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# Erythrocyte membrane polyunsaturated fatty acid (PUFA) levels in a sample of patients with schizophrenia and relation with clinical and progression variables

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**Introduction.** Previous studies have shown that erythrocyte cell membranes in patients with schizophrenia contain considerably less omega-3 fatty acids, particularly EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), reflecting the lower levels present in neuronal and central nervous system membranes. This phenomenon, linked to genetic, metabolic, or dietary factors, has been associated with the development of schizophrenia and the risk of developing and the severity of metabolic syndrome.

**Methods.** This study is an observational study conducted in a sample of 31 patients with schizophrenia treated at the Mataró Mental Health Center (Barcelona). Its aim was to relate the erythrocyte levels of omega 3 with the clinical severity of schizophrenia and dietary habits.

EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid) and other membrane lipid levels were determined, as well as psychopathology, cognitive, and social functioning measures, previous evolution, and finally a survey of dietary habits.

**Results.** Our results did not show a statistically significant correlation between erythrocyte omega-3 levels and psychopathological and clinical severity variables. Higher, statistically significant, levels were found in the group of women and in subjects with more days of admission to the day hospital. In contrast, lower values were obtained in subjects treated with long-acting antipsychotics and in sunflower oil consumers.

**Conclusions.** Despite not being able to demonstrate our working hypothesis, significant correlations were found that were consistent with published findings in the current literature. The need for studies with larger samples and groups of healthy controls is postulated.

**Keywords:** Schizophrenia, Omega-3 polyunsaturated fatty acids, Erythrocyte membranes

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## Niveles de ácidos grasos poliinsaturados (PUFA) en la membrana eritrocitaria de una muestra de pacientes con esquizofrenia y su relación con variables clínicas y evolutivas

**Introducción.** Estudios previos han mostrado que las membranas celulares eritrocitarias de pacientes con esquizofrenia presentan una reducción considerable de ácidos grasos omega-3, particularmente EPA (ácido eicosapentaenoico) y DHA (ácido docosahexaenoico), reflejando niveles disminuidos en las membranas neuronales y del sistema nervioso central. Este fenómeno, ligado a factores genéticos, metabólicos o dietéticos, ha sido relacionado con el desarrollo de la esquizofrenia, con el grado de gravedad y con un mayor riesgo de desarrollar síndrome metabólico.

**Metodología.** El presente estudio, es un estudio observacional que se realizó en una muestra de 31 pacientes con esquizofrenia del Centro de Salud Mental de Mataró (Barcelona). El objetivo fue relacionar los niveles eritrocitarios de omega 3 con la severidad clínica del trastorno y los hábitos dietéticos.

Se determinaron los niveles de EPA (ácido eicosapentaenoico), DHA (ácido docosahexaenoico) y otros lípidos de membrana, así como medidas de estado psicopatológico, funcionamiento cognitivo y social, evolución previa y finalmente una encuesta de hábitos dietéticos.

**Resultados.** Nuestros resultados no mostraron una correlación estadísticamente significativa entre los niveles de ácidos omega 3 eritrocitarios y las variables de gravedad psicopatológica y clínica. No obstante, se hallaron niveles superiores, estadísticamente significativos, en el grupo de muje-

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res y en los pacientes con mayor número de días de ingreso en el hospital de día. De forma opuesta, los valores inferiores se obtuvieron en pacientes tratados con antipsicóticos de acción prolongada y en consumidores de aceite de girasol.

**Conclusiones.** A pesar de no haber podido demostrar nuestra hipótesis de trabajo, se han encontrado correlaciones significativas coherentes con los hallazgos en la literatura actual es por ello que se postula la necesidad de realizar estudios con muestras más amplias y presencia de grupos de controles sanos.

**Palabras clave:** Esquizofrenia, Omega-3, Ácidos grasos poliinsaturados, Membranas eritrocitarias

## INTRODUCTION

Polyunsaturated fatty acids (PUFAs) are essential constituents of cell membrane phospholipids. In the case of neurons, membrane structure and composition can affect key processes like the functionality and sensitivity of neurotransmitter receptors, ion channels, or mitochondrial activity.[1,2] Appropriate amounts of omega 3 and omega 6, as well as several growth factors, are essential for brain development during the perinatal period and adolescence.[3,4] The contributions of docosahexaenoic acid (DHA, of the omega-3 series), arachidonic acid (AA, of the omega-6 series), and nervonic acid (C24:1, of the omega 9 series) are particularly essential. DHA is the major component of excitable membranes, being equivalent to 20% of the brain's dry weight. The deposition of these fatty acids starts during intrauterine brain growth and continues throughout the first months after birth and on to pubertal stages. From an evolutionary perspective, these periods are of particular interest for the development of schizophrenia and other neuropsychiatric diseases as it is postulated that abnormalities in the cytoarchitecture of the phospholipid membrane could be a factor in schizophrenia risk or vulnerability, as Horrobin proposed in the early 1990s.[5] Since omega-3 fatty acids cannot be synthesized in the body, it has been suggested that a decreased dietary supply of these fatty acids, as occurs in industrialized areas, could explain the greater severity of schizophrenia in developed countries.[6]

Erythrocyte membranes have a structure and composition comparable to that of neuronal membranes and are considered to be a good biomarker of the metabolism and intake of omega-3 fatty acids in the body.[7] Studies of PUFAs in erythrocyte membranes have found lower levels in patients with schizophrenia compared to healthy subjects.[8-10] These results have also been reported in subjects with untreated schizophrenia[11] and confirmed by meta-analyses of a total of more than 600 patients and 500 controls.[12,13] Therefore a deficiency of PUFAs, particularly DHA, EPA, and AA, in the erythrocytes of subjects may be a marker of risk for schizophrenia. Since this is a potentially modifiable factor, it would be of the highest scientific and medical interest to confirm it.[14] From this perspective, we studied possible links between lower PUFA levels in erythrocyte membranes and the identification of certain clinical, progression, and prognostic features of the disease, such as the predominance of negative symptoms,[15] deficits in social cognition,[16] or poor response to treatment.[12]

In the present study, we analyzed PUFA levels in erythrocyte membrane in a group of subjects with schizophrenia and explored possible relations between this variable and clinical and developmental variables. The working hypothesis was that subjects with low PUFA levels, specifically of DHA, EPA, and AA, may exhibit worse clinical and cognitive developmental or social functioning results.

## METHODS

### Study Design

In this cross-sectional study we analyzed a sample of 31 subjects diagnosed with schizophrenic or schizoaffective disorder according to DSM-IV criteria.[17] The subjects were recruited from the Mataró Mental Health Center (Barcelona) as participants in a broader study of the efficacy of polyunsaturated fatty acids in the clinical improvement and prevention of metabolic syndrome (multicenter, randomized, controlled, open-label trial) that began in 2009 and ended in 2012. The subjects in the sample were part of the control group (which did not receive supplemental omega-3 treatment). The final stage of the study consisted of the analytical determination of PUFA levels in plasma and erythrocyte membrane. The subjects were of ages between 18 and 45 years old, receiving outpatient treatment with one or more antipsychotic agents, and had signed the informed consent. A diagnosis of diabetes mellitus, organic mental disorder, or intellectual disability was considered an exclusion criterion. The study was approved by the Ethics and Clinical Trial Committee of the Maresme Healthcare Consortium.

### Procedures

#### *Measurement of polyunsaturated fatty acids in erythrocyte membrane*

The extraction and derivatization method used was direct transesterification (Lepage method[18]) and gas chromatography analysis with a flame ionization detector (FID). Erythrocyte membrane levels were obtained for omega-3 (EPA+DHA) fatty acids and some of the fatty acids of the w-3 and w-6 series (linoleic acid [LA], γ-linolenic acid [GLA], α-linolenic acid [ALA], arachidonic acid [AA], total W-6, total W-3, and the W-6/W-3 ratio). Triglyceride, cholesterol,

and glucose levels were measured. Samples were collected under fasting conditions.

### *Assessment*

Clinical assessment consisted, in first place, of a psychopathological interview conducted by an experienced psychiatrist in which the diagnosis of schizophrenia or schizoaffective disorder was confirmed and a psychopathological assessment was made using the Positive and Negative Syndrome Scale (PANSS)[19] and the Clinical Global Impression – Severity Scale (CGI).[20] Other variables assessed were the use of health services during the previous year (number of admissions to the acute and/or subacute care unit, and/or day hospital), current psychopharmacological treatment, and substance use (ascertained by asking directly about different types of substances: alcohol, tobacco, cocaine, and cannabis; and the amount used weekly: standard drinks of alcohol, grams of cocaine, and number of cigarettes and cannabis joints). The cognitive functioning of the subjects was then assessed using the SCIP (Screen for Cognitive Impairment for Psychiatry – Spanish version SCIP-S, Form 3[21]), which uses five subtests to evaluate working memory, immediate and delayed verbal list learning, verbal fluency (executive functions) and, finally, psychomotor speed) and personal and social functioning using the GAF (Global Assessment of Functioning) and PSP scales (Personal and Social Performance, which analyzes issues related to self-care, socially useful activities, personal and social relationships, and disturbing and aggressive behavior).[22]

Finally, a record of dietary habits was kept using the PREDIMED FFQ (a validated food-frequency questionnaire from which the items that best discriminated foods rich in omega-3, omega-6, and omega-9 unsaturated fatty acids were selected).[23]

### *Statistical Analysis*

A descriptive and inferential analysis of data was made using the Student t test and Mann-Whitney U test for the comparison of means when necessary. Pearson correlations were used for the appropriate variables depending on the study objective, and calculated with the IBM SPSS Statistics 21 statistical package.

## RESULTS

The sample consisted of 31 subjects, 20 men (64.5%) and 11 women (35.5%), with a mean age of 35 years. Most were single and had a secondary school education. At the time of the study, 48.4% were in a situation of permanent occupational disability. The most prevalent diagnosis was paranoid schizophrenia (71%), with a mean duration of approximately 10 years (which was more variable in the men than in the women: [*SD*=7.2 *vs. SD*=6.4, respectively]); 42% of the subjects were being treated with a single oral antipsychotic agent at the time of analysis (Table 1).

Regarding the PANSS, the mean total score was 72.4 (*SD*=18.7), with a mean positive PANSS of 14.8 (*SD*=6.2), mean negative PANSS of 21.8 (*SD*=7.6), and mean general psychopathology (PANSS-GP) of 35.4 (*SD*=8). On the CGI scale, 6.4% (*N*=2) of the sample had very low scores, understood as "imperceptible illness"; 3.2% (*N*=1) was assessed as "borderline illness"; 38.8% (*N*=12) obtained a score corresponding to "slightly ill"; 42% (*N*=13) were assessed as "moderately ill"; and 9.6% (*N*=3) were assessed as "markedly ill." The mean SCIP score was 16.2/30 (*SD*=5) for immediate verbal and auditory memory, 16.2/24 (*SD*=4.3) for working memory, 11.1 (*SD*=6) for verbal fluency, 80 (*SD*=48.4) for verbal and auditory list learning, and 9.3/30 (*SD*=4) for psychomotor speed. As regards functionality, the mean PSP score observed was 50 (*SD*=16.3). As for the PSP subscales, 35.4% of the sample (*N*=11) had a clear deficit in self-care, 48.3% (*N*=15) showed a marked deficit in socially useful activities, 42% (*N*=13) had a marked deficit in personal and social relationships, and 58% (*N*=18) scored absence of disturbing and aggressive behaviors. Finally, a mean score of 65.1 (*SD*=5.7) was observed on the Global Assessment of Functioning scale.

The results obtained for erythrocyte membrane PUFA levels in the 31 patients were normally distributed (Kolmogorov-Smirnov test, $p>0.05$) for DHA ($p=0.956$), EPA ($p=0.762$), and AA ($p=0.930$) values. In the overall sample, the levels of DHA, AA, and the rest of the PUFAs showed no significant correlation with the PANSS, SCIP, or GAF scores, or the days of admission other than the days of admission to the day hospital, which had a direct and significant correlation (r=0.4; $p=0.016$). In contrast, the gamma linoleic acid and total omega-6 levels correlated significantly with the number of psychiatric admissions ($r=0.393$, $p=0.042$; $r=0.367$, $p=0.042$, respectively). When the cases of very low DHA levels (DHA<5 mcg/g, *N*=9) were grouped, the PANSS scores obtained were lower in comparison to the subset of subjects with medium or high DHA levels (>0.5, *N*=22), and the difference was statistically significant (57.89 *vs.* 78.57, Mann-Whitney U test, $p=0.008$).

Analysis of the overall omega-3 (DHA+EPA) values revealed significantly lower values in men than in women (*M*=14.1 *vs. M*=19.02, $t=-2.161$, $p=0.039$) (Figure 1); the same tendency was found for HDL cholesterol (men *M*=41.7 and women *M*=59.79). The other variables did not show significant differences (Table 2).

Similarly, no significant differences were found in socio-demographic variables such as race, marital status, level of education, employment status, source of admissions,

**Table 1** Description of the Sample Analyzed

| | | Men | Women | Total |
|---|---|---|---|---|
| Age | | 33.8 (SD 8.2) | 36.5 (SD 5.6) | 35 (SD 7.5) |
| Years of evolution | | 10.9 (SD 7.2) | 10.5 (SD 4.9) | 10.7 (SD 6.4) |
| | | n (%) | n (%) | n (%) |
| Gender | | 20 (64.5%) | 11 (35.5%) | 31 |
| Ethnicity | Caucasian | 17 (85%) | 11 (100%) | 30 |
| | Other | 3 (15%) | 0 (0%) | 3 |
| Diagnosis | Paranoid schizophrenia | 15 (75%) | 7 (63.6%) | 22 (71%) |
| | Undifferentiated schizophrenia | 2 (10%) | 0 (0%) | 2 (6.4%) |
| | Schizoaffective disorder | 3 (15%) | 4 (36.4%) | 7 (22.6%) |
| Treatment | 1 single oral antipsychotic | 7 (35%) | 6 (54.6%) | 13 (42%) |
| | > 1 oral antipsychotic | 1 (5%) | 2 (18.2%) | 3 (9.7%) |
| | Depot antipsychotic[a] | 9 (45%) | 3 (27.2%) | 12 (38.6%) |
| | Depot antipsychotic + Oral antipsychotic | 3 (15%) | 0 (0%) | 3 (9.7%) |
| Substance use | Tobacco | 15 (75%) | 5 (45.4%) | 20 (64.5%) |
| | Alcohol | 4 (20%) | 2 (6.4%) | 6 (19.3%) |
| | Cannabis | 2 (10%) | 0 (0%) | 2 (6.4%) |
| | Cocaine | 1 (5%) | 1 (3.2%) | 2 (6.4%) |

The frequency (%) was calculated for the qualitative variables and the mean and standard deviation (SD) for the quantitative variables. [a]Types of depot antipsychotics: risperidone, fluphenazine decanoate, and zuclopenthixol decanoate

primary diagnosis and treatment with one or more antipsychotics, or smoking. The results of cognitive functioning, such as psychomotor speed and verbal fluency, did have a significant negative correlation with age at onset of the disease ($r$=0.63; $p$=0.000).

The study of dietary habits revealed more consumption of olive oil *(vs.* no consumption), sunflower oil, and nuts in men and more consumption of oily fish and extra virgin olive oil in women; these differences were not significant. Analysis of dietary habits and omega-3 levels in relation to sunflower oil consumption showed significantly lower levels of w-3 ($t$=-2.5; $p$=0.016). Finally, patients treated with depot versus nondepot antipsychotic agents had lower levels of w-3 (depot $M$=13.08 *vs.* nondepot $M$=18.55, $t$=-2.6; $p$=0.014) and slower psychomotor speed (depot 8/30 *vs.* nondepot 11/30; Student $t$=-2.1, $p$=0.045), but better results for clinical variables (except admissions: depot $M$=0.4 *vs.* nondepot $M$=1.4, Mann-Whitney $U$=72.5, $p$=0.06, with a tendency to significance).

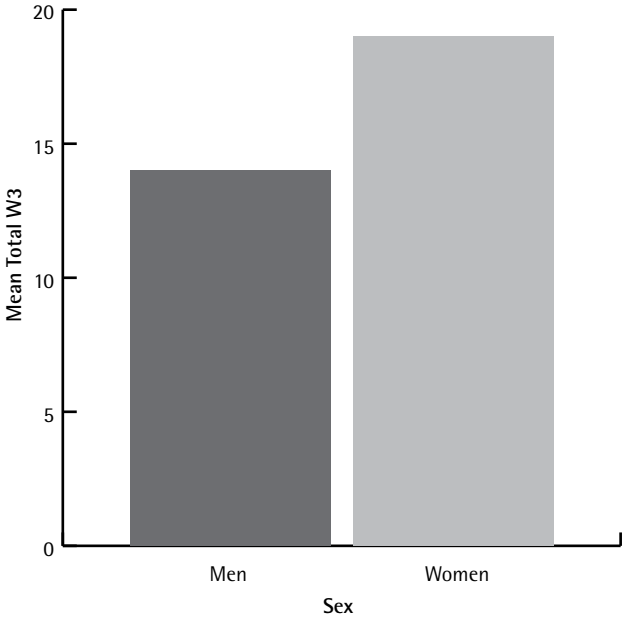


*Figure1* *Relation between w-3 levels and gender*

## DISCUSSION

Since 1994, when Horrobin first formulated the membrane hypothesis of schizophrenia,[5] numerous studies have confirmed the existence of an abnormality in the

**Table 2** Results of Variance Analysis for the Variables Analyzed in Relation to Gender

| | Men (N = 20) Mean (SD) | Women (N = 11) Mean (SD) | t | p |
|---|---|---|---|---|
| Body mass index | 29.86 (7.2) | 28.01(5.7) | 0.723 | NS |
| Glucose[a] | 103 (30.3) | 95.3 (10.8) | 0.812 | NS |
| HDL cholesterol[a] | 41.7 (11.1) | 59.7 (21.2) | -3.120 | 0.004 |
| LDL cholesterol[a] | 113.2 (31.9) | 114.0 (18.7) | -0.080 | NS |
| Triglycerides[a] | 154.3 (86.3) | 143.8 (185.2) | 0.216 | NS |
| Linoleic acid (LA)[b] | 4.8 (1.3) | 5.0 (0.7) | -0.437 | NS |
| γ-Linolenic acid (GLA)[b] | 0.4 (0.7) | 0.2 (0.3) | 0.839 | NS |
| α-Linolenic acid (ALA)[b] | 0.2 (0.32) | 0.2 (0.3) | -0.375 | NS |
| Arachidonic acid (AA)[b] | 14.0 (3.8) | 14.9 (2.7) | -0.707 | NS |
| EPA[b] | 7.1 (3.4) | 9.8 (5.4) | -1.723 | NS |
| DHA[b] | 6.8 (2.7) | 8.9 (3.4) | -1.840 | NS |
| Total W-6[b] | 19.3 (4.8) | 20.2 (3.2) | -0.581 | NS |
| Total W-3[b] | 14.1 (5.6) | 19.0 (6.6) | -2.161 | 0.039 |
| W-6/W-3 ratio | 1.5 (0.5) | 1.2 (0.4) | 1.673 | NS |
| Total PANSS | 71.85 (20.0) | 73.55(17.0) | -0.237 | NS |

Results are expressed as means and standard deviations, in parentheses. [a] Glucose, HDL cholesterol, LDL cholesterol, and triglycerides are measured in mg/dL. [b] The different fatty acids are measured in micrograms of fatty acid per milligram protein. [c] Statistical significance was $p<0.05$. p values>0.05 are marked as NS (nonsignificant).

phospholipid composition and metabolism of neuronal and erythrocyte membranes in patients with schizophrenia. The lower proportion of omega-3 fatty acids (EPA and DHA) in erythrocyte membrane has been linked to more severe forms of schizophrenia with a higher presence of negative symptoms[24] and worse response to treatment,[12] suggesting the existence of an associated endophenotype present in one-third of affected subjects.[15] Our study did not clearly detect this subset of patients in our sample because we did not find a relation between PUFA levels, particularly omega 3 (DHA and EPA), and the clinical and developmental variables analyzed. In addition, subjects with lower DHA, EPA, and AA levels did not show worse results on scales of clinical symptoms, cognitive functioning as assessed by SCIP, or clinical outcome measures (emergencies, admissions, etc.). The small size of our sample and lack of a control group of healthy individuals probably makes it difficult to obtain significant results. However, it is worthwhile to mention certain findings that are consistent with the main working hypothesis. In first place, our data show that erythrocyte membrane PUFA levels, specifically those of omega-3 fatty acids (EPA+DHA), in subjects with schizophrenia were lower in men, independently of smoking and even the regular diet consumed. A possible differential effect of feminine estrogens has been postulated.[25,26] However, it is known that men develop schizophrenia at an earlier age and with a greater impact than women,[27] so low omega-3 levels could be an indicator of greater severity. Data that are suggestive but not significant, possibly due to the small sample size, show an association with age at onset and cognitive processing speed as relevant variables.

An interesting finding of our study was an association between low omega-3 levels and the use of long-acting antipsychotic agents (depot). The use of this formulation is also associated with worse performance on the SCIP processing speed tests compared to other types of antipsychotic agents. This association could also indicate a certain type of severity in these subjects compared to others, since the use of depot drugs usually implies low disease awareness and a protective effect with regard to admissions, as shown by the reduction in days of admission among those using depot drugs compared to other subjects in the sample.

The results that relate current dietary habits, especially the consumption of sunflower oil, also clearly favor low omega-3 levels and higher omega-6 levels with an increased proportion of admissions and, probably, a greater severity of episodes, as well as an imbalance in the omega-6/omega-3 ratio. Such changes, described in the Bentsen series as a low omega-3 endophenotype,[15] have important adverse metabolic effects in this population.[28,29]

In summary, our exploratory study does not confirm the role of PUFAs, specifically omega-3 fatty acids, as markers for risk of schizophrenia, but it yielded some suggestive results that merit further study in larger samples and

comparison with a group of healthy controls. If the possible role of PUFAs in the pathophysiology of the disease is considered, with the important metabolic implications this has for patients already subject to environmental metabolic risk factors (such as sedentary lifestyle, high-carbohydrate diets, antipsychotic use, etc.), we have to conclude that 30 years after the Horrobin membrane hypothesis was formulated it continues to yield new and promising data for research and probably for the prevention and treatment of schizophrenia in the future.

## CONFLICTS OF INTEREST

The authors state that they have no conflicts of interest in relation to the topic and content of this article.

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