Effect of Dietary Fatty Acids on Serum Lipids and Lipoproteins

A Meta-analysis of 27 Trials

Ronald P. Mensink and Martijn B. Katan

To calculate the effect of changes in carbohydrate and fatty acid intake on serum lipid and lipoprotein levels, we reviewed 27 controlled trials published between 1970 and 1991 that met specific inclusion criteria. These studies yielded 65 data points, which were analyzed by multiple regression analysis using isocaloric exchanges of saturated (sat), monounsaturated (mono), and polyunsaturated (poly) fatty acids versus carbohydrates (carb) as the independent variables. For high density lipoprotein (HDL) we found the following equation: ΔHDL cholesterol (mmol/l) = 0.012 × (carb→sat) + 0.009 × (carb→mono) + 0.007 × (carb→poly) or, in milligrams per deciliter, 0.47 × (carb→sat) + 0.34 × (carb→mono) + 0.28 × (carb→poly). Expressions in parentheses denote the percentage of daily energy intake from carbohydrates that is replaced by saturated, *cis*-monounsaturated, or polyunsaturated fatty acids. All fatty acids elevated HDL cholesterol when substituted for carbohydrates, but the effect diminished with increasing unsaturation of the fatty acids. For low density lipoprotein (LDL) the equation was ΔLDL cholesterol (mmol/l) = 0.033 × (carb→sat) − 0.006 × (carb→mono) − 0.014 × (carb→poly) or, in milligrams per deciliter, 1.28 × (carb→sat) − 0.24 × (carb→mono) − 0.55 × (carb→poly). The coefficient for polyunsaturates was significantly different from zero, but that for monounsaturates was not. For triglycerides the equation was Δtriglycerides (mmol/l) = −0.025 × (carb→sat) − 0.022 × (carb→mono) − 0.028 × (carb→poly) or, in milligrams per deciliter, −2.22 × (carb→sat) − 1.99 × (carb→mono) − 2.47 × (carb→poly). Thus, replacement of carbohydrates by fat lowered serum triglycerides independent of the nature of the fat. Replacement of saturated by unsaturated fatty acids raised the HDL to LDL cholesterol ratio, whereas replacement by carbohydrates had no effect. Thus, under isocaloric, metabolic-ward conditions the most favorable lipoprotein risk profile for coronary heart disease was achieved if saturated fatty acids were replaced by unsaturated fatty acids, with no decrease in total fat intake. Extrapolation of our data to free-living populations requires more insight into effects of diet on body weight; if high-oil diets promote obesity, then their favorable effects on serum lipids will be lost. (*Arteriosclerosis and Thrombosis* 1992;12:911–919)

KEY WORDS • meta-analysis • humans • controlled trials • diet studies • fatty acids • cholesterol • high density lipoprotein cholesterol • low density lipoprotein cholesterol • triglycerides

Studies performed in the 1950s and early 1960s have shown that the serum cholesterol level increases when dietary carbohydrates are replaced by certain saturated fatty acids and decreases when carbohydrates are replaced by (n-6) polyunsaturated fatty acids.[1,2] The formulas of Keys et al[1] and Hegsted et al[2] that summarize these studies have formed the basis of policies for the dietary prevention of ischemic heart disease.[3,4] However, these formulas may no longer be adequate. First, they do not differentiate between the effects of diet on low density (LDL) and those on high density (HDL) lipoprotein cholesterol. This distinction is relevant because LDL and HDL cholesterol may have opposite effects on the risk for ischemic heart disease,[5,6] and some studies have suggested that the cholesterol-decreasing effect of (n-6) polyunsaturated fatty acids is not limited to LDL but extends to HDL cholesterol.[7,8] Second, recent studies have failed to show any effect of polyunsaturates on serum total and LDL cholesterol beyond that which could be accounted for by the displacement of saturates from the diet.[9,10]

For these reasons, we have screened the literature for well-controlled trials on the effect of dietary fatty acids on serum lipoproteins in humans. Results were combined to derive equations that relate changes in the dietary fatty acid intake to changes in serum HDL, LDL, and total cholesterol and triglyceride concentrations. We focused on the most common types of fatty acids, fat substances for which most of the evidence is available.

Methods

Selection of Studies

We selected studies that were published as original articles between 1970 and 1991 that met the following criteria: 1) A thorough control of food intake, with

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dietary fatty acids being the sole variable. Specifically, cholesterol intake had to be the same on the various diets. A description of the diet had to be provided that allowed calculation of the intakes of saturated, monounsaturated, and polyunsaturated fatty acids. 2) A design that eliminated the effect of nonspecific drifts of the outcome variables with time. This is accomplished by either feeding different groups of volunteers different diets side by side (parallel design) or feeding each volunteer several diets in random order (crossover or Latin square design). "Before-and-after" designs that lacked a control group were excluded. 3) Feeding periods that were sufficiently long to attain equilibrium, i.e., of 14 days or more.[11,12] 4) Subjects who did not suffer from gross disturbances of lipid metabolism.

We also excluded diets that had been specifically enriched in any of the following classes of fatty acids: 1) Very-long-chain (n-3) polyunsaturated fatty acids (fish oils). There is evidence that these raise rather than decrease the level of LDL cholesterol,[13] but the data on this topic are as yet too contradictory and incomplete. 2) *trans* isomers of unsaturated fatty acids. These probably do not have the same effect on serum cholesterol as oleic acid, the most abundant *cis*-monounsaturated fatty acid, but again the evidence is incomplete.[14] 3) Stearic acid (C18:0). Its effect on serum total and LDL cholesterol is quite different from that of the other common saturated fatty acids: lauric (C12:0), myristic (C14:0), and palmitic (C16:0) acids.[1,15]

Values for total and HDL cholesterol levels in plasma were multiplied by 1.030 and those for triglyceride levels in plasma by 1.029 to convert them to serum values.[16] LDL cholesterol concentrations were calculated from the reported mean concentrations of total and HDL cholesterol and triglycerides by using the Friedewald equation (Friedewald et al[17]). For the sake of uniformity, this was also done for those studies in which LDL cholesterol levels were reported by the authors themselves.

Dietary fat contains, on average, 96% by weight as fatty acids; the other 4% is glycerol and other lipids.[18] For publications in which the intakes of the various fatty acid classes had been normalized to add to 100% of total fat, we converted intakes back into true fatty acid intakes by multiplying them by 0.96.

Not all articles specified the amounts of the individual saturated fatty acids in the diet. Therefore, we could not discriminate, on one hand, between lauric, myristic, and palmitic acids (C12:0, C14:0, and C16:0), which raise total serum cholesterol, and on the other hand, the medium-chain fatty acids and stearic acid (C18:0), which do not.[1,15] In practice the absence of this information is not a problem because in mixed natural diets, the share of C12:0–C16:0 in total saturates is fairly constant at 60–70% by weight.[1,18,19]

The proportion of very-long-chain (n-3) polyunsaturated fatty acids in experimental diets not containing added fish oil is minimal; therefore, in the present article total polyunsaturates may be considered to be equal to polyunsaturates with 18 carbon atoms (linoleic and some α-linolenic acid).

We found 27 trials that met our inclusion criteria (Table 1). These trials yielded 65 data points and included 682 volunteers, 474 men and 208 women. The volunteers in these trials had been recruited in medical centers,[9,28,30,31,33,34] from students or university staff,[10,12,21,22,25–27,29,32,35–44] from mentally retarded men,[20] or from monks in a monastery.[23,24] The youngest volunteers were just below 20 years of age while the oldest were older than 70 years. Sixteen trials were carried out with men only, and the remaining studies included both men and women. However, differences in response between men and women were usually not reported and therefore are not considered in the present report. The test diets were fed for 14–91 days. In 19 studies, mean prestudy levels of total cholesterol were reported; they ranged between 3.81 mmol/l and 6.50 mmol/l (147–251 mg/dl).

The maximum difference in total fatty acid intake between diets within one study was 23.4% of total daily energy[20]; in saturated fatty acids, 20.2% of energy[30]; in monounsaturates, 22.6% of energy[9]; and in polyunsaturates, 24.2% of energy.[9] In all trials fatty acids were exchanged for either other fatty acids or carbohydrates. Possible effects on serum lipids and lipoproteins of the other two energy-yielding substrates, protein and alcohol, therefore did not enter into the present analysis.

Statistical Methods

Each data point consisted of the composition of a particular diet and the mean serum lipid or lipoprotein level of a group of subjects while on that diet. A parallel design with a common baseline diet followed by two test diets yielded two data points. Mean serum lipoprotein levels in such studies were corrected by us for differences in initial levels between treatment groups. For example, Mensink and Katan[32] first fed all of their subjects a baseline diet high in saturated fat for 17 days. Subsequently, one half of the subjects received a test diet high in olive oil and the other half a test diet rich in carbohydrates, both for 5 weeks. At the end of the baseline diet period, the average serum total cholesterol concentration was 0.05 mmol/l lower in the future olive oil group than in the future carbohydrate group. Therefore, we added 0.05 mmol/l to the reported[32] average serum cholesterol concentration of the olive oil group at the end of the olive oil diet period. Such a correction was applied to all lipid and lipoprotein values obtained in parallel-design studies. Any drift in the serum lipoprotein level with time occurred simultaneously in both diet groups and therefore did not affect the differences in final serum lipoprotein levels between the two diet groups. In a crossover, rotating-diet, or Latin square design, every subject received every diet. Such studies yielded as many data points as there were dietary periods, with no need for correction.

Within each experiment, the sum of the intakes of calories from saturated, monounsaturated, and polyunsaturated fatty acids and of carbohydrates was constant because every change in one of these four nutrients was balanced by opposite changes in one or more of the others to maintain caloric balance. For the present purpose, we expressed all effects of fatty acids relative to those of carbohydrates.

The relation of the mean serum lipoprotein level of subjects in study n (n=1, . . ., 27) on diet d (d=1, . . ., 65) with the composition of that particular diet was modeled as follows:

TABLE 1. Characteristics of Studies, Subjects, and Diets

Reference	Year	No. of subjects		Design*	Days of test period	Fatty acid content of test diet (% of daily energy intake)											
						Diet 1			Diet 2			Diet 3			Diet 4		
		Men	Women			Sat	Mono	Poly	Sat	Mono	Poly	Sat	Mono	Poly	Sat	Mono	Poly
Grande et al[20]	1972	38		×	28	2.2	1.5	0.6	3.2	6.5	2.5	5.0	16.1	6.3	8.3	6.7	12.7
Anderson et al[21]	1976	12		×	14	19.6	8.4	5.2	4.8	5.1	22.7						
Brussaard et al[22]	1980	37	23	//	35	8.0	10.0	3.0	10.0	8.0	11.0	11.0	8.0	19.0	18.0	16.0	3.0
Lewis et al[23]	1981	12		×	35	8.7	8.5	8.7	12.7	12.9	12.8						
and McPherson et al[24]	1985																
Brussaard et al[12]	1982	23	12	//	91	7.0	8.0	4.0	9.0	10.0	11.0						
Laine et al[25]	1982	13	11	×	20	16.3	14.1	3.3	7.4	12.4	14.4	7.7	12.7	15.3			
Becker et al[26]	1983	12		×	28	2.7	29.2	6.5	4.0	15.1	17.5						
Harris et al[27†]	1983	3	4	×	28	14.4	16.4	7.2	6.4	10.8	21.6						
Wolf and Grundy[28]	1983	11		×	30	18.8	10.0	9.6	9.3	9.9	9.6	14.1	7.2	7.2			
Mattson and Grundy[9†]	1985	12		×	28	19.1	15.4	3.9	3.3	28.2	6.9	4.3	5.6	28.1			
Reiser et al[29*]	1985	19		×	35	23.7	6.6	3.0	14.3	15.9	3.1	6.5	7.7	18.9			
Grundy[30‡]	1986	6	1	×	28	24.0	7.7	6.7	6.4	6.4	6.4	3.8	26.9	7.7			
Grundy et al[31]	1986	9		×	60	9.6	12.5	16.3	9.6	9.6	9.6						
Mensink and Katan[32]	1987	24	24	//	35	6.7	9.3	5.2	9.8	24.0	5.1						
Bonanome and Grundy[33]	1988	11		×	21	19.6	14.9	3.7	3.1	30.6	4.7						
Grundy et al[34]	1988	10		×	42	6.7	25.9	5.8	6.7	6.7	5.8						
Katan et al[35]	1988	24	23	×	21	10.5	12.0	21.0	23.0	14.5	5.2						
McDonald et al[36]	1989	8		×	18	5.1	20.2	10.3	6.8	7.4	21.6						
Mensink and Katan[10]	1989	29	29	//	35	12.9	15.1	7.9	12.6	10.8	12.7						
Ginsberg et al[37]	1990	24		//	70	9.0	10.6	10.0	8.8	17.2	10.1						
Mensink and Katan[38]	1990	25	34	×	21	9.5	24.1	4.6	19.4	14.7	3.4						
Wardlaw and Snook[39]	1990	20		×	35	6.7	26.9	5.8	7.7	13.4	18.2						
Berry et al[40]	1991	18		×	84	8.7	17.1	6.3	8.3	6.8	17.4						
Chan et al[41]	1991	8		×	18	6.5	18.7	7.4	5.3	18.3	8.5	7.1	8.4	16.8	6.4	9.9	16.1
Wardlaw et al[42]	1991	16		//	56	7.2	22.1	10.7	7.4	8.1	22.2						
Valsta et al[43]	1992	29	30	×	17	12.4	16.2	7.6	12.7	10.2	13.3						
Martin et al[44§]		21	17	×	23	10.1	15.9	4.0	10.0	9.9	10.1						

Sat, saturated fatty acids; mono, monounsaturated fatty acids; poly, polyunsaturated fatty acids.

*×, Rotating-diet, crossover, or Latin square design; //, parallel design.

†The gender of the subjects was not reported in the article but was kindly provided by Prof. W.E. Connor and Prof. S.M. Grundy, respectively.

‡Fourteen percent of daily energy intake from fat in each diet was provided by the same basic foods and the remainder by the test fat. The fatty acid composition was not given for the basic foods, but it was estimated by us using Reference 18 and subsequent issues of handbook 8.

§Published only as an abstract.

$$\text{Serum lipoprotein level } (n,d) = \text{intrinsic level } (n) + a \times [\text{carb} \rightarrow \text{sat}(d)] + b \times [\text{carb} \rightarrow \text{mono}(d)] + c \times [\text{carb} \rightarrow \text{poly}(d)] + \text{error } (d)$$

The intrinsic level is a constant that is characteristic for the group of volunteers participating in study *n*. It can be visualized as the mean lipoprotein level predicted for this particular group when consuming a fat-free, high-carbohydrate diet. In the present model, differences in age or genetic makeup between volunteers in different experiments will result in differences in intrinsic level, as will differences in the average cholesterol or fiber intake between studies or biases in analytical levels between laboratories. "[carb→sat*(d)*]" refers to the isoenergetic replacement of carbohydrates by saturated fatty acids up to the level provided by diet *d*, "[carb→mono*(d)*]" to replacement by *cis*-monounsaturated fatty acids, and "[carb→poly*(d)*]" to replacement by *cis,cis*- or *cis,cis,cis*-polyunsaturated fatty acids. The error term is the difference between the lipid or lipoprotein level predicted by the model and the value actually observed. Amounts of fatty acids and carbohydrates are expressed as percentages of total daily energy intake. The aim of the calculation was to estimate those values of the regression coefficients (slopes) a, b, and c, for which the sum of the squares of the error term for all studies and diets combined was minimized. The regression coefficients can be interpreted as the predicted change in the serum lipoprotein level when dietary carbohydrate intake decreases by 1% of daily energy intake and the intake of a particular fatty acid increases simultaneously by 1% of energy. As the intake of protein, alcohol, and dietary cholesterol did not change within a study, these variables are not featured in the equation.

Analysis of residuals was performed to check the appropriateness of the regression model used. All sta-

TABLE 2. Estimated Multiple Regression Equations for Mean Changes (Δ) in Serum Lipids and Lipoproteins When Carbohydrates in the Diet Are Replaced Isocalorically by Saturated Fatty Acids (carb→sat), *cis*-Monounsaturated Fatty Acids (carb→mono), or Polyunsaturated Fatty Acids (carb→poly) Expressed as Percent Contribution to Total Daily Energy Intake

Lipid or lipoprotein	Change per percent of energy replaced			No.*
ΔHDL cholesterol (mmol/l)	=0.012×(carb→sat) + 0.009×(carb→mono) + 0.007×(carb→poly)			59
95% CI (mmol/l)	+0.007 to +0.017	+0.005 to +0.012	+0.003 to +0.012	
ΔHDL cholesterol (mg/dl)	=0.47×(carb→sat) + 0.34×(carb→mono) + 0.28×(carb→poly)			
	(p<0.001)	(p<0.001)	(p=0.002)	
ΔLDL cholesterol (mmol/l)	=0.033×(carb→sat) − 0.006×(carb→mono) − 0.014×(carb→poly)			57
95% CI (mmol/l)	+0.023 to +0.042	−0.014 to +0.002	−0.023 to −0.006	
ΔLDL cholesterol (mg/dl)	=1.28×(carb→sat) − 0.24×(carb→mono) − 0.55×(carb→poly)			
	(p<0.001)	(p=0.114)	(p=0.002)	
ΔTotal cholesterol (mmol/l)	=0.039×(carb→sat) − 0.003×(carb→mono) − 0.015×(carb→poly)			65
95% CI (mmol/l)	+0.031 to +0.047	−0.010 to +0.004	−0.023 to −0.008	
ΔTotal cholesterol (mg/dl)	= 1.51×(carb→sat) − 0.12×(carb→mono) − 0.60×(carb→poly)			
	(p<0.001)	(p=0.342)	(p<0.001)	
ΔTriglycerides (mmol/l)	=−0.025×(carb→sat) − 0.022×(carb→mono) − 0.028×(carb→poly)			63
95% CI (mmol/l)	−0.033 to −0.017	−0.029 to −0.016	−0.035 to −0.021	
ΔTriglycerides (mg/dl)	= −2.22×(carb→sat) − 1.99×(carb→mono) − 2.47×(carb→poly)			
	(p<0.001)	(p<0.001)	(p<0.001)	
ΔHDL/LDL cholesterol ratio	=0.000×(carb→sat) + 0.003×(carb→mono) + 0.005×(carb→poly)			57
(mg/mg or mmol/mmol)	−0.003 to +0.003	+0.001 to +0.005	+0.003 to +0.007	
	(p=0.968)	(p=0.013)	(p=0.001)	

HDL, high density lipoprotein; LDL, low density lipoprotein. Saturated fatty acids include the contribution of non–cholesterol-raising saturated fatty acids with 18 or <12 carbon atoms. The 95% confidence intervals (CI) and probability values refer to regression coefficients on the line defined by the preceding equation.
*No., number of data points.

tistical analyses were carried out with the general linear model and regression procedure of the Statistical Analysis System (SAS).[45]

Results

All 27 studies reported values for serum total cholesterol, 25 studies reported HDL cholesterol, and 26 studies reported serum triglycerides; for 24 studies, LDL cholesterol concentration could be calculated. The intrinsic levels, i.e., the predicted levels to which the subjects in a particular study would revert on a diet free from fat, ranged for HDL cholesterol from 0.65[31] to 1.46 mmol/l[25] (25–56 mg/dl); for LDL cholesterol, from 1.95[26] to 4.20 mmol/l[28] (76–162 mg/dl); for total serum cholesterol, from 3.42[44] to 6.15 mmol/l[28] (132–238 mg/dl); and for serum triglycerides, from 1.33[12] to 3.08 mmol/l[30] (117–273 mg/dl).

Table 2 and Figure 1 present the regression coefficients for the relation between serum lipid or lipoprotein levels and fatty acid intake. The equations predict the mean change in a particular lipid or lipoprotein concentration when 1% of daily dietary energy intake as carbohydrates is replaced isocalorically by a particular fatty acid. For an "average" group of adult men or women with a daily energy intake of 10 MJ (2,400 kcal), 1% of energy is equivalent to about 6 g of carbohydrates or 2.7 g of fatty acid.

The equations for HDL show that under isocaloric, metabolic-ward-type conditions, all three classes of fatty acids will elevate HDL cholesterol when they replace carbohydrates in the diet. The effect diminishes with increasing unsaturation of the fatty acid. Alternatively, one could say that replacement of fat by carbohydrates will lower HDL cholesterol levels and that the effect is stronger the more saturated the dietary fat that is removed. The coefficient for polyunsaturates was significantly smaller than that for saturated fatty acids (p<0.05). As a result, isocaloric replacement of saturated by polyunsaturated fatty acids is predicted to lead to a fall in HDL cholesterol that is statistically though perhaps not biologically significant, as the extent of this effect is only 0.005 mmol/l (0.2 mg/dl) per percent of energy.

According to the regression equation, saturated fatty acids strongly elevated LDL cholesterol levels. Polyunsaturated fatty acids had a modest but significant LDL cholesterol–lowering effect relative to carbohydrates. Although the coefficient for the effect of monounsaturates on LDL cholesterol was also negative, it was not significantly different from zero. The coefficient for polyunsaturates, however, was not significantly lower than that for monounsaturates. The equation for LDL cholesterol predicts that if 10% of dietary energy provided by saturated fatty acids is replaced by carbohydrates, the LDL cholesterol level will decrease by 0.33 mmol/l (13 mg/dl). For the "average" group of adult men or women, this would imply a replacement of 27 g of saturated fatty acids by 60 g of sugar or starch. If this amount of carbohydrate is then replaced in turn by polyunsaturates, the LDL cholesterol level will decrease by another 0.14 mmol/l (6 mg/dl). Direct replacement of saturates by polyunsaturates will yield the sum of these two effects, namely a fall of 0.47 mmol/l (18 mg/dl) in the LDL cholesterol level. As LDL cholesterol levels were estimated using the Friedewald formula, the analyses were repeated with those 17 studies that had

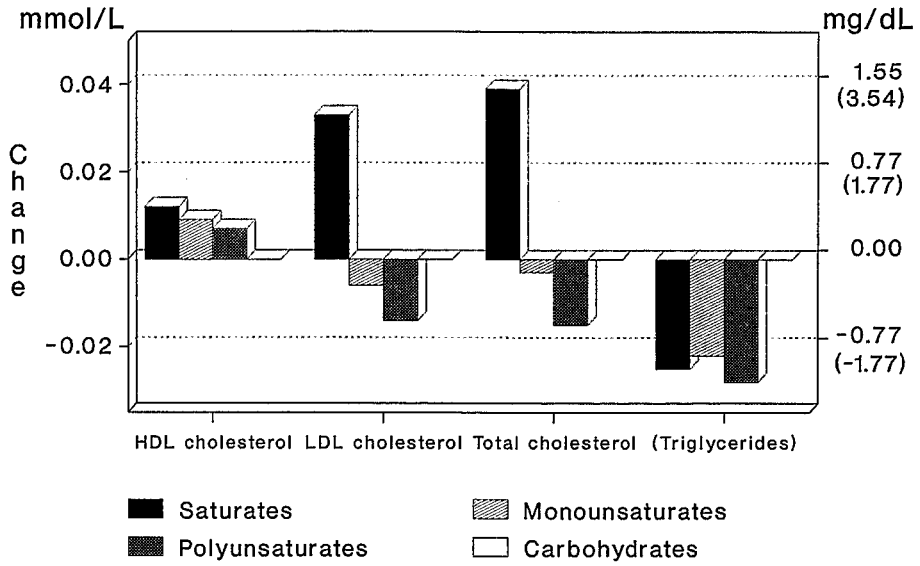

FIGURE 1. *Bar graph showing predicted changes in serum lipids and lipoproteins when 1% of energy as carbohydrate is replaced by fatty acids of a particular class under isocaloric, metabolic-ward or similar conditions. Coefficients are valid both ways; thus, replacement of 1% of energy from saturated fat by carbohydrates will cause a fall in cholesterol or rise in triglycerides equal to the length of the black column. Values between brackets refer to predicted changes in triglycerides, expressed in milligrams per deciliter.*

measured LDL cholesterol directly. The regression coefficient for saturates per 1% of energy was now 0.030 mmol/l (1.16 mg/dl, $p<0.001$); for monounsaturates, −0.009 mmol/l (−0.35 mg/dl, $p=0.076$); and for polyunsaturates, −0.014 mmol/l (−0.54 mg/dl, $p=0.0165$). All of these coefficients are highly similar to those obtained for the full set of studies (Table 2).

The predicted effect on total serum cholesterol largely mirrored that on LDL cholesterol. However, the regression coefficient for polyunsaturated fatty acids was now significantly more negative than that for monounsaturated fatty acids ($p<0.05$), probably because polyunsaturates lowered both LDL and HDL cholesterol relative to monounsaturates. The HDL to LDL cholesterol ratio did not change if saturates were replaced by carbohydrates, but it increased if carbohydrates were replaced by unsaturated fatty acids, the effect being larger for polyunsaturates than for monounsaturates.

Replacement of carbohydrates by fat decreased the level of triglycerides. Although polyunsaturated fatty acids had the greatest triglyceride-lowering effect, the regression coefficients did not differ significantly between the three classes of fatty acids.

To visualize the derived equations, we subtracted from each observed lipid or lipoprotein level on a particular diet the intrinsic level for that particular set of volunteers and plotted the difference against the level predicted for that diet from our equations. This resulted in Figures 2A–2D, in which each point refers to one of the diets studied in the different experiments.

For nine studies,[9,26–28,33,36–38,41] the proportions of the major individual fatty acids in the diets were reported, and for another seven studies,[10,12,22–24,32,35,43] mainly from Wageningen, the complete dietary fatty acid composition could be traced. These 16 studies together yielded 38 data points, which we used to calculate the impact of separate fatty acids. For total cholesterol we now found, per percent of energy, a regression coefficient (with 95% confidence interval) for lauric acid (C12:0) of 0.021 mmol/l or 0.83 mg/dl (−0.058 to 0.101 mmol/l); for myristic acid (C14:0), of 0.124 mmol/l or 4.79 mg/dl (−0.011 to 0.259 mmol/l); for palmitic acid (C16:0), of 0.034 mmol/l or 1.31 mg/dl (0.014 to 0.054 mmol/l); for stearic acid (C18:0), of 0.030 mmol/l or 1.17 mg/dl (−0.029 to 0.090 mmol/l); for oleic acid (C18:1), of −0.007 mmol/l or −0.29 mg/dl (−0.020 to 0.005 mmol/l); for linoleic acid (C18:2), of −0.016 mmol/l or −0.63 mg/dl (−0.029 to −0.004 mmol/l); and for α-linolenic acid (C18:3), a coefficient of −0.023 mmol/l or −0.88 mg/dl (−0.091 to 0.045 mmol/l).

Discussion

Total Cholesterol

Comparison with the Keys equation. Our equation relating changes in serum total cholesterol to changes in fatty acid intake is in remarkably good agreement with a similar equation derived by Keys and coworkers in 1965[1] from an entirely different set of experiments. In turn, this equation was in close agreement with that derived by Hegsted et al[2] from yet another set of experiments. The coefficient for sat′, the sum of the cholesterol-raising saturates (C12:0, C14:0, and C16:0) derived by Keys et al,[1] was 2.4 mg/dl or (2.4/38.67)/0.96=0.065 mmol/l per percent of energy. We divided by 0.96 here because 1 g of fat contains 0.96 g of fatty acids. Our present analysis yielded a coefficient for total saturated fatty acids of 0.039 mmol/l (1.5 mg/dl) per percent of energy (Table 2). The share of the cholesterol-raising saturated fatty acids lauric, myristic, and palmitic in total saturates in the studies reviewed here was about 70%, or in other words, sat′=0.70×sat. When we assume that stearic acid (C18:0) has the same effect on serum cholesterol levels as carbohydrates, then our coefficient in terms of the cholesterol-raising saturates lauric, myristic, and palmitic acids will be about 0.039/0.70=0.056 mmol/l (2.2 mg/dl) per percent of energy. This is somewhat lower than but still in good agreement

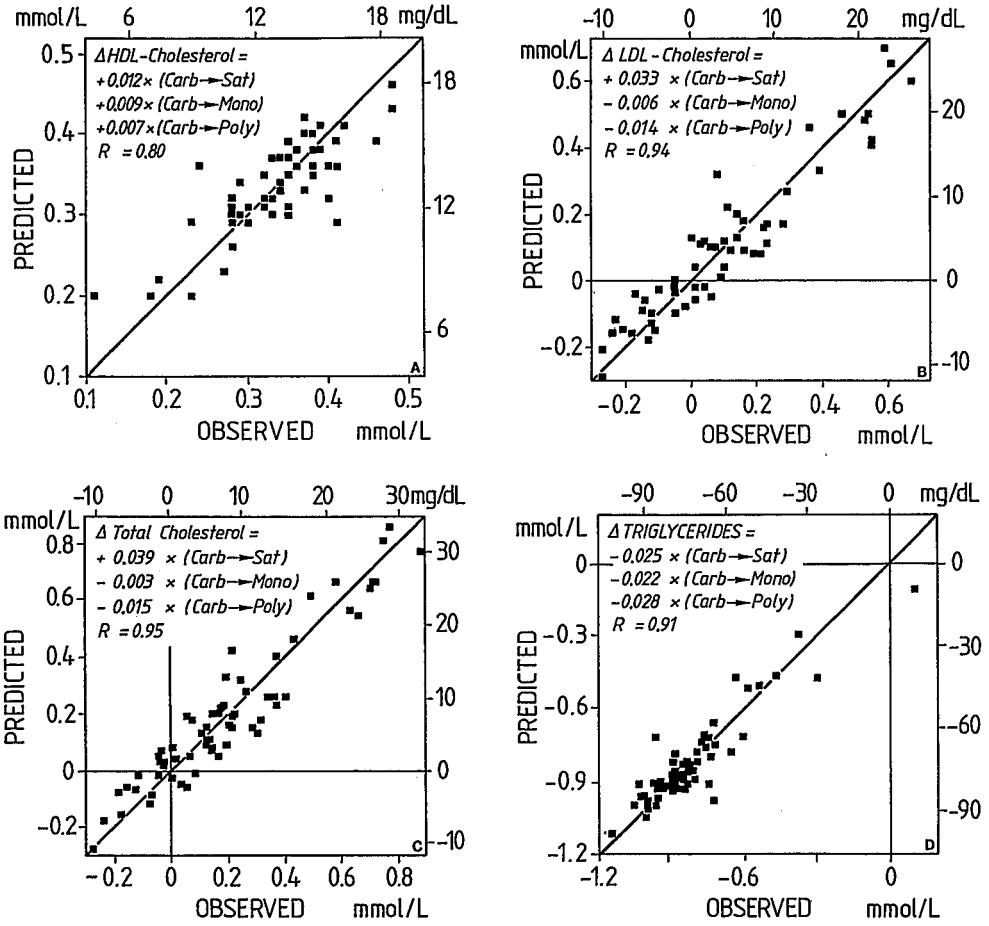

FIGURE 2. Panel A: *Scatterplot showing observed versus predicted changes* (Δ) *in serum high density lipoprotein (HDL) cholesterol levels when carbohydrates are replaced isocalorically by a specific mix of fatty acids under isocaloric, metabolic-ward or similar conditions. Each point refers to a specific test diet in one of the studies of Table 1 (see "Methods"). The "observed" value is the observed absolute concentration on a particular diet minus the "intrinsic" level expected for the same group of volunteers when fed a fat-free, high-carbohydrate diet. "Predicted" values were calculated for each particular diet from the equation in Table 2. R denotes the Pearson correlation coefficient between observed and predicted values.* Panel B: *Scatterplot showing observed versus predicted changes in serum low density lipoprotein (LDL) cholesterol levels.* Panel C: *Scatterplot showing observed versus predicted changes in serum total cholesterol levels.* Panel D: *Scatterplot showing observed versus predicted changes in serum triglyceride levels. Carb, carbohydrates; sat, saturates; mono, monounsaturates; poly, polyunsaturates.*

with the value of 0.065 derived by Keys et al[1] more than 25 years ago.

Our analysis of the newer studies also showed a qualitative agreement with previous studies in that polyunsaturates had a specific cholesterol-lowering effect over and above that of replacing saturates in the diet, even though several of the individual studies, including our own,[10] failed to detect this. However, the effect amounted to only 0.015 mmol/l (0.60 mg/dl) per percent of energy from polyunsaturates, as opposed to 0.031/0.96=0.033 mmol/l (1.28 mg/dl) in the Keys equation.[1] According to the present analysis, the specific effect of polyunsaturated fatty acids on the serum cholesterol level is less than previously thought. With the same assumptions as above, our version would read:

$$\Delta\text{Cholesterol}=1.2\times(1.8\Delta S'-0.1\Delta M-0.5\Delta P)$$

instead of the original

$$1.2\times(2\Delta S'-\Delta P)^{1}$$

where S′ equals lauric plus myristic plus palmitic acids, M is monounsaturates, and P is polyunsaturates.

Interaction with dietary cholesterol. The level of cholesterol in the diet may modify the extent of the change in serum cholesterol induced by the type of dietary fat. In the Second Faribault Study of the National Diet Heart Study, 197 men received in random order four different diets, each for 10 weeks. Addition of 495 mg cholesterol from egg yolk caused a rise in serum total cholesterol of 0.32–0.36 mmol/l (13–14 mg/dl) against a high-saturated-fat background diet, but of only 0.11–0.18 mmol/l (4–7 mg/dl) when the background diet was high in polyunsaturates.[46] Unfortunately, such an interaction effect could not be ascertained from the present study; the number of studies for which cholesterol intake per 1,000 kcal could be calculated (n=16) was too small to allow proper examination of this issue.

Specific saturated fatty acid. Another shortcoming of our model is that the cholesterol-raising effects of the different saturates are assumed to be equal. In agree-

ment with the study of Hegsted et al,[2] our subsidiary analyses (see "Results") suggested that the saturated fatty acid of 14-carbon-atom length, myristic acid, is four to six times as hypercholesterolemic as the other two cholesterol-raising saturates, lauric acid (C14:0) and palmitic acid (C16:0). However, confidence limits were wide, and levels of lauric and palmitic acids in the diets were strongly correlated. Results from a recent study also suggest only a modest cholesterol-raising effect of synthesized fat high in lauric acid.[47] This observation, however, as well as the potent effect of myristic acid, awaits confirmation.

Nonlinearity. Finally, the relation between fatty acid intake and serum lipoprotein levels might not be truly linear. However, inspection of Figures 2A–2D suggests that a simple linear model in which diets are characterized solely by their contents of saturated, monounsaturated, and polyunsaturated fatty acids goes a long way toward predicting group mean changes in serum lipid and lipoprotein levels.

Individual differences in response. In view of the variability between individuals in the response of serum cholesterol levels to diet,[48,49] our data may only be applied to means of groups of subjects. Even then, the relation between diet and serum lipoproteins may be influenced by genetic or environmental factors. However, results from many studies have shown that in humans, the response of serum lipids and lipoproteins to dietary lipids is largely independent of factors such as ethnicity, age, and gender. Antonis and Bersohn[50] fed eight different diets to South African prisoners over a 3-year period and did not observe important differences in responses of serum cholesterol between whites and blacks (Bantus). McMurry et al[51] recently fed a high-fat diet to Mexican Indian men and women with high levels of physical activity. Although cholesterol intake differed between the experimental diets, changes observed in serum HDL and LDL cholesterol levels were in quantitative agreement with the changes predicted by the equations derived above. Changes in triglycerides did not, probably because the subjects were not in caloric equilibrium. Age also seems not to be an important determinant of responsiveness.[52] Gender, however, might affect the magnitude of the response, although not its direction.[10,32] Thus, extrapolation of the equations obtained to other groups of subjects appears warranted, provided that one keeps in mind the imprecision involved, as indicated by the confidence intervals in Table 2.

Our study emphasizes that dietary fatty acids are not the sole or even the most important determinant of serum lipid levels, as shown by the large differences in intrinsic levels (see "Results"). These differences are probably due to other dietary components, age, degree of obesity, and genetic differences in lipid metabolism. Nonetheless, dietary fatty acids modified serum lipid levels, regardless of the intrinsic starting levels.

Effects of Fatty Acids on Lipoprotein Concentrations

LDL. Changes in the level of total cholesterol were largely due to changes in LDL, as shown by the similar coefficients for total and LDL cholesterol in Table 2. This is in agreement with the results of Keys et al[1] and Hegsted et al,[2] who reported that the responses of β-lipoproteins paralleled those of total cholesterol.

HDL. HDL cholesterol levels also changed with diet, especially when fat replaced carbohydrates. The latter effect ranged from a rise of 0.07 mmol/l (2.8 mg/dl) per 10% of energy for polyunsaturated fatty acids to 0.12 mmol/l (4.7 mg/dl) for saturated fatty acids. In epidemiological studies of free-living populations, HDL cholesterol changed by 0.10 mmol/l (3.8 mg/dl) for each 10% of energy from carbohydrates replaced by fat (reviewed by Katan[53]). This value agrees well with the present results.

VLDL and triglycerides. In the controlled trials analyzed here, replacement of carbohydrates by fat caused a fall in the fasting level of triglycerides and thus presumably in the level of very low density lipoproteins (VLDLs) and other triglyceride-rich particles. The ratio of serum total triglycerides to VLDL cholesterol is 2.2 mmol/mmol.[17,54] Application of this ratio to the triglyceride equation of Table 2 shows that replacement of 10% of energy as fat by carbohydrates should be expected to increase VLDL cholesterol by 0.10–0.13 mmol/l (3.9–5.0 mg/dl), which would offset and may indeed obscure the simultaneous fall in HDL cholesterol of 0.07–0.12 mmol/l (2.7–4.6 mg/dl). Recent epidemiological evidence suggests that the rise in serum triglycerides induced by carbohydrates is not transient.[55] The fall in HDL cholesterol and the rise in triglycerides caused by high-carbohydrate diets should thus be of some concern in the dietary treatment of patients prone to hypertriglyceridemia.

Very-long-chain polyunsaturates of the (n-3) family, as found in fish oils, markedly lower serum triglycerides.[13] Several authors have reported that (n-6) polyunsaturates also cause lower serum triglyceride levels than do saturated fatty acids.[7,8] However, in the present analyses the difference in the effect on serum triglycerides between polyunsaturates, i.e., linoleic acid and to a minor extent α-linolenic acid, and other fatty acids was slight and statistically not significant. In view of the contrary reports, this subject needs further study.

Predicted Changes in Risk for Coronary Heart Disease

According to the present analysis replacement of saturated by unsaturated fatty acids produces a more favorable lipoprotein profile than does replacement by carbohydrates, so long as other factors, notably body weight, remain equal. Replacing 10% of energy in the form of saturates by carbohydrates would lower LDL cholesterol by 0.33 mmol/l (13 mg/dl) and HDL by 0.12 mmol/l (4.7 mg/dl), whereas replacement by monounsaturates causes a fall of 0.39 mmol/l (15 mg/dl) in LDL cholesterol and of 0.03 mmol/l (1.2 mg/dl) in HDL cholesterol. Use of polyunsaturates instead of monounsaturates would cause a slight additional fall of 0.08 mmol/l (3 mg/dl) in LDL cholesterol but also an additional decrease of 0.02 mmol/l (1 mg/dl) in HDL cholesterol. Both epidemiological and controlled clinical trials suggest that each 1 mg/dl (0.026 mmol/l) increment in LDL cholesterol causes an increase in coronary risk of 1%. Epidemiological observations also show an increase of 2–3% in risk for each 1 mg/dl (0.026 mmol/l) decrease in HDL cholesterol.[56] A causal relation between changes in HDL cholesterol and changes in risk is credible but not proven. If lowering of HDL cholesterol through diet is *not* detrimental to risk, then

replacement of saturates by polyunsaturates would yield a slightly better risk profile than replacement by monounsaturates. If, however, HDL cholesterol *is* causal, then our findings lead to the prediction that replacing saturates by either monounsaturates or polyunsaturates reduces coronary risk to about the same extent, with a possible slightly beneficial effect of polyunsaturates over monounsaturates.

Surprisingly, our regression equation would predict that replacement of saturates by carbohydrates yields little if any improvement in coronary risk. This is in obvious disagreement with a large body of epidemiological evidence that shows that low-fat diets are associated with low risk for coronary heart disease. This discrepancy might have several explanations. First, we must reemphasize that it has not been proven that changing the level of HDL cholesterol will change risk; HDL might be nothing more than an indicator of some underlying process that itself is not sensitive to dietary manipulation. Alternatively, low HDL cholesterol levels might increase risk only when LDL cholesterol levels are high. A third possibility is that populations with a low fat intake have less body fat[57,58]; this would lower coronary risk by itself and would also counteract the HDL-cholesterol lowering and reinforce the LDL cholesterol-lowering effects of a low-fat, high-carbohydrate diet. Also, effects of diet on other risk factors for coronary heart disease such as blood pressure,[59] platelet function,[60] and LDL oxidizability[40,61] are important. Unfortunately, the extent of these effects in humans is not well defined.

Obviously, these questions about diet and coronary risk cannot be settled by drawing theoretical inferences from short-term dietary trials. However, our analysis does raise the question of whether replacement of fat by carbohydrates rather than replacement of saturated by unsaturated fats is really the optimal strategy for the reduction of coronary risk, a question that probably can only be answered by long-term clinical trials.

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