C O M M U N I C A T I O N S

DE LA FACULTE DES SCIENCES
DE L'UNIVERSITE D'ANKARA

FACULTY OF SCIENCES
UNIVERSITY OF ANKARA

Series B: Chemistry and Chemical Engineering

VOLUME: 54 Number: 2 YEAR: 2008

Faculty of Sciences, Ankara University
06100 Tandoğan, Ankara – TURKEY
ISSN 1303-6017

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This Journal is published two issues in a year by the Faculty of Sciences, University of Ankara. Articles and any other material published in this journal represent the opinions of the author(s) and should not be construed to reflect the opinions of the Editor(s) and the Publisher(s).

Correspondence Address:
COMMUNICATIONS
DERGİ BAŞEDİTÖRLÜĞÜ
Ankara Üniversitesi Fen Fakültesi,
06100 Tandoğan, ANKARA – TURKEY
Tel: (90) 312-212 67 20 Fax: (90) 312-223 23 95
e-mail: commun@science.ankara.edu.tr

Print:
Ankara Üniversitesi Basımevi
İncitaş Sokak No:10 06510 Beşevler
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ANKARA UNIVERSITY PRESS, ANKARA, 2008

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Volume : 54 **Number : 2** **Year : 2008**

Series : B

Commun. Fac. Sci. Univ. Ank. Series B
V.54 (2). pp. 1- 16 (2008)

GAS CHROMATOGRAPHIC ANALYSIS OF PS, PI, SPH AND LPC FATTY ACIDS IN RED BLOOD CELL MEMBRANE OF RAT, RABBIT, HUMAN AND DOG

*SAADET DERMİŞ *, BİLGEHAN DOĞRU***

** Department of Analytical Chemistry, Faculty of Pharmacy, University of Ankara, 06100 Tandoğan, Ankara-TURKEY*

*** Department of Biochemistry, Faculty of Pharmacy, University of Ankara, 06100 Tandoğan, Ankara-TURKEY*

(Received Sept. 16 , 2007; Accepted Dec. 23, 2007)

ABSTRACT

Fatty acid analysis of red blood cell (RBC) membrane PS, PI, LPC and SPH were examined in species like rat, human, dog and rabbit by GC. $C_{16:0}$ and $C_{18:0}$ were the prominent SFA (saturated fatty acids) in species phospholipids studied. $C_{16:0}$ was higher in LPC and SPH whereas $C_{18:0}$ was higher in PS and PI in all species and in dog LPC.

Although evenly spread in phospholipids, MUFA (monounsaturated fatty acid) in PS, PI and LPC seamed to be higher than in SPH. Among MUFA $C_{16:1}$ and $C_{18:1}$ were the prominent ones in the species, $C_{18:1}$ being considerably higher than $C_{16:1}$. PUFAs (polyunsaturated fatty acids) were relatively high in PS and PI compared to LPC and SPH in each species. Arachidonate was one of the most abundant fatty acids in human, rat and dog. Rabbit possessed very low levels of arachidonate in each phospholipid compared to that of other species phospholipids. By contrast linoleic acid was high in rabbit RBC membrane phospholipids. While $C_{18:2}$, $C_{20:4}$ and $C_{22:6}$ were the major PUFAs of human RBC membrane phospholipids studied, $C_{18:2}$ and $C_{20:4}$ were the major ones in the others. 22-Carbon fatty acids were present in small amounts in most species. $C_{22:6}$ was present in rabbit, rat and human RBC membrane phospholipids but very little was present in dog.

KEYWORDS: fatty acids, gas chromatography, analysis, RBC membrane, PS, PI, LPC,SPH, rat, rabbit, human, dog.

Abbreviations: SFA, Saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; LCPUFA, long chain polyunsaturated fatty acid; LCMUFA, long chain monounsaturated fatty acid; RBC, Red blood cell; PS, phosphatidylserine; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; SPH, sphingomyeline; BHT, butylated hydroxy toluene; SDS, sodium dodecyl sulphate; RBC, red blood cell; HPLC, high performance liquid chromatography; GC, gas chromatography

1. INTRODUCTION

There are numerous phospholipids in mammalian cells having different polar head groups such as phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), sphingomyeline (SPH) , lysophosphatidylcholine (LPC) and phosphatidylserine (PS).The most important phospholipids are SPH, PC, PE, and PS which make up 95 % of the total phospholipids [1].

In RBC membrane, phospholipids have a structural role containing a variety of fatty acids varying in chain length and in the number of double bonds[2-9]. They are commonly enriched with polunsaturated fatty acids (PUFA)s having a critical role in regulation of cellular functions and fluidity of cell membranes [10-12] and also function as precursors of oxygenated derivatives called eicosanoids [13,14].

PUFAs are obtained both from the diet and hepatic metabolism. It is also known that PUFA mainly linoleic acid ($C_{18:2}$ n-6) have been recommended for treatment [15,16] and prevention of some diseases [17,18] such as hearth disease[19] and atherosclerosis[20-22] whilst saturated fatty acids increase atherosclerotic tendency **.** It has also been observed that mainly docosahexaenoic acid ($C_{22:6}$ n-3) and eicosapentaenoic acid ($C_{20:5}$ n-3) have triglyceride lowering effect [22-24].

Specific fatty acid alterations have been determined in the blood and tissues of patients with cystic fibrosis[25]. The two major alterations include decreased levels of linoleic acid and docosahexaenoic acid. Changes in arachidonic acid, other monounsaturated and polyunsaturated fatty acids (PUFA) levels have also been described in cystic fibrosis. Increased arachidonic acid release from membrane phospholipids has also been observed. These changes may have an important role in the progression of the disease.

The studies on fatty acids have been performed mostly on RBC membrane and plasma total fatty acids [5, 6, 26-35] but the data concerning the fatty acid of some spesific phospholipids is lacking. The RBC membrane fatty acids of different phospholipids can be compared to find out whether particular phospholipids are rich in particular fatty acids.

The fatty acids of different species could also be compared. Therefore the aim of this study was to compare the relative amounts of fatty acids of PS, PI, LPC and SPH in RBC membrane in different species like rabbits, dogs humans and rats.

2. MATERIALS AND METHODS

2.1. EXPERİMENTAL

2.1.1. Preparation of tissues

Blood samples were collected from male rats (3 months old), male rabbits (10 months old), male humans (30 year old) and male dogs (6 years old). All the species were given the nutrients and energy for a balanced diet to ensure a healthy and active life.

The plasma was separated from RBCs immediately by sentrifugation at 1000 g for 10 min after collecting the blood into a heparinized plastic beaker.

RBC membranes were prepared by modifying the method of Burton et al, [35] and Steck and Kant. [36]. The packed RBCs were washed three times with 3 volumes of 0.89 % NaCl, pH 7.4 solution and haemolyzed by mixing with chilled 5 mM pH 8.0 phosphate buffer. 5 mM Ascorbic acid was added as an antioxidant. The supernatant was removed after centrifugation of the mixture at 22,000 g for 20 min. The pellet was then washed by resuspending in 2.5 mM phosphate buffer, pH 8.0 and centrifuged as before. The pellet became colourless after washing second time with 1.25 mM phosphate buffer, pH 8.0, showing that no haemoglobin remained.

RBC membrane was then extracted immediately.

2.1.2. Lipid extraction and analysis

RBC membranes were extracted twice by the method of Verdon and Blumberg's by modifying the concentrations of SDS, BHT, solvents and avoiding the day light.

RBC membrane was mixed with an equal volume of 0.02 % aqueous SDS in a large volumetric tube. 0.002 % BHT in ethanol (two volumes) containing pentamethyl-6-chromanol (α-tocopherol with no side chain,internal standart, synthesized [25]) was added and mixed. All the procedure was performed on ice. 0.00025 % BHT in 50 % diethylether / hexane (5 volumes) was added, mixed and centrifuged for 3 min at 10,000 x g. The upper layer was removed for washing with glass-distilled water and drying by passage through a filter containing Na_2SO_4. Then it was evaporated to dryness on a rotary evaporator. It was transferred to a vial after dissolving in ether/hexane and evaporated to dryness with nitrogen. Finally it was dissolved in 50 % ether/hexane. The extract was analysed by HPLC for extraction recovery determination. It was 95-100 %. Another portion of the extract was transmethylated for the analysis of phospholipid fatty acids.

2.1.3. Transmethylation of fatty acids

The transmethylation of lipids was carried out by the method of Christie. Lipid sample (1-2 mg) mixed with 2.5% H_2SO_4 in anhydrous methanol (v/v) (2 volume) was incubated for 2 hours at 70^0C. 5 vol of 5% NaCl saturated with $NaHCO_3$ was added, then the mixture was extracted with 3 volume of petroleum ether for three times. After evaporating to dryness it was dissolved in a small volume of HPLC grade hexane.

2.1.4. Purification of fatty acids

The methylated sample extracts and the standards (meythyl esters of fatty acid standards) were applied to Silicagel 60 GF_{254} TLC plates (0.5 mm thick). The developing solvent was petroleum ether : diethyl ether : acetic acid (90 : 10 : 1 by volume) [26]. Various kinds of methylated fatty acid esters were separated from more polar compounds and cholesterol that remain at or near the origin. It was also separated from hydrocarbons and any BHT added as an antioxidant, which migrated ahead. Only the standard fatty acids were made visuable using dodecamolybdophosphoric acid in ethanol spray and by heating which developes a blue colour. Petroleum ether / hexane (50 %) containing 0.01 % BHT was used as an eluent for the elution of the methyl esters of fatty acids from the silicagel. The elution solvent was then evaporated by using a rotary evaporator and dissolved in a small volume of HPLC grade hexane and kept in the deep before the analysis.

2.1.5. Fatty acid analysis by GC

Methyl esters of fatty acid analysis were analysed by using a Hewlett Packard 5890 A gas chromatography with an x-meter Carbowax 20 m capillary column. Carrier gas Helium was used (50 mL/min). The oven temperature rising from 50^oC to 230^o C at 12^oC/min was programmed. The detector temperature was 230^o C. Retention times and peak areas were measured with a reporting integrator Hewlett Packard model, SP 4270. Fatty acids were identified by comparing their retention time with those of standard methyl fatty acid esters. Extraction recovery of fatty acids was determined by adding a known amount of PC with two C_{15} saturated fatty acids attached to the mixture to be transmethylated.

2.1.6. Species diet

Friskies Go-Dog pellets, (Friskies Pet Care, U. K.) (diets of dogs) contained fats and oils, meat and animal derivatives, cereals, derivatives of vegetable origin,, vegetable protein extracts and minerals as ingredients (vitamin E 60 mg/kg, vitamin A 5400 iu/kg, vitamin D 450 iu/kg, copper 11 mg/kg, oil 9 %, protein 24 %, ash 9 %, fibre 5 %).

Rabbits diet were a high fibre diet (Beekay, Bantin and Kingman Ltd, U.K.) contained fats and oils, grass meal, wheatfeed, barley meal, ground oats, linseed meal, fish meal, minerals, vitamins and trace elements (nitrogen free extract 50 %, dry matter 88 %, crude protein 18 %, crude oil 4 %, crude fibre 9 %, ash 7 %,). Saturated fatty acids were were also present as 0.75 %, unsaturated fatty acids as 1.84 % and linoleic acid as 1.24 %. Vitamin A 36000 iu/kg, vitamin D_3 2000 iu/kg, vitamin E 130 mg/kg were added.

Rats had a high quality and a low protein diet (Rat and Mouse No. 1 Modified, SDS Ltd., Witham, Essex) organised to keep rats in good health over a long time of period. Diet of rats were the pellets made up of crude crude fibre 4.3 %, protein 14.6 %, crude oil 2.6 %, ash 5.8 % and nitrogen free extract 62.7 %, palmitoleic acid ($C_{16:1}$) 0.07 %, oleic acid ($C_{18:1}$) 0.74 %, linoleic acid ($C_{18:2}$) 0.56 %, linolenic acid ($C_{18:3}$) 0.05 %, arachidonic acid ($C_{20:4}$), 0.13 %, palmitic acid ($C_{16:0}$), 0.31 % and stearic acid ($C_{18:0}$) 0.04 %. Retinol 1922 μg/kg (1 μg retinol : 3.3 iu vitamin A activity), α-tocopherol 68.3 mg/kg, cholecalciferol 15.1 μg/kg (1 μg cholecalciferol : 40 iu vitamin D_3 activity) were added as vitamins.

Humans had a healthy diet.

3. RESULTS AND DISCUSSION

SFA

Each species had the same major fatty acids in the phospholipids of RBC membrane (Tables 1- 4). $C_{16:0}$ was higher in LPC and SPH in each species. $C_{18:0}$ was higher in PS and PI in all species and in dog LPC as well (tables 2 and 5). Among SFA $C_{16:0}$ and $C_{18:0}$ were the prominent ones. $C_{20:0}$ was found to be high in rat and rabbit LPC and SPH and rabbit PI. Among LCMUFA $C_{22:0}$ was not present in dog RBC membrane phospholipids (Table 2).

MUFA

In the RBC membrane MUFA were fairly evenly spread but MUFA in PS, PI and LPC seamed to be higher than in SPH (Table 5). Among MUFA $C_{16:1}$ and $C_{18:1}$ were the prominent ones in the species (Tables 1- 4). $C_{18:1}$ was found to be considerably higher than $C_{16:1}$ in all species of RBC membrane phospholipids studied.

PUFA

PS and PI had relatively high concentrations of PUFAs compared to LPC and SPH in each species (Tables 1- 5). These results show that PS and PI are important for the membrane fluidity. In terms of arachidonate it was clear that rabbit posessed very low levels in each phospholipid compared to that of other species phospholipids (Table 1). It was one of the most abundant fatty acids in human, rat and dog (tables 2- 4). Conversely linoleic acid was high in rabbit RBC membrane phospholipids (82 %) (Table 1) compared to other species. This probably points to the lower activities of elongase and Δ^5- desaturase in the rabbit to generate arachidonic acid from linoleic acid. $C_{18:2}$, $C_{20:4}$ and $C_{22:6}$ were the major PUFAs of human RBC membrane phospholipids studied (Table 3). $C_{18:2}$ and $C_{20:4}$ were the main ones in the other species. They are probably the major fatty acids contributing to the physicochemical structure or fluidity of the membrane. High levels of $C_{18:2}$ and $C_{20:4}$ in rat RBC membrane phospholipids (Table 4) make rat a convenient candidate for the metabolic study of linoliec and arachidonic acid.

22-Carbon fatty acids were present in small amounts in most species. Some phospholipids of rat RBC membrane had a higer $C_{22:4}$ content (table 4) compared to other species. Rats may have a higher capacity for converting $C_{20.4}$ to $C_{22:4}$ by the elongase enzyme than the others. $C_{22:6}$ was present in rabbit, rat and human RBC membrane phospholipids but very little was present in dog (table 2), showing that the enzymes capable of synthesizing $C_{22:6}$ (elongase and ^{4}- desatures) were lower in dog than other species. $C_{22:4}$ was also found to be $\triangle$ in very small amounts. This could be the reflection of the diet. 22 Carbon fatty acids are found in oil of fish and of some vegetables. $C_{22:6}$ is high in fish oil. Dogs and rats had very little % of 22 C fatty acids in their RBC phospholipids (Tables 2 and 4) because of not consuming food containing appreciable amounts of that fatty acids.

Other workers presented the data that RBC fatty acid composition reflects the type of fat in the diet[37]. Feeding fish oil induces changes in the fatty acid

pattern of rabbit RBC membrane and serum phospholipids. An increase is observed in amounts of $C_{20:5}$, $C_{22:5}$ and $C_{22:6}$ with fish oil[3].These results are in accordance with monkeys [38] , humans [39]and rats [26].

There were similarity between the data of human RBC phospholipids and the data reported by Hsio et al [40]. Their values for $C_{18:0}$ in PS, $C_{16:0}$, $C_{18:1}$ and $C_{20:4}$ in PI and $C_{18:2}$ in SPH were higher. The percentage fatty acids of rat RBC membrane PI were in agreement with those of Williams and Maunder [41] with the exception of $C_{18:0}$ that was found to be a little bit higher than our values, they also found $C_{22:5}$ and $C_{22:6}$ fatty acids in PI. Rat values agreed with those reported by Ghosal et al [27] with the exceptions of $C_{16:1}$, $C_{18:1}$ being a little bit higher in SPH than their values, they found higher percentage of $C_{16:0}$ compared to our data. $C_{16:0}$, $C_{16:1}$ and $C_{20:4}$ in PS were also found to be higher in our study compared to those of Ghosal et al. Rabbit RBC membrane SPH fatty acid levels were in agreement with those of van Den Boom et al[42]. They dedected $C_{24:1}$ fatty acid in RBC membrane SPH. Comparison on this fatty acid can not be made due to the fact that $C_{24:1}$ was not present unfortunately in the standard fatty acid mixture used in this study.

4. CONCLUSION

Each species had the same major fatty acids in the phospholipids of RBC membrane Among MUFA $C_{16:1}$ and $C_{18:1}$ were the prominent ones in the species studied. PS and PI had relatively high concentrations of PUFAs compared to LPC and SPH in each species showing that PS and PI are important for the membrane fluidity. In terms of arachidonate rabbit posessed very low levels in each phospholipid compared to that of human, rat and dog phospholipids. By contrast linoleic acid was high in rabbit RBC membrane phospholipids.

This probably points to the lower activities of elongase and Δ^5- desaturase in the rabbit. $C_{18:2}$, $C_{20:4}$ and $C_{22:6}$ were the major PUFAs of human RBC membrane phospholipids studied. $C_{18:2}$ and $C_{20:4}$ were the main ones in the other species probably the major fatty acids contributing to the physicochemical structure or fluidity of the membrane. 22-Carbon fatty acids were present in small amounts in most species.

$C_{22:6}$ was present in rabbit, rat and human RBC membrane phospholipids but very little was present in dog, showing that the enzymes capable of synthesizing $C_{22:6}$ (elongase and Δ^4- desaturase) were lower in dog than other species. It could also be related to the diet.

ÖZET

İnsan, tavşan, köpek ve sıçan gibi farklı türlerin RBC membranında PS, PI, LPC ve SPH fosfolipidlerinin gaz kromatografik yağ asiti analizleri yapılmıştır. Çalışılan fosfoliliplerde doymuş yağ asitleri içinde en fazla $C_{16:0}$ ve $C_{18:0}$ göze çarpmaktadır. Bütün türlerde $C_{16:0}$ LPC ve SPH de, $C_{18:0}$ ise PS and PI de yüksek düzeyde ve köpekde LPC de yüksek düzeyde bulunmuştur. Türlerde oldukça eşit dağılımış olmasına karşın MUFA PS, PI ve LPC de SPH e göre daha fazladır. Türlerde MUFA içinde de $C_{18:1}$, $C_{16:1}$ den daha fazla olmak kaydıyla $C_{16:1}$ ve$C_{18:1}$ en göze çarpanlarıdır. Bütün türlerde PUFA, PS ve PI de LPC ve SPH e kıyasla daha fazladır. PUFA içinde arachidonat insan, köpek ve sıçanda en çok bulunan PUFA dır. Tavşan RBC membranı fosfolipidlerinde ise arakidonat diğer türlere göre çok düşük düzeydedir. Tersine linoleik asit tavşan fosfolipidlerinde fazla bulunmuştur. $C_{18:2}$, $C_{20:4}$ ve $C_{22:6}$ insan RBC membranı fosfolipidlerinde esas PUFA iken çalışılan diğer türlerde $C_{18:2}$ ve $C_{20:4}$ dır. 22- Karbon yağ asitleri çoğu türde az miktarlarda bulunmuştur. $C_{22:6;}$ tavşan, sıçan ve insan RBC membranı fosfolipidlerinde bulunmuşken; köpekte oldukça az miktarda bulunmuştur.

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Table1. *Fatty acid content of the phospholipids of rabbit red blood cell membrane*

Fatty acids	Phospholipids PS	PI	LPC	SPH
$C_{16:0}$	8.57 ± 0.5	12.6 ± 0.5	30.7 ± 0.6	45.4 ± 1.0
$C_{16:1}$	0.37 ± 0.1	1.23 ± 0.1	7.77 ± 1.7	1.60 ± 0.2
$C_{18:0}$	23.7 ± 0.6	23.7 ± 0.8	29.8 ± 2.0	16.2 ± 1.0
$C_{18:1}$	8.53 ± 1.7	13.0 ± 0.7	8.13 ± 1.0	7.70 ± 1.6
$C_{18:2}$	31.2 ± 1.0	34.5±1.0	9.43 ± 1.1	7.40 ± 1.0
$C_{18:3}$	0.73 ± 0.4	0.53±0.1	N.D	0.63 ± 0.3
$C_{20:0}$	1.27 ± 0.1	1.07 ± 0.4	7.33 ± 0.8	5.15 ± 0.2
$C_{20:1}$	2.17 ± 0.3	2.10±0.4	N.D	N.D.
$C_{20:2}$	2.20 ± 0.2	1.70±0.4	2.90 ± 1.8	N.D.
$C_{20:3}$	1.03 ± 0.1	0.90±0.1	< 0.1	< 0.1
$C_{20:4}$	7.53 ± 0.4	5.70 ± 0.4	2.10 ± 0.2	0.46 ± 0.1
$C_{20:5}$	N. D.	0.19 ± 0.1	< 0.1	N. D.
$C_{22:0}$	3.17 ± 1.0	1.03 ± 0.3	< 0.1	8.20 ± 0.4
$C_{22:1}$	< 0.1	N.D	N.D	2.46± 0.2
$C_{22:4}$	0.53 ± 0.2	< 0.1	< 0.1	< 0.1
$C_{22:6}$	< 0.1	0.57 ± 0.1	< 0.1	3.98 ± 0.8
$C_{23:0}$	N. D.	N. D.	< 0.1	< 0.1

Values are percentages of total.

N.D.: Not detected. Each value represents the mean ± S.E.M. of three individuals.

Table 2. *Fatty acid content of the phospholipids of dog red blood cell membrane*

	Phospholipids			
Fatty acids	PS	PI	LPC	SPH
$C_{16:0}$	4.03 ± 0.4	10.3 ± 0.2	18.0 ± 1.8	57.8 ± 1.1
$C_{16:1}$	0.93 ± 0.2	7.37 ± 0.2	4.90 ± 1.0	1.43 ± 0.3
$C_{18:0}$	28.1 ± 0.3	20.9 ± 1.1	24.8 ± 1.9	16.0 ± 0.2
$C_{18:1}$	21.9 ± 1.9	11.8 ± 0.8	10.1 ± 0.5	12.0 ± 1.0
$C_{18:2}$	1.77 ± 0.5	10.8 ± 2.0	11.7 ± 2.7	7.00 ± 1.0
$C_{18:3}$	N. D.	3.27 ± 0.5	9.03 ± 1.9	0.43 ± 0.1
$C_{20:0}$	1.30 ± 0.1	8.83 ± 0.2	8.26 ± 0. 1	0.27 ± 0.1
$C_{20:1}$	< 0.1	1.99 ± 0.3	4.7 ± 2.9	1.03 ± 0.2
$C_{20:2}$	< 0.1	1.34 ± 0.1	4.97 ± 2.6	N. D.
$C_{20:3}$	7.50 ± 1.8	1.05 ± 0.3	N. D.	< 0.1
$C_{20:4}$	29.6 ± 3.5	20.2 ± 0.6	3.60 ± 1.8	3.60 ± 0.1
$C_{20:5}$	N. D.	N. D.	N. D.	N. D.
$C_{22:0}$	N. D.	N. D.	N. D.	N. D.
$C_{22:4}$	N. D.	0.11 ± 0.1	N. D.	N. D.
$C_{22:6}$	N.D	0.30 ± 0.1	N. D.	N. D.
$C_{23:0}$	N. D.	N. D.	N. D.	N. D.

Values are percentages of total.
N.D.: Not detected. Each value represents the mean ± S.E.M. of three individuals

Table 3. *Fatty acid content of the phospholipids of human red blood cell membrane*

Fatty acids	Phospholipids PS	PI	LPC	SPH
$C_{16:0}$	4.27 ± 0.3	5.47 ± 0.7	41.9 ± 1.8	35.4 ± 6.0
$C_{16:1}$	1.83 ± 1.1	0.30 ± 0.1	13.5 ± 5.3	5.20 ± 1.2
$C_{18:0}$	28.9 ± 1.0	25.6 ± 1.1	18.4 ± 1.0	11.9 ± 1.7
$C_{18:1}$	16.9 ± 0.9	26.9 ± 1.5	10.3 ± 1.1	6.40 ± 1.8
$C_{18:2}$	4.20 ± 0.8	0.75 ± 0.8	5.87 ± 1.7	4.97 ± 1.7
$C_{18:3}$	N. D.	0.23 ± 0.1	N. D.	0.47 ± 0.2
$C_{20:0}$	< 0.1	2.73 ± 0.1	4.70 ± 1. 3	2.16 ± 0.8
$C_{20:1}$	N. D.	< 0.1	< 0.1	< 0.1
$C_{20:2}$	N. D.	N.D	N. D.	N. D.
$C_{20:3}$	1.40 ± 0.5	1.07 ± 0.3	N. D	ND
$C_{20:4}$	27.6 ± 0.6	22.3 ± 3.6	3.30 ± 1. 2	< 0.1
$C_{20:5}$	N. D.	N. D.	< 0.1	2.23 ± 0.3
$C_{22:0}$	< 0.1	< 0. 1	< 0.1	7.07 ± 1.4
$C_{22:1}$	N.D	< 0.1	< 0.1	< 0.1
$C_{22:4}$	N. D.	N.D	N. D.	N.D.
$C_{22:6}$	1.30 ± 0.5	1.40 ± 0.82	N. D.	N.D.

Values are percentages of total.
N.D.: Not detected. Each value represents the mean ⊥ S.E.M. of three individuals.

Table 4. *Fatty acid content of the phospholipids of rat red blood cell membrane*

Fatty acids	Phospholipids			
	PS	PI	LPC	SPH
Fatty acids				
$C_{16:0}$	13.8 ± 0.7	16.3 ± 0.7	24.9 ± 2.2	33.7 ± 1.5
$C_{16:1}$	3.83 ± 0.4	3.48 ± 0.8	9.03 ± 1.9	7.83 ± 2.5
$C_{18:0}$	27.8 ± 0.3	26.5 ± 0.4	17.8 ± 2.5	16.4 ± 0.3
$C_{18:1}$	12.1 ± 1.2	6.37 ± 1.1	8.67 ± 0.5	4.33 ± 0.7
$C_{18:2}$	3.33 ± 0.4	6.30 ± 0.9	7.48 ± 0.3	6.80 ± 0.9
$C_{18:3}$	N. D.	0.87 ± 0.5	1.27 ± 0. 8	1.17 ± 0.1
$C_{20:0}$	0.73 ± 0.2	10.1 ± 0.9	17.1 ± 2.0	18.4 ± 2.4
$C_{20:1}$	N. D.	< 0.1	N. D.	N. D.
$C_{20:2}$	N. D.	< 0.1	N. D.	< 0.1
$C_{20:3}$	N.D	< 0.1	< 0.1	< 0.1
$C_{20:4}$	26.8 ± 1.9	23.2 ± 2.9	2.18 ± 0.4	2.05 ± 0.7
$C_{20:5}$	0.46 ± 0.2	< 0.1	< 0.1	N. D.
$C_{22:0}$	N.D	1.63 ± 0.4	4.80 ± 1.4	3.93 ± 0.7
$C_{22:1}$	N.D.	N.D.	< 0.1	3.67 ± 0.4
$C_{22:4}$	N. D.	2.65 ± 1.0	3.97 ± 1.3	N.D.

Values are percentages of total.
N.D.: Not detected. Each value represents the mean ± S.E.M. of three individuals.

Table 5. Percentage of Monounsaturated Fatty Acids in RBC Membrane

	PS	PI	LPC	SPH
Rabit	11	16	16	12
Dog	23	21	20	15
Human	19	27	24	12
Rat	26	10	18	16

Percentage of PUFA in RBC Membrane

	PS	PI	LPC	SPH
Rabit	43	44	14	13
Dog	39	37	29	11
Human	36	33	9	8
Rat	31	33	15	10

Percentage of Arachidonate in RBC Membrane

	PS	PI	LPC	SPH
Rabit	8	6	2	1
Dog	30	20	4	4
Human	28	22	3	< 0.1
Rat	27	23	2	2

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Volume : 54 **Number : 2** **Year : 2008**

Series : B