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ORIGINAL CONTRIBUTION

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The effect of maternal T1DM on the fatty acid composition of erythrocyte phosphatidylcholine and phosphatidyethanolamine in infants during early life

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Abstract *Background* The risk for type 1 diabetes (T1DM) in children of mothers with T1DM is different to that in children of fathers with T1DM. Fatty acid (FA) intake, in particular EPA and DHA, has been associated with T1DM risk and has been suggested to be inadequate in infants of diabetic mothers. We asked, therefore, whether EPA and DHA FA nutritional status in offspring of mothers with T1DM could contribute to their reduced T1DM risk *Methods* BABYDIET follows children with increased genetic and familial risk for T1DM from birth to age 3 years. FA nutritional state was assessed by determining the erythrocyte membrane phosphatidylethanolamine (PE) and phosphatidylcholine (PC) composition in children of T1DM mothers and children of T1DM fathers or with T1DM siblings participating in the BABYDIET study. Samples for determination of erythrocyte membrane FA composition were collected at ages 3 and 12 months in 48 and 49 infants, respectively. FA measurements were adjusted for breastfeeding duration, FA supplementation, and gluten exposure. *Results* 3-months-old children of T1DM mothers and T1DM fathers/sibs had similar levels of PC DHA and EPA (DHA 1.53 ± 0.23 vs. 1.65 ± 0.11 wt.%; EPA 0.15 ± 0.02 vs. 0.21 ± 0.03 wt.%) and PE DHA and EPA (DHA 7.54 ± 0.37 vs. 7.92 ± 0.38 wt.%; EPA 0.53 ± 0.06 vs. 0.61 ± 0.04 wt.%). No differences were also observed after stratification for breastfeeding. At age 12 months, a minor reduction of PE DHA was observed in children of T1DM mothers. Expected higher levels for DHA and EPA in fully breastfed children and in children of mothers taking fish oil supplementation were observed at 3 months in all children. Other differences included increased levels of the major saturated FA 16:0 in 3-months-old infants from T1DM mothers (PC 35.45 ± 0.35 vs. 33.89 ± 0.26 wt.%, mean ± SEM, $P_{corr} = 0.005$; PE 16.13 ± 0.39 vs. 14.93 ± 0.24 wt.%, $P_{corr} = 0.05$). *Conclusion* Although FA status was not identical in children from T1DM mothers and from T1DM fathers, maternal T1DM was not associated with changes in offspring's EPA and DHA incorporation into erythrocyte membrane.

Key words type 1 diabetes – erythrocyte membrane – DHA and EPA – breastfeeding – fish oil supplementation

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Abbreviation DHA: Docosahexaenoic acid, 22:6 n-3, EPA: Eicosapentaenoic acid, 20:5 n-3, FA: Fatty acid, LC-PUFA: Long chain polyunsaturated fatty acids, MUFA: Monounsaturated fatty acids, PC: Phosphatidylcholine, PE: Phosphatidylethanolamine, PUFA: Polyunsaturated fatty acids, SEM: Standard error of the mean, SFA: Saturated fatty acids, T1DM: Type 1 diabetes mellitus, TLC: Thin layer chromatography

Introduction

The quality of dietary lipid composition during early life has a major impact on body composition, tissue differentiation and organ function. The availability of longchain polyunsaturated fatty acids (LC-PUFA) appears to affect the quality of growth and the development of visual and cognitive function [1, 4, 8, 18]. Furthermore, the intake of fatty acids (FAs), in particular eicosapentaenoic acid (EPA = LC-PUFA = 20:5 n-3) and docosahexaenoic acid (DHA = LC-PUFA = 22:6 $n = 3$), during pregnancy and infancy, has been inversely associated with islet autoimmunity and T1DM risk [20, 23, 24]. The risk for T1DM is also affected by maternal T1DM status; risk in children born to mothers with T1DM is around half that of children born to fathers with T1DM. The reason for this difference is still unexplained [6, 26].

The nutritional status of children born to mothers with T1DM has been suggested to differ to that of other children in several aspects, including FA. In particular, there are several reports of differences in breast milk composition between mothers with and without T1DM [5, 16, 25] suggesting an inadequate supply of FAs such as EPA and DHA to infants of diabetic mothers. We therefore sought to determine whether a difference in EPA and DHA uptake in children of mothers with T1DM could explain their reduced T1DM risk. Since the FA composition of erythrocyte cell membranes is an established marker of FA nutrition status [15, 19, 21], we performed a pilot study in which the FA content of erythrocyte cell membranes from children born to mothers with T1DM were analysed comparatively to those of children without a T1DM mother. Samples were collected at age 3 months in order to examine uptake predominantly from breast milk, and at age 12 months to determine whether any observed differences at 3 months persisted after introduction of milk supplements and solid foods to the diet.

Methods

Subjects

BABYDIET is an ongoing dietary intervention study in newborn children with increased genetic and familial risk for T1DM. The study aims to determine whether delayed introduction of dietary gluten can reduce the incidence of islet autoimmunity in high-risk first degree relatives of patients with T1DM and prospectively follows children from birth to the age of 3 years [22]. Since 2001, 149 newborn were recruited into the study and randomized to exposure to dietary gluten at either 6 (early exposure) or 12 (late exposure) months of age. Children born between 2001 and 2006 in Germany were eligible for this study. Children were monitored intensively with collection of venous blood, urine and stool, and 3-day dietary records of weighed food intake at 3-month intervals for 3 years. Infections, medication and the introduction of new food groups were recorded daily. Written informed consent was obtained from the parents. The study was approved by the ethics committee of the Ludwig-Maximilian-University, Munich, Germany (Ethikkommission der Medizinischen Fakultät der Ludwig-Maximilians Universität No. 329/00).

Samples for determination of erythrocyte membrane phosphatidylethanolamine (PE) and phosphatidylcholine (PC) composition were serially collected since 2003 at the age of 3 months (56 infants), 12 months (66 infants) and 24 months (47 infants); data at 3 and 12 months are discussed here. Food records indicated that gluten was introduced at a median of 6.9 months in the group randomized to early gluten exposure and at 11.7 months in the group randomized to late gluten exposure.

Breastfeeding and fatty acid supplementation

Data on breastfeeding (yes, no) and the duration of full breastfeeding (weeks) and any breastfeeding were obtained through daily food records and by 3-monthly questionnaires (obtained between 3 and 24 months of age). Breastfeeding was defined according to WHO criteria [27] as "full breastfeeding" if the infant received breast milk with or without supplementation with water or water-based drinks, vitamins and medicines, but without formula, or other milk or solids, or as "any breastfeeding" if the infant received breast milk irrespective of any other types of food including full breastfeeding. Maternal FA supplementation during breastfeeding was reported by the mother in a questionnaire administered when their child was 3-months-old.

Fatty acid analysis

The FA composition of the erythrocyte membrane phospholipids was determined as previously described [11]. In brief, erythrocytes and plasma were separated from EDTA-containing blood samples and the erythrocytes were hemolyzed. After lysis, isopropanol (Merck, Darmstadt, Germany) and BHT (50 mg/l) (Fluka, Neu-Ulm, Germany) were added to prevent oxidation and tubes were stored at −80°C until analysis. After defrosting, FAs were extracted twice; first by 10 ml isopropanol/chloroform (3:2 vol/vol), then by 4 ml chloroform. The extracts were then dried under reduced pressure. Thin layer chromatography (TLC) with chloroform, methanol, 25% ammonia solution and distilled water (73:27:2.2:2.8, by vol) as the mobile phase was used to separating red blood cell PE and PC. The remaining lipids were dissolved in 400 µl of chloroform/methanol (1:1 v/v) and deposited on a TLC plate (90 µl). After visualisation of the components with 2′,7′-dichlorofluorescein, bands containing lipid fractions were scraped from the TLC plate and transferred to 4 ml glass tubes equipped with teflon-lined screw caps. Methylesters of FAs from the fractions were obtained by reaction with 1 ml 3 M methanolic hydrochloric acid at 85°C for 45 min in closed glass tubes. After neutralisation with sodium carbonate/sodium hydrogen carbonate/sodium sulfate buffer, 1 ml hexane was added. After centrifugation at 400×*g* for 3 min, the hexane layer was transferred to another vial and the extraction was repeated. The combined extracts were then dried under a stream of nitrogen. Derivatives were stored at −20°C until GLC analysis.

The FA methyl esters (FAME) were analyzed by capillary GLC (Hewlett-Packard 5890 Series II gas chromatograph, equipped with a 60 m × 0.32 mm BPX-70 column from SGE, Weiterstadt, Germany). FID signals were evaluated with EZ-Chrom Elite version 2.61 software (Scientific Software, Pleasanton, CA, USA). FAME peaks were identified by comparison with commercial standards (Nu-Chek-Prep, Elysian, MN, USA). Values were calculated as weight percentages (wt.%) of all FAs determined (14–24 C atoms).

Coefficients of variation (intraassay) were below 10% for all presented FA.

Statistical analysis

Results are expressed as means ± SEM. After checking for the normal distribution of the data, statistical analysis for comparisons between groups was performed by using the two-tailed Student's *t* test for normally distributed variables and the Mann–Whitney *U* test for not normally distributed variables. Adjustment for potential confounder variables was done using General Linear Model multivariate analyses, with FAs as the dependent variable, maternal fish oil supplementation during pregnancy and/or lactation (yes/no) and gluten exposure (6/12 months) as categorical variables, and breast feeding as covariates. For all analyses, *P* values were corrected for the number of comparisons and a two tailed P_{corr} value of 0.05 was considered significant. All statistical analyses were performed using the Statistical Package for Social Science (SPSS 14.0; SPSS, Chicago, IL, USA).

Results

Characteristics of the infants studied

At age 3 months, blood samples of 48 infants were collected for the determination of erythrocyte membrane fatty acid composition (Table 1). Of these, 23 infants had a mother with T1DM and 25 infants had a non-diabetic mother. Median HbA1c of mothers with T1DM during the last trimester of pregnancy was 5.7% (range 5.1–6.2%). Breastfeeding characteristics and fish oil supplementation of the mother during pregnancy and/or lactation is provided in Table 1. At age 12 months, blood samples of 49 infants were collected, including 32 infants who had a previous sample taken at 3 months. Of those, 27 were exposed to gluten at 6 months and 22 at 12 months. The median duration of total breastfeeding was 18.5 weeks in infants of mothers with T1DM compared with 41.3 weeks in infants of non-diabetic mothers.

None of non-fully breastfed infants received LC-PUFA supplemented formulas.

Comparison of the erythrocyte membrane FA composition in 3 months-old infants from mothers with or without T1DM

At age 3 months, no difference between infants of T1DM mothers and infants of non-diabetic mothers

Table 1 Patient characteristics

	Mothers with T1DM	Mothers without T1DM	Total
Age of 3 months	23	25	48
Full breastfeeding (N)	10	17	27
Fish oil supplementation (*N*)	7	6	13
Age of 12 months	25	24	49
Gluten exposure at 6/12 months (*N*)	15/10	12/12	27/22
Median duration of total breastfeeding (weeks)	18.5	41.2	30.2

was found in the levels of erythrocyte EPA (PE 0.53 ± 0.06 vs. 0.61 ± 0.04 wt.%; PC 0.15 ± 0.02 vs. 0.21 ± 0.03 wt.%) and DHA (PE 7.54 ± 0.37 vs. 7.92 ± 0.38 wt.%; PC 1.53 ± 0.13 vs. 1.65 ± 0.11 wt.%; Table 2). Infants from mothers with T1DM had significantly higher levels of the SFA 16:0 compared with infants of non-diabetic mothers (PC fraction of erythrocyte membranes: 16:0: 35.45 ± 0.35 vs. 33.89 ± 0.26 wt.%; $P_{corr} = 0.005$; PE fraction:16:0: 16.13 ± 0.39 vs. 14.93 ± 0.24 wt.%; $P_{corr} = 0.05$; Table 2). Levels of n-3 PUFA 22:5 in the PC fraction were lower in infants from diabetic mothers compared with infants from non-diabetic mothers (0.36 ± 0.03 vs. 0.46 ± 0.02 wt.%; $P_{corr} = 0.025$; Table 2).

Previous studies have shown that breastfeeding and maternal fish oil supplementation may influence the FA composition of erythrocyte membranes in infants [9, 13, 21]. Therefore, these factors were used as covariates in a multivariate analysis. In this multivariate analysis, full breastfeeding and fish oil supplementation during pregnancy and/or lactation significantly affected the FA composition: levels of EPA (20:5 n-3) and DHA (22:6 n-3) were higher in fully breastfed infants compared with non fully breastfed infants (PE: EPA: 0.71 ± 0.05 vs. 0.53 ± 0.06 wt.%, $P = 0.04$; DHA 8.84 ± 0.32 vs. 7.34 ± 0.43 wt.%, $P = 0.008$) and higher in infants from mothers who took fish oil supplements compared with infants from mothers who did not (PE EPA: 0.72 ± 0.07 vs. 0.52 ± 0.04, $P = 0.02$ wt.%; DHA 8.93 ± 0.46 vs. 7.24 ± 0.29 wt.%; $P = 0.008$). Similar to the univariate analysis, in the multivariate analysis, maternal T1DM was associated with higher SFA levels (PC 14:0:

Table 2 Fatty acid composition (wt.%) of erythrocyte membrane PC and PE in 3-months-old infants from mothers with or without T1DM

	PC		PE	
	Mothers with T1DM $n = 23$	Mothers without T1DM $n = 25$	Mothers with T1DM $n = 23$	Mothers without T1DM $n = 25$
SFA				
C14:0	0.54 ± 0.05	0.40 ± 0.03	0.19 ± 0.02	0.16 ± 0.02
C16:0	35.45 ± 0.35*	33.89 ± 0.26*	16.13 ± 0.39*	14.93 ± 0.24*
C18:0	11.92 ± 0.24	12.60 ± 0.28	8.85 ± 0.23	8.95 ± 0.17
C20:0	0.19 ± 0.01	0.19 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
C22:0	0.10 ± 0.02	0.12 ± 0.02	0.02 ± 0.01	0.02 ± 0.00
C24:0	0.29 ± 0.09	0.36 ± 0.09	0.02 ± 0.01	0.02 ± 0.00
Total SFA	48.58 ± 0.37	47.56 ± 0.24	25.32 ± 0.51	24.19 ± 0.31
MUFA				
C16:1 n-7	0.33 ± 0.02	0.31 ± 0.02	0.13 ± 0.01	0.13 ± 0.01
C18:1 n-9	18.65 ± 0.44	17.72 ± 0.29	17.10 ± 0.44	15.79 ± 0.34
C18:1 n-7	2.20 ± 0.06	2.09 ± 0.06	1.27 ± 0.04	1.16 ± 0.03
C20:1 n-9	0.46 ± 0.03	0.41 ± 0.02	0.59 ± 0.03	0.51 ± 0.03
C22:1 n-9	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.07 ± 0.01
C24:1 n-9	0.40 ± 0.07	0.36 ± 0.05	0.11 ± 0.02	0.07 ± 0.02
Total MUFA	22.11 ± 0.49	20.95 ± 0.31	19.28 ± 0.48	17.73 ± 0.38
n-9 PUFA				
C20:3 n-9	0.06 ± 0.00	0.08 ± 0.01	0.20 ± 0.02	0.19 ± 0.01
n-6 PUFA				
C18:2 n-6	17.19 ± 0.52	17.28 ± 0.38	4.94 ± 0.22	4.74 ± 0.17
C18:3 n-6	0.05 ± 0.00	0.05 ± 0.00	0.07 ± 0.01	0.07 ± 0.01
C20:2 n-6	0.40 ± 0.01	0.41 ± 0.01	0.36 ± 0.02	0.32 ± 0.02
C20:3 n-6	1.84 ± 0.10	2.03 ± 0.08	1.75 ± 0.12	1.66 ± 0.06
C20:4 n-6	6.23 ± 0.46	7.23 ± 0.25	26.95 ± 0.76	28.43 ± 0.29
C22:2 n-6	0.07 ± 0.01	0.05 ± 0.01	0.13 ± 0.05	0.12 ± 0.04
C22:4 n-6	0.44 ± 0.02	0.49 ± 0.03	7.97 ± 0.18	7.81 ± 0.17
C22:5 n-6	0.18 ± 0.01	0.19 ± 0.02	1.30 ± 0.06	1.29 ± 0.05
Total n-6 PUFA	26.39 ± 0.69	27.73 ± 0.34	43.48 ± 0.75	44.44 ± 0.39
n-3 PUFA				
C18:3 n-3	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.07 ± 0.01
C20:3 n-3	0.06 ± 0.01	0.06 ± 0.01	0.11 ± 0.03	0.16 ± 0.02
C20:5 n-3 (EPA)	0.15 ± 0.02	0.21 ± 0.03	0.53 ± 0.06	0.61 ± 0.04
C22:5 n-3	0.36 ± 0.03**	0.46 ± 0.02**	3.30 ± 0.19	3.56 ± 0.10
C22:6 n-3 (DHA)	1.53 ± 0.13	1.65 ± 0.11	7.54 ± 0.37	7.92 ± 0.38
Total n-3 PUFA	2.21 ± 0.17	2.47 ± 0.14	11.57 ± 0.55	12.31 ± 0.47

Values are presented as mean ± SEM

DHA docosahexaenoic acid, *EPA* eicosapentaenoic acid, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PUFA* polyunsaturated fatty acids

$P_{corr} \leq 0.05$, $^{**}P_{corr} \leq 0.01$

0.56 ± 0.05 vs. 0.36 ± 0.05 wt.%; $P = 0.007$), but did not affect EPA or DHA levels.

Comparison of the erythrocyte membrane FA composition in 3-months-old fully breastfed infants from mothers with or without T1DM

At age 3 months, infants who were still fully breastfed and had a mother with T1DM showed significantly higher levels of 14:0 in the PC fraction (14:0: 0.63 ± 0.06 vs. 0.42 ± 0.02 wt.%; $P_{corr} = 0.01$) compared with fully breastfed infants of non-diabetic mothers (Table 3). The content of other single FAs was comparable in children of diabetic and non-diabetic mothers. When the FA groups SFA, MUFA, total PUFA and total n-3 and n-6 PUFA were compared, there were no differences between fully breastfed infants from mothers with versus mothers without T1DM (Table 3).

Comparison of the erythrocyte membrane FA composition in 12 months-old infants from mothers with or without T1DM

At 12 months, a minor reduction in PE DHA levels was observed in children of T1DM mothers (4.60 ± 0.24 vs. 5.92 ± 0.36 wt.% $P_{corr} = 0.015$); no other differences in erythrocyte EPA and DHA levels were observed (Table 4). Infants from mothers with T1DM had lower levels of SFA 18:0 ($P_{corr} = 0.025$) in the PE fraction and 22:0 ($P_{corr} = 0.05$) in the PC fraction, as well as lower levels of MUFA 24:1 n-9

Table 3 Fatty acid compositions (wt.%) of erythrocyte membrane PC and PE in fully breastfed, 3-months-old infants from mothers with or without T1DM

	PC		PE	
	Mothers with T1DM $n = 10$	Mothers without T1DM $n = 17$	Mothers with T1DM $n = 10$	Mothers without T1DM n = 17
SFA				
C14:0	0.63 ± 0.06*	0.42 ± 0.02*	0.17 ± 0.02	0.17 ± 0.03
C16:0	34.43 ± 0.40	33.36 ± 0.28	15.40 ± 0.38	15.12 ± 0.25
C18:0	12.82 ± 0.25	13.24 ± 0.16	9.05 ± 0.26	9.14 ± 0.20
C20:0	0.19 ± 0.01	0.17 ± 0.01	0.12 ± 0.02	0.10 ± 0.01
C22:0	0.08 ± 0.02	0.14 ± 0.03	0.02 ± 0.00	0.02 ± 0.01
C24:0	0.25 ± 0.09	0.46 ± 0.12	0.02 ± 0.01	0.02 ± 0.01
Total SFA	48.76 ± 0.41	47.79 ± 0.22	24.76 ± 0.43	24.56 ± 0.31
MUFA				
C16:1 n-7	0.40 ± 0.01	0.35 ± 0.02	0.15 ± 0.03	0.15 ± 0.04
C18:1 n-9	17.54 ± 0.25	17.35 ± 0.30	15.84 ± 0.36	15.05 ± 0.19
C18:1 n-7	2.23 ± 0.06	2.18 ± 0.04	1.23 ± 0.03	1.18 ± 0.03
C20:1 n-9	0.35 ± 0.02	0.34 ± 0.02	0.47 ± 0.02	0.44 ± 0.02
C22:1 n-9	0.07 ± 0.01	0.06 ± 0.00	0.07 ± 0.01	0.07 ± 0.01
C24:1 n-9	0.32 ± 0.07	0.39 ± 0.07	0.12 ± 0.04	0.08 ± 0.03
Total MUFA	20.91 ± 0.26	20.67 ± 0.33	17.88 ± 0.35	16.97 ± 0.22
n-9 PUFA				
C20:3 n-9	0.07 ± 0.01	0.07 ± 0.01	0.20 ± 0.03	0.19 ± 0.02
n-6 PUFA				
C18:2 n-6	16.26 ± 0.45	16.87 ± 0.37	4.38 ± 0.17	4.43 ± 0.12
C18:3 n-6	0.05 ± 0.00	0.04 ± 0.00	0.06 ± 0.01	0.06 ± 0.01
C20:2 n-6	0.40 ± 0.02	0.42 ± 0.02	0.28 ± 0.01	0.29 ± 0.01
C20:3 n-6	2.12 ± 0.12	2.18 ± 0.08	1.63 ± 0.08	1.64 ± 0.08
C20:4 n-6	7.34 ± 0.46	7.67 ± 0.21	28.52 ± 0.81	28.56 ± 0.27
C22:2 n-6	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.12 ± 0.07
C22:4 n-6	0.46 ± 0.03	0.52 ± 0.04	7.81 ± 0.25	7.71 ± 0.19
C22:5 n-6	0.17 ± 0.02	0.17 ± 0.01	1.29 ± 0.08	1.31 ± 0.07
Total n-6 PUFA	26.87 ± 0.60	27.94 ± 0.44	44.03 ± 0.89	44.12 ± 0.44
n-3 PUFA				
C18:3 n-3	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.00	0.06 ± 0.01
C20:3 n-3	0.08 ± 0.01	0.06 ± 0.01	0.15 ± 0.04	0.13 ± 0.03
C20:5 n-3 (EPA)	0.20 ± 0.02	0.19 ± 0.02	0.74 ± 0.10	0.63 ± 0.05
C22:5 n-3	0.45 ± 0.02	0.45 ± 0.02	3.95 ± 0.27	3.61 ± 0.12
C22:6 n-3 (DHA)	1.77 ± 0.11	1.83 ± 0.12	8.05 ± 0.34	8.53 ± 0.12
Total n-3 PUFA	2.59 ± 0.13	2.60 ± 0.16	13.37 ± 0.60	12.95 ± 0.50

Values are presented as mean ± SEM
DHA docosahexaenoic acid, *EPA* eicosapentaenoic acid, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PUFA* polyunsaturated fatty acids; *$P_{corr} \leq 0.05$; **$P_{corr} \leq 0.01$

(P_{corr} = 0.05) in the PC fraction. Furthermore, infants from mothers with T1DM had higher levels of PUFA 18:2 n-6 (P_{corr} = 0.05) and 18:3 n-3 (P_{corr} <0.001) in the PE fraction compared with infants from non-diabetic mothers (Table 4). After adjusting for breastfeeding duration and dietary intervention (i.e. gluten exposure at 6 or 12 months of age) in the multivariate analysis, infants from mothers with T1DM showed a higher MUFA 24:1 n-9 content in the PC fraction (0.31 ± 0.03 vs. 0.17 ± 0.03 wt.%; P_{corr} = 0.025) compared with infants from non-diabetic mothers. Breastfeeding duration influenced levels of MUFA 20:1 n-9 (P_{corr} = 0.005), DHA (P_{corr} < 0.001) and total n-3 PUFA (P_{corr} = 0.005) in the PE fraction of erythrocyte membranes.

Discussion

This is to our knowledge the first report of erythrocyte membrane fatty acid composition in young infants with an increased genetic and familial risk for T1DM. Potentially relevant to T1DM risk, no differences were found in the levels of PUFAs, including EPA and DHA, in these children. Differences were observed in other erythrocyte membranse FA. In particular, 3-months-old infants of T1DM mothers had higher values of the major SFA 16:0 as compared to children of non-diabetic mothers. These differences were also evident when analysing only the fully breastfed infants.

In contrast to the finding reported by Ghebremeskel et al. [12] that healthy neonates born to mothers

Table 4 Fatty acid compositions (wt.%) of erythrocyte membrane PC and PE in 12-months-old infants from mothers with or without T1DM

	PC		PE	
	Mothers with T1DM n = 25	Mothers without T1DM n = 24	Mothers with T1DM n = 25	Mothers without T1DM n = 24
SFA				
C14:0	0.43 ± 0.02	0.41 ± 0.03	0.13 ± 0.01	0.19 ± 0.02
C16:0	35.98 ± 0.57	34.08 ± 1.04	15.04 ± 0.37	14.85 ± 0.46
C18:0	11.03 ± 0.25	11.45 ± 0.25	7.93 ± 0.36*	9.16 ± 0.21*
C20:0	0.15 ± 0.01	0.16 ± 0.01	0.12 ± 0.01	0.13 ± 0.01
C22:0	0.04 ± 0.01*	0.09 ± 0.02*	0.04 ± 0.01	0.08 ± 0.04
C24:0	0.10 ± 0.02	0.26 ± 0.06	0.03 ± 0.01	0.06 ± 0.04
Total SFA	47.74 ± 0.74	46.45 ± 1.24	23.28 ± 0.59	24.47 ± 0.48
MUFA				
C16:1 n-7	0.40 ± 0.03	0.36 ± 0.02	0.13 ± 0.01	0.12 ± 0.01
C18:1 n-9	18.31 ± 0.29	18.34 ± 0.27	17.16 ± 0.36	16.77 ± 0.36
C18:1 n-7	1.99 ± 0.04	1.85 ± 0.05	1.18 ± 0.03	1.11 ± 0.03
C20:1 n-9	0.37 ± 0.02	0.36 ± 0.01	0.63 ± 0.02	0.56 ± 0.02
C22:1 n-9	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
C24:1 n-9	0.19 ± 0.02*	0.30 ± 0.04*	0.08 ± 0.01	0.06 ± 0.01
Total MUFA	21.33 ± 0.33	21.28 ± 0.31	19.25 ± 0.40	18.69 ± 0.38
n-9 PUFA				
C20:3 n-9	0.11 ± 0.02	0.07 ± 0.01	0.13 ± 0.01	0.13 ± 0.02
n-6 PUFA				
C18:2 n-6	20.60 ± 0.44	20.12 ± 0.42	6.68 ± 0.17*	6.00 ± 0.20*
C18:3 n-6	0.06 ± 0.01	0.05 ± 0.00	0.07 ± 0.01	0.07 ± 0.01
C20:2 n-6	0.39 ± 0.02	0.38 ± 0.01	0.43 ± 0.02	0.38 ± 0.02
C20:3 n-6	1.90 ± 0.07	1.65 ± 0.07	1.84 ± 0.12	1.55 ± 0.06
C20:4 n-6	5.30 ± 0.28	5.29 ± 0.34	26.75 ± 0.57	26.91 ± 0.57
C22:2 n-6	0.07 ± 0.01	0.12 ± 0.03	0.13 ± 0.01	0.11 ± 0.01
C22:4 n-6	0.43 ± 0.02	0.68 ± 0.29	9.42 ± 0.24	8.57 ± 0.33
C22:5 n-6	0.21 ± 0.02	0.21 ± 0.03	1.55 ± 0.11	1.23 ± 0.06
Total n-6 PUFA	28.95 ± 0.56	28.49 ± 0.60	46.87 ± 0.70	44.81 ± 0.78
n-3 PUFA				
C18:3 n-3	0.15 ± 0.01	0.14 ± 0.01	0.14 ± 0.01***	0.11 ± 0.01***
C20:3 n-3	0.06 ± 0.01	0.07 ± 0.01	0.21 ± 0.02	0.22 ± 0.02
C20:5 n-3 (EPA)	0.15 ± 0.02	0.21 ± 0.06	0.51 ± 0.04	0.56 ± 0.04
C22:5 n-3	0.42 ± 0.03	0.61 ± 0.22	4.02 ± 0.12	4.01 ± 0.14
C22:6 n-3 (DHA)	0.92 ± 0.07	1.45 ± 0.39	4.60 ± 0.24*	5.92 ± 0.36*
Total n-3 PUFA	1.71 ± 0.10	2.48 ± 0.69	9.48 ± 0.33	10.83 ± 0.40

Values are means ± SEM

DHA docosahexaenoic acid, *EPA* eicosapentaenoic acid, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine, *PUFA* polyunsaturated fatty acids

*P_{corr} ≤ 0.05; **P_{corr} ≤ 0.01; ***P_{corr} ≤ 0.001

with T1DM have highly compromised levels of arachidonic acid (AA) and DHA in plasma lipids of cord blood, our study did not show any differences with respect to the content of EPA, DHA, total n-3 PUFA and total n-6 PUFA in the erythrocyte membrane at 3 and 12 months of age. Apart from the nutritional benefits associated with these FA [1, 4, 8, 18], the findings are also relevant to the T1DM risk in the children since a high EPA and DHA intake has been shown to be associated with decreased risk of islet autoimmunity and T1DM [20, 23, 24]. Our findings suggest that infants of T1DM mothers have no disadvantage with respect to EPA and DHA intake. At the same time, it is unlikely that FA contribute to the T1DM protection seen in children of T1DM mothers as compared to children of T1DM fathers.

In our study, the minor differences of erythrocyte membrane FA composition in infants of mothers with T1DM may be due to differences in the breast milk of mothers with T1DM caused by altered FA metabolism in these women. Previous reports would support this notion. Bitmann et al. [5] examined the milk composition of one diabetic mother at 3–7 days postpartum and observed a lower concentration of medium chain FAs (C10–C14) compared with 13 non-diabetic women, suggesting impaired FA synthesis in the mammary gland. The authors also observed an increased amount of oleic acid (18:1 n-9) and a higher concentration of PUFA in the milk of diabetic mothers, suggesting increased chain elongation compared with the reference women. In contrast, Jackson et al. [16] reported lower levels of PUFA in breast-milk of women with T1DM from 14–84 day postpartum. In another study, no differences could be observed in the milk composition at 3–35 days postpartum of six mothers with tightly controlled T1DM compared with five non-diabetic women [25].

The contrasting results in other studies may be a result of the small sample sizes in the previous studies and heterogeneity between feeding habits of mothers in the various studies. In our present study, we had access to 129 samples taken from children aged 3 and 12 months, and had records of breastfeeding status, fish oil supplementation and gluten exposure. Indeed, for children aged 3 months, full breastfeeding and fish oil supplementation during pregnancy and/or lactation were associated with higher concentrations of EPA and DHA and total n-3 PUFA in the PE fraction. These findings confirm previous reports that fishoil supplementation during pregnancy and/or lactation and breastfeeding has a significant impact on the FA supply of the infants. Fish oil supplementation during pregnancy has been shown to increase the n-3 fatty acid content of breast milk [7, 10, 14] and is associated with increased DHA and EPA content of maternal and infant erythrocyte membranes [9, 14]. A higher percentage of n-3 FAs, in particular DHA, in erythrocyte membrane lipids has previously been reported in breastfed infants compared to formula-fed infants [13, 19, 21]. It has also been shown that infants who receive fish oil supplemented formula achieve a fatty acid status similar to that of breastfed infants [17], but none of non-fully breastfed infants in this study received LC-PUFA supplemented formulas. The dietary intervention of a delayed introduction of gluten did not affect fatty acid levels at 12 months of age.

The strength of our study is that we measured the FA composition of the erythrocyte membranes in the child thereby obtaining a measure of both the intake and the lipometabolic status of the child [2, 3]. A limitation is that we analysed the percentage of the fatty acid and not the absolute concentration.

Conclusion

These data suggest that there are only minor differences in erythrocyte FA composition in infants of mothers with T1DM compared with infants of non-diabetic mothers, in particular if infants are fully breastfed. It seems that mothers with T1DM provide a similar supply of LC-PUFA, in particular of EPA and DHA, in breast milk to that provided by non-diabetic mothers. We conclude, therefore, that EPA and DHA status of the child is unlikely to explain the reduced T1DM risk seen in children of mothers with T1DM. Nevertheless, for infants who were not fully breastfed, erythrocyte LC-PUFA contents were lower than those of fully breastfed infants, and supplementation could be considered for non-fully breastfed children in general.

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References

1. Agostoni C, Trojan S, Bellu R, Riva E, Giovannini M (1995) Neurodevelopmental quotient of healthy term infants at 4 months and feeding practice: the role fo long-chain polyunsaturated fatty acids. Pediatr Res 38:262–266
2. Arab L (2003) Biomarkers of fat and fatty acid intake. J Nutr 133:925S–932S
3. Arab L, Akbar J (2002) Biomarker and the measurement of fatty acids. Pub Health Nutr 5:865–871
4. Birch EE, Garfield S, Hoffmann DR, Uauy R, Birch DG (2000) A randomized controlled trial of early dietary supply of longchain polyunsaturated fatty acids and mental development in term infants. Dev Med Child Neurol 42:171–174
5. Bitman J, Hamosh M, Hamosh P, Lutes V, Neville MC, Seacat J, Wood DJ (1989) Milk composition and volume during the onset of lactation in a diabetic mother. Am J Clin Nutr 50:1364–1369
6. Bleich D, Polak M, Eisenbarth GS, Jackson RA (1993) Decreased risk of type I diabetes in offspring of mothers who acquire diabetes during adrenarchy. Diabetes 42:1433–1439
7. Boris J, Jensen B, Dalby Salvig JD, Secher NJ, Olsen SF (2004) A randomized controlled trial of the effect of fish oil supplementation in late pregnancy and early lactation on the n-3 fatty acid content in human breast milk. Lipids 39:1191–1196
8. Carlson SE, Ford AJ, Werkman SH, Peeples JM, Koo WWK (1996) Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and Arachidonate from egg yolk lecithin. Pedriatr Res 39:882–888
9. Dunstan JA, Mori TA, Barden A, Beilin LJ, Holt PG, Calder PC, Taylor AL, Prescott SL (2004) Effects of n-3 polyunsaturated fatty acid supplementation in pregnancy on maternal and fetal erythrocyte fatty acid composition. Eur J Clin Nutr 58:429–437
10. Dunstan JA, Roper J, Mitoulas L, Hartmann PE, Simmer K, Prescott SL (2004) The effect of supplementation with fish oil during pregnancy on breast milk immunoglobulin A, soluble CD14, cytokine levels and fatty acid composition. Clin Exp Allergy 34:1237–1242
11. Geppert J, Kraft V, Demmelmair H, Koletzko B (2005) Docosahexaenoic acid supplementation in vegetarians effectively increases omega-3 index: a randomized trial. Lipids 40:807–814
12. Ghebremeskel K, Thomas B, Lowy C, Min Y, Crawford MA (2004) Type 1 diabetes compromises plasma arachidonic and docosahexaenoic acid in newborn babies. Lipids 39:335–342
13. Granot E, Golan D, Berry EM (2000) Breast-fed and formula-fed infants do not differ in immunocompetent cell cytokine production despite differences in cell membrane fatty acid composition. Am J Clin Nutr 72:1202–1205
14. Henderson RA, Jensen RG, Lammi-Keefe CJ, Ferris AM, Dardick KR (1992) Effect of fish oil on the fatty acid composition of human milk and maternal and infant erythrocytes. Lipids 27:863–869
15. Innis SM (1992) Plasma and red blood cell fatty acid values as indexes of essential fatty acids in the developing organs of infants fed with milk or formula. J Pediatr 120:S78–S86
16. Jackson MJ, Lammi-Keefe CJ, Jensen RG, Couch SC, Ferries AM (1994) Total lipid and fatty acid composition of milk from women with and without insulin-dependent diabetes mellitus. Am J Clin Nutr 60:353–361
17. Makrides M, Neumann M, Simmer K, Pater J, Gibson R (1995) Are long-chain polyunsaturated fatty acids essential nutrients in infancy? Lancet 345:1463–1468
18. Martinez M, Mougan I (1998) Fatty acid composition of human brain phopholipids during normal development. J Neurochem 71:2528–2533
19. Minda H, Molnar S, Burus I, Decsi T (2002) Effect of different types of feeding on fatty acid composition of erythrocyte membrane lipids in full-term infants. Acta Paediatr 91:874–881
20. Norris JM, Yin X, Lamb MM, Barriga K, Seifert J, Hoffmann M, Orton HD, Baron AE, Clare-Salzler M, Chase HP, Szabo NJ, Erlich H, Eisenbarth GS, Rewers M (2007) Omega-3 polyunsaturated fatty acid intake and islet autoimmunity in children at increased risk for type 1 diabetes. JAMA 298(12):1420–1428
21. Putnam JC, Carlson SE, DeVoe PW, Barness LA (1982) The effect of variations in dietary fatty acids on the fatty acids composition of erythrocyte phosphatidylcholine and phosphatidylethanolamine in human infants. Am J Clin Nutr 36:106–114
22. Schmid S, Buuck D, Knopff A, Bonifacio E, Ziegler AG (2004) BABYDIET, a feasibility study to prevent the appearance of islet autoantibodies in relatives of patients with Type 1 diabetes by delaying exposure to gluten. Diabetologia 47:1130–1131
23. Stene LC, Joner G, the Norwegian Childhood Diabetes Study Group (2003) Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case–control study. Am J Clin Nutr 78:1128–1134
24. Stene LC, Ulriksen J, Magnus P, Joner G (2000) Use of cod liver oil during pregnancy associated with lower risk of Type 1 diabetes in the offspring. Diabetologia 43:1093–1098
25. Van Beusekom CM, Zeegers TA, Martini IA, Velvis HJR, Visser GHA, van Doormaal JJ, Muskiet FAJ (1993) Milk of patients with tightly controlled insulin-depent diabetes mellitus has normal macronutrient and fatty acid composition. Am J Clin Nutr 57:938–943
26. Warram JH, Krolewski AS, Gottlieb MS, Kahn CR (1984) Differences in risk of insulin-dependent diabetes in offspring of diabetic mothers and diabetic fathers. N Engl J Med 311:149–152
27. World Health Organization (1991) Indicators for assessing breastfeeding practices. Geneva, Switzerland: WHO/CDD/SER91