Psychiatria Danubina, 2008; Vol. 20, No. 3, pp 372–383 *Conference paper*
© Medicinska naklada - Zagreb, Croatia

PHOSPHOLIPID MEMBRANE ABNORMALITIES AND REDUCED NIACIN SKIN FLUSH RESPONSE IN SCHIZOPHRENIA

Alena Buretić-Tomljanović[1], Jasminka Giacometti[2], Sergej Nadalin[1], Gordana Rubeša[3], Mirjana Vulin[4] & Draško Tomljanović[5]

[1]*Department of Biology and Medical Genetics, School of Medicine, University of Rijeka, Croatia*
[2]*Department of Chemistry and Biochemistry, School of Medicine, University of Rijeka, Croatia*
[3]*Psychiatry Clinic, Clinical Medical Centre, Rijeka, Croatia*
[4]*Psychiatric Hospital Lopača, Rijeka, Croatia*
[5]*Private psychiatric practice, Rijeka, Croatia*

SUMMARY

Objectives: *Reduced n-3 and n-6 polyunsaturated fatty acids (PUFAs) content in red blood cell (RBC) membranes and abnormal membrane phospholipid metabolism were repeatedly implicated in the etiology of schizophrenia.*

Findings: *Prenatal and perinatal depletion of PUFAs interferes with normal brain development and function. The lack of docosahexaenoic acid - DHA in the brain is reflected in lower membrane DHA/AA (AA - arachidonic acid) ratio, increased activity of AA-metabolizing enzymes, and disturbance of downstream metabolic pathways involved in signaling, growth modulation, brain glucose uptake, immune functions, neurotransmission, synaptogenesis and neurogenesis. Preliminary high-throughput metabolomic studies revealed abnormal biochemical profile in patients with schizophrenia or brief psychotic disorder when compared to healthy controls. The results of both metabolomic and proteomic studies pointed to energy metabolism and lipid biosynthesis being impaired in schizophrenia. The usefulness of antipsychotic medication and supplementation with PUFAs in reverting to the normal metabolic state has been suggested in early treatment of the first psychotic episode. Abnormalities of phospholipid metabolism can be also detected as attenuated niacin skin flush response in the variety of neuropsychiatric disorders.*

Conclusions: *Disturbances of lipid homeostasis could represent biochemical markers in the preclinical phase of neuropsychiatric illnesses and could serve as triggers in genetically vulnerable individuals. The assessment of patients' lipid status may also help in monitoring the course of the disease and treatment response. In this regard, simple, cheap and fast niacin skin flush test might be valuable. It might help in diagnosis of adolescents and young adults with psychotic behaviour, or in defining the necessity for long-term antipsychotic therapy. Along with antipsychotic medication schizophrenic patients need specific medical nutrition therapies.*

Key words: *lipidomics - membrane lipids - brain lipids – phospholipids - polyunsaturated fatty acid metabolism - bioactive lipids - niacin testing - schizophrenia*

* * * * *

CELLULAR LIPIDOMICS AND MEMBRANE LIPIDS

Cellular lipidomics, a discipline within metabolomics, investigates over thousand different lipids in the cell, quantitatively describing their structure and function (van Meer 2005). The vital role of lipid molecules was recognized in health and disease, and can be explored using sophisticated high-throughput technology. Mass spectrometry, for instance, enables contemporary measurement the huge amount of different cellular metabolites. Cellular lipidomics is further interes-

ted in enzymes involved in lipid metabolism and transport, and mechanisms of their regulation. It is expected that lipidomics will provide a molecular signature to a certain pathways or a disease conditions (Adibhatla et al. 2006). Along with lipidomics analyses, the methodology of RNA silencing (that enables selective gene "turning off" by degrading RNA) represents a tool to investigate diverse lipid intermediates' functions. Due to its recent expansion, lipidomics inevitably joins genomics and proteomics, enabling the consideration of the cellular processes as an integrated system.

By a definition, lipids are biological substances generally hydrophobic in nature and principally soluble in organic solvents (Fahy et al., 2005). Most lipids contain a polar head and long, hydrophobic tails (amphipatic molecules). Their hydrophobic nature enables specific forms of aggregation in the cell: i) as lipid droplets in the cytosol or lipoproteins being secreted or endocytosed (consisting mainly of triacylglycerols and cholesterylesters), ii) as membrane bilayers assembling the bulk of cellular lipids. The three classes of lipids are main constituents of membrane bilayers: phospholipids, sphingolipids and cholesterol. Among phospholipids, in most membranes the most numerous are phosphatidylcholine (PC) molecules (50%), followed by phosphatidylethanolamine (PE) (20%), negatively charged and mainly localized in the inner leaflet - phosphatidylserine (PS), and phosphatidylinositol (PI). These molecules (PC, PE and PS) contain different polar headgroups bound to a glycerol with two fatty acid chains (diacylglycerol – DAG). Usually, one fatty acid is saturated and another one is unsaturated contributing to the bilayer's fluidity. The main sphingolipid, sphingomyelin (SM) contains phosphocholine head (like PC), sphingosine tail and one saturated fatty acid (forming ceramide). Sphingolipids make membranes rigid. The third class of lipids, cholesterol (the mammalian sterol) helps maintaining the appropriate level of membranes' fluidity, concurrently contributing to their fluidity and rigidity. Cholesterol keeps adjacent phospholipid molecules altogether, preventing, at the same time, their aggregation.

Cellular membranes differ regarding lipid composition, although the detailed lipid composition of each organellar membrane is not known (van Meer 2005). Similar lipid composition was suggested for lipid droplets, peroxisomes, mitochondria and endoplasmic reticulum (ER). Phospholipids are synthesized on the cytosolic surface of ER and Golgi, and translocated to other organellar or plasma membrane via secretory vesicles. Secretory vesicles beyond Golgi, as well as, endocytic vesicles are 10-fold enriched in sphingolipids and cholesterol. Glycosylated sphingolipids (ceramide carrying carbohydrates) are found only in the outer leaflet of the plasma membrane thus contributing to membrane asymmetry (differences in composition between inner and outer leaflet).

LIPIDOMICS OF THE BRAIN AND VERSATILE ROLES OF N-3 POLYUNSATURATED FATTY ACIDS

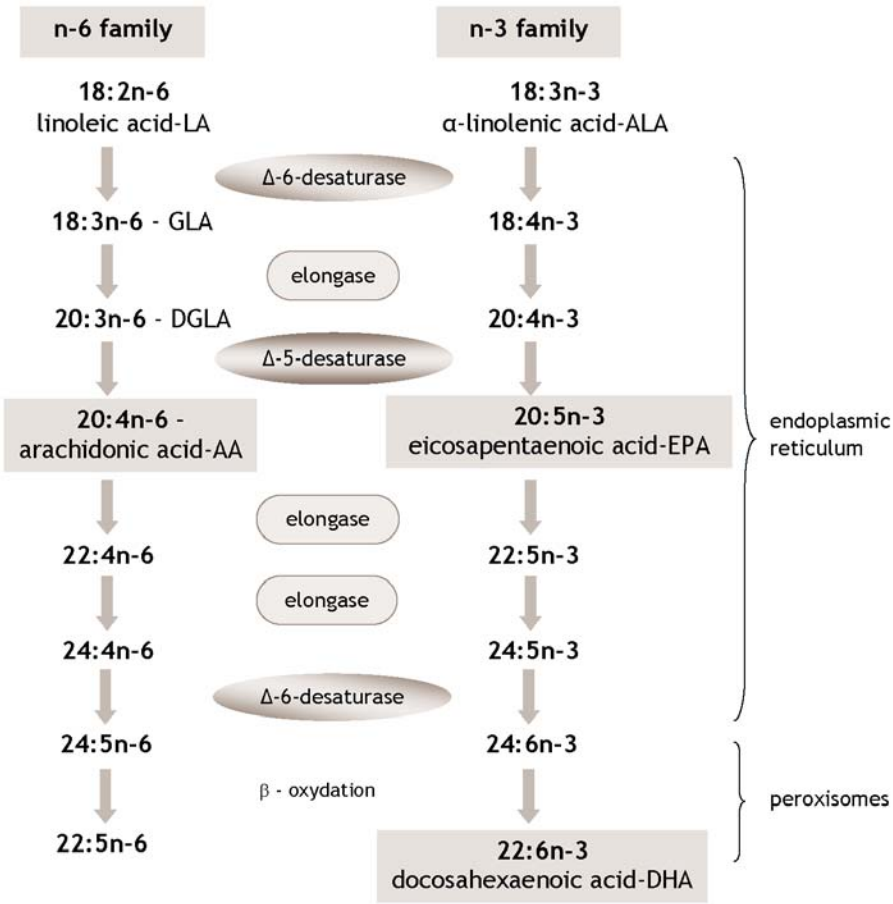
Brain has a high concentration of lipids; therefore lipid metabolism may be of particular importance for brain and central nervous system (CNS) as the whole. Lipids are engaged in numerous physiological processes in the cell, including cell signaling, and lipid dysregulation was implicated in many neurodegenerative diseases. Lipidomic analyses in schizophrenia, bipolar disorder, major depression or Alzheimer disease may help identifying particular lipid intermediates or enzymes that could possibly serve as new drug targets in these conditions (Berger et al. 2002, Adibhatla et al. 2006). Challenges in the brain lipidomic analyses are studies of lipid-to-lipid interactions and lipid-to-protein interactions that are very complex (Rapaka et al. 2005). Particular lipid intermediates (i.e. ceramide phosphate or sphingosine-1-phosphate) may activate a cascade of enzymes working on membrane phospholipids (phospholipases A2 - PLA2s or cyclooxygenase 2 – COX-2, for instance). This may, in turn, activate downstream processes resulting in synthesis of numerous

bioactive lipid metabolites. It is estimated that cytosolic PLA2 (cPLA2) enzyme may recognize approximately 50 lipids while interacting on the membrane bilayers.

Important point for brain lipidomics is to establish a correlation between lipid profile in the brain and various forms of behaviour. Some progress has been already made: i) endocannabinoids were found to regulate sedation, euphoria, appetite, memory, sleep and other processes (Grant & Cahn 2005, Pacher et al. 2006), ii) a connection between lipids and long-term-potentiation in memory formation has been established (Gerdeman & Lovinger 2003, Slanina et al. 2005). Furthermore, the role of particular lipid molecules (polyunsaturated fatty acids - PUFAs) in neurodevelopmental processes (i.e. cell migration, eicosanoid precursors, ligands for transcription factors that regulate gene expression, cellular communication, interaction with proteins) (Innis 2007, Yavin 2006, Bourre 2006) and cognitive aging (Youdim et al. 2000, Borsonelo & Galduróz 2008, McNamara et al. 2008, Das 2008) is emerging. Disturbances of lipid metabolism and deviation of lipid homeostasis are suggested as crucial for abnormal CNS and brain development, thus making the prerequisites for later life appearance of wide array of clinical symptoms. PUFA deficiency and disturbances of lipid homeostasis were observed in many other neuropsychiatric illnesses except schizophrenia: major depression, bipolar disorder, Alzheimer dementia, multiple sclerosis, attention-deficit hyperactive disorder, dyslexia, but also coronary heart disease, hypertension, cancer, diabetes, inflammatory and auto-immune disorders, etc. (Chiu et al. 2002, Frasure-Smith et al. 2004, Zamaria 2004, Richardson 2004, Gerber et al. 2005, Plourde et al. 2007, Su 2008).

The uniqueness of brain lipid content is its heavy concentration of the polyunsaturated docosahexaenoic fatty acid (DHA) from n-3 series (22:6n-3). DHA is accumulating during CNS development and its early deprivation has been associated with vulnerability to neuropathological insult (Innis 2007, Rapoport et al. 2007). The lack of DHA in the brain is probably reflected in lower DHA/AA ratio (AA -polyunsaturated arachidonic acid from the n-6 series; 20:4n-6). The decrease of the DHA/AA ratio imposes a higher activity of AA-metabolizing enzymes (such as cPLA2, COXs, LOXs) that give rise to synthesis of different proinflammatory eicosanoids (20-carbon-atoms-containing lipid metabolites, derivatives of AA) and inflammatory cytokines (tumour necrosis factor, interleukin-1 and interleukin-6) (James et al. 2000, Yao & van Kammen 2004, Calder 2006). In comparison, DHA can be enzymatically converted to various docosanoids (resolvins and neuroprotectins) with anti-inflammatory properties (Das 2004, Calder 2006). PUFAs from n-6 and n-3 series exert these effects through interfering with intracellular signaling pathways and activation of different transcription factors. It follows that membrane PUFA composition, and their n-3/n-6 ratio determines the nature of inflammatory response in the cells and the body. It was earlier shown that increased n-3 PUFA levels in serum might attenuate the pro-inflammatory response to psychological stress (Maes et al. 2000).

The main source of DHA is diet; alternatively DHA can be endogenously synthesized from its precursor, essential alpha-linolenic fatty acid (α-LNA, 18:3n-3) in the liver (Figure 1). Body cannot synthesize α-LNA; therefore, it must be obtained from diet too. Usually, liver produces sufficient DHA to maintain brain demands. This conversion from α-LNA to DHA depends on action of the elongases and desaturases enzymes (Figure 1), whose activity varies individually and by sex (conversion is more efficient in females than males, suggesting the role of sex hormones) (Burdge 2006, Childs et al. 2008). In the brain, DHA is prevalent at the cytofacial site of the membrane where it is involved in numerous intracellular events and functions during prenatal and perinatal life: intracellular signaling, growth modulation, maintenance of balanced oxidative status (Mazza et al. 2007), gene expression (Nakamura et al. 2004), synaptogenesis and



Legend: GLA - γ-linolenic acid; DGLA- dihomo γ-linolenic acid

Figure1. Metabolic pathways from essential to polyunsaturated fatty acids of n-6 and n-3 families

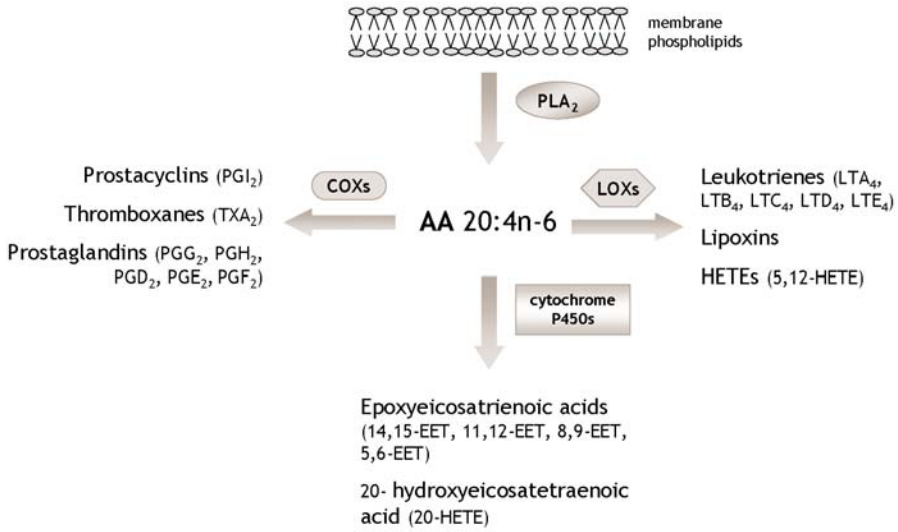
neurogenesis (associated with remarkable DHA accumulation) (Green et al. 1999), and dopaminergic and serotonergic neurotransmission (du Bois et al. 2005, Chalon 2006, Vancassel et al. 2007). Remarkable elevation of dopamine receptor (D1 and D2) genes expression in the postnatal rat brain was found following maternal n-3 fatty acid dietary deficiency (Kuperstein et al. 2005). DHA is also an important regulator of brain glucose uptake. Glucose utilization and glucose transport were impaired in the endothelial cells of the blood-brain barrier in rats deficient in n-3 PUFAs (Pifferi et al. 2007). The finding that dietary supplementation with DHA or eicosapentaenoic fatty acid (EPA, 20:5n-3) increased the glucose uptake while AA had no effect, led to suggestion that membrane n-3 PUFAs may modulate some glucose transport proteins' expression and activity (Pifferi et al. 2007). Interestingly, impairment in brain glucose uptake has been associated with cognitive decline and aging (Freemantle et al. 2006), and impaired glucose tolerance was demonstrated in schizophrenic patients long ago (Peet 2006). According to growing literature, schizophrenia is not solely a brain disease. Findings of disturbed glucose tolerance and immune functions, increased oxidative stress, features of metabolic syndrome, and risk for cardiovascular disease in schizophrenia point to some abnormal basic cellular mechanisms with systemic effects in the body. Disturbance of lipid homeostasis in cellular membranes of schizophrenic patients might represent the underlying cause. Disturbance of lipid homeostasis was determined in neuronal membranes but also plasma membranes in peripheral tissue i.e. red blood cells – RBC, bringing the phospholipid hypothesis of schizophrenia reported by Feldberg (1976) and Horrobin (1977) into light.

THE ROLE OF BIOACTIVE LIPIDS AND PHOSPHOLIPID HYPOTHESIS OF SCHIZOPHRENIA

Feldberg (1976) and Horrobin (1977) proposed schizophrenia as prostaglandin deficiency disease. Prostaglandin deficiency is probably influenced by reduced levels of particular PUFAs (AA and DHA) in the membrane phospholipids of schizophrenic patients (Mahadik et al. 1994, Arvindakshan et al. 2003) and increased PLA2 activity in serum, platelets, and brain tissue of schizophrenic patients (Gattaz et al. 1987, Ross et al. 1997, Tavares et al. 2003). An increased phospholipid breakdown in brains of unmedicated schizophrenic patients was also indicated by magnetic resonance imaging (Pettegrew et al. 1991, Williamson et al. 1996). Plasma membrane PUFAs deficiency causes the increased activity of membrane-associated enzymes, such as cPLA2 or iPLA2 and further PUFAs loss (Ross 1999, Ross 2006, Gattaz et al. 1987, Ross et al. 1997, Barbosa et al. 2007). PLA2s comprise a super-family of enzymes that catalyze the hydrolysis of the sn-2 fatty acyl bond of phospholipids to liberate free fatty acids and generate lysophospholipids (LPs). cPLA2 has a preferance to AA, while iPLA2 mainly liberates DHA from membrane phospholipid molecules (Forlenza et al. 2007, Green et al. 2008). Both free fatty acids and LPs act as potent second messengers at low concentrations, whereas are neurotoxic at high concentrations (Farooqui & Horrocks 2004). Cytosolic PLA2s are involved in the multiple signaling pathways; some of them mediate transient calcium rise in the cytoplasm. Therefore, elevated activity of cPLA2s may cause disturbances of the calcium homeostasis and provoke cell death and neurodegeneration (Thomas et al. 2006). Free fatty acids, liberated by action of PLA2s have many options: they can be used as energy supply by mitochondria and peroxisomes (Prabakaran et al. 2004), can be peroxidized to reactive oxygen species (ROS) (Yao & van Kammen 2004), bind to specific nuclear transcription factors and mediate expression of lipophilic genes (Wolfrum & Spener 2000, Jump 2004, Nakamura et al. 2004, Sampath & Ntambi 2005), or metabolized to prostaglandins, thromboxanes and prostacyclins (by action of COX-1, COX-2, and COX-3 enzymes), leukotrienes (by action of lipoxygenase-5 and lipoxygenase-12 enzymes - LOXs), epoxides and other metabolites (by multiple cytochrome P450 enzymes) (Figure 2). COX-2 metabolizes liberated AA to prostaglandins D2 and E2 (PGD_2 and PGE_2) that exert inflammatory effects. The metabolic pathway including cPLA2 and COX-2 does not function properly in vast majority of schizophrenic patients (Tavares et al. 2003, Bosveld-van Haandel et al. 2006). The abnormal metabolic pathway is expressed as reduced skin redness after topical application of niacin solution in range 0.001M – 0.1M. Niacin (nicotinic acid) causes local skin flush and oedema due to AA release by cPLA2 and subsequent PGD_2 and PGE_2 synthesis by COX-2. It is hypothesized that schizophrenic patients have greatly attenuated niacin skin flush response and low level of PG synthesis due to AA deficiency in the membrane phospholipids. The finding of disturbed prostaglandin synthesis has a meaning since mentioned metabolic pathway is a common phospholipid-dependent signal transduction pathway. Since neurotransmission is associated with PUFA release from the membrane phospholipids, the occurence of such an abnormality in neurons may alter PLA2-dependent monoaminergic neurotransmission (Ross 2003, Chalon 2006).

Other studies of AA metabolism can broaden phospholipid hypothesis of prostaglandin disturbances in schizophrenia. For instance, cytochrome P450 epoxygenase-derived eicosanoids (epoxyeicosatrienoic acids - EETs) have numerous potent biological activities: regulation of peptide hormone secretion, ionic transport, inflammation, hemostasis and other functions (Zeldin, 2001). New data show that n-9 and n-3 PUFAs exert some of their beneficial physiological effects through interfering with cytochrome P450 ω-hydroxylase activity and formation of hydroxylated metabolites of AA (hydroxyeicosatetraenoic acids-HETEs or

oxylipids). EPA, DHA, and particularly eicosatrienoic acid (ETA, 20:3n-3) were recognized as alternative substrates for cytochrome P450 ω-hydroxylase and as potent inhibitors of AA hydroxylation (Fer et al. 2008). Oxylipids also play prominent physiological roles.



Legend: AA – arachidonic acid; PLA_2 – phospholipase A2; COXs – cyclooxygenases; LOXs – lipoxygenases

Figure 2. The three major pathways of arachidonic acid metabolism

Furthermore, EPA and DHA are substrates for a novel group of mediators produced through COX-2/LOX pathways called E-series and D-series resolvins, respectively. DHA is enigmatic molecule since it is also a supstrate for docosatriene (neuroprotectin D1) synthesis (mediator exerting prominent anti-inflammatory effects) (Calder 2006, Das 2008) and isoprostanes which are neurotoxic.

RED BLOOD CELL FATTY ACID CONTENT IN SCHIZOPHRENIA

Reduced levels of certain PUFAs in RBC membranes were repeatedly reported both in drug-naïve first-episode schizophrenic patients and chronically ill, medicated ones (Fenton et al. 2000, Khan et al. 2002, Arvindakshan et al. 2003). Consequently, structural and metabolic changes of plasma membranes in schizophrenic patients are reflected in the surface architectonics of RBCs (Novitskii et al. 2000). Reduced PUFA level was accompanied with higher levels of plasma peroxides, suggesting the increased oxidative stress in schizophrenic patients (Khan et al. 2002). Doris et al. (1998) have found higher concentration of dihomogamma-linolenic acid (DGLA, 20:3n-6) in RBCs of schizophrenic patients compared to healthy controls. DGLA is a precursor of AA synthesis by delta-5-desaturase enzyme (Figure 1). Phospholipid analysis of RBCs indicated the similar levels of PC and PS in schizophrenic patients and controls, while SM level was increased and PE level decreased in patient group (Ponizovsky et al. 2001). SM/PE ratio correlated directly with PANSS Negative Symptom Scale Score enabling authors to suggest that elevated SM level might be a factor of negative syndrome development in schizophrenia. They pointed to ceramide content of SM, known as a potent mediator of apoptosis.

All the findings support a view of reduced level of certain PUFAs, namely DHA and AA, in the brain and RBCs of schizophrenic patients as the consequence of increased oxidative stress (an intrinsic property of the illness) and increased activity of PLA2 enzymes in temporal cortex and serum of schizophrenic patients. Important factor that may contribute to the reduced PUFA levels represent dietary habits. Nutritional patterns of schizophrenic patients are different from those of healthy controls and are principally poor (high in saturated fats and sweet drinks, low in unsaturated

fats) (Amani 2007). Deprivation of n-3 PUFAs from diet altered expression of AA and DHA signaling cascades in rat frontal cortex (Rao et al. 2007) increasing cPLA2, synovial PLA2 and COX-2 activities. Another factor contributing to the reduced PUFA levels is conversion rate from essential fatty acids (EFAs) LA and α-LNA to long-chain PUFAs that depends on the activity of a rate-limiting enzyme delta-6-desaturase and elongases, and is influenced by sex hormones. Delta-6-desaturase's activity and regulation has recently become a subject of intense investigation (Das 2005, Hashimoto et al. 2008). Except delta-6-desaturase, a number of other enzymes involved in the lipid metabolism are of immense interest in lipidomic and genetic analyses: stearoyl-CoA desaturase (an endoplasmic reticulum enzymes that catalyzes the biosynthesis of monounsaturated fatty acids from saturated fatty acids) (Dobrzyn & Ntambi 2005), fatty acid synthase (Wang et al. 2004), long-chain fatty acid-CoA ligase (selectively esterifies AA, EPA and DHA with co-enzyme A forming acyl-CoA that can be incorporated into membrane phospholipids) (Covault et al. 2004), etc.

Reduction of DHA, associated with reduction in grey matter volume of orbitofrontal cortex has been even found in healthy adults of advanced age (McNamara et al. 2008) giving support to the hypothesis of schizophrenia as a syndrome of accelerated aging (Kirkpatrick et al. 2007).

ANTIPSYCHOTIC AND PUFA SUPPLEMENTATION EFFECTS TO LIPID HOMEOSTASIS IN SCHIZOPHRENIA

There are no validated biomarkers of schizophrenia that establish diagnosis or reliably predict response to treatment (Kaddurah-Daouk 2006). By studying the huge number of metabolites in cells, tissues and body fluids, metabolomics results might be representative of the individual's overall health status. In their metabolomics study, Holmes et al. (2006) revealed abnormal biochemical profile in patients with schizophrenia or brief psychotic disorder when compared to healthy controls. Mostly glucose, acetate, alanine, and glutamine separated the two groups. Glucose and acetate abnormalities pointed to pathways of energy metabolism and lipid biosynthesis being impaired in schizophrenia. Their preliminary data suggested usefulness of antipsychotic medication in reverting to the normal metabolic state, particularly in early treatment of the first psychotic episode. The results of proteomic analysis of the human brain tissue of schizophrenic patients coincide with those of mentioned metabolomics study: the enzymes involved in gycolysis, electron-transport chain, ATP synthesis, and lipid biosynthes (including cholesterol synthesis) were significantly down regulated (Prabakaran et al. 2004). Contrary, fatty acid β-oxydation enzymes (including numerous peroxisomal enzymes) were significantly increased at the transcriptional level.

A preliminary lipidomic study of Kaddurah-Daouk et al. (2007) gave first insight into profile of seven different lipid classes in unmedicated schizophrenic patients and after two weeks of treatment with antipsychotics (olanzapine, risperidone and aripiprazole). The authors were able to separate two groups of patients: responders and nonresponders to antipsychotic medication (assessed using Clinical Global Impression score – CGI), based on identifying pre-treatment lipid metabolites that were related to response to medication. Changes in lipid profiles, in this research, differed according to antipsychotic treatment. Olanzapine appeared more potent in modification of plasma phospholipids, while both olanzapine and risperidone caused more changes in n-6 series, than n-3 series of fatty acids. Aripiprazole induced minimal change in plasma free fatty acid composition.

A number of randomized, placebo-controlled studies investigated the effects of EFA, EPA or DHA supplementation on positive and negative symptom improvements and PUFA status of patients with schizophrenia (Peet et al. 2001, Fenton et al. 2001, Emsley et al. 2002, Peet et al. 2002, Peet 2003). All those studies generally revealed improvement in negative or positive psychopathology and total n-3 fatty acid content

after supplementation with EFAs or PUFAs, although were hardly inconsistent regarding patient selection and used neuroleptic medication. Supplementation with pure EPA showed better effect on positive symptoms than supplementation with pure DHA. Improvements with EPA supplementation were mainly achieved in unmedicated or patients with an acute psychotic episode (Peet et al. 2001), while had no obvious effect in chronic, residual type schizophrenia (Fenton et al. 2001).

ATTENUATED NIACIN SKIN FLUSHING – A BIOCHEMICAL MARKER IN SCHIZOPHRENIA

The mechanism of niacin action is a part of AA-cascade pathways. Niacin binds to appropriate receptors in skin macrophages and epidermal Langerhans cells that is followed by synthesis and release of PGD_2 and PGE_2 (Figure 2). Precursor molecule AA is released from membrane phospholipids through activity of PLA2 enzyme; it is further transformed in PG molecule by the action of COX-2 (Tavares et al. 2003, Nilsson et al. 2006). Once released, PGs stimulate relaxation of smooth muscles in skin capillary walls, and consequent vasodilatation, flushing and edema. Appropriate skin response to the application of niacin is only possible if the PLA2/COX-2 cascade pathway works properly (Hudson et al. 1999, Messamore 2003, Maclean et al. 2003).

Attenuated response to local application of niacin was established in schizophrenia, but other neuropsychiatric diseases as well. Nevertheless, niacin response is significantly weaker in schizophrenic patients when compared to bipolar or depressive (unipolar) patients (Maclean et al. 2003, Bosveld-van Haandel et al. 2006, Liu et al. 2007). Reduced volumetric niacin response was also associated with severity of illness (Puri et al. 2007). In healthy individuals, who use cannabis regularly, niacin response was significantly reduced, but there was no effect of cannabis in the group of schizophrenic patients (Smesny et al. 2007a). Considering the suggested modulation of AA metabolic cascade by cannabis, the results indicated already existing metabolic disorder in the schizophrenia group.

Niacin skin response is influenced by age and sex, being weaker in males than females, and diminishing with age (Smesny et al. 2004). Although the cause of disturbance in PGs' signaling cascade may not be necessarily caused by PUFA precursor's deficit (the defect may be in niacin or PG receptors, or in poor vasomotor activity), niacin non-responsiveness may, in majority of cases, indicate the disturbance of membrane phospholipids metabolism and decreased concentration of AA in cellular membranes (Smesny et al. 2005, Smesny et al. 2007b). If this is the case, niacin skin flush testing might serve as a biochemical marker for identifying a subpopulation of patients with a disturbed phospholipids metabolism.

CONCLUSIONS

Membrane phospholipids are the source of important bioactive substances.

Lipid metabolism in the body depends upon diet (environmental factor) (Wahle et al. 2004), hormonal influences, and genetic background (endogenous synthesis of particular metabolites). While enzymes participating in lipid metabolism are well characterized, they provide interesting means for studying gene-environment interactions in pathogenesis of various diseases.

Disturbances of lipid homeostasis could represent biochemical markers in the preclinical phase of neuropsychiatric illnesses and could serve as triggers in genetically vulnerable individuals. The assessment of patients' lipid status may help in monitoring the course of the disease and treatment response. While waiting for more complete and reliable results from complex and expensive lipidomic and metabolomic studies, simple, cheap and fast niacin skin flush test might be valuable in identifying individuals with abnormal phospholipid metabolism. It might help in diagnosis of adolescents and young adults with psychotic behaviour, or in defining the necessity for long-term antipsychotic therapy.

Along with antipsychotic medication, schizophrenic patients need specific medical nutrition therapies.

REFERENCES

1. *Adibhatla RM, Hatcher JF & Dempsey RJ: Lipids and Lipidomics in Brain Injury and Diseases. The AAPS J 2006; 8:E314-21.*
2. *Amani R: Is dietary pattern of schizophrenia patients different from healthy subjects? BMC Psychiatry 2007; 7:15 doi:10.1186/1471-244X-7-15.*
3. *Arvindakshan M, Sitasawad S, Debsikdar V, Ghate M, Evans D, Horrobin DF et al: Essential polyunsaturated fatty acid and lipid peroxide levels in never-medicated and medicated schizophrenia patients. Biol Psychiatry 2003; 53:56-64.*
4. *Barbosa NR, Junqueira RM, Vallada HP & Gattaz WF: Association between BanI genotype and increased phospholipase A2 activity in schizophrenia. Eur Arch Psychiatry Clin Neurosci 2007; 257:340-3.*
5. *Berger GE, Wood SJ, Pantelis C, Velakoulis D, Wellard RM & McGorry PD: Implications of lipid biology for the pathogenesis of schizophrenia. Aust N Z J Psychiatry 2002; 36:355-66.*
6. *Borsonelo EC & Galduróz JCF: The role of polyunsaturated fatty acids (PUFAs) in development, aging and substance abuse disorders: Review and propositions. Prostaglandins Leukot Essent Fatty Acids 2008; doi 10.1016/j.plefa. 2008.03.005.*
7. *Bosveld-van Haandel L, Knegtering R, Kluiter H & van den Bosch RJ: Niacin skin flushing in schizophrenic and depressed patients and healthy controls. Psychiatry Res 2006; 143:303-6.*
8. *Bourre JM: Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain. Part 2: macronutrients. J Nutr Health Aging 2006; 10:386-99.*
9. *Burdge GC: Metabolism of alpha-linolenic acid in humans. Prostaglandins Leukot Essent Fatty Acids 2006; 75:161-8.*
10. *Calder PC: Polyunsaturated fatty acids and inflammation. Prostaglandins Leukot Essent Fatty Acids 2006; 75:197-202.*
11. *Chalon S: Omega-3 fatty acids and monoamine neurotransmission. Prostaglandins Leukot Essent Fatty Acids 2006; 75:259-69*
12. *Childs CE, Romeu-Nadal M, Burdge GC & Calder PC: Gender differences in the n-3 fatty acid content of tissues. Proc Nutr Soc 2008; 67:19-27.*
13. *Chiu C-C, Huang S-Y, Su K-P, Lu M-L, Huang M-C, Chen C-C & Shen WW. Polyunsaturated fatty acid deficit in patients with bipolar mania. Eur Neuropsychopharmacol 2003; 13:99-103.*
14. *Covault J, Pettinati H, Moak D, Mueller T & Kranzker HR: Association of a long-chain fatty acid-CoA ligase 4 gene polymorphism with depression and with enhanced niacin-induced dermal erythema. Am J Med Genet B Neuropsychiatr Genet 2004; 127B:42-7.*
15. *Das U: Perinatal supplementation of long-chain polyunsaturated fatty acids, immune response and adult diseases. Med Sci Monit 2004; 10:HY19-25.*
16. *Das U: A defect in the activity of Delta6 and Delta5 desaturases may be a factor predisposing to the development of insulin resistance syndrome. Prostaglandins Leukot Essent Fatty Acids 2005; 72:343-50.*
17. *Das U: Folic acid and polyunsaturated fatty acids improve cognitive function and prevent depression, dementia, and Alzheimer disease - but how and why? Prostaglandins Leukot Essent Fatty Acids 2008; 78:11-9.*
18. *Dobrzyn A & Ntambi JM: The role of stearoyl-CoA desaturase in the control of metabolism. Prostaglandins Leukot Essent Fatty Acids 2005; 73:35-41.*
19. *Doris AB, Wahle K, MacDonald A, Morris S, Coffey I, Muir W & Blackwood D: Red cell membrane fatty acids, cytosolic phospholipase-A2 and schizophrenia. Schizophr Res 1998; 31:185-96.*
20. *du Bois TM, Deng C & Huang XF: Membrane phospholipid composition, alterations in neurotransmitter systems and schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2005; 29:878-88.*
21. *Emsley R, Myburgh C, Oosthuizen P & van Rensburg SJ: Randomized, placebo-controlled study of ethyl-eicosapentaenoic acid as supplemental treatment in schizophrenia. Am J Psychiatry 2002; 159:1596-98.*
22. *Fahy E, Subramaniam S, Brown HA, Glass CK, Merrill Jr AH, Murphy RC et al: A comprehensive classification system for lipids. J Lipid Res 2005; 46:839-61.*
23. *Farooqui AA & Horrocks LA: Brain phospholipases A2: a perspective on the history. Prostaglandins Leukot Essent Fatty Acids 2004; 71:161-9.*
24. *Feldberg W: Possible association of schizophrenia with a disturbance in prostaglandin metabolism: a physiological hypothesis. Psychol Med 1976; 6:359-69.*
25. *Fenton WS, Hibbeln J & Knaube M: Essential Fatty Acids, Lipid Membrane Abnormalities, and the Diagnosis and Treatment of Schizophrenia. Biol Psychiatry 2000; 47:8-21.*
26. *Fer M, Corcos L, Dréano Y, Plée-Gautier, Salaün J-P, Berthou F & Amet Y. Cytochromes P450 from*

family 4 are the main omega hydroxylating enzymes in humans: CYP4F3B is the prominent player in PUFA metabolism. J Lipid Res 2008; Epub ahead of print.

27. *Forlenza OV, Schaeffer EL & Gattaz WF: The role of phospholipase A2 in neuronal homeostasis and memory formation: implications for the pathogenesis of Alzheimer's disease, J Neural Transm 2007; 114:231-8.*
28. *Frasure-Smith N, Lespérance F & Julien P: Major depression is associated with lower omega-3 fatty acid levels in patients with recent acute coronary syndromes. Biol Psychiatry 2004; 55:891-6.*
29. *Freemantle E, Vandal M, Tremblay-Mercier J, Tremblay S, Blachere J-C, Bégin ME et al:. Omega-3 fatty acids, energy substrates, and brain function during aging. Prostaglandins Leukot Essent Fatty Acids 2006; 75:213-20.*
30. *Gattaz WF, Köllisch M, Thuren T, Virtanen JA & Kinnunen PK: Increased plasma phospholipase-A2 activity in schizophrenic patients: reduction after neuroleptic therapy. Biol Psychiatry1987; 22:421-6.*
31. *Gerber M, Thiébaut A, Astorg P, Clavel-Chapelon F & Combe N: Dietary fat, fatty acid composition and risk of cancer. Eur J Lipid Sci Technol 2005; 107:540-59.*
32. *Gerdeman GL & Lovinger DM. Emerging roles for endocannabinoids in long-term synaptic plasticity. British J Pharmacol 2003; 140:781-9.*
33. *Grant I & Cahn BR. Cannabis and endocannabinoids modulators: Therapeutic promises and challenges. Clin Neurosci Res 2005; 5:185-99.*
34. *Green P, Glozman B, Kamensky B & Yavin E: Developmental changes in rat brain membrane lipids and fatty acids - the preferential prenatal accumulation of docosaheaenoic acid. J Lipid Res 1999; 40:960-6.*
35. *Green JT, Orr SK & Bazinet RP: The emerging role of group VI calcium-independent phospholipase A2 in releasing docosahexaenoic acid from brain phospholipids. J Lipid Res 2008; 49:939-44.*
36. *Hashimoto K, Yoshizawa AC, Okuda S, Kuma K, Goto S & Kanehisa M: The repertoire of desaturases and elongases reveals fatty acid variations in 56 eucaryotic genomes. J Lipid Res 2008; 49:183-91.*
37. *Holmes E, Tsang TM, Huang JTJ, Leweke FM, Koethe D et al: Metabolic profiling of CFS: Evidence that early intervention may impact on disease progression and outcome in schizophrenia. PLoS Med 2006; 3:e327.*
38. *Horrobin DF: Schizophrenia as a prostaglandin deficiency disease. Lancet 1977; 1:936-7.*
39. *Hudson C, Gotowiec A, Seeman M, Warsh J & Ross BM: Clinical Subtyping reveals Significant Differences in Calcium-Dependent Phospholipase A2 Activity in Schizophrenia. Biol Psychiatry 1999; 46:401-5.*
40. *Innis SM: Fatty acids and early human development. Early Hum Dev 2007; 83:761-6.*
41. *James MJ, Gibson RA & Cleland GL: Dietary polyunsaturated fatty acids and inflammatory mediator production. Am J Clin Nutr 2000; 71 (suppl):343S-8S.*
42. *Jump D: Fatty acid regulation of gene transcription. Crit Rev Clin Lab Sci 2004; 41:41-78.*
43. *Kaddurah-Daouk R: Metabolic Profiling of Patients with Schizophrenia. PLoS Med 2006; 3:1222-3.*
44. *Kaddurah-Daouk R, McEvoy J, Baillie RA, Lee D, Yao JK, Doraiswamy PM & Krishnan KRR: Metabolomic mapping of atypical antipsychotic effects in schizophrenia. Mol Psychiatry 2007; 12:934-45.*
45. *Khan MM, Evans DR, Gunna V, Scheffer RE, Parikh VV & Mahadik SP: Reduced erythrocyte membrane essential fatty acids and increased lipid peroxides in schizophrenia at the never-medicated first-episode of psychosis and after years of treatment with antipsychotics. Schizophr Res 2002; 58:1-10.*
46. *Kirkpatrick B, Messias E, Harvey PD, Fernandez-Egea E & Bowie CR: Is schizophrenia a syndrome of accelerated aging? Schizophr Bull 2007; Epub ahead of print.*
47. *Kuperstein F, Yakubov P, Dinerman S, Gil R, Eylam R, Salem Jr. N & Yavin E: Overexpression of dopamine receptor genes and their products in the postnatal rat brain following maternal n-e fatty acid dietary deficiency. J Neurochem 2005; 95:1550-62.*
48. *Little SJ, Lynch MA, Manku M & Nicolau A: Docosahexaenoic acid-induced changes in phospholipids in cortex of young and aged rats: A lipidomic analysis. Prostaglandins Leukot Essent Fatty Acids 2007; 77:155-62.*
49. *Liu CM, Chang SS, Liao SC, Hwang TJ, Shieh MH, Liu SK et al: Absent response to niacin skin patch is specific to schizophrenia and independent of smoking. Psychiatry Res 2007; 152:181-7.*
50. *Maes M, Christophe A, Bosmans E, Lin A & Neels H: In humans, serum polyunsaturated fatty acid levels predict the response of proinflammatory cytokines to psychologic stress. Biol Psychiatry 2000; 47:910-20.*
51. *Mahadik SP, Mukherjee S, Correnti EE, Kelkar HS, Wakade CG, Costa RM & Scheffer R: Plasma membrane phospholipid and cholesterol distribution of skin fibroblasts from drug-naive patients at the onset of psychosis. Schizophr Res 1994; 13:239-47.*
52. *Mazza M, Pomponi M, Janiri L, Bria P & Mazza S: Omega-3 fatty acids and antioxidants in neurological and psychiatric diseases: An overview.*

Prog Neuropsychopharmacol Biol Psychiatry 2007; 31:12-26.

53. *Maclean R, Ward PE, Glen I, Roberts SJ & Ross BM: On the relationship between methylnicotinate-induced skin flush and fatty acids levels in acute psychosis. Prog Neuropsychopharmacol Biol Psychiatry 2003; 27:927-33.*
54. *McNamara RK, Liu Y, Jandacek R, Rider T &Tso P: The aging orbitofrontal cortex: Decreasing polyunsaturated fatty acid composition and associated increases in lipogenic gene expression and stearoyl-CoA desaturase activity. Prostaglandins Leukot Essent Fatty Acids 2008; doi 10.1016/j.plefa.2008.04.001.*
55. *Messamore E: Relationship between the niacin skin flush response and essential fatty acids in schizophrenia. Prostaglandins Leukot Essent Fatty Acids 2003; 69:413-9.*
56. *Nakamura MT, Cheon Y, Li Y & Nara TY: Mechanism of regulation of gene expression by fatty acids. Lipids 2004; 39:1077-82.*
57. *Nilsson BM, Hultman CM & Wiesel FA: Niacin skin flush response and electrodermal activity in patients with schizophrenia and healthy controls. Prostaglandins Leukot Essent Fatty Acids 2006; 74:339-46.*
58. *Novitskii VV, Ryazantseva NV & Semin IR: Surface architectonics of peripheral blood erythrocytes in patients with mental diseases, Bull Exp Biol Med 2000; 130:979-82.*
59. *Pacher P, Bátkai S & Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev 2006; 58:389-462.*
60. *Peet M, Brind J, Ramchand CN, Shah S & Vankar GK: Two double-blind placebo-controlled pilot studies of eicosapentaenoic acid in the treatment of schizophrenia. Schizophr Res 2001; 30:243-51.*
61. *Peet M: Essential fatty acids: theoretical aspects and treatment implications for schizophrenia and depression. Advances in psychiatric treatment 2002; 8:223-9.*
62. *Peet M: Eicosapentaenoic acid in the treatment of schizophrenia and depression: rationale and preliminary double-blind clinical trial results. Prostaglandins Leukot Essent Fatty Acids 2003; 69:477-85.*
63. *Peet M: The metabolic syndrome, omega-3 fatty acids and inflammatory process in relation to schizophrenia. Prostaglandins Leukot Essent Fatty Acids 2006; 75:323-7.*
64. *Pettegrew JW, Keshavan MS, Panchalingam K, Strychor S, Kaplan DB, Tretta MG & Allen M: Alterations in brain high-energy phosphate and membrane phospholipid metabolism in first-episode, drug-naive schizophrenics. A pilot study of the dorsal prefrontal cortex by in vivo phosphorus 31 nuclear magnetic resonance spectroscopy. Arch Gen Psychiatry 1991; 48:563-8.*
65. *Pifferi F, Jouin M, Alessandri JM, Haedke U, Roux F, Perriere N, Denis J, Lavialle M & Guesnet P: n-3 fatty acids modulate brain glucose transport in endothelial cells of blood-brain barrier. Prostaglandins Leukot Essent Fatty Acids 2007; 77:279-86.*
66. *Plourde M, Fortier M, Vandal M, Tremblay-Mercier J, Freemantle E, Bégin M, Pifferi F & Cunnane SC: Unresolved issues in the link between docosahexaenoic acid and Alzheimer disease. Prostaglandins Leukot Essent Fatty Acids 2007; 77:301-8.*
67. *Ponizovsky AM, Modai I, Nechamkin Y, Barshtein G, Ritsner MS, Yedgar S, Lecht S & Bergelson LD: Phospholipid patterns of erythrocytes in schizophrenia: relationships to symptomatology. Schizophr Res 2001;52:121-6.*
68. *Prabakaran S, Swatton JE, Ryan MM, Huffaker SJ, Huang JT-J, Griffin JL et al: Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. Mol Psychiatry 2004; 6:684-97.*
69. *Puri BK, Richardson AJ, Counsell SJ, Ward PE, Bustos MG, Hamilton G et al: Negative correlation between cerebral inorganic phosphate and the volumetric niacin response in male patients with schizophrenia who have seriously and dangerously violently offended: A 31P magnetic resonance spectroscopy study. Prostaglandins Leukot Essent Fatty Acids 2007; 77:97-9.*
70. *Rao JS, DeMar JC, Rapoport SI, Bazinet RP & Lee H-J: Dietary n-3 PUFA deprivation alters expression of enzymes of the arachidonic and docosahexaenoic acid cascades in rat frontal cortex. Mol Psychiatry 2007;12:151-7.*
71. *Rapaka RS, Piomelli D, Spiegel S, Bazan N & Dennis EA: Targeted lipidomics: Signaling lipids and drugs of abuse. Prostaglandins Other Lipid Mediat 2005; 77:223-34.*
72. *Rapoport SI, Rao JS & Igarashi M: Brain metabolism of nutritionally essential polyunsaturated fatty acids depends on both the diet and the liver. Prostaglandins Leukot Essent Fatty Acids 2007; 77:251-61.*
73. *Richardson AJ: Long-Chain Polyunsaturated Fatty Acids in Childhood Developmental and Psychiatric Disorders. Lipids 2004; 39:1215-22.*
74. *Ross BM, Hudson C, Erlich J, Warsh JJ & Kish SJ: Increased phospholipid breakdown in schizophrenia. Evidence for the involvement of a calcium-independent phospholipase A2. Arch Gen Psychiatry 1997; 54:487-94.*

75. *Ross BM, Turenne S, Moszcsynska A, Warsh JJ & Kish SJ. Differential alteration of phospholipase A2 activities in brain of patients with schizophrenia. Brain Res 1999; 821:407-13.*
76. *Ross BM: Phospholipid and eicosanoid signaling disturbances in schizophrenia. Prostaglandins Leukot Essent Fatty Acids 2003; 69:259-69.*
77. *Ross BM, Hughes B, Kish SJ & Warsh JJ: Serum calcium-independent phospholipase A2 activity in bipolar affective disorder. Bipolar Disord 2006; 8:265-70.*
78. *Sampath H & Ntambi JM: Polyunsaturated fatty acid regulation of genes of lipid metabolism. Annu Rev Nutr 2005; 25:317-40.*
79. *Slanina KA, Roberto M & Schweitzer P. Endocannabinoids restrict hippocampal long-term potentiation via CB1. Neuropharmacol 2005; 49:660-8.*
80. *Smesny S, Rosburg T, Klemm S, Riemann S, Baur K, Rudolph N et al: The influence of age and gender on niacin skin test results - implications for the use as a biochemical marker in schizophrenia. J Psychiatr Res 2004; 38:537-43.*
81. *Smesny S, Rosburg T, Riemann S, Baur K, Rudolph N, Berger G & Sauer H: Impaired niacin sensitivity in acute first-episode but non in multi-episode schizophrenia. Prostaglandins Leukot Essent Fatty Acids 2005; 72:393-402.*
82. *Smesny S, Rosburg T, Baur K, Rudolph N & Sauer H: Cannabinoids Influence Lipid-Arachidonic Pathways in Schizophrenia. Neuropsychopharmacol 2007a; 32:2067-73.*
83. *Smesny S, Klemm S, Stockebrand M, Grunwald S, Gerhard UJ, Rosburg T et al: Endophenotype properties of niacin sensitivity as marker of impaired prostaglandin signaling in schizophrenia. Prostaglandins Leukot Essent Fatty Acids 2007b; 77:79-85.*
84. *Su K-P: Mind-body interface: the role of n-3 fatty acids in psychoneuroimmunology, somatic presentation, and medical illness comorbidity of depression. Asia Pac J Clin Nutr 2008; 17 (S1):151-7.*
85. *Tavares H, Yacubian J, Talib LL, Barbosa NR & Gattaz WF: Increased phospholipase A2 activity in schizophrenia with absent response to niacin. Schizophr Res 2003; 61:1-6.*
86. *Thomas W, Coen N, Faherty S, Flatharta CO & Harvey BJ: Estrogen induces phospholipase A2 activation through ERK1/2 to mobilize intracellular calcium in MCF-7 cells. Steroids. 2006; 71:256-65.*
87. *Vancassel S, Leman S, Hanonick L, Denis S, Roger J, Nollet M et al: n-3 polyunsaturated fatty acids supplementation reverses stress-induced modifications on brain monoamine levels in mice. J Lipid Res 2008; 49:340-8.*
88. *van Meer G: Cellular lipidomics. The EMBO Journal 2005; 24:3159-65.*
89. *Wahle KWJ, Caruso D, Ochoa JJ & Quiles JL: Olive Oil and Modulation of Cell Signaling in Disease Prevention. Lipids 2004; 39:1223-31.*
90. *Wang Y, Voy BJ, Urs S, Kim S, Soltani-Bejnood M, Quigley N et al: The Human Fatty Acid Synthase Gene and De Novo Lipogenesis are Coordinately Regulated in Human Adipose Tissue. J Nutr 2004; 134:1032-8.*
91. *Williamson PC, Brauer M, Leonard S, Thompson T & Drost D: 31P magnetic resonance spectroscopy studies in schizophrenia. Prostaglandins Leukot Essent Fatty Acids 1996; 55:115-8.*
92. *Wolfrum C & Spener F: Fatty acid as regulators of lipid metabolism. Eur J Lipid SciTechnol 2000; 102:746-62.*
93. *Yao JK & van Kammen DP: Membrane phospholipids and cytokine interaction in schizophrenia. Int Rev Neurobiol 2004; 59:297-326.*
94. *Yavin E: Versatile roles of docosahexaenoic acid in the prenatal brain: From pro- and anti-oxidant features to regulation of gene expression. Prostaglandins Leukot Essent Fatty Acids 2006; 75:203-11.*
95. *Youdim KA, Martin A & Joseph JA: Essential fatty acids and the brain: possible health implications. Int J Dev Neurosci 2000; 18:383-99.*
96. *Zamaria N. Alteration of polyunsaturated fatty acid status and metabolism in health and disease. Reprod Nutr Dev 2004; 44:273-82.*
97. *Zeldin D: Epoxygenase Pathways of Arachidonic Acid Metabolism. J Biol Chem 2001; 276:36059-62.*

Acknowledgements:
This work was supported by grants No. 062-0982522-0369 and No. 062-0000000-0221 from the Ministry of Science, Education, and Sports, Zagreb, Croatia.

Correspondence:
Alena Buretić-Tomljanović, PhD, Associate Professor
Dept. of Biology and Medical Genetics, University of Rijeka
Braće Branchetta 22, Rijeka, Croatia
E-mail: alena@medri.hr