

**Intervento al convegno  
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Sorghum Food for Celiac Patients  
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## **THE LIPIDOMIC APPROACH IN THE EVALUATION OF NUTRITIONAL STATUS AND ITS CLINICAL APPLICATION**

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### **INTRODUCTION**

The current use of single biomarkers as indicators of disease are nowadays replaced by comprehensive profiling of individual metabolites linked to an understanding of health and human metabolism. The emerging science is known as metabolomics.[1-3] Within metabolomics, lipidomics [4] plays an important role for the understanding of dynamic changes occurring to lipids in physiological and pathological conditions, and the present intervention will show how lipidomics can be used into clinical practice.

Lipids are small molecules that share common physical and chemical properties as a class, whose presence and abundance are key to much of metabolic regulation, from subcellular compartments to whole body energy control and signaling. The chemical structure of lipids is well known, but the importance of some constituents of lipids, as fatty acids, has been stressed only in the recent past.[5] By measuring changes in fatty acids concentrations in the cell membranes and the effects induced by drugs, diet and diseases on their levels, some important correlations have been made and others will be in the future.

Furthermore, the discovery of trans lipids in the membranes as a new biomarker of radical stress has also modified the significance of cell membrane modification and the strategy to control free radical damages.[6,7]

### **FA AND CELIAC DISEASE**

The correlation between fatty acids and celiac disease can be based on the functions of cell membranes. In particular:

- The fatty acid (FA) composition of intestinal membranes is important for epithelial function, and disturbances may contribute to the pathophysiology of many diseases. Altered

fatty acid content may also contribute to the pathophysiology of the celiac disease because FAs are important for enzymes and for the transport and receptor functions of epithelial membranes.

- Some recent studies suggest an omega-6 fatty acid deficiency, at least in the intestinal mucosa, meanwhile there is an improved fat absorption of omega-3 fatty acids due to a gluten-free diet.[2]

Any other studies of essential fatty acid levels in celiac disease are available. To cover this lack, lipidomics can be suggested as the best approach that allows a follow up of the patient status and, as it will be shown further, to intervene by dietary and nutraceutical protocols.

### **LIPIDOMIC BASELINES**

Lipidomic analysis can focus on different targets. In particular the fatty acid residues present in membrane phospholipids can be considered as a very appropriate target for different reasons:

- the fatty acids are located in the hydrophobic tails of membrane phospholipids. If they change, also membrane properties and functions will be different. Membrane changes can be considered as a basic contribution to the change of the whole cell metabolism. In this respect, fatty acids have not yet been fully considered for their fundamental contribution.

- Fatty acids derive from biosynthesis and diet. They are present as different structures: saturated fatty acids (SFA) and unsaturated fatty acids (UFA), which contain one or more double bonds. UFA are divided into monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), the latter being essential fatty acids (EFA) for humans, since they have to be introduced by the diet.

The double bond in MUFA and PUFA is very important. In Figure 1 it can be seen that the double bond creates a bent in the structure, whereas the saturated fatty acid is a linear molecule. This simple feature is the most important driving force for the assembly of the phospholipids in forming cell membranes, and it contribute enormously to the membrane properties in order to have good interactions and exchanges between the extra- and intracellular environment.

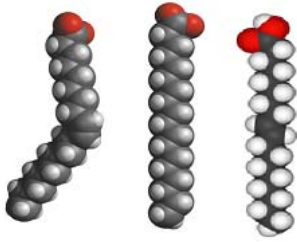


Figure 1 - Unsaturated (left) and saturated fatty acid structure compared to trans fatty acid (right)

As previously noted, the recent discovery on the double bond reactivity to free radicals in cell membranes put forward the hypothesis of a geometrical change of the fatty acid structure. In fact, the double bond geometry can be converted by radical attack from cis (the natural configuration which produces a bent) to trans isomer (Figure 1, right), with a dramatic effects on the molecular arrangement that loses the bent and becomes more similar to saturated fatty acids.

Trans lipids have been recognized by the BioFreeRadicals group at ISOF- CNR in Bologna (Italy) in cell cultures and rats as consequence of a radical stress,[8,9] but also in several pathological disorders, such as dermatitis.[10]

## **THE LIPIDOMIC PROFILE**

Fatty acids in membranes are the target of the lipidomic profile described in this work. As previously explained, the different types of fatty acids have to stay in an ideal balance to preserve membrane functionality.

By the lipidomic profile, this balance is studied, not only as a numerical value obtained from the lipid analysis, but also as a complex result of the metabolism, diet, and familiarity, which are typical for each individual. In the study the contribution of free radicals is taken into account, that can disturb the normal quantity and type of fatty acids, through the process of peroxidation and isomerization. A picture of the individual status emerges, either in physiological or pathological conditions. This approach is in line with several reports on the membrane status and free radical involvement in the aging process.[11,12]

The lipidomic profile has become the leading product of a spin-off company born in December 2005 at the Consiglio Nazionale delle Ricerche in Bologna. The spin-off is Lipinutragen srl and the product is the FAT PROFILE<sup>®</sup>, which is a registered European Community trade mark.

FAT PROFILE<sup>®</sup> is very easy to perform. It starts from a venous blood sample (2cc in EDTA as anticoagulant) sent to the Lipinutragen laboratories, that will be processed and analysed. The result consists of:

- A table with the fatty acid values of the patient membrane, in comparison with normal values coming from the available scientific literature of healthy subjects;
- A graph with an easy-to-read chromatic scale, representing the membrane equilibrium of the patient.
- A lipidomic profile, that brings to the Medical Doctor an explanation of the values found in the patient taking into account the data from his/her style of life and familiarities (the patient has to fill out a questionnaire). Upon request the response proposes nutraceutical/dietary suggestions in order to help the recovery of the membrane equilibrium.

FAT PROFILE® is an advanced scientific tool for Medical Doctors in the field of cell membranes and their relationship with health. It also represents a help for the personalization of therapies and the satisfaction of the individual needs. In fact, nutraceutical supplementation, such as the pharmacological one, must be regulated by an effective need, because the effects of a wrong supplementation are similar in both cases. FAT PROFILE® allows the individual needs to be envisaged and the appropriate nutraceutical supplementation to be individuated.

The physiological situations where the FAT PROFILE® can be useful are:

*DIET AND NUTRITION*

*AGING*

*SPORT ACTIVITY*

*MONITORING OF PREGNANCY AND LACTATION*

But it can be important also in pathologies:

*DERMATOLOGICAL PROBLEMS*

*IDENTIFICATION OF RISK FACTORS FOR CARDIOVASCULAR DISEASES*

*METABOLIC DISORDERS AND DISLIPIDEMIA*

*OBESITY*

*OCULISTICS*

*AUTISM AND DEPRESSION*

*CYSTIC FIBROSIS*

*and many others.*

A typical report of the values found in erythrocyte membranes is shown in Figure 2



RTD	LIPIDI TOTALI (% rel.)		
ACIDI GRASSI	Valori Trovati	Valori Normali (a)	Percentuale
Palmitico (16:0)	25	17 - 27	
Palmitoleico (16:1)	0,5	0,2 - 0,5	
Stearico (18:0)	20	13 - 20	
Oleico (18:1)	18,7	9 - 18	+ 3,59 %
Trans 18:1	1,1	0 - 0,3	+ 266,67 %
Vaccenico (18:1)	2	0,7 - 1,3	+ 53,85 %
Linoleico omega6 (18:2)	10,6	9 - 16	
Eicosatrienoico omega6 (20:3)	2,1	1,9 - 2,4	
Arachidonico omega6(20:4)	19,8	13 - 17	+ 15,47 %
Trans-ARA	0,1	0 - 0,4	
EPA omega3 (20:5)	0,1	0,5 - 0,9	- 80,00 %
DHA omega3 (22:6)	0,4	5 - 7	- 92,00 %
Tot. saturi (SFA)	45	30 - 45	
Tot. monoinsaturi (MUFA)	19,7	13 - 23	
Tot. poliinsaturi (PUFA)	32,9	28 - 39	
INDICE DI SQUILIBRIO NELLA BIOSINTESI LIPIDICA			
SFA / MUFA	2,3	1,7 - 2	+ 15,00 %
omega6 / omega3	51	3,5 - 5,5	>100%
EFA deficiency	0,53	>0,4	

**SPHEROCYTOSIS:**  
Lack of omega 3  
Excess of ac. Oleic- TRANS  
Excess of ARA  
Supplementation with:omega 3,  
A,C,E ,flavonoids, folic acid,biliary acids  
Probiotic  
Soy lecithin  
Every day fish ( salmon ) and legumes  
Clinical improvement

Figure 4- Report of fatty acid status from a case of spherocytosis

The intervention consisted of:

Omega 3 (1 g/d), antioxidant vitamins (ACE complex), flavonoids, folic acid, biliary acids and soy lecithin. His dietary habits were also modified: probiotics, fish as salmon, legumes, vegetables have been introduced.

After 1 month, bilirubin decreased to 2 mg/L and the patient reported to feel himself stronger, so that he could start busy activity (running on bicycle).

Another example is a girl, aged 11, with Friederich ataxia, a genetic disease with neurological symptoms. Her lipidomic profile suggested a reduced omega-3 pathway and an inflammatory trend, with a borderline membrane radical stress (Figure 5). The treatment was done with idebenone, a potent antioxidant with specific activity on brain, with an omega-3 supplementation covered also by anti-radical protection. Her diet was modified to use more fish and legumes, avoiding saturated fats, eggs, red meat and vegetable seed oils.

Her mother called me one week ago after 1 month, and the girl seems to improve her walk and attention.

Paziente / Data /3/2016 Esplendi/Strasburgo	LIPIDI TOTALI (% rel.)		
ACIDI GRASSI	Valori Trovati	Valori Normali (a)	Percentuale
Palmitico (16:0)	29,1	17 - 27	+ 7,78 %
Palmitoleico (16:1)	0,3	0,2 - 0,5	
Stearico (18:0)	15,9	13 - 20	
Oleico (18:1)	16,9	9 - 18	
Trans 18:1	0,3	0 - 0,3	
Vaccenico (18:1)	1	0,7 - 1,3	
Linoleico omega6 (18:2)	12,3	9 - 16	
Elcosatrenico omega6 (20:3)	2,2	1,9 - 2,4	
Arachidonico omega6(20:4)	17,4	13 - 17	+ 2,35 %
Trans-ARA	0,2	0 - 0,4	
EPA omega3 (20:5)	0,4	0,5 - 0,9	-20,00 %
DHA omega3 (22:6)	4	5 - 7	-20,00 %
Tot. saturi (SFA)	45	30 - 45	
Tot. monoinsaturi (MUFA)	17,9	13 - 23	
Tot. poliinsaturi (PUFA)	36,3	29 - 39	
INDICE DI SQUILIBRIO NELLA BIOSINTESI LIPIDICA			
SFA / MUFA	2,5	1,7 - 2	+ 25,00 %
omega6 / omega3	7,2	3,5 - 5,5	+ 30,91 %
EFA deficiency	0,8	>0,4	

Girl, Aged 11 y., Friederich ataxia:  
 Omega 3 reduction  
 ARA increase  
 Supplementation with:  
 Antioxidant and Q101  
 ( IDEBENON), n-3 and DHA  
 DIET:  
 Avoid: meat, seeds,wheat  
 flor,sweets  
 Improve: fish, legumes and  
 fruit/vegetables

Figure 5- Report of a case of Friederich ataxia

The last example is a woman that suffered from the death of his son during the Iraq war. From then she started to feel weak, depressed, and to have bone pains and respiratory distress. In her analyses there was a high ESR rate, high cholesterol and very low gamma globulin levels.

****	LIPIDI TOTALI (% rel.)		
ACIDI GRASSI	Valori Trovati	Valori Normali (a)	Percentuale
Palmitico (16:0)	31,7	17 - 27	+45%
Palmitoleico (16:1)	1,7	0,2 - 0,5	>100%
Stearico (18:0)	16,1	13 - 20	
Oleico (18:1)	20,4	9 - 18	+20%
Trans 18:1	0,2	0 - 0,3	
Vaccenico (18:1)	1,3	0,7 - 1,3	
Linoleico omega6 (18:2)	8,6	9 - 16	-15%
Elcosatrenico omega6 (20:3)	1,2	1,9 - 2,4	-30%
Arachidonico omega6(20:4)	13,4	13 - 17	
Trans-ARA	0,1	0 - 0,4	
EPA omega3 (20:5)	0,9	0,5 - 0,9	
DHA omega3 (22:6)	0,1	5 - 7	-92,00 %
Tot. saturi (SFA)	47,8	30 - 45	+10%
Tot. monoinsaturi (MUFA)	21,7	13 - 23	
Tot. poliinsaturi (PUFA)	24,1	29 - 39	
INDICE DI SQUILIBRIO NELLA BIOSINTESI LIPIDICA			
SFA / MUFA	2,2	1,7 - 2	+ 15,00 %
omega6 / omega3	23,2	3,5 - 5,5	>100%
EFA deficiency	0,53	>0,4	

B.C. aged 65 ( first sample):  
 Hard smoker- little overweight-  
 respiratory disease-weakness-  
 depression  
 In the blood: Low gamma-globulins  
 level,high cholesterol with low  
 HDL,and high ESR  
 LIPIDOMICS highlights: high SFA,  
 low PUFA especially omega -6 and  
 DHA, low ARA -  
 Supplementation with: vit. A,C,E-  
 flavonoids, DHA , zinc and soy lecithin  
 DIET NOTES:  
 Carboirdates reduction  
 Avoid beef meat  
 Increase fish and legumes  
 Every day :PROBIOTIC with plants  
 sterols

Figure 6- Report of a case of depression and wrong diet

Her diet was also unbalance, with high carbohydrate and saturated fat intake compared to fish and legumes, and with abnormal intake of fruits and vegetables.

Her lipidomic profile showed high levels of saturated fatty acids, low levels of omega-6 and of DHA (Figure 6).

She was given DHA and soy lecithin, together with flavonoids and an antioxidant complex. The diet was modified, lowering carbohydrates and fats and introducing more vegetables and fruits with high ORAC rates and plant sterols to reduce cholesterol.

LIPIDI TOTALI (% rel.)			
ACIDI GRASSI	Valori Trovati	Valori Normali (a)	Percentuale
Palmitico (16:0)	26,5	17 - 27	
Palmitoleico (16:1)	0,3	0,2 - 0,5	
Stearico (18:0)	16,3	13 - 20	
Oleico (18:1)	17,1	9 - 18	
Trans 18:1	0	0 - 0,3	
Vaccenico (18:1)	1,4	0,7 - 1,3	+ 7,69 %
Linoleico omega6 (18:2)	13,3	9 - 16	
Eicosatetraenoico omega6 (20:3)	1,3	1,9 - 2,4	- 31,68 %
Arachidonico omega6 (20:4)	14,5	13 - 17	
Trans-ARA	0,1	0 - 0,4	
EPA omega3 (20:5)	0,3	0,5 - 0,9	- 40,00 %
DHA omega3 (22:6)	7,8	5 - 7	+ 11,43 %
Tot. saturi (SFA)	42,8	30 - 45	
Tot. monoinsaturi (MUFA)	18,5	13 - 23	
Tot. poliinsaturi (PUFA)	37,2	28 - 39	
INDICE DI SQUILIBRIO NELLA BIOSINTESI LIPIDICA			
SFA / MUFA	2,3	1,7 - 2	+ 15,00 %
omega6 / omega3	3,6	3,5 - 5,5	
EFA deficiency	0,8	>0,4	

Figure 7- The profile of Figure 6 changed after 8 months of therapy

In Figure 7 there is the control panel after 8 months that showed the increase of DHA, the reactivation of omega-6 pathway, the reduction of saturated fatty acids. She felt much better, stronger and less depressed, her cholesterol lowered, the ESR rate normalized, with absence of respiratory diseases in the winter.

In Figure 8 there is also a comparison of chromatic index which helped the patients to visualize her improvement

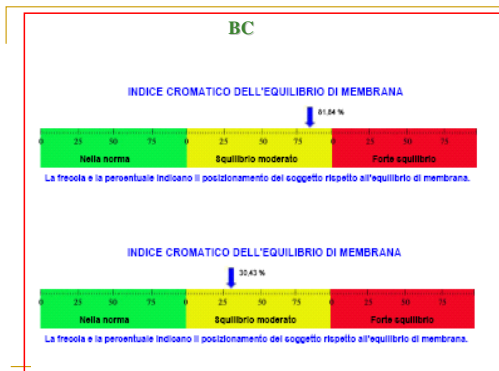


Figure 8 - Improvement of the chromatic index

## CONCLUSION

It is worldwide recognized that our health is influenced mostly by the dietary habits and that the "fat family" needs much more respect,[13] as recognized by the new food pyramid.

The nutritional therapies have a very important opportunity in applying the approach of lipidomics.

We end with the famous Feuerbach byword "we are what we eat" that means the cultural aspect of the food and the choices that we can do according to our habits, but includes also a better knowledge of the real needs of our body to work in the proper way.

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