J. Biol. Res. 2006 n. I - Vol. LXXXI - Rubbettino - Soveria Mannelli

Biological, biochemical and mathematical considerations about the use of an Artificial Neural Network (ANN) for the study of the connection between platelet fatty acids and major depression Technical report

M. Cocchi, L. Tonello

Fondazione Villa Maria, Centro di Biologia Molecolare

KEY WORDS: platelet, fatty acids, Major Depression, Artificial Neural Network

Abstract

During the last years a great deal of scientific research has been spent to identify biochemical markers (mainly fatty acids) in the erythrocyte membrane and in plasma phospholipids with the purpose of a biochemical characterization of the Major Depression.
As we didn't consider fully satisfying the above research models, we have performed the study of the fatty acids in a very particular and complex tissue as the platelets are.
It is very well known that the platelets have metabolic capacities for the fatty acids synthesis, and they have receptors for the neurotransmitters.
The research of a mathematical way able to perform an efficient biochemical diagnosis of Major Depression has convinced us to realize an ANN (Self Organizing Map – SOM), the so called Kohonen network.
A number of continuous and independent proofs has highlighted the SOM discriminating capacity, selecting only three parameters among all ones studied: Arachidonic Acid, Linoleic Acid and Palmitic Acid.
The deep analysis of the meanings of ADAM has given to us the opportunity to check if biochemical and nutritional strategies are possible to modify, in a significant way, the conditions of the depressive patients.
We have hypothesized that the position of the cases could be linked to a different physical-chemical condition of the platelet membrane, determined by different ratios among the three fatty acids.
It is likely that within the circumstances witch discriminate an healthy from a pathologic subject, the Linoleic Acid gives a biochemical modification of the factors involved in the determination of the biochemical aspect of the pathology.
We must consider, further, that the effects of the Linoleic Acid on the pathology are relevant to condition of balance between Arachidonic Acid and Palmitic Acid.
Practically, with reference to the position of the subjects over ADAM map, when the Arachidonic Acid percent is over a certain value, the subject is certainly depressed, in the same way for the values of the Palmitic Acid.

During the last years a great deal of scientific research has been spent to identify biochemical markers (mainly fatty acids) in the erythrocyte membrane and in plasma phospholipids with the purpose of a biochemical characterization of the Major Depression.
In particular, a great attention has been paid to the study of the n-6 and n-3 fatty acids and their ratio n-6/n-3 (Adams et al., 1996; Green et al., 2005; Hakkarainen et al., 2004; Maes et al., 1996; Mischoulou et al., 2000; Passi et al., 2003; Peet, 2003; Peet et al. 1998; Puri et al., 2001).
As we didn't consider fully satisfying the above research models, we have performed the study of the fatty acids in a very particular and complex tissue as the platelets are (Cocchi et al., 2006).
It is very well known that the platelets have metabolic capacities for the fatty acids synthesis, and they have receptors for the neurotransmitters.

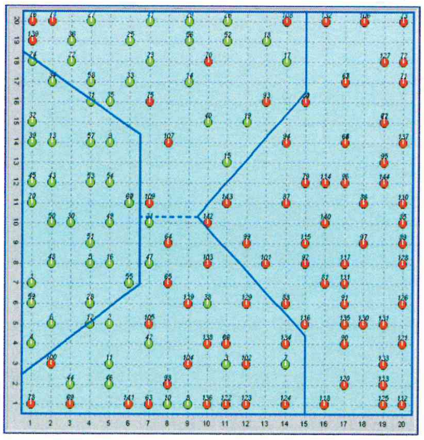
These characteristics show the platelets as an element very similar to the neuron. With scientific simplification "a fragment of circulating brain".
According to our idea we have recruited 84 patients (51 females and 33 males, mean age: 60.21, SD: ± 12.27) in an outpatient and inpatient Psychiatric Clinic (Villa Baruzziana) in Bologna (Italy); 60 controls (38 Males and 22 Females, mean age: 33.97, SD: ± 12.40) from the community.
We have decided for ethical reasons to not submit the normal subjects to a proper psychiatric visit. The un-balance of the numeric density between patients and controls was kept in order to strengthen the ANN learning to reduce the false negatives.
All patients were interviewed to confirm the diagnosis of major depression. The tools used were: Clinical Global Impression (CGI), Symptoms Check List-90 (SCL-90), Medical and pharmacological history, BMI, Structured Clinical Interview DSM-IV-SCID-IV (American Psychiatric Association 2000), (Diagnostic and Statistical Manual of Mental Disorders, 2000) Hamilton Rating Scale of Depression (HRSD).
The severity of depressive symptoms in the patients group was assessed with the Hamilton Rating Scale of Depression 21 items version HRSD-21 (Hamilton, 1960)
The study was approved by the Ethic Committee of the local Health Authorities.
We have investigated the platelet fatty acids and some antioxidants (Vit. E and CoQ_{10}), in total 19 parameters; the statistic analysis has found a significant difference in 8 of them (Table 1).

VARIABLE	p
C20:4	< 0.01
C18:2	< 0.01
C16:0	< 0.01
C17:1	< 0.01
C22:6	< 0.01
C18:0	< 0.01
VitE	< 0.01
C16:1	< 0.05

Tab. 1 - Significance levels of the ANOVA between normal and depressive subjects

The research of a mathematical way able to perform an efficient biochemical diagnosis of Major Depression has convinced us to realize an ANN (Self Organizing Map – SOM), the so called Kohonen network (Kohonen, 1995; 1997)
A number of continuous and independent proofs has highlighted the SOM discriminating capacity selecting only three parameters among all ones studied: Arachidonic Acid, Linoleic Acid and Palmitic Acid.

The network has been named "ADAM" (Analysis of Depression with Artificial neural network Mapping).



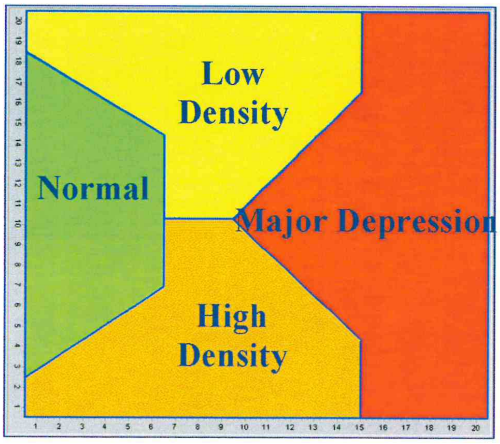


Fig. 1, 2

The SOM has distributed the 144 subjects (administered in an hidden form) over the map and has allowed us to recognize four sub – maps:
Two specifics (green = exclusively normal, red = exclusively major depression) and two mixed (yellow and orange respectively with high density of normal and high density of pathologic). The two intermediate sub – map (yellow and orange) have been considered, at the beginning, as expression of possible different degrees of depressive pathology (for the people with major depression diagnosis) and of a non diagnosed pathological condition in the case of the normal people, moreover, in agreement to the scientific literature. (Bellantuomo et al., 2002; Poluzzi et al., 2004)
It must be underlined that none of the pathological subjects is in the green map and none of the "normal" is in

the red map. This evidence shows that the platelet fatty acids can characterize the condition of the subject and that they can interpret the variations of the fatty acids administered to the SOM.

The three fatty acids have a constant sum in all the subjects investigated (53,33 $\pm$ 3,43 with a correlation coefficient of – 0,66).

We have hypothesized that the position of the cases could be linked to a different physical-chemical condition of the platelet membrane, determined by different ratios among the three fatty acids.

We have calculated a ratio index, B1, between Palmitic Acid (saturated) and Arachidonic Acid + Linoleic Acid (polyunsaturated)

$$\mathbf{B1} = \frac{\textbf{Palmitic Acid}}{\textbf{Linoleic Acid + Arachidonic Acid}}$$

The ratio has given an average index which, in each fraction of the general map has showed an increasing degree of unsaturation (green – yellow – red) and of saturation (green – orange and red – orange), confirming the particular characteristic of the orange fraction. Fig. 3, 4

A further in depth study has allowed us to recognize a more efficient index, B2, with the capacity to correlate the characteristics of saturation and of unsaturation of the three fatty acids together.

Following a discussion with Prof. Gianni Lercker to whom we are gratefully indebted, the index has been identified:

$$B_2 = \sum_{i=1}^{3} \left(A_i \frac{mp_i}{mw_i}\right)$$

i	Name	
1	Palmitic A.	C 16:0
2	Linoleic A.	C 18:2
3	Arachidonic A.	C 20:4

where:

$\boldsymbol{A_i}$ = percent of *i* Fatty Acid

$\boldsymbol{mw_i}$ = molecular weight of *i* Fatty Acid

$\boldsymbol{mp_i}$ = melting point of *i* Fatty Acid

It represents the melting coefficient obtained by the sum of the three fatty acids melting points.

The B2 coefficient has been calculated for each one of the 144 subjects.

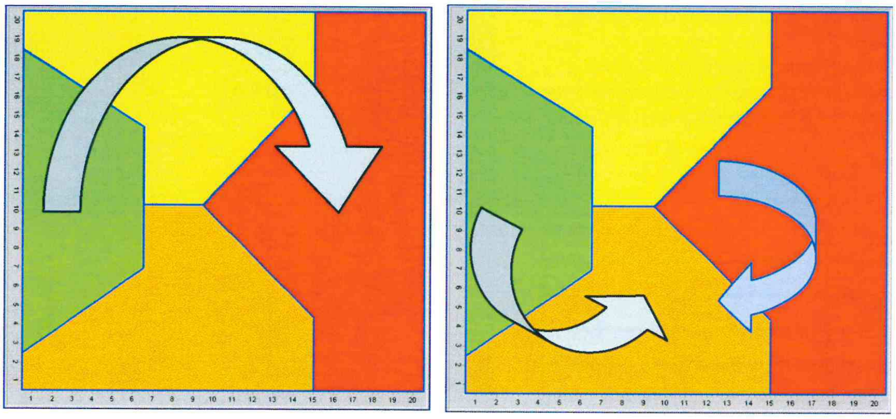


Fig. 3, 4

Mappa B2

20	2,24	1,17	1,92	1,89	1,92	2,04	2,11	1,85	1,68	1,59	1,58	1,56	1,64	1,60	1,30	0,99	0,50	0,02	-0,49	-1,02
19	1,92	1,94	2,25	2,08	1,98	1,98	2,00	1,71	1,53	1,44	1,37	1,53	1,70	1,54	1,32	0,96	0,48	0,10	0,12	-0,21
18	2,34	2,28	2,21	2,20	2,11	2,00	1,91	1,83	1,55	1,34	1,36	1,50	1,51	1,33	1,29	0,52	0,39	0,36	0,35	0,44
17	2,36	2,37	2,27	2,19	2,22	2,12	2,04	1,85	1,77	1,55	1,39	1,32	1,31	1,30	1,23	0,49	0,39	0,38	0,43	0,51
16	3,03	2,57	2,35	2,38	2,31	2,18	2,09	2,03	1,71	1,47	1,29	1,23	1,31	1,27	1,25	0,97	0,40	0,43	0,46	0,47
15	3,21	3,19	2,59	2,47	2,53	2,39	2,21	2,41	1,67	1,40	1,27	1,14	1,22	1,21	1,22	0,72	0,53	0,47	0,47	0,15
14	3,13	3,24	2,76	2,55	2,79	2,73	2,55	2,57	2,27	1,36	1,15	1,13	1,16	1,16	1,08	0,55	0,54	0,48	0,11	-0,49
13	2,99	3,05	2,53	2,44	2,53	2,47	2,42	2,53	2,17	1,17	1,09	1,11	1,02	1,01	0,67	0,49	0,51	0,18	-0,01	-0,24
12	2,91	2,83	2,35	2,28	2,34	2,18	2,01	2,06	1,97	1,63	1,55	1,48	0,79	0,67	0,60	0,43	0,49	0,12	-0,21	-0,27
11	2,39	2,54	2,44	2,31	2,22	2,12	1,93	1,97	2,22	2,20	2,01	1,91	0,79	0,73	0,63	0,48	0,31	0,07	-0,18	-0,38
10	2,49	2,55	2,45	2,41	2,28	2,13	2,01	1,97	2,27	2,40	2,15	1,68	1,07	0,69	0,55	0,50	0,35	0,05	-0,30	-0,42
9	2,83	2,78	2,54	2,52	2,46	2,26	2,05	1,91	2,02	2,21	1,81	1,57	1,37	0,69	0,56	0,51	0,22	0,04	-0,24	-0,40
8	3,28	3,10	2,78	2,53	2,57	2,43	2,23	2,03	2,01	2,05	1,91	1,41	1,23	0,87	0,58	0,51	0,32	0,19	-0,41	-0,57
7	3,45	3,33	2,88	2,69	2,57	2,50	2,22	1,93	1,97	1,82	1,66	1,38	1,19	0,85	0,63	0,64	0,38	0,32	-0,37	-0,51
6	3,78	3,74	3,07	2,86	2,88	2,59	2,43	2,06	2,07	1,54	1,50	1,40	1,11	0,83	0,76	0,44	0,26	0,08	-0,36	-0,46
5	4,05	3,80	3,40	3,13	2,93	2,76	2,54	2,49	1,99	1,81	1,83	1,57	1,12	0,87	0,75	0,33	-0,05	-0,05	-0,35	-0,54
4	4,60	4,20	3,75	3,18	3,18	2,91	2,85	2,71	2,20	1,99	1,89	1,85	1,23	1,01	0,91	0,10	-0,03	-0,14	-0,70	-0,80
3	4,45	4,20	4,19	3,68	3,45	3,36	2,90	2,69	2,42	2,09	1,93	1,77	1,51	1,34	1,18	-0,01	-0,16	-0,82	-1,07	-1,01
2	7,10	4,47	4,14	3,96	3,63	3,40	3,34	2,91	2,75	2,43	2,08	2,05	1,66	1,19	0,73	-0,14	-0,29	-1,00	-1,53	-1,95
1	8,23	6,58	4,26	3,98	3,36	3,06	3,89	3,07	2,90	2,50	2,16	2,34	1,72	1,03	0,50	-0,03	-0,19	-1,48	-1,79	-2,64
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**

Table 2

B2 < 1
1 < B2 < 2
2 < B2 < 3
B2 > 3

Tab. 2

This brought to a further classification of the subjects with statistic significance.
When the SOM was asked to map the B2 expected in each point we obtained (Tab.2):
As a result we obtained a distribution of the coefficients in increasing order, from the right to the left of the SOM (from -2,64 to 8,23) with a typical fan form. Fig. 5

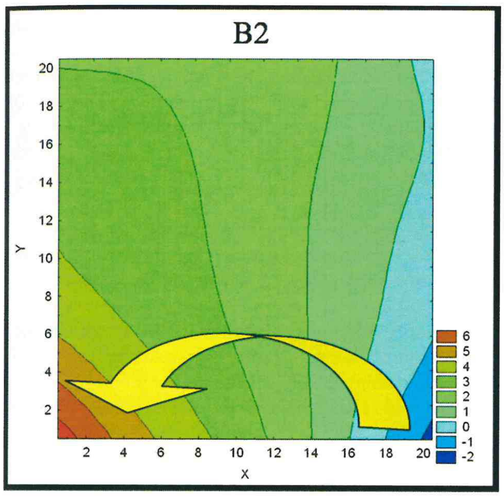


Fig. 5

$$B_2 \approx +0.25PA\,\% -0.02LA\% -0.16AA\%$$

The subjects of the green fraction have a B2 average value of 2,80 and this value is the middle point between – 2,64 (absolute minimum of the map) and 8,23 (absolute maximum of the map).
We must highlight the orange fraction where, excluding the red fraction, there are reflected all the B2, of positive mark, of the whole map.
A careful analysis of the mathematical formulation of the B2 shows that it is ruled out, almost completely, by the Arachidonic Acid and by the Palmitic Acid. Fig. 6, 7

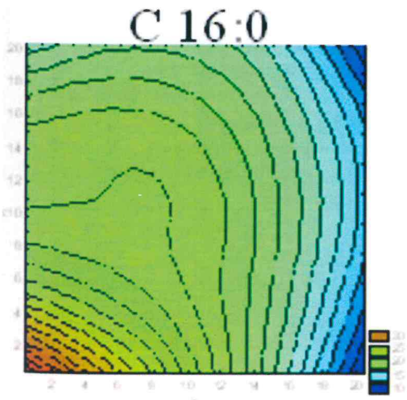


Fig. 6

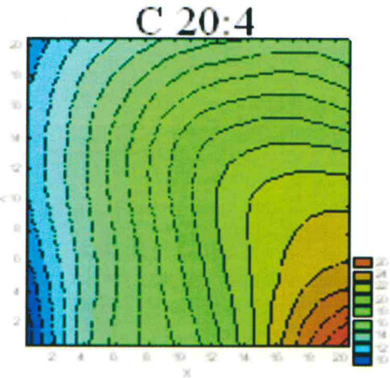
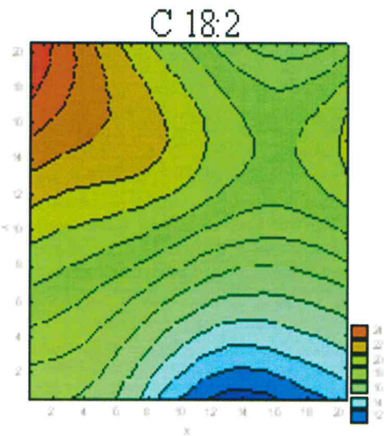


Fig. 7

These two fatty acids define the belonging macro area of a subject. Within a macro area it is the Linoleic Acid that modulates with precision the B2 position and consequently the position of the subjects, Fig. 8, 9



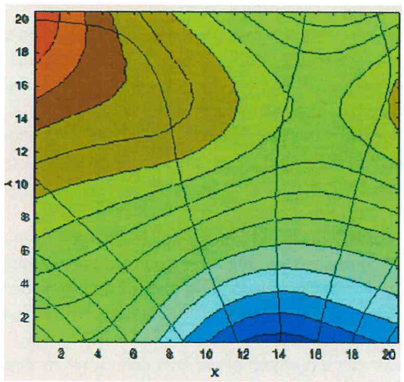


Fig. 8, 9

e. g.: two subjects with the same B2, one diagnosed as depressive the other normal differ by the percent of Linoleic Acid. This observation is plausible if we think to the

very low melting coefficient of Linoleic Acid.
It is likely that within the circumstances witch discriminate an healthy from a pathologic subject, the Linoleic Acid gives a biochemical modification of the factors involved in the determination of the biochemical aspect of the pathology.
We must consider, further, that the effects of the Linoleic Acid on the pathology are relevant to condition of balance between Arachidonic Acid and Palmitic Acid.
Practically, with reference to the position of the subjects over ADAM map, when the Arachidonic Acid percent is over a certain value, the subject is certainly depressed, in the same way for the values of the Palmitic Acid.
When the B2 is close to its normal value, the percentage of Linoleic Acid becomes very important
These aspects introduce, moreover, to the concept of hyper-saturation of the platelets in the case of the Palmitic Acid and of hyper-un-saturation in the case of the Arachidonic Acid.
A further consideration, for the characterization of the pathology, must be done for the Linoleic Acid.
It's excess can, further, determine the biochemical conditions for the development of the pathology (as verified in the depressive patients).
Certainly the network develops mathematical operations, not yet known, among the fatty acid values administered to the SOM.
We have investigated the platelet fatty acids of other subjects and ADAM gave us the corresponding level of incidence of depression and of normality according to the scientific literature:

1) subjects with Morphoea (n=80)
2) subjects with Scleroderma (n=31)
3) subjects with Ischemic Cardiovascular Pathology (n=31)
4) subjects with Obesity (children, 11-13 years old; n=23)
5) young adult (18-24 years old; n=45)

It has been impossible to us, for the obvious ethic difficulties, to determine the psychiatric profile.
Nevertheless, the percentage of depression according to ADAM is very close to the epidemiological data of the international literature. (Gruck et al., 2001;Lima, 2004; Crowe et al., 2006)
The deep analysis of the meanings of ADAM has given to us the opportunity to check if biochemical and nutritional strategies are possible to modify, in a significant way, the conditions of the depressive patients.
In other words, if it is possible to modify the ADAM position of the patient changing the fatty acid profile within a possible and compatible range.
The researches are running.
From the simulations we have tried until now, we have assumed that changes of the ADAM patient position are possible and, virtually, it is possible to recover the patient to a normal condition or to maintain him in the pathological condition.
This means that a number of patients cannot be recovered to a normal biochemical condition and, perhaps to a normal clinical condition.
If drugs are utilized and their effect, among the others, is to modify the Arachidonic Acid (e.g. Lithium), we think that it is absolutely necessary to know the platelet Arachidonic Acid level (the same for the other fatty acids) to not create dangerous consequences.
It has been possible to observe, through simulations, that, changes of similar amount of the fatty acid can significantly better or worsening the position of the subject.
We have thought also, if ADAM could be a network able to classify animal and human brains according to the same three fatty acids.
Literature and personal brain fatty acid composition data have been administered to the network.(Svennerohlm, 1968; Cocchi et al., 1993; Maldjian et al., 1995)
All the data have confirmed that the brain Linoleic Acid is very low if compared to the other tissues (from 0.5% to 2.0).
We thought that the low level of Linoleic Acid could wrong the network, instead, the three fatty acid found their position in 14:1 with a range from 14:1 to 11:1 (11:1 represents very few cases according to the age and the animal kind).
The brain B2 values are compatible with the data of the subjects who have the same position in ADAM even though the Linoleic Acid values are significantly different from the brain ones.
The 14:1 coordinate is very characteristic for some reasons:

1) since its position belongs to a restricted area where the B2 has a strong gap with the red area.
2) Since it is the pivot of the curves of the B2 which, in turn, is the starting point of the above mentioned fan.
3) Since it has been demonstrated that the brain fatty acid coordinates don't change their position also in spite of Linoleic Acid modifications up to 10% of absolute value.

According to the mathematical obviousness which has supported the experimental work, we want to elaborate some conjectures.
The brain, evaluated through ADAM, seems to have similar biochemical platelets characteristics as those of the patient with depression.
Nevertheless the brain is guarantee by an unquestioned and strong stability given by the great range of tolerance to the Linoleic Acid modifications, before it is possible to induce substantial changes from its original position.
It is not thinkable to easily fill this gap.
On the contrary, it is plausible to think that such a low concentration of brain Linoleic Acid could be in related to a rigorous mechanism of metabolic control.
The central role of the Linoleic Acid seems to be confirmed, also in platelets, since it becomes the element which regulates the distribution of all the map values starting from 14:1 which is the absolute minimum of the map.
Increasing its value, with a different degree of intensity, it is possible to reach each point of the map.
We must still understand how it is possible such a precise correspondence between the mathematical calculation of the network and the biological event.

References

Adams PB, Lawson S, Sanigorski A, Sinclair AJ. 1996. Arachidonic acid to eicosapentaenoic acid ratio in blood correlates positively with clinical symptoms of depression. Lipids 31 (suppl): S157–61.

Bellantuono C, Mazzi MA, Tansella M, Rizzo R., Goldberg D. 2002. The identification of depression and the coverage of antidepressant drug prescriptions in Italian general practice. J Affect Disord, Oct; 72 (1): 53-9.

Cocchi M, Noble RC, Fallowfield H, Speake B, Turchetto E. 1993. The significance of n-3 fatty acids in fetal and neonatal development and some alternative source. Proceeding of the Nutrition Society - Abstract of Communication, 224/A.

Cocchi M, Tonello L et al. 2006. Biochemical Markers in Major Depression as interface between Neuronal Network and Artificial Neural Network. Journal of Biological Research, in press.

Crowe M, Ward N, Dunnachie B, Roberts M. 2006. Characteristics of adolescent depression. International Journal of Mental Health Nursing, 15: 10-18.

Diagnostic and Statistical Manual of Mental Disorders (2000) Fourth Edition, Text Revision (DSM-IV-TR®).

Green P, Gispan-Herman I and Yadid G. 2005. Increased arachidonic acid concentration in the brain of flinders sensitive line rats, an animal model of depression. Journal of Lipid Research, Vol. 46: 1093-1096.

Gruck TP, Kavan MG, Elsasser GN, Barone EJ. 2001. Assesment and tretment of depression following myocardial infarction. Am Fam Physician, 64: 641-648.

Hakkarainen R, Partonen T, Haukka J, Virtamo J, Albanes D, Lönnqvist J. 2004. Is low dietary intake of omega-3 fatty acids associated with depression? Am J Psychiatry 161: 567–569.

Hamilton M. 1960. A rating scale for depression. J Neurol Neurosurg Psychiat. Feb; 23:56-62.

Kohonen T. 1995(1st ed), 1997 (2nd ed). "Self Organizing Maps" Berlin: Springer-Verlag.

Lima D. 2004. Bipolar disorder and depression in childhood and adolescence. Jornal de Pediatria, 80 (2 suppl): S11 - S20.

Maes M, Smith R, Christophe A, Cosyns P, Desnyder R, Meltzer H. 1996. Fatty acid composition in major depression: decreased omega 3 fractions in cholesteryl esters and increased C20:4 omega 6/C20:5 omega 3 ratio in cholesteryl esters and phospholipids. J Affect Disord 38:35–46.

Maldjian A, Farkas K, Noble RC, Cocchi M, Speake BK. 1995. The transfer of docosahexaenoic acid from the yolk to the tissues of the chick embryo. Biochim. Biophys. Acta, Sep 14; 1258 (2): 81-9.

Mischoulon D, Fava M. 2000. Docosahexanoic acid and omega-3 fatty acids in depression. Psychiatr Clin North Am 23:785–94.

Passi S, Stancato A, Aleo E, Dmitrieva A, Littarru GP. 2003. Statins lower plasma and lymphocyte ubiquinol/ubiquinone without affecting other antioxidants and PUFA. Biofactors, 18(1-4):113-124.

Peet M, Murphy B, Shay J, Horrobin D. 1998. Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. Biol Psychiatry 43:315–319.

Peet M. 2003. Eicosapentaenoic acid in the treatment of schizophrenia and depression: rationale and preliminary double-blind clinical trial results. Prostaglandins Leukot Essent Fatty Acids (Scotland), 69(6) p477-85.

Poluzzi E et al. 2004. Prescriptions of antidepressants in primary care in Italy: pattern of use after admission of selective serotonin reuptake inhibitors for reimbursement. Eur J Clin Pharmacol. Jan, 59 (11): 825-31.

Puri BK, Counsell SJ, Hamilton G et al. 2001. Eicosapentaenoic acid in treatment-resistant depression associated with symptom remission, structural brain changes and reduced neuronal phospholipid turnover. Int J Clin Pract (England), 55(8) p560-3.

Svennerohlm L. 1968. Distribution and fatty acid composition of phosphoglycerides in normal human brain. Journal of Lipid Research, Volume 9.