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Familial aggregation of red blood cell membrane fatty acid composition: the Kibbutzim Family Study

Rozenn N. Lemaitre[a,*], David S. Siscovick[a,b], Elliot M. Berry[c], Jeremy D. Kark[d], Yechiel Friedlander[d]

[a]*Department of Medicine, Cardiovascular Health Research Unit, University of Washington, Seattle, WA 98101, USA*
[b]*Department of Epidemiology, University of Washington, Seattle, WA 98101, USA*
[c]*Department of Human Nutrition and Metabolism, Hebrew University-Hadassah School of Public Health, Jerusalem*
[d]*Unit of Epidemiology, Hebrew University-Hadassah School of Public Health, Jerusalem*

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Abstract

The fatty acid composition of membranes plays an important role in health and diseases. Whether genetic factors play a role in interindividual variability in membrane fatty acid levels has received limited attention. Using variance decomposition methods, we estimated the heritability of red blood cell (RBC) membrane fatty acids in an unselected population sample of 80 families (205 male and 212 female subjects) living in kibbutz settlements in Israel. Fatty acid levels were measured by gas chromatography. We estimated that polygenes explained 40% to 70% of the sex- and age-adjusted interindividual variability in all RBC fatty acids: saturated, monounsaturated, and polyunsaturated. The heritability estimates remained very similar after further adjustment for smoking, alcohol consumption, physical activity, lipoproteins, body mass index, waist to hip ratio, education, and religiosity. In bivariate genetic analyses, we observed positive genetic correlations for the fatty acid pairs 20:4n6-22:6n3 and 20:5n3-22:6n3, and negative genetic correlations for the pairs 16:0-20:4n6, 16:0-22:6n3, 18:1n9-20:3n6, 18:2n6-20:4n6, 18:2n6-24:0, and 20:3n6-20:4n6, suggesting that shared effects of the same sets of loci account for 12% to 30% of the additive genetic variance in these pairs of fatty acids. This study suggests a considerable polygenic component for all RBC membrane fatty acids and provides evidence that shared genetic effects account for the additive genetic variance in various fatty acid pairs. Future studies are needed to map the genes underlying the interindividual variation in these inherited phenotypes.

There is increasing evidence for the importance of the fatty acid composition of membranes in health and disease. For example, higher membrane levels of long-chain n-3 fatty acids from fatty fish are associated with lower risk of sudden cardiac arrest [1], preeclampsia [2], and cognitive decline [3,4]; higher membrane levels of trans isomers of linoleic acid are associated with higher risk of sudden cardiac arrest [5]; and several membrane fatty acids appear to influence the risk of breast cancer [6].

The influence of diet on specific membrane fatty acids is well known. In particular, membrane fatty acids that derive from the dietary intake of polyunsaturated fat exclusively (eg, linoleic acid) or mostly (eg, long-chain n-3 fatty acids) are markers of dietary intake [7-9]. In addition, we recently demonstrated that total fat intake influences the overall membrane fatty acid composition [10].

Whether genetic factors also play a role in interindividual variability in membrane fatty acid composition has received limited attention [11]. Our interest in this question comes in part from the observation of familial aggregation of sudden cardiac arrest [12,13] and the search for candidate genes that might account for this cluster within families. If heritability of membrane fatty acids can be demonstrated, it might explain in part the familial component of sudden cardiac arrest and help direct the search for new candidate genes.

We took advantage of a family study conducted in a setting with limited dietary variation and with measures of cell membrane fatty acid composition, the Kibbutzim Family Study, to investigate the heritability of red blood cell (RBC) membrane fatty acid composition.

* Corresponding author. Cardiovascular Health Research Unit, University of Washington, Seattle, WA 98101, USA. Tel.: +1 206 287 2780; fax: +1 206 287 2662.
E-mail address: rozenl@u.washington.edu (R.N. Lemaitre).

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1. Materials and methods

1.1. Families

The recruitment of families in the Kibbutzim Family Study took place in 6 kibbutz settlements located in the northern area of Israel. The probands were randomly selected from among 465 healthy subjects aged 45 to 64 years who previously participated in a cross-sectional study of risk factors for coronary heart disease in these kibbutz settlements [14]. The families of the probands were invited to participate in the study if there were at least 4 individuals aged 15 years or older, belonging to 2 or more generations, living within the same kibbutz. Institutionalized people and women who were pregnant or had given birth within the previous 3 months were excluded. Eighty kindreds were recruited for this study between 1992 and 1993. The kindreds ranged in size from 2 to >30 individuals. A total of 476 individuals were examined, corresponding to an 80% response rate among those eligible. Signed informed consent was obtained from the family members, including the probands. All participants were asked to complete a medical questionnaire. After a 12-hour overnight fast, blood samples were obtained from each subject from an antecubital vein for biochemical measurements, DNA extraction, and storage of biological material. This report includes 417 subjects (205 male and 212 female) with RBC membrane fatty acid measurement.

1.2. RBC membrane fatty acid measurements

Blood specimens were processed [15] and submitted to gas chromatography according to published methods [16]. The fatty acid methyl esters were separated using a Tracor 565 column (1.85 m × 4 mm internal diameter, 10% SP-2330 on 100/120 Chromosorb in WAW 1-1851; Alltech, Deerfield, IL). Temperature programming was used from 185°C to 220°C. Individual fatty acids were identified by comparing retention times with known standards (Supelco, Bellefonte, PA). We integrated all peaks in the range from palmitic acid (16:0) to docosahexaenoic acid (DHA) (22:6n3); however, several minor fatty acids, including 16:1n7, 18:3n6, and 18:3n3, were not identified. Fatty acids were expressed as percentages of total fatty acids by weight.

1.2.1. Validation of RBC membrane n-3 polyunsaturated fatty acid measurements

In the previous cross-sectional study from which the probands were selected, dietary information was obtained through the administration of a food-frequency instrument in addition to the erythrocyte membrane fatty acid composition. The nutrient content of all food items was derived from Israeli and US tables, supplemented by special laboratory analysis of many items. Among the 465 subjects of the cross-sectional study, the correlation coefficients of dietary DHA (22:6n3), expressed as a percentage of total fat intake, with RBC DHA was 0.47; and that with total n-3 polyunsaturated fatty acids (PUFA) measured in RBC was 0.45. The correlation coefficients of dietary eicosapentaenoic acid (20:5n3) with RBC eicosapentaenoic acid was 0.20, and that with total n-3 PUFA measured in RBC was 0.45. The correlation coefficient between total n-3 PUFA estimated from the dietary questionnaire with total n-3 PUFA in RBC was 0.47.

1.3. Statistical analysis

Levels of RBC fatty acids were first adjusted for the effects of age and sex through stepwise multiple regression. Phenotype levels were modeled as a function of sex, age, age^2, age^3, sex × age, sex × age^2, and sex × age^3. Only significant terms were retained. Criteria for entry and exit into the stepwise models were $P < .1$ and $P > .15$, respectively. The appropriate estimated partial regression coefficients were used to adjust the dependent variable for each individual. In addition to sex and age, we considered the effects of level of *religiosity* (defined as belonging to a secular vs an orthodox kibbutz settlement), *education* (defined as number of years of formal schooling), *smoking* (defined as a categorical variable —current smoker vs nonsmoker—and as the average number of cigarettes smoked per day), *alcohol consumption* (defined as number of alcoholic drinks consumed by the individual in a typical week, as well as during the week before the interview), *physical activity* (coded as a categorical dichotomous variable, indicating if the subject engaged in strenuous physical activity for a minimum of 20 minutes weekly), lipids, lipoproteins, body mass index (BMI) (weight/$height^2$), and the ratio of waist to hip circumferences (W/H ratio) as an index of body fat distribution. The adjustment for sex and age accounted for 0.33% to 12.7 % of the total variation seen in plasma RBC fatty acids. Adjustment for the additional covariates added 5.8% to 22.3 % to the explained variability.

The degree of resemblance among family members was expressed by interclass and intraclass correlation coefficients. Because of the varying sibship size, we used an equal family weight as defined by Karlin et al [17]. This method reduces the effect of large families on the familial correlations. Interclass and intraclass correlations were estimated using a Box and Cox [18] transformation of RBC fatty acid variables, which reduced the skewness and the impact of outliers on the correlation analysis. Hypotheses testing of inter- and intraclass correlations was performed using the Fisher [19] z transformation. Because of the varying sibship size within families, the "effective sibship size" was computed to estimate the appropriate degrees of freedom associated with each family [20,21].

Variance components decomposition method, as implemented in SOLAR software (Southwest Foundation for Biomedical Research, San Antonio, TX) [22,23], was then used to estimate the unmeasured additive genetic and shared environmental components of the variability in RBC fatty acids traits. The likelihood of the phenotypes of the family members is assumed to follow a multivariate normal

distribution with a phenotypic covariance matrix that is a function of kinship between individuals and the additive genetic, household, and environmental variances. Once the expected means and covariance matrix of each pedigree are defined, the likelihood of a pedigree is evaluated with the multivariate normal density function and cumulated over all of the pedigrees. Although we assume multivariate normality, this assumption is robust; and consistent parameter estimates are obtained when the assumption is violated [23].

Maximum-likelihood methods were used to simultaneously estimate mean and variance values as well as the effects of covariates, heredity, and environment components. The significance of covariate effects was assessed with a Wald test. The relative proportions of the residual variance in a trait explained by genetic determinants were calculated as the variance attributable to that component divided for the residual phenotypic variance after adjustment for covariates. The significance of genetic effects was assessed by comparing the likelihoods of models in which these parameters were estimated with models in which they were constrained to zero. Twice the difference in ln likelihood between these models is asymptotically distributed as 1/2:1/2 mixture of χ^2_1 and χ^2_0 [24]. Because probands were selected randomly and independent of their fatty acids values, there was no need to make any corrections for ascertainment.

Finally, a bivariate genetic analysis was implemented to examine the possible pleiotropic genetic effects and shared environmental influences on RBC fatty acid phenotypes. These analyses were maximum-likelihood based and were implemented in SOLAR software. This approach uses estimated heritabilities of the 2 traits (h^2_1 and h^2_2) to partition the phenotypic correlations (ρ_P) between the RBC pairs of phenotypes into 2 additive components attributable to pleiotropic genetic (ρ_G) effects and common environmental (ρ_E) effects so that these will sum up to:

$$\rho_P = \left(\sqrt{h^1_2} + \sqrt{h^2_2}\rho_G\right) + \left[\sqrt{(1-h^1_2)} + \sqrt{(1-h^2_2)}\rho_E\right]$$

From this model, the genetic and environmental correlations can be estimated and compared with models in which the genetic and environmental correlations are constrained to zero. A correlation coefficient differing from zero indicates genetic pleiotropy and/or shared environmental effects on a trait pair. The square correlation is an estimate of the proportion of the additive genetic (environmental) variance in each trait of the RBC pair phenotypes that is due to the effects of the same set of genes (environmental factors).

2. Results

The study included 205 male and 212 female family members, on average 43.9 years old (range, 15-97 years). Table 1 summarizes the distribution of RBC membrane fatty

Table 1
Red blood cell fatty acids by sex and age groups among family members in the Kibbutz Family Study

Sex and age	n	16:0	18:0	18:1n9	18:2n6	20:3n6	20:4n6	22:4n6	20:5n3	22:6n3	24:0	Total SFA	Total PUFA	Total n-3	Total n-6
Male															
<45	112	23.3 ± 1.6	17.6 ± 1.1	16.8 ± 1.3	16.1 ± 1.6	1.8 ± 0.7	14.4 ± 1.4	0.9 ± 0.4	0.3 ± 0.1	4.0 ± 1.1	2.7 ± 0.6	43.5 ± 1.6	37.2 ± 1.8	4.3 ± 1.0	32.7 ± 1.7
45-60	49	23.2 ± 1.7	17.7 ± 0.8	16.5 ± 1.0	15.6 ± 1.5	1.9 ± 0.8	14.1 ± 1.4	0.9 ± 0.4	0.4 ± 0.3	4.1 ± 1.1	2.7 ± 0.7	43.7 ± 1.4	37.2 ± 1.7	4.4 ± 1.1	32.8 ± 1.5
≥60	44	23.6 ± 1.8	17.5 ± 1.2	16.7 ± 3.0	14.8 ± 1.8	1.6 ± 0.4	14.0 ± 1.7	0.9 ± 0.4	0.3 ± 0.3	4.1 ± 1.5	2.7 ± 0.7	44.1 ± 2.0	36.6 ± 1.9	4.5 ± 1.7	32.5 ± 2.2
Total	205	23.4 ± 1.7	17.6 ± 1.1	16.7 ± 1.7	15.7 ± 1.7	1.8 ± 0.7	14.2 ± 1.5	0.9 ± 0.4	0.3 ± 0.2	4.0 ± 1.1	2.7 ± 0.7	43.6 ± 1.7	37.1 ± 1.8	4.4 ± 1.2	32.7 ± 1.8
Female															
<45	118	23.2 ± 2.2	17.6 ± 1.6	16.7 ± 1.5	16.2 ± 1.7	1.8 ± 1.6	15.3 ± 2.3	0.9 ± 0.4	0.3 ± 0.2	4.1 ± 1.1	2.8 ± 0.7	43.6 ± 2.6	37.5 ± 2.4	4.5 ± 1.2	32.3 ± 2.3
45-60	42	23.2 ± 1.8	17.4 ± 1.2	16.4 ± 1.1	15.5 ± 1.7	1.7 ± 0.4	15.5 ± 1.6	0.9 ± 0.3	0.4 ± 0.2	4.5 ± 1.3	2.6 ± 0.6	43.4 ± 1.6	37.9 ± 1.6	4.9 ± 1.3	32.9 ± 1.3
≥60	52	23.1 ± 2.0	17.5 ± 1.4	17.0 ± 1.7	15.0 ± 1.9	1.7 ± 0.5	14.8 ± 1.9	0.9 ± 0.6	0.3 ± 0.2	4.1 ± 1.3	2.6 ± 0.8	43.6 ± 1.4	36.9 ± 2.4	4.6 ± 1.4	32.7 ± 1.9
Total	212	23.2 ± 2.1	17.6 ± 1.4	16.7 ± 1.5	15.7 ± 1.8	1.8 ± 1.3	15.2 ± 2.1	0.9 ± 0.4	0.3 ± 0.2	4.2 ± 1.2	2.7 ± 0.7	43.6 ± 2.4	37.4 ± 2.3	4.6 ± 1.3	32.7 ± 2.0

Mean ± standard deviation, expressed as percentage of total fatty acids.

acid measurements in sex and age strata. Whereas mean levels of total saturated fatty acids (SFA) and 18:2n6 were similar in male and female subjects, total PUFA, 20:4n6, and n-3 fatty acids (20:5n3, 22:6n3, total n-3) were higher in female subjects. In both male and female subjects, no clear effect of age on RBC fatty acid measurements was evident. In male subjects, total SFA and total n-3 fatty acids tended to increase whereas total PUFA tended to decrease with age. Similarly, in female subjects, total PUFA levels were lowest in the 60+ age group. In both male and female subjects, 18:2n6 decreased with age.

Table 2 presents the familial correlation coefficients for sex- and age-adjusted RBC fatty acid measurements. We observed modest, significant correlations of total SFA, total PUFA, total n-3, and total n-6 between spouses, suggesting an influence of shared environment. These correlations tended to be higher between parent-child and between siblings. Among individual fatty acids, fatty acids exclusively (18:2n6) or mostly (n-3 fatty acids) from the diet were correlated between spouses as well as between blood-related family members. However, fatty acids derived from metabolism as well as diet (18:1n9, 20:4n6 and 20:3n6) were only correlated between parent-child and between siblings.

For total RBC measurements, there was a suggestion of asymmetry in parent-child correlations, with mother-child correlations higher than father-child correlations (Table 2). Total RBC measurements appeared higher between mother and daughter (r = 0.38-0.58), intermediate between mother and son (r = 0.25-0.44) and father and son (r = 0.03-0.48), and lowest between father and daughter (r = 0.20-0.36). The sibling correlations were similar to the parent-child correlation coefficients, suggesting similar inter- (parent-offspring) and intragenerational (sibling) influence on fatty acid levels.

The sources of variation in RBC fatty acid measurements are presented in Table 3. Polygenes explained 73% to 75% of the sex- and age-adjusted interindividual variability in RBC total SFA, total PUFA, and total n-3 levels. The heritability estimate for total n-6 was somewhat lower (h^2 = 0.52). Similarly, heritability estimates for individual RBC fatty acids were relatively high; and all estimates differed significantly from zero. The lowest estimates were obtained for 18:0 and 18:1n9 (h^2 = 0.41-0.46) and the highest estimates for 22:6n3 (h^2 = 0.71). To examine whether the heritability estimate parameters may change upon further adjustment for a set of environmental covariates, we repeated the analysis on RBC fatty acids levels adjusted for sex, age, religiosity, education, cigarette smoking, alcohol consumption, physical activity, lipids and lipoproteins, BMI, and W/H ratio. Results under these models were very similar to those obtained by using the sex- and age-adjusted values (Table 3).

We next examined whether pairs of RBC fatty acids were influenced by shared effects of additive genetic factors and/or unmeasured environmental characteristics. Modest positive genetic correlations were observed for 20:4n6 with 22:6n3 and 20:5n3 with 22:6n3, suggesting that shared genetic effects account for 20% to 30% of the additive genetic variance in these fatty acid pairs (Table 4). Negative genetic correlations were observed for the pairs 16:0-20:4n6, 16:0-22:6n3, 18:1n9-20:3n6, 18:2n6-20:4n6, 18:2n6-24:0,

Table 3
Genetic determinants of RBC fatty acids: the Kibbutz Family Study

Variable	Heritability estimates [a]	
	Univariate model	Multivariate model
16:0	0.68 ± 0.08	0.67 ± 0.09
18:0	0.46 ± 0.11	0.45 ± 0.11
18:1n9	0.41 ± 0.11	0.36 ± 0.12
18:2n6	0.51 ± 0.10	0.51 ± 0.11
20:3n6	0.62 ± 0.11	0.63 ± 0.11
20:4n6	0.53 ± 0.11	0.60 ± 0.11
22:4n6	0.62 ± 0.09	0.51 ± 0.11
20:5n3	0.56 ± 0.11	0.52 ± 0.11
22:6n3	0.71 ± 0.08	0.65 ± 0.09
24:0	0.53 ± 0.09	0.59 ± 0.10
Total SFA	0.73 ± 0.08	0.69 ± 0.08
Total PUFA	0.75 ± 0.08	0.63 ± 0.09
Total n-3	0.75 ± 0.08	0.66 ± 0.11
Total n-6	0.52 ± 0.10	0.73 ± 0.08

[a] In the univariate model, values were adjusted to sex and age. In the multivariate model, religiosity, education level, smoking status, alcohol consumption, physical activity, lipids and lipoproteins, BMI, and W/H ratio were also included.

Table 2
Familial correlations of RBC fatty acids: the Kibbutz Family Study

Familial relationship	n	16:0	18:0	18:1n9	18:2n6	20:3n6	20:4n6	22:4n6	20:5n3	22:6n3	24:0	Total SFA	Total PUFA	Total n-3	Total n-6
F-M	106	0.28	0.15	−0.03	0.17	0.07	0.08	0.31	0.31	0.42	0.16	0.22	0.27	0.33	0.06
P-C	296	0.40	0.25	0.21	0.24	0.24	0.18	0.31	0.30	0.44	0.34	0.38	0.41	0.46	0.21
M-D	79	0.56	0.28	0.19	0.25	0.26	0.29	0.34	0.09	0.59	0.47	0.44	0.58	0.56	0.38
M-S	78	0.42	0.30	0.22	0.38	0.46	0.28	0.41	0.38	0.38	0.30	0.44	0.43	0.41	0.25
F-D	67	0.24	0.23	0.36	0.20	0.14	0.16	0.31	0.26	0.34	0.24	0.20	0.30	0.36	0.25
F-S	72	0.30	0.21	0.11	0.13	0.48	−0.08	0.23	0.45	0.42	0.32	0.46	0.29	0.48	−0.03
Sib-Sib	118	0.41	0.26	0.28	0.36	0.42	0.41	0.41	0.40	0.53	0.27	0.47	0.46	0.54	0.52
S-S	34	0.30	0.19	0. 08	0.46	0.40	0.36	0.59	0.36	0.58	0.25	0.42	0.36	0.61	0.42
S-B	58	0.39	0.35	0.42	0.39	0.49	0.54	0.26	0.45	0.56	0.23	0.51	0.39	0.52	0.48
B-B	26	0.61	0.17	0.26	0.19	0.25	0.22	0.55	0.37	0.45	0.38	0.44	0.77	0.53	0.77

Sex- and age-adjusted values. F-M indicates father-mother; P-C, parent-child; M-D, mother-daughter; M-S, mother-son; F-D, father-daughter; F-S, father-son; S-S, sister-sister; S-B, sister-brother; B-B, brother-brother.

Table 4
Maximum-likelihood estimates of the additive genetic (ρ_G) and environmental (ρ_E) correlations from the bivariate genetic analysis of RBC fatty acids[a]: the Kibbutz Family Study

Phenotype pairs	$\rho_G \pm$ SE	$\rho_E \pm$ SE	ρ_p
16:0-18:0	0.16 + 0.15	−0.36 ± 0.14	−0.07
16:0-18:2n6	0.26 ± 0.13	0.13 ± 0.15	0.21
16:0-20:4n6	−0.36 ± 0.12	−0.56 ± 0.14	−0.43
16:0-20:5n3	−0.09 ± 0.14	0.29 ± 0.16	0.06
16:0-22:6n3	−0.34 ± 0.13	0.04 ± 0.17	−0.20
18:1n9-18:2n6	−0.14 ± 0.25	0.18 ± 0.13	0.05
18:1n9-20:3n6	−0.55 ± 0.18	0.25 ± 0.16	−0.15
18:2n6-20:4n6	−0.50 ± 0.12	−0.44 ± 0.13	−0.47
18:2n6-20:5n3	−0.36 ± 0.17	0.15 ± 0.15	−0.11
18:2n6-22:6n3	−0.19 ± 0.15	−0.22 ± 0.15	−0.20
18:2n6-24:0	−0.44 ± 0.13	−0.19 ± 0.14	−0.33
20:3n6-20:4n6	−0.50 ± 0.15	0.48 ± 0.18	−0.15
20:4n6-20:5n3	0.26 ± 0.18	−0.46 ± 0.15	−0.06
20:4n6-22:6n3	0.44 ± 0.15	−0.14 ± 0.17	0.21
20:5n3-22:6n3	0.54 ± 0.12	0.29 ± 0.14	0.43

[a] In the bivariate model, values were adjusted to sex, age, religiosity, education level, smoking status, alcohol consumption, physical activity, lipids and lipoproteins, BMI, and W/H ratio.

and 20:3n6-20:4n6, suggesting that shared effects of the same sets of loci account for 12% to 31% of the additive genetic variance in these pairs of fatty acids.

Significant influence of the shared environmental factors on the relationship between several pairs of fatty acids was also observed (Table 4). For example, a positive environmental correlation was observed between 20:5n3 and 22:6n3, as would be expected if the main source of these 2 fatty acids was fatty fish. Negative correlations were observed for the pairs 20:4n6-16:0, 20:4n6-18:2n6, and 20:4n6-20:5n3.

3. Discussion

This study brings evidence of widespread heritability of RBC fatty acid membrane composition in humans. We estimated that genetic variation explained 40% or more of interindividual variability in levels of all the fatty acids we measured, including saturated, monounsaturated, and polyunsaturated fatty acids. In addition, we found evidence for shared genetic effects (positive and negative) between several fatty acids.

The main limitation of this family study is the absence of dietary information on the individual family members. Similarity of diet within families, coupled with dietary differences between families, would lead to overestimates of heritability. However, the family members in the study all lived in kibbutzim, where people often dine in a central dining room and share a relatively homogeneous environment. In fact, the overall limited dietary variation in the kibbutzim environment was the reason why we chose this setting because it would allow detection of genetic influence above dietary noise. Several observations suggest that diet alone could not explain study results: (1) Spousal correlations for 18:1n9, 20:3n6, and 20:4n6 were low and not significantly different from zero; yet heritability estimates were 41% to 62% for these fatty acids. (2) High heritability estimates were obtained for fatty acids that are not good markers of diet, such as 16:0, and fatty acids that are found in low amount in foods, such as 20:3n6. (3) In bivariate analyses that differentiated genetic and environmental effects, shared additive genetic effects explained 11% to 33% of variability of fatty acid phenotype pairs.

Evidence of heritability of fatty acid composition of serum phospholipids was obtained in a small study of obese identical female twins [11]. Serum phospholipids are precursors of membrane fatty acids. In the twin study, intrapair correlations of fatty acid levels were high for all the serum fatty acids. Our study extends these results to membrane fatty acids and healthy men and women. Additional evidence for heritability of specific fatty acids comes from candidate gene studies. In particular, variation in the Δ-5 and Δ-6 desaturase genes was shown to influence the levels of several serum phospholipid fatty acids, including 20:4n6, 18:2n6, 20:3n6, and 20:5n3, explaining 28% of the variation in 20:4n6 and 9% of the variation in 18:2n6 [25]. Variation in the intestinal fatty acid binding gene is also associated with variation in plasma 20:4n6 in obese children [26]. Finally, there is evidence of heritability of membrane fatty acid composition in both sheep and pig animal models [27-29].

Linoleic acid (18:2n6) is an essential fatty acid, originating exclusively from the diet; and membrane levels of 18:2n6 are a well-documented marker of dietary intake [7]. The PUFA 18:2n6 is particularly abundant in the Israeli diet [30]; and this was reflected by higher levels in RBC membranes than observed, for example, in a US study [5].

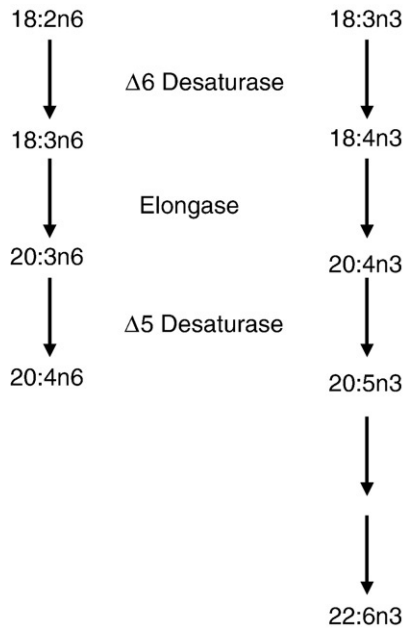

Fig. 1. Major metabolic pathways of n-6 and n-3 fatty acids.

Whether dietary characteristics of the study population also explained the observed low levels of RBC 22:4n6 is unclear. The evidence of a high heritability estimate for RBC membrane 18:2n6 provided by this study suggests that pathways leading to the incorporation into membranes and possibly alternate pathways using 18:2n6 (eg, oxidation for energy, storage into triacylglycerols) have a heritable component. In addition, we found evidence for heritability within the pathway leading to the production of 20:4n6 from 18:2n6 depicted in the figure. First, 2 fatty acids derived from 18:2n6, 20:3n6, and 20:4n6 also showed high heritability. Second, 18:2n6 shared negative genetic effects with 20:4n6, suggesting that the known inhibition of the desaturases by high levels of 18:2n6 [31,32] has a genetic component. Because the desaturases and elongases are shared by the metabolic pathways of 18:2n6 and 18:3n3 (Fig. 1), high levels of 18:2n6 also inhibit the production of both 20:5n3 and 20:4n6. Consistent with a genetic component, we also observed shared negative genetic effects of 18:2n6 with 20:5n3 in our study. In agreement with these observations, variation in the 2 genes coding for the desaturases, *FADS1* and *FADS2*, was associated with higher levels of 18:2n6 and lower levels of 20:4n6 and, to a smaller extent, 20:5n3 [25].

In summary, we observed significant heritability estimates for all RBC membrane fatty acids; and the evidence presented herein suggests a genetic component within multiple fatty acid pathways. Future studies are needed to investigate the genes responsible for the heritability and whether variation in these genes influences the risk/benefit of specific dietary components.

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