



Essential Fatty Acids in Human Breastmilk

Center for Studies of Sensory Impairment, Ageing and Metabolism (CeSSIAM)

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Overview

(1) About me

(2) Essential Fatty Acids

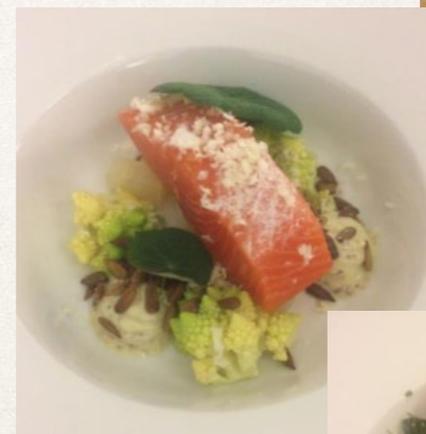
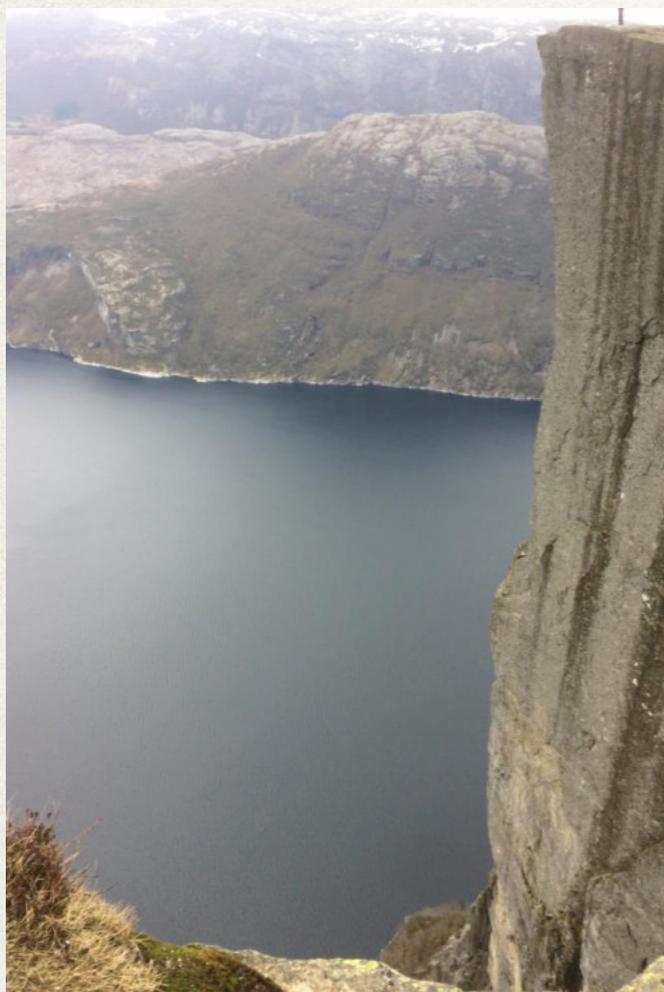
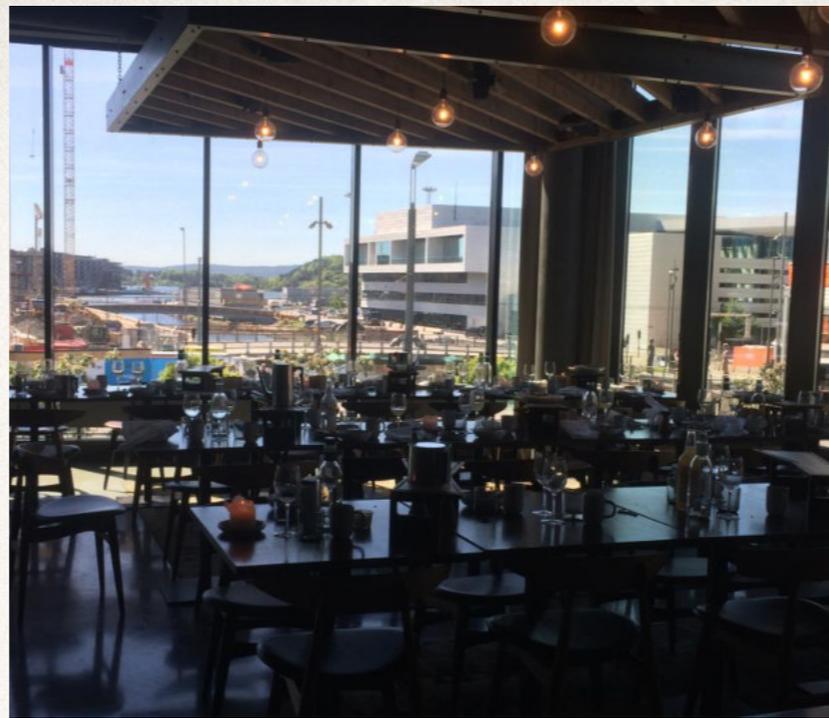
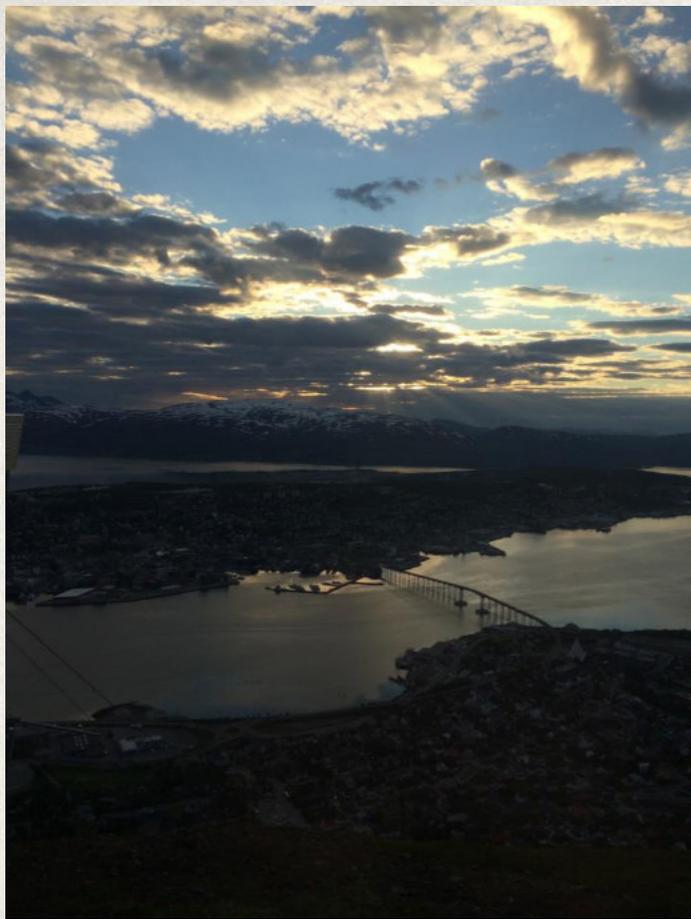
a. In Human Nutrition

b. In Infant Development

c. Concentration in Human Breastmilk

(3) Quetzaltenango Study

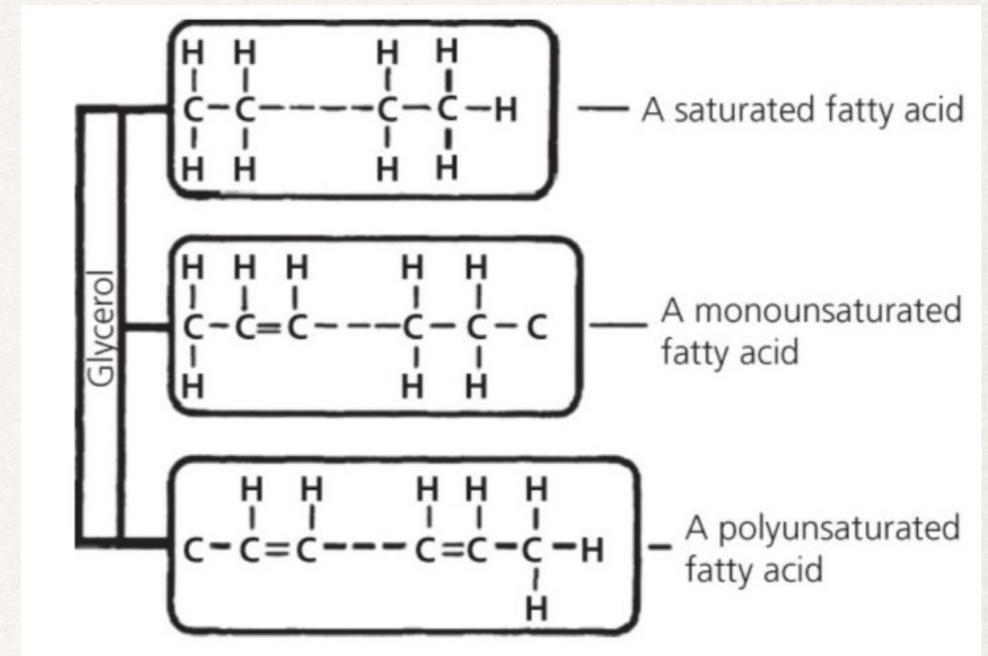
(4) My Work with CeSSIAM in 2017



Basics on Dietary Fats

❖ Structure

- ❖ glycerol + fatty acid
- ❖ 40 different fatty acids, consisting of carbon chain with hydrogen attached
 - ❖ different length
 - ❖ double bonds
 - ❖ cis or trans formation
- ❖ Saturation, number of double bonds
 - ❖ *saturated fatty acid (SFA)*
 - ❖ *monounsaturated fatty acid (MUFA)*
 - ❖ *polyunsaturated fatty acid (PUFA)*



Example of a triglyceride molecule made of glycerol and one SFA, one MUFA, and one PUFA. Source: Felicity Savage King "Nutrition for Developing Countries"

Essential Fatty Acids

- ❖ Omega-6 PUFA

- ❖ *linoleic acid (LA)*

- ❖ **Arachidonic acid (AA/ARA), 20:4 (n-6)**

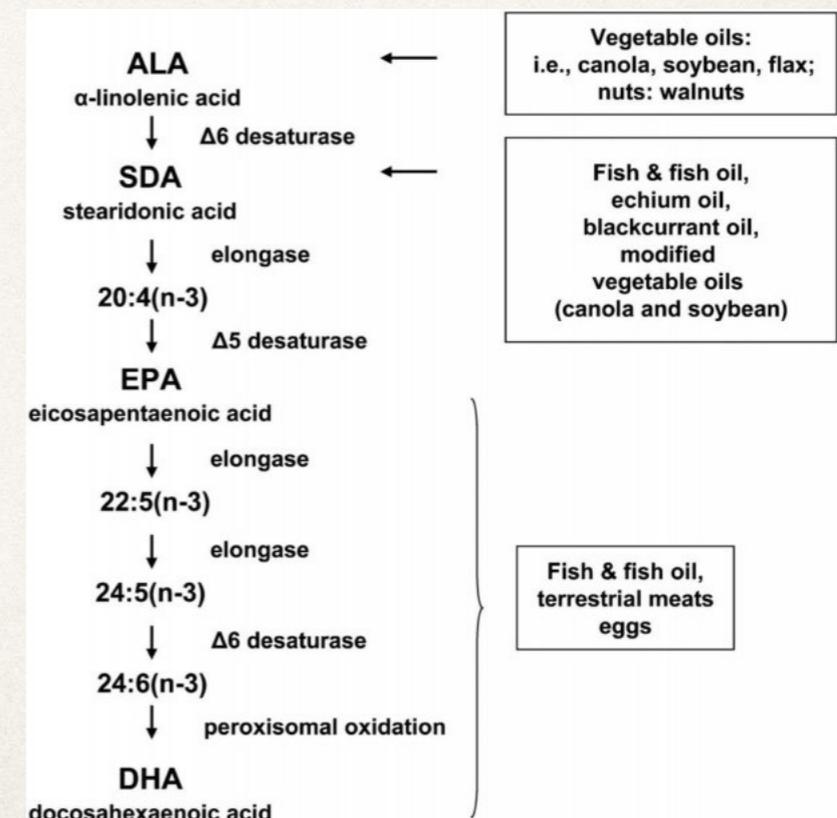
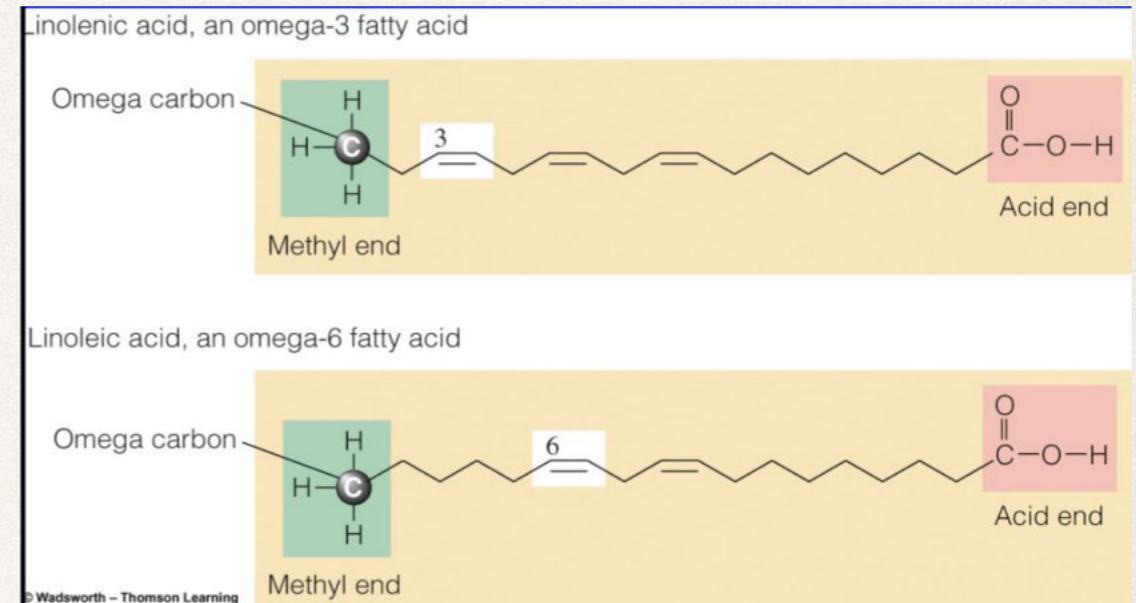
- ❖ Omega-3 PUFA

- ❖ *alpha-linolenic acid (ALA), 18:3 (n-3)*

- ❖ *eicosapentaenoic acid (EPA), 20:5 (n-3)*

- ❖ **docosahexaenoic acid (DHA), 22:6 (n-3)**

- ❖ synthesis of DHA and AA from precursor FA is limited but generally higher in women



Essential Fatty Acids in Nutrition

- ❖ ***Arachidonic acid (AA)***

- ❖ meats, poultry, eggs, bryophytes (mosses, ferns)
- ❖ precursor LA: vegetables oils (corn, sunflower, soybean), poultry fat, nuts, seeds



- ❖ ***docosahexaenoic acid (DHA)***

- ❖ human milk
- ❖ pacific oysters and fish: mackerel, salmon, sardines, trout,...
- ❖ precursor ALA: oils (flaxseed, canola, walnut), nuts and seeds, soybeans



FAs and Infant Development

- ❖ DHA accumulation in the brain 27 weeks of gestation until 18 years
- ❖ *n*-3 LC-PUFA
 - ❖ increased cognitive function at age 4
 - ❖ brain growth
 - ❖ improved immune function
 - ❖ breastmilk DHA and AA are associated with immune factors
 - ❖ improved vision



DHA and AA in Human Breastmilk

- ❖ Fat content: 3-4% by weight → 50-60% of calories
- ❖ main LC-PUFA: DHA and AA
- ❖ Brenna et al. 2007
 - ❖ Meta-analysis of 65 studies: LC-PUFA, DHA, AA in human breastmilk
 - ❖ capillary GC column analysis: full resolution of FA methyl esters
- ❖ Factors influencing PUFA and LC-PUFA concentration
 - ❖ diet (short-term and habitual)
 - ❖ nutritional status of the mother
 - ❖ parity, gestational age, smoking
 - ❖ lifestyle of the given population, *thus* region



Results and Conclusion

- ❖ lactation period 1-18 months

	DHA	AA
mean \pm SD % of total FA	0.32 \pm 0.22%	0.47 \pm 0.13%

- ❖ highest DHA concentrations 1.4-0.6%

- ❖ Canadian Arctic, Japan, Dominican Republic, Philippines, Congo

- ❖ lowest DHA concentrations 0.06-0.14%

- ❖ Pakistan, rural South Africa, Canada, the Netherlands, France

- 
- fish-eating coastal populations have higher DHA concentrations
 - AA in breastmilk is under tighter control and less predictable
 - linolenic acid supplementation **did not increase** breast milk DHA
 - dietary DHA intake of 200mg/d

Geographical Variation

❖ Urwin et al. 2013

❖ Objective:

- ❖ determine FA composition and immune factor concentration in river/lake, coastal and inland regions in China

❖ Methodology:

- ❖ colostrum, transition milk, mature milk
- ❖ Analysis: lipid composition, immune factors (CD14, TGF-beta1/2, sIgA), breast milk protein

Geographical Variation cont.

- ❖ PUFA in breast milk
 - ❖ river/lake region: highest total $n-3$ PUFA intake
 - ❖ coastal region: lowest total $n-3$ PUFA intake, but highest DHA
 - ❖ inland region: highest total $n-6$ PUFA intake
 - ❖ milk DHA positive predictor of all immune factors
-
- ➔
 - breast milk fatty acids and immune factors differ across region and time and depend on fish consumption patterns
 - DHA and AA concentration is associated with immune factor concentration

Quetzaltenango Study Overview

- ❖ Objective:
 - ❖ determine macro- and micronutrient composition in breastmilk at varying points in time
- ❖ lactating women at 40, 80, 120 and 180 days postpartum with a subset of n=10
- ❖ recruitment in health clinics in Quetzaltenango
- ❖ collection: breastmilk sample, BIA measurements and urine
- ❖ analysed by Dried Milk Spot Technique (DMS)

Sample descriptives

Table 1. Sample descriptives of participants
(n=40)

Mean age of mother y (SD)	
24.55 (5.08)	
Civil status n (%)	
single	5 (12.5)
married	35 (87.5)
Education n (%)	
primary	13 (32.5)
secondary	23 (57.5)
superior	4 (10)
Traditional clothing n (%)	
6 (15)	
Sex of the child n(%)	
female	18 (45)
male	22 (55)

Sample descriptives per subgroup

Table 2. Sample descriptives per subgroup (n=10, total n=40)

	40 d (n=10)	80 d (n=10)	120 d (n=10)	180 d (n=10)
Mean age mother in years (SD)	27.4 (5.5)	23.2 (5.8)	22.7 (3.7)	22.63 (4.3)
Mean age child in days (SD)	41 (2.9)	73 (4.2)	120 (7.7)	186 (4.5)
Feeding frequency, times per day (SD)	9.1 (5.1)	8.6 (4.2)	10.9 (2.7)	8.8 (2.2)
median	7,5	8.0	10.0	10.0
Receiving complementary foods (n)	2	1	2	7

Breastmilk content

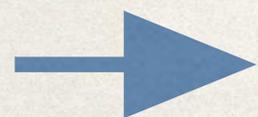
Table 3. Breastmilk Content (n=40)

Whey mm	
Mean (SD)	6.79 (0.59)
Range (min-max)	3.7-7.2
Cream mm	
Mean (SD)	0.35 (0.17)
Range (min-max)	0.1-0.7
% Cream of total	
Mean (SD)	5.19 (2.44)
Range (min-max)	1.39-10.77
% Fat of total	
Mean (SD)	3.15 (1.67)
Range (min-max)	0.55-6.97
kcal/l	
Mean (SD)	636.67 (162.78)
Range (min-max)	382.78-1009.38

Breastmilk content per subgroup

Table 4 Comparison of means of breastmilk contents per subgroups (n=40)

	40 d (n=10)	80 d (n=10)	120 d (n=10)	180 d (n=10)
Whey mm (SD)	6.82 (0.33)	7 (0.13)	6.62 (1.03)	6.72 (0.49)
Cream mm (SD)	0.39 (0.20)	0.36 (0.18)	0.38 (0.17)	0.28 (0.13)
% cream of total (SD)	5.71 (2.93)	5.16 (2.62)	5.72 (2.23)	4.17 (1.88)
% fat of cream (SD)	3.51 (2)	3.13 (1.8)	3.51 (1.53)	2.46 (1.29)
kcal/l (SD)	671.31 (195.45)	634.47 (175.26)	672.03 (149.16)	568.86 (125.76)



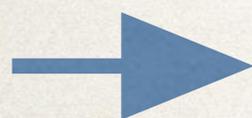
• **no significant difference** between any of the milk components

DHA content of breastmilk

Table 5. DHA content of breastmilk total (n=39) and per subgroup (in g%)

	DHA 22:6 n-3			
Mean (SD)	0.13 (0.05)			
Range (min-max)	0.06-0.32			
Median	0.12			
	40 d (n=10)	80 d (n=10)	120 d (n=10)	180 d (n=9)
Mean (SD)	0.14 (0.03) ^{a,b}	0.16 (0.07) ^a	0.10 (0.03) ^b	0.12 (0.02) ^{a,b}
Range	0.1-0.19	0.09-0.32	0.06-0.16	0.09-0.16
Median	0.14	0.14	0.09	0.12

Groups compared by ANOVA Tukey post-hoc analysis p<0.05



- DHA content of breastmilk differs **significantly** between 80 and 120 days postpartum

Conclusions on DHA

- ❖ similar results to the findings in the meta-analysis by Brenna et al. (2007) and Urwin et al. (2013) in China
 - ❖ DHA content is time-dependent, falling significantly between 80 and 120 days postpartum
- ❖ mean DHA levels (n=39) across the 6-month period is 0.13% of fat, corresponding to about 7th percentile in distribution of international study-values
- ❖ mean DHA at 120-day lactation of 0.10% corresponds to second lowest values reported in literature reviewed by Brenna et al. (2014)

Length measurements of newborns



Geographical Variation in Breastmilk Polyunsaturated Fatty Acid Profile with Emphasis on Omega-3 Fatty Acids using Dried Milk Spot Technique



Overview

- ❖ Objective:

- ❖ Determine geographical variation of essential fatty acids in human breastmilk in Guatemala

- ❖ Hypothesis:

- ❖ The DHA and EPA content of the breast milk is associated with geographical distance to the ocean

Guatemalan Sites

1) Quetzaltenango

- A. low SES, public health clinics
- B. middle SES, public health clinics
- C. high SES, private health clinics

2) San Juan Ostuncalco: low-income women

3) Champerico in Retalhuleu, a coastal port with ocean fish

4) Santa Maria Chiquimula in Totonicapan province, imbedded highland municipality - *pending*

5) Sololá, lake region

- 1) Municipality
- 2) Lake villages (Panajachel and Santiago Atitlán)



Subjects

- ❖ Subset of $n=15$ per site
- ❖ Inclusion criteria
 - ❖ predominately breastfed infants ages 26-64 days
 - ❖ healthy, or at least breastfed at normal intervals
- ❖ Exclusion criteria
 - ❖ diseased mother or child with altered feeding frequency
 - ❖ mothers with children born preterm

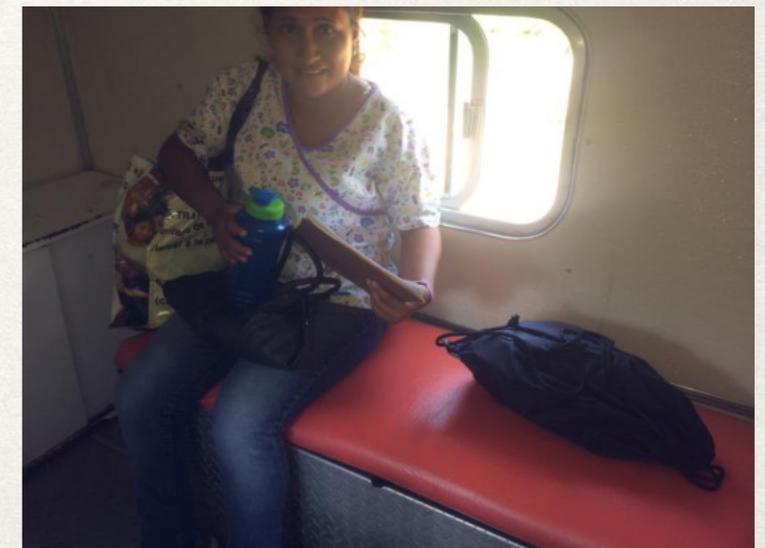
Recruitment in Sololá

- ❖ Municipio de Sololá
 - ❖ Centro de Salud
- ❖ Lake villages Panajachel and Santiago Atitlán
 - ❖ Centro de Salud Dr Eduardo Hernandez-Soto, Panajachel
 - ❖ Home visits
 - ❖ Centro de Salud, Santiago Atitlán
 - ❖ Hospitalito Santiago Atitlán



Recruitment in Champerico

- ❖ Centro de Salud Champerico
- ❖ Home visits



Procedure

- ❖ Questionnaire and breastmilk sample collection
- ❖ Dried Milk Spot Technique



Sololá

Municipio			Código			Fecha		
1	01/09	San Juan	01-1	01/09	San Juan	02	02/09	San Juan
2	02/09	San Juan	02-1	02/09	San Juan	03	03/09	San Juan
3	03/09	San Juan	03-1	03/09	San Juan	04	04/09	San Juan
4	04/09	San Juan	04-1	04/09	San Juan	05	05/09	San Juan
5	05/09	San Juan	05-1	05/09	San Juan	06	06/09	San Juan
6	06/09	San Juan	06-1	06/09	San Juan	07	07/09	San Juan
7	07/09	San Juan	07-1	07/09	San Juan	08	08/09	San Juan
8	08/09	San Juan	08-1	08/09	San Juan	09	09/09	San Juan
9	09/09	San Juan	09-1	09/09	San Juan	10	10/09	San Juan
10	10/09	San Juan	10-1	10/09	San Juan	11	11/09	San Juan
11	11/09	San Juan	11-1	11/09	San Juan	12	12/09	San Juan
12	12/09	San Juan	12-1	12/09	San Juan	13	13/09	San Juan
13	13/09	San Juan	13-1	13/09	San Juan	14	14/09	San Juan
14	14/09	San Juan	14-1	14/09	San Juan	15	15/09	San Juan
15	15/09	San Juan	15-1	15/09	San Juan	16	16/09	San Juan
16	16/09	San Juan	16-1	16/09	San Juan	17	17/09	San Juan
17	17/09	San Juan	17-1	17/09	San Juan	18	18/09	San Juan
18	18/09	San Juan	18-1	18/09	San Juan	19	19/09	San Juan
19	19/09	San Juan	19-1	19/09	San Juan	20	20/09	San Juan
20	20/09	San Juan	20-1	20/09	San Juan	21	21/09	San Juan
21	21/09	San Juan	21-1	21/09	San Juan	22	22/09	San Juan
22	22/09	San Juan	22-1	22/09	San Juan	23	23/09	San Juan
23	23/09	San Juan	23-1	23/09	San Juan	24	24/09	San Juan
24	24/09	San Juan	24-1	24/09	San Juan	25	25/09	San Juan
25	25/09	San Juan	25-1	25/09	San Juan	26	26/09	San Juan
26	26/09	San Juan	26-1	26/09	San Juan	27	27/09	San Juan
27	27/09	San Juan	27-1	27/09	San Juan	28	28/09	San Juan
28	28/09	San Juan	28-1	28/09	San Juan	29	29/09	San Juan
29	29/09	San Juan	29-1	29/09	San Juan	30	30/09	San Juan
30	30/09	San Juan	30-1	30/09	San Juan	31	31/09	San Juan

Champerico

Código	Fecha	Nombre
CH-01	30/09	CAP Champerico
CH-02	30/09	CAP Champerico
CH-03	30/09	CAP Champerico
CH-04	30/09	CAP Champerico
CH-05	30/09	CAP Champerico
CH-06	04/09	CAP Champerico
CH-07	04/09	CAP Champerico
CH-08	04/09	CAP Champerico
CH-09	04/09	CAP Champerico
CH-10	05/09	CAP Champerico
CH-11	05/09	CAP Champerico
CH-12	05/09	CAP Champerico
CH-13	05/09	CAP Champerico
CH-14	05/09	CAP Champerico
CH-15	05/09	CAP Champerico

Results

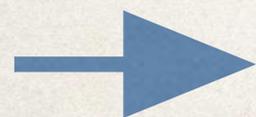
Table 6 Sample descriptives of participants per sub-group (n=15, total n=45)

	Sololá municipality	Sololá lake village	Champerico
Age of mother y			
mean (SD)	26.5 (7.1)	25.5 (6.9)	25.5 (5.4)
median	25	25	26
Civil Status n (%)			
single	2 (13)	2 (13)	4 (27)
married	13 (86)	13 (86)	11 (73)
Education n (%)			
none	2 (13)	3 (20)	3 (20)
primary	3 (20)	6 (40)	8 (53)
secondary	7 (47)	2 (13)	4 (27)
superior	3 (20)	4 (27)	0
Traditional clothing n (%)			
	14 (93)	12 (80)	0

Results cont.

Table 7 Sample descriptives of participants per sub-group (n=15, total n=45)

	Sololá municipality	Sololá lake village	Champerico
Age of child d			
mean (SD)	43.1 (12.6)	42.9 (12.4)	49.2 (14.9)
median	42	38	54
Sex n(%)			
female	6 (40)	5 (33)	5 (33)
male	9 (60)	10 (67)	10 (67)
Mean feeding frequency per day			
n (SD)	10 (5)	13.2 (3.9)	15.1 (6.7)
median	9	12	15



Daily feeding frequency differs **significantly** between Sololá municipality and Champerico ($p < 0.05$)

Indicators of dietary PUFA Intake

- ❖ none of the participants consumed Omega 3 supplements
- ❖ women tend to avoid fish during pregnancy and lactation period
- ❖ monthly fish consumption is lowest in Sololá municipality (3.67 ± 3.6); Sololá lake villages (4.33 ± 3.5) and highest in Champerico (5.53 ± 8.15)
- ❖ median monthly fish consumption is 3 in SM and CH, and 5 in SL

Table 8 PUFA intake in subgroups (n=15, total n=45)

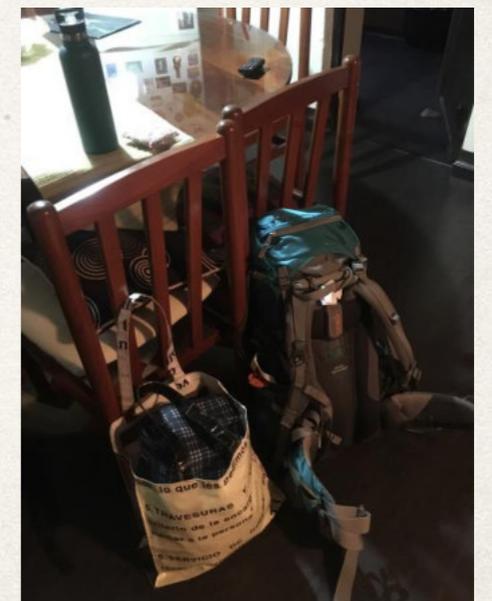
	Sololá municipality	Sololá lake village	Champerico
Omega 3 supplement			
no	15	15	15
Fish consumption per month			
Mean (SD)	3.67 (3.6)	4.33 (3.5)	5.53 (8.15)
Median	3	5	3

Statistical Analysis

- ❖ Significant differences between the subgroups in terms of:
 - ❖ fish consumption
 - ❖ feeding frequency
 - ❖ fatty acid content
- ❖ Correlations
 - ❖ fatty acids and age of mother
 - ❖ fatty acids and socio-economic status

Challenges

- ❖ women stay at home after giving birth (*la cuarentena*)
- ❖ vaccination schemes and days differ across communities
- ❖ carrying the material
- ❖ locating and contacting potential participants, lack of phone numbers
- ❖ language barrier
- ❖ questionnaire: recall bias
- ❖ breastmilk extraction with breastmilk pump
- ❖ results take a long time
- ❖ climate in Champerico
- ❖ occasional power outages



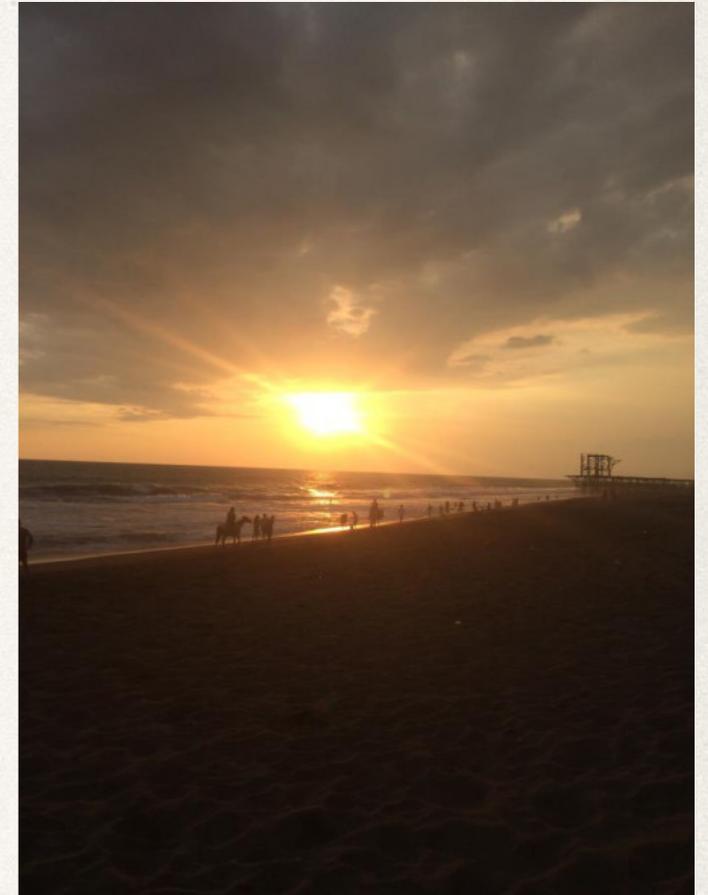
Lessons learned

- ❖ a good relation with the *centros de salud* is pivotal:
 - ❖ access to data
 - ❖ they know their municipality: manpower, trust and logistics
 - ❖ they are interested in the results and can further act on them
- ❖ timing: *censo*, public and local holidays
- ❖ breastmilk extraction technique
- ❖ always being prepared to collect samples and being well organised
- ❖ interview technique and approaching potential participants
- ❖ standardizing interviewers (a straight forward questionnaire is essential)



Temporary Conclusions

- ❖ believes about food consumption in lactation phase are strong
- ❖ restriction of foods in the lactation period possibly constitute an additional strain on FA deficient mother and *thus* the infant



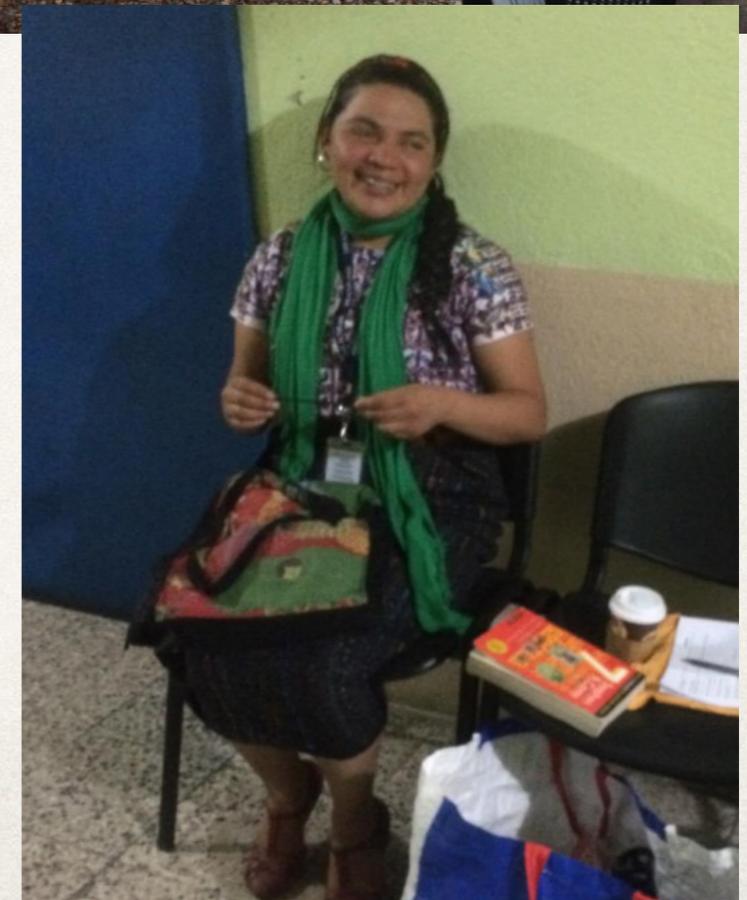
➔ for the statistical analysis the laboratory results are needed

Collaborators

- ❖ Centro de Salud Panajachel
- ❖ Centro de Desarrollo Social (CDS), Oficina de la Mujer, Panajachel
- ❖ Centro de Salud Sololá
- ❖ Centro de Salud Santiago Atitlán
- ❖ Hospitalito Santiago Atitlán
- ❖ CAP Champerico

Thanks to

- ❖ All the women participating in the study
- ❖ The health centres and their staff
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- ❖ Celeste and Rosario for helping with interviews and translations
- ❖ Dr Noel Solomons
- ❖ The CeSSIAM team



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Thank you for your attention. Questions?



