25th ESPEN Meeting
21-24 Sept. 2003, Cannes

Insights from tracer studies in metabolic research

Perinatal fatty acid metabolism

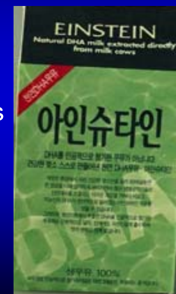
Berthold Koletzko, Hans Demmelmair, Elvira Larqué

Dr. von Hauner Children's Hospital
Ludwig-Maximilians-University of Munich
München, Germany

Importance of LC-PUFA metabolism in the perinatal period

LC-PUFA:

- High accretion in membranes (e.g. brain, synapses, photoreceptors)
- Influence on membrane properties
- Precursors for eicosanoids
- Modulate immune phenotypes
- AA enhances infant growth
- DHA enhances visual, cognitive & motor development



LC-PUFA metabolism in the perinatal period: burning questions

- Characterization of human placental transfer
- Transfer into human milk, contribution of maternal diet, endogenous synthesis, and stores
- Infant endogenous synthesis and turnover, effects of diet

Stable Isotope Tracer Studies

- Application of stable isotope labelled analogues of natural substrates

Natural ^{13}C-content: ≈ 1.11 %
Technically 99 % ^{13}C-content achievable,

- Subsequent determination of tracer content in selected compartments
- Mathematical evaluation of the results
  → Turnover, flux rates, fractional transfer rates
- Quantitative results
  → Physiological interpretation

Analytical Principle

plasma
tissue
milk
protein
amino acid
breath-CO_2

metabolite

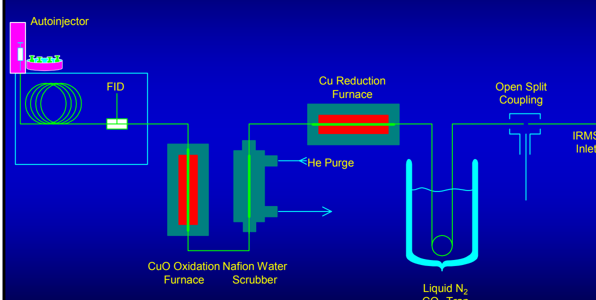
sample preparation: extraction, hydrolysis, precipitation, derivatisation

component isolation: chromatography

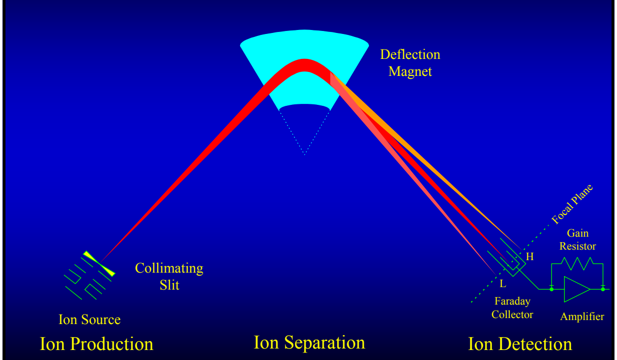
spectrometry: GC-MS, IRMS, IR, LC-MS

Isotope or Isotopomer Ratio

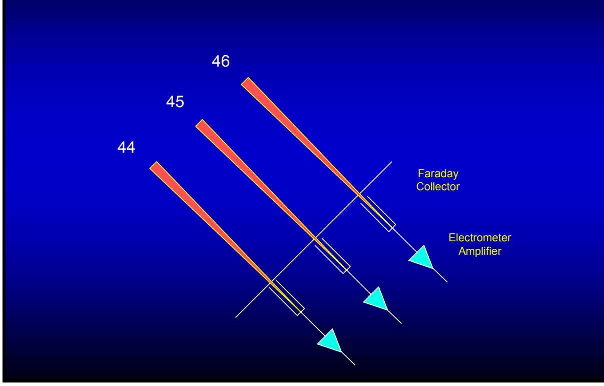
GC-C-IRMS System Diagram



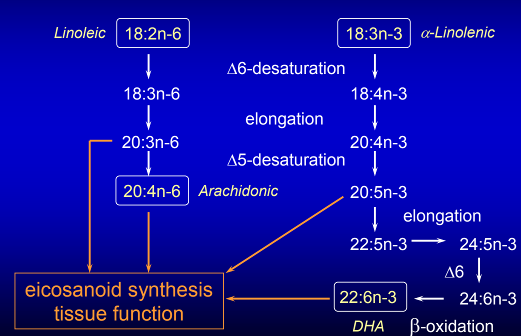
Multi-collector Magnetic Isotope Ratio Mass Spectrometer



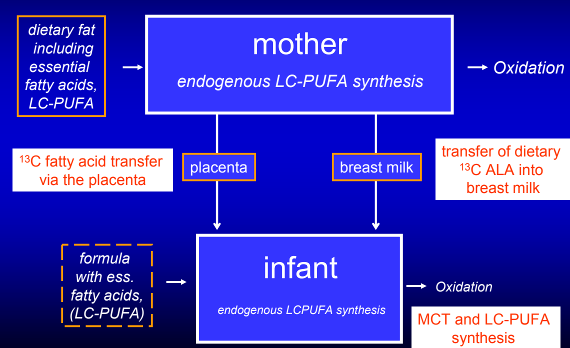
Projection of CO_2 Ions on a Multicollector Array



Synthesis of LC-PUFA from essential fatty acids

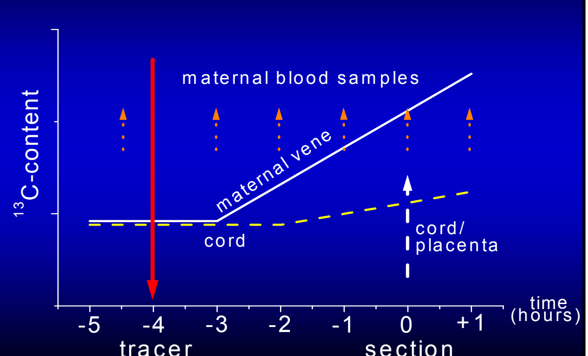


Perinatal fatty acid metabolism



Human placental ^{13}C fatty acid transfer *in vivo*

Larqué et al, J Lipid Res 2003;44:49-55



^{13}C fatty acid transfer across the human placenta *in vivo*

Larqué et al, J Lipid Res 2003;44:49-55

Study details

- four pregnant women, elective caesarian section
- gestation: 39±0.6 w
- weight: 83±20 kg
- age: 34±2 years

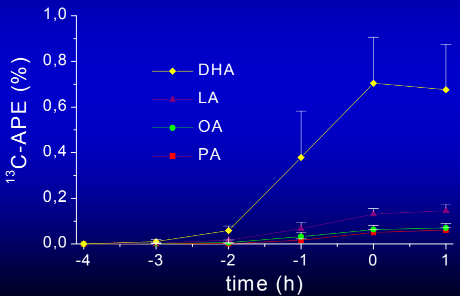
oral application of stable isotope tracer:

- palmitic acid: 0.5 mg/kg
- oleic acid: 0.5 mg/kg
- linoleic acid: 0.5 mg/kg
- docosahexaenoic acid: 0.1 mg/kg

^{13}C fatty acid transfer across the human placenta *in vivo*

Larqué et al, J Lipid Res 2003;44:49-55

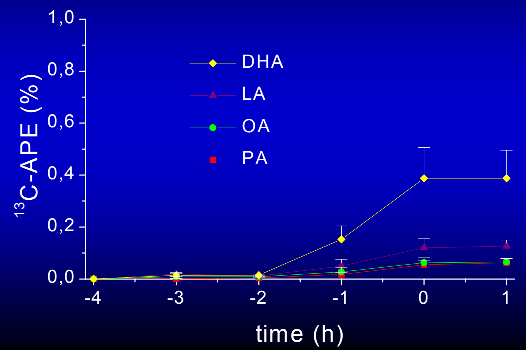
^{13}C-enrichment in maternal triglycerides (M+SE)



^{13}C fatty acid transfer across the human placenta *in vivo*

Larqué et al, J Lipid Res 2003;44:49-55

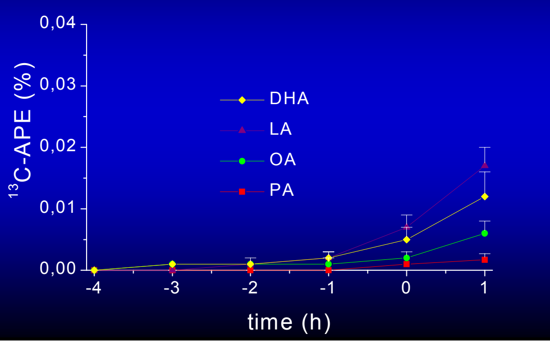
^{13}C-enrichment in maternal NEFA (M+SE)



^{13}C fatty acid transfer across the human placenta *in vivo*

Larqué et al, J Lipid Res 2003;44:49-55

^{13}C-enrichmen in maternal phospholipids (M+SE)



^{13}C fatty acid transfer across the human placenta *in vivo*

Larqué et al, J Lipid Res 2003;44:49-55

Estimation of tracer fatty acids transferred from the mother to the neonate (M±SE)

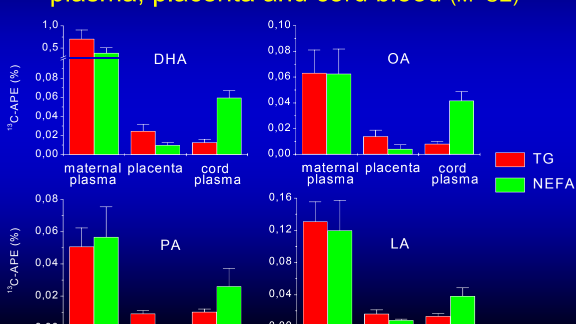
% of µmol/l tracer cord plasma/AUC maternal plasma

	PA	OA	LA	DHA
PL	1.63 ± 1.45	0.36 ± 0.43	0.38 ± 0.34	1.17 ± 0.63
TG	1.92 ± 1.30	0.81 ± 0.46	0.70 ± 0.49	0.70 ± 0.53
NEFA	3.03 ± 2.54	1.97 ± 1.48	1.13 ± 0.90	1.14 ± 0.89
Total	6.59 ± 4.94	3.14 ± 2.08	2.22 ± 1.83	3.01 ± 1.59

^{13}C fatty acid transfer across the human placenta *in vivo*

Larqué et al, J Lipid Res 2003;44:49-55

^{13}C-enrichment at time of delivery in maternal plasma, placenta and cord blood (M+SE)

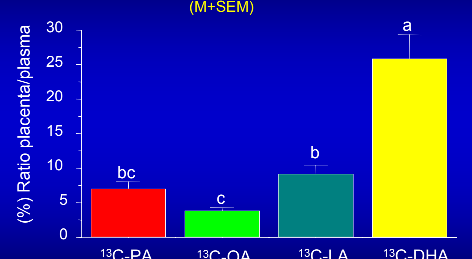


^{13}C fatty acid transfer across the human placenta *in vivo*

Larqué et al, J Lipid Res 2003;44:49-55

Tracer distribution between plasma and placenta

(M+SEM)



$$\text{(\%) Ratio Placenta/plasma} = \frac{\text{fatty acid concentration in placenta (µmol/g) * APE-placenta}}{\text{fatty acid concentration in plasma (µmol/ml) * APE-plasma}} \times 100$$

Conclusions

- Trend (n.s.) to preferential DHA incorporation into infantile PL
- Higher ^{13}C-enrichment in cord blood NEFA than in placental lipids
  → different placental NEFA compartments
- Preferential DHA incorporation into placental tissue, relative to LA, OA and PA, at the studied time point

Transfer of dietary ^{13}C essential fatty acids into breast milk

- PUFA transfer from diet into milk
- PUFA conversion to milk LC-PUFA

Demmelmair et al, J Lipid Res1998;39:1389-96
Del Prado et al, Am J Clin Nutr 2001;74:242-7
Fidler et al, J Lipid Res 2000;41:1376-83
Kuhn et al, submitted

calculation of fatty acid transfer into milk [%-dose]:

$$\frac{\text{volume} * \text{fat content} * \text{fatty acid-\%} * {}^{13}\text{C-excess}}{\text{dose of } {}^{13}\text{C-ALA tracer}}$$

Linoleic Acid Transfer into Human Milk

J Lipid Res. 1998;39:1389-96

6 breastfeeding mothers each studied at 2, 6 and 12 weeks of lactation, dietary protocols

↓

Oral tracer dose of uniformly ^{13}C-labeled linoleic acid (1mg/kg)

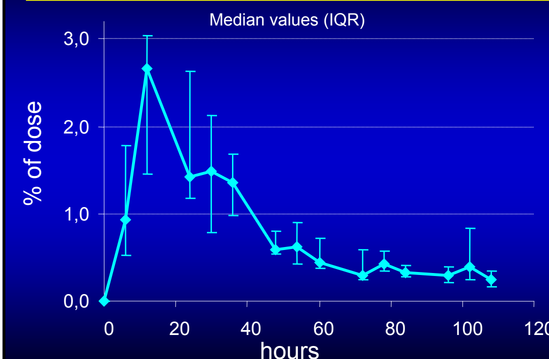
↓

Milk collection and breath samples at defined time points before and after the tracer application over a period of 5 days

↓

Fatty acid composition in human milk measured by GC, isotopic enrichments of fatty acids + breath CO_2 measured by GC-IRMS

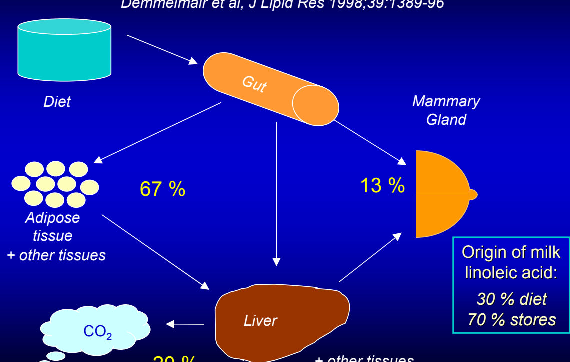
Linoleic Acid Transfer from Diet to Milk



J Lipid Res. 1998;39:1389-96

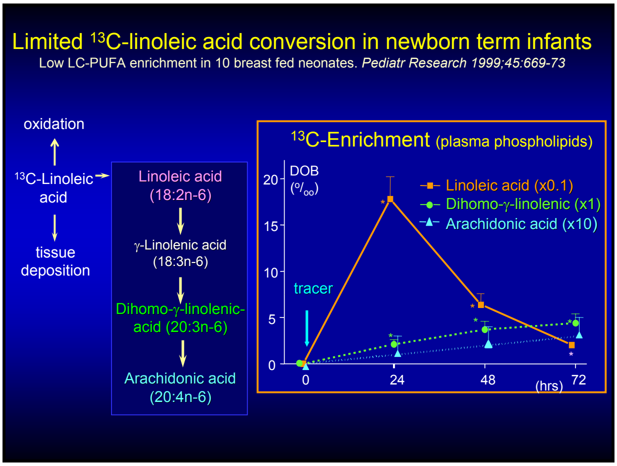
Linoleic Acid Flux during Lactation

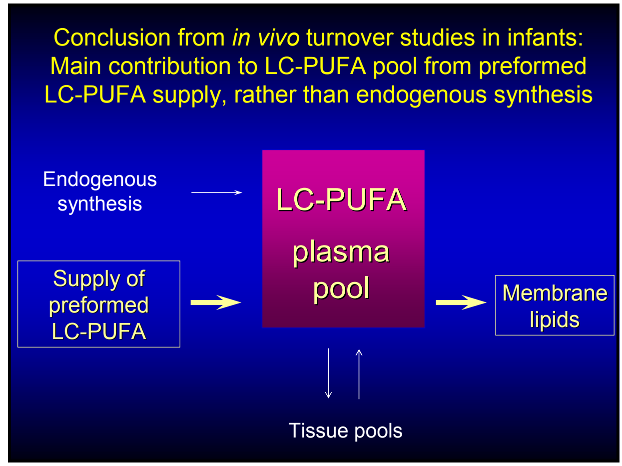
Demmelmair et al, J Lipid Res 1998;39:1389-96



Conclusions

- Metabolic disposal differs markedly between ALA and LA:
- The major part of milk linoleic acid is derived from maternal stores
- The major part of milk α-linolenic acid is directly derived from maternal diet
- Immediate dietary precursor intake is of marginal importance for synthesis of milk LC-PUFA





MCT and LC-PUFA synthesis

- medium chain triglycerides (MCT) are very well absorbed
- MCT are rapidly oxidised
- MCT improve absorption of minerals

→ most preterm infant formulae contain MCT

? Influence of MCT on PUFA status and metabolism ?

Conversion of ^{13}C-linoleic acid (LA) to ^{13}C-arachidonic acid (AA)

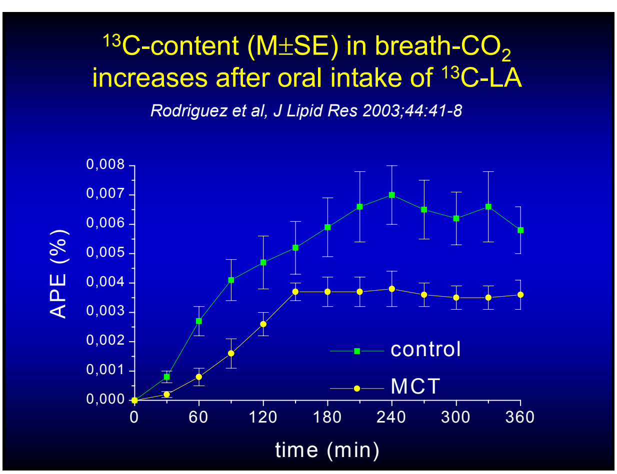
Study Design

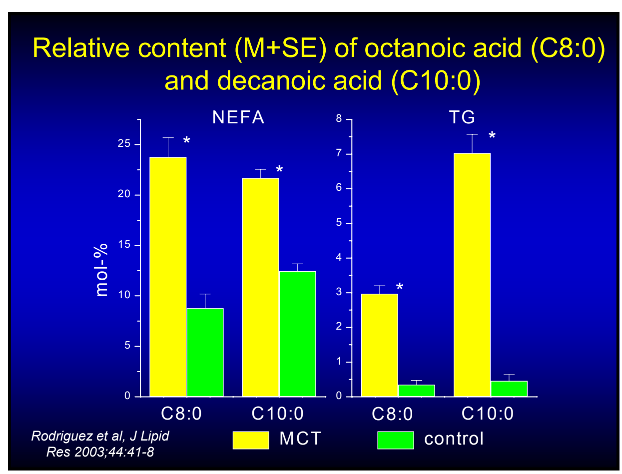
Rodriguez et al, J Lipid Res 2003;44:41-8

diets: MCT = 40 % of lipids MCT
control = lipids without MCT
almost identical in essential fatty acid content

subjects: preterm infants
MCT (n=10): weight: 1,9±0,1 kg, gestation: 31±0,6 weeks, postnatal age: 35±5 d
control (n=9): weight: 1,8±0,1 kg, gestation: 30±0,8 weeks, postnatal age: 35±5 d

procedure: feeding of study diets for seven days
study day 5: oral intake of 2mg U^{13}C-LA/kg
after tracer intake: ^{13}C-breath test
study days 6 and 7: blood sampling
analysis of phospholipid fatty acids (^{13}C-content) and of triglyceride fatty acids





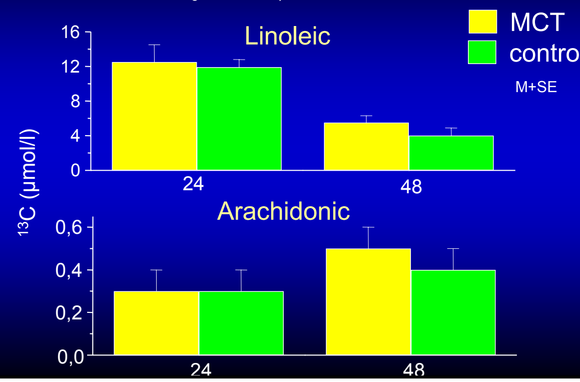
Relative content of essential fatty acids and their major derivatives in plasma lipids

	Controls	MCT
Phospholipids (mol-%)		
LA	19,75±0,57	19,58±1,08
AA	6,61±0,33	6,77±0,31
ALA	0,10±0,00	0,15±0,01
DHA	1,50±0,13	1,36±0,06
Triglycerides (mol-%)		
LA	12,41±0,13	13,28±1,06
AA	0,49±0,06	0,54±0,05
ALA	0,75±0,03	1,05±0,08*
DHA	0,12±0,02	0,13±0,01

(M±SE)

^{13}C-concentration in linoleic and arachidonic acids 4 and 48 h after tracer intake

Rodriguez et al, J Lipid Res 2003;44:41-8



Conclusions

- Higher MCT intake decreases LA oxidation
- Hardly any influence on PUFA concentrations, in spite of major difference in dietary intake
- No detectable influence of dietary MCT on the relative intensity of endogenous AA synthesis from LA
- MCT tend to improve PUFA availability, probably through reduced PUFA oxidation

Overall Summary

- Fatty acid transfer via the placenta
  - More than one NEFA pool in the placenta
  - Preferential uptake of DHA by the placenta
- Transfer of dietary fatty acids into breast milk
  - Most breast milk ALA directly transferred from the diet
  - Major source of milk n-3 LC-PUFA: body pools with low turnover
- Endogenous synthesis of LC-PUFA in preterm neonates
  - Relative LC-PUFA synthesis is not influenced by dietary MCT content
  - MCT reduce PUFA oxidation

European Commission sponsored Scientific Conference

Early Nutrition and its Later Consequences: New Opportunities

2-3 July 2004

Satellite Meeting to World Congress on Pediatric Gastroenterology & Nutrition, Paris

www.metabolic-programming.org

EU Project Number: QLK1-CT-2002-30582