FATTY ACIDS PROFILE IN PHOSPHOLIPIDS OF THE ERYTHROCYTE MEMBRANES IN SWIMMING RATS

Joanna GDULA-ARGASIŃSKA[1], Małgorzata TYSZKA-CZOCHARA[1], Aneta TELEGŁÓW[3], Zbigniew DĄBROWSKI[3], Paweł PAŚKO[2], Tadeusz LIBROWSKI[1], Magdalena GAWEŁ[1], Magdalena OLBERT[1], Anna LIPKOWSKA[1]

1. *Department of Radioligands, Medical College, Jagiellonian University, Medyczna 9, 30-688 Krakow, Poland*
2. *Department of Food Chemistry and Nutri tion, Medical College, Jagiellonian University, Medyczna 9, 30-688 Krakow, Poland*
3. *Department of Clinical Rehabilitation, University School of Physical Education, Jana Pawła II 78, 31-571 Kraków, Poland.*

Abstract

Alterations in membrane lipid composition, particularly fatty acids content is known to result in functional and structural changes. Dietary lipids and physical activity or stress play an important role in this change. It was of interest to study the influence of swimming in different temperatures on the content of fatty acids in the phospholipids of the erythrocyte membranes of rats. Our research demonstrated a significant influence of swimming on the content of fatty acids in the phospholipids of the erythrocyte membranes of rats. These changes of fatty acids, particularly the decrease of arachidonic acid and significant increase of saturated fatty acids in the phospholipids of the erythrocyte membranes in swimming rats may be induced by oxygen radicals generated in different kinds of stress.

Keywords: fatty acids, erythrocyte membranes, physical activity, rats

Corresponding author: Tadeusz Librowski, mflibrow@cyf-kr.edu.pl

Introduction

The mechanisms by which erythrocyte cells regulate membrane phospholipid fatty acid turnover and membrane function are not yet fully understood. Elucidation of the relationship between phospholipid fatty acid composition and plasma membrane function in the red blood cells remains a complex problem because these cells have no ability to alter membrane phospholipid fatty acid composition by phospholipid synthesis de novo. Therefore, they depend on lipid exchange and acylation of fatty acids as the mechanisms for phospholipid repair and renewal. Alterations in membrane lipid composition, particularly fatty acids content is known to result in functional and structural changes in the membrane. Dietary lipids, physical activity or stress play an important role in this change. It was of interest to study the influence of swimming in different temperatures on the content of fatty acids in the phospholipids of the erythrocyte membranes of rats

The deformability of red blood cells in the circulatory system is crucial for the flow of blood through blood vessels. Membrane lipid fluidity basically depends on the type of cholesterol (free or esterified), the class of phospholipids, the molar ratio of cholesterol to phospholipids, the degree of saturation of fatty acids, the length of the acyl chains and the presence or absence of amphipathic compounds such as lysophosphatides [1].

Lipids are crucial structural components of cell membranes and they dictate the integrity of the membranes. Membrane fatty acids composition directly reflects membrane properties. Its fluidity is influenced by changes in the level of unsaturation of the phospholipid fatty acyl chains, cholesterol to phospholipid ratio and fatty acyl chain length. Membrane lipids are susceptible to peroxidation induced damage as they are largely composed of polyunsaturated fatty acids. Peroxidative reactions involving free radicals in lipid domains result in damage to integral membrane proteins, leading to alteration of membrane dynamics and function [1, 2, 3, 4].

Materials and methods

Chemicals

Methanol LiChrosolv®, chlorophorm LiChrosolv®, n-hexane LiChrosolv®, heptane LiChrosolv®, boron trifluoride – methanol complex 20%, acetone were purchased from Merck.

Animals

Thirty 36-week old Wistar male rats were used in the experiment and randomly allocated to three research groups: (i) non-swimming controls kept in cages at room temperature, (ii) rats swimming in water at 4°C for 10 min, and (iii) rats swimming in water at 25oC for 30 min, as described above [5]. After completion of the experiment, the animals were killed by cervical dislocation. The femoral artery and vein were cut open and 2ml of blood was extracted and placed in Vacuette-type test tubes with potassium EDTA. Permission for this study was obtained from the Local Ethics Committee of the Jagiellonian University.

Erythrocyte ghosts

Hemoglobin-free erythrocyte membranes were prepared by hypotonic hemolysis at 4°C in 40 volumes 10 mM Tris pH 7.4, isolated by centrifugation (16,000g x 15 min) and washed several times to eliminate haemoglobin residues.

Determination of fatty acids profile

Lipids from the erythrocyte membranes were extracted with chlorophorm – methanol solution (2:1 v/v). The individual fractions of the lipids were dertemined by the technique of thin-layered liquid chromatography (TLC) according to Chedid [6]. Fatty acid methyl esters (FAME) were synthesized with use 20% BF3 w in methanol at the 100°C. FAME analyses were done by gas chromatography (Agilent 6890N with capillary column DB- 23 (60m, ID 0,25mm, 0,25 μm) and FID detector). Chromatograph parameters: FID 260°C, injector 250°C, split ratio 50:1, oven 140°C for 5 min, 140-190°C at 4°C/min, 190°C for 15 min, 190-240°C at 2,75°C/min, 240°C for 4min, carrier gas - Helium at 43cm/sec, constant pressure mode. For the identification of fatty acids, retention times of standards FAME from Supelco (47801) were used. Peak areas were measured with an integrator (ChemStation). The sum of all peak areas of the fatty acids identified was taken as 100%.

For all fatty acids the mean value in percent was calculated. The total sum of saturated and unsaturated fatty acids, as index SAT and UNSAT was evaluated.

Statistical analysis

After logarithmic transformation the data were normally distributed and significant differences in the fatty acids content between

groups were assessed by use of a one-way analysis of variance (ANOVA) using Tukey's test at the 95% confidence level ($p<0,05$). All statistical calculations were carried out using the commercially available packages Statistica 8.0.

Results

In phospholipids of the erythrocyte membranes from control rats arachidonic acid dominated (41,7%) and was significantly higher that in experimental probes. Ahigh amount of stearic (27,8%) and palmitic (19,3%) acids was observed. The total SAT (saturated) index was 47,4% while the index of UNSAT acids was 52,6% (Table1, Fig.1).

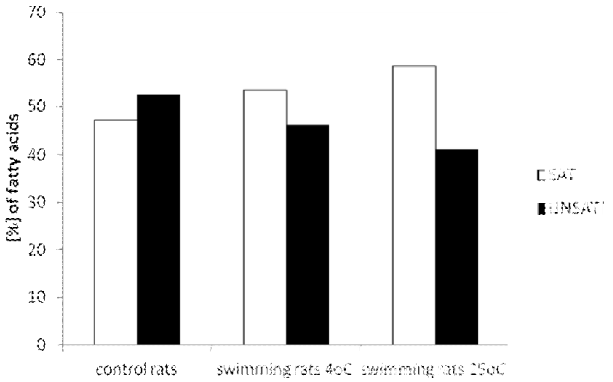

Fig.1 Total sum of saturated (index SAT) and unsaturated (index UNSAT) fatty acids in phospholipids of the erythrocyte membranes in the control and swimming groups

The profile of the phospholipids of the erythrocyte membranes of rats swimming at 4°C differed from the control data. Index SAT (53,3%) was higher compared to control. Palmitic acid dominated in erythrocyte membranes from these group of animals. Asignificant decrease the content of arachidonic acid was observed, and an increase of the amount of oleic and linolenic acids compared to the control group. Interestingly it was observed that elaidic acid (trans -isomer) was increased compared to the data from other mammals (Table1, Fig.1).

In phospholipids of the erythrocyte membranes from rats swimming at 25°C index SAT (58,8%) was higher than in the other group of rats. Stearic acid dominated (33,2%) in theerythrocytes of the rats (Table 1, Fig. 1).

Table 1. Fatty acids profile expressed as percent [%] of total phospholipids of the erythrocyte membranes from control and experimental groups

	Control rats	Rats swimming at 4°C	Rats swimming at 25°C
Lauric acid C12:0	0,1^{a}±0,01	0,7^{b}±0,2	0,3^{a}±0,04
Myristoleic acid C14:1	2,5^{a}±0,6	0,5^{b}±0,11	2,5^{a}±1,0
Pentadecanoic acid C15:0	0,1^{a}±0,02	0,7^{b}±0,1	0,1^{a}±0,01
Palmitic acid C16:0	19,3^{a}±2,70	27,1^{b}±4,2	25,6^{a,b}±2,2
Palmitoleic acid C16:1	0,5^{a}±0,08	0,7^{a}±0,1	0,3^{a}±0,1
Heptadecanoic acid C17:0	0,3^{a}±0,02	1,3^{a}±0,5	0,3^{a}±0,08
Stearic acid C18:0	27,8^{a}±3,10	24,8^{a}±3,5	33,2^{a}±3,9
Elaidic acid C18:1n9t	0,8^{a}±0,22	8,0^{c}±1,5	3,1^{b}±1,1
Oleic acid C18:1n9c	4,2^{a}±0,53	12,0^{b}±2,1	4,8^{a}±0,9
Linolic acid C18:2	0,8^{a}±0,15	1,7^{a}±0,8	2,9^{a,b}±0,7
Arachidic acid C20:0	0,2^{a}±0,04	0,3^{a}±0,1	0,2^{a}±0,01
Linolenic acid C18:3n3	2,0^{a}±0,61	8,5^{b}±1,4	7,0^{b}±2,2
Arachidonic acid C20:4n6	41,7^{a}±4,52	13,7^{b}±4,1	20,0^{b}±5,2

Mean values ±SD, N=10, different letters denote significant statistical differences between groups, $p<0,05$

Discussion

We showed the significant rise of palmitic and stearic acids in the material from rats subjected to a physical effort. Statistically an essential fall in the arachidonic acid content was observed in phospholipids of erythrocyte

membranes in experimental rats, compared to the data for control group.

A physical effort causes a significant increase in body temperature as a result of increasing metabolic processes. The generated heat in muscles is spread in the entire organism by theblood flow. As a result the internal temperature increases. The mechanism of the temperature adjustmentduring physical strain is likely to be resolved by signals released in form of metabolic and neurohormone factors. In the resting state heat loss through the skin takes place mainly through convection and evaporating.

A physical effort can negatively influence the red blood cells. Intravascular hemolysis is one of the most pronounced mechanisms for destruction of erythrocytes during and after physical activity. Exercise-induced oxidative stress has been proposed among other factors as an explanation of exercise-induced hemolysis. Exercise induced a significant increase in thiobarbituric acid-reactive substance and protein carbonyl content levels in sedentary subjects and resulted in an increase of osmotic fragility and decrease in deformability of erythrocytes, accompanied by signs for intravascular hemolysis [5, 7, 8, 9, 10, 11]. Yoshida observed a higher level of oxidizing lipids in erythrocyte membranes than in plasma. Petridou and coworkers examined the effect of a physical effort to the composition of fatty acids in the liver, skeletal muscles and the adipose tissue of rats. They showed that a physical effort had modified the composition of fatty acids of the phospholipid fraction in the liver and skeletal muscles and the triglyceride fraction in the adipose tissue [4, 12]. Previous authors, however, did not perform examinations with using RBC membranes. Based on findings of their examinations it is possible to conclude that a physical effort can influence to the content of fatty acids in erythrocytes.

Free radicals are known to play a pivotal role in tissue damage, as well as have an disturbing effect on erythrocytes. Taking this into consideration, the composition of fatty acids in the RBC membranes is translating to the resistance of erythrocytes to the effect of free oxygen radicals, rheological parameters as well as their length of life. . Our research demonstrated a significant influence of swimming on the content of fatty acids in the phospholipids of the erythrocyte membranes of rats. These changes of fatty acids, particularly decrease of arachidonic acid and significant increase of saturated fatty acids in the phospholipids of the erythrocyte membranes in swimming rats may be induced by oxygen radicals generated in different kinds of stress.

Resumo

Oni scias, ke perturboj de la lipida kompono de membranoj, speciale la enhavo de grasacidoj, rezultigas funkciajn kaj strukturajn ŝanĝojn. Dietaj lipidoj, korpa aktiveco kaj streĉo ludas gravan rolon koncerne tiujn ŝanĝojn. Estis interese studi la influon de naĝado en akvo kun diversaj temperaturoj al la kvanto de grasacidoj en fosfolipidoj de eritrocitaj membranoj de ratoj. Nia esploro montris konsiderindan influon de naĝado al la kvanto de grasacidoj en fosfolipidoj de eritrocitaj membranoj de ratoj. Tiu ŝanĝoj de grasacidoj, speciale la malaltiĝo de arakidonata acido kaj la signifa altiĝo de saturataj grasacidoj en fosfolipidoj de eritrocitaj membranoj de naĝintaj ratoj povus esti efikitaj per oksigenaj radikaloj, kiuj ekestas dum diversaj formoj de streĉo.

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