Fatty acid-based lipidomics: the concept of membrane remodeling applied to molecular diagnostics

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Seminar for

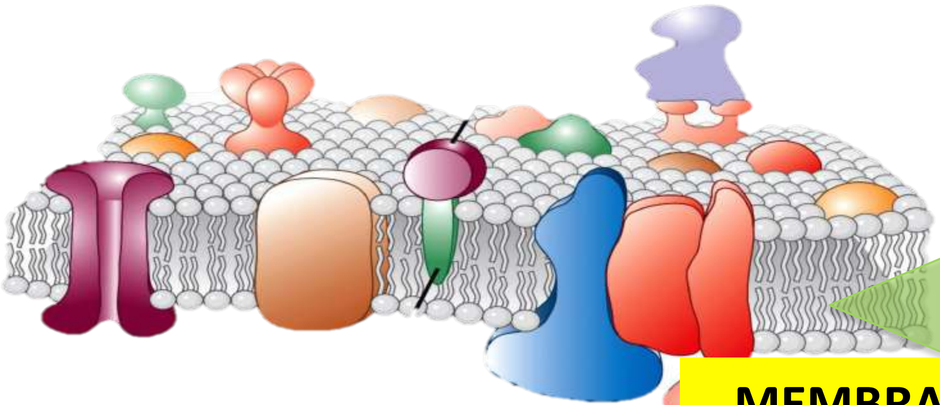
REP-EAT

Horizon 2020 MSCA-COFUND
Rep-Eat - G.A. n. 713714

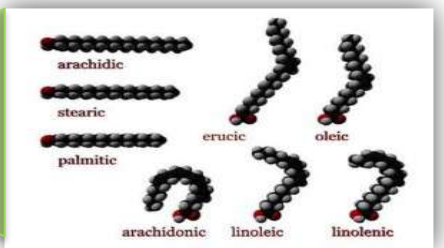
Membrane LIPIDOME: Composition of membrane phospholipids

The natural lipid composition of cell membranes in all tissues ensures the cell functionality. It is formed by fatty acids : SATURATED, MONOUNSATURATED, POLYUNSATURATED FATTY ACIDS

THIS IS A FLEXIBLE COMPOSITION, FOLLOWING THE LIFE AND NUTRITION STYLES OF THE SUBJECT- THERE ARE INTERVALS OF TOLERABILITY FOR EACH FATTY ACID IN THE TISSUE

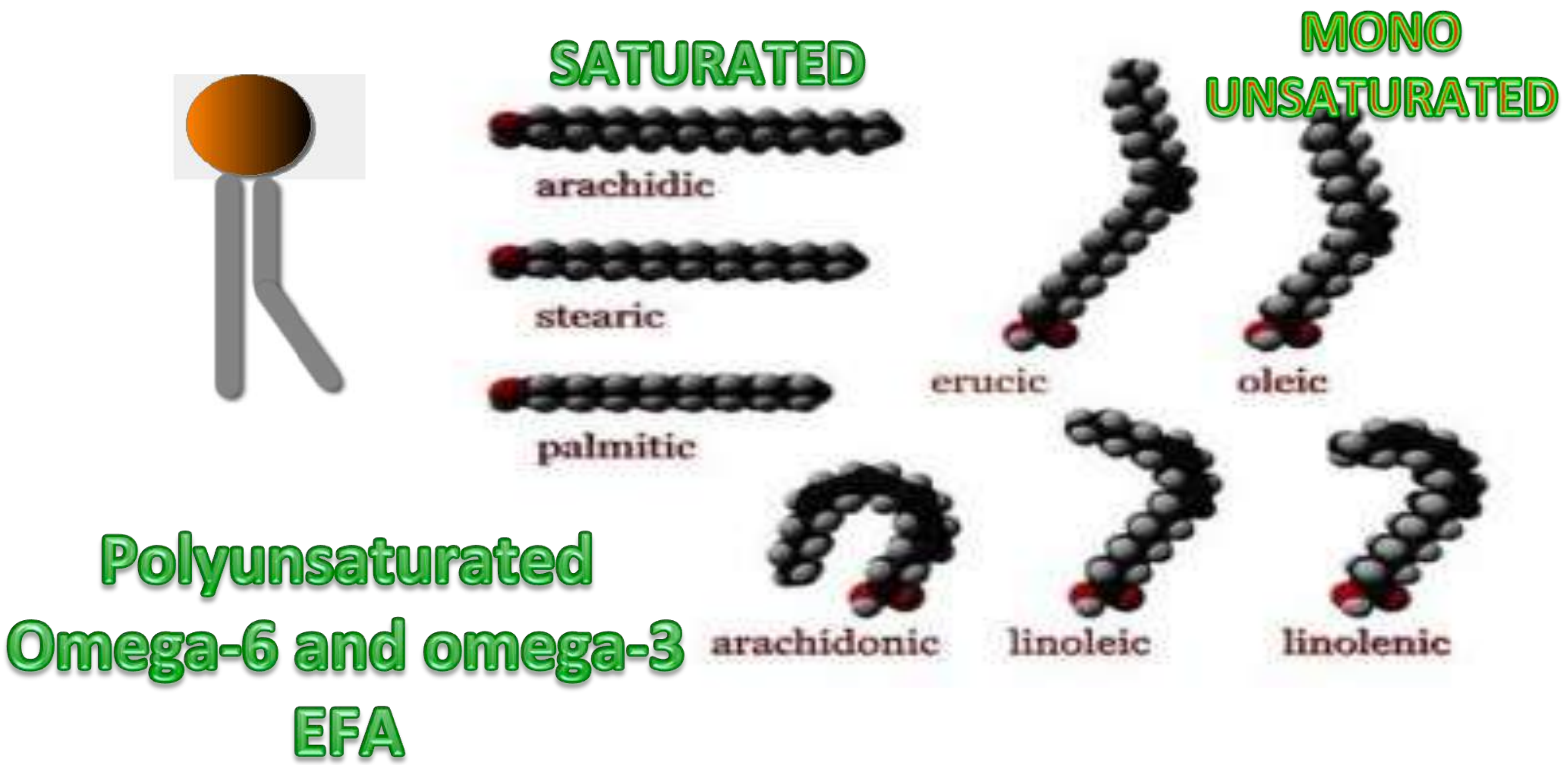


THE LIPID POOL HAS ITS OWN COMPOSITION IN EACH TISSUE



MEMBRANE LIPIDOME

Lipid diversity and essentiality: homeostatic conditions



EVERY TISSUE HAS ITS OWN COMPOSITION TO RESPECT

FATTY ACID	Adipose tissue (%rel)	RBC (%rel)	LIVER (%rel)	RETINA (%rel)	BRAIN (%rel)
18:2, omega-6, LIN	10.5	9.3	17.5	1.4	0.6
20:4 , omega-6, AA	**0.3**	**15.2**	**7.7**	**9.6**	**7.7**
20:3, omega-6, DGLA	0.2	1.5	1.6	nd	1.2
20:5, omega-3, EPA	traces	0.7	0.4	0.1	traces
22:6, omega-3, DHA	**0.3**	**3.2**	**3.4**	**19.7**	**7.2**
SFA	27.2	43.1	42.0	48.2	45.9
MUFA	59.7	23.0	23.8	14.2	29.7
PUFA	13.1	33.3	32.0	37.2	23.4

AA = arachidonic acid = inflammatory mediator

Adapted from Lauritzen, Chem. Phys Lipids 1999

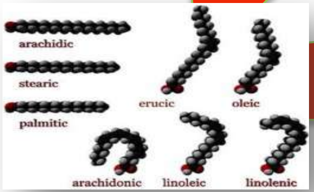
Signaling and remodeling

LAND'S CYCLE

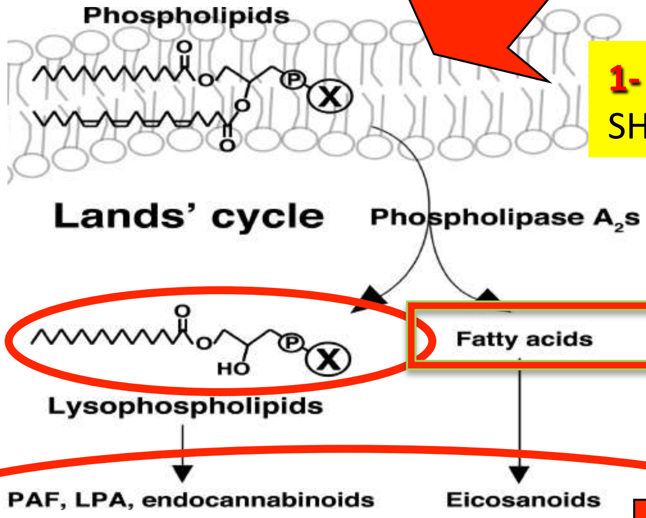
STIMULI

3- REMODELING

1- Phospholipase activation
SHORT TERM RESPONSE



POOL

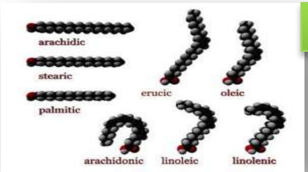


- FORMATION OF LIPID MEDIATORS
flammation, immunity, pain, lipogenesis, lipolysis)

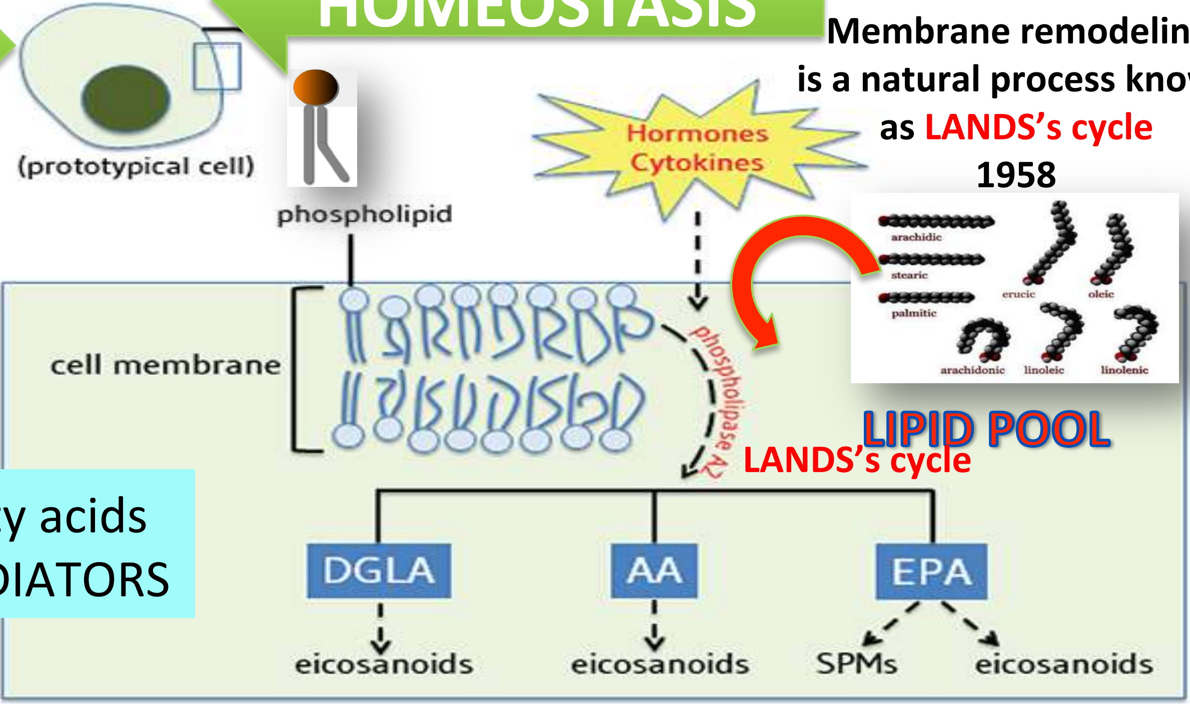
4- availability of fatty acids (nutrition, metabolism)

The equilibrium of cell signaling and responses

MEMBRANE LIPIDOME



HOMEOSTASIS



Membrane remodeling is a natural process known as LANDS's cycle 1958

LIPID POOL

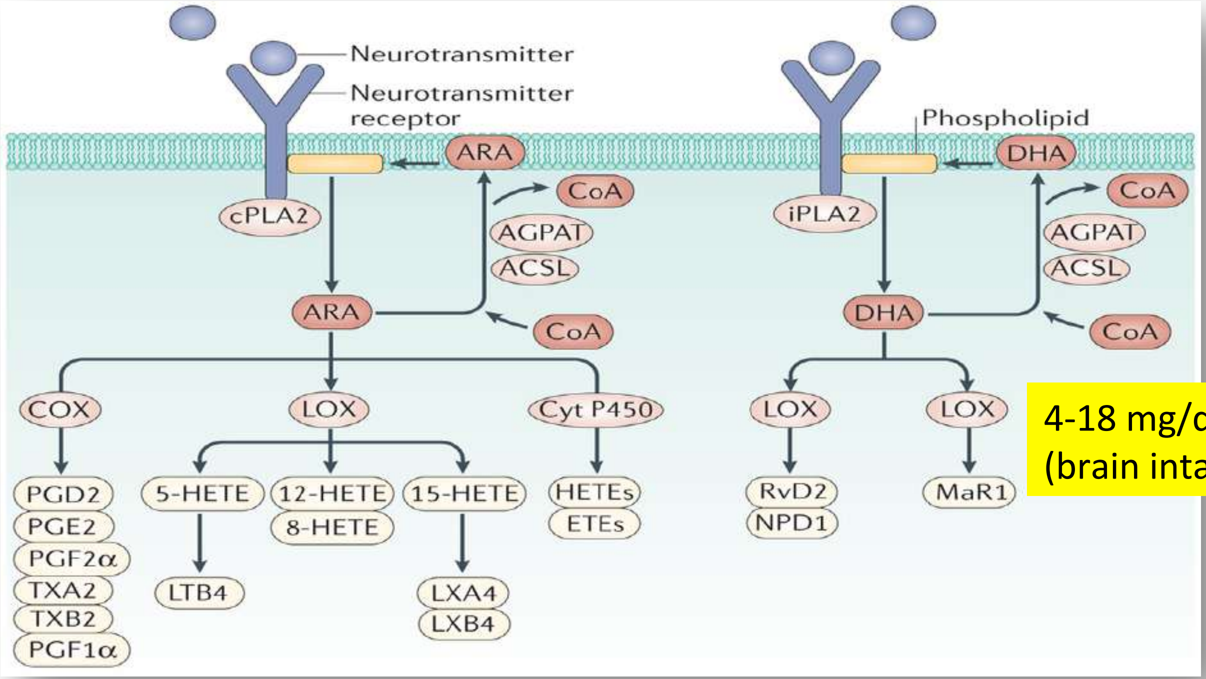
LANDS's cycle

Status of the ω6 – ω3 fatty acids
Response of the LIPID MEDIATORS

http://lpi.oregonstate.edu/mic/other-nutrients/essential-fatty-acids

C. Ferreri et al, Diagnostics, 2017

Neuronal equilibrium

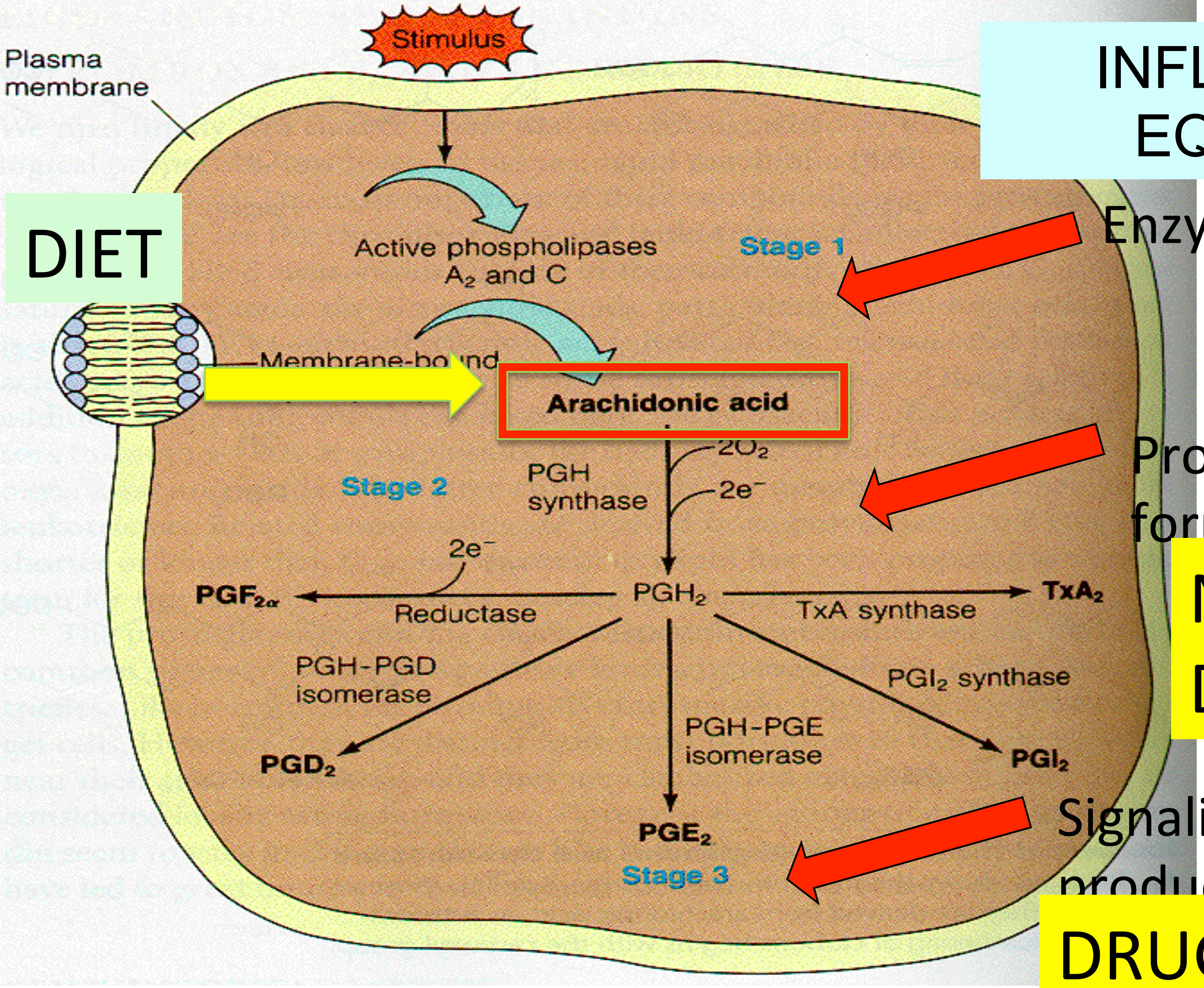


4-18 mg/day of DHA (brain intake)

Nature Reviews Neuroscience **15**, 771–785 (2014)

INFLAMMATORY EQUILIBRIUM

DIET



Enzyme activation

DRUGS

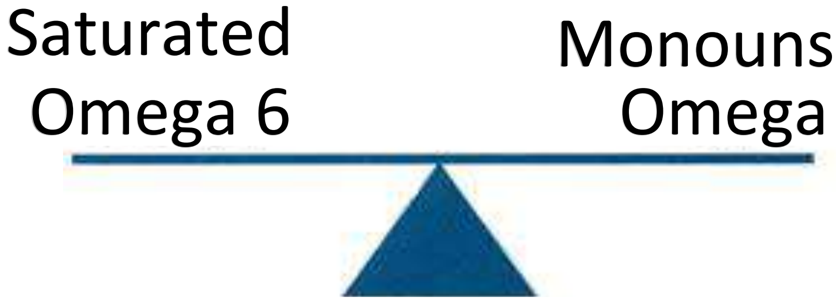
Prostaglandin formation

NSAID DRUGS

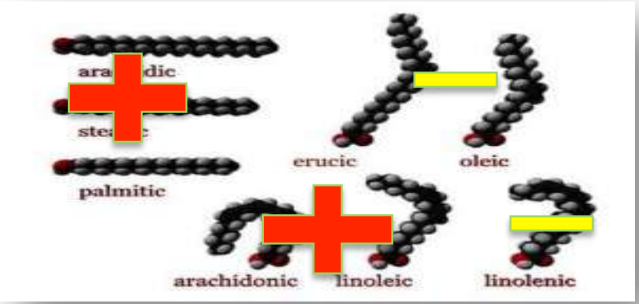
Signaling and cytokine production

DRUGS

The reasons for unbalance

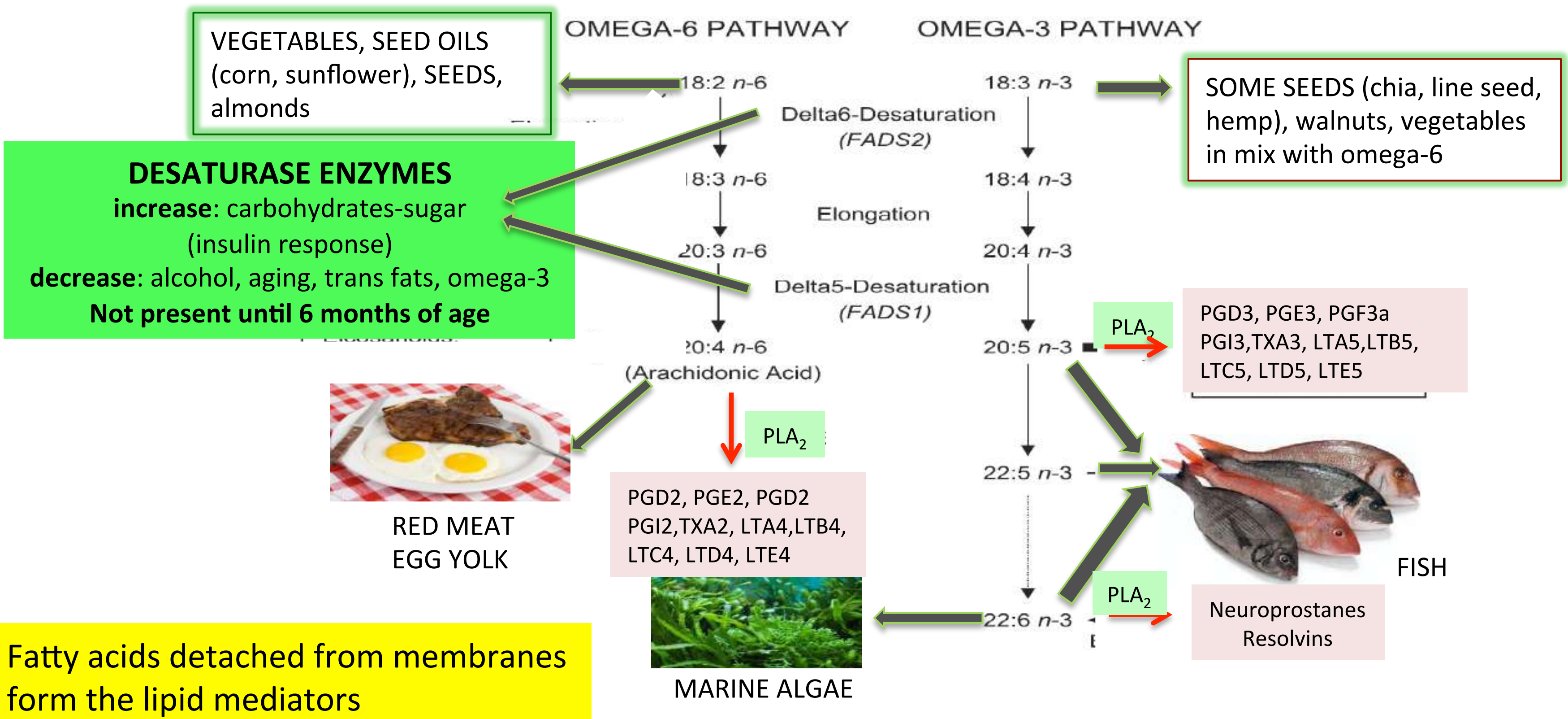


ARCAIC DIET



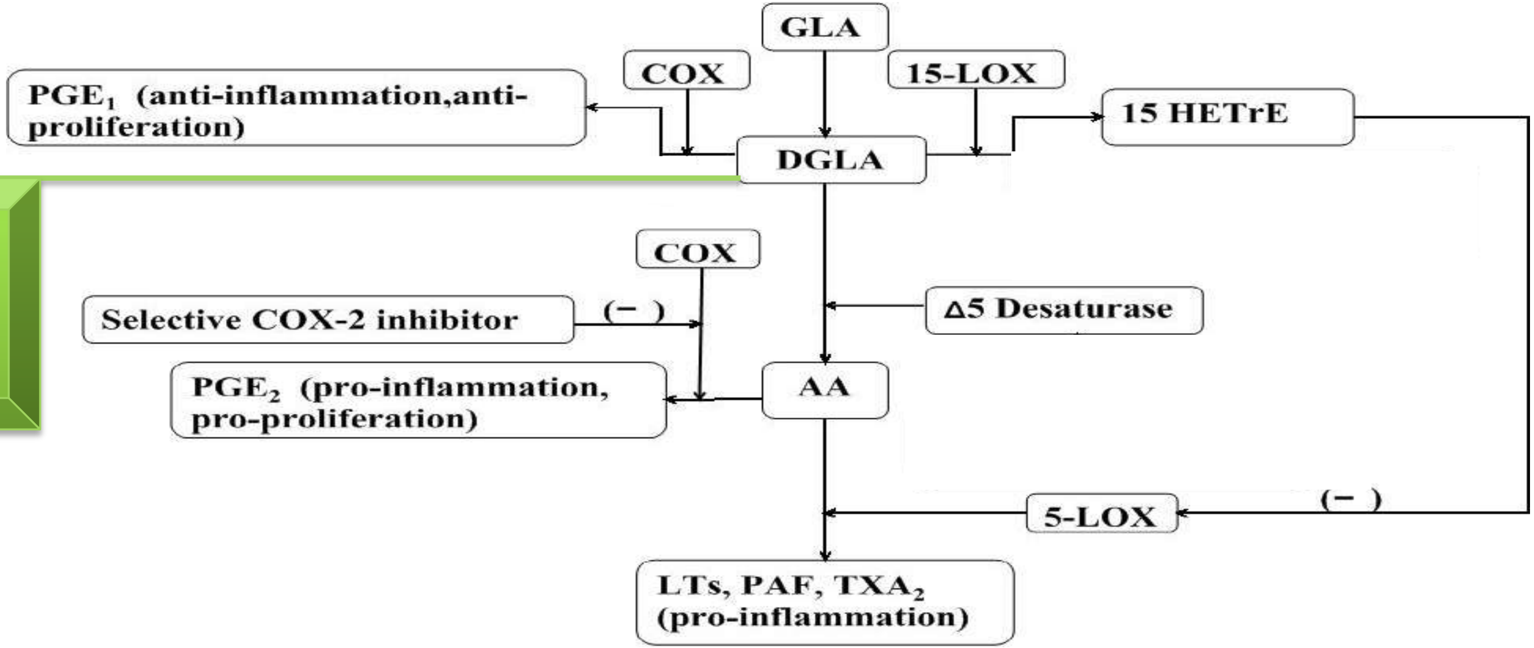
MODERN DIET

PUFA, intake and metabolism



Fatty acids detached from membranes form the lipid mediators

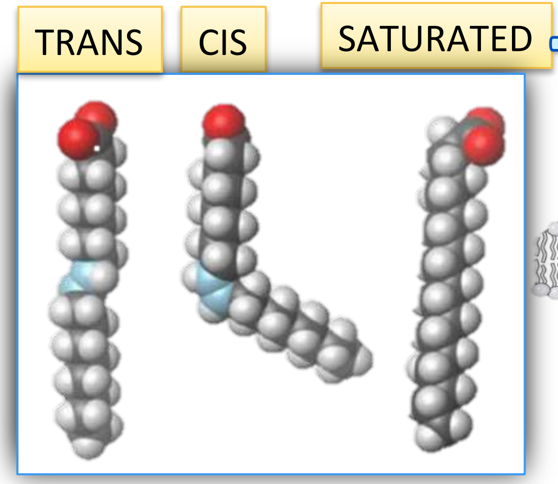
BORAGO officinalis - Oenothera biennis



Control of kinases and liberation of PLA2

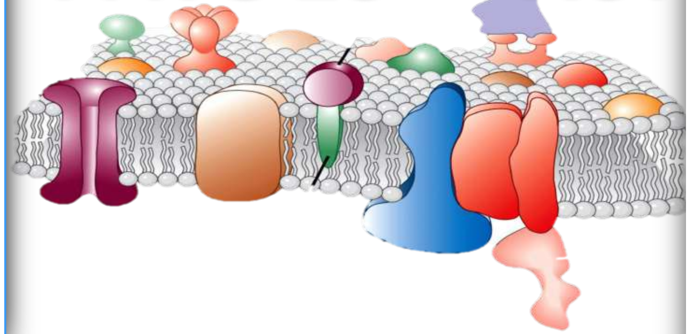
Wang et al. Lipids in Health and Disease 2012, 11:25

The unnatural lipids: **trans** lipids



THIOLS = RSH

STRESS



- Trans fatty acids are present in cell membranes and **cause a change** in the quality of the natural lipids
- They can be obtained from diet (junk and processed foods) as well as metabolism (free radical stress)
- In particular free radicals – obtained during the metabolism from substances like thiols – are able to transform cis lipids into trans lipids. Therefore, they can measure indirectly **the exposition of MEMBRANE to STRESS**. We evidenced the trans FA range = 0.1- 0.4% for a normal condition. **The ZERO VALUE (no reactivity) as well as values > 0.4% (high reactivity) are out of normal**, and a strategy can be performed to bring the values to normal conditions.

Reading human lipidome

FAT PROFILE®

PATIENT:
ID:
DATE:

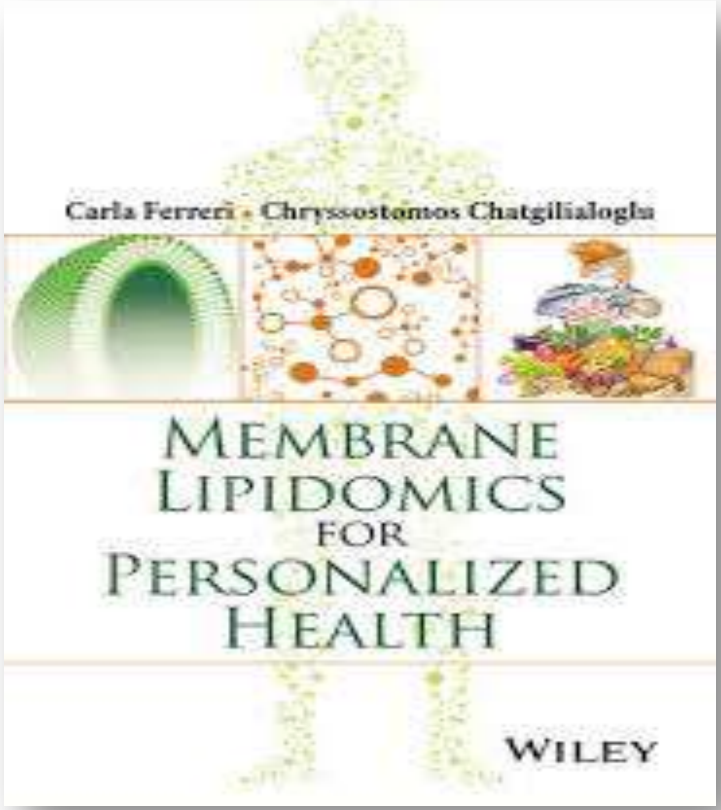
Fatty acids		Value	Optimal range	Variation	Optimal range
Saturated fatty acids	Palmitic (16:0)	23.0	17 - 27		
	Stearic (18:0)	20.3	13 - 20	+1.5 %	
Monounsaturated fatty acids	Palmitoleic (16:1)	0.4	0.2 - 0.5		
	Oleic (9c 18:1)	14.9	9 - 18		
	Vaccenic (11c 18:1)	2.5	0.7 - 1.3	+84.62 %	
PolyUnsaturated Fatty Acids omega-6	Linoleic (18:2)	11.9	9 - 16		
	DGLA (20:3)	1.7	1.9 - 2.4	-10.53 %	
	Arachidonic (20:4)	19.4	13 - 17	+14.12 %	
PolyUnsaturated Fatty Acids omega-3	EPA (20:5)	1.5	0.5 - 0.9	+66.67 %	
	DHA (22:6)	2.9	5 - 7	-42 %	
Trans fatty acids - Free radical stress	TRANS 18:1	0.1	0.1 - 0.3		
	TRANS 20:4	0.7	0.1 - 0.4	+75 %	
FAMILIES	Tot. SFA	43.4	34 - 45		
	Tot. MUFA	15.0	15 - 23		
	Tot. PUFA	37.4	30 - 43		

LIPID BIOSYNTHESIS INDICATORS	Value	Optimal range	Variation	Optimal range
SFA / MUFA	2.8	1.7 - 2	+40 %	
omega6 / omega3	7.5	3.5 - 5.5	+36.36 %	
Omega 3 Index Cardiovascular Risk *	4.4	0-4% undesirable 4-8% intermediate >8% minimum risk		

(a) Optimal value intervals in healthy controls described in: Ferreri C., Chatgilialoglu C. (2012) Role of fatty acid-based functional lipidomics in the development of molecular diagnostics tools. Expert Review of Molecular Diagnostics, 12, 767-780.

* According to Harris SW Pharmacol. Res. 2007 for people not having cardiovascular pathologies

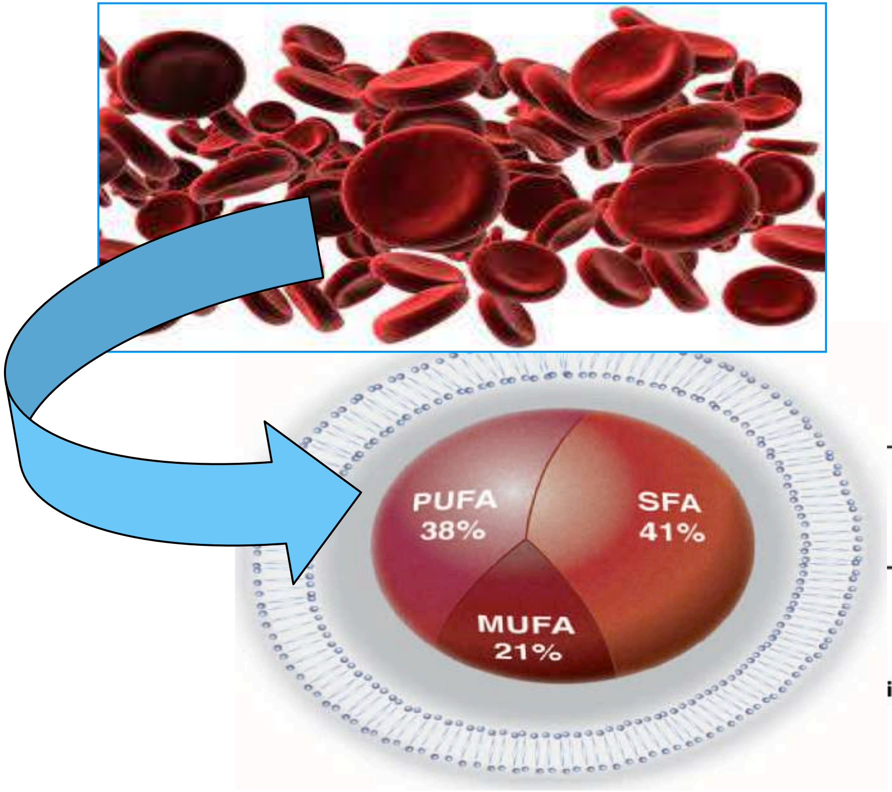
Membrane lipidomics for molecular medicine

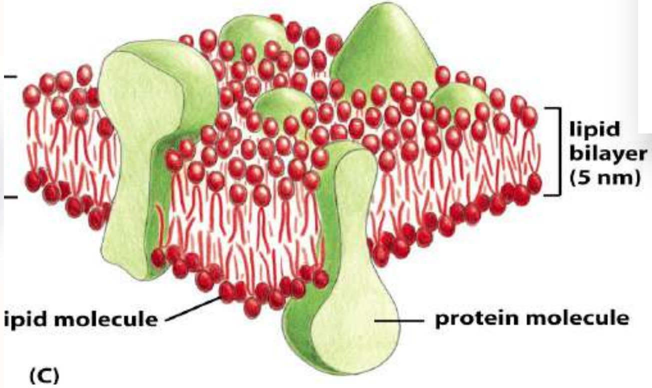


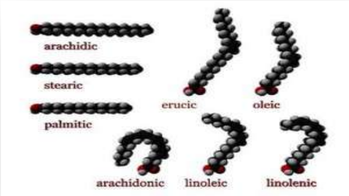
August 2015

Erythrocyte membrane lipidome

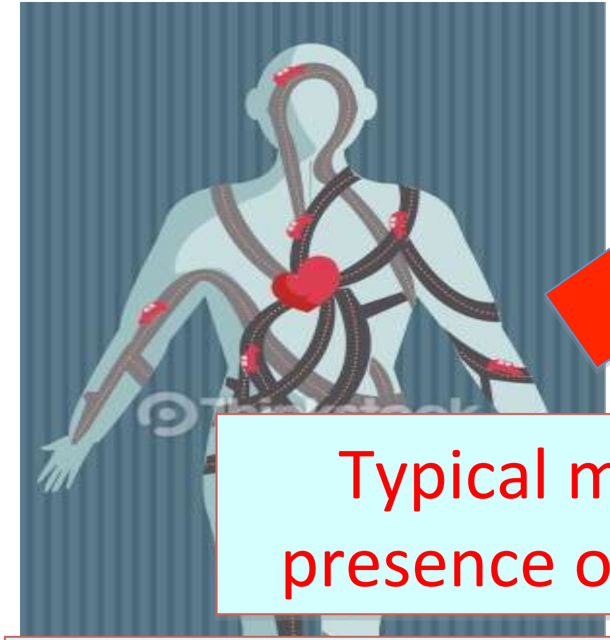
Erythrocyte fatty acid cluster as comprehensive metabolic-nutritional biomarker







Why RBC membranes?



Known reporter for nutrition and metabolism of all tissues

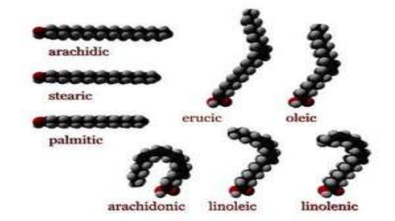
Typical membrane composition with the presence of ALL fatty acid types and families

Mean life time = 120 days

Follow up of changes = every 6 months - once a year

The protocol choice

SAMPLING: ISOLATION of mature RBC (>3 mo. old)



Individuation of a fatty acid CLUSTER (10 cis FA + 2 trans FA)

Determination of mean values in healthy population

EXPERT REVIEWS

Role of fatty acid-based functional lipidomics in the development of molecular diagnostic tools

Expert Rev. Mol. Diagn. 12(7), 767–780 (2012)

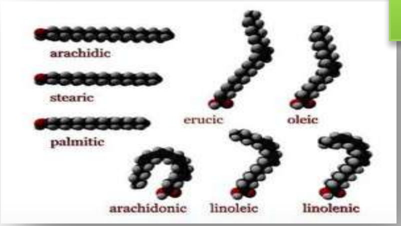
Table 1. Fatty acids and index intervals in the erythrocyte membrane phospholipids of healthy Italian population (relative % upon the total fatty acid content).

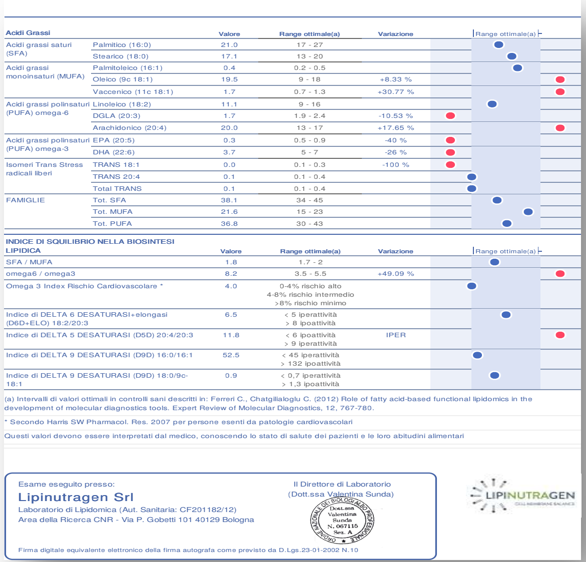
FA residues	Acronym	Normal values
C16:0		17–27
C16:1-Δ9		0.2–0.5
C18:0		13–20
C18:1-Δ9		9–18
C18:1-Δ11		0.7–1.3
C18:2-Δ9,12	LA	9–16
C20:3-Δ8,11,14	DGLA	1.9–2.4
C20:4-Δ5,8,11,14	AA	13–17
C20:5-Δ5,8,11,14,17	EPA	0.5–0.9
C22:6-Δ4,7,10,13,16,19	DHA	5–7
Total saturated FA	SFA	30–45
Total MUFA	MUFA	13–23
Total PUFA	PUFA	28–39
SFA/MUFA		1.7–2
ω-6/ω-3		3.5–5.5
Sum of mono-*trans*	*TRANS*	≤0.4

AA: Arachidonic acid; DGLA: Dihomo-γ-linolenic acid; DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; FA: Fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; SFA: Saturated fatty acid.
Data taken with permission from [52].

Reference values

MEMBRANE LIPIDOME CLUSTER





Acidi Grassi		Valore	Range ottimale(a)	Variazione
Acidi grassi saturi (SFA)	Palmitico (16:0)	21.0	17 - 27	
	Stearico (18:0)	17.1	13 - 20	
Acidi grassi monoinsaturi (MUFA)	Palmitoleico (16:1)	0.4	0.2 - 0.5	
	Oleico (9c 18:1)	19.5	9 - 18	+8.33 %
	Vaccenico (11c 18:1)	1.7	0.7 - 1.3	+30.77 %
Acidi grassi polinsaturi (PUFA) omega-6	Linoleico (18:2)	11.1	9 - 16	
	DGLA (20:3)	1.7	1.9 - 2.4	-10.53 %
	Arachidonico (20:4)	20.0	13 - 17	+17.65 %
Acidi grassi polinsaturi (PUFA) omega-3	EPA (20:5)	0.3	0.5 - 0.9	-40 %
	DHA (22:6)	3.7	5 - 7	-26 %
Isomeri Trans Stress radicali liberi	TRANS 18:1	0.0	0.1 - 0.3	-100 %
	TRANS 20:4	0.1	0.1 - 0.4	
	Total TRANS	0.1	0.1 - 0.4	
FAMIGLIE	Tot. SFA	38.1	34 - 45	
	Tot. MUFA	21.6	15 - 23	
	Tot. PUFA	36.8	30 - 43	

INDICE DI SQUILIBRIO NELLA BIOSINTESI LIPIDICA	Valore	Range ottimale(a)	Variazione
SFA / MUFA	1.8	1.7 - 2	
omega6 / omega3	8.2	3.5 - 5.5	+49.09 %
Omega 3 Index Rischio Cardiovascolare *	4.0	0-4% rischio alto 4-8% rischio intermedio >8% rischio minimo	
Indice di DELTA 6 DESATURASI+elongasi (D6D+ELO) 18:2/20:3	6.5	< 5 iperattività > 8 ipoattività	
Indice di DELTA 5 DESATURASI (D5D) 20:4/20:3	11.8	< 6 ipoattività > 9 iperattività	IPER
Indice di DELTA 9 DESATURASI (D9D) 16:0/16:1	52.5	< 45 iperattività > 132 ipoattività	
Indice di DELTA 9 DESATURASI (D9D) 18:0/9c-18:1	0.9	< 0,7 iperattività > 1,3 ipoattività	

(a) Intervalli di valori ottimali in controlli sani descritti in: Ferreri C., Chatgilialoglu C. (2012) Role of fatty acid-based functional lipidomics in the development of molecular diagnostics tools. Expert Review of Molecular Diagnostics, 12, 767-780.

* Secondo Harris SW Pharmacol. Res. 2007 per persone esenti da patologie cardiovascolari

Questi valori devono essere interpretati dal medico, conoscendo lo stato di salute dei pazienti e le loro abitudini alimentari

Esame eseguito presso:
Lipinutragen Srl
Laboratorio di Lipidomica (Aut. Sanitaria: CF201182/12)
Area della Ricerca CNR - Via P. Gobetti 101 40129 Bologna

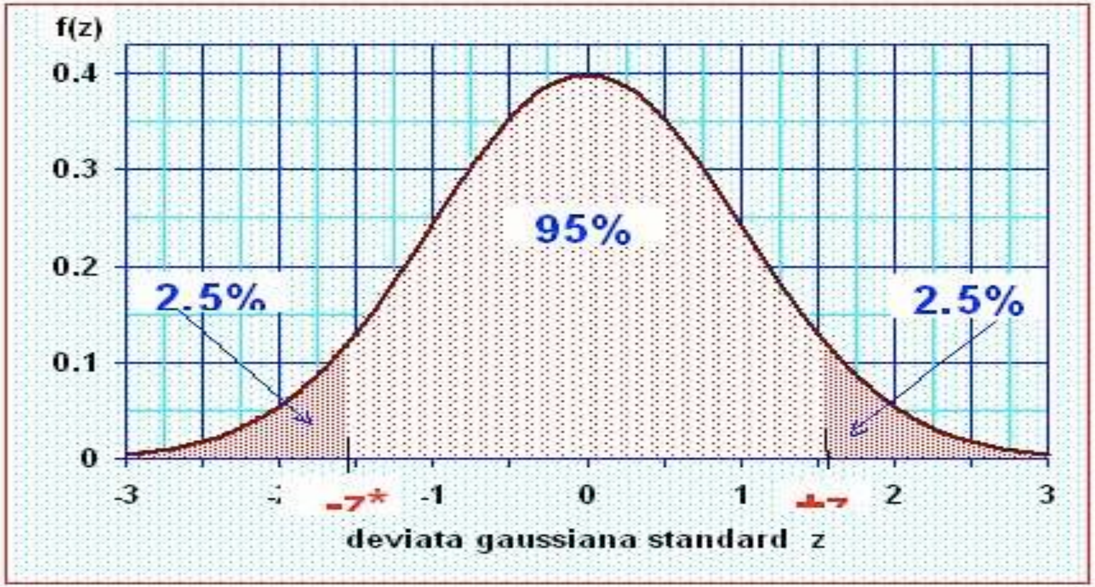
Il Direttore di Laboratorio
(Dott.ssa Valentina Sunda)

Ordine Nazionale dei Biologi Albo Professionale — Dott.ssa Valentina Sunda N. 067115 Sez. A

Firma digitale equivalente elettronico della firma autografa come previsto da D.Lgs.23-01-2002 N.10

LIPINUTRAGEN

Distribution of the values found in populations
Normal range calculation
Like cholesterol levels



Amelioration along the way

The protocol choice: automatization

20 blood drops (0.5 mL)

Lipidomic Laboratory
authorized by the Italian Health Ministry

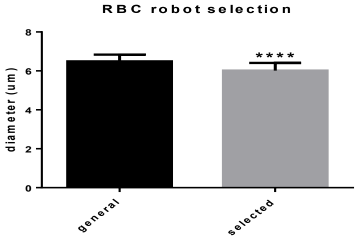
High-throughput

Cell selection
MATURE ERYTHROCYTES

Membrane isolation
Fatty acid work-up and samples for GC (and GC/MS) analysis

Pipeline for full characterization of ROBOT selection

1. Cell diameter



7.2% smaller

2. Distinct lipidomic profile

Fatty Acid Family	RBC (%) [§, a]	Mature RBC (%) [#, a]
SFA	40.6–49.8	34–45
MUFA	14.4–20.3	15–23
PUFA omega-6	27.4–34.0	24–34
PUFA omega-3	3.2–7.8	5.7–9
Total PUFA	32.2–40.2	30–43

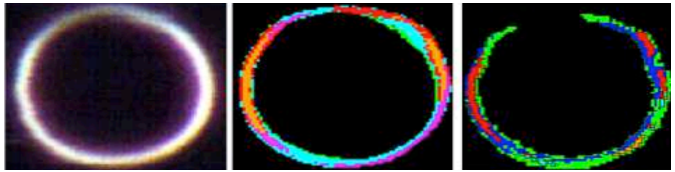
Diagnostics 2017, 7, 1; doi:10.3390/diagnostics7010001

3. Surface antigen markers

CD235a+/CD71-

- Expression of glycophorin A
- Downregulation of transferrin receptor

4. HDFM spectral signature



RBC Spectral library for cell recognition

Biomed. Spectr. Imaging, 2016, 5 , 175-184; *Sci. Rep* 2017, 7:9854.

Science Innovation to the Market

National Research Council of Italy

LIPINUTRAGEN

CELL MEMBRANE BALANCE

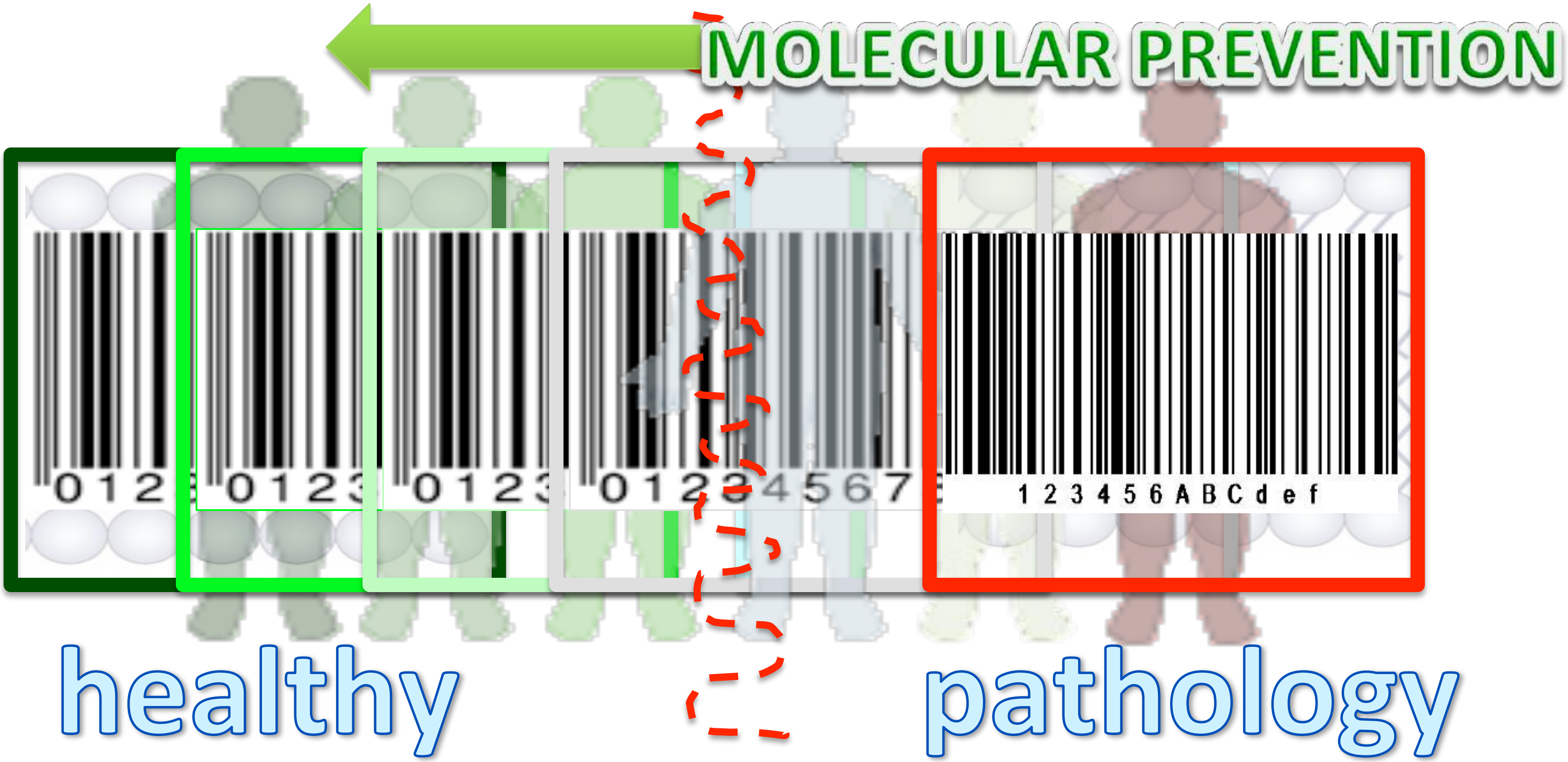
Data base of 25000 analyses (2017)
Healthy and unhealthy individuals

Molecular profiles

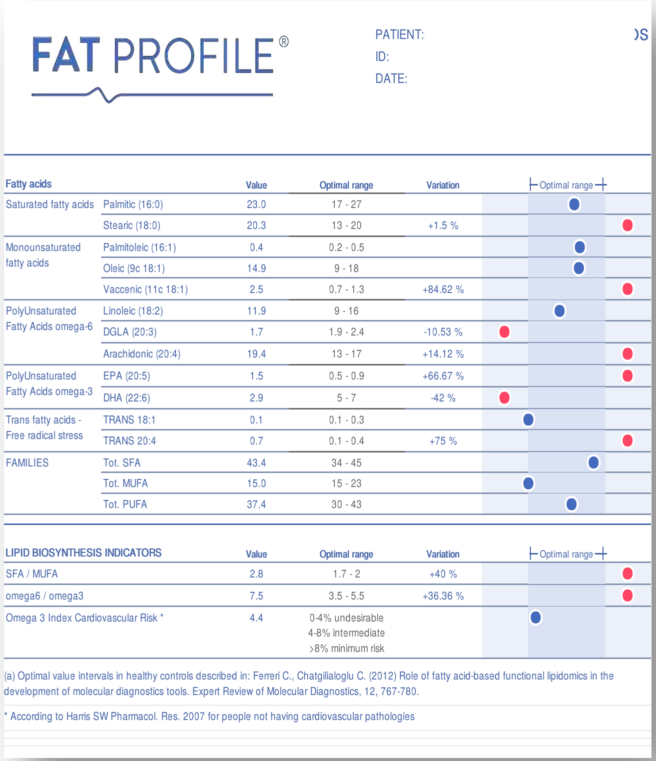
DATABASE

healthy

pathology


MOLECULAR PREVENTION
healthy
pathology

MAIN METABOLIC/DIETARY PATHWAYS



FAT PROFILE®

PATIENT:
ID:
DATE:

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	Oleic (9c 18:1)	14.9	9 - 18	
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	DGLA (20:3)	1.7	1.9 - 2.4	-10.53 %
	Arachidonic (20:4)	19.4	13 - 17	+14.12 %
PolyUnsaturated Fatty Acids omega-3	EPA (20:5)	1.5	0.5 - 0.9	+66.67 %
	DHA (22:6)	2.9	5 - 7	-42 %
Trans fatty acids - Free radical stress	TRANS 18:1	0.1	0.1 - 0.3	
	TRANS 20:4	0.7	0.1 - 0.4	+75 %
FAMILIES	Tot. SFA	43.4	34 - 45	
	Tot. MUFA	15.0	15 - 23	
	Tot. PUFA	37.4	30 - 43	

Optimal range

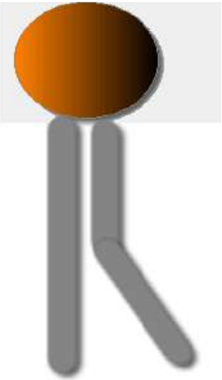
LIPID BIOSYNTHESIS INDICATORS	Value	Optimal range	Variation
SFA / MUFA	2.8	1.7 - 2	+40 %
omega6 / omega3	7.5	3.5 - 5.5	+36.36 %
Omega 3 Index Cardiovascular Risk *	4.4	0-4% undesirable 4-8% intermediate >8% minimum risk	

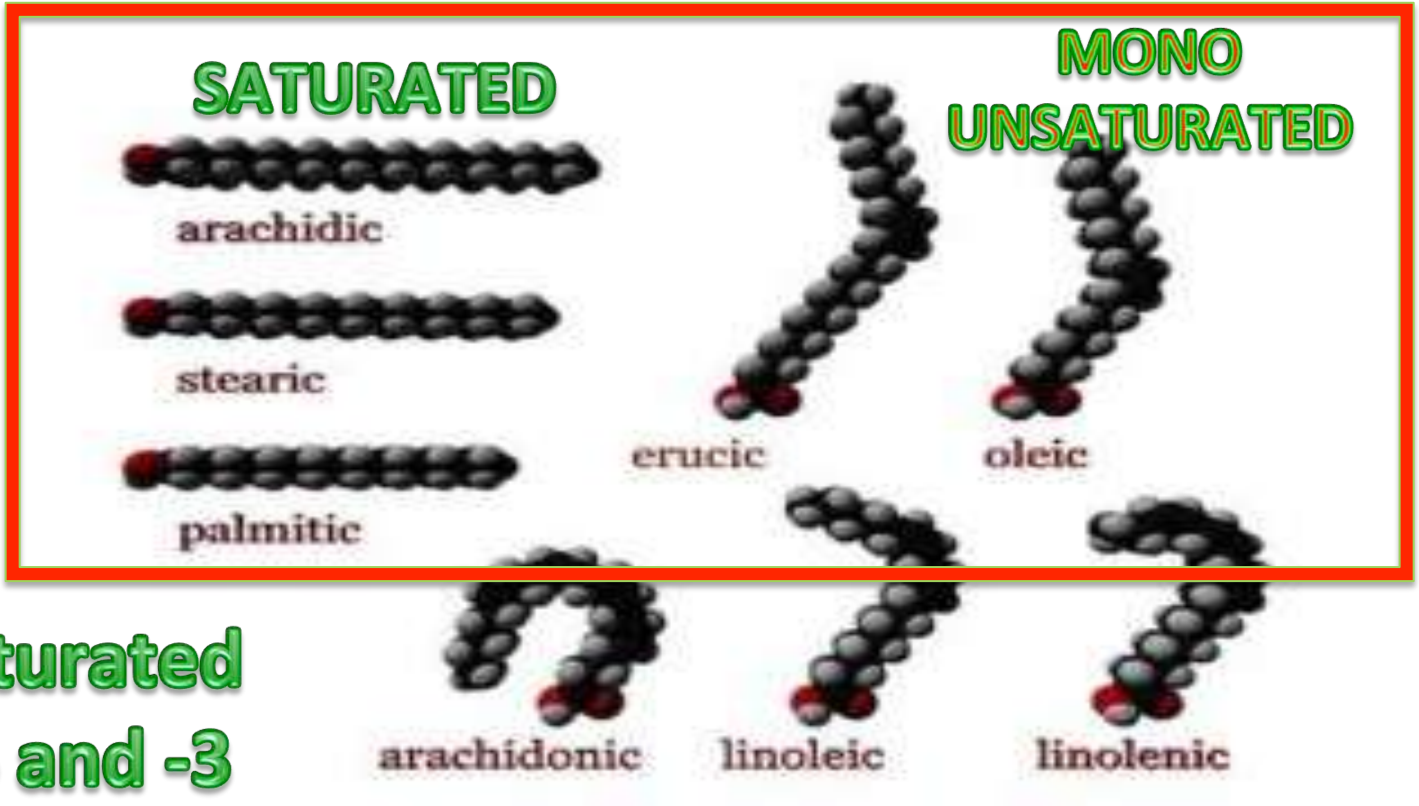
Optimal range

(a) Optimal value intervals in healthy controls described in: Ferreri C., Chatgilialoglu C. (2012) Role of fatty acid-based functional lipidomics in the development of molecular diagnostics tools. Expert Review of Molecular Diagnostics, 12, 767-780.

* According to Harris SW Pharmacol. Res. 2007 for people not having cardiovascular pathologies

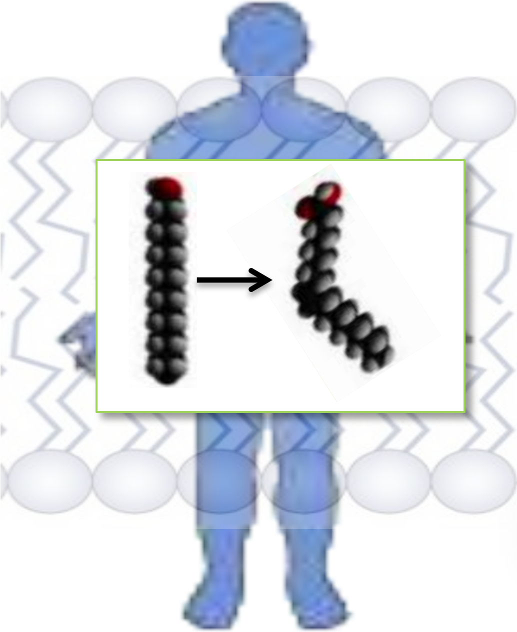
Lipid diversity and essentiality





Polyunsaturated
Omega-6 and -3

SATURATED - MONOUNSATURATED FATS



BIOSYNTHESIS + DIET

C16:0
Palmitic acid

$\xrightarrow{\Delta 9 \text{ desaturase}}$

(C16:1-Δ9)
Palmitoleic acid

NO DIET

$\downarrow$ **Elongase**

(C18:1-Δ11)
Vaccenic acid

$\downarrow$ **Elongase**

(C18:0)
Stearic acid

Δ9 desaturase $\downarrow$

(C18:1-Δ9)
Oleic acid

METABOLIC PROFILE

Nutrition Facts

Serving Size 2 tbsp. (30g)
Servings Per Container 23
Calories 240
Fat Cal. 230

Amount/serving	%DV*	Amount/serving	%DV*
Total Fat 26g	40%	**Total Carb.** 2g	1%
Sat. Fat 7g	35%	Fiber 0g	0%
Polyunsat. Fat 14g		Sugars 0g	0%
Monounsat. Fat 5g		**Protein** 5g	10%
Cholest. 0mg	0%		
Sodium 40mg	2%		

Vitamin A 0% • Vitamin C 0% • Calcium 0% • Iron 0%

*Percent Daily Values (DV) are based on a 2,000 calorie diet.

Ingredients: refined soybean oil, vinigar, garlic, salt, pepper and seasoning

DIETARY INTAKE (FOR HEALTHY HUMANS 10-15 G/DAY)

C16:0
Palmitic acid

Δ9 desaturance →

(C16:1-Δ9)
Palmitoleic acid

↓ **Elongase**

(C18:1-Δ11)
Vaccenic acid

↓ **Elongase**

(C18:0)
Stearic acid

↓ **Δ9 desaturase**

(C18:1-Δ9)
Oleic acid

METABOLIC PROFILE

Nutrition Facts

Serving Size 2 tbsp. (30g)
Servings Per Container 23
Calories 240
Fat Cal. 230

Amount/serving	%DV*	Amount/serving	%DV*
Total Fat 26g	40%	**Total Carb.** 2g	1%
Sat. Fat 7g	35%	Fiber 0g	0%
Polyunsat. Fat 14g		Sugars 0g	0%
Monounsat. Fat 5g		**Protein** 5g	10%
Cholest. 0mg	0%		
Sodium 40mg	2%		

Vitamin A 0% • Vitamin C 0% • Calcium 0% • Iron 0%

*Percent Daily Values (DV) are based on a 2,000 calorie diet.

Ingredients: refined soybean oil, vinigar, garlic, salt, pepper and seasoning

+ fatty acid synthase

C16:0 Palmitic acid $\xrightarrow{\Delta 9 \text{ desaturase}}$ (C16:1-Δ9) Palmitoleic acid

C16:0 Palmitic acid $\xrightarrow{\text{Elongase}}$ (C18:0) Stearic acid

(C18:0) Stearic acid $\xrightarrow{\Delta 9 \text{ desaturase}}$ (C18:1-Δ9) Oleic acid

(C16:1-Δ9) Palmitoleic acid $\xrightarrow{\text{Elongase}}$ (C18:1-Δ11) Vaccenic acid

METABOLIC PROFILE

Nutrition Facts

Serving Size 2 tbsp. (30g)
Servings Per Container 23
Calories 240
Fat Cal. 230

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Vitamin A 0% • Vitamin C 0% • Calcium 0% • Iron 0%

*Percent Daily Values (DV) are based on a 2,000 calorie diet.

Ingredients: refined soybean oil, vinigar, garlic, salt, pepper and seasoning

+ FAS

C16:0
Palmitic acid

Δ9 desaturase →

(C16:1-Δ9)
Palmitoleic acid

↓ **Elongase**

(C18:1-Δ11)
Vaccenic acid

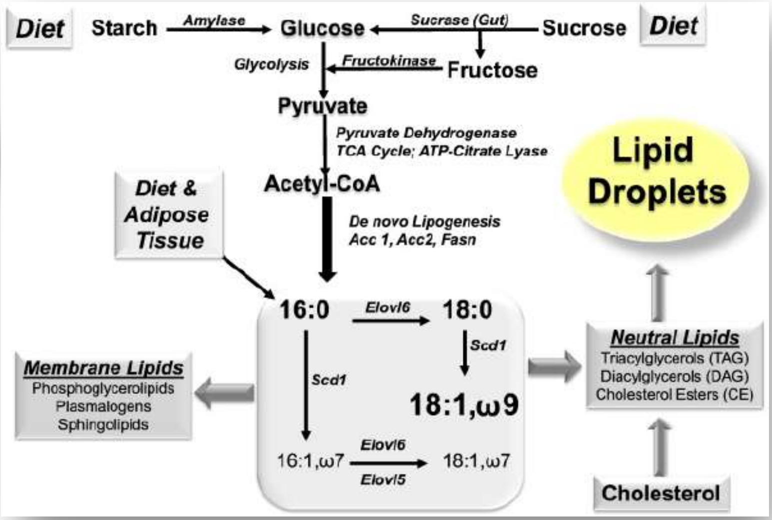
↓ **Elongase**

(C18:0)
Stearic acid

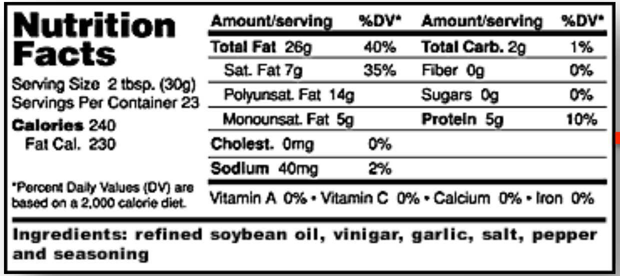
↓ **Δ9 desaturase**

(C18:1-Δ9)
Oleic acid

CONNECTION WITH GLUCOSE METABOLISM



METABOLIC PROFILE



Nutrition Facts

Serving Size 2 tbsp. (30g)
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Calories 240
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Amount/serving	%DV*	Amount/serving	%DV*
Total Fat 26g	40%	**Total Carb.** 2g	1%
Sat. Fat 7g	35%	Fiber 0g	0%
Polyunsat. Fat 14g		Sugars 0g	0%
Monounsat. Fat 5g		**Protein** 5g	10%
Cholest. 0mg	0%		
Sodium 40mg	2%		

*Percent Daily Values (DV) are based on a 2,000 calorie diet.

Vitamin A 0% • Vitamin C 0% • Calcium 0% • Iron 0%

Ingredients: refined soybean oil, vinigar, garlic, salt, pepper and seasoning

C16:0
Palmitic acid

Δ9 desaturase

(C16:1-Δ9)
Palmitoleic acid

Elongase

(C18:0)
Stearic acid

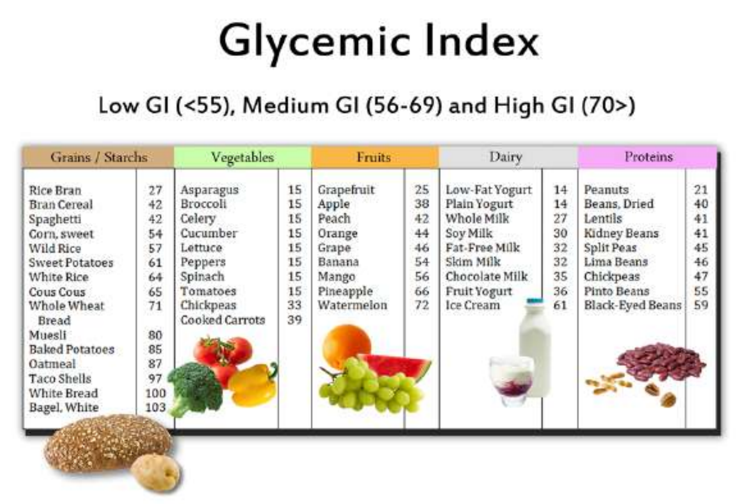
Elongase

(C18:1-Δ11)
Vaccenic acid

Δ9 desaturase

(C18:1-Δ9)
Oleic acid

CARBOHYDRATES AND INSULINIC RESPONSE

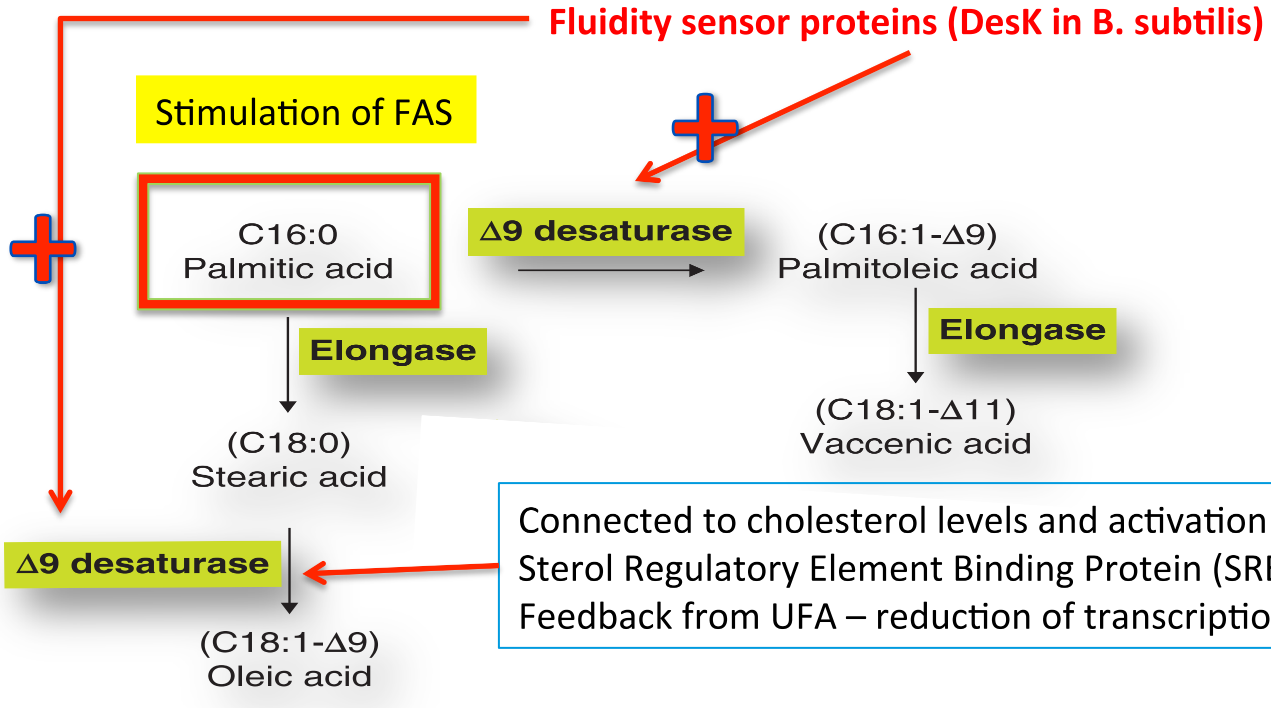


Glycemic Index

Low GI (<55), Medium GI (56-69) and High GI (70>)

Grains / Starchs		Vegetables		Fruits		Dairy		Proteins	
Rice Bran	27	Asparagus	15	Grapefruit	25	Low-Fat Yogurt	14	Peanuts	21
Bran Cereal	42	Broccoli	15	Apple	38	Plain Yogurt	14	Beans, Dried	40
Spaghetti	42	Celery	15	Peach	42	Whole Milk	27	Lentils	41
Corn, sweet	54	Cucumber	15	Orange	44	Soy Milk	30	Kidney Beans	41
Wild Rice	57	Lettuce	15	Grape	46	Fat-Free Milk	32	Split Peas	45
Sweet Potatoes	61	Peppers	15	Banana	54	Skim Milk	32	Lima Beans	46
White Rice	64	Spinach	15	Mango	56	Chocolate Milk	35	Chickpeas	47
Cous Cous	65	Tomatoes	15	Pineapple	66	Fruit Yogurt	36	Pinto Beans	55
Whole Wheat Bread	71	Chickpeas	33	Watermelon	72	Ice Cream	61	Black-Eyed Beans	59
Muesli	80	Cooked Carrots	39						
Baked Potatoes	85								
Oatmeal	87								
Taco Shells	97								
White Bread	100								
Bagel, White	103								

METABOLIC PROFILE

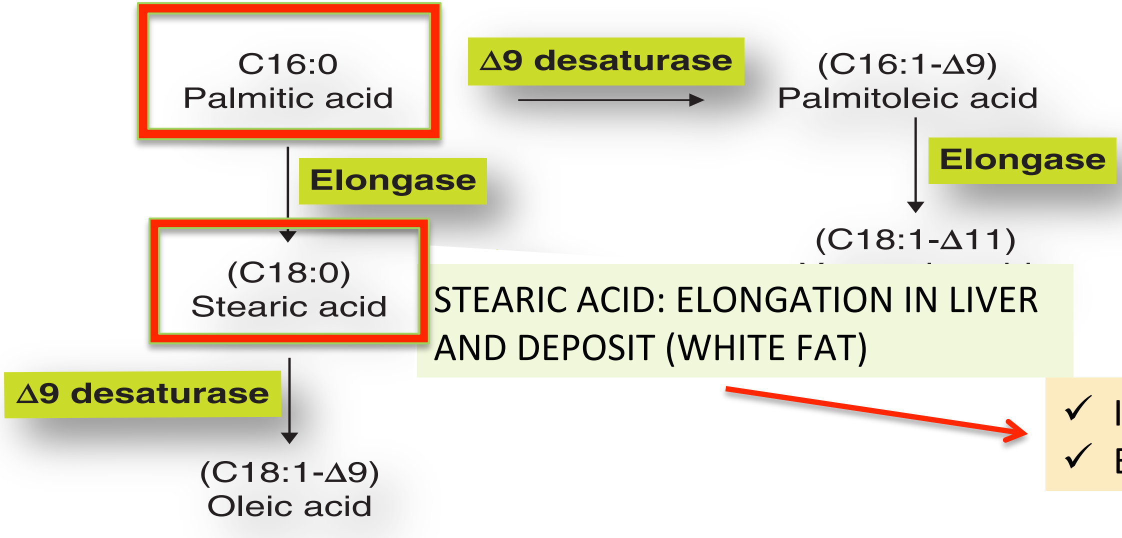


Connected to cholesterol levels and activation of OLE1 gene
Sterol Regulatory Element Binding Protein (SREBP)
Feedback from UFA – reduction of transcription

MOL. MICROBIOL 2006, 62, 1507-1514

METABOLIC PROFILE

PALMITIC ACID LEVEL



STEARIC ACID: ELONGATION IN LIVER AND DEPOSIT (WHITE FAT)

- ✓ INCREASE CHOLESTEROL LEVELS
- ✓ ENERGY PRODUCTION

METABOLIC PROFILE

C16:0
Palmitic acid

$\xrightarrow{\Delta 9 \text{ desaturase}}$

(C16:1-Δ9)
Palmitoleic acid

↓ **Elongase**

(C18:1-Δ11)

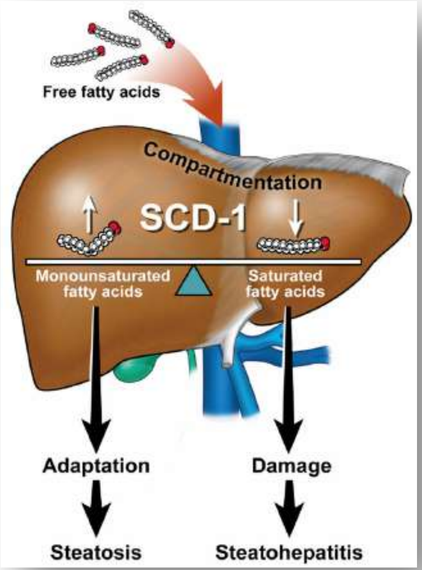
↓ **Elongase**

(C18:0)
Stearic acid

↓ **Δ9 desaturase**

(C18:1-Δ9)
Oleic acid

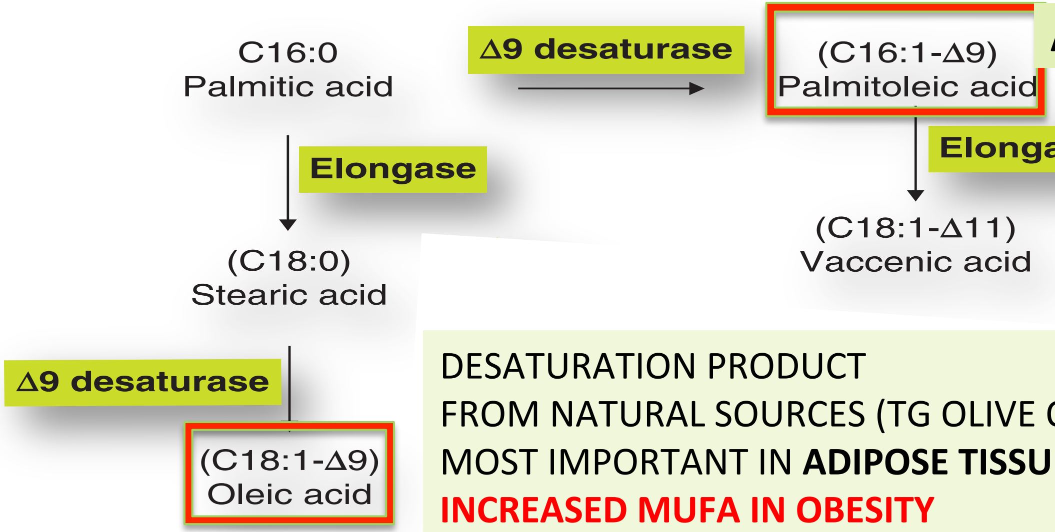
STEARIC ACID: ELONGATION IN LIVER AND DEPOSIT (WHITE FAT)



- ✓ HEPATIC FUNCTION
- ✓ MATURE RBC EVALUATION

METABOLIC PROFILE

LIPID ACCUMULATION

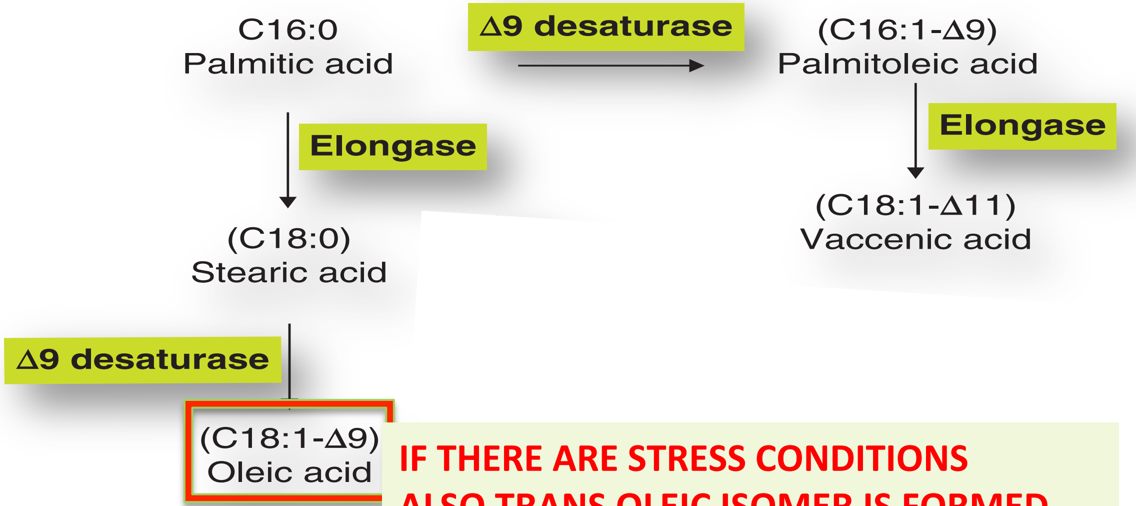


ADIPOSE TISSUE: OBESITY MARKER

DESATURATION PRODUCT
FROM NATURAL SOURCES (TG OLIVE OIL)
MOST IMPORTANT IN **ADIPOSE TISSUE**
INCREASED MUFA IN OBESITY
AND IN CELLPROLIFERATION
(TUMORIGENESIS MARKER)

METABOLIC PROFILE

LIPID ACCUMULATION



IF THERE ARE STRESS CONDITIONS
ALSO TRANS OLEIC ISOMER IS FORMED

METABOLIC PROFILE: INTERVENTION

FAT AND CARBOHYDRATE CONTROL FROM DIET

C16:0
Palmitic acid

$\xrightarrow{\Delta 9 \text{ desaturase}}$

(C16:1-Δ9)
Palmitoleic acid

Elongase

(C18:1-Δ11)
Vaccenic acid

Elongase

(C18:0)
Stearic acid

Δ9 desaturase

(C18:1-Δ9)
Oleic acid

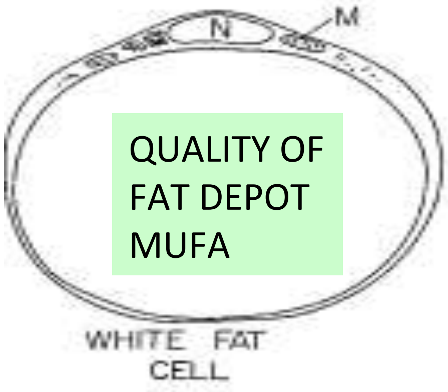
CONTROL OF FAT vs LEAN MASS

CONTROL OF HEPATIC FUNCTION

METABOLISM ACTIVATION

ACTIVATION/CONTROL DESATURASE

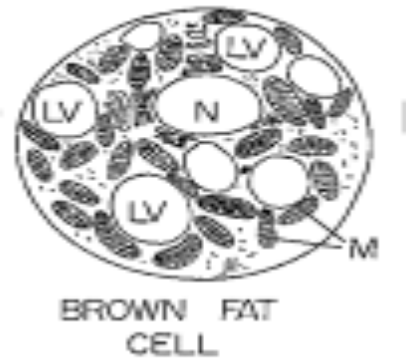
The adipocyte shift: white to brown cells



QUALITY OF
FAT DEPOT
MUFA

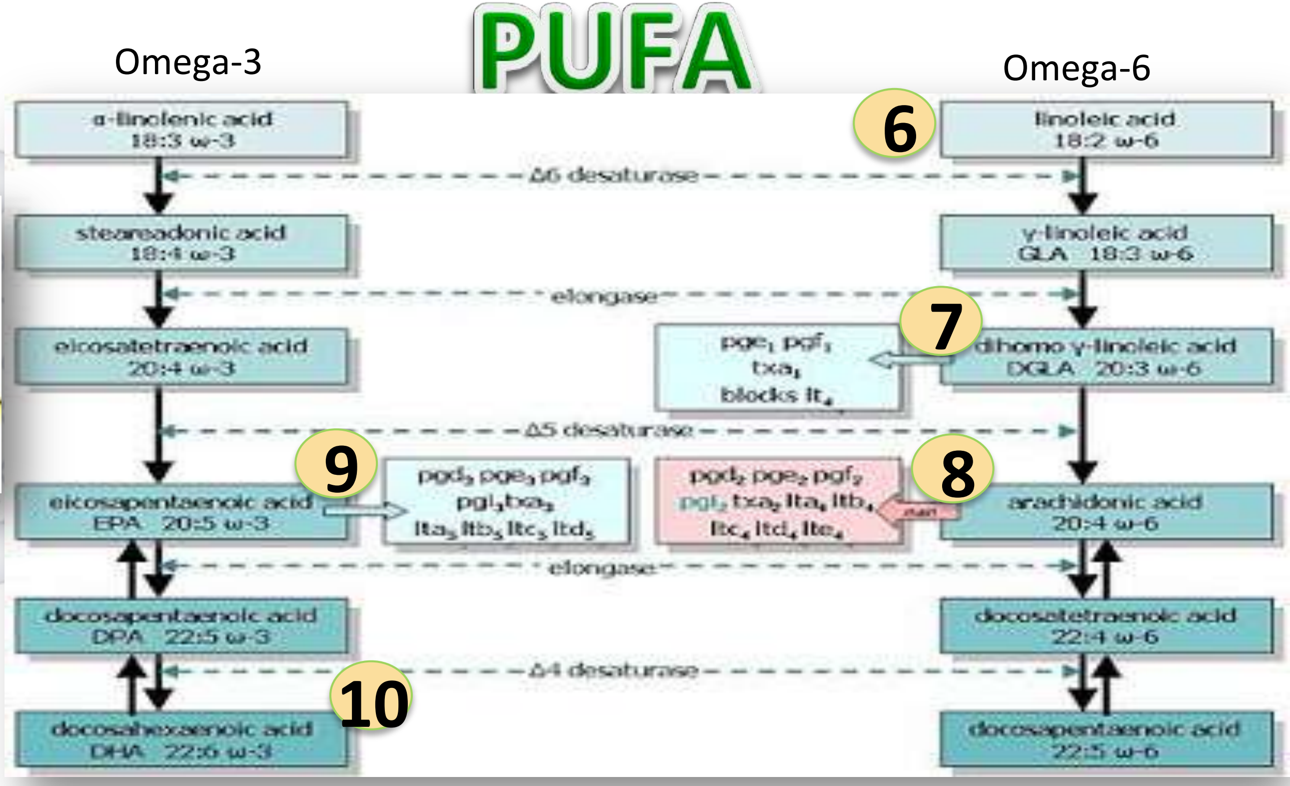
COLD
20-22 °C FOR HUMANS

NORADRENALINE



DIET can alter this shift (**fat depot**: SATURATED vs UNSATURATED FAT ratio)
changing the cell signaling for lipolysis and cell shift

Molecular profile for the "reactive/inflammatory status":
PUFA pathways



Lipidomic evaluation of inflammatory condition

DIET

Linoleic acid (LA, 18:2- Δ9,12) 6

↓ **Δ6 desaturase**

γ-linolenic acid (18:3- Δ6,9,12)

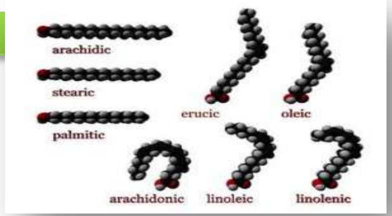
↓ **elongase**

Dihomo–γ-linolenic acid (20:3- Δ8,11,14) 7

↓ **Δ5 desaturase**

Arachidonic acid (AA, 20:4- Δ5,8,11,14) 8

MEMBRANE LIPIDOMICS



Control the starting point

Lipidomic evaluation of inflammatory condition

DIET

Linoleic acid (LA, 18:2- Δ9,12) 6

↓ **Δ6 desaturase**

γ-linolenic acid (18:3- Δ6,9,12)

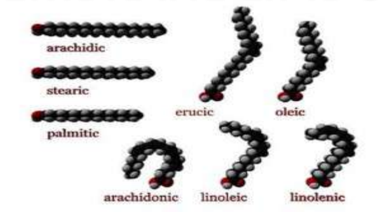
↓ **elongase**

Dihomo–γ-linolenic acid (20:3- Δ8,11,1 7

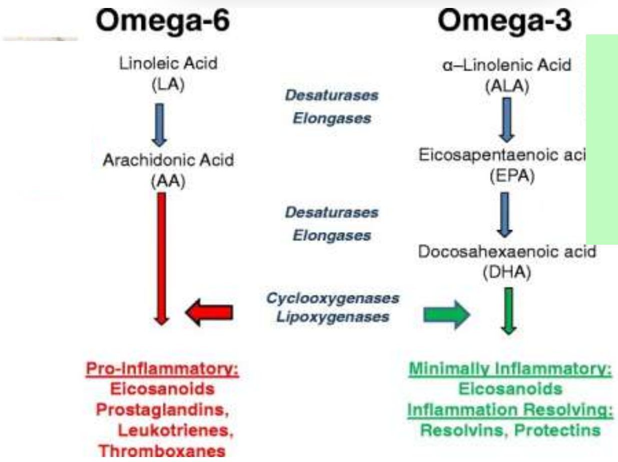
↓ **Δ5 desaturase**

Arachidonic acid (AA, 20:4- Δ5,8,11,1 8

MEMBRANE LIPIDOMICS



- ✓ Control the starting point for omega-6
- ✓ Control omega-3 not ALA in RBC



NOT MEASURED because is too variable

Lipidomic evaluation of inflammatory condition

DIET

Linoleic acid (LA, 18:2- Δ9,12) 6

Δ6 desaturase

γ-linolenic acid (18:3- Δ6,9,12)

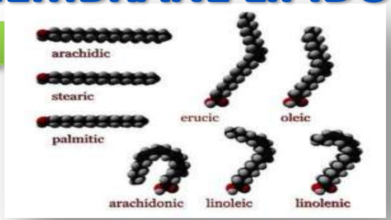
elongase

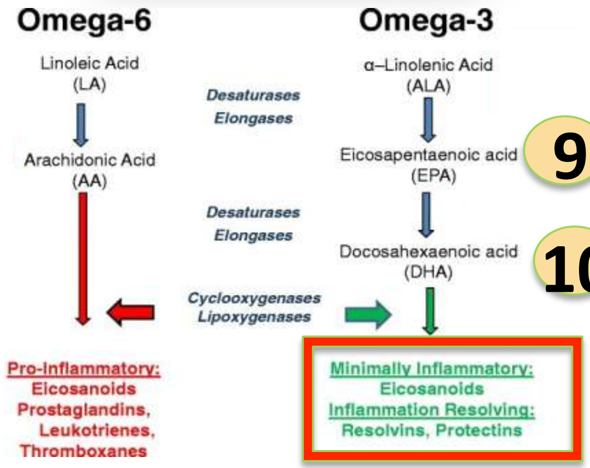
Dihomo−γ-linolenic acid (20:3- Δ8,11,1 7

Δ5 desaturase

Arachidonic acid (AA, 20:4- Δ5,8,11,1 8

MEMBRANE LIPIDOMICS





9

10

Two long chain PUFA

Lipidomic evaluation of inflammatory condition

DIET

Linoleic acid (LA, 18:2- Δ9,12)

↓ **Δ6 desaturase**

γ-linolenic acid (18:3- Δ6,9,12)

↓ **elongase**

Dihomo−γ-linolenic acid (20:3- Δ8,11,14) 7

↓ **Δ5 desaturase**

Arachidonic acid (AA, 20:4- Δ5,8,11,14)

OMEGA-6 precursor of LIPID MEDIATORS

Control the
anti-inflammatory
Omega-6 FA

Lipidomic evaluation of inflammatory condition

DIET

Linoleic acid (LA, 18:2- Δ9,12)

↓ **Δ6 desaturase**

γ-linolenic acid (18:3- Δ6,9,12)

↓ **elongase**

Dihomo−γ-linolenic acid (20:3- Δ8,11,14)

↓ **Δ5 desaturase**

Arachidonic acid (AA, 20:4- Δ5,8,11,14) **8**

OMEGA-6 precursor of LIPID MEDIATORS

Control the pro-inflammatory Omega-6 FA

WHAT IS THE LEVEL when you assign the anti-inflammatory strategy?

Nutraceutical correction for inflammatory conditions

DIET

Linoleic acid (LA, 18:2- Δ9,12)

↓ **Δ6 desaturase**

γ-linolenic acid (18:3- Δ6,9,12)

↓ **elongase**

Dihomo-γ-linolenic acid (20:3- Δ8,11,14)

↓ **Δ5 desaturase**

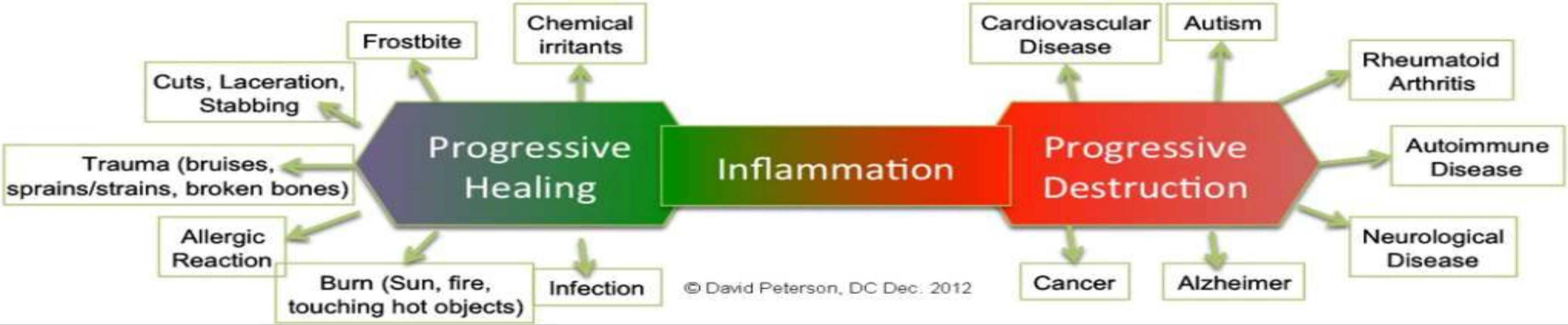
Arachidonic acid (AA, 20:4- Δ5,8,11,14)

Increase competition from omega-3: alfa-linolenic acid (omega-3) – SOURCES (hemp, lineseed oils; spinach, walnuts)

Insert SOURCES of GLA as precursor of anti-inflammatory mediators: **NUTRACEUTICALS** (Oenothera biennis, Borrago oils)

Increase competition for delta-6 desaturase and lipid mediators from the long chain omega-3 PUFA EPA e DHA –
SOURCES: fish, algae
REDUCE intakes of omega-6: read meat, poultry, egg yolk

Inflammation: REGENERATIVE or DEGENERATIVE process?

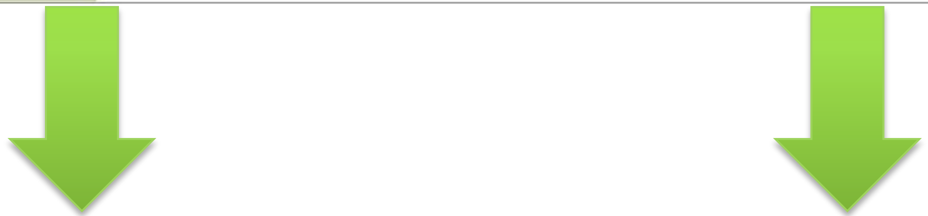


Cell reactivity toward regeneration

Cell reactivity toward degeneration

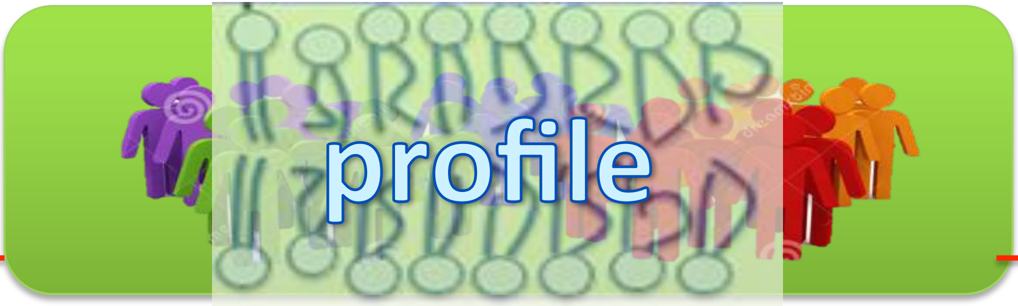
When the inflammation shifts from healing to destruction?

Inflammation: REGENERATIVE or DEGENERATIVE process?



Cell reactivity toward regeneration

Cell reactivity toward degeneration



Expressing the status of membrane homeostasis and signals

World Journal of Gastroenterology

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ORIGINAL ARTICLE

Observational Study

Functional lipidomics in patients on home parenteral nutrition: Effect of lipid emulsions

Loris Pironi, Mariacristina Guidetti, Ornella Verrastro, Claudia Iacona, Federica Agostini, Caterina Pazzeschi, Anna Simona Sasdelli, Michele Melchiorre, Carla Ferreri

Lipid emulsions and mature RBC membranes

Table 2 Fatty acid profile of the intravenous lipid emulsions and of the red blood cell membrane in the study groups

Fatty acids, % total FAs	OO-IVLE	SMOF-IVLE	OO-IVLE group	SMOF-IVLE group	No-IVLE group	HC group	*P* value
Palmitic (16:0)	12.8	14.1	26.2 (23.1-30.6)	28.1 (24.8-30.6)	28.1 (26.1-29.4)	26.6 (20.3-29.6)	0.040
Stearic (18:0)	3.7	4.4	16.7 (14.3-18.3)	16.8 (15.9-18.3)	16.1 (14.2-16.7)	18.1 (13.4-20.7)	< 0.001
Palmitoleic (16:1)	0	0	0.5 (0.4-0.9)	0.5 (0.2-0.8)	1.0 (0.3-1.8)	0.4 (0.2-0.7)	< 0.001
Oleic (18:1; 9c)	61.8	43.3	18.9 (17.8-23.5)	18.6 (17.3-19.9)	18.9 (16.6-22.5)	17.1 (14.2-22.2)	< 0.001
Vaccenic (18:1; 11c)	2.3	2.5	1.9 (1.6-2.7)	1.7 (1.5-2.3)	2.2 (1.7-3.3)	1.4 (0.9-2.6)	< 0.001
Linoleic (18:2; n-6)	17.7	26.9	8.7 (5.5-10.4)	9.2 (7.2-10.9)	7.7 (3.9-9.6)	11.6 (8.8-14.4)	< 0.001
DGLA (20:3; n-6)	0	0	2.2 (1.5-3.9)	1.9 (1.1-2.1)	2.2 (1.5-2.9)	2.0 (1.5-2.8)	0.049
Arachidonic (20:4; n-6)	0	0	18.3 (10.0-21.1)	12.2 (10.7-14.4)	19.2 (18.4-20.3)	16.8 (13.1-21.4)	< 0.001
Alfa-linoleic (18:3; n-3)	1.7	2.4	< 0.01	< 0.01	< 0.01	< 0.01	
EPA (20:5; n-3)	0	3.4	0.6 (0.4-4.5)	3.2 (2.1-5.0)	0.5 (0.4-1.0)	0.7 (0.2-6.0)	< 0.001
DHA (22:6; n-3)	0	3	4.8 (3.4-8.4)	7.6 (6.2-10.4)	3.9 (3.0-4.7)	5.2 (2.3-8.4)	< 0.001
trans-Oleic	0	0	0.1 (0.1-0.2)	0.1 (0.0-0.2)	0.2 (0.0-0.2)	0.1 (0.0-0.2)	0.275
5-8-trans-Arachidonic	0	0	0.1 (0.1-0.2)	0.1 (0.0-0.2)	0.2 (0.0-0.1)	0.1 (0.0-0.3)	0.109
Total SFAs	16.5	18.5	43.5 (41.1-46.4)	44.9 (40.7-47.4)	44.0 (40.8-45.6)	44.3 (37.7-50.1)	0.786
Total MUFAs	65.1	45.8	21.5 (20.7-25.6)	20.9 (19.7-21.9)	21.9 (20.7-27.1)	19.5 (15.8-24.8)	0.001
Total PUFAs	19.4	35.7	34.4 (30.6-37.1)	33.7 (31.0-39.2)	33.8 (28.9-35.7)	35.7 (24.4-39.2)	0.937

Lipid emulsions and mature RBC membranes

Fatty acids, % total FAs	OO-IVLE	SMOF-IVLE
Palmitic (16:0)	12.8	14.1
Stearic (18:0)	3.7	4.4
Palmitoleic (16:1)	0	0
Oleic (18:1; 9c)	61.8	43.3
Vaccenic (18:1; 11c)	2.3	2.5
Linoleic (18:2; n-6)	17.7	26.9
DGLA (20:3; n-6)	0	0
Arachidonic (20:4; n-6)	0	0
Alfa-linoleic (18:3; n-3)	1.7	2.4
EPA (20:5; n-3)	0	3.4
DHA (22:6; n-3)	0	3
trans-Oleic	0	0
5-8-trans-Arachidonic	0	0
Total SFAs	16.5	18.5
Total MUFAs	65.1	45.8
Total PUFAs	19.4	35.7

E
F
A

Lipid emulsions and mature RBC membranes

Fatty acids, % total FAs	OO-IVLE	SMOF-IVLE	OO-IVLE group	SMOF-IVLE group
Palmitic (16:0)	12.8	14.1	26.2 (23.1-30.6)	28.1 (24.8-30.6)
Stearic (18:0)	3.7	4.4	16.7 (14.3-18.3)	16.8 (15.9-18.3)
Palmitoleic (16:1)	0	0	0.5 (0.4-0.9)	0.5 (0.2-0.8)
Oleic (18:1; 9c)	61.8	43.3	18.9 (17.8-23.5)	18.6 (17.3-19.9)
Vaccenic (18:1; 11c)	2.3	2.5	1.9 (1.6-2.7)	1.7 (1.5-2.3)
Linoleic (18:2; n-6)	17.7	26.9	8.7 (5.5-10.4)	9.2 (7.2-10.9)
DGLA (20:3; n-6)	0	0	2.2 (1.5-3.9)	1.9 (1.1-2.1)
Arachidonic (20:4; n-6)	0	0	18.3 (10.0-21.1)	12.2 (10.7-14.4)
Alfa-linoleic (18:3; n-3)	1.7	2.4	< 0.01	< 0.01
EPA (20:5; n-3)	0	3.4	0.6 (0.4-4.5)	3.2 (2.1-5.0)
DHA (22:6; n-3)	0	3	4.8 (3.4-8.4)	7.6 (6.2-10.4)
trans-Oleic	0	0	0.1 (0.1-0.2)	0.1 (0.0-0.2)
5-8-trans-Arachidonic	0	0	0.1 (0.1-0.2)	0.1 (0.0-0.2)
Total SFAs	16.5	18.5	43.5 (41.1-46.4)	44.9 (40.7-47.4)
Total MUFAs	65.1	45.8	21.5 (20.7-25.6)	20.9 (19.7-21.9)
Total PUFAs	19.4	35.7	34.4 (30.6-37.1)	33.7 (31.0-39.2)

E
F
A

OMEGA-6

OMEGA-3

ANIMAL STUDIES

Ann. Rev. Nutr. 1984. 4:521–62

NUTRITION OF THE DOMESTIC CAT, A MAMMALIAN CARNIVORE

EFA OMEGA-6 IMPAIRMENT CAUSES:
- SKIN PROBLEMS
- THROMBOCYTOPENIA
- REPRODUCTIVE PROBLEMS

EVALUATE THE LINOLEATE/ARACHIDONATE INTAKES FROM THE DIET

DIET

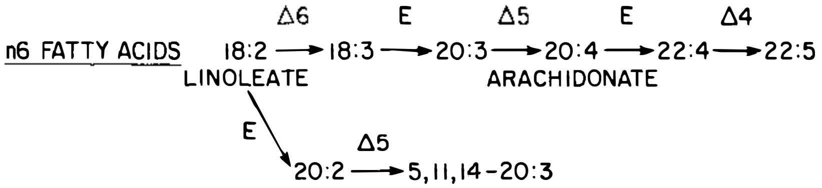


Figure 3 Pathways of linoleate metabolism in mammals. In the liver of the cat there is almost no Δ6-desaturase activity, so arachidonate is a dietary essential. E= elongation, Δ= desaturation.

NOT A VERY HIGH CONVERSION OF LINOLEATE INTO ARACHIDONATE: CONVERSION OCCURS AFTER FEEDING 20:3 FOR MORE THAN 10 WEEKS

REVISTA BRASILEIRA
DE ZOOTECNIA

BRAZILIAN JOURNAL OF ANIMAL SCIENCE

DOG & CAT RECOMMENDED

R. Bras. Zootec., v.37, *suplemento especial* p. 20-27, 2008

Table 1 - Summary of the Essential and Conditionally Essential Fatty Acids for Dog and Cat Life Stages.

Nutrient	Cats		
	Growth	Adult	Gest/Lact
Omega-6			
LA	X	X	X
AA	C	--	C
Omega-3			
ALA	Rec[b]	--	Rec[b]
EPA[c]	--	--	--
DHA[c]	C	Rec[b]	C

Fish oil rich in EPA

a) X, Essential fatty acid; C, Conditionally essential for the respective life stage.
b) Recommended but no requirement established
c) Many n-3 LCPUFA sources contain both EPA and DHA; EPA should not exceed 60% of EPA + DHA total.

- LINOLEIC ALFA-LINOLENIC ACID ARE ESSENTIAL FA, THE SECOND ONE NOT ESTABLISHED AS REQUIREMENTS
- SUPPLEMENTATIONS OF OMEGA-3 ONLY FOR AMELIORATION OF SPECIFIC PERFORMANCES

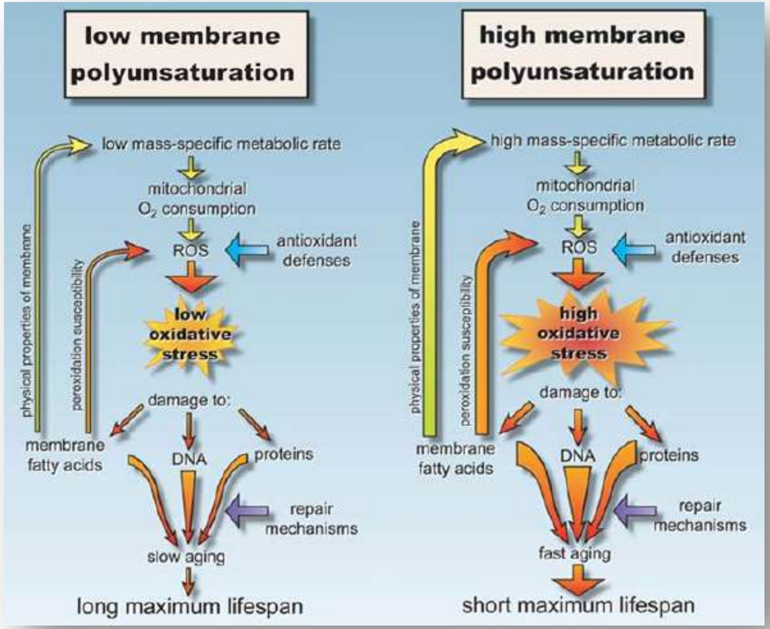
PUFA AND LIFE SPAN

AGE (2008) 30:89–97
DOI 10.1007/s11357-008-9055-2

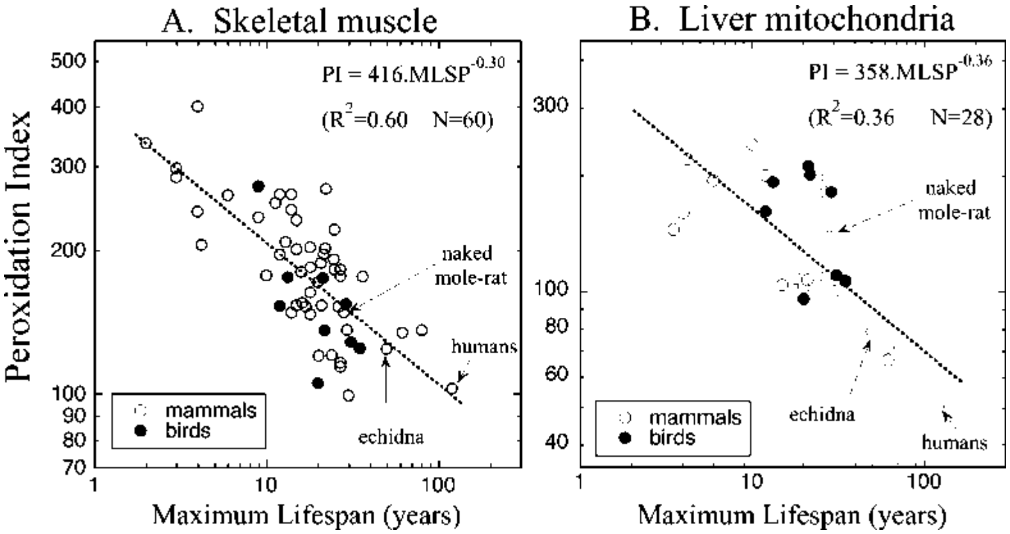
Explaining longevity of different animals: is membrane fatty acid composition the missing link?

A. J. Hulbert

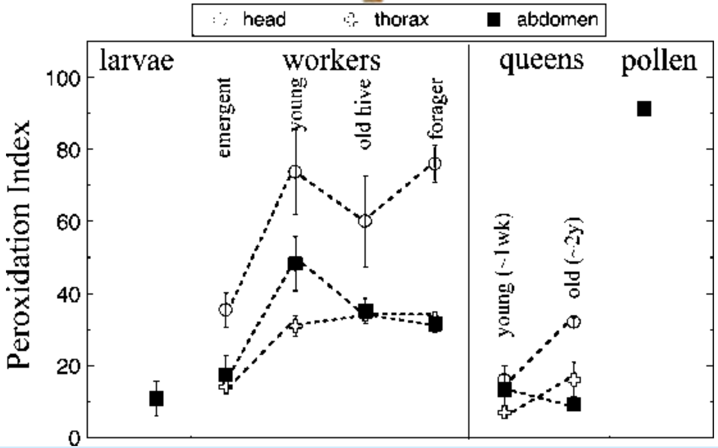
The membrane pacemaker theory of aging



PUFA AND LIFE SPAN



- SIMILARITY OF MAXIMUM LIFE SPAN POTENTIAL (MLSP) IN SKELETAL MUSCLE AND MITOCHONDRIA
- THE INCREASE OF MLSP IS CONNECTED TO REDUCTION OF PEROXIDATION INDEX (PUFA)



- THE PI OF PHOSPHOLIPIDS IS HIGHER IN LESS-LIVING ANIMALS
- QUEENS LIVE 3 TIMES MORE

A PROPOSITION FOR HUMANS

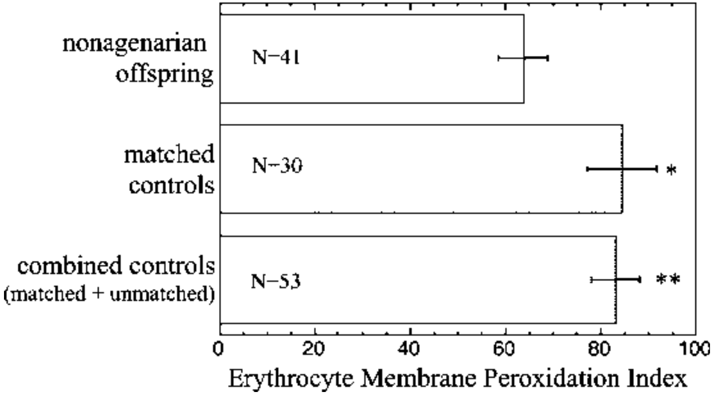


Fig. 6 Comparison of the PI of human erythrocyte membrane fatty acids. Data are taken from Puca et al. (2008). *Error bars* ± 1 SEM; sample size shown in each bar. Both controls are significantly different (*P=0.02 and **P=0.009) from nonagenarian offspring

OUR WORK ON LONGEVITY OFFSPRING AND NORMAL CONTROLS
REJUVEN. RES.2008, 11, 1-10

ANIMALS LIFE?

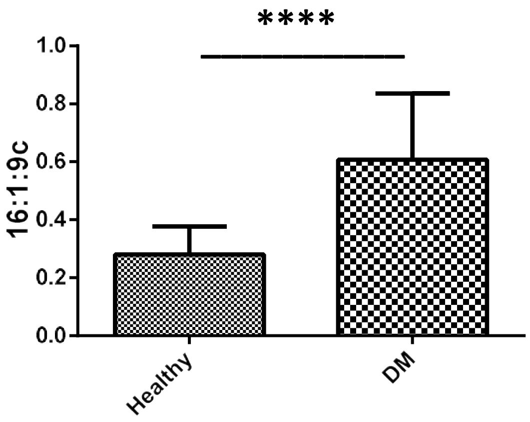
Experimental models

Animal lipidomics

- ✓ Rats fed a trans-free diet
  Free Radic Biol Med 2005
- ✓ Environmental radiations to animals (frogs, trouts) *Rad. Res. 2012, CNL Nuclear Rev 2016*
- ✓ Sportive rats *Lipids in Health and Disease, 2012*
- ✓ Rats with different diets, *Br J Nutr 2013, Chem Res Toxicol 2013 , BBA Bioenergetics 2015, Br J Nutr 2016*
- ✓ Tumor-injected mice, *submitted*

INITIAL WORK FOR dog RBC MEMBRANE PROFILE

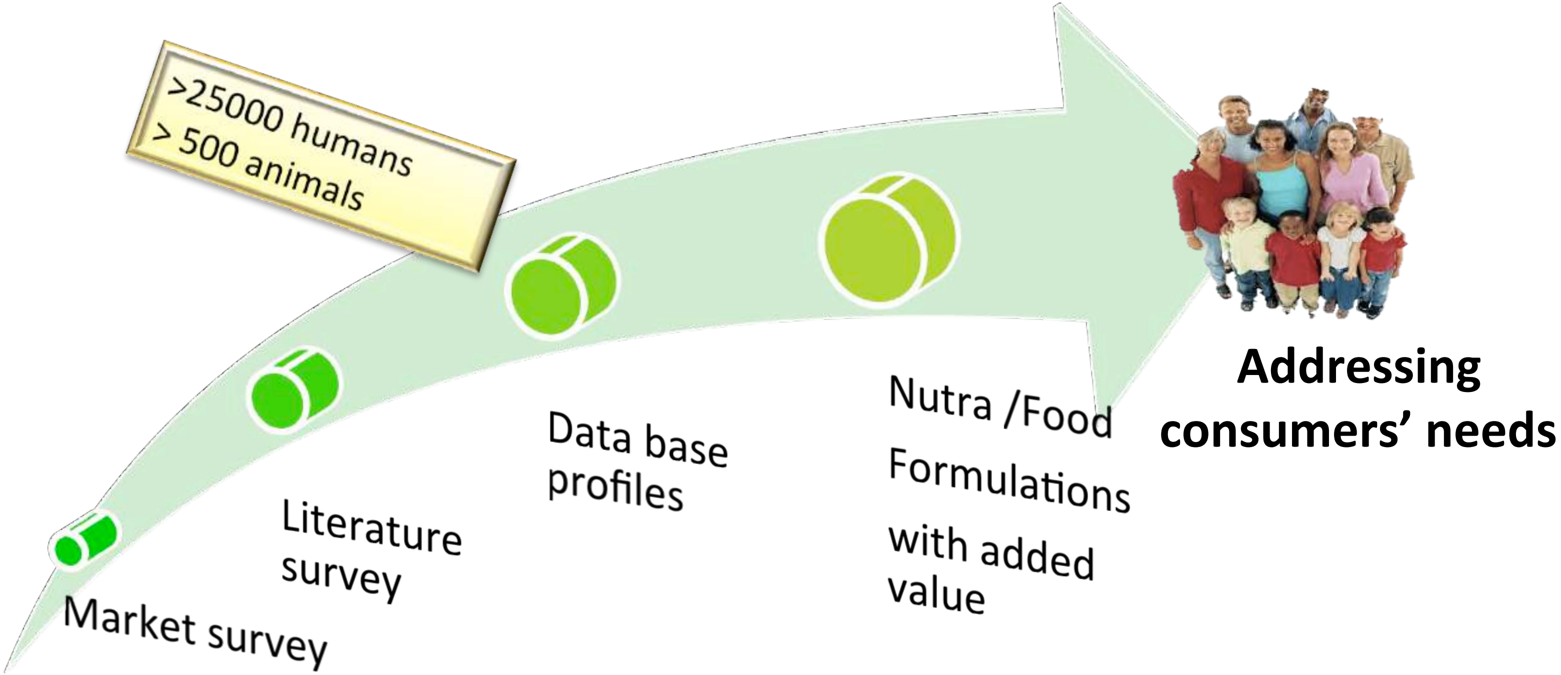
FAME	OPTIMAL VALUES for dogs
SFA (16:0-palmiticand18:0-stearic)	39.5 - 42.0
MUFA (palmitoleic16:1,oleic18:1n-9andvaccenic18:1n-7)	13.5 - 14.0
18:2 (n6) linoleic	11.5 - 12.0
20:3(n-6) diomogamma-linoleico	1.9 - 3.4
20:4 (n-6) arachidonic	28 - 29.6
18:3 (n-3) alpha-linoleic	0.1 - 0.2
20:5 (n-3) EPA	0.2 - 0.4
22:6 (n-3) DHA	0.1 - 0.2
Σtrans	0 - 0.1
SFA/MUFA	2.9 - 3.5
ω6/ω3	27.5 - 34.2



N=24 N=7

PALMITOLEIC ACID
SIGNIFICANTLY DIFFERENT
DESATURASE ACTIVATION
IN METABOLIC SITUATIONS

Innovative productive chain for NUTRA-products



Nutritional claims addressing human profiles according to EFSA
Monitoring effects in humans for further claims

Create the healthy balance through innovation

Thank you for your attention!