C	ΔHDL-C
Clinical variables			
Age (years)	0·335***	0·357**	−0·129
Sex (M, F 0, 1)	−0·240	−0·216	0·261*
BMI (kg/m²)	0·029	0·066	−0·121
Energy intake (kJ/d)	0·232	0·188	−0·080
Diet variables			
% Δ Total fat	0·231	0·180	0·243*
Fatty acids composition			
% ΔSFA	0·374**	0·240*	0·233*
% ΔPUFA	−0·314**	−0·268*	0·344**
% ΔMUFA	−0·090	0·070	0·247*
Cholesterol (SQRT (C_1–C_2))	−0·005	0·121	0·241*
Carbohydrates	0·051	0·085	−0·396***
Protein	0·000	0·000	0·000
Baseline lipids (mg/l)			
TC	0·439***	0·421***	0·001
TG	0·164	0·123	−0·127
VLDL-C	0·220	0·096	0·578***
LDL-C	0·431***	0·490***	0·166
HDL-C	−0·039	−0·146	0·460***

ΔTC, change in total cholesterol; % ΔSFA, percentage change in saturated fatty acids; % ΔPUFA, percentage change in polyunsaturated fatty acids; % ΔMUFA, percentage change in monounsaturated fatty acids; BMI, body mass index (kg/m²); SQRT (C1–C2), square root difference in dietary cholesterol; TG, triacylglycerols; VLDL-C, very-low-density-lipoprotein-cholesterol; LDL-C, low-density-lipoprotein-cholesterol; HDL-C, high-density-lipoprotein-cholesterol.

* $P < 0.05$; ** $P < 0.025$; *** $P < 0.01$.

† For details of subjects and dietary changes, see Table 1 and pp. 272–276.

in addition to % ΔSFA and %ΔPUFA, but not greater than baseline HDL-C levels (values not presented).

DISCUSSION

We found that age (years), % ΔSFA, and baseline LDL-C (mg/l) levels determine ΔTC and ΔLDL, while % ΔSFA and % ΔPUFA and baseline HDL-C (mg/l) levels predict ΔHDL-C. Each 10 % reduction in SFA results in a decrease in LDL-C and HDL-C of approximately 100 and 30 mg/l respectively in an average member of the normolipidaemic population. The common term in both these equations is SFA, explaining the positive correlation between ΔLDL-C and ΔHDL-C. Although Keys (1984) proposed manipulation of only one dietary variable at a time, in clinical practice this is not feasible. In fact, the Nutrition Committee, American Heart Association (1988) recommends that 'all Americans from age 2 years and older consume a diet lower in total fat (to 30 %), decreased in SFA (< 10 %), and less than 300 mg cholesterol/d'. The National Cholesterol Education Program Expert Panel (1988) guidelines further recommend that PUFA should not exceed 10 % of energy and MUFA should make up 10–15 % of total energy.

The first algorithm (equation 1) shows that within this subject population ΔTC is best

predicted by age and a specific % ΔSFA. Hypothetically, if a patient aged 20 years presents with a TC of 2100 mg/l and the physician wishes to effect a decrease in this concentration of 300 mg/l to a final level of 1800 mg/l, the resultant necessary reduction in percentage of total energy intake from SFA is 13·8. This patient, under rigorous guidance, would need to reduce his SFA intake from that of about 24% to a level of approximately 10% total energy from SFA. This reduction appears rather unrealistic with the patient's young age contributing to the necessity for such a severe dietary manipulation. For example, a patient aged 30 years who desired the same reduction in TC of 300 mg/l would require a drop in SFA intake of considerably less, namely a reduction of only 6·1% total energy intake from 24 to 18% total energy intake from SFA. In agreement with our findings, others have shown suppression of LDL-receptor activity by SFA and ageing (Grundy *et al.* 1985; Spady & Dietschy, 1985).

The previously described example demonstrates the utility of these equations and allows a health care professional to calculate the average % ΔSFA modification to achieve a treatment goal. However, we would like to underscore that any estimate of the average amount of dietary change has a confidence interval about the mean and, therefore, must be interpreted within a range. These results, however, compared favourably with the earliest reported of such equations derived by others (Keys *et al.* 1957, 1965*a, b*; Hegsted *et al.* 1965). We found a highly significant correlation between our equation and the Minnesota equation (Keys *et al.* 1957, 1965*a, b*; Hegsted *et al.* 1965; $P < 0.0001$). However, the predicted ΔTC of the Minnesota equation tended to overestimate somewhat the observed changes in ΔTC in our group, especially for the younger patients. This small overestimation is not surprising as it was derived from a data set including older male subjects, and may not be applicable to younger subjects. Our equation may be advantageous in clinical practice, since the patient's age is easily obtained, and estimation of a % ΔPUFA would not be required. Thus, this equation may have special applicability in younger persons.

The crossover to a diet high in carbohydrate and low in total fat content has long been shown to lower plasma cholesterol levels (Keys *et al.* 1965*a*). However, dietary carbohydrates and oleic acid have been shown to produce a 'neutral' effect on serum cholesterol levels. In this analysis, neither the change in dietary carbohydrate nor total dietary fat were significant predictors of ΔTC beyond age and dietary SFA content. This is not surprising as total fat and carbohydrate were maintained in four of the six protocols, whereas dietary SFA content was dramatically reduced.

More recent evidence suggests that all SFA are not equal in their ability to elevate plasma cholesterol concentrations (Cobb, 1992). Stearic acid ($C_{18:0}$) appears to affect plasma cholesterol less than other SFA (Bonanome & Grundy, 1988). Keys *et al.* (1965*a*) recommended that $C_{18:0}$ be subtracted from the dietary SFA content when calculating the plasma cholesterol change. In practice, however, $C_{18:0}$ is rarely removed when the equation is used to predict dietary responsiveness (Grundy & Denke, 1990). In the present study, with mixed natural diets, subtraction of $C_{18:0}$ content did not significantly alter the predictive equations.

Our second algorithm (equation 2) indicates that a unit desired reduction in LDL-C is dependent on dietary % ΔSFA, age and baseline LDL-C. Applying this equation, we assume a case patient aged 20 years has a baseline LDL-C of 1000 mg/l, with the goal being to reduce this concentration by 300–700 mg/l. Thus, this patient would require a change in dietary SFA of 21·6% total energy intake. Supposing a patient of similar age and desired drop in LDL-C has a higher baseline LDL-C of 1300 mg/l, the required dietary % ΔSFA would be a reduction of only 15·2 for this patient. This is a less severe requirement than the first patient of the same age and desired LDL-C reduction. The latter patient,

requiring less change in dietary SFA, is more responsive to dietary manipulation. Grundy & Vega (1988) have demonstrated a positive correlation between baseline LDL-C levels and ΔLDL to diet.

The third algorithm (equation 3) predicts responsiveness for a given desired change (presumably, an increase in HDL-C). This algorithm can be solved for the individual subject only after equations 1 and 2 have been solved as % ΔPUFA remains the only unknown variable in the algebraic solution. For example, taking the 20-year-old male with a LDL-C of 1000 mg/l and the 30-year-old male with a LDL-C of 1300 mg/l (equation 2), assume both have a baseline HDL-C level of 450 mg/l with a ΔHDL-C of zero, the near optimal case, the resultant required % Δ total energy intake from PUFA would be a reduction of 31·3 and 21·5 respectively.

Given the current typical PUFA intake in Western societies of 6% total energy intake/d (National Center for Health Statistics, 1981), such a reduction in PUFA would be unrealistic. Adjustment to a more sensible clinical dimension could be achieved by substituting a reasonable ΔHDL-C or known change in % ΔPUFA-fat. Since a 10% ΔPUFA is reasonable, substituting this value into equation 3 for these same two hypothetical subjects yields a decrease in plasma HDL-C of approximately 90 and 40 mg/l for the younger and older patient respectively.

The fact that females generally present with higher baseline HDL-C than males (Albers *et al.* 1976) necessitates the input of typical female baseline HDL-C levels to determine the effect of sex on the responsiveness of HDL-C, as expressed in equation 3. Assuming our two hypothetical patients to be female, with baseline HDL-C levels of 600 mg/l, and keeping all other known terms similar to those of the two male subjects described previously, a 10% increase in PUFA yields a HDL-C decrease of about 110 and 60 mg/l respectively for the younger and older females. Compared with the two male counterparts (90 and 40 mg HDL-C/l decrease), the decrease in HDL-C for the two females was greater and, theoretically, less desirable.

In the present study the change in MUFA was not a statistically important predictor of HDL-C decrease, compared with SFA and PUFA composition. Although some investigators (Grundy *et al.* 1986; Grundy, 1989), using formula diets containing very high amounts of MUFA (28% total energy intake), reported that replacement of SFA with MUFA prevents the ΔHDL-C, this cannot be interpreted from our study. More recent studies (Dreon *et al.* 1990), using solid food diets with SFA composition held constant, showed that both PUFA- and MUFA-fat replacement produced the same HDL-C changes in subjects after a diet-crossover. In our composite diet analysis SFA was not held constant and relatively small changes in MUFA were employed in most studies, thus, potentially limiting interpretation of the role of MUFA in predicting ΔHDL.

Conclusions

The findings of the present study have potential implications for epidemiological intervention in the normolipidaemic population. As illustrated in equations 2 and 3, the amount of change in LDL-C, and per unit change in HDL-C fraction in normolipidaemic subjects, is partially dictated by the dietary SFA content. Currently, the Nutrition Committee, American Heart Association (1988) recommends diet modification for all Americans. Broad-sweeping preventive approaches include reduction of TC levels in the general population *v.* a more-high-risk strategy targeted at subjects with elevated LDL-C levels. Results from the present study in normolipidaemic subjects support the latter approach. Younger subjects with lower LDL-C levels are not as diet responsive. Furthermore, young women with low LDL-C and high HDL-C may, in fact, not benefit from this dietary intervention.

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