Is the Erythrocyte Membrane Fatty Acid Composition a Valid Index of Skeletal Muscle Membrane Fatty Acid Composition?

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Recent studies suggest that insulin sensitivity is related to the fatty acid composition of phospholipids in skeletal muscle (SM) membranes. Since it is difficult to obtain muscle biopsies, it may be useful to have information on the fatty acid composition using more accessible cells such as erythrocytes. This would be possible only if the composition of erythrocyte and muscle membranes are very similar. Since no comparative data are available, we evaluated the phospholipid fatty acid composition of erythrocyte and SM membranes in 16 individuals, 10 nondiabetics (male to female ratio, 4:6; age, 50 ± 11 years; body mass index, 27 ± 5 kg/m^2; mean ± SD) and 6 type 2 diabetic patients (male to female ratio, 2:4; age, 64 ± 5 years; body mass index, 27 ± 4 kg/m^2). All patients underwent abdominal surgery, during which a biopsy of the abdominal rectus muscle (50 to 100 mg) was obtained. Erythrocyte and SM phospholipid fatty acids were extracted and then methylated; the methyl fatty acids were separated and quantified by gas chromatography. Compared with erythrocyte membranes, muscle membranes showed a significantly higher proportion of ω-6 polyunsaturated fatty acid ([PUFA] 43.0% ± 3.1% *v* 29.7% ± 1.6%, *P* < .001) and lower saturated fatty acid ([SFA] 41.1% ± 1.5% *v* 43.4% ± 1.2%, *P* < .001), monounsaturated fatty acid ([MUFA] 11.5% ± 1.7% *v* 20.0% ± 1.9%, *P* < .001), and ω-3 PUFA (3.8% ± 0.6% *v* 7.4% ± 1.0%, *P* < .001). The greatest increase involved linoleic acid (26.9% ± 2.8% *v* 10.3% ± 1.6%, *P* < .001), whereas lignoceric acid (0.8% ± 0.2% *v* 5.0% ± 0.6%, *P* < .001) and oleic acid (10.4% ± 1.6% *v* 13.5% ± 1.3%, *P* < .001) were significantly lower. These results show that erythrocyte and muscle membrane phospholipid fatty acids are significantly different. Therefore, data on SM membranes cannot be extrapolated on the basis of measures of erythrocyte phospholipid fatty acid composition.

RECENT STUDIES indicate that peripheral insulin sensitivity can be influenced by the dietary fatty acid composition and consequently by the phospholipid fatty acid composition of skeletal muscle (SM) membranes. Studies in rats[1] have shown that a diet rich in ω-3 polyunsaturated fatty acids (PUFA) has a beneficial effect on insulin resistance and that insulin sensitivity is strongly related to the proportion of SM phospholipid long-chain ω-3 fatty acids. The few studies in humans have shown, on one hand, a positive relationship between insulin sensitivity and the percentage of phospholipid long-chain PUFA in SM membranes[2,3] and, on the other hand, an inverse relationship with the percentage of saturated fatty acids (SFA).[2,4]

Since cellular membrane fatty acids are derived from the plasma pool of fatty acids—which itself derives mainly from dietary fats—it may be hypothesized that all cellular membranes have a similar fatty acid composition. Since it is difficult to obtain SM specimens, it would be easier to study more accessible cells such as red blood cells (RBCs). This hypothesis must be validated by comparative data, which are not currently available. Therefore, the aim of this study was to compare the membrane fatty acid composition of SM and RBCs. As a secondary aim, we also tried to verify whether the SM membrane fatty acid composition is related to indices of insulin sensitivity, as previously reported.[2-4]

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SUBJECTS AND METHODS

Patients

After provision of informed consent, 16 subjects (10 nondiabetics and 6 type 2 diabetic patients according to the World Health Organization classification)[5] requiring abdominal surgery participated in the study. Patients with neoplasms, cachexia, severe infection, jaundice, or severe cardiovascular disease were excluded from the study. Diabetic subjects were treated with diet alone (n = 2) or diet plus glucose-lowering medications (n = 4) and were in good metabolic control. The protocol was approved by the Ethics Committee of Federico II University (Naples, Italy).

Biological Samples

The day before surgery, fasting blood samples were collected to evaluate serum cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol, plasma glucose and insulin, and erythrocyte fatty acid composition. During surgery, specimens (50 to 100 mg) of the abdominal rectus muscle were also obtained from each subject. Packed RBCs, obtained from EDTA-K_3 venous blood samples after isolating the plasma and buffy coat, were immediately frozen at −80°C after addition of 5 μL/mL butylated hydroxytoluene 2% (BHT) in methanol (wt/vol) + N_2.[6] Skeletal muscle samples were washed in a saline solution and immediately stored in N_2 at −80°C until analysis.

Assays

Serum total cholesterol and triglycerides were analyzed on a Cobas Mira (Roche, Basel, Switzerland) analyzer with enzymatic colorimetric methods using commercially available kits (Boehringer, Mannheim, Germany).[7,8] HDL cholesterol was evaluated after precipitation of all other lipoprotein classes with phosphotungstate sodium and magnesium chloride.[9] Blood glucose was evaluated with the enzymatic glucose-hexokinase method (Roche).[10] Insulin was evaluated using a radioimmunologic method (Technogenetics, Milan, Italy).[11]

Membrane phospholipid fatty acid. Specimens of muscle (30 to 50 mg) were homogenated in a chloroform:methanol (2:1 vol/vol) and BHT 0.01% (wt/vol) ice-cold solution, and total lipids were extracted by the Folch procedure.[12] Phospholipids were then isolated by thin-layer chromatography.[13] Two hundred microliters of RBCs were

hemolyzed with distilled water, and total lipids were extracted with the same procedure as for muscle samples. Both muscle and erythrocyte phospholipids were transmethylated with 1 mL benzene and 5 mL 2% H_2SO_4 in methanol at 65° to 70°C for 18 hours[14]; the fatty acid methylesters (FAMEs) were then extracted with *n*-hexane.

Both muscle and erythrocyte FAMEs were separated and measured on a Hewlett-Packard (Palo Alto, CA) 5890 gas chromatograph equipped with a 30-m Omegawax 320 capillary column (Supelco, Bellefonte, PA). Dried helium was used as a carrier gas (linear velocity, 24 cm/s; split ratio, RBCs 30:1 and SM 5:1). The initial column temperature was 200°C for 18 minutes and the increase rate was 1°C/min until 215°C for 10 minutes. The temperature was 250°C at the injection port and 260°C at the hydrogen flame ionization detector. Fatty acids were identified by comparing their retention times against those of the Supelco standard mixtures (PUFA, GLC 1100, and rapeseed oil).

All RBC and SM samples were examined in triplicate. A coefficient of variation (CV) less than 3% was accepted for fatty acids at concentrations greater than 5%; for lower concentrations (<5% and <1%), a CV less than 5% and less than 10%, respectively, was accepted.

Statistical Analysis

Results for fatty acids are expressed as a percentage of total fatty acids. Differences between SM and erythrocytes were evaluated using the *t* test for paired data.[15] Furthermore, linear regression analysis was used to evaluate the possible relationship between muscle fatty acids and insulin resistance, evaluated as both fasting serum insulin and homeostasis model assessment (HOMA).[16]

RESULTS

The clinical characteristics of the subjects are shown in Table 1. There were no significant differences in the 2 groups, although diabetic patients tended to be older and to have higher serum cholesterol, triglycerides, and plasma glucose. All data concerning SM and RBC fatty acid composition for diabetic and nondiabetic patients were completely superimposable, and they were therefore considered as 1 group. The content of total SFA was higher in RBCs versus SM cells (43.4% ± 1.2% *v* 41.1% ± 1.5%, $P < .001$; Table 2). This was mostly due to an increase in long-chain SFA (behenic and lignoceric acid). Total monounsaturated fatty acids (MUFAs) were higher in RBCs than in SM cells (20.0% ± 1.9% *v* 11.5% ± 1.7%, $P < .001$); the increase involving mainly oleic and nervonic acid (Table 2). Total ω-3 PUFAs were significantly higher in RBCs versus SM cells (7.4% ± 1.0% *v* 3.8% ± 0.6%, $P < .001$) due to significant enrichment in long-chain fatty acids (docosapentaenoic and docosahexaenoic acid).

A significant reduction of ω-6 PUFA was found in RBCs compared with SM cells (29.7% ± 1.6% *v* 43.0% ± 3.1%, $P < .001$), which is mostly accounted for by significantly lower concentrations of linoleic acid (10.3% ± 1.6% *v* 26.9% ± 2.8%, $P < .001$). Also for this class of fatty acids, RBCs are characterized by higher concentrations of long-chain fatty acids (docosatetraenoic and docosapentaenoic acid) compared with SM cells.

There were no significant correlations between the percentage of fatty acids largely present in SM and insulin resistance evaluated as fasting insulin and as HOMA, both in the study population as a whole (Table 3) and in the 2 groups (diabetic and nondiabetic patients) considered separately (data not shown).

Table 1. Characteristics of the Participants (mean ± SD)

Characteristic	Controls	Type 2 Diabetics
No. of subjects	10	6
Male/female ratio	4/6	2/4
Age (yr)	50 ± 11	64 ± 5
BMI (kg/m^2)	27 ± 5	27 ± 4
Fasting plasma glucose (mmol/L)	5.5 ± 1.1	6.7 ± 1.8
Plasma insulin (μU/mL)	8 ± 3	10 ± 5
Serum cholesterol (mmol/L)	5.3 ± 1.6	6.4 ± 1.0
Serum triglycerides (mmol/L)	1.5 ± 0.7	1.9 ± 1.1
HDL cholesterol (mmol/L)	1.1 ± 0.3	1.1 ± 0.2

Table 2. Fatty Acid Composition of RBCs and SM Cells (%, mean ± SD)

Fatty Acid	RBCs	SM Cells
Palmitic acid C16:0	22.5 ± 1.0	22.7 ± 1.4
Stearic acid C18:0	13.4 ± 0.8	16.3 ± 1.6*
Behenic acid C22:0	1.9 ± 0.2	0.7 ± 0.1*
Lignoceric acid C24:0	5.0 ± 0.6	0.8 ± 0.2*
Total SFA	43.4 ± 1.2	41.1 ± 1.5*
Palmitoleic acid C16:1 ω-7	0.7 ± 0.2	1.0 ± 0.2*
Oleic acid C18:1 ω-9	13.5 ± 1.3	10.4 ± 1.6*
Nervonic acid C24:1 ω-9	5.5 ± 1.0	NF*
Total MUFA	20.0 ± 1.9	11.5 ± 1.7*
Linoleic acid C18:2 ω-6	10.3 ± 1.6	26.9 ± 2.8*
Arachidonic acid C20:4 ω-6	14.1 ± 1.0	12.7 ± 2.0†
Docosatetraenoic acid C22:4 ω-6	2.8 ± 0.6	0.9 ± 0.2*
Docosapentaenoic acid C22:5 ω-6	0.4 ± 0.1	0.3 ± 0.1*
Total PUFA ω-6	29.7 ± 1.6	43.0 ± 3.1*
Eicosapentaenoic acid C20:5 ω-3	0.5 ± 0.2	0.5 ± 0.1
Docosapentaenoic acid C22:5 ω-3	1.6 ± 0.2	1.2 ± 0.2*
Docosahexaenoic acid C22:6 ω-3	5.2 ± 0.9	1.9 ± 0.6*
Total PUFA ω-3	7.4 ± 1.0	3.8 ± 0.6*

Abbreviation: NF, not found.
*$P < .001$, †$P < .01$, SM *v* RBCs.

DISCUSSION

This study clearly demonstrates that the fatty acid composition of RBC and SM membrane phospholipids is significantly different. The former are characterized by significantly lower concentrations of ω-6 PUFAs, mainly due to a reduction of linoleic acid by almost one third. On the other hand, compared with SM,

Table 3. Simple Linear Correlations (*r*) Between SM Fatty Acids and Indices of Insulin Resistance

SM Fatty Acid	Fasting Serum Insulin	HOMA
SFA	−.011	.030
C16:0	.045	−.042
C18:0	−.004	.124
MUFA	−.065	.093
C16:1 ω-7	.093	.150
C18:1 ω-9	−.077	.082
ω-6 PUFA	.008	−.080
C18:2	.108	−.064
C20:3	.384	.396
C20:4	−.236	−.153
C22:4	.167	.260
C22:5	.136	.168
ω-3 PUFA	−.054	−.104
C20:5	−.030	−.031
C22:5	.177	.181
C22:6	−.106	−.160

RBC membranes have a higher concentration of long-chain fatty acids (>18C) in SFA, MUFA, and ω-3 or ω-6, PUFA.

Since the fatty acids of different membranes of the body are derived from the same pool of fatty acids, ie, plasma—which itself derives mainly from dietary fats—the clear differences in the fatty acid composition of RBCs and SM cells found in this study were unexpected. These data imply that the uptake of fatty acids from the plasma pool is specific for different tissue types and depends on their function. Recently, it has been shown that there are different families of fatty acid–binding proteins in humans, with differing ability to bind fatty acids. More specifically, it has been shown that the fatty acid–binding protein in muscles is able to bind a greater amount of some fatty acids, including linoleic acid, compared with the same protein of hepatic origin.[17,18] These differences could explain the increased content of linoleic acid in SM in our study.

Moreover, the differences in fatty acid composition between RBCs and SM cells may also be due, at least in part, to the different phospholipid composition of these 2 types of cells. In act, RBCs are richer in sphingomyelin, which preferentially esterifies long-chain fatty acids, while SM cells are richer in cardiolipin, which instead preferentially esterifies linoleic acid.[19-21]

Whatever the explanation for the variations between RBC and SM fatty acids, our results clearly demonstrate many differences in RBC and SM fatty acid composition, and thus, SM membrane composition cannot be studied using the more accessible RBCs. This conclusion may be less valid for 2 fatty acids, palmitic and eicosapentaenoic, whose content is very similar in RBCs and SM. Therefore, RBC composition may be considered a satisfactory index of SM composition only for these 2 fatty acids.

Concerning the secondary aim of the study, we did not observe any relation between SM fatty acids and indices of insulin resistance (both fasting plasma insulin and HOMA). This result is in disagreement with the few data in the literature. As a matter of fact, other studies show an inverse relationship between the percentage of palmitic acid in the SM and insulin sensitivity in the elderly.[4] Furthermore, they show a direct correlation between insulin sensitivity and the percentage of PUFAs in the muscle of both normal and insulin-resistant subjects (affected by coronary heart disease).[2]

Such differences could be due to the fact that the relation between SFA and PUFA and insulin sensitivity may be evident only in populations with a high dietary intake of these fatty acids. In our population, the dietary intake of both SFA and PUFA is lower versus Australian and Scandinavian populations.[22] Therefore, at least at these low levels, SFA and PUFA may not be important for the regulation of insulin action. However, it would be important to study a larger number of people with a much more variable fatty acid intake.

In conclusion, our study clearly demonstrates that the RBC membrane fatty acid composition is not a valid index of the fatty acid composition of SM membranes. Therefore, further studies to evaluate the possible relationship between membrane composition and insulin action should use SM cells directly.

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