Erythrocyte Membrane Fatty Acids Profile in Colorectal Cancer Patients: A Preliminary Study

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Abstract. *Background/Aim: The study of fatty acid composition of the erythrocytes' membrane can be considered an appropriate biomarker for investigating the relations of the pattern of fatty acids metabolism to a specific disease. The aim of the present study was to analyze the fatty acid profile in red blood cell membranes of patients with colorectal cancer (CRC). Materials and Methods: Thirteen patients with CRC and 13 patients with no malignant disease were enrolled in the study. Fatty acids were extracted from erythrocytes' membranes and quantified by gas chromatography. The data were analyzed using the Mann-Whitney test. Results: Patients with CRC showed significantly lower mean percentage of n-3 polynunsatured fatty acids (n-3 PUFAs) than controls (5.1% vs. 8.0%, respectively). This finding was reflected in the higher ratio n-6-PUFA/n-3-PUFA observed in cancer patients. Conclusion: Lipidomic analysis of erythrocyte membranes confirmed the presence of an altered fatty acid profile in patients with CRC.*

Colorectal cancer (CRC) evolves through a multi-step process and is a metabolic disease, since cancer cells re-program their metabolic pathways to support their accelerated proliferation rate (1). One of the most well-known metabolic changes in colon cancer is alteration of lipid metabolism, believed to be important for the initiation and progression of tumor (2-4).

To date, the majority of research on lipid metabolism in CRC has focused on the increase of fatty acid synthesis (1-3). The alteration of lipid metabolism in cancer is down-stream of many oncogenes promoting the expression and activity of enzymes involved in fatty acid synthesis (4-8). Fatty acid synthase (FAS) is a key enzyme in *de novo* lipogenesis (8). Altered expression of FAS appears to be an important process required by many transformed cells for growth and survival in different types of tumor (9-11), including colon cancer (11, 12).

Previously, we have demonstrated that FAS activity levels, as well as the expression of its mRNA are up-regulated in colorectal cancer tissues (5). There are several lines of evidence suggesting that FAS and other lipogenic enzymes, such as lipoprotein lipase (LPL), have a role in tumor progression (13). LPL activity has been shown to be highest in areas containing actively proliferating cells in a variety of human sarcomas and carcinomas (14).

Essential fatty acids and their polyunsaturated derivatives have a structural role in cell membranes, influencing their fluidity and membrane enzyme activities (15). The study of fatty acid composition of the erythrocyte membranes can be considered an appropriate biomarker for investigating not only dietary intake but also the relations between the patterns of fatty acid metabolism and specific diseases. The polyunsaturated fatty acid (PUFA) content of red blood cell membranes has been demonstrated to be related with breast cancer (16-18).

Decreased levels of essential fatty acids, linoleic acid and α-linolenic acid, as well as of their derivatives have been found in plasma and red blood cells from patients with gastrointestinal cancer (15).

Moreover, dietary consumption of fish oil, rich in n-3-PUFAs is associated with a reduced risk of human colorectal cancer (19, 20). These findings are supported by studies showing a growth inhibitory effect of n-3-PUFAs in cancer cells (21-23) and in patients with familial adenomatous polyposis (FAP) (20). Recently, we demonstrated that PUFAs administration in the diet was responsible for reduction of polyp numbers and volume in $Apc^{Min/+}$ mice (24, 25).

The present study was designed to analyze the fatty acid profile in red blood cell membranes of patients with CRC, considering that changes in fatty acids composition of erythrocyte membranes may be related to illness status.

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Materials and Methods

Patients and blood sampling. Twenty-six consecutive patients (9 females and 17 males, mean age 64.6±10.8 years) were recruited from subjects hospitalized at the Division of Surgery of our Institute.

Thirteen patients with histologically-proven colorectal cancer (cases) and 13 patients with no malignant disease (controls) were invited to give a blood sample. The controls included 3 subjects with a diagnosis of inguinal hernia, 4 with a diagnosis of gallbladder stones, 3 with a colon diverticulosis and 3 with a hemorrhoidal prolapse. Participants were fasted for 12 h prior to examination. Blood samples taken from the subjects by venous puncture were collected in tubes containing Ethylenediaminetetraacetic Acid (K-EDTA) anticoagulant. For *in vitro* isolation of erythrocytes, blood samples with K-EDTA were quickly layered on a Ficoll-Paque solution and centrifuged at 400 × *g* for 40 min at 20˚C. The lymphocytes and plasma were then removed and the erythrocytes were recovered from the bottom layer and washed with 4-volumes of phosphate-buffered saline. Isolated red blood cells were stored at –80˚C until assayed. All the analyses were performed within 3 months.

No study participant took drugs known to affect lipid metabolism or hormone replacement therapy. No patients enrolled in the study had subjective complaints of appetite loss, reduced food intake, weight loss or abnormal liver function tests. All subjects underwent a complete medical examination including weight and height measurements. Body-mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2). The study was approved by the Ethics Committee of IRCCS "S. de Bellis", Castellana Grotte (BA-Italy) and all patients gave informed consent.

Reagents and standards. Chloroform, methanol, toluene, n-hexane, hydrochloric acid ACS reagent 37%, boron trifluoride in methanol ≥99.5%, sodium sulfate and sodium chloride were obtained from Sigma-Aldrich (Milan, Italy). The Ficoll-Paque PLUS reagent was obtained from GE Heathcare (Uppsala, Sweden).

Larodan 37 FAME Mix (Larodan Fine Chemicals, 90-1100 Mixture ME 100), methyl hexadecenoate 7(Z) (Larodan Fine Chemicals), methyl palmitelaidate (9,E) (Larodan Fine Chemicals), methyl vaccinate (11,Z) (Larodan Fine Chemicals), methyl docosapentanoate (7Z,10Z,13Z,16Z,19Z) (Larodan Fine Chemicals) were provided from LABSERVICE srl (Rome, Italy) Fatty acid methyl ester isomer mix was prepared by dissolving fatty acids methyl esters in n-hexane, stored at –20˚C and used for qualitative and quantitative analysis.

Fatty acids extraction, purification and preparation of Fatty Acid Methyl Esters. We used the modified method of Moilanen (26), that is itself a modification of the method described by Folch (27).

Each sample of red blood cells (RBC) was thawed to room temperature. Fatty acids were hydrolyzed from phospholipids of RBC membranes by adding 0,9 ml of an acidified salt solution (H_2SO_4 $2 \cdot 10^{-4}$ M, NaCl 0,1%). The samples were extracted by centrifugation at 1,500 ×*g* for 10 min with 3 ml of chloroform and 1.5 ml of methanol. The lower layer, containing fatty acids, was removed with care, replaced in a new tube and dried by a centrifugal evaporator (Bio-Rad, Milan, Italy).

Preparation of fatty acid methyl esters was carried out by adding 1 ml of toluene and 1,5 ml of BF_3·MeOH 14% and incubating for 2 h at 90˚C. To the samples were added 2,5 ml of 5% aqueous sodium chloride solution and they were centrifuged at 900 × *g* for 10 min. The upper toluene layer was recorded, drown off into a vial and analyzed.

Table I. *Clinical characteristics of subjects enrolled in the study.*

	Cancer patients (n=13)	Control subjects (n=13)
Age (years)	67.08±10.5	62.1±11.2
Female/male	4/9	5/8
BMI (Kg/m^2)*	27.97±4.2	27.87±7.2
Total proteins (g/l)	6.4±0.5	6.3±0.5
Albumin (g/l)	3.4±0.5	3.5±0.2

*BMI: Body mass index. Values expressed as mean±SD.

Gas chromatography and fatty acids quantification. Gas chromatography analyses were performed by a Thermo Scientific instrument with auto-sampler, a split/splitless injector, FID detector and an hydrogen gas generator (Thermo Fisher Scientific, Milan Italy). Separation of FAME was carried-out on a BPX 70 capillary column (SGE Analytical Science, P/N SGE054623, 60 m × 0,25 mm ID – BPX70 0,25UM (SGE Europe Ltd, United Kingdom). Hydrogen was used as carrier gas, 3.0 ml min^{-1}, constant flow mode; the amount injected was 1 μl in splitless mode (split flow 50 ml min^{-1}, splitless time 1 min). The temperature of the injector and the FID detector were 250˚C and 270˚C, respectively. The initial temperature of the oven was 40˚C, then it increased to 170˚C at 10˚C min^{-1} for 5 min, then to 200˚C at 4˚C min^{-1} for another 5 min, and finally the temperature increased to 255˚C, at 50˚C min^{-1} and held for 4,5 min.

Quantification of the methyl esters of fatty acids extracted was performed by the use of a mixture of the above-mentioned standards. Four solutions of increasing concentration of the mix of standards were prepared; each solution was analyzed three times and the resulting chromatograms, were analyzed using the Clarity Lite software, were used to build the calibration curve for each component of the mixture. The standard of C22:5n3 (DPA) was not available but it was quantified using the same response factor of the calibration curve of C22:6n3 (DHA).

Statistical analysis. Median, mean and standard deviation (SD) were calculated for each group. Data were analyzed using the Mann-Whitney test. Differences were considered significant at $p<0.05$.

Results

Clinical characteristics of the patients enrolled in the study are shown in Table I. Histologically, all tumors were adenocarcinomas and they were classified according to their grade of histologic differentiation and tumor stage. All tumors were moderately differentiated, G2; clinical staging was performed using the staging system of the International Union against Cancer. Two patients were in Stage I, 4 in Stage II and 7 in Stage III. BMI, age and nutritional status,

Table II. *Mean percentage of fatty acid composition of red blood cell membranes in cancer patients and controls.*

			Cancer patients (n=13)		Controls (n=13)		*p*-Value*
Lipid name	Common name	Systematic name	Mean %	SD	Mean %	SD	
C14:0	Myristic acid	tetradecanoic acid	0.45	0.14	0.46	0.1	ns
C14:1	Myristoleic acid	9-tetradecenoic acid					
C15:0	Pentadecanoic acid		0.15	0.1	0.2	0.1	ns
C16:0	Palmitic acid	hexadecanoate	31.8	3.4	30.00	4.1	ns
C16:1(trans)	Palmitelaidic acid	*trans*-9-hexadecenoic acid	nd		0.01	0,01	
C16:1n7	Palmitoleic acid	*cis*-9-hexadecenoic acid	0.29	0.16	0.29	0.1	ns
C17:0	Margaric acid	heptadecanoic acid	0.37	0.08	0.41	0.10	ns
C18:0	Stearic acid	octadecanoic acid	17.2	3.2	16.2	1.6	ns
C18:1(trans)	Elaidic acid	*trans*-octadec-9-enoic acid	0.2	0.1	0.15	0.1	ns
C18:1n9	Oleic acid	*cis*-9-octadecenoic acid	14.60	1.86	14.00	2.5	ns
C18:1n7	Vaccenic acid	*trans*-octadec-11-enoic acid	1.2	0.2	1.1	0.2	ns
C18:2(trans)	Linoelaidic acid	all-*trans*-octadeca-9,12-dienoic acid	0.43	1.45	5.28	6.1	0.02
C18:2n6	Linoleic acid	all-*cis*-9,12-octadecadienoic acid	7.6	1.41	7.3	1.7	ns
C18:3n6	γ-Linolenic acid (GLA)	all-*cis*-6,9,12-octadecatrienoic acid	0.02	0.04	4.1	6.3	0.009
C18:3n3	α-Linolenic acid (ALA)	all-*cis*-9,12,15-octadecatrienoic acid	0.05	0.04	0.12	0.1	ns
C20:0	Arachidic acid	eicosanoic acid	0.31	0.24	0.41	0.13	ns
C20:2n6	Eicosadienoic acid all-*cis*-11,14-eicosadienoic acid		0.13	0.27	0.11	0.10	ns
C20:3n6	Dihomo-gamma-linolenic acid (DGLA)	all-*cis*-8,11,14-eicosatrienoic acid	1.27	0.39	1.17	0.47	ns
C20:4n6	Arachidonic acid (AA)	all-*cis*-5,8,11,14-eicosatetraenoic acid	11.10	4.14	10.05	4.58	ns
C20:3n3	Eicosatrienoic acid (ETE)	all-*cis*-11,14,17-eicosatrienoic acid	0.00	0.00	0.07	0.09	ns
C20:5n3	Eicosapentaenoic acid (EPA, Timnodonic acid)	all-*cis*-5,8,11,14,17-eicosapentaenoic acid	0.46	0.36	0.44	0.24	ns
C22:0	Behenic acid	docosanoic acid	0.75	0.21	1.03	0.60	ns
C22:2n6	Docosadienoic acid	all-*cis*-13,16-docosadienoic acid	0.1	0.09	0.18	0.07	0.04
C22:5n3	Docosapentaenoic acid (DPA,Clupanodonic acid)	all-*cis*-7,10,13,16,19-docosapentaenoic acid	1.35	0.63	4.00	2.03	0.0003
C22:6n3	Docosahexaenoic acid (DHA, Cervonic acid)	all-*cis*-4,7,10,13,16,19-docosahexaenoic acid	3.63	1.80	3.22	1.83	ns
C24:0	Lignoceric acid	tetracosanoic acid	2.05	0.61	2.61	1.22	ns
Saturated fatty acids (SFAs)			53.7	7.8	51.5	5.9	ns
Monounsaturated fatty acids (MUFAs)			19.9	3.3	20.7	5.0	ns
Polyunsaturated fatty acids (PUFAs)			26.4	7.7	27.8	7.1	ns
n-9 PUFAs			1.0	2.1	0.6	1.5	ns
n-6 PUFAs			20.3	5.7	19.2	5.9	ns
n-3 PUFAs			5.1	2.5	8.0	1.9	0.008
Saturation index			1.2	0.4	1.2	0.3	ns
SFA/MUFA			2.8	0.6	2.7	0.7	ns
AA/DHA			3.05	2.3	3.1	2.1	ns
n-6/n-3 (ω-6/ω-3)			4.0	1.2	2.7	1.0	0.01

*Mann-Whitney test, differences were considered significant at $p<0.05$; ns: no significant; nd: no detected.

evaluated using biochemical parameters, were not different between case and control subjects (Table I).

Table II shows the mean percentage fatty acid composition of red blood cell membranes in cases and controls.

Compared to control subjects, patients with CRC had lower percentages of linoelaidic acid, α- and γ-linolenic acid (ALA and GLA, respectively), docosadienoic acid and docosapentaenoic acid (Table II).

CRC patients showed significantly lower percentage of n-3-PUFA composition than controls (5.1% *vs.* 8.0%, respectively) (Table II). This finding was reflected in the higher ratio n-6-PUFA/n-3-PUFA observed in cancer patients with respect to control subjects (Table II). No difference was observed in the percentage of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and total PUFAs between CRC patients and controls (Table II). Consequently, the ratio SFAs/MUFAs was similar between the two groups.

Moreover, we determined the saturation index (SI) as the ratio between stearic acid and oleic acid (Table II). No difference was detected between cases and controls in SI value, known to be an indicator of membrane fluidity.

Discussion

The most significant finding of the study was that the fatty acid profile of the erythrocyte membranes differed in patients with CRC in comparison to subjects with no malignant disease.

CRC patients showed a 2.5-times lower percentage of ALA than controls, justifying a decrease of total *n*-3-series PUFAs. Consequently a higher ratio n-6-PUFA/n-3-PUFA was present in cancer patients in respect to control subjects.

It is well-known that a high n-6/n-3 ratio predisposes to inflammation (28-30) and this evidence let us hypothesize that intrinsic abnormalities in fatty acid profile could support the inflammation linked to cancer development.

Our results are consistent with those of other studies demonstrating low levels of red blood cell membrane n-3 PUFAs in patients with gastrointestinal cancers or colon adenomas (15, 31).

Our cancer patients showed significantly lower levels of DPA in erythrocyte membranes, suggesting a role for this fatty acid in neoplastic transformation of colonic mucosa.

The interesting question, whether the fatty acid composition of erythrocyte membranes reflects the fatty acid composition of other tissues, remains largely unclear. Different studies demonstrate that the fatty acid composition of membranes differed from tissue to tissue (32), while others suggest that the fatty acid composition of human erythrocyte membranes may reflect fatty acid composition in other body tissue (33).

The differences in membrane composition detected in this study between cases and controls are certainly indexes of an altered lipid metabolism in cancer patients, explaining an overall reduction of total n-3 PUFAs. The change in n-3 PUFAs levels in these patients appears to be related to metabolic alterations caused by the cancer *per se* rather than to malnutrition as biochemical and anthropometric parameters were similar in the groups considered.

In conclusion, lipidomic analysis of erythrocyte membranes confirmed the presence of an altered fatty acid profile in patients with CRC.

Clearly, this is a preliminary study and further studies on a larger number of patients are required to evaluate the relationship between red blood cell membrane fatty acid composition and colon cancer pathogenesis.

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References

1 Zhang F and Du G: Dysregulated lipid metabolism in cancer. World J Biol Chem *3(8)*: 167-174, 2012.
2 Menendez JA and Lupu R: Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. Nat Rev Cancer *7*: 763-777, 2007.
3 Cairns RA, Harris IS and Mak TW: Regulation of cancer cell metabolism. Nat Rev Cancer *11*: 85-95, 2011.
4 Tennant DA, Duran RV and Gottlieb E: Targeting metabolic transformation for cancer therapy. Nat Rev Cancer *10*: 267-277, 2010.
5 Notarnicola M, Altomare DF, Calvani M, Orlando A, Bifulco M, D'Attoma B and Caruso MG: Fatty acid synthase hyperactivation in human colorectal cancer: relationship with tumor side and sex. Oncology *71*: 327-332, 2007.
6 Notarnicola M, Tutino V, Calvani M, Lorusso D, Guerra V and Caruso MG: Serum levels of Fatty Acid Synthase in colorectal cancer patients are associated with tumor stage. J Gastrointestinal Cancer *43(3)*: 508-511, 2012.
7 Notarnicola M, Miccolis A, Tutino V, Lorusso D and Caruso MG: Low levels of lipogenic enzymes in peritumoral adipose tissue of colorectal cancer patients. Lipids *47(1)*: 59-63, 2012.
8 Kuhajda FP: Fatty acid synthase and human cancer: new perspectives on its role in tumor biology. Nutrition *16*: 202-208, 2000.
9 Kuhajda FP: Fatty acid synthase and cancer: new application of an old pathway. Cancer Res *66*: 5977-5980, 2006.
10 Van de Sande T, Roskams T, Lerut E, Joniau S, Van Poppel H, Verhoeven G and Swinnen GV: High levels expression of fatty acid synthase in human prostate cancer tissues is linked to activation and nuclear localization of Akt/PKB. J Pathol *206*: 214– 219, 2005.
11 Rashid A, Pizer ES, Moga M, Milgraum LZ, Zahurak M, Pastermack GR, Kuhajda FP and Hamilton SR: Elevated expression of fatty acid synthase and fatty acid synthetic activity in colorectal neoplasia. Am J Pathol *150*: 201-208, 1997.
12 Visca P, Alo PL, Del Nonno F, Botti C, Trombetta G, Marandino F, Filippi S, Di Tondo U and Perrone Donnorso R: Immunohistochemical expression of fatty acid synthase, apoptotic-regulating genes, proliferating factors, and Ras protein product in nonneoplastic mucosa. Clin Cancer Res *5*: 4111-4118, 1999.
13 Notarnicola M, Messa C and Caruso MG: A significant role of lipogenic enzymes in colorectal cancer. Anticancer Res *32(7)*: 2585-2590, 2012.

14 Sakayama K, Masuno H, Miyazaki T, Okumura H, Shibata T and Okuda H: Existence of lipoprotein lipase in human sarcomas and carcinomas. Jpn J Cancer Res *85*: 515-521, 1994.
15 Barò L, Hermoso MC, Nuñez MC, Jiménez-Rios JA and Gil A: Abnormalities in plasma and red blood cell fatty acid profiles of patients with colorectal cancer. British J Cancer *77*: 1978-1983, 1998.
16 Pala V, Krogh V, Muti P, Chajès V, Riboli E, Micheli A, Saadatian M, Sieri S and Berrino F: Erythrocyte membrane fatty acids and subsequent breast cancer: a prospective Italian Study. J Natl Cancer Inst *93*: 1088-1095, 2001.
17 Pender-Cudlip MC, Krag KJ, Martini D, Yu J, Guidi A, Skinner SS, Zhang Y, Qu X, He C, Xu Y, Qian SY and Kang JX: Delta-6-desaturase activity and arachidonic acid synthesis are increased in human breast cancer tissue. Cancer Sci *104*: 760-764, 2013.
18 Bartsch H, Nair J and Owen RW: Dietary polyunsaturated fatty acids and cancer of the breast and colorectum: emerging evidence for their role as risk modifiers. Carcinogenesis *20*: 2209-22018, 1999.
19 Fini L, Piazzi G, Ceccarelli C, Daould Y, Belluzzi A, Munarini A, Graziani G, Fogliano V, Selgrad M, Garcia M and Gasbarrini A: Highly purified Eicosapentaenoic acid as free fatty acids strongly suppresses polyps in $Apc^{Min//+}$ mice. Clin Cancer Res *16(23)*: 5703-5711, 2010.
20 West NJ, Clark SK, Philips RKS, Hutchinson JM, Leicester RJ, Belluzzi A and Hull MA: Eicosapentaenoic acid reduces rectal polyp number and size in familial adenomatous polyposis. Gut *59*: 918-925, 2010.
21 Notarnicola M, Messa C, Refolo MG, Tutino V, Miccolis A and Caruso MG: Synergic effect of Eicosanpetaenoic acid and lovastatin on gene expression of HMGCoA reductase and LDL receptor in cultured HepG2 cells. Lipids in Health and Disease *9(1)*: 135, 2010.
22 Notarnicola M, Messa C, Refolo MG, Tutino V, Miccolis A and Caruso MG: Polyunsaturated fatty acids reduce Fatty Acid Synthase and Hydroxy-Methyl-Glutaryl CoA-Reductase gene expression and promote apoptosis in HepG2 cell line. Lipids in Health and Disease *10(1)*: 10, 2011.
23 Cao W, Ma Z, Rasenick MM, Yeh S and Yu J: n-3 poly-unsaturated fatty acids shift estrogen signaling to inhibit human breast cancer cell growth. PLoS One *7*: e52838, 2012.
24 Notarnicola M, Tutino V, Tafaro A and Caruso MG: Antitumorigenic Effect of Dietary Natural Compounds Is via Lipid Metabolism Modulation in $Apc^{Min/+}$ Mice. Anticancer Res *33(9)*: 3739-3744, 2013.
25 Barone M, Notarnicola M, Caruso MG, Scavo MP, Viggiani MT, Tutino V, Polimeno L, Pesetti B, Di Leo A and Francavilla A: Olive oil and omega-3 polyunsaturated fatty acids suppress intestinal polyp growth by modulating the apoptotic process in ApcMin/+ mice. Carcinogenesis April 7 [Epub ahead of print], PMID 24632492, 2014.
26 Moilanen T and Nikkari T: The effect of storage on the fatty acid composition of human serum. Clinica Chimica Acta *114*: 111-116, 1981.
27 J. Folch, M. Lees and Stanley GHS: A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem *226(1)*: 497-509, 1957.
28 Riediger ND, Othman R, Fitz E, Pierce GN, Suh M and Moghadasian MH: Low n-6:n-3 fatty acid ratio, with fish- or flaxseed oil, in a high fat diet improves plasma lipids and beneficially alters tissue fatty acid composition in mice. Eur J Nutrition *47*: 153-160, 2008.
29 Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Willett WC and Rimm EB: Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. Circulation *108*: 155-160, 2003.
30 Tulk HM and Robinson LE: Modifying the n-6/n-3 polyunsaturated fatty acid ratio of a high saturated fat challenge does not acutely attenuate postprandial changes in inflammatory markers in men with metabolic syndrome. Metabolism: clinical and experimental *58*: 1709-1716, 2009.
31 Kodba ZC, Voncima DB, Novic M and Potocnik U: Multivariate data analysis of erythrocyte membrane phospholipid fatty acid profiles in the discrimination between normal blood tissue and various disease state. Acta Chim Slov *57*: 571-580, 2010.
32 Di Marino L, Maffettone A, Cipriano P, Sacco M, Di Palma R, Amato B, Quarto G, Riccardi G and Rivellese AA: Is the erythrocyte membrane fatty acid composition a valid index of skeletal muscle membrane fatty acid composition? Metabolism *49*: 1164-1166, 2000.
33 Baur LA, O'Connor J, Pan DA, Wu BJ, O'Connor MJ and Storlien LH: Relationships between the fatty acid composition of muscle and erythrocyte membrane phospholipid in young children and the effect of type of infant feeding. Lipids *35*: 77-82, 2000.

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