Erythrocyte-Derived Measures of Membrane Lipid Composition in Healthy Men: Associations With Arachidonic Acid at Low to Moderate But Not High Insulin Sensitivity

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The lipid composition of erythrocyte membranes was explored as a surrogate for that of skeletal muscle in investigations into the influence of membrane fatty acid composition on insulin sensitivity. In a preliminary study (study 1), erythrocyte and monocyte/platelet membrane fatty acid percentages were compared with those of muscle membrane in 10 otherwise healthy men undergoing orthopedic surgery. In a further study (study 2), relationships between erythrocyte membrane fatty acid concentrations and insulin sensitivity, S_I, measured using the intravenous glucose tolerance test (IVGTT), were evaluated in 30 asymptomatic men. In study 1, significant positive correlations were found between muscle and erythrocyte membrane fatty acid percentages for 16:0 saturated fatty acid ($r = 0.92$, $P < .001$), and for the 18:2n-6, 20:4n-6, 20:5n-3, and 22:5n-3 polyunsaturated fatty acids (PUFAs) ($r = 0.67$ to 0.83, $P < .05$ to $.01$). There were fewer and weaker associations between muscle and monocyte/platelet membrane fatty acid compositions. In study 2, highly insulin-sensitive individuals (n = 8) had significantly lower erythrocyte membrane fatty acid concentrations than those with low/normal S_I. Among those with low/normal S_I (n = 22), S_I correlated positively with erythrocyte membrane arachidonic acid concentration ($r = 0.57$, $P < .01$) and with total PUFAs ($r = 0.46$, $P < .05$). Indices of delta 6 and delta 5 desaturase activities were significantly higher and lower, respectively, in high compared with low/normal S_I individuals. For a range of fatty acids, erythrocyte membrane fatty acid composition shows close associations with that of muscle membranes. Measurements in erythrocyte membranes support a role for membrane arachidonic acid content in the modulation of insulin sensitivity, specifically at low/normal insulin sensitivities.

SKELETAL MUSCLE is the principal site of insulin-mediated glucose disposal and has provided a focus for investigations into mechanisms that might influence the development of insulin resistance.[1] Several areas of muscle physiology and metabolism have been considered, including the membrane environment in which the insulin receptor is embedded.

In vivo investigations have shown that the fatty acid composition of muscle membrane can provide significant correlates of insulin resistance. Specifically, an increase in saturated fatty acid content is associated with an increase in insulin resistance while an increase in polyunsaturated fatty acid (PUFA) content is associated with lesser degrees of insulin resistance.[2-4] It has been proposed that the association between increasing insulin resistance and decreasing membrane PUFA content is mediated by decreasing membrane fluidity and there is evidence to support links between these factors.[5-7] However, attention has also been given to metabolic mechanisms that could cause either changing insulin resistance to affect membrane fatty acid composition or changes in fatty acids to affect insulin responsiveness. Desaturase and elongase enzymes mediate in vivo synthesis of PUFAs from linoleic (C18:2 n-6) and alpha linolenic (C18:3 n-3) acids and the delta 6 and delta 5 desaturases catalyse rate-limiting steps in this processes. The activities of both these enzymes have been linked with variation in insulin sensitivity[2,4,8] or with insulin itself.[9] There is also evidence that fatty acids can affect insulin sensitivity at the cellular level.[10] In these studies, a positive association between insulin sensitivity and membrane arachidonic acid has been one of the most consistently observed associations.

Evaluation of muscle membrane fatty acid composition is clearly important in understanding the etiology of insulin resistance and its clinical consequences. However, studies, particularly in larger groups, are hindered by the relative difficulty of obtaining biopsy samples for muscle membrane analysis. Prior to the in vivo studies of muscle lipids and insulin sensitivity mentioned above, significant associations between membrane lipid content and insulin action had been repeatedly described for in vitro studies carried out on other cell types.[11-14] However, the extent to which studies of insulin resistance in such alternative cell types offer an effective surrogate for studies in muscle needs to be considered. We therefore evaluated variation in membrane lipid composition in erythrocytes and monocytes/platelets, ie, relatively accessible cell types, in relation to muscle cell membrane lipid composition (study 1). We then explored the associations between erythrocyte membrane fatty acid concentrations and insulin sensitivity measured by minimal model analysis of intravenous glucose tolerance test (IVGTT) glucose and insulin concentrations (study 2).

MATERIALS AND METHODS

Study 1

Samples of skeletal muscle (~10 to 20 mg) and heparinized whole blood (10 mL, for isolation of erythrocytes and mononuclear leukocytes/platelets) were taken from 10 male patients, aged 30 to 55, undergoing routine orthopedic surgery. These samples were immedi-

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ately placed on ice and were received in the laboratory within 3 hours of being taken. Blood samples were processed on receipt (see below), while skeletal muscle samples were stored at −80°C before processing. Full informed consent was given in each case and local ethics committee approval was given.

Study 2

Thirty apparently healthy, non-obese men (aged 25 to 76) participating in an ongoing cohort study (The Heart Disease and Diabetes Risk Indicators in a Screened Cohort [HDDRISC] Study) underwent an IVGTT and had blood taken for the measurement of individual fatty acid concentrations in erythrocyte membranes. Full informed consent was obtained in each case and local ethics committee approval was given. In preparation for the IVGTT, participants were instructed to consume more than 200 g/d carbohydrate in their diet for the previous 3 days, to have fasted overnight (>12 hours), and to have taken only water and refrained from cigarette smoking on the morning of the test. After resting for 15 minutes in a semi-recumbent position, an indwelling cannula was inserted into an antecubital vein in each arm. Prolonged venous stasis was avoided. Prior to glucose infusion, blood samples were taken for the measurement of fasting plasma glucose, insulin, and serum lipids, and for the isolation of erythrocytes. All samples was kept on ice and processed within 1 hour of being taken. An intravenous glucose injection was then given (0.5 g glucose/kg body weight as a 50% [wt/vol] solution of dextrose, given over 3 minutes), via the cannula in the opposite arm to the sampling arm. Blood samples (10 mL) were then taken at 3, 5, 7, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, and 180 minutes for the measurement of plasma glucose and insulin.

Laboratory Measurements

Plasma glucose concentrations were measured on the day of sampling by a glucose oxidase procedure.[15] Samples for measurement of plasma insulin were stored at −20°C prior to analysis. Measurement of plasma specific insulin was by a microtiter well chemiluminescent immunometric system (Molecular Light Technology Research Ltd, Cardiff, UK), based on the antibody system described by Sobey et al.[16] This assay is specific for bioactive insulin with minimal cross-reactivity (<2%) from intact proinsulin, 32-33 split proinsulin, and C-peptide.

Serum for measurement of cholesterol, triglyceride, high-density lipoprotein (HDL)-cholesterol, HDL_3-cholesterol, and apolipoproteins were stored at 2 to 6°C and assayed within 5 days of collection. Cholesterol, triglycerides, and apolipoproteins were measured using a Cobas Mira discrete analyzer (Roche Diagnostics, Basel, Switzerland). Cholesterol and triglycerides were measured using a colorimetric method described by Siedel et al[17]; HDL-, HDL_2-, and HDL_3-cholesterol levels using standard precipitation techniques[18,19]; and low-density lipoprotein (LDL)-cholesterol using the Friedwald method.[20] Apolipoproteins (apo)AI and apoB were determined using immunoturbidometric methods,[21] while apoAII was determined using an in-house method incorporating antisera from Boehringer Mannheim (Mannheim, Germany; catalog no. 726478).

Quality control was monitored by use of commercially available lyophilized sera and by participation in relevant National Quality Assurance schemes (UK Radioimmunoassay Quality Assurance Scheme [RIQAS] for C-peptide and insulin, and UK National External Quality Assurance Scheme (NEQAS] for all other analytes). Within- and between-assay coefficients of variation are typically less than 3% (plasma glucose), less than 7 % (plasma insulin, range 10 to 110 μU/mL), less than 3% (cholesterol), less than 4% (triglycerides), less than 5% (HDL), less than 8% (HDL_3), less than 8% (apoAI), less than 6% (apoAII), and less than 7% (apoB).

Isolation of Blood Cells for Lipid Extraction

Blood cells were isolated from whole blood on a Ficoll density gradient.[22] Using 15 mL quickfit tubes, 6 mL of whole blood was gently layered onto a density gradient of 3 mL Histopaque 1077 over 3 mL Histopaque 1119 (Sigma Chemical Co, Poole, UK). The tubes were then centrifuged at 700 × *g* for 30 minutes at room temperature. For study 1, mononuclear leukocytes/platelets were isolated from the plasma/Histopaque 1077 interface, while erythrocytes were sedimented at the base of the tube. The mononuclear fraction was carefully decanted into another quickfit tube and the remaining supernatant discarded from above the erythrocyte fraction. Both isolates were then resuspended and washed 3 times with 5 mL ice-cold isotonic saline containing 20 mmol/L EDTA (NaCl, 9 g/L; EDTA, 7.45 g/L). Following each wash, the tubes were centrifuged for 10 minutes at 200 × *g* at room temperature. Following the third wash, the cells were resuspended in 3 mL ice-cold isotonic saline containing 20 mmol/L EDTA and frozen at −80°C to lyse the cells. For study 2, the supernatant, which contained all other cells except for erythrocytes, was discarded and the erythrocytes harvested from the base of the tube. The erythrocyte fraction was then resuspended and prepared as described above.

Lipid Extraction and Chromatographic Analyses

Blood cells. Blood cell samples were thawed at room temperature and centrifuged at 2,900 × *g* for 30 minutes at 4°C to sediment the membrane pellets. The supernatant was discarded and lipids extracted from the pellets according to Radin[23] with addition to the extraction mixture of internal standards triheptadecanoate, phosphatidylcholine heptadecanoate, and cholesterol heptadecanoate (Sigma Chemical Co; 100 μL of a 2.6-mmol/L solution, previously stored in amber glass vials at −40°C). The phospholipid fraction was isolated using thin-layer chromatography. Component fatty acids of all fractions were transmethylated with methanolic HCl at 50°C for 12 hours.[24] Under these conditions, this reagent readily trans-esterifies free fatty acids, O-acyl lipids, and N-acyl lipids such as sphingolipids.[25] The fatty acid methyl esters were extracted into chloroform and resolved using a PU4400 gas-liquid chromatograph (Phillips Scientific, Cambridge, UK) fitted with a new 0.53 mm × 30 m SP-2380 wide-bore capillary column (Supelco Ltd, Essex, UK).

Skeletal muscle. Samples were washed in ice-cold isotonic saline, blotted, and homogenized using a glass homogenizer until no visible particles remained. Lipids were then extracted using redistilled chloroform/methanol (2:1 vol/vol) containing 0.45 mmol/L butylated hydroxytoluene as antioxidant and with addition to the extraction mixture of internal standards triheptadecanoate, phosphatidylcholine heptadecanoate, and cholesterol heptadecanoate (Sigma Chemical Co; 100 μL of a 2.6-mmol/L solution, previously stored in amber glass vials at −40°C). This was followed by a Folch wash to remove non-lipid contaminants.[26] The lipid extracts were subjected to thin-layer chromatography. The phospholipid fraction was then transmethylated and the component fatty acids resolved and identified as described above. In the muscle preparations, the SP-2380 column completely resolved all fatty acid peaks.

Retention times were compared with those of high purity (>99%) standards (Sigma Chemical Co). Peaks were analyzed using an IBM PS/2 microcomputer and Nelson 2600 software (Perkin-Elmer Nelson Systems Inc, Buckinghamshire, UK). Intra-assay coefficients of variation were derived for all fatty acids of interest by repeat analysis (n = 10) of a single lipid extract. At weight percentages of 0.2%, 1.5%, 6.0%, and 24%, the coefficients of variation were 18.0%, 3.4%, 2.7%, and 1.8%, respectively. A mean percent recovery of 100% ± 6.0% was determined over the fatty acid range C16-C20 by analyzing known amounts of palmitic (16:0), linoleic (18:2n-6), and eicosapentanoic (20:5n-3) acid.

Table 1. Skeletal Muscle Cell and Red Blood Cell Membrane Fatty Acid Composition and Their Relationships in 10 Men Undergoing Orthopedic Surgery

Fatty Acid	Fatty Acid Composition (%)		Correlation (rho) Between RBC and SMC Fatty Acids
	SMC	RBC	
16:0	18.1 (1.9)	20.3 (1.0)	0.92‡
18:0	11.1 (1.3)	11.5 (0.7)	−0.85†
18:1n9	18.0 (2.5)	26.7 (1.6)	−0.48
18:2n-6	31.9 (3.7)	13.0 (1.3)	0.83†
18:3n-6	0.2 (0.1)	0.3 (0.1)	0.29
18:3n3	0.4 (0.1)	3.5 (0.9)	−0.15
20:3n-6	1.1 (0.5)	1.6 (0.5)	−0.32
20:4n-6	13.2 (1.8)	13.9 (1.3)	0.77†
20:5n3	1.3 (0.3)	1.1 (0.3)	0.67*
22:5n3	1.8 (0.5)	2.8 (0.8)	0.76†
22:6n3	3.0 (0.5)	5.5 (1.1)	0.43
All SFA	29.2 (0.9)	32.1 (1.7)	0.50
All n-6-PUFA	46.3 (3.7)	28.5 (1.9)	0.67*
All n3-PUFA	6.4 (0.8)	12.7 (1.2)	0.68*
n-6 + n3 PUFA	52.8 (3.3)	41.3 (1.1)	0.33
C20-C22 PUFA	20.3 (1.9)	24.7 (1.6)	0.71*

NOTE. Values are mean (%) total fatty acids (SD).

Significant correlations (Spearman rho): *$P < .05$, †$P < .01$, ‡$P < .001$

Abbreviations: RBC, red blood cells; SMC, skeletal muscle cells; SFA, saturated fatty acids; n-6-PUFA, n3-PUFA series 6 and series 3 polyunsaturated fatty acids, respectively.

Data Analysis

Insulin sensitivity, S_I, was determined using the minimal model of glucose disappearance,[27] using programs written in Fortran 77. The relatively high glucose dose (0.5 g/kg) used in this study provides for a sufficient endogenous insulin response in nondiabetic volunteers without recourse to additional augmentation of pancreatic insulin secretion. This is reflected in the high rate of model identification and the good correlation obtained with measures of insulin sensitivity derived from the euglycemic clamp ($r = 0.92$).[28,29] In the present analysis, values of S_I were multiplied by a factor of 0.67, which is the slope of the regression line for S_I values derived from insulin concentrations measured using our previously employed radioimmunoassay and insulin concentrations measured by the chemiluminesce-based assay in the present study (unpublished results). This enabled S_I values in the present study to be interpreted with reference to S_I values in the HDDRISC cohort as a whole (see below).

In study 1, the percentage proportion of each individual fatty acid in muscle and erythrocyte lipid extracts was determined relative to the total fatty acids identified in the sample. In study 2, erythrocyte fatty acid concentrations were derived using the red blood cell count and were expressed as nanograms per 10^6 cells. Proportions of each fatty acid were then derived relative to sum of the concentrations of all fatty acids identified. In study 2, indices of the delta 6 and delta 5 desaturase enzyme activities were derived as the erythrocyte membrane concentration ratios, C18:3 n-6/C18:2 n-6 and C20:4 n-6/C18:3 n-6 and, respectively. Statistical analyses were performed using the SYSTAT statistical package (SYSTAT Inc, Evanston, IL). Correlations in study 1 were performed using Spearman nonparametric correlation. Preliminary analysis of data from study 2 revealed a marked dissociation in the characteristics of erythrocyte membrane fatty acids at low/normal insulin sensitivities and at high insulin sensitivities. Quintile cut points for S_I in 552 men from the HDDRISC study, from which men in study 2 were recruited, were 1.32, 2.17, 3.03, and 4.52 $min^{-1} \cdot mU^{-1} \cdot L$.[30] Men in study 2 were assigned to 2 groups according to these quintile cut points: those with S_I in the bottom 3 quintiles were assigned to the low/normal S_I group and those with S_I in the top 2 quintiles to the high S_I group (in practice, had the men in the present study been divided according to low, middle, and upper tertiles of S_I in the HDDRISC cohort, they would have been assigned to identical groupings, ie, low/middle and upper).

RESULTS

Study 1

Skeletal muscle cell and erythrocyte membrane mean percentage fatty acid contents and the Spearman correlation observed between skeletal muscle and erythrocyte membrane fatty acid composition are shown in Table 1. Significant correlations were found for palmitic (16:0), stearic (18:0: an inverse correlation), linoleic (18:2n-6), arachidonic (20:4n-6), eicosapentanoic (20:5n3), and docosapentanoic (22:5n3) acid and also for n3-PUFA, n-6-PUFA, and total C20-C22 PUFA. The 2 principal positive correlations among the PUFAs (for 18:2 n-6 and 20:4 n-6) are illustrated in Fig 1. There were no correlations for the other fatty acids, including oleic acid (18:1n9), the most abundant in erythrocytes (26.7%). The only significant correlations found between skeletal muscle cell and leukocyte/platelet membrane mean percentage fatty acid contents were for alpha-linolenic (18:3n3, $r = 0.79$) and docosapentanoic (22:5n3, $r = 0.64$) acid.

Study 2

The characteristics of the 30 men in study 2 are shown in Table 2. Those in the high S_I group had significantly lower

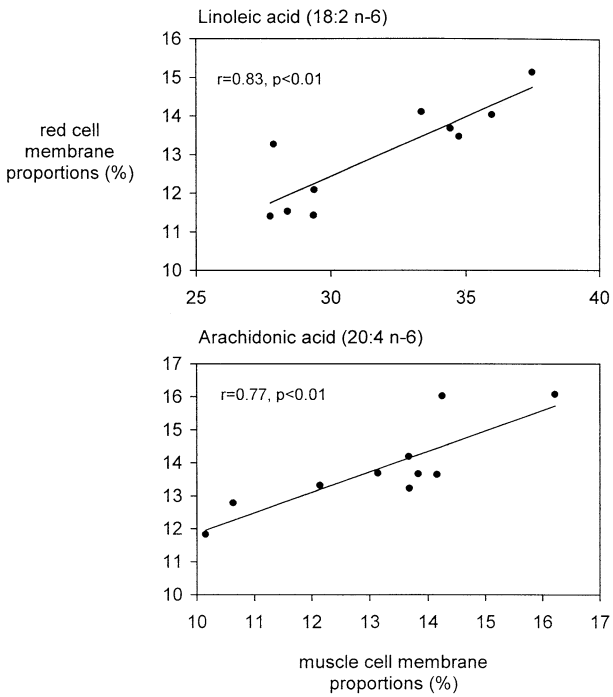

Fig 1. Correlations between fatty acid proportions in muscle and erythrocyte membranes for linoleic (18:2 n-6) and arachidonic (20:4 n-6) acids in 10 men undergoing elective surgery (study 1).

Table 2. Study 2 Group Characteristics

Characteristic	Group 1: Low/Moderate S_I (n = 22)	Group 2: High S_I (n = 8)
Age (yr)	44.6 (7.3)	50.9 (11.5)
Body mass index (kg/m^2)	27.8 (3.4)	24.8 (1.8)†
Weight (kg)	89.1 (12.4)	75.0 (7.4)†
Systolic blood pressure (mm Hg)	133.2 (14.1)	113.0 (46.3)
Diastolic blood pressure (mm Hg)	85.6 (9.5)	71.3 (29.1)*
Fasting insulin (mU/L)	7.3 (3.1)	3.3 (1.3)‡
Fasting glucose (mmol/L)	5.2 (0.5)	5.3 (0.5)
S_1 (min^{-1}·mU^{-1}·L)	1.6 (0.8)	4.6 (0.8)‡
Cholesterol (mmol/L)	5.7 (1.2)	5.3 (0.7)
Triglycerides (mmol/L)	2.2 (1.5)	1.3 (0.6)*
HDL (mmol/L)	1.3 (0.3)	1.5 (0.3)*
HDL_2 (mmol/L)	0.4 (0.2)	0.6 (0.2)*
HDL_3 (mmol/L)	0.9 (0.1)	0.9 (0.2)
LDL (mmol/L)	3.4 (0.9)	3.1 (0.6)
apoAI (mg/dL)	119.9 (16.6)	130.9 (19.3)
apoAII (mg/dL)	40.4 (6.3)	39.6 (7.3)
apoB (mg/dL)	83.0 (21.0)	76.4 (14.6)

NOTE. Values are mean (SD). Significant differences: *$P < .05$, †$P < .01$, ‡$P < .001$

body mass index ($P < .01$), plasma triglycerides ($P < .05$), fasting insulin levels ($P < .001$), systolic ($P < .1$), and diastolic blood pressure ($P < .05$) and higher HDL-cholesterol ($P < .05$) and HDL_2-cholesterol ($P < .05$) concentrations. It was also noted that these individuals tended to smoke less (no current smokers in the high S_I group compared with 4 in the low/moderate S_I group), exercise more (regular aerobic exercise was taken by 50% in the high S_I group compared with 32% in the low/moderate S_I group) and have lower alcohol intake (average weekly intake 10 units in the high S_I group compared with 14 units in the low/moderate S_I group).

Erythrocyte membrane fatty acid concentrations for the low/normal and high S_I subgroups are shown in Table 3. The predominant fatty acids were palmitic (16:0), stearic (18:0), oleic (18:1 n-9), linoleic (18:2 n-6), and arachidonic (20:4 n-6) acids. Concentrations of each of these were significantly lower in the high S_I group. The index of delta 6 desaturase activity was significantly higher in the high S_I group and that of delta 5 desaturase significantly lower.

Among the 22 men with low or normal S_I, erythrocyte membrane lipid concentration and S_I were positively correlated for arachidonic acid ($r = 0.57$, $P < .01$) acid (Table 3 and Fig 2). Significant associations were also found for the concentrations in erythrocyte membranes of total n-6-PUFAs ($r = 0.47$, $P < .05$), total n-6 + n3-PUFA ($r = 0.46$, $P < .05$), and total C20-22 PUFA ($r = 0.46$, $P < .05$). Associations between erythrocyte membrane fatty acid concentrations and S_I in the high S_I group tended to be negative and were significant for palmitic (16:0) and borderline significant for total fatty acids and total saturated fatty acids.

Erythrocyte membrane fatty acid proportions for the low/normal and high S_I subgroups are shown in Table 4. Proportions of the minor fatty acid fractions, 18:3 n-6 and 18:3 n3, were significantly higher in the high S_I group compared with the low/normal S_I group. Otherwise there were no significant differences in fatty acid proportions. Neither were there any significant correlations between S_I and fatty acid proportions in the low/normal or high S_I groups.

Investigation of associations between erythrocyte membrane fatty acids and metabolic variables other than S_I revealed that a high concentration of arachidonic acid in cell membranes was

Table 3. Erythrocyte Fatty Acid Concentrations Among Healthy Men With Low/Normal S_I (n = 22) and High S_I (n = 8) and Their Associations with S_I

Fatty Acid	Erythrocyte Fatty Acid Concentration (ng/10^6 cells)		Correlation for S_I and Erythrocyte Membrane Lipid Concentration	
	Low/Normal S_I	High S_I	Low/Normal S_I	High S_I
16:0	35.1 (2.2)	25.6 (1.5)*	0.23	−0.71*
18:0	21.4 (1.1)	17.1 (1.1)*	0.23	−0.36
18:1n9	29.2 (1.4)	23.0 (1.6)*	0.31	−0.43
18:2n-6	17.6 (0.7)	12.5 (0.8)†	0.37	−0.37
18:3n-6	0.1 (0.0)	0.1 (0.0)	−0.08	0.08
18:3n3	0.3 (0.0)	0.3 (0.1)	0.22	0.12
20:4n-6	23.3 (1.2)	15.8 (1.2)‡	0.57†	−0.36
20:5n3	1.5 (0.2)	1.3 (0.1)	0.09	−0.57
22:5n3	3.6 (0.3)	2.8 (0.3)	0.29	−0.45
22:6n3	7.1 (0.6)	5.6 (0.6)	0.14	−0.31
delta 6 desaturase	0.007 (0.004)	0.014 (0.009)*	−0.22	0.38
delta 5 desaturase	215 (105)	142 (144)*	0.32	−0.31
All fatty acids	139.3 (6.6)	104.3 (6.1)‡	0.33	$-0.64^{P=.086}$
All SFA	56.5 (3.2)	42.7 (2.2)‡	0.23	$-0.69^{P=.058}$
All n-6-PUFA	41.0 (1.8)	28.4 (1.8)‡	0.47*	−0.55
All n3-PUFA	12.5 (1.0)	10.0 (0.9)	0.17	−0.33
n-6 + n3 PUFA	53.5 (2.4)	38.4 (2.5)‡	0.46*	−0.60
C20-C22 PUFA	35.5 (1.9)	25.5 (2.0)‡	0.46*	−0.36

Significant differences (Mann-Whitney) and correlations (Spearman rho): *$P < .05$, †$P < .01$, ‡$P < .001$.
Borderline significances ($P = .05$–$.1$) are given in full.

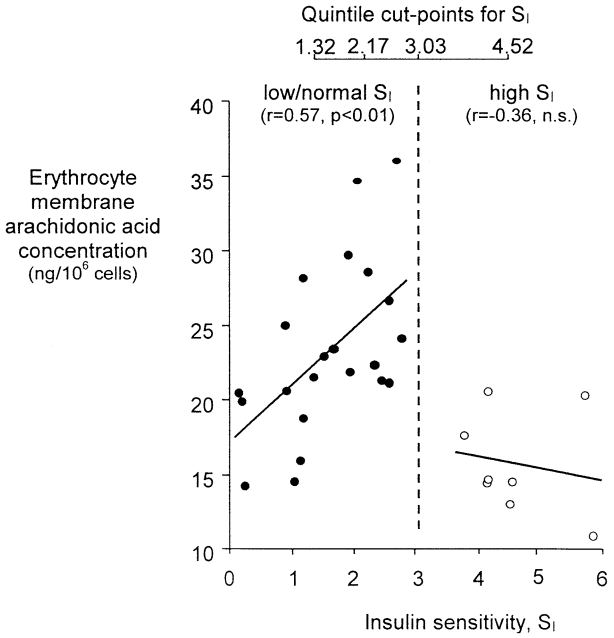

Fig 2. Variation in arachidonic acid concentrations in erythrocyte membranes according to S_I, S_I ($min^{-1} \cdot mU^{-1} \cdot L$) in 30 healthy men (study 2). Quintile cut-points for S_I from Godsland et al[30] are shown. Low/normal and high S_I are distinguished by the division between third and fourth quintiles. Spearman rho and its significance and regression lines for the 22 men with low/normal S_I and the 8 with high S_I are shown.

associated with low plasma triglyceride concentrations ($r = -0.63$, $P < .01$). No such association could be found with the concentration of other erythrocyte fatty acids (results not shown).

DISCUSSION

In this study, the fatty acid composition of erythrocyte membrane phospholipids compared well with that reported in the literature both in terms of percent composition[31] and concentration.[32] Associations between proportions of fatty acids in erythrocyte and muscle cell membranes nevertheless varied according to the type of fatty acid. The strongest positive associations were seen with the unsaturated fatty acid palmitic (16:0) and the PUFAs linoleic (18:2n-6), arachidonic (20:4n-6), eicosapentanoic (20:5n-3), and docosapentanoic (22:5n-3) acids. One other study, recently published, has correlated muscle and erythocyte membrane fatty acid compositions, but found weaker correlations, the strongest coefficients being 0.39 (total n-3 PUFAs and the n-3/n-6 PUFA ratio) and 0.44 (22:6 n-3, docosahexaenoic acid).[33] This study concerned unweaned infants aged less than 2 years, so it is possible that closer relationships exist between muscle and erythrocyte membrane composition in adults than in infants.

The original study of muscle membrane lipid composition and insulin sensitivity by Borkman et al reported the strongest correlates of S_I in muscle membrane to be the n-6 PUFAs, in particular arachidonic acid.[2] The present finding of a strong correlation between these species in muscle and erythrocyte membranes shows that measurement of the fatty acid composition of erythrocyte membranes can provide an effective surrogate for skeletal muscle cell membranes. The relevance of such measurements to studies of insulin resistance is further supported by our observation that, for those with low/normal S_I, the strongest correlate of S_I was erythrocyte membrane arachidonic acid concentration. The importance of arachidonic acid in these associations is also apparent in the study of Clifton and Nestel in which a high proportion of arachidonic acid in erythrocyte membranes was found to be associated with low fasting insulin concentrations.[34] Our study extends this observation with the finding that proportions of arachidonic acid in

Table 4. Erythrocyte Fatty Acid Proportions Among Healthy Men With Low/Normal S_I (n = 22) and High S_I (n = 8) and Their Associations With S_I

	Erythrocyte Fatty Acid Proportion (%)		Correlation for S_I and Erythrocyte Membrane Lipid Proportion	
Fatty Acid	Low/Normal S_I	High S_I	Low/Normal S_I	High S_I
16:0	24.9 (3.1)	24.7 (2.9)	−0.06	−0.14
18:0	15.4 (2.2)	16.4 (1.2)	−0.32	0.07
18:1n9	21.0 (1.2)	22.0 (1.8)	0.12	0.05
18:2n-6	12.9 (1.9)	12.0 (1.3)	−0.05	0.17
18:3n-6	0.1 (0.0)	0.2 (0.1)*	−0.20	0.31
18:3n3	0.2 (0.1)	0.3 (0.2)*	0.15	0.36
20:4n-6	16.9 (3.4)	15.1 (1.4)	0.18	0.07
20:5n3	1.1 (0.4)	1.2 (0.2)	−0.04	−0.35
22:5n3	2.5 (0.5)	2.7 (0.4)	0.12	−0.10
22:6n3	5.0 (1.1)	5.3 (1.1)	−0.14	0.00
All SFA	40.3 (3.9)	41.1 (2.0)	−0.21	0.07
All n-6-PUFA	29.9 (4.8)	27.3 (0.8)	0.05	0.29
All n3-PUFA	8.9 (1.8)	9.6 (1.4)	0.02	−0.17
n-6 + n3 PUFA	38.2 (2.9)	36.9 (1.5)	0.11	0.12
C20-C22 PUFA	25.5 (2.6)	24.3 (2.4)	0.28	0.07

Significant differences (Mann-Whitney): *$P < .05$.

erythrocyte membranes correlate well with those of muscle membranes, and that erythrocyte membrane arachidonic acid content correlates with a direct measure of insulin sensitivity.

Surprisingly, however, we found no association between S_I and the proportions of arachidonic acid, or any other fatty acid, in erythrocyte membranes. It might be expected that higher concentrations of arachidonic acid would parallel higher proportions, but this assumes a relatively constant total fatty acid concentration in erythrocyte membranes. A novel observation in our study was that the total amounts of fatty acids in erythrocyte membranes varied. We also found (results not shown) that concentrations of individual fatty acids increased in proportion to that of total fatty acids. Therefore, arachidonic acid concentrations rose with total fatty acid concentrations. Consequently, arachidonic acid proportions, and proportions of other fatty acids, remained relatively unchanged. The effect of this is apparent in our observation of significantly lower fatty acid concentrations in people with high S_I compared with those with low/normal S_I, but no difference in fatty acid proportions. The association between erythrocyte membrane fatty acid concentration and insulin sensitivity is itself a novel observation, for which we have no ready explanation. Circulating very-low-density lipoprotein concentrations decrease with increasing insulin sensitivity,[35] which might lead to decreasing availability of phospholipids. However, appreciably more investigation of this issue will be necessary before a clear understanding emerges.

Several factors might contribute to the dichotomy we observed between the positive association between erythrocyte membrane arachidonic acid concentration and S_I in individuals with low/normal S_I and the very low arachidonic acid concentrations in those with high S_I. Dietary influences among health-conscious individuals with high S_I could include increased proportions of oleic acid in the diet at the expense of the arachidonic acid precursor, linoleic acid. Alternatively, increased consumption of n-3 fish oils could diminish arachidonic acid availability by competition for the desaturase/elongase pathways that n-3 and n-6 fatty acid metabolism share. We also found evidence for significantly lower delta 5 desaturase activity in individuals with high S_I, which would have the effect of impairing conversion of the 20:3 n-6 precursor to arachidonic acid. However, our finding of a negative relationship between delta 5 desaturase activity and S_I does contrast with the significant positive association reported by others.[2,4] In these studies, desaturase activities were estimated on the basis of proportions rather than concentrations of membrane fatty acids. A better understanding of variation in membrane fatty acid concentration and its influence on proportions of membrane fatty acids may be necessary before these discrepancies can be resolved. Further clarification is also needed with regard to the role of insulin in the control of desaturase activity. For example, in experimental studies, insulin has been shown to induce delta 6 desaturase gene expression,[9] whereas insulin sensitization appears to have the opposite effect.[8]

It has been proposed that increases in membrane fluidity associated with increasing degrees of membrane fatty acid unsaturation affect responsiveness to insulin. However, if this were the principal mediator of the associations seen, a continuous increase would be expected in the magnitude of the correlation between insulin sensitivity and membrane fatty acids of successively increasing unsaturation. This was not apparent. Instead, it was specifically the concentration of arachidonic acid in the membrane that correlated with insulin sensitivity. This accords with the observation of Nugent et al that, although there appears to be a generally stimulatory effect of PUFAs on insulin-stimulated glucose uptake by isolated adipocytes, arachidonic acids have a markedly greater effect than other PUFAS and this involves peroxisome proliferator-activator receptor gamma (PPARγ) rather than changes in membrane fluidity.[10]

Insulin resistance is a risk factor for diabetes. Accordingly, given the extent to which insulin resistance and membrane fatty acid content are related, variation in membrane fatty acids might influence the risk of diabetes. In a large prospective study, Vessby et al compared serum cholesterol ester fatty acid composition between those who did and did not develop diabetes and found a higher proportion of unsaturated fatty acids in those who developed the disease.[36] However, the principal fatty acids concerned were 18:3n-6, gamma linolenic acid, and 20:3n-6, di-homo-gamma linolenic acid, which have not been consistently linked with variation in insulin sensitivity. A better understanding of the relationships between the fatty acid composition of the different serum lipids and the fatty acid composition of different tissues will be necessary to resolve these issues. Moreover, our finding that cell membrane fatty acid concentrations can vary and that this variation is linked with variation in insulin sensitivity suggests that greater attention will need to be given to different quantitative measures of membrane fatty acids and their inter-relationships. Finally, our finding of low arachidonic acid concentrations in individuals with high S_I, despite a positive association between arachidonic acid concentration and S_I in those with low/normal S_I, suggests a need for studies to define the relative ranges of insulin sensitivities being investigated.

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