Feature

Maastricht essential fatty acid birth cohort

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Supporting Table S1 is available online http://dx.doi.org/10.1002/lite.201500007

Summary

The Maastricht Essential Fatty Acid Birth cohort (MEFAB) was established in 1989 to study the changes in fatty acid concentration during pregnancy and how this related to the fatty acid concentrations of the neonate. The original sample contains data of 1203 subjects. Some participants whom participated in the original MEFAB study also participated in follow-up studies at age 4, 7 or 12; 41%, 52% and 35% of eligible participants, respectively. Collected data include maternal fatty acid concentrations at multiple points during pregnancy and at birth and at age 7 for the child, but also anthropometric measurements during development, assessments of cognitive development, asthma, atopy and cardiovascular risk factors. Data of MEFAB have been used in 37 articles in peer-reviewed journals and 4 doctoral theses have been completed. Data of MEFAB is upon request available for new research questions.

Why was the cohort set up?

From the 1970s onwards essential fatty acids (EFA) and especially their longer-chain, more unsaturated derivatives, the long-chain polyunsaturated fatty acids (LCPUFA) have been a topic of research and discussion because of their potential beneficial effects on numerous health conditions. In the past decades beneficial associations have been found with for example heart disease, metabolic syndrome, cognitive decline, asthma, immune support, visual development and depression (for a review see for example [1]).

In the late 1980's it was serendipitously found that the walls of umbilical arteries and the veins demonstrate biochemical signs of EFA deficiency [2]. This led to the question whether the suboptimal supply of EFA during foetal life would exert an influence on development. Moreover, it was not known how maternal availability of LCPUFA during pregnancy related to the concentrations of the infants and whether a particular period in pregnancy or a specific fatty acid had a distinct importance. It was hypothesized that the maternal EFA and LCPUFA status was inadequate to support optimal tissue concentrations in the developing foetus. Because of the important biological activities of these fatty acids and the inability of the foetus to produce the n-3 LCPUFA in sufficient amount itself, this insufficient supply could have important implications for later health and functioning.

The Maastricht Essential Fatty Acid Birth cohort (MEFAB) was established in 1989 to study the changes in fatty acid concentration during pregnancy and how this related to the fatty acid concentrations of the neonate. Furthermore the association between LCPUFA status of mothers during pregnancy and of their infants at birth in relation to various birth outcomes (weight, length and head circumference) was studied. For this purpose 42 different fatty acids were determined in maternal plasma in early, middle and late pregnancy.

Furthermore, these fatty acids were determined in the blood vessels of the umbilical cord and plasma of the child. Thus the association between the status of mother and the status of the child could be determined.

Later three follow-up studies were added at age 4, 7 and 12 where further assessments of cognitive development, asthma/atopy, growth and cardiovascular disease risks were performed.
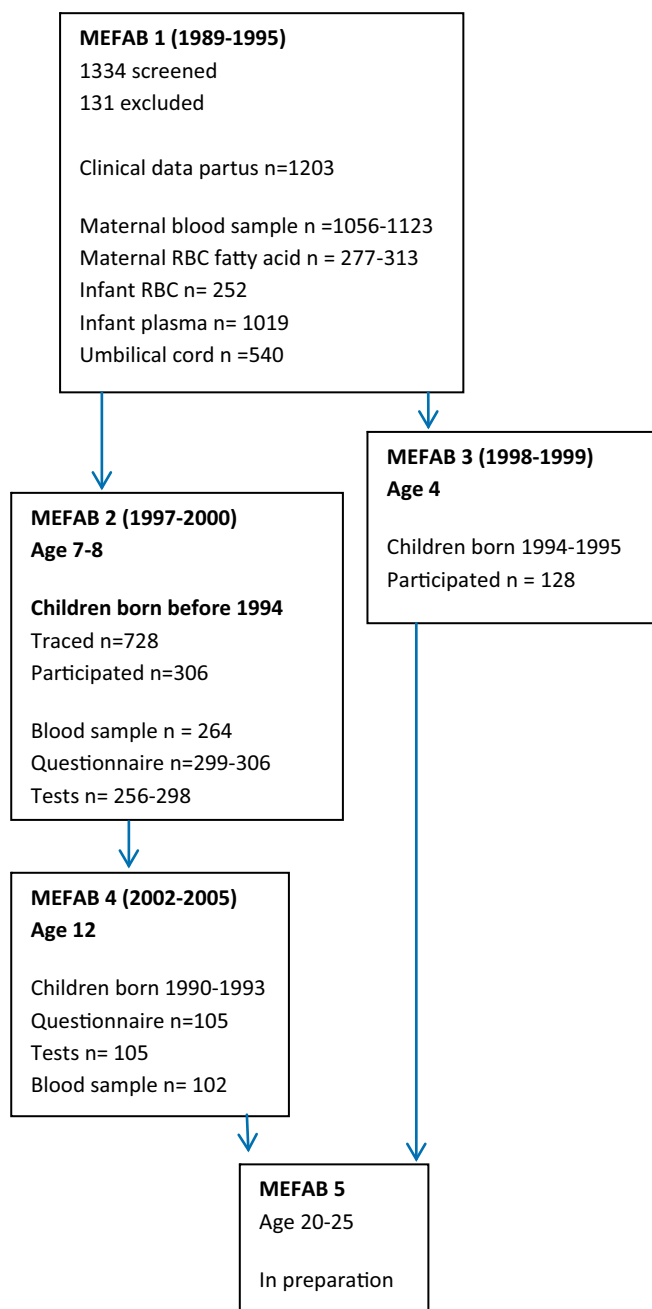
MEFAB was originally founded by Professor Gerard Hornstra at the department of Human Biology, Maastricht University. After his retirement in 2003, the NUTRIM School for Nutrition, Toxicology and Metabolism at Maastricht University took over the MEFAB cohort. A multidisciplinary steering committee continues the follow-up of this cohort for its 25th year now.

Who is in the cohort?

Pregnant women attending one of 3 antenatal clinics in the South of Limburg (a province located in the South of the Netherlands) at the time of their first antenatal visit between 1989 and 1995 were asked to participate in the study. Women were eligible to participate if they were less than 16 weeks pregnant and did not suffer from any cardiovascular, neurological, renal or metabolic condition. A total of 1334 pregnant women were screened for this study. 131 women were either excluded or dropped out before partus, leaving 1203 subjects available for analyses (90%).

How often have they been followed-up

For the flow diagram see **Figure 1**. The pregnant women were followed during pregnancy and after pregnancy and their neonates were studied at birth (MEFAB 1). Between 1997 and 2000, when the children were approximately 7 years old a follow-up study was

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MEFAB 1 (1989-1995)

1334 screened

131 excluded

Clinical data partus n=1203

Maternal blood sample n =1056-1123
Maternal RBC fatty acid n = 277-313
Infant RBC n= 252
Infant plasma n= 1019
Umbilical cord n =540

MEFAB 2 (1997-2000)
Age 7-8

Children born before 1994
Traced n=728
Participated n=306

Blood sample n = 264
Questionnaire n=299-306
Tests n= 256-298

MEFAB 3 (1998-1999)
Age 4

Children born 1994-1995
Participated n = 128

MEFAB 4 (2002-2005)
Age 12

Children born 1990-1993
Questionnaire n=105
Tests n= 105
Blood sample n= 102

MEFAB 5
Age 20-25

In preparation

Figure 1. Flow-chart.

executed to investigate the longer term associations between their prenatal and perinatal exposure to EFA and LCPUFA and selected aspects of their mental and physical development (MEFAB 2). For this follow-up singletons born before 1994 of whom an umbilical blood sample was available, were eligible for participation.

Parents of children born between January 1994 and September 1995 were asked to let their children participate in a follow-up when they were approximately 4 years old (MEFAB 3). The main focus of MEFAB 3 was cognitive development. Parents of children born between 1990 and 1993 who participated in both MEFAB 1 and MEFAB 2 were asked to let their child participate in MEFAB 4 between 2002 and 2005. The focus of MEFAB 4 was body composition and obesity. Exclusion criteria for MEFAB 4 were any chronic illnesses or depression in the child.

Currently a 25-year follow-up is in preparation, which will focus on cardio metabolic risk factors and brain functioning.

Ethics

Pregnant woman who entered the study gave written consent for their participation and for the use of the plasma, red blood cells and/or umbilical cord of their neonate (MEFAB 1). For both ME-FAB 2 and MEFAB 3 both parents (if possible) gave written consent for their own participation and for that of their child in the study. For MEFAB 2 consent was obtained for questionnaires, blood sampling, and for the execution of cognitive function tests, motor function tests, physical fitness tests and peak flow measurements. Some parents only gave consent for either the tests or the questionnaires. For MEFAB 3 parents gave their informed consent for both the questionnaires and the cognitive development test or for the questionnaires only. For MEFAB 4 both parents and children gave their informed consent for participation in the study and for the use of the questionnaires, blood sampling and body composition measurements.

The initial study and all follow-up studies were approved by the Ethics committee of the University hospital Maastricht/Maastricht University.

Attendance

The number of available maternal blood samples during pregnancy and immediately after delivery differed per time point and varied between 1057 (79%) and 1123 (84%).

For MEFAB 2 750 children were eligible to participate. Of these 728 (97%) were traced and 691 (92%) could be invited. A total of 364 parents did not reply or did not consent, 21 children dropped out during the study. The final study population eventually consisted of 306 children (41%), with blood samples of 265 children (39%).

For MEFAB 3 246 children were eligible. Of these children, 1 had passed away, 38 could not be localized, 36 parents only consented to the questionnaires, 39 parents did not consent at all and 4 children dropped out, leaving 128 (52%) children in the study population.

For MEFAB 4 305 children were eligible (participated both in MEFAB 1 and 2), 105 (35%) of these children participated (See **Figure 1**).

What has been measured?

In **Table 1** all measurements from MEFAB 1 to MEFAB 4 are listed.

Fatty acid composition of maternal plasma phospholipids was assessed in blood collected at study entry (approximately 14 weeks of pregnancy), 22 and 32 weeks of pregnancy and immediately after delivery. Additionally, fatty acid measurements were performed in umbilical cord plasma phospholipids of their neonates and in phospholipids isolated from the walls of umbilical arteries and veins. The standardized methods used to measure plasma fatty acid concentrations and the other study parameters at baseline and during follow-ups have been described in detail by Al et al. [3].

During the follow-up of the children, anthropometric parameters (height, weight, skin fold thickness measurements, and waist circumference) were collected at age 0.5, 1, 2, 3, 4 (data received from local health services) and at age 7. Children who participated in MEFAB 2 donated blood for the determination of the plasma lipoprotein profile, haematology profile, clotting profile, apolipoprotein E (ApoE) and cholesteryl ester transfer protein (CETP) and polymorphisms and plasma leptin, C reactive protein, glucose, insulin and proinsulin concentrations. Children who participated in ME-

Table 1. Data available at the different parts of the MEFAB cohort.

MEFAB 1 1989–1995	Maternal	Social economic status (based on postal code). Clinical data during pregnancy and delivery from hospital records. Fatty acid profile of phospholipids (PL) during pregnancy (<16, 22, 32 weeks) and at delivery or maternal fatty acid composition of erythrocyte PL at same time points.
	Neonatal	Birth outcome (weight, length and head circumference) and conditions at birth (Apgar score, and pH and pCO2 of cord blood). Fatty acid profile of cord plasma PL, umbilical artery wall PL, umbilical vein wall PL or cord blood erythrocyte PL (multiple possible).
MEFAB 2 1997–2000	Maternal	Medical questionnaire related to pregnancy and birth and family anamnesis. Intelligence (RAVEN), Fatty acid profile of maternal plasma PL at follow up. Growth (weight and height) during early childhood.
	Children	Body composition based on skin folds (body fat, fat mass, fat free mass). Early development, medical history and medical examination of children at age 7. Atopy questionnaire. Mental processing (Kaufman ABC). School results (spelling, reading, arithmetic and vocabulary). Motor functions (Kaufman motor ABC and Maastricht motor test). Child Behaviour Checklist. Neurological examination. Physical fitness at age 7 (blood pressure heart rate, exercise test). Peak flow measurement at rest and at physical exhaustion. *Blood sample*: Hematology profile. Clotting profile. Plasma lipoprotein profile, ApoE, CETP polymorphisms. Leptin and C reactive protein in plasma. Fatty acid composition of children's plasma PL. Fasting plasma glucose, insulin and proinsulin concentrations. *Subsample*: Language (Revision Amsterdam child intelligence test) and mental fatigue score.
MEFAB 3 1998–1999	Parental	Maternal intelligence measured with Progressive Matrices test of Raven. Maternal and paternal education (8 point scale).
	Children	Kaufman Assessment Battery for Children. Groningen Developmental Scale. McCarthy Scales of Children's Mental Ability (motor scale only).
MEFAB 4 2002–2005	Parental	Weight and height Attitude towards eating (Three factor eating Questionnaire).
	Children	Weight, height and BMI at age 1, 7, 12. Body composition (body fat, fat mass and fat free mass). Attitude towards eating (Three factor eating Questionnaire). Physical activity (Baecke questionnaire). *Blood sample*: Serum leptin concentration, polymorphism of obesity related genes coding for PPARγ2, glucocorticoid receptor and ciliary neurotrophic factor.

FAB 2 also underwent a Bruce treadmill test to measure physical fitness. They also performed extensive peak flow measurements. For children who participated in MEFAB 3 information on children's neurodevelopment was collected at age 4 (Groningen Developmental Scale, motor scale of the McCarthy Scales of Children's Mental Abilities) for those who participated in MEFAB 2 this information was collected at age 7 (Kaufman Assessment Battery for Children, Maastricht Motor Test, Revised Amsterdam Child Intelligence Test, Child Behavior Checklist). Mothers of the children who underwent the neurodevelopment test completed the RAVEN test to measure intelligence.

Participants in MEFAB 4 again donated blood in which serum leptin concentration, polymorphism of obesity related genes coding for PPARγ2, glucocorticoid receptor and ciliary neurotrophic factor were determined. Furthermore they filled out a questionnaire about attitude towards eating and a questionnaire about their physical activity.

What has been found? Key findings and publications

At this moment 37 articles using MEFAB data have been published in peer reviewed journals and 4 doctoral theses have been completed (for all articles see **Supporting Table S1**; these articles are referred to with author's name and year in the brackets).

MEFAB 1

Results concerning MEFAB 1 have been summarized by Al and Hornstra [4–6]. In this paper we will focus on the findings of the follow-up studies; however all articles can be found in **Supporting Table S1**. In the current paper we will shortly summarize the most important findings from MEFAB 1, for more in depth information the reviews can be consulted.

The maternal plasma concentration of phospholipid-associated essential PUFA increased during pregnancy by on average 40%, however the non-essential fatty acids increased more than 65%. Furthermore, it was shown that absolute and relative amount of DHA in the maternal plasma phospholipids were significantly lower in multigravidae compared to primigravidae. Strong correlations between maternal and foetal EFA and LCPUFA were observed. Presence of trans fatty acids in the cord tissue was associated with proportional lower amount of essential PUFA. Preterm infants had a significantly lower LCPUFA status compared to term neonates, but this appeared a physiological phenomenon. Significant positive associations were found between the maternal intake of n-3 fatty acids plus arachidonic acid (AA) and birth length (BL), whereas the intake of linoleic acid (LA) was negatively related to head circumference (HC) (Badart Smook 1997). In addition, a positive association was observed between maternal DHA concentrations early in pregnancy and birth weight (BW) and HC (Dirix 2009a). In contrast, maternal concentrations in late pregnancy and/or at delivery of AA and dihomo γ-linolenic acid (DGLA, the direct precursor of AA) were negatively related to BW and BL. This suggests that maternal docosahexaenoic acid (DHA) content may program foetal growth in a positive way and that the maternal AA may be involved in foetal growth limitation. Interestingly, significant negative associations were observed between BW and umbilical plasma DHA concentration (Dirix 2009c). There is evidence to suggest that

this is caused by a limited DHA transfer from mother to foetus, resulting in lower DHA concentrations in larger foetuses (Rump 2001a). This would imply that relationships with function variables are less convincing for neonatal than for maternal fatty acid concentrations.

Metabolic syndrome

In MEFAB 2 the association between early life time exposure to LCPUFA and markers of the metabolic syndrome (e.g. obesity and insulin resistance) was studied. Rump (2002a) showed that umbilical cord plasma phospholipid concentrations of γ-linolenic acid and DGLA were negatively related to plasma insulin concentrations and the calculated insulin resistance at age 7. Gamma-linoleic acid was also negatively related to body fatness, pro-insulin levels and leptin concentrations at age 7. Interestingly, in the study of De Vries (2014) it was shown that *maternal* DGLA concentrations throughout pregnancy were *positively* associated with an increased BMI at age 7.

Neural development

Another focus of MEFAB 2 (and MEFAB 3) was the association between early LCPUFA exposure (as reflected by umbilical plasma and red cell AA and DHA concentrations) and later brain function, cognition and behaviour. Neither at age 4 (Ghys 2002) nor at age 7 (Bakker 2003) were significant associations found between LCPUFA exposure during pregnancy and cognition. Bakker (2009) did observe that movement quality (a measure of brain-muscle interaction and a low score of which may predict ADHD and later learning problems) at age 7 was positively associated with plasma phospholipid DHA levels measured at birth, but were unrelated to DHA concentrations at follow-up. This suggests that prenatal LCPUFA availability may be more important for later motor function than childhood dietary LCPUFA intake. In contrast, associations with early AA levels tended to be negative, but were not significant. When looking at behaviour, research has found that higher levels of DHA at birth are associated with lower levels of internalizing behaviour at age 7 (Krabbendam 2007). Relationships with DHA status at follow-up were, again, not significant. This could imply that the perinatal exposure to higher DHA levels may reduce the risk for depression in adult life.

Other measurements

No clear and consistent associations were found between prenatal AA status and lung function or plasma inflammation markers at age 7 (Dirix 2009b).

What are the main strengths and weaknesses?

One of the main strengths of the MEFAB study and database, is that it is, as far as we know, world-wide the only prospective mother-child cohort containing extensive data on maternal plasma fatty acid composition at various time points during pregnancy and at delivery. In addition, fatty acid data are available from umbilical plasma as well as from the walls of umbilical veins and arteries. This enables the assessment of perinatal (umbilical plasma) and earlier (umbilical vessel walls) fatty acid exposure of the developing foetus. MEFAB is one of the few fatty acid related cohorts that is being followed up for a long period of time and has presently the potential to contact participants 25 years after birth. Another strength of the cohort is the fact that all common essential and non-

essential fatty acids have been measured, including the non-essential longer-chain, more unsaturated derivatives. Since some of these latter fatty acids (20:3n-9 and 22:3n-9) are essential fatty acid status markers, MEFAB data can be used to relate later functions to pre and perinatal EFA and LCPUFAs statuses. Furthermore, fatty acid data are available from different time points during pregnancy; consequently, later functional conditions can be related to fatty acid exposures during early or later fetal development. Alongside the measurements of fatty acids, a vast array of other measurements were taken at different follow-ups which are now available to study the influence of pre- and perinatal LCPUFA exposure to a large number of health outcomes. Data collection in the MEFAB cohort started around 25 years ago, follow-ups provide cost-effective opportunities to examine the long-term impact of maternal LCPUFA status in early, mid- and late pregnancy on future offspring health and function. Such data may assist in determining critical development periods during which dietary fat modulation has the potential to influence later function and health.

Weaknesses include the loss to follow-up over time. However, this issue affects many observational studies and clinical trials with a long follow-up, especially those covering such an extended time period as MEFAB. This leads to a relative small cohort, findings should thus be interpreted with caution. Another weakness is the lack of data on other dietary factors, which might exert a confounding role in the association of prenatal LPUFA status with later health. Lastly, the study was executed in the the South of Limburg, a province of the Netherlands, which has a predominantly Caucasian population. Therefore results are not generalizable to more multicultural populations. But the homogeneity of the study population does add to the validity of the studies.

Can I get hold of the data? Where can I find out more?

One of our goals is to encourage collaboration by making MEFAB data available to other investigators, while at the same time ensuring data integrity and respecting the privacy of our participants. Please contact the corresponding author for further information.

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