# IMPORTANCE OF CIS AND *TRANS* ISOMERIC POLYUNSATURATED FATTY ACIDS IN PAEDIATRICS

**Ph.D. Thesis**


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# Abbreviations

n-6 fatty acids:

| | |
|---|---|
| C18:2n-6: | linoleic acid (LA) |
| C18:3n-6: | γ-linolenic acid (GLA) |
| C20:3n-6: | dihomo-γ-linolenic acid (DHGLA) |
| C20:4n-6: | arachidonic acid (AA) |

n-3 fatty acids:

| | |
|---|---|
| C18:3n-3: | α-linolenic acid (ALA) |
| C20:5n-3: | eicosapentaenoic acid (EPA) |
| C22:5n-3: | docosapentaenoic acid (DPA) |
| C22:6n-3: | docosahexaenoic acid (DHA) |

*trans* fatty acids:

| | |
|---|---|
| C16:1n-7*t*: | *trans* hexadecenoic acid |
| C18:1n-7/9*t*: | *trans* octadecenoic acid |
| C18:2n-6*tt*: | *trans* octadecadienoic acid |

| | |
|---|---|
| DKA: | diabetic ketoacidosis |
| EFA: | essential fatty acid |
| LCPUFA: | long-chain polyunsaturated fatty acid |
| NEFA: | non-esterified fatty acid |
| PC: | phosphatidylcholine |
| PE: | phosphatidylethanolamine |
| PL: | phospholipid |
| PUFA: | polyunsaturated fatty acid |
| STE: | sterol esther |
| TG: | triacyl-glycerol |

# Introduction

Long-chain polyunsaturated fatty acids (LCPUFAs) play an important role in the human body in building up cell membranes and in maintaining of their fluidity. The most important fatty acids are the n-3 essential fatty acid, α-linolenic acid (C18:3n-3, ALA) and the n-6 essential fatty acid, linoleic acid (C18:2n-6, LA), as well as their two long-chain metabolites, docosahexaenoic acid (C22:6n-3, DHA) and arachidonic acid (C20:4n-6, AA). DHA and AA play an important role in the perinatal maturation of the nervous system, and as precursors of prostaglandins, thromboxans and leucotriens.

Unsaturated fatty acids are usually found in the nature in cis configuration. *Trans* fatty acids are produced in the stomach of ruminants during the fermentation in their rumen, and during hydrogenization of oils to increase melting point of vegetable oils during food industrial activities. Cis double bond breaks the molecule, while *trans* isomers (similar to saturated fatty acids) are linear in space. Mostly from this different configuration arise their different physiological effects: *trans* fatty acids have similar atherogenic effects like saturated fatty acids, while cis isomers have more beneficial physiological effects. Cis and *trans* isomers use the same enzymes during their metabolism, so *trans* fatty acids may disturb the metabolism of the physiologically important n-3 and n-6 fatty acids.

The potential effect that *trans* fatty acids may disturb the metabolism of essential fatty acids may have great importance in the perinatal period fastest neural growth and differention of the organism. During this perinatal period, the growing organism needs great amounts of DHA and AA for building up different tissues, first of all for building up neural tissues rich in LCPUFA.

## Background and aims

The principal aim of our studies was to investigate, on the one hand, the possible correlation between essential fatty acids and their LCPUFA metabolites and *trans* isomeric fatty acids, on the other hand, the for us most exciting investigations concerning the perinatal period had to be preceded by data collection on fatty acid supply in more stable later ages. So as first step we determined the fatty acid composition of plasma and erythrocyte membrane lipids of diabetic children and young adults. This was followed by our studies on the relationship between *trans* fatty acids and LCPUFA supply in the perinatal period. The work consists of three topics:

1.) Influence of the acute and chronic disturbance of carbohydrate metabolism on fatty acid metabolism. Because data about the relationship between *trans* fatty acids and polyunsaturated fatty acids in the literature are contradictory, first we investigated the possible effect in young diabetic adults and controls. We also aimed to compare fatty acid composition of plasma lipids in children hospitalized in our department because of diabetic ketoacidosis at the beginning of the therapy and after ketoacidosis.

2.) *Trans* fatty acids and LCPUFAs in expecting mothers and their newborns: European multicentre study. Because LCPUFAs play an important role in the perinatal development of the neural system, our aim was to investigate, whether *trans* fatty acids are related to LCPUFA supply during pregnancy in mothers, and at birth in their newborns. Because the supposed relationship can be profoundly influenced by the maternal diet, we used the possibility to study this question with the support of the EU scientific research framework not only in Hungarian, but in German and Spanish expecting mothers too.

3.) *Trans* fatty acids during lactation. Many studies investigated the relationship between the fatty acid composition of human milk, fatty acid composition of the infant's blood and the neurological development of the infant, but the *trans* fatty acid content of human milk was investigated only in few studies covering low number of subjects. This relationship was also investigated in an international collaboration in a sizeable birth cohort study both at the $6^{th}$ week and the $6^{th}$ month of lactation.

# Methods

## *Processing of plasma samples*

Blood samples were drawn into tubes containing 2 mg/ml EDTA as anticoagulant. Samples were centrifuged on 3000 RPM for 10 minutes, and after that the plasma samples were stored in Eppendorf tubes on -80°C until use. For analysis lipids were extracted from 100 µl plasma with a mixture of 3 ml chloroform and 1 ml methanol after melting. Plasma lipids were separated with thin layer chromatography to phospholipid (PL), sterol esther (STE), triacyl-glycerol (TG) and non-esterified fatty acid (NEFA) fractions. During transesterification fatty acid methyl esters were made with a hydrochloric acid methanol. Identification and quantitative determination of fatty acids was made with high resolution capillary gas-liquid chromatography, with the use of internal standard.

## *Processing of erythrocyte membranes*

Erythrocyte mass was washed with isotonic sodium chloride solution three times, and then haemolysed with distillated water at room temperature. We added ice-cold isopropyl alcohol containing 0.5% BHT (butilated hydroxitoluol, antioxidant). Samples were stored on -80°C until further analysis. Erythrocyte membrane lipids were separated with the help of thin layer chromatography to phosphatidylcholine (PC) and phosphatidylethanolamine (PE) fractions. The further processing and chromatographic evaluation of the samples was done according to the methodology of the analysis of plasma samples.

## *Processing of human milk samples*

Human milk samples were stored on -80ºC until analysis. We pipetted the content from the samples into capillary tubes that were centrifuged for 5 minutes in a capillary centrifuge. After 5 minutes we measured the ratio between fat and the sum of milk. The crematocrit and fat content of human milk was calculated with the help of the following formulas:

*Crematocrit: milk fat*100/sum of milk*

*Fat (g/dl): (crematocrit-0.59)/1.46*

In the case when the crematocrit of a human milk sample was over 2%, it was attenuated on the basis of the following formula:

*Fat (g/dl): ((crematocrit*10)-10)*10*

Milk samples needed to be attenuated were added 60ºC isotonic sodium chloride solution. We made methyl esthers from the fatty acids with hydrochloric acid methanol, and chromatographic evaluation of the samples was done according to that written down by plasma samples.

### *Statistical analysis*

For statistical analysis of the data we used Windows SPSS 11.5 program. We used two-sided Student t-test for calculating statistical differences between anthropometrical data of two different groups, while differences between fatty acid data were calculated with Mann-Whitney test. Anthropometrical data of the same group between two timepoints was analysed with one-sided t-test, whereas fatty acid values were compared with Wilcoxon test. Correlations between *trans* fatty acids and cis isomers were calculated with Spearman's linear correlation analysis.

# 1. Cis and trans isomeric polyunsaturated fatty acids in diabetes mellitus

## *1.1 Cis and trans fatty acids in diabetic young adults and controls*

### Persons

Thirtyfour diabetic young adults and thirtysix controls were enrolled in our study. We investigated the fatty acid composition of four plasma (PL, TG, STE and NEFA) and two erythrocyte membrane fractions (PC, PE).

### Results

Values of *trans* hexadecenoic acid (C16:1n-7*t*) were significantly lower in diabetic young adults than in controls in the PL, TG and PE fractions. We did not found any significant differences in the values of *trans* octadecenoic acid (C18:1n-7/9*t*) and *trans* octadecadienoic acid (C18:2n-6*tt*), but the sum of *trans* fatty acids was significantly lower in the diabetic group than in controls in the STE and NEFA fractions.

Values of LA were significantly higher in the STE, but significantly lower in the NEFA and PC lipids in diabetic young adults than in controls. Values of AA and the n-6 LCPUFA were significantly higher in the diabetics than in controls in the PL fraction. The n-3 essential fatty acid, ALA was significantly lower in the NEFA fraction in the diabetic group than in controls. Values of DHA were similarly significantly lower in the STE, PC and PE lipids in diabetics than in controls. Values of the n-3 LCPUFA were significantly lower in the STE, TG, NEFA, PC and PE lipids in diabetic young adults than in controls.

The ratios of n-3 to n-6 PUFA were significantly lower in the PL, STE, PC and PE fractions in diabetic young adults than in controls. The ratios of n-3 to n-6 LCPUFA were also significantly lower in all of the investigated lipid fractions in diabetics than in controls.

In diabetic young adults we found significant negative correlations between *trans* octadecenoic acid and the most important n-6 metabolite, AA in the PL and PE fractions, and between the values of *trans* octadecenoic acid and DHA in the PE fraction. Values of docosapentaenoic acid (DPA, C22:5n-3) correlated significantly and inversely to the values of *trans* octadecenoic acid in the PL and TG fractions, to the

values of *trans* octadecadienoic acid in the TG fraction and to the sum of *trans* fatty acids in the PL and TG fractions. We found significant inverse correlation between values of *trans* octadecenoic acid and n-3 PUFA and n-3 LCPUFA in the PE fraction.

In controls we found significant negative correlations between, on the one hand, *trans* hexadecenoic acid and, on the other hand, values of AA, n-6 PUFA, n-6 LCPUFA, DPA, n-3 PUFA and n-3 LCPUFA in the PC fraction. Values of *trans* octadecenoic acid and n-6 PUFA were correlated also significantly and inversely to each other in the PL and NEFA fractions. We found significant negative correlation between, on the one hand, AA and, on the other hand, values of *trans* octadecenoic acid, and the sum of *trans* fatty acids in the PC fraction.

### Conclusions

In our present study we found significantly higher LA and AA values in diabetic young adults, while values of the n-3 essential fatty acid, ALA, and its most important metabolite DHA were significantly lower in the diabetic group than in controls. Ratios of n-3 polyunsaturated fatty acids to n-6 polyunsaturated fatty acids, and ratios of n-3 long-chain polyunsaturated fatty acids to n-6 long-chain polyunsaturated fatty acids were significantly lower in diabetics than in controls. This observation suggests that it might be useful to supplement the diet of diabetic patients 1-2 times per week with sea fish containing n-3 polyunsaturated fatty acids.

Values of *trans* isomers were significantly lower in the diabetics, than in controls. The human organism cannot synthesise *trans* fatty acids, we ingest them with our food, so the differences seen in the values of *trans* fatty acids could be explained with great probability by the different dietary intake of the two groups. We saw significant and inverse correlations between values of *trans* fatty acids and long-chain polyunsaturated fatty acids in both diabetic patients and healthy controls.

## *1.2 Changes in cis and trans fatty acids during diabetic ketoacidosis in children*

We investigated the relationship between diabetic ketoacidosis and fatty acid composition of plasma lipids in two studies. First we determined the changes in fatty acid values in a small group of children suffering from diabetic ketoacidosis (DKA). After that we followed up the changes in a diabetic child, in that multiple, repeated attacks of diabetic ketoacidosis occurred before the carbohydrate metabolism could permanently be stabilized.

### *1.2.1 Changes in fatty acid composition of plasma lipids in children suffering diabetic ketoacidosis*

#### Persons

We investigated nine children, who were treated because of diabetic ketoacidosis at the Department of Paediatrics in Pécs. Venous blood samples were taken on admission and following the successful treatment of DKA. We analyzed the fatty acid composition of four plasma fractions (PL, TG, STE and NEFA).

#### Results

Values of the n-6 essential fatty acid, LA were significantly decreased after DKA in PL, TG and NEFA fractions. Values of the most important metabolite, AA were significantly higher in the TG fraction after DKA, while values of two 22-carbon metabolites derived from AA (C22:4n-6 and C22:5n-6) significantly increased in PL and NEFA fractions after DKA. Values of the n-3 essential fatty acid, ALA did not change in any fractions significantly, but its most important metabolite, DHA rose significantly in the TG after DKA. The AA/LA ratio reflecting the activity of Δ-5 and Δ-6 desaturase together, and (GLA+DHGLA)/LA ratio characterising the Δ-6 desaturation step rose significantly after DKA in the PL fraction.

There were no significant changes in the values of *trans* fatty acids after DKA in the PL, TG and STE fractions, but values of *trans* octadecenoic acid and the sum of *trans* fatty acids increased significantly after DKA in the NEFA fraction. We found significant negative correlation between, on the one hand, LA and, on the other hand, values of *trans* octadecenoic acid and the sum of *trans* fatty acids in the TG fraction during DKA, and after DKA between values of LA and *trans* octadecenoic acid in the PL fraction and values of LA and the sum of *trans* fatty acids in the NEFA fraction. Values of AA correlated significantly and inversely to values of *trans* octadecadienoic acid in the TG fraction, and to the sum of *trans* fatty acids in the STE fraction during DKA, and to values of *trans* octadecenoic acid in PL, *trans* octadecadienoic acid in STE, and the sum of *trans* fatty acids in PL fraction after DKA. Values of ALA were correlated significantly and inversely to *trans* octadecenoic acid, *trans* octadecadienoic acid and the sum of *trans* fatty acids in TG fraction during DKA and after DKA to values of *trans* octadecenoic acid in NEFA fraction and to values of the sum of *trans* fatty acids in the PL and NEFA fractions.

### *1.2.2 Changes in fatty acid composition of plasma lipids in a diabetic child during and after repeated episodes of ketoacidosis*

#### Person

We investigated blood samples of a diabetic young adult during nine different ketoacidosis at admission and after recovery from DKA, that is, after finishing intravenous treatment. We investigated four plasma fractions (PL, TG, STE and NEFA). By the end of the 16$^{th}$ month of our observation we were finally able to convince the patient about the need of intensification of her therapy by subcutaneous continuous insulin infusion that led to the cessation of the need for repeated admission for DKA.

#### Results

Changes in fatty acid values were significant in the PL fraction. Values of the n-6 essential fatty acid, LA decreased significantly after DKA, while values of the metabolite dihomo-γ-linolenic acid (DHGLA, C20:3n-6) rose significantly. Although we did not find significant differences in the values of the most important metabolite, AA, but values of the from AA synthesized C22:4n-6 were significantly higher after than during DKA. Values of the n-3 essential fatty acid, ALA did not change significantly during the recovery from DKA, but values of its most important metabolite, DHA increased significantly after DKA.

Values of the AA/DHGLA ratio reflecting the Δ-5 desaturational step and the (GLA+DHGLA)/LA ratio reflecting the Δ-6 desaturational step were significantly higher after than during DKA. The AA/LA ratio reflecting the activity of Δ-5 and Δ-6 desaturase together was also significantly higher after than during DKA.

#### Conclusions

After diabetic ketoacidosis the values of the n-6 essential fatty acid, LA decreased significantly, while values of the from it synthesized n-6 polyunsaturated fatty acids increased significantly. These results can be explained with the increased transformation of LA. On the basis of product/substrate ratio it seems to be probable, that during the recovery from DKA the activity of Δ-6 desaturase enzyme is rising. Our results support our previous hypothesis that the differences seen in the availability of fatty acids in diabetic children could be connected to the non-physiological insulin status.

Fatty acid composition of plasma lipids after DKA came closer to values characteristic for healthy people. Therefore our results raise the possibility that differences in plasma fatty acid composition may play an additional role in the complex metabolic disturbance of diabetic ketoacidosis.

Observing nine subsequent ketoacidosis of a diabetic child we found similar result as investigating ketoacidosis of nine different children. So the phenomenon that values of LCPUFA are increasing in the plasma of diabetic children after recovery from DKA seems to be independent from the individual differences in fatty acid supply. This phenomenon observed several times in the same patient corroborates our previous observation that disturbances of fatty acid supply in diabetes is in strong correlation with insulin supply.

# 2. Cis and trans isomeric polyunsaturated fatty acids in expecting mothers and their newborns

## *2.1 Cis and trans polyunsaturated fatty acids in European expecting women during pregnancy and at delivery*

### Persons

In this study Spanish, German and Hungarian expecting mothers were included at the 20$^{th}$ week of gestation. At inclusion, at the 30$^{th}$ week of gestation and at delivery in all three countries colleagues measured the height, body weight, blood pressure of the mothers and they filled in detailed social and food frequency questionnaires and drew venous blood samples. In this study only healthy expecting mothers were included who had uncomplicated singleton pregnancy.

Part of the investigation was a supplementation study where women were divided into four groups and they received from the 20$^{th}$ week of gestation either 500 mg docosahexaenoic acid or 400 mg 5-metil-tetrahydro-folic acid or 500 mg docosahexaenoic acid and 400 mg 5-metil-tetrahydro-folic acid or placebo until delivery. Because supplementation may change the relationship between *trans* fatty acids and polyunsaturated fatty acids, so at the 30$^{th}$ week of gestation and at delivery we only evaluated the blood samples of the placebo group.

### Results

At the 20$^{th}$ week of gestation values of *trans* hexadecenoic acid were significantly higher in German than in Spanish and Hungarian expecting mothers in the PC fraction. In the PE fraction in Hungarian expecting women values of all the investigated *trans* fatty acids were significantly lower, than in the mothers of the two other nations.

At the 20$^{th}$ week of gestation values of the essential fatty acid, LA correlated significantly and inversely to values of *trans* octadecadienoic acid in Spanish expecting mothers in the PC fraction. However, there were significant inverse correlations between the values of the most important n-6 metabolite, AA and almost all of the investigated *trans* fatty acids in all three nations in both erythrocyte membrane fractions.

We found no correlation between the n-3 essential fatty acid, ALA and *trans* fatty acids in the PC fraction, whereas in the PE fraction we found significant inverse correlation with all the investigated *trans* fatty acids in Spanish, and with *trans*

octadecenoic acid and the sum of *trans* fatty acids in German expecting women. Values of the most important n-3 metabolite, DHA correlated significantly and inversely to all the investigated *trans* fatty acids in German expecting mothers in the PC fraction, and in both German and Spanish expecting mother in the PE fraction. We found significant negative correlations between, on the one hand, values of DHA and, on the other hand, *trans* octadecenoic acid and the sum of *trans* fatty acids in Spanish and Hungarian expecting mothers in the PC fraction, and we also found negative correlation with the values of *trans* octadecadienoic acid in Spanish expecting mothers in the PC fraction.

At the 30$^{th}$ week of gestation values of *trans* hexadecenoic acid were significantly higher in Spanish than in Hungarian expecting mothers in the PC fraction. Values of *trans* octadecenoic acid were significantly higher in Spanish than in German and Hungarian expecting mothers. In the PE fraction values of *trans* octadecenoic acid in Spanish expecting mothers were again the highest. Values of the sum of *trans* fatty acids were also significantly higher in Spanish than in German expecting mothers.

At the 30$^{th}$ week of gestation we found significant negative correlations between, on the one hand, values of the essential fatty acid, LA and, on the other hand, *trans* octadecenoic acid, *trans* octadecadienoic acid and the sum of *trans* fatty acids in Spanish expecting mothers in the PC fraction, while we found significant positive correlations with *trans* octadecenoic acid and the sum of *trans* fatty acids in Hungarian expecting mothers in the PE fraction. We found significant negative correlations between the values of the most important n-6 metabolite, AA and all the investigated *trans* fatty acids in German expecting mother in both PC and PE fractions. We did not found any negative correlations between *trans* isomers and values of AA in the PC fraction of the two other nations, but in the PE fraction we found significant negative correlations with values of *trans* octadecenoic acid an the sum of *trans* fatty acids in German, and with values of *trans* octadecadienoic acid in Hungarian expecting women. Values of the most important n-3 metabolite, DHA correlated significantly and inversely to all of the investigated *trans* fatty acids in German expecting women in both PC and PE fractions. We also found significant inverse correlations with *trans* octadecenoic acid in Spanish mothers in the PC and PE fractions and with the sum of *trans* fatty acids in the PE fraction.

At delivery we found no significant differences between the values of *trans* fatty acids in either PC, or PE fractions in the mothers of the three nations.

At delivery we found no significant correlations between the essential fatty acid, LA and the investigated *trans* fatty acids in any of the nations. In contrast, values of the most important n-6 metabolite, AA correlated significantly and inversely to all the investigated *trans* fatty acids in Spanish mothers in the PE fraction. We found significant negative correlations between values of, on the one hand, ALA and, on the other hand, *trans* octadecenoic acid and the sum of *trans* fatty acids in Spanish mothers in the PE fraction. Values of the most important n-3 metabolite, DHA correlated significantly and inversely to *trans* hexadecenoic acid in the PC fraction, and to *trans* octadecenoic acid and the sum of *trans* fatty acids in the PE fraction.

### Conclusions

In our present study we found significant negative correlations between values of *trans* fatty acids and the physiologically important n-3 and n-6 long chain polyunsaturated fatty acids at the 20$^{th}$ week of gestation. This negative correlation was observed also at the 30$^{th}$ week of gestation and at delivery. Because *trans* fatty acids are not synthesized in the human body, *trans* fatty acids detected in the maternal circulation should come from diet (differences seen in *trans* fatty acid content of blood samples of Spanish, German and Hungarian mothers could be explained with the different dietary intakes). Because AA and DHA play an important role in the development of fetal nervous system, higher maternal *trans* fatty acid intake could disturb the n-3 and n-6 polyunsaturated fatty acid supply of the mother and the fetus too.

## *2.2 Cis and trans fatty acids in cord blood of European newborns*

### Persons

We investigated newborns of the mothers participated who received placebo during pregnancy.

### Results

We found no significant differences among the *trans* fatty acid values in cord blood of Spanish, German and Hungarian newborns either in PC or PE fractions.

Values of the essential fatty acid, LA correlated significantly and inversely to *trans* octadecadienoic acid in the PC fraction in cord blood lipids of German newborns. Values of the most important n-6 metabolite, AA correlated significantly and inversely to values of all the investigated *trans* fatty acids in Spanish newborns in the PC and PE

fractions. We also found significant negative correlations with *trans* hexadecenoic acid in Hungarian newborns in the PC fraction, and in German newborns with the sum of *trans* fatty acids in the PE fraction.

We found significant negative correlation between the essential ALA and the *trans* octadecenoic acid in German newborns in the PC fraction. Values of DHA correlated significantly and negatively to *trans* octadecenoic acid, *trans* octadecadienoic acid and the sum of *trans* fatty acids in Spanish newborns in the PC fraction.

## Conclusions

*Trans* fatty acids found in the cord blood of newborns come from the maternal blood, so they reflect maternal diet. Similarly to expecting women, we found significant negative correlations between *trans* isomers and the values of the two most important long-chain metabolites, AA and DHA in newborns. These results suggest that *trans* fatty acid intake during pregnancy could disturb the availability of those metabolites that play an important role in the perinatal development of the nervous system in the fetus.

From the further scientific research point of view our results show that *trans* isomeric polyunsaturated fatty acids should be taken into account as confounders in studies investigating DHA supplementation in expecting women.

# 3. Cis and trans polyunsaturated fatty acids in human milk

## *3.1 Cis and trans polyunsaturated fatty acids at the 6th week of lactation*

### Persons

In this prospective birth cohort study we enrolled those expecting women who came to the Department of Gynecology and Obstetrics at the University of Ulm and gave birth to healthy, term newborns. We excluded those mothers from the further analysis, whose child was born before the 32nd week of gestation, or whose birth weight was under 2500 g or the newborn was transferred to neonatal intensive care unit after birth.

Six weeks postpartum German colleagues contacted by telephone 1024 mothers (96%) successfully, 786 (76.7%) were still breastfeeding their infants, and 769 milk samples (that is 97.8% of the previous value) could be collected. A trained nurse visited all women who were still breastfeeding and collected 10 ml human milk, which was immediately frozen.

### Results

Although we did not find significant correlations between the 16-carbon *trans* hexadecenoic acid and the values of the n-6 essential fatty acid, LA, values of the from it synthesized metabolites, DHGLA and AA showed weak, but significant positive correlations with *trans* hexadecenoic acid. We also found significant positive correlation between the values of *trans* hexadecenoic acid and the n-3 essential fatty acid, ALA.

We found significant and strong negative correlations between the values of, on the one hand, the 18-carbon *trans* polyunsaturated fatty acid, *trans* octadecenoic acid and, on the other hand, the n-6 essential fatty acid, LA and the from its synthesized γ-linolenic acid (C18:3n-6, GLA), DHGLA and AA. Values of the n-3 essential fatty acid, ALA and the from its synthesized eicosapentaenoic acid (C20:5n-3, EPA), DPA and DHA were also significantly and inversely correlated to *trans* octadecenoic acid. Similarly, we found significant negative correlations between the *trans* octadecadienoic acid and the values of LA and the from it synthesized DHGLA and AA. Values of *trans* octadecadienoic acid and the n-3 essential fatty acid, ALA and the from it synthesized EPA, DPA and DHA correlated significantly and inversely.

The sum of *trans* fatty acids showed significant negative correlations with the values of the essential n-6 fatty acid, LA and the from it synthesized DHGLA and AA. We also found significant inverse correlation between the sum of *trans* fatty acids and the values of essential n-3 fatty acid, ALA and the from it synthesized EPA and DHA. On the one hand, the sum of *trans* fatty acids and, on the other hand, values of n-6 PUFA and the sum of n-6 LCPUFA correlated significantly and inversely. The inverse correlation between the sum of *trans* fatty acids and the sum of n-6 LCPUFAs proved to be stronger than the negative correlation found between the sum of *trans* fatty acids and n-6 PUFAs.

## *3.2 Cis and trans polyunsaturated fatty acids at the 6th month of lactation*

### Persons

We investigated the mothers who participated in the birth cohort study in Ulm. Six months postpartum German colleagues contacted the mothers by telephone and 462 milk samples could be collected.

### Results

We did not find any significant correlation between the 16-carbon *trans* hexadecenoic acid and values of the n-6 essential fatty acid, LA, and the from it synthesized most important metabolite, AA, but we found significant positive correlation between the values of *trans* hexadecenoic acid and GLA. We did not found significant correlations between values of *trans* hexadecenoic acid and the values of the n-3 essential fatty acid, ALA and its most important metabolite, DHA, but values of *trans* hexadecenoic acid and EPA correlated significantly and positively, while with DPA they were correlated significantly and negatively.

We found significant and strong negative correlations between the 18-carbon *trans* octadecenoic acid and values of the essential fatty acid, LA and the from it synthesized GLA, DHGLA and AA. We also found significant negative correlations between *trans* octadecenoic acid and values of the n-3 essential fatty acid, ALA and its two metabolites, EPA and DPA. We found significant negative correlations between the *trans* octadecadienoic acid and values of the essential fatty acid, LA, and the from it synthesized GLA and AA. We found no significant correlation between *trans* octadecadienoic acid and values of the essential fatty acid, ALA, but values of *trans*

octadecadienoic acid and the most important n-3 metabolites, EPA, DPA and DHA correlated significantly and inversely.

Values of the sum of *trans* fatty acids showed significant negative correlations with the n-6 essential fatty acid, LA and its most important metabolite, AA, and the n-3 essential fatty acid, ALA. However we did not find significant correlation between the sum of *trans* fatty acids and DHA. The sum of *trans* fatty acids and values of both n-6 PUFA and the n-6 LCPUFA correlated significantly and inversely.

## Conclusions

Several factors support the streght of the present study. First we investigated a sizeable group of lactating mothers: the number of investigated mothers was almost twice as high as the cumulative number of the mothers investigated in the 3 previous studies that reported associations between *trans* fatty acids and polyunsaturated fatty acids in human milk. Second we investigated a relatively homogenous group of lactating mothers both at the 6$^{th}$ week and 6$^{th}$ month of lactation. Third, we were able to control for the confounding factor of nationality.

In our present birth cohort study we found significant inverse correlations between the 18-carbon *trans* polyunsaturated fatty acids, the *trans* octadecenoic acid and *trans* octadecadienoic acid and the long chain polyunsaturated fatty acids at the 6$^{th}$ week of lactation in human milk. However, we did not find negative correlations between the 16-carbon *trans* polyunsaturated fatty acid and the long chain, polyunsaturated metabolites. Because cis and *trans* isomers use the same enzymes during their metabolism, our results raise the possibility that 18-carbon *trans* isomers may reduce long chain polyunsaturated fatty acid supply in human milk.

At the 6$^{th}$ month of lactation we still found strong negative correlations between *trans* octadecenoic acid and *trans* octadecadienoic acid and values of the most important n-3 and n-6 fatty acids.

Because *trans* fatty acids found in the human milk must originate from the maternal diet, our present results raise the possibility that maternal *trans* fatty acid intake may disturb in breastfed infants the long chain, polyunsaturated fatty acid supply that play an important role in the development of nervous system.

## New results

1. We found significantly lower n-3 and higher n-6 fatty acid values in diabetic young adults than in healthy controls.
2. At studying the effect of diabetic ketoacidosis on essential fatty acid metabolism we found that during recovery from ketoacidosis the percentage contribution of essential fatty acids to plasma lipids is decreasing, while the percentage contribution of long-chain polyunsaturated fatty acids is increasing. These changes indicate that the metabolism of essential fatty acids is influenced the effect of insulin.
3. By investigation the course of repeated ketoacidosis in a young adult, i. e. by excluding the effect of diet among individuals, we corroborated the observation on the role of insulinemia in essential fatty acid metabolism.
4. We found significant negative correlations between values of *trans* fatty acids and long-chain polyunsaturated fatty acids in erythrocyte membrane lipids in expecting mothers both at the $20^{th}$ and $30^{th}$ week of pregnancy and at delivery.
5. We also found significant negative correlations between *trans* fatty acids and long-chain polyunsaturated fatty acids in erythrocyte membrane lipids in cord blood samples of newborns.
6. In human milk samples of in a sizeable group of lactating mothers, values of *trans* isomers and polyunsaturated fatty acids correlated significantly and inversely at the $6^{th}$ week of lactation.
7. We still found negative correlations between *trans* isomers and values of polyunsaturated fatty acids at the $6^{th}$ month of lactation.

## Practical use of the results

1. Lower n-3 polyunsaturated fatty acids found in young diabetic adults than in controls and the decreasing ratios of n-6 polyunsaturated fatty acids to n-3 polyunsaturated fatty acids during recovery of diabetic ketoacidosis suggest that the supplementation of the diet of diabetics with food rich in n-3 fatty acids may be beneficial.
2. During pregnancy *trans* fatty acid intake may influence the long chain polyunsaturated fatty acid supply that play an important role in the maturation of fetal nervous system, so it might be useful to give the following advices to expecting women: they should reduce in their diet the quantity of margarines, fried food, biscuits and cakes containing hydrogenized oils in large amounts.
3. On the basis of the negative correlations between *trans* fatty acids and long chain polyunsaturated fatty acids in human milk samples of lactating women it might be useful to advise also lactating women to lower the dietary intake of *trans* fatty acids.
4. At planning each scientific study in that fatty acid supply is investigated in expecting or lactating women supplemented with long chain polyunsaturated fatty acids, dietary intake of *trans* fatty acids have to be considered as an independent confounding variable.

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