Comparison Between Fat Intake Assessed by a 3-Day Food Record and Phospholipid Fatty Acid Composition of Red Blood Cells: Results From the Monitoring of Cardiovascular Disease-Lille Study

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We investigated the relationship between assessment of fatty acid intake by a 3-day food record and by capillary gas chromatography of erythrocyte phospholipid fatty acid. The study was performed in a sample of 244 men aged 45 to 66 years from the general population who were participating in the Monitoring of Cardiovascular Disease (MONICA)-Lille survey. The relationship between each nutrient and food item and erythrocyte phospholipid fatty acid was investigated by a regression model on proportion including each food item and nutrient as a dependent variable and percentage of fatty acid and covariables (nonalcoholic energy intake, age, alcohol intake, and smoking) as independent variables. Polyunsaturated fat and linoleic acid intake were positively correlated with linoleic acid content of erythrocytes ($\beta = 0.641$ and 0.604, respectively, $P < .001$). Monounsaturated and saturated fat intake were correlated with oleic acid ($\beta = 0.375$ and 0.373, respectively, $P < .01$). Fish intake correlated positively with docosahexaenoic acid (DHA) ($\beta = 0.383$, $P < .001$) and negatively with arachidonic acid ($\beta = -0.509$, $P < .01$). These data confirm, on a group level, a good relationship between assessment of polyunsaturated fat intake by a 3-day record and linoleic acid content of erythrocyte membranes. These data suggest that erythrocyte oleic acid content is a marker of both saturated and monounsaturated fat intake.

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ALTHOUGH THERE IS abundant experimental evidence of the influence of diet on cardiovascular risk factors, epidemiologic studies have produced more conflicting results. High levels of fat intake are associated with increased coronary heart disease (CHD) risks.[1,2] The most important descriptive data on diet and CHD were reported by Keys,[1] who showed among seven countries that intake of saturated fat as a percentage of calories was strongly correlated with CHD death rates. If a high intake of saturated fatty acids is now considered a positive risk factor for CHDs, the role of unsaturated fatty acids is less clear. For instance, whereas some studies found a favorable influence of monounsaturated fatty acids on CHD risk, others suggest that higher levels of monounsaturated fatty acids are associated with higher CHD morbidity in young male cohorts.

These discrepancies might be explained, among other reasons, by the inaccuracy in assessing fatty acid intake due to two main limitations: the shortcomings of food composition tables and the difficulty in evaluating true fat intake because of wide intraindividual variation. It has been calculated that to rank subjects with a high degree of accuracy, a 15-day study of polyunsaturated fat intake is necessary.[3] In epidemiologic studies involving several hundred subjects, the number of recording days is limited. In the Monitoring of Cardiovascular Disease (MONICA) survey, a 3-day record was chosen as a compromise to maintain a high participation rate,[4] but it was necessary to validate the results against a reference method, especially for fat intake.

Specific fatty acid levels in blood cell membranes and subcutaneous fat have been proposed as indicators of dietary fat intake. It has been shown that adipose tissue fatty acid content is a good marker of long-term intake of linoleic acid,[5] but it requires aspirating fat, which is hardly acceptable in field studies. Red blood cells lack the capacity for de novo fatty acid synthesis or modification by desaturation or elongation. They are thus a good reflection of midterm fat intake: it takes 4 to 6 weeks to equilibrate erythrocyte fatty acid content with the diet,[6] but changes in the dietary polyunsaturated to saturated fat ratio (P/S) induce significant changes in the linoleic to oleic acid ratio in erythrocyte membranes after 8 days.[7] Dougherty et al,[8] in a transcultural study conducted among 40- to 45-year-old men, found that fatty acid patterns in erythrocyte phospholipids were in accordance with the type of fat consumed as assessed by a 7-day record validated by duplicate portion analysis. Glatz et al,[7] in an experimental study, gave a mixed diet with either a low or high P/S for 3 weeks and found that linoleic to oleic acid ratio changes in erythrocytes were proportional to absolute changes in the P/S in the diet. Hill et al[9] observed among 10 male subjects given either a high saturated or high polyunsaturated fat diet for 16 days that subjects on polyunsaturated fat diets had higher levels of linoleic acid and lower levels of oleic acid. Stanford et al,[10] in a pilot cross-sectional study conducted among 15 women, found a high correlation coefficient (.8) between oleic and linoleic acid intake as assessed by a 4-day record and erythrocyte content of these fatty acids. It appears that, at least for linoleic acid and the P/S, erythrocytes seem to be a good indicator and reflect the type of fat consumed by different populations in international comparisons.

Few studies exist for comparison on a large scale between fat intake as assessed by a 3-day food record and fatty acid composition of erythrocytes. The aim of this study was first

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to confirm that polyunsaturated fat intake evaluated by a 3-day record was correlated with polyunsaturated fat content of phospholipid erythrocytes, the latter considered a good marker of polyunsaturated fat intake; and second, to identify if other fatty acids such as oleic acid and n-3 fatty acids could potentially be useful markers of fat intake.

SUBJECTS AND METHODS

Population and Study Design

Data for this study were collected during the first MONICA population survey conducted from June 1986 to February 1989 in the urban community of Lille, France.

According to recommendations on nutritional studies of the MONICA Project provided by a Concerted Action Project on Nutrition in the European Community (EURONUT) Workshop in 1984,[4] a subsample of men aged 45 to 64 years was selected for the nutritional survey. The sample was obtained by a multistage random-sampling method using the 1982 national population census. The group was representative of the population of the same age with respect to socioeconomic status. Five hundred seventy-six were selected, and 389 accepted (participation rate, 68%). Of 389 initial participants, 88 men were excluded, 39 because they were taking hypolipidemic drugs and 49 because of missing data. Fifty-seven samples with an arachidonic acid content less than 10%, indicating oxidation of polyunsaturated fatty acids during storage,[7] were eliminated. Therefore, final analysis was performed on 244 subjects.

Participants were seen at home and submitted to a standard interview and physical measurements as recommended in the MONICA Manual.[11] The interview, which was conducted by specially trained personnel, provided information on medical history, socioeconomic variables, and smoking habits. Height and body weight were measured on subjects in ordinary indoor clothing with jacket and shoes removed. Body mass index was calculated according to the Quetelet equation (weight divided by height squared expressed as kilograms and meters, respectively). During the examination, a fasting blood sample was drawn, and an extensive set of biochemical measurements were performed, including determination of the phospholipid fatty acid composition of red blood cells. Subjects participating in the dietary survey were given oral and written instructions on how and when to complete a 3-day food intake record.

Dietary Records

Dietary intake was assessed by a 3-day record. Each subject was asked to estimate the amount of food and beverage consumed over a period of 3 days. The start of the record was arranged so that each day of the week was adequately represented. When the record was completed, a home visit was made by a trained dietician to check the information recorded and to quantify household measures and portion sizes. When necessary, portion size photographs were used.

Nutrient intake was calculated using a food composition table based on the table described by Renaud and Attie[12] completed by other sources (manufacturer and laboratory).

A double validation was conducted. The first was a validation of portion size quantification among 21 subjects. They conducted a 3-day precise-weighing survey after the 3-day record. In comparison to the weighing record, the 3-day record showed an insignificant overestimation of fatty acids. Saturated and monounsaturated fatty acids were overestimated by 1% and 2%, respectively. Polyunsaturated and linoleic fatty acids were overestimated by 17%.

The second was a validation of the food composition table among 30 subjects.[13] For these subjects, duplicate portions of all food consumed during 1 day were collected, weighed, and homogenized. Aliquots were taken, frozen, and sent to the laboratory for chemical analysis. Comparison of the weighed record to chemical analysis showed a significant overestimation of saturated fatty acids of 15%. Monounsaturated fatty acids were overestimated by 4% (nonsignificant). Polyunsaturated and linoleic fatty acids were not different (0%).

Fatty Acid Analysis

Five milliliters of blood was centrifuged at 2,000 rpm for 10 minutes to isolate red blood cells within 2 hours after sampling. The cell pellet was washed three times with 10 mL phosphate buffer containing 0.1 mol/L Na_2HPO_4 and 0.1 mol/L NaH_2PO_4 (5/1 vol/vol), and then homogenized in buffer diluted twofold. After extraction and methylation as described previously,[14] methyl esters of the fatty acids were kept under nitrogen atmosphere before being analyzed by gas-liquid chromatography. In these conditions, there is no peroxidation during storage; stability of methyl esters was checked, and over 8 months' storage there was no change in the ratio of 20:4n-6 to 20:2n-6 (0.6 *v* 0.5).

Statistical Analysis

Independent associations between each of the phospholipid fatty acids of red blood cells and the other relevant variables cannot be investigated simply by linear regression of these variables on the set of fatty acids, since the latter are expressed as percentages, which clearly constrains their range and sum.

A mathematical model has been reported by Aitchison and Bacon-Shone[15] to circumvent this problem. According to this model, regression of a variable y on a proportion (eg, x1, x2, and x3) can be expressed as $y = b0 + b1 \log(x1/x3) + b2 \log(x2/x3)$. The partial association between a dependent variable and a particular proportion is assessed by maintaining constant the subcomposition from which the particular proportion is removed.

This model was used to analyze data of the present study. With the analytic procedure used, 21 fatty acids were identified and expressed as percentages. In the present analysis, we have studied the relationship between fat intake and eight of these fatty acids. (When one of these fatty acids was undetected in a particular subject, a conventional level of 0.01% was fixed to allow log-transformation.) The remaining fatty acids (21.4% of total) were used as the denominator to compute log ratios, as in the earlier equation, for each fatty acid. Multiple regression analysis of each fat intake and food items on the transformed percentages was performed using a linear regression model.[16]

The value of the standardized regression coefficient indicates the absolute change of the dependent variable corresponding to a change of one standard deviation of the transformed percentage. Since distributions of the log-transformed percentages are close to normality, it is then possible to compare the magnitude of the mean change of the dependent variables associated with a common unit of change of each transformed fatty acid.

Adjustment on covariates (age, nonalcoholic energy intake, alcohol intake, and smoking consumption) was performed by introducing the covariates and transformed proportions into the regression models.

These analyses have two stages: an overall test considering the contribution of all fatty acids together and a second stage, performed if the first test is significant, which tests the contribution of each fatty acid. Since in this second stage many tests are generated, P values were calculated with the Bonferroni adjustment.

Table 1. Age, Body Mass Index, Alcohol and Smoking Consumption, and Nonalcoholic Energy Intake Among 244 Men Aged 45 to 64 Years

Characteristic	Mean ± SD
Age (yr)	56.1 ± 5.9
Body mass index (kg/m²)	26.5 ± 3.5
Alcohol consumption (g/d)	33.3 ± 28.7
Smoking consumption (g/d)	7.5 ± 11.4
Nonalcoholic energy intake (MJ/d)	8.2 ± 0.21

RESULTS

The main characteristics of the study population are listed in Table 1. Mean daily fat intake and mean daily consumption of various food items are listed in Tables 2 and 3, respectively. Percentage composition of fatty acids of red blood cell phospholipids is listed in Table 4.

There was no significant association between age, body mass index, smoking consumption, and phospholipid erythrocyte fatty acids. The estimate of alcohol consumption obtained by the 3-day record is positively associated with 16:1n-7 ($P < .05$ with Bonferroni's adjustment).

Fat Intake

Polyunsaturated and linoleic fatty acids expressed as a percentage of total fat intake or as grams per day were strongly correlated with erythrocyte linoleic acid. On the other hand, polyunsaturated and linoleic fatty acids expressed as a percentage of total fat intake were negatively correlated with erythrocyte oleic acid. Monounsaturated and saturated fat intakes (grams per day) were positively correlated with oleic acid. Moreover, saturated fat intake (as a percentage of total fat) was negatively correlated with erythrocyte linoleic acid. P/S was positively correlated with the linoleic to oleic acid ratio ($r = .32, P = .0001$; Table 5).

Percentages of 18:2n-6 and 18:1n-9 in quintiles of intake of polyunsaturated, monosaturated, and saturated fats are presented in Fig 1, which shows that whereas the correlation between 18:2n-6 and polyunsaturated fat holds at the entire range of intake, it is not true at the lowest quintile for 18:1n-9.

Table 2. Fat Intake Assessed by a 3-Day Record Among 244 Men Aged 45 to 64 Years

Fat Type	Mean ± SD
Total	
g/d	92.4 ± 29.6
% nonalcoholic energy	42.0 ± 6.8
Polyunsaturated	
g/d	13.9 ± 7.9
% of total fat	15.0 ± 6.5
Linoleic acid	
g/d	11.9 ± 7.4
% of total fat	12.9 ± 6.4
Monounsaturated	
g/d	31.8 ± 10.9
% of total fat	34.4 ± 4.2
Saturated	
g/d	38.9 ± 14.4
% of total fat	41.8 ± 6.5
P/S	0.39 ± 0.24

Table 3. Daily Consumption of Various Food Items by a 3-Day Record Among 244 Men Aged 45 to 64 Years

Food Item	Mean ± SD (g/d)
Meat	173.9 ± 79.2
Fish	30.1 ± 43.2
Cheese	40.2 ± 30.0
Cooking fat and spreads	
Butter	20.3 ± 16.4
Margarine	10.4 ± 11.7
Sunflower oil	6.3 ± 9.3
Peanut oil	5.1 ± 7.4
Olive oil	0.4 ± 1.7

When correction factors from the results of validation of the nutritional study are applied, the results remain unchanged.

Food Items

Fish intake was correlated positively with docosahexaenoic acid ([DHA] 22:6n-3) and negatively with arachidonic acid. There was no significant association between meat and cheese intake and phospholipid erythrocyte fatty acids. Butter intake was positively correlated with oleic acid. There was no significant association between intake of the other cooking fats or spreads and phospholipid erythrocyte fatty acids (Table 6).

DISCUSSION

Our study confirms in free-living subjects that a 3-day record of food intake is sufficient on a group level to detect the correlation between linoleic acid intake and linoleic acid content of erythrocyte phospholipids demonstrated either in experimental studies[7,9] or in epidemiologic studies conducted with a 7-day record.[8]

We found that both saturated and monounsaturated fat were positively correlated with oleic acid. These correlations were weaker that those seen with linoleic acid. This could be explained by the fact that oleic acid is not specific; it could be considered a marker of both saturated and monounsaturated fat intake because stearic acid, which provides 23% of Western saturated acid intake,[17] is rapidly converted to oleic acid.[18-19] Furthermore, these correlations do not hold for the entire range of fat intake—the lowest quintile of intake is in both cases clearly not related to erythrocyte content. This is probably due to an increased fat

Table 4. Percentage Composition of Red Blood Cell Phospholipid Fatty Acids Among 244 Men Aged 45 to 64 Years

Phospholipid Fatty Acid	Mean ± SD (%)
Palmitic (C16:0)	23.73 ± 3.24
Palmitoleic (C16:1n-7)	1.53 ± 1.59
Stearic (C18:0)	11.51 ± 3.02
Oleic (C18:1n-9)	13.40 ± 2.15
Linoleic (C18:2n-6)	9.72 ± 2.88
Arachidonic (C20:4n-6)	14.00 ± 2.41
EPA (C20:5n-3)	0.74 ± 0.50
DHA (C22:6n-3)	3.98 ± 1.59
Linoleic/oleic ratio	0.73 ± 0.22

Table 5. Multiple Regression Analysis of Fat Intake Assessed by a 3-Day Record of Red Blood Cell Phospholipid Fatty Acids Among 301 Men Aged 45 to 64 Years

Phospholipid Fatty Acid	Polyunsaturated		Linoleic Acid		Monounsaturated		Saturated	
	% of Fat	g/d	% of Fat	g/d	% of Fat	g/d	% of Fat	g/d
Palmitic (C16:0)	+.143	−.030	+.088	−.078	−.098	−.230	−.030	−.212
Palmitoleic (C16:1n-7)	−.116	−.106	−.109	−.106	+.043	−.037	+.109	−.005
Stearic (C18:0)	+.175	+.130	+.195	+.154	−.156	−.036	−.084	−.019
Oleic (C18:1n-9)	−.712*	−.308	−.630*	−.262	+.349	+.388*	+.499†	+.467*
Linoleic (C18:2n-6)	+.660*	+.432*	+.636*	+.444*	−.234	−.133	−.537*	−.263†
Arachidonic (C20:4n-6)	+.031	+.048	+.058	+.077	+.077	+.003	+.090	−.052
EPA (C20:5n-3)	−.196‡	−.089	−.211‡	−.112	+.016	+.049	+.156	+.121‡
DHA (C22:6n-3)	−.018	−.081	−.083	−.146	+.004	−.016	+.020	−.040

NOTE. Values are standardized multiple regression coefficients of the dependent variables on the logit of the proportions of fatty acids, adjusted for age, nonalcoholic energy, and alcohol and smoking consumption.

*$P < .001$.

†$P < .01$.

‡$P < .05$.

synthesis in the presence of a low level of fat intake,[20] which maintains a relatively stable membrane composition. Few studies examined the relationship between saturated fat intake and oleic acid content of biologic samples. Lopez et al,[21] in studying plasma fatty acids, found a positive relationship for men and a negative one for women. For platelets, Renaud et al[22] found a relationship between 16:1n-9 and saturated fat intake. The fact that oleic acid could be a marker of saturated fat intake, especially when the latter is high, would explain the apparently surprising findings of Tavendale et al[23] and Wood et al[24] in Scotland. The first group found that oleic content of adipose tissue was highly correlated with the district-standardized mortality ratio for CHD, and in the latter study, adipose tissue and platelet levels of oleic acid were higher in subjects with angina pectoris and infarction than in controls.

The use of raw data (food items) provides an interesting insight into our results. Fish intake is correlated, as expected, with DHA (22:6n-3) but not with eicosapentaenoic acid (EPA) (20:5n-3). It has been suggested that adipose tissue EPA was a reasonable marker of even moderate fish intake.[25] Katan et al,[26] in a supplementation study, found that the amount of EPA supplemented was reflected in the proportion of EPA in erythrocytes, but they did not study DHA. Bjerve et al[27] observed a positive correlation between an increasing number of fish meals and plasma concentrations of EPA and DHA of serum phospholipids. An interesting finding is the negative correlation of fish intake with arachidonic acid, the same phenomenon observed by Houwelingen et al[28] in serum phospholipids. This fact is in accordance with other findings. Eskimos who have a diet high in n-3 fatty acids[29] have low lipid levels of arachidonic acid,[30] and in experimental studies, feeding-increased levels of n-3 fatty acids decrease arachidonic acid content of membrane phospholipids in man[31] and animals.[32-33] This effect is more important when fish oil is associated with high levels of dietary saturated fatty acids.[34]

When investigating relationships between fat intake and fatty acids used as biomarkers, we have to take into account three kinds of facts in the interpretation of data.

1. In vivo metabolism of fatty acids. Membrane fatty acid composition is determined by the interplay between available fatty acids from a dietary source and further metabolism of these fatty acids, such as conversion of stearic acid to oleic acid and competition between n-6 and n-3. Metabolic interaction also occurs with ethanol: we found that ethanol intake was correlated with 16:1n-7, and this confirms results from other studies in platelets,[22] cholesteryl esters,[35] and triglycerides,[21] suggesting that ethanol has to be taken into account in the interpretation of data.

2. Technical difficulties arising from assays of fatty acids based on the quantification of chromatographic peaks, which is difficult in the case of small peaks such as EPA and DHA, leading to a higher variability. Less reliable results may be expected for other reasons, such as oxidation of polyunsaturated fatty acids upon storage. Stanford et al[10] repeated fatty acid determinations after 12 months' storage at −70°C among 15 healthy women. They found for the majority of fatty acids, except 20:1(n-9) and 24:1(n-9), a correlation between baseline and 12-month measurements; however, they did not show individual values. In adipose tissue, Deslypere et al[36] showed the stability of seven n-3 polyunsaturated fatty acids over a period of 7 months. However, blood contamination of the samples resulted in

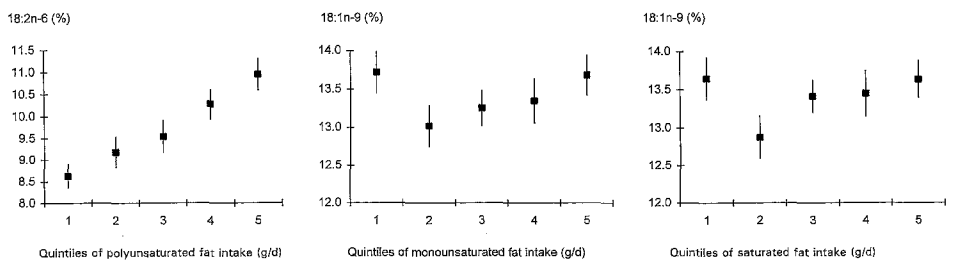

Fig 1. Percentage of 18:2n-6 and 18:1n-9 of red blood cells in quintiles of polyunsaturated, monounsaturated, and saturated fat intake among 244 samples (mean and 95% confidence interval).

Table 6. Multiple Regression Analysis of Various Food Items (g/d) Assessed by a 3-Day Record of Red Blood Cell Phospholipid Fatty Acids Among 301 Men Aged 45 to 64 Years

Phospholipid Fatty Acid	Butter	Vegetable Fat	Sunflower Oil	Peanut Oil	Meat	Fish	Cheese
Palmitic (C16:0)	−.178	−.107	+.091	−.220	−.051	+.255	−.128
Palmitoleic (C16:1n-7)	+.030	+.039	−.139	+.065	−.025	−.029	+.027
Stearic (C18:0)	−.050	+.072	+.114	−.155	−.050	+.001	+.069
Oleic (C18:1n-9)	+.517†	−.227	−.247	+.355	−.039	−.173	+.279
Linoleic (C18:2n-6)	−.345*	+.337	+.280	−.086	+.159	−.072	−.199
Arachidonic (C20:4n-6)	+.029	−.019	+.079	+.078	+.073	−.184	−.019
EPA (C20:5n-3)	+.089	+.031	−.149	−.096	−.104	−.217*	+.055
DHA (C22:6n-3)	−.095	−.047	−.070	−.066	+.022	+.319*	−.101

NOTE. Values are standardized multiple regression coefficients of the dependent variables on the logit of the proportions of fatty acids, adjusted for age, nonalcoholic energy, and alcohol and smoking consumption.

*$P < .05$.

†$P < .01$.

higher coefficients of variation. In fact, oxidation of polyunsaturated fatty acids upon storage is promoted by hemoglobin in some hemolysates, whereas others are not affected.[37] The only way to prevent oxidation is to prepare erythrocytes immediately after blood sampling, which is sometimes difficult in epidemiologic studies.

This was performed within 2 hours in our study. There are a great number of factors that may influence oxidation of blood fatty acids (whether this auto-oxidation of fatty acids happens in vivo or in vitro remains to be proven). With increasing age, the range of susceptibility to auto-oxidation is wider,[38] antioxidant status influences the susceptibility to oxidation of erythrocyte lipids,[38] and it has been found that the serum concentration of selenium was directly associated with the percentage of essential fatty acids in plasma phospholipids.[39] Susceptibility to auto-oxidation is also increased by increasing polyunsaturated fatty acids in the diet.[40] Thus, in patients with low antioxidant status, erythrocyte fatty acids may not reflect food intake. We have not studied the antioxidant status of our patients. We have chosen to eliminate samples with an arachidonic acid level less than 10%, first because, when they had validated erythrocyte phospholipids as a marker of linoleic acid intake, Glatz et al[7] had eliminated samples with an arachidonic acid content less than 10% because they hypothesized on the basis of previous studies[37] that in these samples polyunsaturated fatty acids had been oxidized. Since the first aim of our study was to validate the 3-day record used in the MONICA study with a biomarker of food intake, we had to use this biomarker in the conditions of the validation study. We have been secondarily confirmed in this attitude when we have looked at the percentages of 18:2n-6 and 18:1n-9 in quintiles of intakes of polyunsaturated fat among these 57 subjects (Fig 2). The figure clearly shows that the relationship between intake and erythrocyte content of linoleic acid is not found in this population. We compared 57 subjects with low linoleic and arachidonic acid erythrocyte content with the rest of the population. They did not differ with regard to smoking status, alcohol intake, or vegetable, fish, and fruit intake. Despite their lower erythrocyte linoleic acid content (7.9% *v* 9.7%, $P < .0001$), they had a marginally higher linoleic acid intake (13.1 *v* 11.9 g, $P = .08$). If we analyze our results including these 57 samples, we do not find the negative correlation between fish intake and arachidonic acid. The other results are similar.

3. Uncertainties of food composition tables relating to some fatty acids, especially n-3. We can only relate markers to intake of their known food sources, such as fish. However, fish is not the only source of n-3. Houwelingen et al[41] found that in volunteers reporting no fish consumption, the amount of n-3 fatty acids of serum lipids varied widely, probably due to hidden sources of n-3 such as chicken, which are often fed fish-meal diets.

Our study confirms that a 3-day record of food intake may provide a good reflection of linoleic acid and P/S intake. It suggests that erythrocyte DHA might be a good marker of fish intake, but this latter point needs further study. It emphasizes that among some subjects, may be those with low antioxidant status, erythrocyte phospholipids could not be a good marker of fat intake. This point was beyond the scope of this study, but it has to be taken into account in studies involving subjects, such as cancer patients, known to have a low antioxidant status.[42]

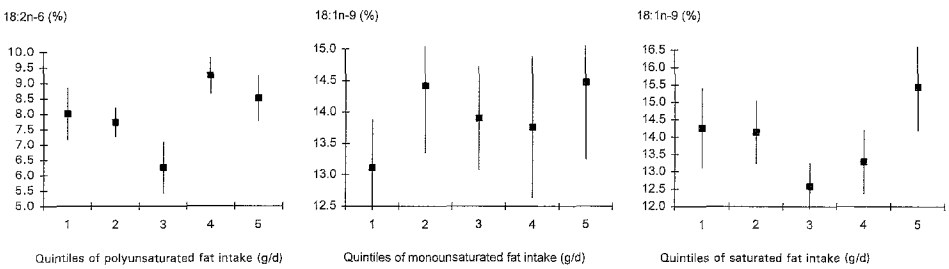

Fig 2. Percentage of 18:2n-6 and 18:1n-9 of red blood cells in quintiles of polyunsaturated, monounsaturated, and saturated fat intake among 57 samples with arachidonic acid content less than 10% (mean and 95% confidence interval).

REFERENCES

1. Keys A: Seven Countries: A Multivariate Analysis of Death and Coronary Heart Disease. Cambridge, MA, Harvard University Press, 1980, p 252
2. Stallones RA: Ischaemic heart disease and lipids in blood and diet. Annu Rev Nutr 3:155-158, 1983
3. Nelson M, Block AE, Morris JA, et al: Between and within subject variation in nutrient intake from infancy to old age: Estimating the number of days required to rank dietary intakes with desired precision. Am J Clin Nutr 50:155-167, 1989
4. Knutman JT, Pietinen P, Debacker GG, et al: The MONICA-Project: Optional Study on the Surveillance of the Dietary Intake of the Population With Regard to Cardiovascular Diseases. EURO-NUT Report, Wageningen, 1985
5. Dayton S, Hashimoto S, Dixon W, et al: Composition of lipids in human serum and adipose tissue during prolonged feeding of a diet high in unsaturated fat. J Lipid Res 7:103-111, 1966
6. Farquhar JW, Ahrens EH: Effects of dietary fats on human erythrocyte fatty acid patterns. J Clin Invest 42:675-685, 1963
7. Glatz JFC, Soffers AEMF, Katan MB: Fatty acid composition of serum cholesteryl esters and erythrocyte membranes as indicators of linoleic acid intake in man. Am J Clin Nutr 49:269-276, 1989
8. Dougherty RM, Galli C, Ferro-Luzzi A, et al: Lipid and phospholipid fatty acid composition of plasma red blood cells and platelets and how they are affected by dietary lipids: A study of normal subjects from Italy, Finland and the USA. Am J Clin Nutr 4:443-455, 1987
9. Hill JG, Kuksis A, Bevreridge JMF: The effect of diet on the phospholipid composition of the red blood cells in man. J Am Oil Chem Soc 42:137-141, 1965
10. Stanford JL, King I, Kristal AR: Long-term storage of red blood cells and correlations between red cell and dietary fatty acids: Results from a pilot study. Nutr Cancer 16:183-188, 1991
11. World Health Organization: MONICA Manual/CVD/MNC/ Version 1.1. Geneva, Switzerland, WHO, 1987
12. Renaud S, Attie MC: La composition des Aliments. Paris, France, Astra-Clave Information, 1986
13. Cournot MP, Nicaud V, Romon M, et al: Comparaison des résultats obtenus par pesée et par analyse chimique de repas dupliqués en cours d'enquêtes alimentaires dans trois régions françaises. Cah Nutr Diet 28:286-292, 1993
14. Theret H, Bard JM, Nuttens MC, et al: The relationship between the phospholipid fatty acid composition of red blood cells, plasma lipids and apolipoproteins. Metabolism 4:562-568, 1993
15. Aitchison J, Bacon-Shone J: Log contrast models for experiments with mixtures. Biometrika 71:323-330, 1984
16. SAS Institute: SAS User's Guide: Statistics (ed 5). Cary, NC, SAS Institute, 1985, pp 1-956
17. Bull ML, Day JL, Burt R, et al: Individual fatty acids in the British household food supply. Hum Nutr Appl Nutr 371:373-377, 1983
18. Elovson J: Immediate fate of albumin bound (1-^{14}C) stearic acid and following its intraportal injection into carbohydrate refed rats. Early course of desaturation and esterification. Biochim Biophys Acta 106:480-484, 1965
19. Bonamone A, Bennett M, Grundy SM: Metabolic effects of dietary stearic acid in mice: Changes in the fatty acid composition of triglycerides and phospholipids in various tissues. Atherosclerosis 94:119-127, 1992
20. Herzberg GR: The 1990 Borden Award Lecture: Dietary regulation of fatty acid and triglyceride metabolism. Can J Physiol Pharmacol 60:1637-1647, 1991
21. Lopez SM, Trembo SL, Mascioli E, et al: Human plasma fatty acid variations and how they are related to dietary intake. Am J Clin Nutr 53:628-637, 1991
22. Renaud S, De Backer G, Thevenon C, et al: Platelet fatty acids and function in two distinct regions of Belgium: Relationship to age and dietary habits. J Int Med 229:79-88, 1991
23. Tavendale R, Lee AJ, Smith CS, et al: Adipose tissue fatty acids in Scottish men and women: Results from the Scottish Heart Health Study. Atherosclerosis 94:161-165, 1992
24. Wood DA, Riemersma RA, Butler S, et al: Linoleic acid eicosapentaenoic acids in adipose tissue and platelets and risk of coronary heart disease. Lancet 1:177-182, 1987
25. Hunter DJ, Rinn EB, Sacks FM, et al: Comparison of measures of fatty acid intake by subcutaneous fat aspirate, food frequency questionnaire, and diet records in a free-living population of US men. Am J Epidemiol 135:418-427, 1992
26. Katan MB, van Birgelen A, Deslypere JP, et al: Biological markers of dietary intake, with emphasis on fatty acids. Ann Nutr Metab 35:249-252, 1991
27. Bjerve KS, Brubakk AM, Fougner KS, et al: Omega 3 fatty acids: Essential fatty acids with important biological effects and serum phospholipids fatty acids as markers of dietary ω3 fatty acid intake. Am J Clin Nutr 57:801S-806S, 1993 (suppl)
28. Houwelingen AC, Hornstra G, Kromhout D, et al: Habitual fish consumption, fatty acids of serum phospholipids and platelet function. Atherosclerosis 75:157-165, 1989
29. Bang HO, Dyerberg J, Sinclair HM: The composition of the Eskimo food in north western Greenland. Am J Clin Nutr 33:657-661, 1980
30. Gibson RA, Sinclair AJ: Are Eskimos obligate carnivores? Lancet 1:1100, 1981 (letter)
31. Wander RC, Patton BD: Comparison of three species of fish consumed as part of a Western diet: Effects on platelet fatty acids and function, hemostasis and production of thromboxane.[1-3] Am J Clin Nutr 54:326-333, 1991
32. Venkatraman JT, Jaohey T, Clandinum MT: Does a threshold for the effect of dietary omega 3 fatty acids on the fatty acid composition of nuclear envelope phospholipids exist? Lipids 27:94-97, 1992
33. Rubin D, Laposata M: Cellular interactions between n-6 and n-3 fatty acids: A mass analysis of fatty acid elongation/ desaturation, distribution among complex lipids, and conversion to eicosanoids. J Lipid Res 33:1431-1440, 1992
34. Garg ML, Wierzbicki AA, Thomson ABR, et al: Fish oil reduces cholesterol and arachidonic acid content more efficiently in rats fed diets containing low linoleic acid to saturated fatty acid ratios. Biochim Biophys Acta 962:337-344, 1988
35. Cambien F, Warnet JM, Verrier V, et al: An epidemiologic appraisal of the associations between the fatty acid esterifying serum cholesterol and some cardiovascular risk factors in middle aged men. Am J Epidemiol 127:75-86, 1988
36. Deslypere JP, van de Bovenkamp P, Harryvan JL, et al: Stability of n-3 fatty acids in human fat tissue aspirates during storage. Am J Clin Nutr 57:884-888, 1993
37. Nelson GJ: Isolation and purification of lipids from animal tissues, in Perkins EG (ed): Analysis of Lipids and Lipoproteins. Champaign, IL, American Oil and Chemical Society, 1975, pp 1-22
38. Stocks J, Offerman EL, Modell CB, et al: The susceptibility to autoxidation of human red cell lipids in health and disease. Br J Haematol 23:713-724, 1972
39. Cabre E, Periago J, Mingorence F, et al: Factors related to the plasma fatty acid profile in healthy subjects with special reference to antioxidant micronutrient status: A multivariate analysis. Am J Clin Nutr 55:831-837, 1992

40. Fraga CG, Tappel AL, Leibovitz BE, et al: Lability of red blood cell membranes to lipid peroxidation: Application to human fed polyunsaturated lipids. Lipids 25:111-114, 1990

41. Houwelingen AC, Kester ADM, Kromhout D, et al: Comparison between habitual intake of polyunsaturated fatty acids and their concentration in serum phospholipids. Eur J Clin Nutr 43:1-20, 1989

42. Hietanen E, Bartsch H, Bereziat JC, et al: Diet and oxidative stress in breast, colon and prostate cancer patients: A case control study. Eur J Clin Nutr 48:575-586, 1994