Fatty acid composition of serum cholesteryl esters and erythrocyte membranes as indicators of linoleic acid intake in man[1-3]

Jan FC Glatz, Ans EMF Soffers, and Martijn B Katan

ABSTRACT We compared the suitability of the linoleic-oleic acid ratio (L:O) of serum cholesteryl esters and erythrocyte membranes as indicators of dietary fatty acid composition. Forty-seven volunteers received a mixed natural diet with a polyunsaturated-saturated fatty acids ratio (P:S) of 0.2 for 3 wk and with P:S 2.0 for another 3 wk (HA group, 24 subjects) or vice versa (HY group, 23 subjects). Duplicate portion analysis revealed that dietary fat type was the only variable. The change in L:O in cholesteryl esters relative to the low P:S diet was 91.3 ± 25.9% ($\bar{x}$ ± SD) in the HA group and 85.1 ± 18.8% in the HY group. The changes in erythrocyte membranes were 33.0 ± 7.9% in the HA group and 22.8 ± 4.9% in the HY group. Thus the effect on erythrocyte fatty acids was smaller but also less variable and the precision of the two measures was similar. Therefore the L:O of either blood component can be used as a marker of a subject's adherence to experimental diets differing in type of fat. *Am J Clin Nutr* 1989;49:269–76.

KEY WORDS Linoleic acid, dietary adherence, fatty acid composition, serum cholesteryl esters, erythrocyte membranes

Introduction

Methodology is urgently needed for assessment of adherence to fat-controlled diets in free-living subjects, both long-term and daily or even from meal to meal. In this respect analysis of body lipids offers great promise. The fatty acid composition of the dietary fat markedly influences the fatty acid composition of lipids of blood components and of adipose tissue. Long-term linoleic acid intake is positively correlated with linoleic acid content of serum triacylglycerols, cholesteryl esters, and phospholipids (1–3) of erythrocytes and platelets (2–5) and adipose tissue (2, 6–8). Because of different rates of turnover of fatty acids in these constituents, differences arise in the time required for a change in dietary fat type to be totally reflected in the fatty acid pattern. Thus, the fatty acid composition of human serum triacylglycerols reflects the composition of the last few meals, that of cholesteryl esters and of erythrocyte membranes reflect the dietary intake of the preceding weeks or months (1, 9, 10), whereas that of human subcutaneous adipose tissue is a valid index of the fatty acid composition of the habitual diet over the past 2–3 y (7, 11, 12).

The fatty acid pattern of lipid components is being used increasingly as an objective biochemical indicator of adherence to experimental diets during dietary intervention studies (2), especially to assigned cholesterol-lowering diets rich in polyunsaturated fatty acids (13–15). In addition, the linoleic acid content of human subcutaneous adipose tissue can be applied as an index for the habitual dietary fatty acid composition thereby replacing dietary recalls or food consumption records (8, 16, 17).

For the short-term assessment of the quality of the dietary fat consumed, the fatty acid composition of cholesteryl esters is commonly used (18–20) whereas that of erythrocyte membranes, although proposed in 1963 (9), is applied infrequently (2, 13, 14). The aim of this study was to find out whether the linoleic acid content of erythrocyte membranes is as good an indicator of a change in the fatty acid composition of the diet as the linoleic acid content of cholesteryl esters.

Subjects and methods

Subjects

All subjects were female or male healthy volunteers who had earlier participated in trials involving dietary cholesterol (21).

[1] From the Department of Human Nutrition, Agricultural University, Wageningen, The Netherlands.

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[3] Address reprint requests to MB Katan, Department of Human Nutrition, Agricultural University, De Dreijen 12, 6703 BC Wageningen, The Netherlands.

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TABLE 1
Base-line characteristics for all participants who successfully completed the study*

Variable	HA group	HY group
n	24	23
Age at entry (y)	54 ± 13	34 ± 13
Height (m)	1.72 ± 0.07	1.78 ± 0.09
Weight (kg)	73.7 ± 15.2	71.4 ± 9.5
Body mass index (kg/m^2)	24.4 ± 3.8	22.5 ± 2.2
Serum total cholesterol (mmol/L)	6.29 ± 1.24	5.01 ± 0.75
Serum HDL cholesterol (mmol/L)	1.48 ± 0.37	1.36 ± 0.25
Serum triacylglycerols (mmol/L)	1.51 ± 0.86	1.12 ± 0.55
Habitual diet		
Energy		
MJ/d	10.1 ± 2.5	9.7 ± 2.8
kcal/d	2414 ± 609	2331 ± 669
Protein (% of energy)	14.2 ± 2.6	15.1 ± 2.8
Fat (% of energy)	44.6 ± 6.5	35.5 ± 6.3
Polyunsaturated fatty acids (% of energy)	6.3 ± 2.1	6.3 ± 2.6
Saturated fatty acids (% of energy)	18.9 ± 2.4	16.2 ± 3.3
P:S	0.34 ± 0.13	0.41 ± 0.23
Carbohydrates (% of energy)	38.5 ± 7.0	46.0 ± 7.1
Cholesterol		
mg/MJ	61 ± 22	28 ± 14
mg/1000 kcal	255 ± 92	117 ± 59
Alcohol (% of energy)	2.5 ± 3.2	3.0 ± 3.2

* $\bar{x}$ ± SD. HDL, high-density lipoprotein cholesterol; P:S, ratio of polyunsaturated to saturated fatty acids.

They were from the general population of Wageningen and surroundings. HA subjects had a high habitual consumption of eggs; HY subjects had normal dietary habits. Baseline data were obtained in October or December 1983. Standing height was measured without shoes and weights were measured to the nearest 0.1 kg after breakfast and without shoes, sweaters, jackets, key rings, etc. Habitual dietary intake was estimated using the dietary history method, and nutrient intakes were calculated using the 1984 edition of the computerized extended Dutch food composition table (22). Their base-line characteristics are given in **Table 1.** Details on the selection of the subjects are described elsewhere (21).

All participants showed a considerable interest in the study and informed consent was obtained from them. Prior approval was obtained from the Research Ethical Committee of the Department of Human Nutrition. The dietary trial was performed in the winter of 1983–1984.

Experimental design

The study was originally designed to investigate the effect of the dietary fatty acid composition on human cholesterol metabolism. Subjects received a mixed natural diet low in polyunsaturated and high in saturated fatty acids (low-P:S diet) for 3 wk and a diet high in polyunsaturated and low in saturated fatty acids (high-P:S diet) for another 3 wk (HA group), or vice versa (HY group). The diets were formulated at 15 levels of energy intake, ranging from 7.7 to 16.1 MJ/d (1840 to 3990 kcal/d). The diets were composed of natural foodstuffs. Total diets were provided except for free-choice items described below. All foodstuffs were weighed out for each person in quantities appropriate to his or her energy needs. On weekdays subjects from the HY group and some from the HA group came in at noon for their hot meal, which was served in a special dining room at the department. Evening bread, breakfast, and other foods were distributed to them as packages each weekday at noon and consumed at home. Food for the weekend including ingredients for the hot meals was provided each Friday. For the other subjects from the HA group, food packages were delivered by dietitians to the participants' homes two or three times a week.

For the high-P:S diet a day's menu providing 10 MJ (2390 kcal) consisted of six slices of whole-meal bread baked with sunflower oil (8 g oil/100 g bread), 30 g margarine rich in linoleic acid, 15 g moderate-fat Gouda cheese, 30 g sliced meat, 15 g low-fat cheese spread, 350 g low-fat milk, one piece of fruit, 20 g selected cookies, 30 g nuts, 8 g egg, 8 g egg yolk, 4 g sunflower oil, 6 g sugar, 200 g potatoes, 200 g vegetables, 60 g gravy rich in linoleic acid, 17 g salad dressing, and 200 g dessert prepared with low-fat milk. In the low-P:S diet, oils and fats rich in linoleic acid were replaced by butter, low-fat milk was replaced by full-cream milk, and 75–100 g meat/d was added. Slight adjustments were made in other items. Egg and egg-yolk consumption were reduced to equalize the cholesterol content of the two diets. Each subject was allowed to consume 1 MJ/d (240 kcal/d) of self-selected foodstuffs free from fat and cholesterol as specified in a list. Typical selections were alcoholic and soft drinks, fruit, and candy. Subjects recorded these free-choice items daily in a diary. No special instructions were given regarding caffeine-containing beverages. Subjects were repeatedly urged not to change their free-choice selections and smoking and exercise habits over the course of the experiment; inspection of the diaries suggested that they had complied with these demands. Actual nutrient composition of the diets (**Table 2**) was measured by analysis of duplicate portions collected for one imaginary HA and one imaginary HY person with average

TABLE 2
Composition of the low-P:S and the high-P:S diets

	HA group		HY group	
Dietary component	Low-P:S diet	High-P:S diet	Low-P:S diet	High-P:S diet
Energy*				
MJ/d	10.6	10.8	12.5	12.4
kcal/d	2532	2577	2961	2980
Protein (% of energy)	14	13	14	13
Fat, total (% of energy)	45	45	44	45
Saturated fatty acids (% of energy)	23	10	23	11
Monounsaturated fatty acids (% of energy)	14	12	14	12
Polyunsaturated fatty acids† (% of energy)	5	21	5	21
P:S	0.2	2.0	0.2	1.9
Carbohydrates (% of energy)	39	40	39	39
Alcohol (% of energy)	2	2	2	2
Cholesterol				
mg/MJ	40	44	40	40
mg/1000 kcal	167	184	167	167
mg/d*	424	474	495	498

* Mean intakes.
† Mainly linoleic acid.

energy intake on each diet supplemented with data from the 1984 edition of the computerized Dutch food table (22).

Body weights were checked to the nearest 100 g twice a week and energy intake was adjusted when necessary to counter weight changes.

At the start of the experiment both groups consisted of 27 subjects. Of these, 24 participants from the HA group and 23 from the HY group successfully completed the study. Reasons for withdrawal of a subjects' data were weight loss or illness (3 HA and 3 HY subjects) or poor dietary adherence as judged by the dietitians and confirmed by a lack of change in the linoleic-oleic acid ratio (L:O) of serum cholesteryl esters (1 HY subject).

Blood sampling and storage

Blood was collected 3 d before the end and on the final day of each test period. Thus, four samples were obtained from each of the 47 subjects except 1 for whom only one blood sample was available for each test period. After an overnight fast venous blood was drawn into one vacutainer tube with and another one without EDTA as anticoagulant. Serum obtained from the latter collections was stored at −20 °C and used for the analysis of cholesteryl esters. The EDTA tubes were immediately centrifuged at low speed and 4 °C to separate the red blood cells. The cells were washed twice with ice-cold isotonic saline, transferred into stoppered glass tubes, and hemolyzed by freezing at −20 °C. All further analyses were performed after the dietary trial was finished.

Determination of the fatty acid composition of cholesteryl esters and erythrocyte membranes

For analysis of cholesteryl esters, lipids were extracted from serum by a procedure modified from that described by Wang and Peter (23). Analysis of all four sera of 13 subjects showed that the variation within subjects was very small. Because the cholesteryl ester fatty acid analysis is rather laborious, we pooled the duplicate sera obtained per subject per diet period for the remaining subjects. Four milliliters of isopropanol and 1.8 mL of distilled water were added to 0.4 mL serum. The sample was mixed on a Vortex mixer (Eckli, SA, Neuchâtel, Switzerland) for 30 s and then 4 mL of *n*-octane were added; this mixture was shaken for 10 min. After centrifugation for 3 min at 600 × *g* the (upper) octane layer was removed and taken to dryness in a stream of nitrogen. The lipids were redissolved in chloroform and separated by thin-layer chromatography on precoated silica gel 60F-254 plates (Merck, Darmstadt, FRG) with petroleum ether (boiling range 40–60 °C)-diethyl ether-glacial acetic acid (160:30:5 by vol) as solvent. The components were made visible under ultraviolet light by spraying the plate with a solution of 2.5 g Rhodamine 6G® (BDH Chemicals, Poole, UK) in 1 L methanol. The area of silica gel corresponding to cholesteryl esters was scraped off the plate and the cholesteryl esters were hydrolyzed with 2 mL of 0.5 mol NaOH/L in methanol for 30 min at 80 °C. The component fatty acids were methylated with BF_3 in methanol according to Metcalfe et al (24) and analyzed with a gas chromatograph (model 433, Packard Instrument Co, Downers Grove, IL) with a 1.8-m glass column (inner diameter 2 mm) packed with 15% CP Sil 84 on chromosorb WHP 100–120 mesh (Chrompack, Middelburg, The Netherlands). The injection volume was 1 μL (containing 2–4 μg of individual fatty acids). N_2 was used as carrier gas at a flow rate of 20 mL/min. The oven temperature was programmed to rise from 170 to 210 °C with a rate of 3°/min and then kept constant at 210 °C thus yielding a good separation of fatty acid methyl esters from $C_{14:0}$ to $C_{22:6}$. The temperature of the injector was 200 °C and that of the hydrogen flame ionization detector was 240 °C. An identical second column was used to correct for base-line drift. Peak areas were measured with an integrator (SP 4100, Spectra Physics, Santa Clara, CA). Identification of individual components was made by comparison of their retention times with those of known standards (Supelco Inc, Bellefonte, PA). Fatty acid composition data are expressed as g/100 g fatty acid methyl esters; values < 0.5 g/100 g are not specified. The sum of all peak areas of the fatty acids identified was taken as 100%.

For analysis of erythrocytes, lipids were extracted from the hemolyzed material by the method of Folch et al (25). The antioxidant 2,6-di-*tert*-butyl-*p*-cresol (BHT; BDH Biochemicals, Poole, UK) was added to the chloroform (50 mg/mL). Phospholipids were transmethylated with 40 mL H_2SO_4/L methanol at 60 °C for 18 h to form fatty acid methyl esters. These methyl esters were extracted with hexane and analyzed by gas-liquid chromatography as described above except for the operating conditions which were as follows: the oven temperature was programmed to increase from 150 to 230 °C at a rate of 3°/min, injector temperature was 230 °C, and detector temperature was 260 °C.

To estimate the reproducibility of the methods, one sample of pooled serum and one of pooled hemolyzed erythrocytes were analyzed in duplicate or triplicate in each run (a total 26 and 16 determinations, respectively). For these samples the combined within- and between-run coefficients of variation (CVs) over a 3-mo period were ~1.5% for major and ~2.5% for minor peaks. The L:O showed CVs within and between runs of 3.2 and 2.0%, respectively, for cholesteryl esters and of 3.1 and 2.1%, respectively, for erythrocyte membranes.

In the course of the analysis of the erythrocyte-membrane fatty acids, we observed a very low content of polyunsaturated fatty acids in a number of samples. Only then did we become aware that the presence of hemoglobin may promote the oxidation of polyunsaturated fatty acids even at −20 °C (4) in some hemolysates whereas others are hardly affected (26). We therefore decided to eliminate the data of all 57 erythrocyte samples that had a $C_{20:4}$ (arachidonic acid) content of < 10%. The mean ± SD content of arachidonic acid amounted to only 4.2 ± 2.3% in the 57 rejected samples and to 12.9 ± 1.0% in the 129 samples that were regarded as being not affected. For two more samples not enough blood was available for analysis. As a result, data on the fatty acid composition of erythrocyte membranes were unavailable for 6 of 24 subjects in the HA group and for 9 of 23 in the HY group.

Statistics

For the calculation of the coefficients of variation we used the combined data obtained for the two groups of subjects because a separate calculation revealed no differences between the two groups (data not shown). Within-person variation was derived from analysis of four samples from 13 persons for cholesteryl esters and from 32 persons for erythrocytes; between-subject variation was based on data from 47 subjects for cholesteryl esters and 32 for erythrocytes. Values include the laboratory error, which corresponded to a coefficient of variation of ~3.5% for both methods. Statistical difference was analyzed by Student's paired *t* test of observations on the low-P:S and high-P:S diets. Pearson correlation coefficients and their significance were calculated according to a two-tailed test (27). The level of significance was set at $p < 0.05$.

Results

Subject participation

Throughout the entire 6-wk experiment the subjects showed a generally good compliance with the diets as

judged from the stability of their body weights and from the observed changes in the level of total serum cholesterol. Differences in body weight between the high- and low-P:S periods were not significant; the average changes ($\bar{x} \pm$ SD) amounted to 0.43 ± 0.84 kg in the HA group and −0.28 ± 0.54 kg in the HY group. Changing from the low-P:S to the high-P:S diet in the HA group caused the serum cholesterol level to decrease from 6.26 ± 1.13 to 5.34 ± 0.96 mmol/L whereas the reverse dietary manipulation in the HY group caused an increase of serum cholesterol from 4.43 ± 0.72 to 5.53 ± 0.96 mmol/L.

Individual adherence to the diets was further estimated by inquiring about possible problems when our dietitians visited the subjects three times a week (HA group) or when the authors shared lunch with them on weekdays (HY group). The data from one HY subject who, in the authors' opinion, did not comply well to the diets were left out. The poor compliance was confirmed by a relatively small change in cholesteryl ester L:O.

From these observations we conclude that for all subjects considered the level of adherence to the experimental diets can be rated as excellent. This allows a meaningful comparison of fatty acid composition of serum cholesteryl esters and erythrocyte membranes as objective markers to assess dietary adherence.

Fatty acid composition of serum cholesteryl esters and erythrocyte membranes

Alterations in the fatty acid composition of both cholesteryl esters and erythrocyte membranes, as induced by changing of the P:S of the diet, were predominantly seen in the linoleic and oleic acid content (**Table 3**). Because the contents of these fatty acids always change in opposite direction of each other, the diet-induced changes are best expressed by the L:O. Together these fatty acids made up 75–80% of the fatty acids in cholesteryl esters and 22–24% of those in erythrocyte membranes.

Analysis of two blood samples taken at a 3-d interval at the end of both periods revealed that in both groups the L:O of cholesteryl esters and of erythrocyte membranes did not change systematically over the last 3 d of each dietary period (data not shown). Therefore, we could use these data to calculate the CVs of the L:O within and between persons (**Table 4**). The observed within-persons variance in L:O of both indices appears of magnitude similar to that of laboratory error, indicating that the true within-person variance is comparatively small. Thus, for a constant diet, the fatty acid composition of serum cholesteryl esters and erythrocyte membranes within one person remain rather stable over a few days and a single blood sample will suffice.

In both blood constituents the observed variability of the L:O was caused almost entirely by between-person variation (Table 4). For serum cholesteryl esters the between-person CV was 1.5-fold higher than that for erythrocyte membranes.

For all subjects taken together the L:O of the cholesteryl esters was positively correlated with that of the erythrocyte membranes both on the low-P:S diet ($r = 0.65$, $n = 32$; $p < 0.01$) and on the high-P:S diet ($r = 0.55$, $n = 32$; $p < 0.01$).

Diet-induced changes in the linoleic-oleic acid ratio of cholesteryl esters and erythrocyte membranes

Changing the diet from low to high P:S in the HA group caused the L:O of serum cholesteryl esters (Table 3) to increase by 2.64 ± 0.71 whereas the reverse dietary manipulation in the HY group caused this ratio to decrease by 2.97 ± 0.68. In the erythrocyte membranes (Table 3) the L:O increased by 0.228 ± 0.073 in the HA group and decreased by 0.193 ± 0.048 in the HY group. Thus, for both blood components the absolute magnitude of the observed mean changes did not depend on the order in which the diets were provided.

When expressed as a percentage of the L:O on the low-P:S diet, the diet-induced changes in cholesteryl esters amounted to 91.3 ± 25.9% for the HA group and 85.1 ± 18.8% for the HY group. The change in erythrocyte membrane L:O was 33.0 ± 7.9% for the HA group ($n = 18$ valid observations) and 22.8 ± 4.9% for the HY group ($n = 14$ valid observations). Note again that the two groups received the diets in reverse order. The fact that these relative changes in erythrocytes are not of similar magnitude is caused by a higher L:O ratio on the low-P:S diet in the HY group than in the HA group (Table 3). From the above data it appears that in both groups of subjects the three- to fourfold larger mean relative changes observed in the cholesteryl esters than in the erythrocyte membranes are counterbalanced by a similarly larger variability between subjects so that there is little difference between the two indices in the precision with which these changes are estimated.

When the individual changes observed in the L:Os of cholesteryl esters and erythrocyte membranes are compared in a scattergram (**Fig 1**), it is also clear that for all subjects both indices change markedly and in the same direction. In addition, for the HA group the absolute increase in the L:O of cholesteryl esters was positively correlated ($r = 0.76$, $n = 18$; $p < 0.01$) with that of erythrocyte membranes. Such a correlation was not seen with the data from the HY group; an explanation for this is lacking.

The interindividual differences in diet-induced changes in L:O (Fig 1) were not associated with differences in the response of serum cholesterol to the dietary manipulation; the correlation coefficients were −0.10 (HA group, $n = 24$) and 0.18 (HY group, $n = 23$) for cholesteryl esters and −0.06 (HA group, $n = 18$) and 0.09 (HY group, $n = 14$) for erythrocyte membranes.

Discussion

The average serum cholesterol responses to manipulation of the dietary fatty acid composition of −0.92 mmol/L in the HA group and of +1.10 mmol/L in the HY group are quite similar to those found by other investigators but lower than the value of 1.40 mmol/L that would be predicted by the equation of Keys et al (29).

TABLE 3
Fatty acid composition of serum cholesteryl esters and erythrocyte membranes in healthy volunteers on diets low or high in linoleic acid (g/100 g fatty acid methyl esters)*

	HA group		HY group	
Fatty acid	Low-P:S diet	High-P:S diet	Low-P:S diet	High-P:S diet
Cholesteryl esters†				
14:0	1.0 ± 0.3	0.5 ± 0.2	1.1 ± 0.2	0.5 ± 0.1
16:0	11.7 ± 0.1	9.5 ± 0.9	10.9 ± 0.5	8.9 ± 0.6
16:1	3.4 ± 1.6	1.5 ± 1.1	2.9 ± 0.8	1.2 ± 0.5
18:0	1.0 ± 0.3	0.8 ± 0.1	1.0 ± 0.1	0.9 ± 0.2
18:1	19.5 ± 2.0	12.6 ± 1.7‡	16.8 ± 1.3	10.9 ± 1.3‡
18:2	55.2 ± 3.8	68.2 ± 3.8‡	58.3 ± 2.0	69.9 ± 2.6‡
20:4	5.3 ± 1.1	5.2 ± 1.6	5.7 ± 0.8	5.8 ± 1.3
Others	2.6 ± 0.4	1.8 ± 0.3	3.0 ± 0.5	1.9 ± 0.4
18:2/18:1	2.89 ± 0.44	5.53 ± 0.92‡	3.49 ± 0.36	6.46 ± 0.88‡
Erythrocyte membranes§				
14:1‖	1.6 ± 0.2	1.6 ± 0.2	1.3 ± 0.2	1.3 ± 0.2
16:0	21.0 ± 1.0	19.8 ± 1.0	20.4 ± 0.8	19.7 ± 0.9
17:0‖	2.6 ± 0.3	2.7 ± 0.3	2.4 ± 0.2	2.4 ± 0.2
18:0	14.1 ± 0.8	14.1 ± 0.7	14.7 ± 0.6	14.6 ± 0.8
18:1	13.4 ± 1.1	11.8 ± 1.1‡	12.6 ± 0.7	11.8 ± 1.1‡
18:2	9.1 ± 1.0	10.9 ± 1.2‡	10.4 ± 0.8	11.9 ± 1.0‡
20:3	1.5 ± 0.4	1.4 ± 0.4	1.6 ± 0.3	1.5 ± 0.3
20:4	12.9 ± 1.0	13.4 ± 0.9	12.8 ± 0.8	12.6 ± 0.7
22:0	2.4 ± 0.3	2.4 ± 0.3	2.3 ± 0.3	2.3 ± 0.2
22:4	2.6 ± 0.7	2.8 ± 0.6	2.9 ± 0.4	2.9 ± 0.6
22:5	1.9 ± 0.5	2.2 ± 0.9	1.8 ± 0.3	1.9 ± 0.4
22:6	3.5 ± 1.0	3.6 ± 1.0	3.3 ± 0.6	3.6 ± 0.9
24:0¶	5.4 ± 0.5	5.4 ± 0.4	5.6 ± 0.7	5.5 ± 1.0
24:1	5.3 ± 0.7	5.2 ± 0.6	4.7 ± 0.4	4.9 ± 0.5
Others	2.9 ± 0.5	2.8 ± 0.4	3.3 ± 0.4	2.9 ± 0.3
18:2/18:1	0.69 ± 0.12	0.92 ± 0.18‡	0.83 ± 0.09	1.02 ± 0.12‡

* $\bar{x}$ ± SD.
† For HA group, $n = 24$; for HY group, $n = 23$.
‡ Significantly different from the low-P:S diet, $p < 0.01$.
§ For HA group, $n = 18$; for HY group, $n = 14$.
‖ Recent studies (Van Birgelen APJM, Soffers AEMF, unpublished observations, 1988) suggest that the $C_{14:1}$ peak consists largely of $C_{16:0}$ dimethylacetal and the $C_{17:0}$ peak consists largely of $C_{18:0}$ dimethylacetal.
¶ Also contains 20:5.

However, the equation of Keys et al may not be valid for high levels of polyunsaturated fatty acids such as those applied in our experiment (30).

The individual serum cholesterol response was not correlated with the extent of the individual change in L:O in erythrocytes or cholesteryl esters. This could suggest that the observed variability in the response of the L:O is not due to differences in dietary adherence but relates to interindividual differences in linoleate metabolism.

TABLE 4
Variability of the linoleic-oleic acid ratio in serum cholesteryl esters and erythrocyte membranes in healthy volunteers on controlled diets low or high in linoleic acid

		Coefficient of variation (%)	
	Diet	Serum cholesteryl esters	Erythrocyte membranes
Within persons	Low P:S	3.7	2.3
	High P:S	2.7	2.2
Between persons	Low P:S	15.1	9.8
	High P:S	16.4	11.5

Choice between erythrocytes and cholesteryl esters

Erythrocyte and cholesteryl ester L:O were found to differ little in their precision for estimating a change in the linoleic acid intake of individual subjects. The observed relative changes in L:O in cholesteryl esters were much larger than those in erythrocyte membranes but so was the interindividual variability of these changes. In our study, diet-induced changes were assessed after subjects had subsisted for almost three weeks on each assigned diet. This is sufficient for the fatty acid composition of the cholesteryl esters to equilibrate with the diet because this takes ~1–2 wk (10, 19) but it is not sufficient for erythrocyte membranes, which require 4–6 wk

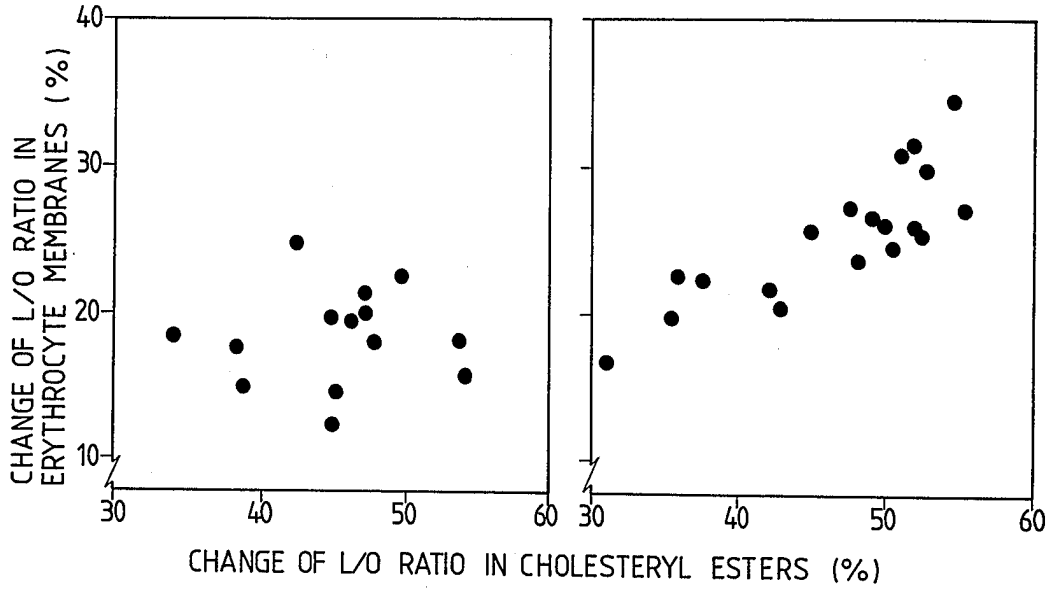

FIG 1. Relationship between individual percentage changes in the linoleic-oleic acid ratio (L:O) of cholesteryl esters and of erythrocyte membranes as induced by changing from a high-P:S diet to a low-P:S diet (HY group, left panel), or vice versa (HA group, right panel).

(9). However, significant changes in the latter do occur after 8 d (9). It is likely that if the diet-induced changes had been measured after a period > 3 wk, the relative changes in L:O would have increased further in erythrocyte membranes but not in cholesteryl esters.

The choice between the use of the L:O of cholesteryl esters and erythrocyte membranes as the index of adherence to diets rich in polyunsaturated fatty acids will depend on several factors. One is the duration of the dietary intervention, erythrocyte membranes possibly being more suitable for studies lasting several months. The ease and reproducibility of the methods for analyzing fatty acid patterns of serum components may be crucial. In our laboratory the combined within- and between-run CVs for the determination of the L:Os were similar, amounting to 3.5% for both cholesteryl esters and erythrocyte membranes. The latter can be assayed by a less elaborate and less expensive method because the erythrocyte lipid extract can be analyzed directly by gas-liquid chromatography without the prior thin-layer chromatography necessary for the isolation of cholesteryl esters. In our hands the overall numbers of samples processed per day was two to three times as high for erythrocyte membranes as for cholesteryl esters. The more complicated fatty acid pattern of erythrocyte membranes did demand a gas chromatographic separation time of 50 min vs 40 min for cholesteryl ester fatty acids but this disadvantage was largely overcome by use of an automatic sampler.

A more serious problem is the oxidation of polyunsaturates in erythrocytes upon storage. Such oxidation might also explain the arachidonic acid levels in normal humans of < 5% reported by some investigators (13, 31). From our experience, the only way to eliminate oxidation is to prepare erythrocytes immediately after blood sampling, hemolyze them by freezing at −20 °C for 2 h, and analyze their lipids within 1 wk. Serum for cholesteryl ester analysis, on the other hand, can be stored until analysis is convenient.

Other comparative data on the monitoring of dietary adherence by use of the L:O of cholesteryl esters and erythrocyte membranes are available from the National Diet-Heart Study (2). Using the combined data from the various centers participating in this study, we calculated that the change in dietary P:S was proportional to the absolute change in L:O of both cholesteryl esters and erythrocyte membranes; each increment of the P:S by one unit causes the L:O ratio of cholesteryl esters to increase by 1.2 and that of erythrocyte membranes by 0.10 (2). The absolute changes per unit P:S found in our study (1.4–1.6 for cholesteryl esters and 0.11–0.13 for erythrocyte membranes) agree quite well with these data. For cholesteryl esters similar changes were reported by Dayton et al (6) and De Gennes et al (18) but others (20, 21, 32) found an absolute change per unit of P:S of only 0.3–0.5. In the Diet-Heart Study (2) the between-subject variation of the relative changes in L:O of cholesteryl esters and erythrocyte membranes were not reported so that we cannot infer from this study which of the two indices is the more precise.

McMurchie et al (33, 34) recently introduced the use of the fatty acid composition of human cheek cell phospholipids as a noninvasive manner for the qualitative estimation of dietary lipid intake. However, the validity of this method needs confirmation because during a 12-wk trial the proportion of linoleic acid in the cheek cell phospholipids responded to a change from a low- to a high-P:S diet but not to the reverse manipulation (34).

Applicability to the monitoring of dietary adherence in individual patients

The results of our study show that the L:O of erythrocyte membranes can be a good alternative for that of se-

rum cholesteryl esters as a biochemical index of adherence of individual subjects to diets rich in polyunsaturated fatty acids. Because the fatty acid composition of erythrocyte membranes when compared with that of cholesteryl esters equilibrates with the fatty acid pattern of the diet at a slower rate and is analytically less demanding, the L:O of erythrocyte membranes should be an attractive index for monitoring compliance to dietary changes over periods on the order of months, provided that analysis is not delayed long enough to allow oxidative damage to take place.

However, will the erythrocyte L:O be useful to monitor adherence when the change in dietary fatty acids is less extreme than in our study? A hypothetical example will clarify this. Suppose a group of patients is bled for erythrocyte fatty acid analysis and is then switched from a habitual diet with a P:S of 0.2 (eg, 40 en% fat, 20 en% saturates, 4 en% polyunsaturates) to a diet with a P:S of 1.0 (eg, 40 en% fat, 12 en% saturates, 12 en% polyunsaturates). After 1 or 2 mo on the fat-modified diet, red blood cells are collected and analyzed for the second time. The expected percentage change in L:O would then be less than that in our experiment. The variance of the change between patients would also be less if the dietary change is less because in the extreme case of no change in diet, the expected change in L:O ratio would be reduced to 0 with an SD equal to the within-subject SD $\times 2^{0.5}$; with a within-person SD of 2.3 (Table 4) this works out to some 3.3 percentage points. Thus the expected change in L:O would lie anywhere between $0 \pm 3.3\%$ when the diet remains constant and the $33 \pm 7.9\%$ observed in our HA group when the group was switched from a P:S 0.2 to a P:S 2.0 diet. For our hypothetical patients going from a P:S 0.2 to a P:S 1.0 diet, a change in erythrocyte L:O of 16% would appear a reasonable expectation. Such a change would have an SD between patients of $\sim$5 percentage points (*see* Appendix). In that case, 97.5% of all patients should show an increase in L:O of 6% or more, 6% being the mean change minus 2 SDs; any change of $< 6\%$ would raise a suspicion of poor compliance.

Thus our results predict that even for modest changes in dietary fatty acid composition, measuring erythrocyte (or, for that matter, cholesteryl ester) fatty acid composition before and after the change in diet will provide crude but useful information on dietary adherence in individual patients.

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Appendix

Estimation of the standard deviation of the change in linoleic-oleic acid ratio (L:O) in red blood cells for patients going from a diet with P:S 0.2 to a diet with P:S 1.0

The combined biological and laboratory SD of the L:O within patients equalled 2.3% on the low- and 2.2% on the high-P:S diet. (Here and elsewhere % stands for 0.01 × L:O on Low-P:S diet.) Changing the dietary P:S from 0.2 to 2.0 caused a change in L:O of 33 ± 7.9% for the HA group. (For the present calculation using the values of the HY group led to essentially the same conclusions.) The variance of the change still includes the within-subject variance of the two measurements on which the change was based; subtracting these yields the pure SD between subjects:

$$SD_{between} = (7.9^2 - 2.3^2 - 2.2^2)^{0.5} = 7.2 \quad (1)$$

It is now assumed somewhat arbitrarily that changing the dietary P:S from 0.2 to 1.0 instead of 2.0 will produce an average increase in L:O of 16% instead of 33%. The associated SD between subjects will be proportionally less, and will be

$$SD_{between} = 7.2 \times 16/33 = 3.3 \quad (2)$$

The SD between patients as actually measured will again contain the within-subject variance and will be

$$SD_{between} = (3.3^2 + 2.3^2 + 2.2^2)^{0.5} = 4.6 \quad (3)$$

In the Discussion this value was rounded to 5.