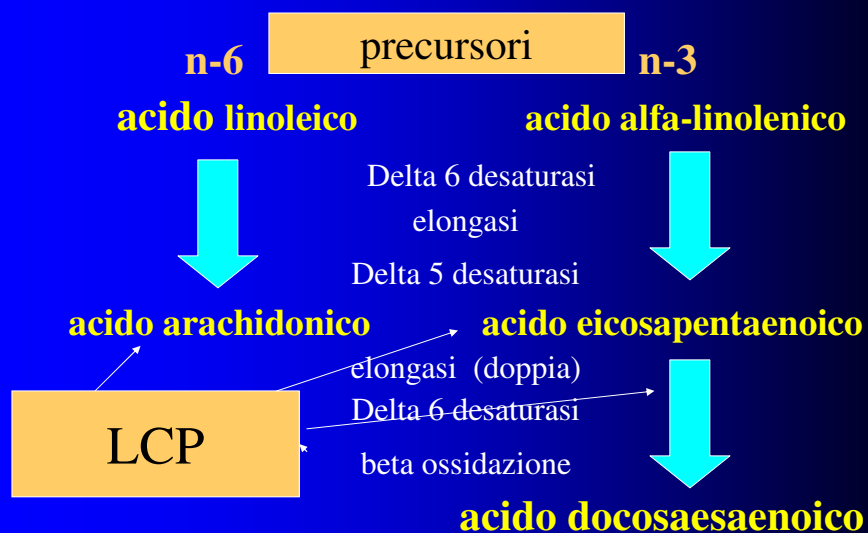


UTILIZZO DEGLI LCPUFA nelle malattie metaboliche ereditarie

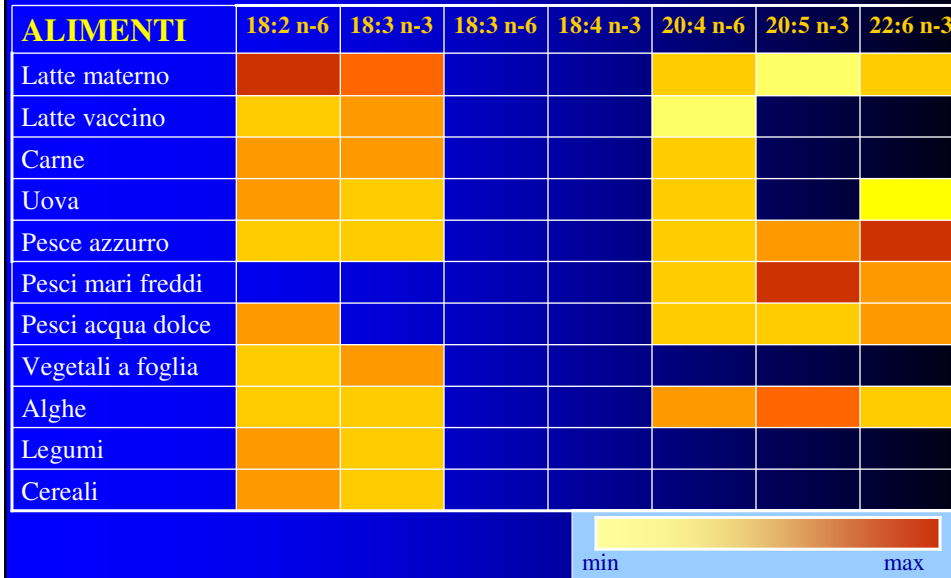
Carlo Agostoni

Clinica Pediatrica
Ospedale San Paolo
Università degli Studi di Milano

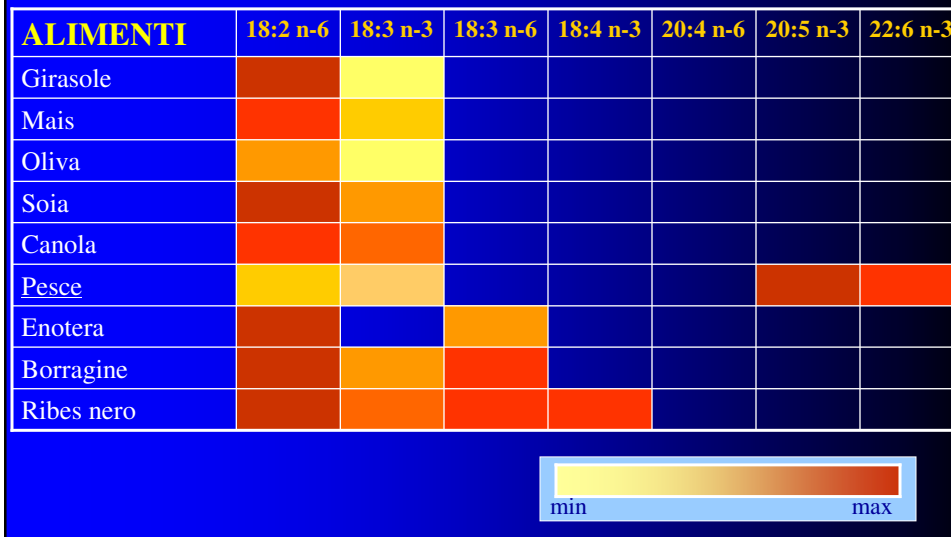
ACIDI GRASSI POLINSATURATI



CONTENUTO DI ACIDI GRASSI NEGLI ALIMENTI



CONTENUTO DI ACIDI GRASSI NEGLI OLI



Differenze strutturali tra BF (+ DHA) e FF (- DHA) in bambini deceduti per “morte in culla”

The mean weight percentage of docosahexaenoic acid was significantly greater ($p < 0.02$) in 5 breast-milk-fed infants (9.7%) than in 5 age-comparable formula-milk-fed infants (7.6%).

Farquharson et al, Lancet 1992; 340:810

Breast-fed infants had a greater proportion of DHA in their erythrocytes and brain cortex relative to those fed formula ($P < 0.005$) but differences were not observed in retina. Cortex DHA increased in breast-fed (but not formula-fed) infants with age ($r^2 = 0.72$, $P < 0.01$, $n = 15$), largely an effect of length of feeding ($r^2 = 0.62$, $P < 0.01$, $n = 35$).

There was an association between age at death and erythrocyte DHA with cortex DHA ($r^2 = 0.50$, $P < 0.01$).

Makrides et al, Am J Clin Nutr 1994;60:189

Rilievi autoptici sulla composizione del cervello

Long-chain fatty acids are analyzed in tissues from infants whose cause of death was not neurologically related.

Total **n-3** and **n-6** polyunsaturated and **n-9** monounsaturated fatty acid amounts increased in the whole forebrain during the prenatal and postnatal periods up to at least 2 years of age.

The most abundant brain polyunsaturated fatty acids were docosahexaenoic acid (DHA) (22:6n-3), arachidonic acid (20:4n-6), and adrenic acid (22:4n-6).

Martinez M, J Pediatr 1992, 120: S129

LCPUFA : Structural correlates from experimental models (mostly cultured cells) of neural functional effects in infancy -1

- altered membrane fluidity, volume and packing
- changed lipid phase properties
- modified membrane lipid-protein interactions within specific microdomains

TARGET:
cell membrane composition and properties

LCPUFA : Structural correlates from experimental models (mostly cultured cells) of neural functional effects in infancy - 2

- → changed physical properties and membrane excitability
- → modified membrane proteins' ability to bind ligands and activate enzymes
- → altered receptor activity, antigenic recognition, signal transduction
- → modified electrical properties of membranes

EFFECTS: on membrane activities

LCPUFA : Structural correlates from experimental models (mostly cultured cells) of neural functional effects in infancy - 3

- → development of synaptic processes (ARA)
- → modulation of neurotransmitter uptake and release (DHA)
- → direct effect on the expression of genes regulating cell differentiation and growth

EFFECTS: on cell to cell signalling and gene expression

LCPUFA : Structural correlates from experimental models (mostly cultured cells) of neural functional effects in infancy - 4

- → growth stimulation on retinal neurons, higher rhodopsin concentrations (DHA)
- → overexpression of retinal genes (DHA)
 - overexpression of ion channels involved in retinal synaptogenesis (DHA)
- → overall contribution to the development and maturation of retina (other brain regions?)

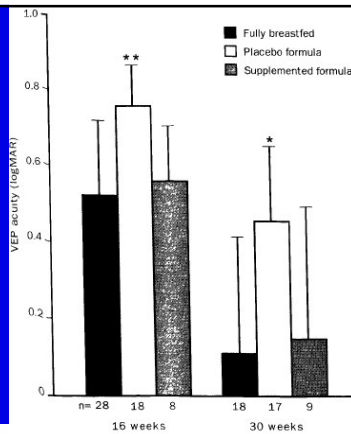
THE CLEAREST EFFECTS: on retinal function

Are long-chain polyunsaturated fatty acids essential nutrients in infancy?

Lancet 1995; **345**: 1463–68

Maria Makrides, Mark Neumann, Karen Simmer, John Pater, Robert Gibson

Fatty acid	Breastmilk (n=23)*	Placebo formula (n=14)†	Supplemented formula (n=12)‡
Saturated	45.33 (3.33)	50.60 (0.32)	48.99 (0.22)
Monosaturated	38.11 (2.66)	30.97 (0.27)	30.75 (0.17)
18:2 ω 6	13.92 (3.02)	16.79 (0.08)	17.44 (0.16)
18:3 ω 6	0.16 (0.04)	0.05 (0.02)	0.27 (0.03)
20:4 ω 6	0.40 (0.07)	ND	0.01 (0.01)
22:4 ω 6	0.07 (0.02)	ND	ND
22:5 ω 6	0.03 (0.01)	ND	ND
Total ω 6	15.13 (3.12)	16.85 (0.09)	17.73 (0.16)
18:3 ω 3	0.94 (0.25)	1.58 (0.01)	1.52 (0.02)
20:5 ω 3	0.07 (0.04)	ND	0.58 (0.04)
22:5 ω 3	0.16 (0.04)	ND	0.07 (0.01)
22:6 ω 3	0.21 (0.13)	ND	0.36 (0.03)
Total ω 3	1.43 (0.35)	1.58 (0.01)	2.53 (0.07)



VEP acuity and erythrocyte fatty acids

Erythrocyte DHA correlated with VEP acuity at 16 and 30 weeks ($r^2=0.23$, $p<0.001$; $r^2=0.12$, $p<0.005$). These correlations persisted when only data from randomised (formula-fed) infants were analysed ($r^2=0.37$, $p<0.001$; $r^2=0.17$, $p<0.05$ at 16 and 30 weeks, respectively). No other fatty acid consistently correlated with VEP acuity.

Docosahexaenoic acid status and developmental quotient of healthy term infants

THE LANCET

*Carlo Agostoni, Enrica Riva, Sabina Trojan, Roberto Bellù, Vol 346 • September 2, 1995
Marcello Giovannini

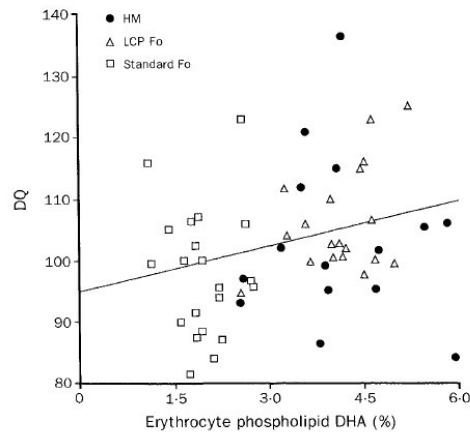


Figure: Relation between DHA in erythrocyte phospholipids and DQ at age 4 months

LCPUFA : Structural correlates from experimental models (mostly cultured cells) of other functional effects in infancy - 5

- → modification of eicosanoid function
- → decreased inflammatory processes and platelet aggregation
- → influence on arterial wall compliance and blood pressure

OTHER FUNCTIONAL EFFECTS:
widespread through the organism
(in particular: cardiovascular system)

LCPUFA nelle malattie metaboliche

- Patofisiologia: fibrosi cistica, malattie perossisomiali
- Bassi livelli: PKU, varie malattie degli aminoacidi (indotti da dietoterapia), fibrosi cistica (dieta ed associati alla malattia), malattie perossisomiali (associati alla malattia)
- Nella terapia: malattie perossisomiali, PKU (neuroprotezione), glicogenosi (prevenzione cardiovascolare)

In particolare: bassi livelli di DHA



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Molecular Genetics and Metabolism 88 (2006) 159–165

Molecular Genetics
and Metabolism

www.elsevier.com/locate/ymgme

Essential polyunsaturated fatty acids in plasma and erythrocytes
of children with inborn errors of amino acid metabolism

H. Vlaardingerbroek^a, G. Hornstra^b, T.J. de Koning^c, J.A.M. Smeitink^d, H.D. Bakker^e,
H.B.C. de Klerk^f, M.E. Rubio-Gozalbo^{g,*}

DHA in plasma phospholipids (% of total FA)

INTAKES: traces of ARA and DHA in all patients

Subgroups of amino acid disorders					
	Urea cycle disorders (n = 10) ^c	Branched-chain organic acidurias (n = 7) ^d	Disorders of tyrosine metabolism (n = 5) ^e	Disorders of sulphur aa metabolism (n = 2) ^f	Other (n = 4) ^g
ARA	9.01 ± 1.23	9.91 ± 1.13	10.36 ± 1.45	8.14 ± 0.19	6.56 ± 1.04
DHA	1.78 ± 0.03	2.12 ± 0.22	2.42 ± 0.20	2.01 ± 0.06	1.66 ± 0.44

Fatty acids ^a	Controls (n = 37)			P
		→ 20:4n-6	8.46 ± 0.33	0.61
		22:6n-3	2.45 ± 0.14	0.03

LCP e PKU

- Latte materno e PKU
- LCP nei lattanti PKU
- LCP nei bambini PK

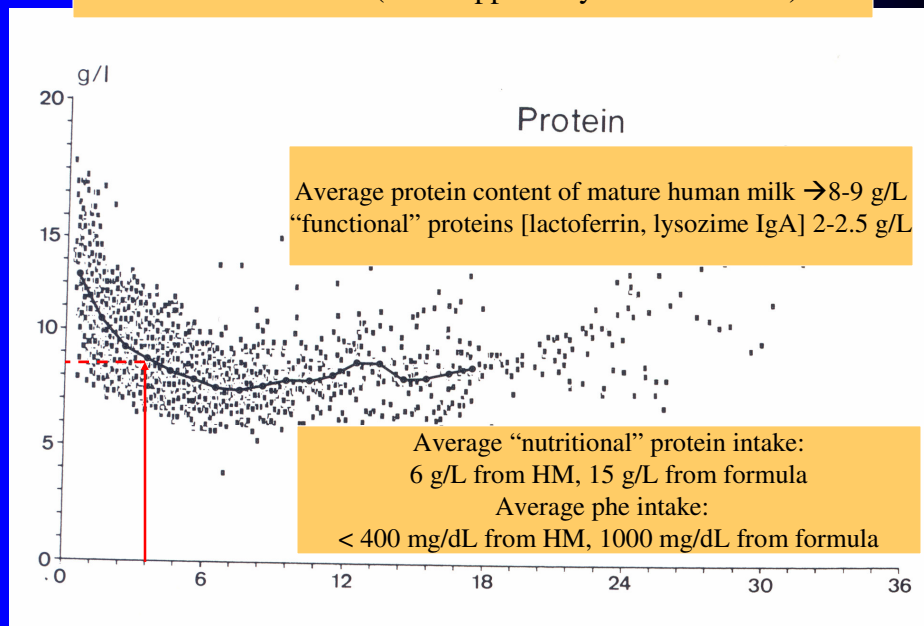
Breastfeeding in PKU

- 26 children affected by PKU at 9 years - 13 breastfed and 13 bottle-fed before diagnosis (26 days on average)
- 14 points IQ (WISC-R) advantage for those who had been breastfed, 12.9 after adjusting for major confounders (social class and maternal education)
- Plasma phenylalanine levels comparable through the years between the two groups (lower, $P = 0.09$, at diagnosis in those who were breastfed)

Riva E et al, *Acta Paediatr* 1996;85:56

SPECULATION: a role for the low Phe and/or the LCPUFA content of human milk?

Protein content of human milk in a large sample of Danish donors (data supplied by K. Michaelsen)



Polyunsaturated fatty acid concentrations in human hindmilk are stable throughout 12-months of lactation and provide a sustained intake to the infant during exclusive breastfeeding: an Italian study

Franca Marangoni^{1*}, Carlo Agostoni², Anna M. Lammardo², Marcello Giovannini², Claudio Galli¹ and Enrica Riva²

ARA % at colostrum 0.5-0.6% 1 to 12 ms

DHA 0.5% at colostrum 0.25-0.35% 1 to 12 ms

Breastfeeding rates among hyperphenylalaninemic infants

ACTA PÆDIATR 89 (2000)

Correspondence section 367

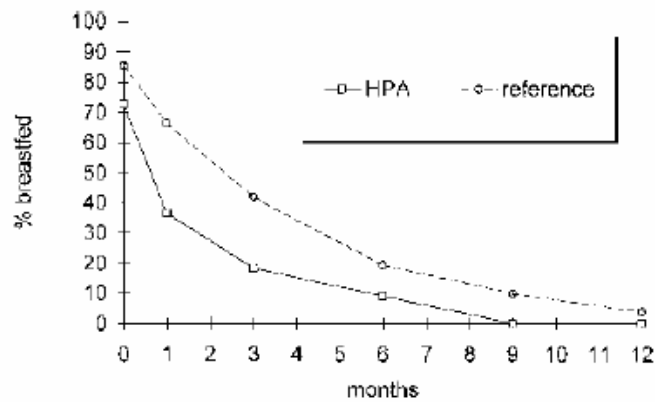


Fig. 1. Rates of any type of breastfeeding.

Low breastfeeding rates in PKU!

LCP e PKU

- Latte materno e PKU
- LCP nei lattanti PKU
- LCP nei bambini PK

Plasma long-chain polyunsaturated fatty acids and neurodevelopment through the first 12 months of life in phenylketonuria

Carlo Agostoni* MD;
Elvira Verduci MD, Department of Paediatrics;
Nicoletta Massetto MD, Department of Neurology;
Giovanni Radaelli PhD, Unit of Medical Statistics;
Enrica Riva MD;
Marcello Giovannini MD, Department of Paediatrics, San
Paolo Hospital, University of Milan, Milan, Italy.

Developmental Medicine & Child Neurology 2003, 45: 257–261

Table III: Plasma phenylalanine [$\mu\text{mol/L}$; mean (SD)] through first 12 months of life, in breastfed and bottlefed infants

<i>Plasma phenylalanine^a</i>	<i>All infants (n=20)</i>	<i>Breastfed (n=12)</i>	<i>Bottlefed (n=8)</i>	<i>p^b</i>
At diagnosis	1077 (470)	1398 (444)	1260 (588)	0.79
At 5 months	471 (376)	558 (416)	340 (280)	0.18
At 12 months	346 (165)	285 (151)	437 (168)	0.04 ^c
Average baseline, 5 months	150 (74)	164 (82)	128 (57)	0.42
Average baseline, 12 months	265 (68)	264 (73)	267 (64)	0.73

^aSignificance of within-group phenylalanine variation (ANOVA for repeated measures): all infants, $p=0.002$; breastfed, $p=0.005$; bottlefed, $p=0.07$.

^bBreastfed versus bottlefed (Mann–Whitney U test).

^cStatistically significant at $p<0.05$.

No differences of Phe concentrations between BF and FF at 18 days (lower protein content in human milk, higher intakes?)

Table II: Plasma lipids and essential fatty acids [percentage of total lipids; mean (SD)] at diagnosis, in breastfed and bottlefed infants

<i>Lipid parameter</i>	<i>All infants (n=20)</i>	<i>Breastfed (n=12)</i>	<i>Bottlefed (n=8)</i>	<i>p^a</i>
Saturated lipids	36 (5)	36 (2)	37 (7)	0.85
Monounsaturated lipids	28 (4)	28 (4)	28 (4)	0.91
Polyunsaturated lipids	36 (4)	36 (3)	35 (5)	0.52
Linoleic acid	22.3 (3.9)	21.0 (3.2)	24.2 (4.3)	0.08
α -Linolenic acid	0.4 (0.2)	0.3 (0.2)	0.5 (0.2)	0.04 ^b
Arachidonic acid	7.7 (2.8)	9.0 (2.6)	5.6 (1.4)	0.007 ^b
Eicosapentaenoic acid	0.2 (0.07)	0.2 (0.07)	0.2 (0.08)	0.30
Docosahexaenoic acid	2.0 (0.7)	2.3 (0.8)	1.6 (0.4)	0.03 ^b

^aBreastfed versus bottlefed (Mann–Whitney U test).

^bStatistically significant at $p<0.05$.

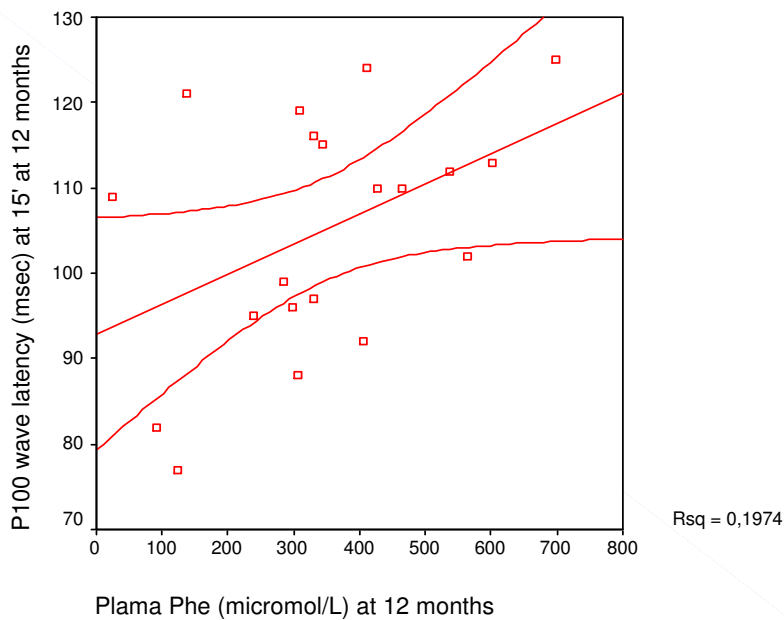
Clear differences of LCP concentrations between BF and FF already evident at 18 days!

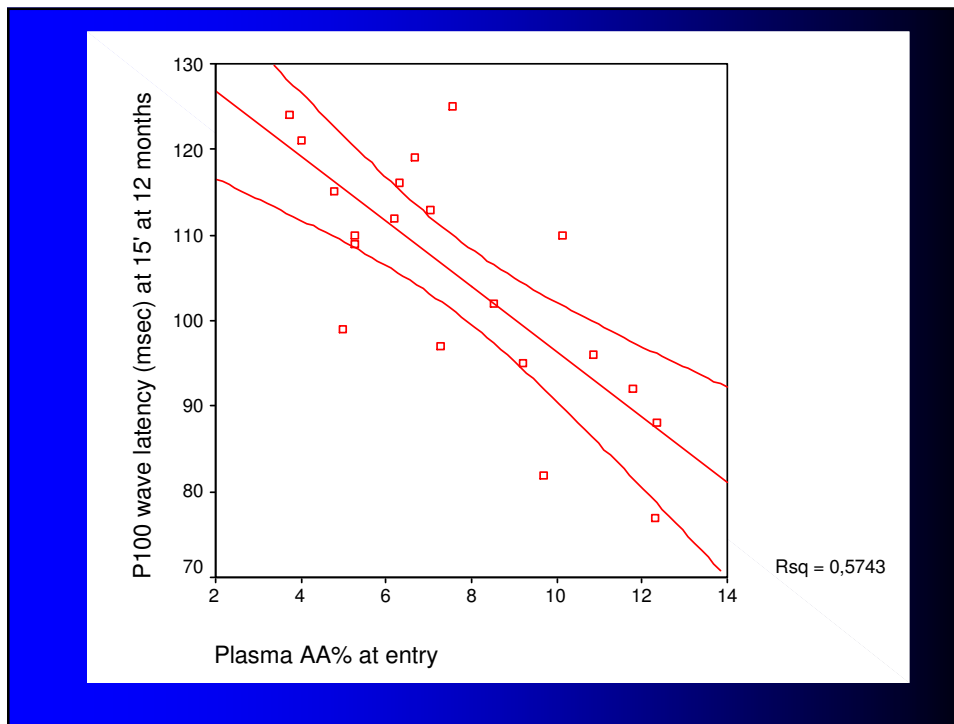
Table IV: Developmental outcomes [Bayley indices and P100 wave latencies; mean (SD)] through first 12 months of life, in breastfed and bottlefed infants

Developmental outcome ^a	All infants (n=20)	Breastfed (n=12)	Bottlefed (n=8)	p ^b
At 5 months				
MDI	90 (7)	91 (5)	88 (9)	0.13
PDI	90 (11)	94 (10)	84 (11)	0.04 ^c
At 12 months				
MDI	82 (11)	85 (10)	78 (11)	0.52
PDI	91 (16)	94 (16)	85 (16)	0.47
P100 wave latency (ms)				
At 15 minutes of arc	98 (8)	96 (11)	117 (6)	<0.001 ^c
At 60 minutes of arc	99 (8)	99 (10)	98 (7)	0.97

MDI, Mental Development Index; PDI, Psychomotor Developmental Index (Bayley 1993).

- Better developmental performance
- Faster recovery of VEPs (shorter P100 wave latencies) for BF at 5 and 12 months, respectively





A randomized trial of long-chain polyunsaturated fatty acid supplementation in infants with phenylketonuria

Carlo Agostoni* MD, Department of Pediatrics, University of Milan Medical School, Milan, Italy.
 Ann Harvie MB ChB, Study Co-ordinator, formerly Paediatric Registrar, Department of Child Health, Royal Hospital for Sick Children;
 Daphne L McCulloch OD PhD, Co-ordinator of vision development aspects of study, Department of Vision Sciences, Glasgow Caledonian University, Glasgow;
 Colin Demellweek PhD, Co-ordinator of mental and physical development aspects of study, Roald Dahl EEG unit, Alder Hey Children's Hospital, Liverpool;
 Forrester Cockburn MD, Formerly Consultant Paediatrician at Department of Child Health, Royal Hospital for Sick Children, Glasgow, UK.
 Marcello Giovannini MD, Department of Pediatrics, University of Milan Medical School, Milan, Italy.
 Gordon Murray PhD, Statistician, Public Health Sciences;
 R Angus Harkness PhD, Biochemist, Department of Child Life and Health, University of Edinburgh, Edinburgh, UK.
 Enrica Riva MD, Department of Pediatrics, University of Milan Medical School, Milan, Italy; on behalf of the European PKU LC-PUFA Supplementation Trial Group.

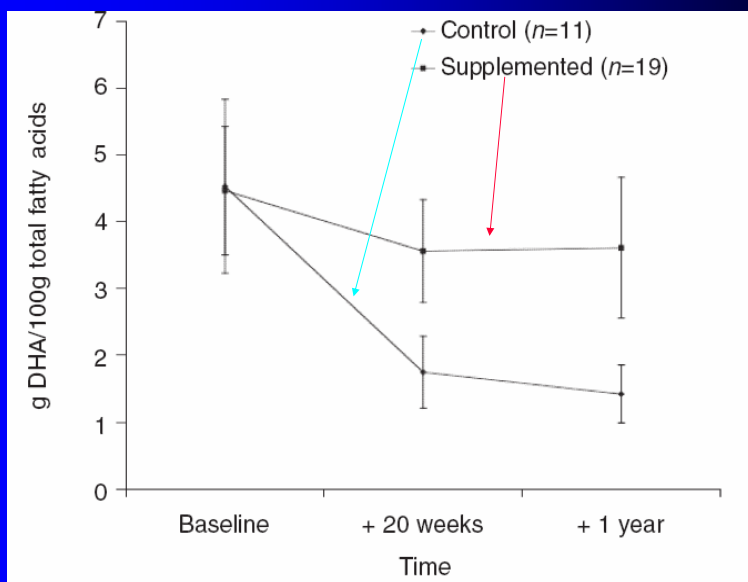
Developmental Medicine & Child Neurology 2006, 48: 207–212

RCT on formula-fed PKU infants

Table I: Characteristics of study population at entry

<i>Characteristic</i>	<i>Control group</i>	<i>Supplemented group</i>	<i>t-test (p)</i>
Number (M/F)	21 (12/9)	21 (8/13)	–
Number of infants			
Italy (M/F)	9 (7/2)	8 (2/6)	–
UK (M/F)	7 (3/4)	9 (4/5)	–
Spain (M/F)	3 (2/1)	2 (1/1)	–
France (M/F)	2 (0/2)	2 (1/1)	–
Birthweight in kg, mean (SD); range	3.32 (0.28); 2.90–3.93	3.35 (0.43); 2.61–3.87	0.80
Age at entry in days, mean (SD); range	20 (6.9); 8–33	18 (5.9); 10–39	0.54
Weight at entry in kg, mean (SD); range	3.70 (0.44); 3.0–4.67	3.60 (0.49); 2.68–4.5	0.50

M, male; F, female.



DHA in erythrocyte phospholipids

No between-group differences of P100 latencies with flash-VEPs and pattern-reversal VEPs at baseline, 12 ws, 20-26 ws and 52 ws BUT:

DHA levels as percentage Pattern VEP^b P100, mean (SD)

Low, <2	1.7 (2.5)
Medium, 2–3.5	0.84 (2.7)
Medium-high, 3.5–4.5	1.1 (3.5)
High, >4.5	-1.8 (5.0)

Higher DHA levels (irrespective of diet) were associated with more mature VEPs as demonstrated by lower z-scores (P = 0.02)

Possibili spiegazioni:

- Differente patrimonio di DHA alla nascita (→ dieta materna, status materno relativo a DHA)
- Differenza interindividuale della capacità di sintesi di DHA

Human Molecular Genetics, 2006, Vol. 15, No. 11 1745–1756
doi:10.1093/hmg/ddl117
Advanced Access Published on May 2, 2006

Common genetic variants of the *FADS1 FADS2* gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids

Linda Schaeffer¹, Henning Gohlke¹, Martina Müller^{1,2}, Iris M. Heid^{1,2}, Lyle J. Palmer³, Iris Kompauer¹, Hans Demmelmair², Thomas Illig¹, Berthold Koletzko⁴ and Joachim Heinrich^{1,*}

LCP e PKU

- Latte materno e PKU
- LCP nei lattanti PKU
- LCP nei bambini PKU

Juan P. Infante¹ and Virginia A. Huszagh

Institute for Theoretical Biochemistry and Molecular Biology, P.O. Box 4512, Ithaca, New York 14852

Molecular Genetics and Metabolism 72, 185–198 (2001)

Decreased Phe hydroxylase in PKU



Increased phenylpyruvate and phenyllactate concentrations



Inhibition of 4-hydroxyphenylpyruvate dioxygenase



Decreased homogentisate synthesis



Decreased α -tocopherolquinone synthesis



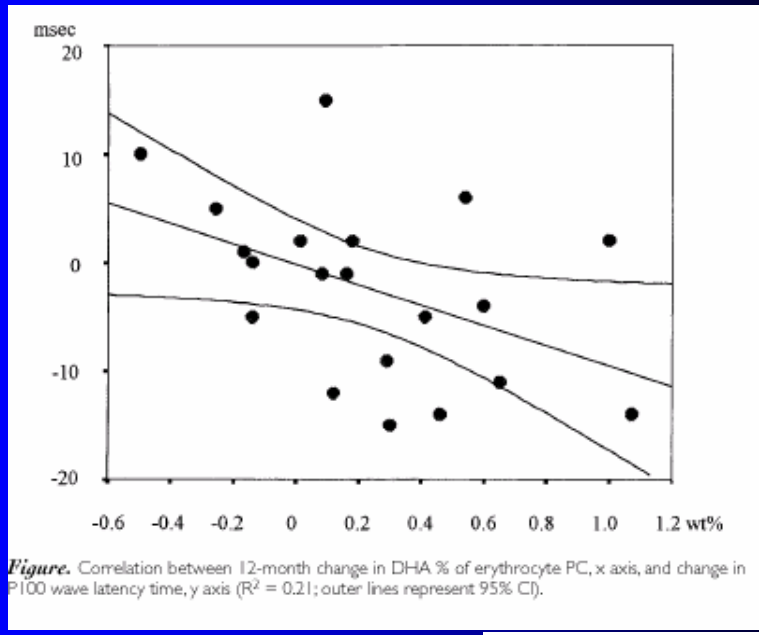
Impaired mitochondrial desaturases



Decreased 20:4n-6 and 22:6n-3 synthesis



Impaired brain growth and development



(J Pediatr 2000;137:504-9)

SHORT REPORT

Long term effects of long chain polyunsaturated fats in hyperphenylalaninemic children

C Agostoni, E Verduci, N Massetto, L Fiori, G Radaelli, E Riva, M Giovannini

Arch Dis Child 2003;88:582-583

Blood fatty acid status and visual function of 20 treated hyperphenylalaninemic (HPA) children, randomly allocated into two groups to receive supplementation of either long chain polyunsaturated fatty acids (LCPUFA), including docosahexaenoic acid (DHA), or placebo for 12 months, have been investigated three years after the end of the treatment. Although in the LCPUFA group blood DHA levels and P100 wave latency improved at the end of supplementation, they had returned to baseline after three years.

life-long LCPUFA dietary supplementation?

LCP e PKU

1. Dietoterapia associata a basso/nullo apporto di LCP e bassi livelli di DHA circolanti
2. L'allattamento al seno nelle prime epoche di vita (con apporto di LCP/DHA) sembra connesso ad outcome più favorevoli nella PKU
3. Lo "status" relativo al DHA è connesso a parametri neurofunzionali più favorevoli nei lattanti (non solo nella PKU)
4. Nei bambini con PKU l'apporto di LCP/DHA sembra connesso a un miglioramento dei parametri neurofunzionali nel corso della supplementazione
5. **Il ruolo degli LCP e della PKU materna va ancora studiato!**

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DI NUTRIZIONE PEDIATRICA (SINUPE)

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SOCIETÀ ITALIANA DI PEDIATRIA

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ASSOCIAZIONE ITALIANA RETT (AIR)
DOCTOR PEDIATRIA

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- MALATTIE CRONICHE E NUTRIZIONE
- OBESITÀ
- PROBIOTICI E SALUTE
- INTEGRATORI E NUTRIZIONE
- SICUREZZA ALIMENTARE
- ALLERGIE ED INTOLLERANZE ALIMENTARI
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- IL PEDIATRA NEI PAESI IN VIA DI SVILUPPO

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