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Fatty Acids in Serum Cholesteryl Esters as Quantitative Biomarkers of Dietary Intake in Humans

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The fatty acid composition of serum cholesteryl esters is used as a qualitative biomarker of fatty acid intake, but quantitative data are scarce. Between 1987 and 1992, the authors fed various fatty acids in four controlled trials to 232 healthy Dutch volunteers and measured the proportion of fatty acids in participants' cholesteryl esters. Each 10% of energy fed as linoleic acid (18:2) raised the proportion of linoleic acid in cholesteryl esters by 9.3 g per 100 g of fatty acids (standard deviation (SD) 3.1). For oleic acid (*cis*-18:1), this figure was 6.5 g/100 g (SD 1.7); for *trans* fatty acids (*trans*-18:1), it was 1.1 (SD 0.5); for stearic acid (18:0), 1.0 (SD 0.4); for palmitic acid (16:0), 1.7 (SD 0.5); for myristic acid (14:0), 2.1 (SD 0.7); and for a mixture of saturated fatty acids (12:0, 14:0, and 16:0), it was 2.2 g/100 g (SD 1.0). The coefficient of variation of the responses was fairly constant, indicating that changes in intake for each of these fatty acids can be monitored with similar precision. These data can be used to estimate the degree of compliance in experimental studies involving exchanges of single dietary fatty acids. Most fatty acids in cholesteryl esters may also be used in observational studies to estimate differences in intake. However, because of multiple simultaneous differences in fatty acid intake between free-living individuals and between populations, such data cannot provide information on absolute intake of fatty acids. *Am J Epidemiol* 1997;145:1114–22.

biological markers; cholesterol; compliance; dietary fats; esters; fatty acids; human body

The pattern of fatty acids in the blood is widely used as a biomarker to assess differences in fatty acid intake between individuals or populations, both in observational studies (1–9) and in controlled trials (10–18). The proportions of fatty acids in cholesteryl esters are correlated with serum lipid levels (1, 5, 7), and they can predict the incidence of diabetes mellitus (19), coronary heart disease (8), or cancer mortality (9). However, little is known about the quantitative relation between the amount of a particular fatty acid in the diet and its proportion in cholesteryl esters; it is thus unclear with what difference in intake a certain difference in cholesteryl ester fatty acid composition corresponds.

In controlled trials, changes in the fatty acid composition of triglycerides, cholesteryl esters, phospholipids, erythrocytes, or platelets are often interpreted as proof of dietary compliance (12, 14). However, a rise in a certain fatty acid in the blood indicates only that a subject has eaten at least some of the food prescribed or provided, not how much.

Here we describe the relations between the amounts of various fatty acids in the diet and levels of cholesteryl esters, as assessed experimentally in healthy Dutch volunteers. Serum lipid and lipoprotein levels in these persons were published previously (20–23). These data may help researchers to interpret differences in cholesteryl ester fatty acid levels between free-living subjects or between populations in observational studies, and to quantitate compliance in trials involving modification of fatty acid intake.

MATERIALS AND METHODS

Subjects and methods

A total of 232 men and women took part in four separate controlled dietary experiments. At the start of the studies, mean serum cholesterol levels per experiment ranged from 4.75 mmol/liter to 5.06 mmol/liter. Mean ages ranged from 24 years to 29 years, and body mass indices (weight (kg)/height $(m)^2$) ranged from 21.5 to 22.4. Average changes in body weight during the trials were between +0.1 and −0.4 kg. Participants were healthy, as indicated by their responses to a medical questionnaire and by the absence of anemia, glucosuria, and proteinuria. The protocols, which had

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Abbreviation: SD, standard deviation.

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been approved by the Ethical Committee of Wageningen Agricultural University, were explained to the subjects, and informed consent was obtained. No payment was offered for participation, but the food was provided free of charge.

Diets consisted of mixed solid foods and were formulated at levels of energy intake ranging from 5.5 MJ/day to 20 MJ/day; each subject received a diet that maintained his or her body weight. Energy requirements were estimated from 3-day food records. On weekdays, hot meals were served at noon in the university's Department of Human Nutrition. Other food was provided daily in a package. Food for weekends was provided on Fridays. In addition, each subject had to select foods and beverages from a list of "free-choice" items that contained no fat or cholesterol. These items provided 9 percent of his or her energy intake. Body weight was recorded twice weekly, and energy intake was adjusted when necessary.

Subjects were asked to maintain their usual pattern of activity and not to change their smoking habits or use of oral contraceptives. They recorded in diaries any sign of illness, any medications or oral contraceptives used, the free-choice items selected, numbers of cigarettes smoked, and deviations from their diets. Inspection of the diaries showed that the participants followed the instructions carefully.

Duplicate portions of the diets were collected each day for an imaginary participant with an energy intake of 10 or 11 MJ/day (2,390 or 2,630 kcal/day), and data were pooled and analyzed after the study. The composition of the free-choice items was calculated (24), and the results were combined with the analyzed values (20–23).

Fasting blood specimens were collected after participants had spent 2.5–5 weeks on each diet. Serum was stored at −80°C.

Study design and diets

Experiment 1 ran from October 4 to November 27, 1987 (20). Fifty-eight healthy men and women first consumed for 17 days a control diet high in saturated fat. For the next 36 days, 14 men and 15 women received a diet enriched with olive oil and sunflower oil (termed the oleic + linoleic diet). The other 13 men and 16 women received a diet enriched with sunflower oil alone (the linoleic diet). The proportion of energy from saturated fatty acids (12:0 + 14:0 + 16:0) decreased by 5.2 percent on the oleic + linoleic diet and by 6.0 percent on the linoleic diet. These decreases were compensated for by an increased intake of both oleic acid (*cis*-18:1, 4.5 percent) and linoleic acid (18:2, 3.5 percent) on the oleic + linoleic diet and by linoleic acid alone (18:2, 8.4 percent) on the linoleic diet. Intakes of total fat and other nutrients were constant. Blood was sampled after 2.5 weeks on the control diet (days 14 and 17) and after 5 weeks on the test diets (days 50 and 53). For each subject, equal volumes of the two blood samples taken per diet period were pooled. Responses to test diets were calculated as the change from the end of the control diet to the end of the test diet.

Experiment 2 ran from September 26 to November 28, 1988 (21). Twenty-five men and 34 women consumed foods from three different diets for 3 weeks each, in random order (multiple crossover). The intake of oleic acid (*cis*-18:1) decreased by 10.5 percent of total energy on a *trans* fatty acid diet and by 10.2 percent on a saturated fat diet in comparison with an oleic acid diet. It was replaced by 10.9 percent of energy as *trans* monounsaturated fatty acids (*trans*-18:1) on the *trans* diet and by 8.8 percent saturated fatty acids (12:0 + 14:0 + 16:0) on the saturated fat diet. Intakes of total fat and other nutrients were constant. Blood was sampled on the 21st day of each diet period.

Experiment 3 (22) had the same design as experiment 2. Between January 29 and April 2, 1990, 26 men and 30 women participated. Eight percent of energy from linoleic acid (18:2) was replaced either by 7.5 percent of energy as *trans* fatty acids (*trans*-18:1) or by 9.0 percent of energy as stearic acid (18:0). Intakes of saturated fatty acids (12:0 + 14:0 + 16:0) and oleic acid (*cis*-18:1) differed by less than 1 percent of energy.

Experiment 4 (23) ran from January 27 to March 30, 1992. It involved 23 men and 36 women and employed the same design as experiments 2 and 3. The intake of oleic acid (*cis*-18:1) decreased by 10.1 percent of energy on a myristic acid (14:0) diet and by 9.3 percent of energy on a palmitic acid (16:0) diet in comparison with the oleic acid diet. It was replaced by either 10.4 percent of energy as myristic acid (14:0) or 9.9 percent as palmitic acid (16:0).

All experiments used special fats developed by the Unilever Research Laboratory (Vlaardingen, The Netherlands). Details have been provided elsewhere (20–23).

Analysis of cholesteryl ester fatty acids

All samples from a particular subject were analyzed in one run and within 8 months after the end of each study. Fatty acids in serum cholesteryl esters were determined as described previously (25), except that gas chromatographic analysis for experiments 2 and 3 was performed on Sil-88 columns (Chrompack, Middelburg, The Netherlands) to obtain separation of *trans* fatty acids and Bond Elute solid-phase extraction

columns (Varian Ltd., Walton-on-Thames, England) were used in experiments 3 and 4 to separate cholesteryl esters and triglycerides. Further details have been given elsewhere (26). The coefficients of variation within runs were, on average, as follows: myristate (14:0), 4.3 percent; palmitate (16:0), 1.9 percent; stearate (18:0), 9.2 percent; *trans*-18:1 fatty acids, 8.9 percent; oleate (*cis*-18:1), 3.1 percent; and linoleate (18:2), 0.8 percent.

Data analysis

To compare the responses of a particular cholesteryl ester fatty acid (Δ) across trials, we recalculated all responses to an intake of 10 percent of energy (Δ_{10}), assuming that response had a linear relation with dosage. For example, if the increase in a fatty acid was 4 g per 100 g of cholesteryl ester fatty acids at a dietary increase of 8 percent of energy, then its response at 10 percent of energy was set at 5 g/100 g. The actual amount of fatty acids exchanged between diets was 5–8 percent of energy in experiment 1 and 8–11 percent of energy in the other trials.

The variance of the mean response (SD_Δ^2), i.e., the variation in response between persons receiving the same dietary modification, can be considered the "noise" of the biomarker. The following sources of variance add to this noise:

$$SD_\Delta^2 = 2 \times SD^2\text{-(within-person)} + SD^2\text{-(response between persons)}.$$

SD^2-(within-person) includes biologic within-subject fluctuations plus laboratory error. It contributes twice, because two blood samples were needed to determine the individual change. Differences in intrinsic level between persons do not add to SD_Δ^2, because individual changes are calculated. However, subjects may differ in their sensitivity to diet; SD^2-(response between persons) is the variation due to these differences in responsiveness. It necessarily depends on the amount of dietary fatty acid exchanged; if the diet is not modified, this component will be zero. As a result, the SD_Δ^2 for the difference between two measurements made in subjects on a constant diet (SD_0^2) will be smaller than the SD_Δ^2 when diets are changed. We assumed that differences in responsiveness increased linearly with differences in exposure. As a result, the SD_Δ^2 increases with the size of dietary difference.

For each dietary comparison, the mean standard deviation (SD) of the responses at a change in intake of 10 percent of energy (SD_{10}) was calculated by multiplying the mean change at this level of intake, Δ_{10}, by the coefficients of variation observed at the actual exchanges of 7.5–11 percent of energy. We estimated SD_0 from within-person fluctuations observed when subjects were fed on diets that contained similar amounts of the fatty acid of interest. "Average" SD_{10} or SD_0 refers to the square root of the respective mean SD^2's.

RESULTS

Table 1 gives the proportions of the six fatty acids of interest in serum cholesteryl esters on each study diet. Together these six fatty acids made up 84.4–88.4 g per 100 g of fatty acids. The other major fatty acids were arachidonic acid (20:4*n*-6; 6–7 g/100 g) and palmitoleic acid (16:1*n*-7; 1.5–4 g/100 g).

Table 2 gives the average changes seen in levels of cholesteryl ester fatty acids when subjects were switched from one diet to another. These changes uniformly followed the changes in dietary fatty acid composition, except for cholesteryl oleate (*cis*-18:1) in experiment 1, which did not increase when a mixture of oleic plus linoleic acid (*cis*-18:1 + 18:2) replaced saturated fatty acids (12:0 + 14:0 + 16:0). The largest responses were seen in levels of cholesteryl linoleate (18:2) and oleate (*cis*-18:1), but the responses of *trans* fatty acids (*trans*-18:1) and saturated fatty acids (14:0 + 16:0 + 18:0) were also very consistent.

Table 3 cross-tabulates the average effects of diets in which one class of dietary fatty acids replaced another; the comparison of oleic plus linoleic acid (*cis*-18:1 + 18:2) with saturated fatty acids (12:0 + 14:0 + 16:0) was excluded. The values represent the change in a cholesteryl ester fatty acid that occurs when that particular fatty acid replaces 10 percent of energy from some other fatty acid in the diet. The type of dietary fatty acid for which a particular fatty acid was substituted had remarkably little effect. The only exception was oleic acid (*cis*-18:1); the response of cholesteryl oleate was smaller when dietary oleic acid replaced a mixture of saturated fatty acids (12:0 + 14:0 + 16:0) than when it replaced other fatty acids. The coefficients of variation of the responses were similar and ranged from 26 percent for oleic acid (*cis*-18:1) to 45 percent for myristic plus palmitic acid (14:0 + 16:0). However, the sizes of the effects were quite different (table 3). Thus, replacement of 8 percent of energy as *trans* fatty acids (*trans*-18:1) by 8 percent linoleic acid (18:2) lowered the proportion of *trans* fatty acids in cholesteryl esters by 0.8 g/100 g and increased that of linoleic acid by 8.2 g/100 g (experiment 3, tables 1 and 2). This left a difference of 7.4 g/100 g which was made up for by a decrease of 5.6 g/100 g in oleic acid (*cis*-18:1), plus smaller decreases in other fatty acids, such that the total was still 100 g/100 g. Similarly, replacement of 10 percent of energy as palmitic acid (16:0) by oleic acid (*cis*-18:1)

TABLE 1. Proportions of fatty acids in serum cholesteryl esters of healthy men and women after they had been placed on diets enriched in these particular fatty acids for 17–36 days in controlled dietary experiments, Wageningen, The Netherlands, 1987–1992

Study diet	Mean proportion (g/100 g of cholesteryl ester fatty acids)							
	Myristate (14:0)	Palmitate (16:0)	Stearate (18:0)	*trans*-18:1* fatty acids	Oleate* (*cis*-18:1)	Linoleate (18:2)	Other	All
Experiment 1								
Saturated fatty acids†	1.1 (0.3)‡	10.8 (0.7)	0.8 (0.2)	ND§	17.9 (1.2)	54.7 (2.7)	14.6	100
Oleic acid + linoleic acid	0.7 (0.2)	10.0 (0.8)	0.7 (0.1)	ND	17.2 (2.0)	59.0 (3.6)	12.4	100
Linoleic acid	0.6 (0.1)	9.7 (0.6)	0.7 (0.1)	ND	13.3 (1.6)	63.4 (2.7)	12.4	100
Experiment 2								
Saturated fatty acids†	1.6 (0.5)	10.2 (0.6)	0.8 (0.1)	0.2 (0.2)	20.3 (1.4)	51.3 (2.6)	15.6	100
trans fatty acids	1.2 (0.6)	8.7 (0.7)	0.9 (0.1)	1.3 (0.3)	17.2 (1.2)	56.0 (2.7)	14.7	100
Oleic acid	1.2 (0.6)	9.1 (0.6)	0.7 (0.1)	0.1 (0.1)	24.3 (1.4)	50.5 (2.5)	14.2	100
Experiment 3								
Stearic acid	2.3 (1.2)	8.4 (0.6)	1.7 (0.4)	0.1 (0.2)	20.2 (1.4)	54.4 (3.2)	12.9	100
trans fatty acids	2.5 (1.1)	9.1 (0.6)	0.9 (0.3)	0.9 (0.3)	20.6 (1.4)	52.8 (3.4)	13.1	100
Linoleic acid	2.7 (0.8)	8.8 (0.6)	0.8 (0.1)	0.1 (0.2)	15.1 (1.6)	61.0 (3.9)	11.6	100
Experiment 4								
Myristic acid	2.8 (0.6)	9.3 (0.6)	0.9 (0.2)	ND	17.9 (1.2)	54.6 (2.9)	14.5	100
Palmitic acid	0.7 (0.2)	10.9 (0.5)	0.9 (0.2)	ND	17.8 (1.1)	56.1 (2.8)	13.6	100
Oleic acid	0.7 (0.2)	9.2 (0.7)	0.8 (0.1)	ND	24.2 (1.8)	51.3 (3.1)	13.7	100

* Proportions of *trans*-18:1 isomers were not determined in experiments 1 and 4; corresponding values in the "Oleate (*cis*-18:1)" column therefore indicate the total level of 18:1 fatty acids.
† A mixture of lauric acid (12:0), myristic acid (14:0), and palmitic acid (16:0).
‡ Numbers in parentheses, standard deviation.
§ ND, not determined.

lowered the level of cholesteryl palmitate by 1.7 g/100 g and raised the level of cholesteryl oleate by 6.3 g/100 g. The remaining difference of 4.7 g/100 g was made for up mainly by linoleic acid (18:2).

DISCUSSION

Our data, based on an aggregate of four carefully controlled trials conducted among 101 men and 131 women, provide stable estimates of the relations between amounts of fatty acids in the diet and their proportions in cholesteryl esters.

Changes in dietary linoleic acid (18:2) caused the largest changes in cholesteryl esters; an increase of 10 percent of energy from linoleic acid increased the level of cholesteryl linoleate (18:2) by 9.3 g/100 g of fatty acids (table 3). Figure 1 shows that this aligns perfectly with the responses of cholesteryl linoleate to linoleic acid feeding seen by other investigators in well-controlled studies (18, 27–35). Similar responses were observed in less controlled studies (36–38). These effects thus appear to be highly reproducible. Linoleic acid diets fed for less than 15 days (29, 31, 35) or for more than 3 months (28, 32) have produced the same responses. Therefore, the periods of 21 days in our trials were apparently long enough to reach a maximum response of cholesteryl linoleate (18:2). Data on the kinetics of the incorporation of other fatty acids are sparse, but a controlled study from the Department of Human Nutrition (39) showed that the incorporation half-life of eicosapentaenoic acid (20:5*n*-3) was 5 days, which implies that after 21 days more than 90 percent of the maximum response is reached (unpublished data). We therefore assume that responses to fatty acids other than linoleic acid also reached a plateau within 21 days. If incorporation was not yet at its maximum after this period, our coefficients may have somewhat underestimated cholesteryl ester response.

Changes in oleic acid (*cis*-18:1) intake had less impact than changes in linoleic acid (18:2) intake on proportions of fatty acids in cholesteryl esters, and the impact of saturated fatty acids and *trans*-18:1 fatty acids was still smaller. Thus, the "signal" of these cholesteryl esters as indicators of changes in intake was weaker than that for linoleic acid (18:2). However, the variability of the changes, or "noise," was also much smaller (table 2), resulting in similar coefficients of variation (table 3). Figure 2 illustrates this point. Therefore, even these smaller changes are reliable indicators of changes in intake.

We note that our data were derived from young, healthy volunteers and may not apply to older or diseased persons.

TABLE 2. Within-subject changes in proportions of serum cholesteryl ester fatty acids of healthy men and women when dietary fatty acids were substituted for each other for 17–36 days in controlled dietary experiments, Wageningen, The Netherlands, 1987–1992

Dietary comparison	Mean difference (diet 2 minus diet 1) (g/100 g of cholesteryl ester fatty acids)							
	Myristate (14:0)	Palmitate (16:0)	Stearate (18:0)	*trans*-18:1* fatty acids	Oleate* (*cis*-18:1)	Linoleate (18:2)	Other	All
Experiment 1								
Saturated fatty acids† → oleic acid + linoleic acid	−0.5 (0.3)‡	−0.8 (0.7)	−0.1 (0.2)	ND§	−0.8 (1.9)	4.2 (3.3)	−2.0	0
Saturated fatty acids† → linoleic acid	−0.5 (0.3)	−1.1 (1.0)	−0.1 (0.2)	ND	−4.6 (1.6)	8.7 (3.3)	−2.4	0
Experiment 2								
trans fatty acids → oleic acid	−0.1 (0.3)	0.4 (0.4)	−0.2 (0.1)	−1.2 (0.3)	7.1 (1.3)	−5.5 (1.9)	−0.5	0
Saturated fatty acids† → oleic acid	−0.4 (0.4)	−1.0 (0.4)	−0.1 (0.1)	−0.1 (0.2)	3.9 (1.3)	−0.8 (1.9)	−1.5	0
trans fatty acids → saturated fatty acids	0.3 (0.4)	1.5 (0.6)	−0.0 (0.1)	−1.1 (0.5)	3.1 (1.2)	−4.7 (1.9)	0.9	0
Experiment 3								
trans fatty acids → linoleic acid	0.2 (0.9)	−0.3 (0.4)	−0.1 (0.2)	−0.8 (0.4)	−5.6 (1.3)	8.2 (2.5)	−1.6	0
Stearic acid → linoleic acid	0.4 (1.1)	0.4 (0.4)	−1.0 (0.4)	−0.0 (0.3)	−5.1 (1.6)	6.6 (2.4)	−1.3	0
trans fatty acids → stearic acid	−0.2 (1.1)	−0.7 (0.5)	0.9 (0.4)	−0.8 (0.4)	−0.5 (1.3)	1.6 (1.9)	−0.3	0
Experiment 4								
Myristic acid → oleic acid	−2.2 (0.7)	−0.1 (0.4)	−0.1 (0.1)	ND	6.3 (1.7)	−3.2 (1.9)	−0.7	0
Palmitic acid → oleic acid	0.0 (0.1)	−1.7 (0.6)	−0.1 (0.1)	ND	6.3 (1.6)	−4.8 (1.8)	0.3	0
Myristic acid → palmitic acid	−2.2 (0.7)	1.6 (0.5)	0.0 (0.1)	ND	−0.1 (0.7)	1.6 (1.6)	−1.1	0

* Proportions of *trans*-18:1 isomers were not determined in experiments 1 and 4; corresponding values in the "Oleate (*cis*-18:1)" column therefore indicate the total level of 18:1 fatty acids.
† A mixture of lauric acid (12:0), myristic acid (14:0), and palmitic acid (16:0).
‡ Numbers in parentheses, standard deviation.
§ ND, not determined.

TABLE 3. Impact of specific dietary intakes of fatty acids on their serum cholesteryl ester content when intake was increased by 10% of energy at the expense of other dietary fatty acids, Wageningen, The Netherlands, 1987–1992

Dietary component replaced	Mean response* (g/100 g of cholesteryl ester fatty acids) of a particular fatty acid in cholesteryl esters per 10% of energy provided by this fatty acid in the diet						
	Myristate (14:0)	Palmitate (16:0)	Saturated fatty acids† (14:0 + 16:0)	Stearate (18:0)	*trans*-18:1 fatty acids	Oleate (*cis*-18:1)	Linoleate (18:2)
Myristic acid (14:0)		1.6 (32)‡				6.2 (27)	
Palmitic acid (16:0)	2.2 (31)					6.8 (25)	
Saturated fatty acids (12:0 + 14:0 + 16:0)					1.1 (40)	3.9 (33)	10.4 (38)
					1.1 (48)		8.1 (37)
Stearic acid (18:0)						6.7 (19)	9.8 (30)
trans-18:1 fatty acids			2.1 (40)	1.0 (49)			8.9‖
Oleic acid (*cis*-18:1)	2.1 (31)	1.7 (33)	1.8§ (37)		1.1 (28)		
Linoleic acid (18:2)			3.1 (66)	1.1 (36)	1.1 (46)	8.6‖	
Mean	2.1 (31)	1.7 (32)	2.2 (45)	1.0 (43)	1.1 (41)	6.5 (26)	9.3 (33)

* For example, when 10% of dietary energy from palmitic acid (16:0) was replaced by myristic acid (14:0), the proportion of myristic acid in cholesteryl esters increased by 2.2 g/100 g of fatty acids, with a coefficient of variation of 31% or a standard deviation of 0.68 g/100 g of fatty acids.
† Response of myristic acid plus palmitic acid (14:0 + 16:0) in cholesteryl esters to changes in intake of lauric acid plus myristic acid plus palmitic acid (12:0 + 14:0 + 16:0).
‡ Numbers in parentheses, coefficient of variation ((standard deviation/mean response) × 100%). Values given are those actually observed at exchanges ranging from 7.5% to 11% of dietary energy (see "Materials and Methods").
§ Mean of three comparisons: a mixture of saturated fatty acids (12:0 + 14:0 + 16:0) vs. oleic acid (18:1) (experiment 2), myristic acid (14:0) vs. oleic acid (18:1), and palmitic acid (16:0) vs. oleic acid (18:1) (experiment 4).
‖ Coefficients of variation are not given because *cis*-18:1 and linoleic acid (18:2) were not compared within persons (experiment 1; parallel design).

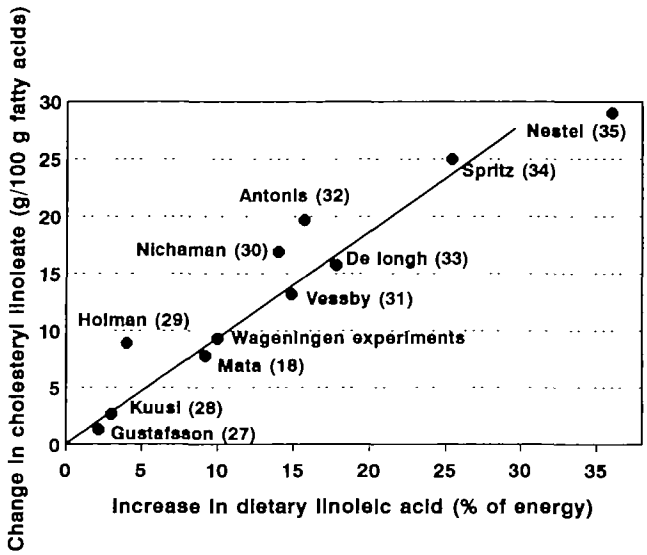

FIGURE 1. Change in the proportion of linoleic acid (18:2) in cholesteryl esters by change in dietary linoleic acid intake. Each point represents the mean result of a strictly controlled study in which linoleic acid was isocalorically exchanged for other fatty acids. Regression analysis indicated an increase in cholesteryl linoleate of 0.93 g per 100 g of fatty acids for every percentage-of-energy increase in linoleic acid intake. "Wageningen experiments" represents the mean result of two trials described in the present paper.

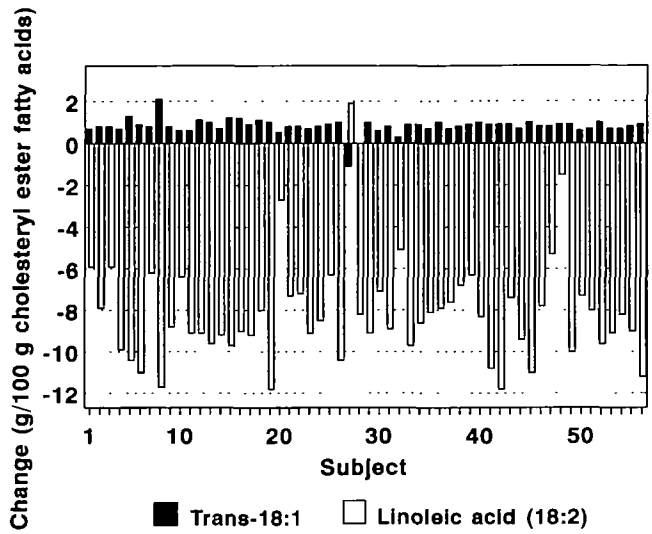

FIGURE 2. Individual differences in the proportions of linoleic acid (18:2) and *trans* fatty acids (*trans*-18:1) in the cholesteryl esters of 56 subjects when 8% of their dietary energy intake was changed from linoleic acid to *trans* fatty acids (experiment 3), Wageningen, The Netherlands, 1987–1992. Responses to linoleic acid were much greater than those to *trans* fatty acids. Nevertheless, proportions of *trans* fatty acids were increased to approximately the same extent in all subjects, while the decrease in cholesteryl linoleate (18:2) varied much more. This resulted in similar coefficients of variation: 33 percent for linoleic acid and 41 percent for *trans* fatty acids.

Cholesteryl esters as indicators of intake in observational studies

Our data show that when one fatty acid replaces another, the relation between the proportion of a fatty acid in cholesteryl esters and its amount in the diet is straightforward. In observational studies, however, people usually differ in their intakes of several fatty acids at the same time. This may distort the relations between cholesteryl ester fatty acids and intake, because differences in a particular cholesteryl ester fatty acid between individuals may result from differences in the intakes of other fatty acids. Some fatty acids are metabolized to longer, more unsaturated fatty acids, and intake of one of these can inhibit elongation and desaturation of others (40). Yet other fatty acids may be endogenously synthesized from carbohydrates. Thus, differences between free-living people in the proportion of a certain cholesteryl ester fatty acid do not necessarily reflect differences in intake of that fatty acid.

Another potential problem is that cholesteryl ester fatty acids are expressed as a proportion of total fatty acids, and if the proportion of one fatty acid is increased through dietary intake, proportions of some or all other fatty acids must inevitably go down. This may cause problems when dietary fatty acids with a large effect on cholesteryl ester fatty acids (18:2 or 18:1) replace fatty acids with relatively small effects (14:0, 16:0, 18:0, or *trans*-18:1). The resulting difference in net effect sizes must then be compensated for by other fatty acids, because the total cholesteryl ester fatty acid proportion, by definition, is 100 g/100 g. This difference is mainly compensated for by either linoleic acid (18:2) or oleic acid (*cis*-18:2), which is in line with the preference of the enzyme lecithin: cholesterol acyl transferase for these fatty acids in phospholipids (41).

The following example illustrates how differences in the proportions of particular fatty acids in cholesteryl esters may result from differences in intake of other fatty acids. Sandker et al. (5) found that the average cholesteryl linoleate (18:2) proportion in elderly men was 11 g/100 g lower in Crete than in Zutphen, The Netherlands. Our coefficients (table 3) would at first sight suggest that the Cretan men consumed approximately 12 percent less of their energy as linoleic acid (18:2) than the Dutch men. However, chemical analysis of food composites (42) indicated that the difference was only 3 percent of energy. This discrepancy can be explained by the 10 percent-of-energy higher saturated fat and 10 percent-of-energy lower oleic acid consumption in Zutphen than in Crete. Our coefficients predict that these dietary differences will decrease the proportion of cholesteryl oleate by 6.5 g/100 g and increase the proportion of cholesteryl ester saturated fats by 2.2 g/100 g, leaving

a difference of 4.3 g/100 g, which will largely be compensated for by an increase in cholesteryl linoleate (18:2). This secondary effect explains the much higher proportion of linoleic acid in the cholesteryl esters of the Zutphen men.

Thus, differences in cholesteryl oleate (*cis*-18:1) or linoleate (18:2) may result from differences in consumption of other fatty acids. Therefore, absolute differences in fatty acid intake between free-living subjects or between populations are not adequately described by the coefficients for cholesteryl ester fatty acids that we derived from one-to-one comparisons of dietary fatty acids. Hence, these coefficients cannot be applied as such to the estimation of quantitative difference in fatty acid intake in observational studies. Nevertheless, cross-sectional studies consistently show correlations between recorded intake and cholesteryl ester proportion for linoleic acid (18:2) (r = 0.25–0.67) and, to a lesser extent, for saturated fatty acids (12:0–18:0) (r = 0–0.57) (4, 16, 43–47). Except for the findings of one study (48), this association seems to be absent for oleic acid (*cis*-18:1) (4, 43, 46, 47), which may be explained by endogenous synthesis and further metabolism and by the compensatory effects described above. Observational data on the relation between *trans* fatty acid (*trans*-18:1) levels in the diet and levels in cholesteryl esters are lacking, but substantial correlations have been found between *trans* fatty acid intake and the proportions of *trans* fatty acids in serum triglycerides, platelet phospholipids (49), and fat tissue (50). It is therefore likely that cholesteryl ester *trans* fatty acids (*trans*-18:1) will also reflect *trans* fatty acid intake in the population.

Thus, proportions of linoleic acid (18:2), saturated fatty acids (14:0, 16:0, and 18:0), and *trans* fatty acids (*trans*-18:1), but not of oleic acid (*cis*-18:1), in cholesteryl esters may be used in observational studies to indicate relative patterns or differences in fatty acid intake. Although cholesteryl esters cannot provide information on absolute fatty acid intake, their use provides a definite advantage over dietary recalls, food records, and food frequency methods in that intake can be measured more objectively.

Application to dietary trials

Our estimates can be used to calculate intervals within which the cholesteryl ester responses are expected to fall in similar trials. Figure 3 shows 95 percent prediction intervals for the average change in the proportion of a cholesteryl ester fatty acid expected when one, 10, or 50 subjects increase their fatty acid intake by 10 percent of energy. The larger the group, the narrower the interval. For example, if a group of 10 persons fully adheres to a diet enriched with 10 percent of energy as oleic acid (*cis*-18:1), the average rise in cholesteryl oleate is expected to be between 5.4 and 7.5 g/100 g on 95 out of 100 occasions. If the average

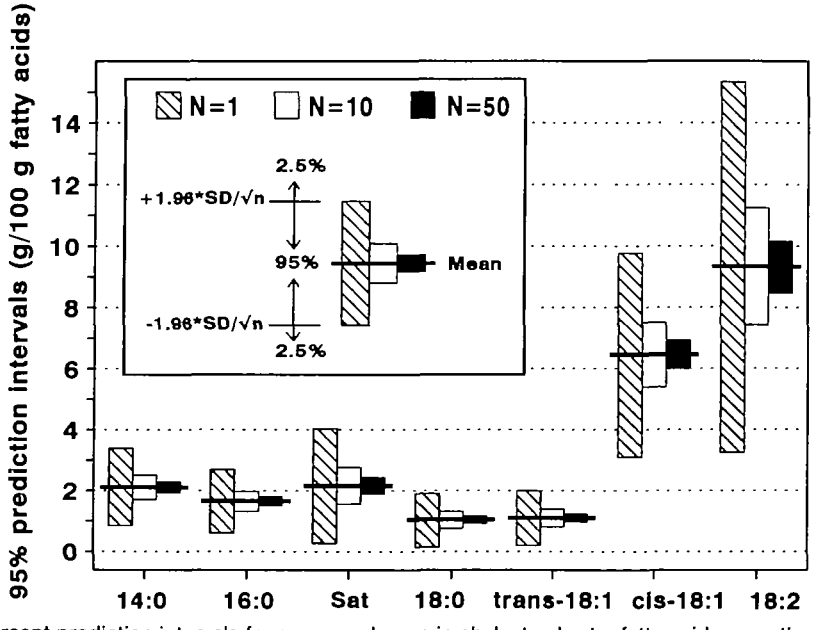

FIGURE 3. Ninety-five percent prediction intervals for average change in cholesteryl ester fatty acid proportions when intake of fatty acids is increased by 10% of energy in participant groups of different sizes, Wageningen, The Netherlands, 1987–1992. It was assumed that the mean changes in proportions of cholesteryl esters (and their standard deviations) observed in these trials represent the mean (μ) changes (and standard deviations (σ)) in cholesteryl ester responses in the population. SD, standard deviation; Sat, saturated fatty acids (response of myristic acid plus palmitic acid (14:0 + 16:0) in cholesteryl esters to changes in intake of lauric acid plus myristic acid plus palmitic acid (12:0 + 14:0 + 16:0)).

rise is less than 5.4 g/100 g, there is reason to doubt compliance. When one person increases his or her intake of a particular fatty acid by 10 percent of energy, the chance that the corresponding fatty acid in cholesteryl esters will show an increase is 99 percent for any of the six fatty acids studied here. Thus, changes in the proportions of cholesteryl esters provide a useful tool for evaluating the average dietary adherence of a group of subjects, and they also seem to be reasonable indicators of individual compliance, provided that laboratory error is minimized.

Our data allow quantification of the degree of compliance in trials involving modifications of fatty acid intake. Proportions of linoleic acid (18:2), saturated fatty acids (14:0, 16:0, and 18:0), and *trans* fatty acids (*trans*-18:1), but not of oleic acid (*cis*-18:1), in cholesteryl esters may be used in observational studies to indicate relative patterns in intake, but they cannot provide information on absolute differences in fatty acid intake.

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