



EARNEST

EARly Nutrition programming- long term follow up of Efficacy and Safety Trials and integrated epidemiological, genetic, animal, consumer and economic research

Instrument: Integrated Project

Thematic Priority 5.4.3.1: Food Quality and Safety

Final Executive Summary

Start date of project: 15.04.2005
End date of project: 14.10.2010

Duration: 5,5 Years

Efficacy and Safety Trials and integrated epidemiological, genetic, animal, consumer and economic research (EARNEST)

Co-ordinator:

Professor Berthold Koletzko, MD, PhD (Dr med Dr med habil)

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

E-mail: programming@med.uni-muenchen.de

Introduction

Differences in nutritional experience during sensitive periods in early life, both before and after birth can programme a person's future development, metabolism and health. Better scientific understanding of Early Nutrition Programming holds enormous potential for implementing preventive strategies to enhance long-term health, well-being and performance. This could not only reduce costs of health care and social services, but may also improve wealth of societies. The Early Nutrition Programming Project brought together a multi-disciplinary team of international scientists and leaders in key areas of the early nutrition programming field from 40 major research centres across 16 European countries. The project was funded by the EC under the Sixth Framework Programme for Research and Technical Development and was coordinated by the Ludwig-Maximilians-University Munich. The integrated programme of work combines experimental studies in humans, prospective observational studies and mechanistic animal work including physiological studies, cell culture models, and molecular biology techniques.

Pre- and post-natal nutrition programme long-term adult health. This conclusion has been well-established in animal studies, and there is a large amount of data from retrospective observational studies suggesting that similar effects are seen in humans. There have been less data available from contemporary prospective studies and randomised controlled trials because these studies have not been running long enough. The Early Nutrition Programming Project enabled follow-up of participants of RCTs into early adulthood, to investigate whether differences induced by pre- or postnatal dietary modification persist later ages and into adulthood.

The implications of early nutrition programming are huge - differences in risk factors for cardiovascular disease, diabetes and obesity, in immune function and allergy risk, in bone health, and in cognitive, neuro-motor and behavioural outcomes have all been seen in children. The potential for improving the health of future generations is enormous.

This EARNEST project also addressed other areas where more information is required about early nutrition programming to inform public health policies and recommendations. The project gave insights on sensitive time periods, on the mechanisms how effects are mediated, and on whether or not, and to which extent, early programming effects can be reversed.

Key objectives of the project

The integrated programme was made up of 9 themes that were devoted to different approaches to study early nutrition programming. Key objectives were:

- Quantification of the effects of early programming on later cardiovascular diseases, obesity, diabetes, cognitive and mental disorders, bone health and some cancers (Themes 1-3).
- Definition of the relative importance of critical periods in foetal and early life on later disease (Themes 1-3).
- Exploration of the impact of genetic determinants on early programming effects and on subsequent outcomes (Theme 3).
- Understanding the role of specific nutrients and their interactions in the maternal and infant diet on programming effects on disease and their risk factors (Themes 1-3).
- Understanding mechanisms for early programming on later disease and their risk factors (Theme 3).
- Development of appropriate strategies for treating and especially for preventing the amplification of adverse programming effects of early nutrition (Theme 1).
- Exploration of the public health impact of how knowledge about early programming affects consumer behaviour (Theme 4).
- Quantification of the impact of early nutrition on the economic burden of adult ill-health (Theme 5).
- Demonstration projects to test the viability of new technologies that offer a potential economic advantage, but which cannot be commercialised directly (Theme 6)
- Improvement of training and enhancement of training opportunities for all partners including those from accession countries (Theme 8).
- Project Management - provision of a structure that addressed the complexity of the project and ensured the effective integration of each of the activities within the different Themes (Theme 9).

Summary Reports of Individual Themes

Theme 1: Follow-up of major intervention trials in pregnancy and early life

Studies in Theme 1 followed-up people who took part in previous intervention trials carried out during pregnancy and the first few years of life. This enabled the effects of the interventions to be tested over a longer period.

The results obtained from the 14 projects developed within the Theme 1 of EARNEST programme had shown important results, which surely will have major impact for EU populations, for future research and development, and for public health.

The main hypotheses that were tested in Theme 1 are listed below:

- Does maternal diet have beneficial long term effects on visual, motor and cognitive development, and cardiovascular risk factors, including obesity propensity and type II diabetes for the child?
- Is prenatal docosahexaenoic acid (DHA) supply safe with regard to growth, infection rates, and occurrence of other potential adverse effects?
- Does infant feeding affect long-term adult health outcomes (atherosclerotic cardiovascular disease and its risk factors, obesity, development of allergies, development of food preferences, and bone health)?

- Do neonatal fatty acid status and supplementation of formula feeds with long chain polyunsaturated fatty acids (LCPUFA) affect specific measures of neurodevelopmental outcome at the age of 8-12 years in term and preterm infants?
- Are gut bacteria different between children developing and not developing allergic disease?

The NUHEAL multicenter intervention study has demonstrated that DHA and/or 5-MTHF supplementation during pregnancy increases DHA plasma and erythrocyte membrane levels in the mother and offspring; this effect has also been shown in the INFAT study, where LCPUFA supplementation during the 2nd half of pregnancy determines also a longer duration of pregnancy of 4 days, significantly higher birth weight, and less increase of triglycerides during pregnancy. DHA and/or 5-MTHF supplementation during pregnancy (NUHEAL study) have not shown any long-term influence in the neuropsychomotor, cognitive and behaviour development up to 6.5 y (*Hempel, Touwen, Kaufmann tests, CBCL*); however the level of maternal & cord blood DHA in plasma phospholipids and its content in PE and PC erythrocyte membrane, have a *major influence on the neurological and cognition outcome* of their offspring from 4y to 6.5 y of life. It also seems that maternal age and the ratio AA/DHA in erythrocyte membrane are major determinants of alterations in *behavior* development.

The Groningen LCPUFA study suggests that the effect of prenatal fatty acid status on developmental outcome is greater than that of early postnatal fatty acid status. The precise nature of the relationships needs to be further elucidated. After birth, the effect of 2 months of postnatal LCPUFA supplementation in term infants on neurodevelopmental outcome largely depends on maternal smoking during pregnancy: in children prenatally exposed to maternal smoking, LCPUFA supplementation of infant formula will be related to *an improvement of cognitive development*. Two months of postnatal LCPUFA supplementation did not affect neurological condition, behaviour, anthropometrics and parameters of cardiovascular health at 9 years. *Breastfeeding is consistently associated with a better outcome at 9 years*. This holds true for neurological condition, cognitive outcome, behaviour, and heart rate and also when maternal IQ is taken into account. The IPD meta-analyses of considerable size performed in Theme 1, provided no evidence for beneficial or harmful effects of LCPUFA supplementation of formula milk on either growth or neurodevelopment at the age of 18 months, neither overall nor in specific subgroups. These findings are important since individual trials of lower size had shown conflicting results.

In preterm infants, the longest follow-up known of an LCPUFA supplementation in early life (UCLON), has shown no significant effect of supplementation on cognitive outcome, including global and specific tests at 10 years. Although overall there were no differences in cognitive function between randomised groups, in the sub-group of infants who received no breast milk in the neonatal period, LCPUFA-supplementation was associated with beneficial effects on *verbal IQ*, which was reflected in full scale IQ cognitive function. In addition, there was also a significant difference in the *word-pair learning scaled score* from the Children's Memory Scale, which is believed to be related the hippocampus function. However, supplemented girls showed benefits for some aspects of literacy and infants who received no human milk showed at 10 yrs some benefit from supplementation early in infancy; those who received human milk showed no benefit. Girls who received LCPUFA supplemented formulas were *fatter and had higher blood pressure at age 10 yrs*. *The effect on blood pressure was mediated by higher weight*. *No effect was seen in boys*. These unexpected results emphasise the importance of longer-term follow-up of these cohorts, and the need to

measure health outcomes such as body composition and blood pressure as well as cognitive function.

No evidence that maternal prenatal protein-energy or calcium supplementation of 1500 mg/d from 20 wk of gestation until delivery influences offspring blood pressure measured at 5–17 y of age in a rural area of Gambia had been found. Data from the Aarhus trial on the effect of fish oil in pregnancy on the risk of offspring allergy, overweight and blood pressure at 17-18 years did not show significant differences in front of the mothers who received olive oil supplement during pregnancy. However, the Aarhus study has demonstrated that 2.7 grams daily of LCPUFA in 3rd trimester may confer protection against asthma in the offspring.

The results emerging from PROBIT III (17046 children) have shown that there is no long-term beneficial effect of prolonged and exclusive breastfeeding on adiposity, stature or blood pressure in childhood at 11.5y. Moreover, no evidence that a successful breastfeeding promotion intervention resulted in lower maternal adiposity or blood pressure at 11.5 years postpartum.

By using an RCT (UCLON), designed to compare an enriched infant formula to promote growth with a control infant formula, it was shown that rapid growth in first 6 months of life is associated with increased fat mass at 6 years. Moreover, accelerated growth in the first four weeks of life has a strong effect on components of body composition, with the strongest effect seen on fat mass index at 19 years. Results emerging from the ALSPAC study have shown that a rapid weight gain after birth was not positively associated with IQ scores at either 49 months or 8 years in non-small for gestational age children. Subgroup analyses with stratification by child's sex yielded similar results.

In the CHOP study, 1138 formula fed infants were randomly assigned to receive a formula with higher or lower protein content; additionally a reference group of 619 exclusively breastfed infants were recruited. The results from this multicenter study has shown that the length z-score was not different between the higher and lower protein group at any time; but at 24 months weight-for-length z scores of infants in the lower protein formula group were 0.2 lower than those of the higher protein group and did not differ from that of the breastfed reference group, probably induced by the different protein intake. It is estimated that an increase of 0.2 SD in weight-for-length during the first 2 years of life is associated with a 13% higher risk for obesity at the age of 14-16 years. Moreover, the effect of the early protein intake on the metabolic-endocrine response showed that higher protein intake stimulates the IGF axis and insulin release in infancy, which is considered to be important for modulating later body size and adiposity risk.

The NUHEAL study demonstrates that Spanish children at 4y have higher daily intake of energy, fat (including DHA and EPA), total protein, carbohydrates, iron, but at the same time have higher BMI in front of German or Hungarian children.

The GINI study has confirmed a long-term allergy-preventive effect of hydrolyzed infant formulas on asthma and atopic eczema until 10 years of age. Moreover, infants fed with extensively-hydrolyzed-casein formula show a lower BMI-gain in the first year of life than those fed with hydrolyzed-whey and cow's-milk formula. However, no long-term effect on BMI up to age 6 was found with the extensively hydrolysed casein formula (eHF-C) or any other formula groups. A general programming effect of early infant feeding on food preference later in life could not be detected by this study.

Related to bone health, the studies developed suggest that the benefits seen early in life associated with the use of infant formulas designed to improve bone outcomes do not appear to extend into mid-childhood and beyond. The study conducted in UK (UCLON) in preterm and term babies has shown that prematurity may have long-term effects on peak bone mass, but this may not relate to dietary intake in infancy. So, increasing dietary nutrient calcium and phosphorus intake for preterm infants whilst on the neonatal unit had no influence on the bone health and markers of turnover by early adulthood. For instance, subjects born preterm are shorter and have lower lumbar spine bone mass in early adult life compared to population reference data; deficits are most marked in those born small for gestational age before 30 weeks. However, the intake of human milk (either as mother's breast milk or banked breast milk) appears to provide beneficial effects by increasing the whole body bone area (WBBA) and bone mineral content (BMC) despite the lower intake of early calcium and phosphorus associated with the provision of un-supplemented breast milk in this study. So, human milk has a positive long-term effect on later bone mass despite its low mineral content which may reflect the presence of *non-nutritional factors in human milk*, such as growth factors or other hormones, that will improve bone health; these findings have implications for *recommended mineral intakes for preterm infants*; this is important taken into account that current high recommended intakes are often difficult to achieve in practice. The short-term positive benefits for bone mass shown in term infants fed with an infant formula with synthetic triglyceride (sn-2-palmitate) to enhance calcium absorption are not present at 10 years. In addition, there are no differences in bone mass between breast-fed and formula fed term infants at age 10 years. So, sensitive period for long-term effects in bone health may not be restricted to a critical window in infancy.

The long-term effect and safety of the approach in the follow-up of the cohort in infants receiving probiotics perinatally was of specific focus. The study performed using a perinatal probiotic supplementation show that this intervention is safe in terms of the growth of the 159 children up to 10 years of age and may confer beneficial effects in terms of the weight regulation. Probiotics intervention resulted in no permanent alterations in the compositional development of the gut microbiota without permanent colonization of the administered strain. Moreover, probiotics could offer a useful intervention strategy against allergy epidemic, because the long-term benefits demonstrated here as a reduced risk of atopic eczema up to 7 years of age, i.e. beyond the intervention period and infancy.

The new study developed within EARNEST with an infant formula supplemented with oligosaccharide prebiotics (scGOS/lcFOS) demonstrated that when this intervention started early in life have a *protective effect against allergic manifestations and infections* during the first 2 years of life. This *long-lasting protection against allergy-associated symptoms* is still present at 5 years of life, probably due a modulation of the immune system.

The NUHEAL follow-up study also has shown that exposure to conventional household cleaning products represents a risk factor for obstructive airway disease in children (4y) with pre-existing risk for allergic disease and the application of room disinfectants was significantly associated with an increased effect for atopic dermatitis and itchy rash. Future preventive strategies might aim to restrict use of common room disinfectants.

The CHOP study has advanced the knowledge about the common solid introduction in different European Countries. Summarizing the results, solids were introduced earlier in formula fed (median 19 weeks) than in breastfed (median 21 weeks) infants. Earlier

introduction of solids (at 3 and 4 completed months) is associated with maternal age, low education level and maternal smoking. Significant differences were detected between countries for both, formula fed and breastfed infants, with infants in Belgium receiving solids earliest. Although recommendations are similar across countries and are the same for formula and breastfed infants, there are differences in the time point of introducing complementary foods. These data inform approaches towards more targeted strategies for more intensive counselling of families at high risk for inappropriate complementary feeding.

Regarding the energy providing liquids (EPL), 30% of breastfed and 43% of formula fed infants received EPL at the age of 4 months with formula fed infants starting earlier (median 17 weeks in formula fed infants and 30 weeks in breastfed infants). Significant differences were also observed between countries. Intake of EPL was associated with a lower intake of formula milk and solids in the first year of life. Since regular intake of EPL not only reduces the intake of formula or breast milk but also increases the risk for later development of dental caries or obesity, better and more extensive counselling regarding the use of EPL should be given to the caregivers.

Maternal postnatal depression was assessed in the CHOP cohort using the Edinburgh postnatal depression scale (EPDS) at an infant's age of 2 and 6 months, respectively. Postpartum depression has a prevalence of about 10%. Infantile colic and prolonged crying are known risk factors for maternal postnatal depression. This association was also studied in the CHOP population where data of 1015 mothers and infants at the age of 2 and 6 months were available. Mothers with a score higher than 12 were identified at risk of depression. Infant crying was associated with high EPDS scores at 2 months and 6 months postpartum. Even if crying had resolved mothers of infants with infantile colic still had a higher risk of depression at 6 months postpartum. No effects of postnatal maternal depression on anthropometric indices in the first two years of life could be found. Thus, a high postnatal maternal depression score is not a risk factor for under- or overweight in childhood in affluent societies.

Finally, the concept of early nutrition programming have been explored in humans in Theme 1, and the concept of „re-programming“ has been suggested as a new goal for nutritional treatment of common and chronic pathologies; the findings achieved will be the baseline for *new products* for infant, children and probably for adolescents, which will open *new windows for research and new advanced therapies* based on functional nutrients. Theme 1 has shown the capacity of different teams and companies from EU countries to develop successfully important and interesting studies with common goals.

Theme 2: Prospective epidemiology

Theme 2 addressed a series of objectives using epidemiological cohorts which had already been established or were newly set up.

The main objectives are listed below:

- To identify cohort databases in Europe which contain information on early dietary and other exposures, and those where such information is lacking.
- To evaluate the similarities and differences between these cohorts and organise an international meeting with the participation of all key persons in EU birth cohorts.
- To assess the scientific potential of undertaking combined analyses of the Danish National Birth Cohort (DNBC) and Norwegian Mother and Child Cohort (NMCC) databases.

- To collect post-pubertal data on body composition and blood pressure in the Avon Longitudinal Study of Parents and Children (ALSPAC).
- To study the relation between prenatal (maternal) and postnatal (infant) dietary factors and how they predict early disease markers and risk factors for chronic diseases, including cryptorchism, early growth patterns, psychomotor developmental milestones, atopic diseases and cognitive development.
- To disentangle the effects of prenatal and postnatal dietary factors on early disease markers.
- To collect biological samples and detailed data on foetal growth in the NMCC to enable triad analyses of the genetic basis of low birth weight.

Theme 2 was based on a unique set of prospective pregnancy cohorts. The British ALSPAC cohort is following offspring from around 14,000 pregnancies and was established in 1991-1992. The Danish National Birth Cohort is following offspring from around 100,000 pregnancies and was established in 1996-2002. The Norwegian MoBa cohort is following offspring also from around 100,000 and was established in 1999-2008. Furthermore a collaborative framework principally involved all existing pregnancy cohorts in Europe. The work in the theme was divided into five workpackages. The first developed the knowledge database regarding existing cohorts in Europe. The second, third and fourth workpackages undertook separate analyses based on the ALSPAC, DNBC and MoBa databases exploiting their unique respective strengths; thus, in ALSPAC the analyses focused on impact of early diet on blood pressure and anthropometric measures at 15 years; in the DNBC the analyses focused on impact of early diet on cryptorchidism, neurodevelopment, atopic diseases and growth up to 7 years; whereas the analyses in MoBa focused on impact of genes and diet on birth weight and foetal growth. The fifth, and last, workpackage undertook coordinated analyses based on DNBC and MoBa.

Developing a knowledge base regarding European cohorts holding information on early diet: This workpackage aimed at creating an overview of, collecting information on, and undertaking comparisons of cohorts in Europe that could be used to examine impact of such exposures on health and development.

The identification of birth cohort databases in Europe followed different approaches. A first list of cohorts was created by researchers participating in EARNEST through personal knowledge of research in the area of mother child cohorts. This list was supplemented through PubMed searches and personal contacts. A second step was the development of a one page questionnaire that was easily completed and that included basic information for each cohort. Cohorts identified were asked to register in the birthcohorts.net website and include the relevant information. At present the birth cohorts website www.birthcohorts.net includes information of practically all mother-child cohorts in Europe and is the main source of systematic essential information of these cohorts.

To assess similarities and differences across cohorts a protocol was developed which involved carefully constructed guidelines on the range and variety of foods to be included in constructing standardized food group and subgroup definitions, and collected data on the number and types of foods included in base questionnaires, which was influential in estimates of means and variability in intakes. Differences in intakes across European countries were described, as well as among women with different socio-demographic and health profiles within countries, to better understand how disparities in dietary intake may influence health outcomes among European women. Focus was on four broad food groups: fruits and vegetables, essential sources of micronutrients such as folate and antioxidants; meats, a major source of bioavailable iron as well as less beneficial compounds such as saturated fat;

seafood, the main source of several essential fatty acids as iron and other micronutrients; and milk and yogurt, important contributors to adequate intakes of calcium in pregnancy.

There were clear regional differences in the intake of fruits and vegetables with highest intakes in Southern European countries. Lower intakes in some countries such as Italy or Spain are above the highest intakes in other countries such as England. There were also clear heterogeneous patterns within regions. For example, fruit and vegetable intakes among pregnant women in Norway are higher than those of other Northern European countries such as Denmark or England.

With the exception of most Southern/Mediterranean countries, where fruit and vegetable intakes were typically highest, median intakes were generally lower among smokers, younger ages and particularly <20y, less educated women, as well as among those with higher pre-pregnancy BMIs. These disparities in intake across these groups were generally about 40-70g/day—about half a serving daily. However, in some cases, differences in median intakes associated with these factors exceeded 90-100g/day, equivalent to a full vegetable serving or a small serving of fruit.

In contrast to the rest of Europe, in Southern countries where intakes were highest, maternal education was not strongly related to increased fruit and vegetable intakes. In Spain and Portugal, fruit and vegetable intakes were markedly lower among smokers and younger women. However, these relationships were not observed in Italy or Greece. While in other regions a declining gradient in fruit and vegetable intakes was typically observed with increasing BMI, pre-pregnancy overweight and obesity were associated with lower reported intakes of fruits only in Greece.

This is the first study showing comparative, pan-European data on dietary intakes in this population with special dietary needs, and provides useful information for the development and targeting of effective food-based dietary guidelines in Europe. The data demonstrate that key dietary components co-vary with other risk factors related to reproductive health outcomes: diet may be an important confounder of, or an underlying contributing cause, linking these factors to reproductive health outcomes.

Early determinants of blood pressure and soft tissue at 15 years of age: The studies in this workpackage were based on The Avon Longitudinal Study of Parents and Children (ALSPAC). ALSPAC is a geographically-based birth cohort investigating the health and development of children. All pregnant women living in three health districts of Bristol (formerly known as the Avon Health Area), England with expected delivery dates between April 1991 and December 1992 were eligible to take part in the study. A total of 14,541 were enrolled, and 13,678 had a singleton, live born child. Detailed data have been collected by self-completed questionnaires (relating to the mother, her partner, and her offspring) from pregnancy onwards. From the age of seven, all children have been invited to regular research clinics.

The main tasks of this workpackage included collection of systolic and diastolic blood pressure data on 5091 children and of body composition data measured by DXA on 5134 children, when they were 15 years.

As regards early determinants of blood pressure at 15 years of age, none of the maternal exposures were associated with offspring systolic and diastolic blood pressure, after adjusting for gender, offspring age and maternal energy intake (prenatal diet variables only).

As regards early determinants of fat mass at 15 years of age, maternal (but also paternal) smoking, and reduced infant sleep were both associated with higher offspring fat mass, but the association with breastfeeding was lost after adjustment for potentially confounding factors. Maternal smoking was the only factor of those explored that is associated with offspring lean mass at age 15.

The ALSPAC team conducted a highly successful teaching event which was set up as an Epidemiology and Statistics Workshop during the 9th meeting in the EARNEST consortium held in Krakow in September 2009. The workshop was later made available as video-broadcast within the consortium.

Early dietary determinants of growth, asthma and allergies, psychomotor development, and cognitive performance: Studies in the third workpackage were based on the Danish National Birth Cohort (DNBC), which is the first large prospective birth cohort holding extensive information on maternal and infant diet. During 1996-2002, 101,046 Danish pregnant women were recruited into this cohort for long-term follow up of themselves and their subsequent children. Information on a broad range of lifestyle and other factors was collected through 1) the recruitment form completed in weeks 6-10 of gestation, 2) telephone interviews of the women in gestation weeks 12 and 30 (prenatal interviews) and when the children were 6 and 18 (postnatal interviews) months old, and 3) through a comprehensive dietary questionnaire sent by postal mail to the women around gestation week 25. Three blood samples were collected, two from the mother (around gestation weeks 6-10 and 25 of gestation) and one from the umbilical cord. The database could be linked to Danish registries containing information on socio-economic and health related variables. Among the registries available was the medical birth registry, which is recording information on birth weight and other variables recorded by health personnel around the time of birth, and another the mandatory National Patient Registry with ICD based diagnoses given during hospital contacts. Information on maternal diet was recorded in the interviews and in the mid-pregnancy 300-item food frequency questionnaire, whereas information on infant diet was recorded in the postnatal interviews.

The objectives were to study associations between maternal and infant dietary exposures on the one hand and early markers of chronic diseases and cognitive development during the first years of life on the other.

A comprehensive series of analyses were undertaken. The main conclusions included the following: As regards cryptorchidism, which is a strong risk factor for testicle cancer, no specific foods or nutrients in the maternal diet could be convincingly pointed out to be determinants of cryptorchidism. Maternal milk intake in pregnancy seemed to have stimulating effect on fetal growth, an effect which tended to beyond the prenatal period up to the age of 7 years. Maternal fish intake during pregnancy and the duration of breastfeeding were independently associated with better early child development. As regards asthma, a protective association seemed to exist for higher intake of fish, whereas results for allergies were less clear. Higher consumption of both peanut, nut, and total nut in mid-pregnancy was inversely associated with wheeze at 18 months and a diagnosis of asthma at 7 years, which is contrary to the current hypotheses and some recommendations which advise lower intakes of peanuts during pregnancy.

Genetic determinants of fetal growth: Studies in the third workpackage were based on the Norwegian Mother and Child Cohort (MoBa). MoBa is a large family-structured cohort that aims to find causes of diseases and explain trajectories and variability of health-related traits over a life course span. In the period 1999-2009, pregnant women were recruited to the study around the time of the ultrasound examination in gestation weeks 16 to 18. The fathers of the children have also been invited to participate. Biological material was collected from mothers, fathers and children and stored in a biobank. Data was assembled from questionnaires, analyses of biological material and linkages to health and exposure information. The cohort included data from more than 108,000 children, 90,700 women and 71,500 men.

An important task was to extract DNA from blood samples from the child at birth and from the two parents obtained from the whole MoBa cohort. This enabled the study of candidate genes for fetal growth; moreover, based on an additional grant given to study candidate genes for preterm birth, further studies could be undertaken of genes related to fetal growth. The studies have led to the identification of a maternal allele in the prostaglandin E receptor 3 gene with apparent protective effect, and a fetal allele in the paraoxonase 1 gene with a risk increasing effect. Other studies undertaken by the MoBa team related prenatal diet to pregnancy outcomes and child development. Thus, fetal growth did not seem to be associated with taking folate supplements or with the folate plasma level in pregnancy. On the other hand, a protective effect of taking vitamin D supplements was found for preeclampsia. Another study showed that folate supplements taken early in pregnancy may have a protective influence on language delay measured when the child is three years, and still another suggested that a particular pre-pregnant healthy diet may have a protective effect on the risk of hyperemesis that is a potentially serious disease of pregnancy.

Coordinated studies based on DNBC and MoBa: The last workpackage of Theme 2 aiming at paving the way for coordinated analyses based on both databases, DNBC and MoBa.

Three different purposes could be identified of undertaking coordinated analyses across the two cohort databases. One purpose is to obtain greater power to study effects of rare exposures or effects on rare outcomes; a second to check out findings obtained in one cohort by undertaking subsequent analyses in the other cohort; and a third to widen exposure ranges compared to what might be possible to obtain in each cohort studied separately. All three purposes were addressed in the collaboration reported on here.

As regards comparisons of design and structures of the two cohort databases, we concluded that MoBA and DNBC are compatible enough to undertake detailed comparisons across the two cohort databases. A specific outcome was the important conclusion that the food frequency questionnaires used by DNBC and MoBa are highly comparable, and that the dietary data can be pooled for specific purposes.

As regards comparison of empirical characteristics across the two cohorts, the clearly share many basic characteristics, such as woman's age, parity, civil status and BMI. However, they also differed distinctly with respect to specific characteristics. Thus, socio-economic status and urbanisation degree of area of residence was greater in the Norwegian women, and there were remarkably fewer women who smoked and used alcohol in Norway. Furthermore, in DNBC compared to MoBa, intakes of fish, vegetables, and fruit were substantially lower, whereas intakes of whole grain products, milk and butter were higher, and substantially more women had preferences for organic foods compared to conventional foods.

As regards coordinated analyses addressing specific scientific questions, the two teams succeeded in addressing three important issues. *Fish intake in pregnancy and fetal growth:* Earlier work by researchers on the team had suggested the hypothesis that fish intake in pregnancy stimulates fetal growth. It was decided quite early in the collaboration to examine this hypothesis on the basis of both cohorts, which had very different fish intake. A main result from the Danish study was identification of an inverse association between total fish consumption and fetal growth, another that this association reflected an association with consumption of fatty fish but not with lean fish. Contrary to the Danish findings, the Norwegian data showed a consistent increase in birth weight with increasing maternal seafood consumption in Norwegian women, but this association seemed only to exist for lean fish and not for fatty fish or fish liver. These findings warrant further explorations of the two datasets that take into account the shifts in the underlying exposure distributions in the various seafood variables. *Mediterranean diet pattern and risk of preterm birth:* The background for this work was a randomised controlled trial testing the effect of a Mediterranean type diet in Norwegian

women, which had, unexpectedly, shown lower occurrences of preterm birth the intervention group. A common analytic strategy was decided upon a priori, which implied defining groups in the cohorts that could mimic the comparison groups created in the trial. After accounting for potential confounding, both cohorts suggested a reduced preterm risk in the 'Mediterranean type diet' compared to the 'control' group. However, neither estimate from the two cohorts reached statistical significance, but pooling of the pooled estimate was statistically significant. The findings warrant further explorations of the two datasets that go deeper into which factors in the 'Mediterranean type diet' could be underlying causal factors.

Physical activity in pregnancy and risk of preeclampsia: An analysis based on the Danish dataset had, unexpectedly, shown a substantially increased risk of preeclampsia in women reporting moderate to relatively high levels of leisure time physical activity in pregnancy. An analysis run on the MoBa dataset could not identify the pattern seen in DNBC of increased risk with higher physical activity level. However, there was not either any clear reduction in risk with higher physical activity. The results from these two large cohorts do put a question mark against the basis for the recommendation, recently implemented in many countries, to exercise 30 min or more in pregnancy.

These activities all necessitated close interaction between the teams in order to discuss and decide upon which scientific issues should be addressed in the collaboration and thereafter to prepare for the coordinated analyses or the writing of the protocols. This interaction and highly positive experience is in itself likely to facilitate coordinated studies in the future.

Theme 3: Animal, cell and molecular studies

Theme 3 covered animal studies which were designed to identify the primary pathways by which altered maternal nutrition either during pregnancy or lactation, results in offspring being at increased risk of later disease. These studies underpinned the findings from the clinical (Theme 1) and observational (Theme 2) themes. At the same time they generated new questions that are applicable to each of these themes. By using a range of large and small animal models that are appropriate to each disease, Theme 3 not only defined specific mechanisms by which later disease is programmed but determined the precise nutritional conditions that contribute to these processes.

The main objectives are outlined below:

- To determine when the critical windows during early development are when maternal nutrition programmes one or more of the following chronic degenerative diseases: obesity, cardiovascular disease, metabolic syndrome, diabetes, renal disease, immune function and cancer.
- To examine the effects of both macro and micronutrients on multiple organ systems and pathways which are implicated within each disease process.
- To elucidate whether these outcomes are genotype dependent (e.g. by using transgenic models) or/and are reversible and can be overcome by later nutritional or pharmacological interventions.

High-protein diets have been shown to cause weight loss by inducing longer postprandial satiety followed by lower subsequent food intake, and increasing dietary thermogenesis. In obese and diabetic people consuming diets high in protein, a reduction of body fat tissue and improved glucose homeostasis was reported. Similar results were obtained in rats fed HP diets with reduced carbohydrate content. Thus, due to its metabolic effects helping to control body

mass gain high protein diets are popular and may be consumed also in women in childbearing age even before they consciously know they are pregnant and during pregnancy.

Reports on the impact of an increased dietary protein intake during pregnancy and lactation on maternal and offspring health and development are scarce. However, there seems to be a similarity with the effects of a low-protein diet that also causes decreased birth weight and altered body mass development of the offspring during infancy. Epidemiological studies in women show that high protein intakes during pregnancy also resulted in growth retardation of the baby. Excess of nutrients in reproducing females during pregnancy and lactation can lead to altered milk production and milk composition, due to alterations in mammary gland structure and gene expression. Thus, high protein diets may also affect early life nutrition of infants when nourished by mother's milk exclusively.

Body mass development, body fat accretion, food intake, blood metabolites, muscle characteristics and locomotive activity were studied in male offspring from birth to 58 weeks to explore changes induced by pre- or postnatal exposure to a maternal high protein diet. We further investigated whether prenatal or early postnatal exposure (suckling period) to a maternal high protein diet has a larger effect on offspring development in mice. Mouse dams were fed a high protein (HP, 40%) or a control protein (C, 20%) diet from mating to weaning (21d). After birth litters were standardized to 10 pups and were cross-fostered to different dams fed C or HP, and three groups of offspring that were exposed to different prenatal/pre-weaning diet combinations: C-C, C-HP, and HP-C. Pregnant dams fed a HP diet had a 25-40% lower body mass gain and gave birth to 13% lighter litters with one pup less (11 vs. 12) whereas individual birth mass was not always lower ($P < 0.05$). At weaning until age 175 days C-HP pups had decreased body mass which did not persist until 1 y of age. At weaning nose to anus length was lower, and relative kidney and heart mass was higher in C-HP offspring compared to the other two groups; differences disappeared thereafter. Total body fat % as determined by DEXA was irrespective of the maternal diet but body fat accretion was less in C-HP offspring between 29 and 370 dpn ($P < 0.05$). Plasma triglyceride and cholesterol values numerically decreased with age in C-HP offspring in contrast to the other groups where no change or an increase was observed. At 23 d, offspring exposed to a maternal HP diet during suckling experienced a reduced muscle growth whereas possibly negative effects due to exposure to a HP diet in utero can be ameliorated by rearing at a C dam. The effect of early life HP maternal diet exposure on muscle in 180 d old mice was small. Locomotive activity decreased from 185 to 360 dpn with no diet effect. We conclude that a maternal HP diet has immediate negative effects on maternal body mass and rearing performance as well as on offspring early postnatal development when suckled by a dam fed HP diet during pregnancy and lactation. However, a maternal HP diet during pregnancy or lactation did not cause persistent adverse effects.

The strength of this component was the consistency between models of maternal obesity (mice and rat) conducted in the two different centres (Kings' College London and Katholieke Universiteit Leuven). First, we have confirmed that maternal obesity induced by diet results in persistent metabolic dysfunctions in both rat and mice. Exposure to maternal obesity during gestation and suckling produced a hyperphagic metabolic syndrome-like phenotype in the offspring, characterized by obesity, and abnormal glucose and insulin homeostasis. Second, we have demonstrated that offspring of obese mice become hypertensive in adulthood with impaired endothelial function. Preliminary finding suggest blood pressure accompanied by increased sympathoexcitatory activation. Sympathetically mediated hypertension has been shown in several models of obesity related hypertension but not if offspring of obese dams. In

addition, juvenile OffOb mice demonstrated cardiac hypertrophy. This altered heart structure arises as a direct consequence of fetal and neonatal exposure to maternal obesity and not as consequence of offspring obesity.

Experiments on small laboratory animals, mainly rats, mice and guinea pigs, were devoted to the characterization of the acute and lasting effects of micronutrients administered during perinatal period on several phenotypes related to metabolic syndrome in human adults

One example was iron deficiency anaemia, as a common nutritional deficiency during pregnancy, with serious consequences, increased risk of neonatal morbidity, mortality, developmental delay, and an increased risk of cardiovascular disease in adult offspring. The data indicates that the involvement of one of the more common mechanism of blood regulation being responsible for the hypertension seen in the offspring of iron deficient mothers is unlikely. By using the less targeted approaches of DNA microarray analysis and proteomics a new hypothesis has been generated linking both the physiological and molecular findings. The common factor is the involvement of process, pathways and proteins involved in cell and organ structure.

In the studies focused on the pathogenesis of hypertension, it was found that several interventions directed at inhibiting factors that promote vasoconstriction and inflammation in the kidney during early life were effective in persistently reducing blood pressure in spontaneously hypertensive rats. Interestingly, several of these interventions also induced a wave of sodium excretion (natriuresis) at weaning (4 wk of age) suggesting a reset of extracellular fluid volume or sodium stores.

In the studies focused on the role of lipids in nutritional programming, it was found that AMP-activated protein kinase is involved in imprinting of “healthy” phenotype by lipids in early nutrition and that this mechanism is controlled by adipocyte hormone leptin. In addition, a novel physiological murine model was developed to study lasting effects of dietary lipids administered during the perinatal period on the neural development. It was demonstrated that increased adiposity later in life is promoted by early low alpha-linolenic acid intake in the guinea pig but not by high arachidonic acid intake. Therefore quantity of fatty acids is as relevant as n-6/n-3 ratio in the diet. It was shown the importance of the fat/carbohydrate content of the complementary diet and its relation to susceptibility of developing adiposity later in life. These results, if relevant to infants, are in line with the current dietary recommendations specifying that the fat intake should not be restricted until 2 years of age.

A period of energy deprivation coinciding with early organogenesis in the fetus has multiple consequences which are particularly marked when studied in species such as the sheep that has a long gestation. It impacts offspring metabolism in a tissue specific manner. Some of the effects have been found to aggravate the impact of juvenile obesity whereas in certain organ such as the kidney and the hypothalamus, energy restriction may be protective.

Aside from such multi-organ responses we established that common factors could be responsible for such long term outcomes. Nonetheless due to organ specific cell regulation it has organ specific consequences. In fact, the mechanisms that impact oxidative stress and inflammation are consistently reset. In addition cellular factors such as PPAR-gamma and its co-activator (PGC1-Alpha) together with FTO are affected in association with modulation of ectopic lipid infiltration.

Such constant alterations upon cellular oxidative stress and energy metabolism may be of critical importance in the understanding in the early programming of long term metabolic outcomes. It suggests that the early nutritional environment acts to determine cellular energy homeostasis. We therefore hypothesise that the energetic environment during development acts on the long term setting of energy metabolism primarily through changes upon intracellular energy balance. The balance between cellular anabolism and catabolism is respectively regulated by the AMP activate kinase (AMPK) and the mammalian target of rapamycin (mTOR). These two key proteins are indeed able to gauge the energy that is available to a cell and to influence metabolism accordingly. Such energy sensing pathways appeared critical in the control of whole body energy homeostasis and can play pivotal functions in the regulation of food intake. As a main research objective we further demonstrated the importance of the early environment in the setting of energy sensing pathways in tissues and organs that controls appetite both centrally and in the periphery.

The results generated by the proteomic analysis of the placentas from copper and iron deficient pregnancies will form the basis for future study. By using the whole proteome approach, as opposed to studying the expression of target proteins individually, we have been able to identify proteins and pathways that would never have been on our initial target list. For example, the pathway analysis carried out on the iron deficient placenta indicates that five of the most significantly affected pathways involve lipid metabolism; indicating that the placental lipid metabolism/transport could be significantly affected by maternal iron deficiency. This in turn could have significant effects on the growth and development of the fetus. Therefore this deliverable has provided us with a testable hypothesis for determining the link between maternal diet and offspring health.

Interestingly, when you compare the proteins which show differential expression in the copper deficient and iron deficient models, four proteins are common to both lists. The regulation of these proteins is also consistent between the two models. These proteins are alpha-enolase, alpha-fetoprotein, transferrin and the tubulin beta chain. Studies are now planned to verify the differential expression of these four proteins.

Theme 4: Consumer attitudes

Little is known about consumer attitudes to the concept of early nutrition programming and this has implications for how the results from the project are publicised. Theme 4 investigated the possibilities for disseminating information acquired during the project life time among consumers.

The main objectives are listed below:

- To determine the information sources used by parents to acquire knowledge about matters related to infant care and nutrition in selected EU countries.
- To evaluate the accuracy of available information on early nutritional programming.
- To assess parental knowledge about links between early nutrition and childhood/adult health and how this varies with age, gender, education, socio-economic status, health status, health-related behaviours (diet, exercise, smoking), and cultural background.
- To explore the relationship between infant feeding and weaning behaviours in the first year of life and parental knowledge about links between early nutrition and childhood/adult health.

- To explore differences between countries in parental knowledge about links between early nutrition and childhood/adult health and their origins in healthcare systems and/or socio-cultural factors.
- To identify the scope for targeted promotions to improve knowledge about the links between early nutrition and lifelong health.

Study 1 investigated how the lifetime health implications for the baby of the choice between breast- and formula-feeding are represented in policy documents in a sample of European countries. The overall aim of the study was to compare the citing of specific health outcomes in policy documents within and between geographically dispersed European countries with diverse public health nutrition traditions, and to consider the findings in the context of the policy making in Europe. Twenty-six policy documents were included in the analysis: four from England, two from Finland, nine from Germany, six from Hungary and five from Spain. There is variation within and between countries in the character of documents, which ranged from being substantial evidence-based reviews to concise summaries.

National governments dominated the policy arena in England and Finland. In these countries the search identified substantial expert reports providing the background and evidence-based recommendations for health-care professionals. In England, two summary documents in support of the WHO global recommendation of exclusive breast-feeding for 6 months were also available. In Germany, Hungary and Spain, most guidance for frontline health-care staff is provided by professional associations. Documents in Hungary tended to be shorter, and not to cite references in support of recommendations. All documents except two in Hungary and one in England had been published in the five years prior to the study. A main policy document was identifiable in four countries, but in Germany all documents exist in parallel.

A total of 203 statements about the health implications for the baby of the choice between breast- and formula feeding were extracted from the policy documents that were included in the study. Seven of the policy documents that were identified contained no such statements. The representation of individual health outcomes varied between documents, both within and between countries. Most statements referred to protection afforded by breastfeeding against infections and longer-term conditions. About a quarter of statements referred to the general health benefits of breastfeeding (compared with formula) and about an eighth to protection against allergy. Considering only those documents containing any health outcome statements, those from Germany and Finland had the highest number of statements per document and Hungary had the lowest.

Generic statements about the health benefits of breastfeeding compared with formula-feeding were most common in Finnish documents, and effects on neurological and mental development were the most frequently cited advantages in this group. Statements that referred to reduced risk of infection mainly concerned the protection provided by breast-feeding against gastrointestinal and respiratory/chest infections. All documents in England and Finland mentioned gastric infections, but this was not the case in the other three countries. Infection was mentioned less in documents from Hungary than in those of other countries. The protection afforded by breast-feeding against allergy was mostly presented in general terms. Reduced allergy risk was often linked to exclusive breast-feeding (although required duration was not usually stated) and familial history of atopy. Protection against gastrointestinal conditions, such as Crohn's disease, irritable bowel syndrome and ulcerative colitis, were most frequently mentioned among the long-term conditions. There were no statements in any documents about the effect of milk feeding choice on bone health. Outcomes were often simply expressed, without explanation or reference to the evidence base.

The study found that current documents on infant feeding policies in five European countries vary in authorship, date of publication, length and character. Analysis of the documents showed no consistency in the way in which health outcomes for the baby are cited as factors in the recommendations for breast- rather than formula-feeding. In more than a quarter of the policy documents, health effects of breast-feeding were not mentioned at all, and in the remaining documents they were often described only in general terms. This finding agrees with other recent research which concludes that health risks of feeding infant formula are poorly represented in journal articles. The health outcomes most consistently mentioned in policy documents in favour of breast-feeding were in the areas of mental/neurological development, protection of immune function, and reduced risk of gastrointestinal and respiratory/chest infections, allergy problems, long-term gastrointestinal conditions and diabetes. Most statements about the health implications of infant feeding choice were not precise about the duration of breast-feeding that is required for benefit, and whether or not the advantage is dependent on exclusive breast-feeding.

Differences between the portfolios of policy documents of individual countries may reflect variations in the structure of health services, resources, history and culture. More substantive policy documents on infant feeding are available in England and Finland, where health care is financed and delivered through public sector arrangements. The greater diversity of agencies producing policy documents on infant feeding in Hungary, Germany and Spain may reflect the more pluralistic nature of their health-care systems. Moreover, professional associations are more important in the policy arena in these countries because maternity and infant services are routinely provided by specialist obstetricians and paediatricians, rather than by general practitioners and primary care teams, as occurs in England and Finland. In all countries, publication of policy documents on important public health issues such as infant feeding is likely to be the product of some degree of dialogue between government agencies and professional associations.

Lack of consistency between documents and countries in the representation of the health outcomes of breast-feeding will limit effective promotion by health professionals and should be a cause for concern among policy makers. It may reflect uncertainties in the scientific evidence on the health outcomes associated with infant feeding choices in the developed world. Systematic assessments of the available evidence base show differences in interpretation. The findings of this study suggest that there is a scope to synchronise evidence and approaches to policy formulation across Europe and to ensure that recommendations reflect new knowledge. Future developments in the area of infant feeding policy are likely to be influenced by output from ongoing research on metabolic programming and the effect of the early nutrition environment on lifelong health. As research findings are disseminated, it is important that policy makers are able to evaluate the scientific evidence and provide clear guidance for health-care professionals about what is known about the lifelong health implications and the associated areas of uncertainty. In this way, consumers will receive full and balanced information on which to make choices about feeding their infant, and public health considerations will be properly addressed.

In **Study 2**, carried out in four of the above five countries (England, Germany, Hungary, and Spain), we established the degree of reflection and the impact of the concept of early nutrition programming among key persons from government organisations and agencies with their remit in infant nutrition in England, Germany, Hungary and Spain. We established the degree of reflection and the impact of the concept of nutrition programming among the different stakeholders of infant nutrition in 17 standardised face-to-face or phone interviews. In summary, the concept of early nutrition programming was widely recognized among the interviewed key persons from government organisations and agencies responsible for the

remit of infant nutrition in England, Germany, Hungary and Spain. However, the concept of early nutrition programming was rarely integrated in the produced documents.

Study 3 explored what parents can learn from available leaflets and magazine articles in five European countries (England, Finland, Germany, Hungary and Spain) about the health implications of infant feeding choices in the first year of life. The objectives were to investigate the availability of leaflets and magazine articles on infant feeding in each country, and to examine the content of materials for statements that associated infant feeding behaviours (milk feeding and introduction of complementary foods) and health outcomes for the baby.

An analysis of leaflets and magazines on infant feeding in the first year of life in each country revealed that the health implications of breast vs formula feeding and of complementary feeding practices were not widely or consistently portrayed, and this may weaken the impact of the messages. Nearly one quarter of leaflets related to milk feeding, and two thirds of those related to complementary feeding, did not include any statements about the health effects for the baby of food choices. Where statements were made, they mostly focussed on generic health benefits and immediate short term implications such as protection against infections (milk feeding) and protection against allergy (complementary feeding). There was less mention of possible long term effects of feeding choices. The main focus of most of the consumer materials was on the practicalities of breastfeeding and of introducing new foods and beverages, rather than the consequences for lifelong health. Whilst understanding feeding processes has been shown to be important to encourage low income mothers to breastfeed, further explanation of the health implications of their decisions may also be warranted to help with altering behaviours.

Written materials for consumers about health issues may not always reflect contemporary science appropriately, and tools have been developed to evaluate nutrition information that is in the public domain. The evidence base of the statements in the leaflets and magazines in the study was not checked because each country had recognised international and national infant feeding guidelines, and it was assumed that written materials produced by reputable agencies would reflect these. Moreover, assessment of the quality of infant nutrition statements is complicated because the scientific evidence on health outcomes is not definitive. Differences exist in the extent to which the protective effect of breast feeding is endorsed with respect to allergy, and long term conditions. Reservations have been expressed about whether the WHO global recommendation of exclusive breast feeding for six months is optimal for all babies, and this may be one reason why it is featured in a minority of written materials.

The search for leaflets in the study countries showed variability in provision, and scope may exist to further exploit this means of conveying health promoting messages to parents. Similarly articles in parenting magazines have the potential to reach target groups. Readership exceeds published circulation figures through placement of magazines in locations such as doctors' waiting rooms. Although space given over to infant feeding is a small proportion of the total content, and longitudinal studies have found no relationship between the number of magazine articles on breastfeeding and breastfeeding rates, carefully designed and delivered messages could influence behaviours.

Study 4 focussed on first time mothers in England, Finland, Germany, Hungary and Spain, because it was felt that they were likely to have recently collected information on infant feeding. Moreover, the views of mothers having their second or subsequent child may be influenced by their previous feeding experiences, and by the extra time pressures they faced caring for a larger family. The questionnaire asked respondents for their perceptions of the importance of infant nutrition in the first year of life. First time mothers were invited to

complete a questionnaire whilst they were in the maternity hospital shortly after the delivery of their babies, a total of 2071 new mothers completed the baseline questionnaire across the five countries. All mothers who provided data at baseline were mailed a follow up questionnaire when their baby was eight months old. This asked them to self report their milk feeding and weaning behaviours, and repeated the baseline question on sources of information on infant feeding.

Over 80% of all mothers stated an intention to exclusively breastfeed their baby, ranging from 68% in England to 97% in Hungary. A total of 70% of mothers stated that they would not start to wean onto solid foods until their baby was six months old, ranging from just over one half in Finland and Spain to over 80% in Germany and Hungary. About 70% of the mothers stating an intention to wean onto solid foods at six months or later cited 'recommendation' as the underlying reason. Whilst 46% did not specify the source of the recommendation, 8% reported that it came from a health professional, and 16% from an informal source such as family members or friends. Only 5% of mothers mentioned a health benefit for the baby as the explicit reason for delaying weaning until the baby reached six months. A relatively small number of mothers declared an intention to wean early (at 4 months or before) and the most important reasons underlying this were returning to work, wanting to involve others, having insufficient milk, wanting to introduce variety and because they thought the baby would be hungry.

A total of 95.6% of mothers agreed that how a baby is fed affects its health over the first years of life. Slightly lower proportions thought that infant feeding affected health in years to come. The specific reasons stated for why a baby's diet influences its health in the first year, and in years to come related to: benefits from breastfeeding in, and for immune function; reduced risk of infection and disease or health in general; the importance of nutrition in general, and of particular nutrients or the composition of breast milk; impact on growth and development; protection against allergy, and other long term conditions (obesity, diabetes, cancer, cardiovascular disease, bone, food preferences).

Although differences exist between countries, when considering the whole sample, more mothers thought that adult health is influenced by physical activity, diet in childhood, adolescence and adulthood, genetics and exposure to cigarette smoke than thought it is affected by diet as a baby, (which was viewed as having a similar influence as environmental pollution). Only family income was rated as significantly less important than diet as a baby as an influence on adult health. When considering a range of diseases and conditions, food allergy was cited by the largest proportion of mothers as being extremely or very much influenced by diet as a baby. In general, higher proportions of mothers in Spain than in the other countries thought that baby diet affected lifelong risks of chronic conditions. Mothers in Germany particularly attributed food and other allergies to diet as a baby.

Over 80% of first time mothers in the study countries who took part in the survey stated an intention, shortly after birth, to exclusively breastfeed their baby. A range of general explanations were offered for this decision, and 25% of these mothers stated a specific reason related to the health of their baby. Most frequently, and particularly in the German and English samples, these reasons were that breastfeeding boosts the immune function and helps prevent allergy. Although 70% of mothers stated an intention to wait until their baby was at least six months old before starting to wean onto solid foods, less than 5% mentioned a reason for this associated with the health of the infant. Most stated that they were following recommendations.

The sample of mothers in this study considers diet as a baby to be a less important influence on lifelong health than many lifestyle and behavioural factors and genetics. Overall, risk of food allergy is the health issue associated with baby diet by the largest number of mothers, but this is due to the high proportion of mothers in Germany who hold this belief. Less than

one half of mothers consider baby diet to have an extremely or very important impact on lifelong risk of overweight, eczema/asthma/hay fever, diabetes, osteoporosis, high blood pressure, heart disease, cancer, or the development of unhealthy food preferences. These relatively low levels of recognition of the impact of early nutrition on lifelong health may reflect the limited attention paid to this issue in consumer information and the policy documents and guidelines for health professionals in the study countries. The evidence on the link between infant nutrition and lifelong health is incomplete and sometimes inconsistent, and expert debate remains about the details of the health effects of infant feeding and the need to delay weaning onto solid foods until the baby is six months of age. Until the scientific basis is fully established, accepted and disseminated, coherent messages will not reach consumers. Books, partners and health professionals were the most influences on infant feeding decisions for the first time mothers in this study, followed by leaflets, magazines, parents and the internet. Advertising, TV, DVD and radio media were reported to be the least important sources. The influence of almost all sources was greater at eight months than at birth. The findings are consistent with previous research showing that mothers access multiple and concurrent sources of information, that the relative importance of these vary over time (and that cross cultural differences exist. In England and Finland, for example, GPs/doctors play a smaller role than in the other countries. Our data revealed no consistent associations between the characteristics of mothers and use of written materials to inform infant feeding decisions. This study confirms earlier work that shows that parents use multiple sources of information when making feeding choices for their infants, and that reliance on different sources varies according to social, cultural and health system factors. Mothers in the current study reported that they were influenced most by health professionals, partners, books and leaflets. Attitudes of physicians and hospital staff, and interventions aimed at improving the knowledge and understanding of partners and other family members, are known to be instrumental in improving breastfeeding rates. It is important that each source accessed by parents passes on evidence-based messages. However, the quality of available information can vary. Health professionals may have gaps in knowledge, differences in opinions and attitudes or poor communication skills. Multifaceted approaches, incorporating written materials, face-to-face advice and institutional change have been shown most effective at improving breastfeeding rates.

Good nutrition is paramount for physical and mental development, and promotion of healthy infant feeding behaviours is a major public health challenge. Parental awareness of the influence of early nutrition on lifelong health is required if they are to make health protecting choices for their infants. Pragmatic factors such as comfort, convenience and cost may override health considerations, particularly if parents do not understand the potential seriousness of the consequences of their decisions, or they assess the likelihood of personal susceptibility to be low. In this study, many mothers were aware of recommendations regarding the duration of exclusive breastfeeding, but did not articulate the reasons underlying this. Improved knowledge of the possible future impact of early nutritional experiences, and the mechanisms involved, may be needed to encourage health enhancing behaviours. Lay knowledge of issues surrounding food and health has been shown to vary with socio-economic status, but education and support can improve decisions regarding breastfeeding, including amongst mothers from lower income groups. The policy and information environment is central to ensuring that scientific findings are disseminated to health professionals in an effective manner, and that clear and consistent messages are communicated to consumers about healthy infant feeding behaviours.

Theme 5: Economic/public health impact studies

The objective of Theme 5 was the evaluation of health economic effects for the society that can be gained by appropriate infant nutrition. The relation of costs and benefits should be the basis for any decision that has to be made about the realisation of a medical intervention strategy, with the possibility to reduce future healthcare costs and welfare losses due to morbidity and mortality. Especially for a preventive intervention like nutrition programming, with effects that will occur in the far future, health economic analyses are essential.

The activity was split in two phases of the project. In the first phase (project months 1 - 18) the tasks were the conception and realisation of a health economic evaluation based on a proposed nutritional programming effect. At the end of the project Workpackage 5 was involved again (project months 55-65) to adjust the previous economic model and calculation to the current state of research that was generated from the project's research activities and calculate the cost-effectiveness of new found early nutrition interventions with programming effects.

In the first phase of the Workpackage, the existing literature was reviewed. One intervention strategy identified as medically effective in a randomised clinical trial was the supplementation of infant formula with long-chain poly-unsaturated fatty acids (LC-PUFA) for long term reduction of blood pressure. Even a small decrease in diastolic blood pressure by few mmHg could result in an explicitly lower risk for high blood pressure and hypertonus-related diseases like coronary heart diseases (CHD) and cerebrovascular diseases (stroke). Therefore, an lcpufa-supplementation of newborn nutrition could obtain a great preventive potential for cardiovascular disease (CVD) which is the main cause of the disease burden (illness and death) in Europe and one of the most important causes of healthcare costs.

An economic analysis of this intervention strategy was performed, taking the social costs and benefits over the whole human lifecycle into account. To assess the programming effect of lcpufa inclusion into infant formulas, a systematic review on the explanatory power of a blood pressure lowering effect in childhood for later life (tracking) was carried out, since the diseases that result from raised blood pressure, like myocardial infarction or strokes, will only develop in old age. Available data regarding baseline exposures, morbidity and mortality by hypertension-related diseases (ischaemic and cerebrovascular diseases) were collected for different European member states and the relevant economic factors (direct healthcare costs, direct non-medical costs, indirect costs, quality adjusted life years) had to be identified and compiled. The health-economic benefit was analysed in a Markov-Model, regarding the nutrition's blood pressure lowering effect and its impact on the probability to develop a CHD or stroke in later life. Other effects that occur from the supplemented nutrition, like the better cognitive ability or visual functions, are not considered in the model, as well as additional benefits resulting from a decreased blood pressure, so that the model's results will rather be an underestimation of the actual social benefits.

This Markov-Model calculation shows that the incremental effectiveness of the LC-PUFA supplementation is 1.20 quality adjusted life years (QALYs) in comparison with the standard-formula. In terms of cost effectiveness, the LC-PUFA supplemented formula is the strictly dominant strategy. This is because it not only leads to extended life expectancy, but simultaneously the total lifetime costs deriving from CHD-related diseases are lower than those obtained with the standard formula, by 630 Euros per child. The conclusion from this health economic evaluation is that the supplementation of formula with long-chain

polyunsaturated fatty acids represents an economically worthwhile prevention strategy. Since the intervention leads to an economic and medical improvement in comparison to the status quo with the standard formula for infants, the supplemented formula is the more cost-effective infant nutrition strategy (table 1).

A sensitivity analysis of the estimated parameters confirmed this result as significant. Even if the blood pressure lowering and CVD-risk reducing effect of the lcpufa supplemented nutrition is lower than the lower 95% Confidence Interval in the RCT and the intervention costs for lcpufa are ten times higher than estimated, the supplemented formula is the more cost-effective infant nutrition strategy.

Table 1: Incremental cost effectiveness, lcpufa

	<i>Cost (Euro)</i>	<i>Incr Cost (Euro)</i>	<i>Eff (QALY)</i>	<i>Incr Eff (QALY)</i>	<i>C/E (Euro / QALY)</i>	<i>Incr C/E (ICER)</i>
<i>lcpufa formula</i>	3.250,37		72,303		44,955	
<i>standard formula</i>	3.879,96	629,58	71,099	-1,204	54,571	(Dominated)

In the second part of the Workpackage, the previous health economical evaluation was adjusted to new found results within the Earnest Project. After identifying the different project outcomes, it was decided to analyse the health economic potential of lower protein in infant formula and its effect on lower weight gain in early life. In Earnest Project Theme 1, the European Childhood Obesity Trial Study Group (CHOP-Study) identified in a randomized clinical trial this significant effect, that is likely to have a long term programming effect on obesity in later life. With obesity being one of the main risk factors for cardiovascular diseases, diminishing the risk for obesity by reducing the protein level in infant formula seems to be a suitable prevention strategy for negative health effects in later life.

In the health economic evaluation of this nutritional intervention strategy, the previously constructed Markov Model had to be adjusted, with CVD and Stroke staying the relevant health states, but changed possibilities. Based on the results of the CHOP-Study, a higher protein content of infant formula is associated with an odds ratio of 1.13 (1.02, 1.25) for obesity in later life, compared with low protein formula. The correlation of bodyweight and CVD was assessed by data from systematic literature reviews that showed a significantly higher risk of obese person (BMI > 30) for a coronary heart disease or stroke.

The result of the adjusted Markov Model analysis is the low protein formula nutrition being the strictly dominant strategy. The cost effectiveness calculation shows an additional benefit in form of an extended life expectancy by 0.04 QALYs and simultaneously the incremental total lifetime costs are (discounted to present value) 20 Euro lower than with the standard formula alternative. (table 2).

The Markov model calculates a cohort simulation with the average economic effect per individual presented as result. But if we only look at the relevant obese children in a birth cohort, the average saved costs are 1,882 Euro (discounted from future) and 3.48 QALYs (only from lowered cvd-risk) for every child whose obesity in early childhood was prevented by the modified formula. With regard to the likely increased cvd-risk of overweight children, the effect for the society will even be multiplied.

Table 2: Incremental cost effectiveness, low protein formula

	<i>Cost (Euro)</i>	<i>Incr Cost (Euro)</i>	<i>Eff (QALY)</i>	<i>Incr Eff (QALY)</i>	<i>Incr C/E (ICER)</i>
<i>Low protein formula</i>	4.031		70,810		
<i>standard formula</i>	4.051	20	70,772	-0,038	(Dominated)

The presented health economic analyses in this Workpackage are approaches to evaluate the economic consequences and health effects that occur from infant nutrition over a whole lifetime. The model is based on several assumptions, simplifications and hypotheses, e.g. the future development of morbidity, mortality and cost parameters of the diseases relevant to the model over the next 100 years can only be predicted with considerable uncertainty.

However, regardless of these limitations and insecurities, the results of the health economic evaluations clearly show the strictly dominance of LC-PUFA-supplemented formula and low protein formula compared with standard nutrition, not only in the medical effectiveness but also in the economic outcome for the society. This supports the supposed high potential of early nutrition interventions with modest programming effects. Preventive effects of early nutrition may increase people's quality adjusted life expectancy and simultaneously save money and support a more sustainable economical welfare.

These results from Earnest's economic impact studies should help to promote this improved infant nutrition and to encourage further research on early nutrition interventions and long-term health outcome.

Theme 6: Demonstration studies

The aim of Theme 6 was to test whether the modification of infant formulae using new technologies and knowledge evolving from the investigation of programming effects offers the potential for commercial application. In particular, this Theme has evaluated innovative approaches to modification of early nutrition with newly developed inulin-type oligosaccharides and a recombinant human protein, both of which are natural constituents of breast milk.

Theme 6 encompasses two workpackages:

- Oligosaccharides, gut colonization and nutritional programming of immune-mediated health
- Impact of bile salt-stimulated lipase (BSSL) on LCPUFA bioavailability

Oligosaccharides, gut colonization and nutritional programming of immune-mediated health

The objective of this demonstration activity was to investigate the effects of prebiotic inulin-type fructans (in particular, oligofructose-enriched inulin) added to infant formula on immunological health-related outcomes in infants.

As a first step in this workpackage, a development phase took place in which the infant formula supplemented with oligofructose enriched inulin in an amount 0.8 g/100 mL was formulated and produced. The rest of nutrients in the infant formula were according to the recommendations of ESPGHAN (European Society for Paediatric Gastroenterology, Hepatology and Nutrition) and the European's Commission Directive on infant formulae and follow-on formulae.

After formula development was completed, a multicenter, randomised, double-blind, placebo-controlled study in healthy term infants was conducted to evaluate the effects of a formula supplemented with oligofructose-enriched inulin on the prevention of infections. Inclusion criteria were: healthy term infants with ages between 0 and 4 months, who are exclusively fed infant formula at study entry. Participating centres were:

- Hospital San Cecilio (Granada) (Main investigator: Prof. C. Campoy)
- Hospital de Nens and Hospital Sant Joan de Deu (Barcelona),
- CAP Llefià (Barcelona)
- Equipo Pediátrico San Francisco (Madrid)
- ZNA Koningin Paola Kinderziekenhuis (Antwerp)
- Universitair Ziekenhuis (Brussels) in Belgium (Main Investigator: Prof. Y. Vandenplas)

Infant follow-up was carried out up to the age of 12 months. Study visits with pediatricians were scheduled at 2, 4, 6, 9 and 12 months of age of the infants. Among variables recorded were number of days with fever, number and type of infections episodes (respiratory, gastrointestinal, other types), and data related with product tolerance and digestibility. Data were collected in Case Report Forms by the pediatricians, as well as in Diaries filled in by parents each month throughout all the study period. Data collected in parent's diaries were checked by the pediatricians at study visits.

In addition, stool samples were collected at 2, 6 and 12 months of age for analysis of microbial composition, calprotectin and total DNA. Saliva samples were collected at the same study times for analysis of IgA and other cytokines. 181 infants were recruited in different clinical centres of Spain and Belgium. During follow-up, 33 infants abandoned the study for different reasons. Follow-up of the infants was completed in October 2010. Data entry in an electronic database has been performed, and a report with demographic pooled data analysis has been delivered, whereas results per intervention group will be available in the next months.

Impact of bile salt-stimulated lipase (BSSL) on LCPUFA bioavailability

The objective of this study was to evaluate the effects of recombinant human enzyme bile-salt stimulated lipase (rhBSSL) on fat absorption in preterm infants.

In the first project year, activities were focused in developing a suitable formulation of rhBSSL for its administration to preterm infants during the clinical study.

Development of a BSSL manufacturing process involved the selection of a production cell line, purification methods, methods for virus clearance and methods for analysis of the final product. All steps to produce rhBSSL were according to Good Manufacturing Practices.

BSSL was formulated in an appropriate pharmaceutical form, stable, soluble in water and easy to dose. BSSL was delivered as a frozen oral solution in a 10 mL glass vial containing 15 mg/mL BSSL.

Toxicology studies with rhBSSL in different animal models such as juvenile rats and marmosets were also performed, and generated data to support safety of rhBSSL. In such studies, no local or systemic toxicity was observed after doses up to 115 mg/kg/day. It was established that for clinical use, BSSL solution should be added to the study preterm formula prior administration to the preterm infant. Stability tests of BSSL at different conditions of temperature and time were done.

Once rhBSSL was available, a Phase II double-blind crossover study was initiated to compare the absorption of total fat and of selected fatty acids, as well as growth and safety in preterm infants treated with 0.15 g/L rhBSSL or placebo. Patients were randomized to receive infant formula supplemented with rhBSSL at a concentration of 0.15 g/L, or infant formula with placebo for the first 7 days. After a washout period of 2 days, the patients crossed over to the other treatment regimen during a second 7-day treatment period. Collection of feces for assessment of the fat absorption was performed during the last 3 days of each treatment period. Patients were enrolled and randomized into the study at the neonatal intensive care unit after fulfilling the inclusion and exclusion criteria. To ensure that all patients received a comparable treatment regimen at baseline, infants receiving another formula prior to study entry were switched to the study formula (produced by Ordesa) at enrollment. The infant formula chosen for use in this study has been developed to replicate, as closely as possible, the fat content and composition found in mother's milk. . 33 infants with a mean gestational age of 32.6 weeks were randomised to receive one-week treatment with rhBSSL and placebo. 32 infants completed the study.

Five study centers in Italy recruited preterm infants to the study: The study centers were:

- Polytechnic University of Marche and Salesi Children's Hospital, University of Ancona, Ancona, Italy (Principal Investigator: Professor Carnielli)
- Policlinico 'Agostino Gemelli' Catholic University of the Sacred Heart, Rome, Italy
- Azienda Ospedaliera di Padova-Dipartimento di Pediatria Padova, Italy
- U.O. Neonatologia, Patologia Neonatale e Terapia Intensiva – Azienda Universitaria Policlinico Umberto Rome, Italy
- Azienda Ospedaliera "Ospedale Policlinico Consorziale"-U.O. Neonatologia e Terapia Intensiva Neonatale Bari, Italy

After all data had been collected and analysed the following results were reported: weight improved with 3.7 g/kg/day in the rhBSSL group compared to the placebo group. The difference was statistically significant. No difference in tolerability between rhBSSL and placebo was seen.

Conclusions

In conclusion, Theme 6 has demonstrated the viability of new technologies applied to infant formula that offer potential economic advantages. The clinical study to evaluate long term effects of oligofructose enriched inulin on the immune-mediated health has been successfully performed and data per intervention groups will be available in the next months. Also, the results about the effects of rhBSSL on preterm infant growth are promising and future phase III studies are in plan. Thus, studies conducted in Theme 6 have provided new and relevant information about effects of these new compounds on markers of long-term health of the recipient infant.

Theme 7: Communication and outreach

Theme 7 raised awareness of the Early Nutrition Programming Project, disseminated findings from the project and promoted the exploitation of new strategies and technologies arising from the project. These aims were carried out through work packages focusing on dissemination, exploitation and media relations.

Dissemination

Throughout the EARNEST project Theme 7 partners have been responsible for producing the following on a regular basis:

- Glossy brochure and updates
- Includes aims of EARNEST, background on science of early nutrition programming, details of partners, plans for implementation of project
- Website design and updates (external)
- The external website has been top of the list in a Google search on early nutrition or early nutrition programming.
- Newsletters for general dissemination
- The six-monthly newsletters provide a mixture of news about activity in the project and a more in-depth look at different parts of the project and the people involved. They also reflect what is going on in the early nutrition research field with reviews of important publications and news of research grants awarded.
- Newsletters for trial participants and their families
- Containing news of EARNEST to ensure continued compliance in trials
- A searchable publications database of publications on early nutrition programming, including those from EARNEST on the external website
- An extension of the monthly PubMed searches to allow more focussed searches in the area of the developmental origins of adult health and disease than is possible with PubMed. The database contains around 500 publications in the field of early nutrition programming. The use of filters enables people to selectively identify publications by members of the Earnest consortium or research coming out of the Earnest project as well as particular health outcomes.
- Email alerts
- About EARNEST publications, events, conferences, training opportunities sent to the media and dissemination databases developed by MIN and designed to drive more visitors to the website
- Media releases (produced and dispatched in conjunction with MIN in Workpackage 7.2- see later report)

Materials have been distributed directly to interested parties on our dissemination database (c 1500 contacts), as well as being viewable and downloadable from the public website, and disseminated further by partners direct and via their websites.

At strategic points we have produced major articles about the EARNEST project aimed at health professionals and the scientific research community (eg Ashwell M, de la Hunty A, Demmelair H, Koletzko B. EARNEST - the Early Nutrition Programming Project *Standardy Medyczne* 2009;37:1-73; de la Hunty A (2008) EARNEST - the early nutrition programming project. *Nutrition Bulletin* 33, 382-386. de la Hunty A (2009) Too much too young: why lower protein and slower growth are better for infants' long term health. CN

Focus 1, 17 – 19; de la Hunty A (2009) The EU Childhood Obesity Project. Nutrition Bulletin 34, 403 - 406.).

Theme 7 partners have been responsible for promotion of the international conferences held as part of EARNEST and for the preparation of conference materials and the published proceedings arising from them:

Koletzko B, Decsi T, Molnar D, de la Hunty A. Early Nutrition programming and Health Outcomes in Later Life: Obesity and Beyond. New York: Springer. 2009.

Koletzko B, The Power of Programming – Conference Proceedings, Amer J Clin Nutr 2011 – in press.

AA and MIN have ensured integration with other EC funded projects through our active membership of and engagement with COMMNET- a network for dissemination officers within of EC FP5, 6 and 7 projects (2005 to 2010). Training elements delivered by AA and MIN to the CommNet community have included Developing Strategic Plans for Dissemination, Brainstorming techniques, Event management, Interaction with Stakeholders and Risk Communication, Public Understanding of Science and Media Relations.

Exploitation

At the outset of the project, we contacted representatives from different stakeholder groups to be part of a panel to advise the EARNEST partners about dissemination and exploitation opportunities. (Ashwell M, de la Hunty A. What is the EARNEST Dissemination and Exploitation Consensus Panel (DECP)? Adv Exp Med Biol 2009;646:189-94). Four DECP meetings were held throughout the life of the project led by AA with support from MIN.

Four main topics have been extensively discussed at the annual DECP meetings and revisited frequently during the project to support the focus of EARNEST partners:

1. Dissemination opportunities for EARNEST results
2. Possible exploitable outcomes from EARNEST - what outcomes from EARNEST will dovetail with stakeholder interests?
3. Global Future forecasting using a PESTLE analysis (political, economic, social, technological, legal and environmental) - what drivers/trends will increase the importance of the concept of Early Nutrition Programming?
4. Overview of possible future claims arising out of EARNEST studies - the usefulness of health claims approved via the EC/EFSA route in communicating health benefits of food from the point of view of the infant food industry and from the point of view of the health professionals. What alternatives might be better?

Media Relations

To deliver an effective media relations three elements are necessary:

1. a strategic plan to provide a road map of activity,
2. content for media releases that will interest media targets and their readers/viewers,
3. a media list of contacts to which to send information and to monitor results.

MIN provided all three elements for EARNEST ensuring a successful and effective campaign.

The strategic plan was developed at the outset and reviewed and updated as necessary each year. At the start of the project a micro-site www.earlynutrition.org was created to enable journalists unfamiliar with the scientific technical terms to engage with the topic and see the relevance to their readers/viewers. Video interviews with key Workpackage leaders were

created by MIN and uploaded to that micro-site which linked directly back to the public website.

Media releases were created and issued on a regular basis and sent to a growing number of media contacts who had opted to 'stay in touch' with EARNEST news. Food, health, consumer interest, women and health, parenting, dietetic, nutrition and policy media outlets – print, online and broadcast – became increasingly interested in the work of EARNEST and its results, publications and interactions with health issues of worldwide concern.

Annual media coverage booklets were produced by MIN and made available for each EC Review meeting. Key highlights of media coverage achieved include a double page spread in New Scientist, BBC television news coverage of the Cambridge meeting, articles in research news and Cordis, Spanish TV and radio coverage of the Granada meeting, quality daily newspaper coverage in UK, US, Germany, Denmark, Australia, MumsNet and on trade websites such as NutraIngredients.

In addition, MIN provided media training and guidance on media protocol for partners throughout the project to enable more individuals to become involved and comfortable with media dissemination activity. As the project progressed more partners translated media materials which ensured higher pick up in a wider range of countries. Increasing co-operation with LMU press team ensured high visibility of EARNEST and its work in Germany. MIN also took an active role in co-operating with the Communications teams of FP6 project Helena and FP7 project Nutrimenthe at the Granada meeting in 2008.

Conclusions from Theme 7

The efforts of Theme 7 have been continually praised at EARNEST annual review meetings and by the Consortium at annual meetings. Our attempts to alert a wide range of stakeholders to the aims and achievements of EARNEST have been hugely successful, mainly due to an active media campaign for targeted dissemination. Our DECP has allowed fruitful preliminary discussions about various forms of exploitation and EARNEST partners have expressed satisfaction with the DECP advice which has supported critical decision-making.

Theme 8- Training

The scientific projects were guided and supported by training activities and activities to support interaction and exchange between partners. Procedures were established to ensure that trainings followed common guide lines, were well suitable for graduate students and contract research staff. Already at the first meetings training programs were included. This formed the basis for subsequent training activities that were both generic and across disciplines. Training brought together the complementary and unique expertise within the EARNEST community. Training packages have been designed to promote excellence between countries as well as within and across partners. This aimed at a new generation of researchers with a blend of complementary skills drawn from each discipline encompassed within all of the EARNEST Themes.

Each partner has allocated a portion of the budget to facilitate travel and maintenance of researchers to ensure adequate skills transfer across the project. Typical examples of the types of training EARNEST are detailed below.

Workshop "Epidemiology and Statistics for Early life Researchers":
 Practical Training on 'Current Techniques in Measuring Body Composition'
 Practical Training on Physical activity measurement in children in free living conditions
 Practical Training on Methods for the investigation of energy and substrate metabolism in pigs and mice
 Media training, including interviews by print media, live interviews by broadcast media
 Workshop: Neurodevelopmental testing (Hempel) at preschool age
 One week course "Pregnancy and Programming
 Nutrition Summer School: Paediatric Nutrition: An Evidence-Based Approach (jointly with ESPGHAN)

Furthermore, many of the partners opened training programs within their own structures, which were attractive to students and researchers from other EARNEST partners, e.g. partner DESC-SSI and the University of Aarhus Basic offered participation in their course: Principles of Epidemiology

In total 46 training activities have been performed during the 5.5. years of EARNEST, which significantly contributed to the acquisition of specific skills of individuals, which were required to achieve the scientific project objectives, and improved the knowledge about the different aspects of metabolic programming in the EARNEST community and also beyond, as some of the training activities were opened for interested parties outside EARNEST. With the advancement of the project specific training were complemented by inter Theme training activities, which brought in the form of workshops scientists from the different themes together. Thus, clinicians, epidemiologists and biologists shared their expertise in the field of programming research for mutual benefit.

Continuation of the training activities and the established interaction between the different early nutrition experts beyond the EARNEST funding period has been enabled by the foundation of the Early Nutrition Academy (ENA; www.early-nutrition.org)), a non profit organization with the aim to support research, training and wider dissemination of knowledge in the area of nutrition and programming. Some of the workshops and courses during EARNEST were already organized as ENA activities, e.g. the ENA postgraduate course "Early nutrition and physical activity: determinants for metabolic programming". Sustainability of ENA could be ensured by establishing links to other ongoing EU funded projects and training programs of individual partners. This promises a long term benefit of EARNEST for the European research community.

Overall conclusions

The partnership of the EARNEST consortium, as well as the External Advisory Board and the Dissemination, Exploitation and Communication Panel, regard the project as highly successful. Major progress in advancing scientific knowledge has been achieved, with an already high number of publications often in high ranking scientific journals at the time of project end, and many further publications still to come. The project has led to very strong collaborations among partner institutions, including a large extent of multidisciplinary collaborative work, which continues in a sustainable manner after the end of the funding period and has led to numerous new research projects. A strong, successful training element of the project has enhanced the career opportunities of new investigators, many of whom are now well on track in their academic development. Dissemination beyond scientific publications was highly successful, with enormous response in the scientific community, for

example through the two public conferences organized in Budapest and Munich and the ensuing published conference reports, as well as very wide and visible media coverage and public outreach. Already at this stage, a large extent of translational application of scientific outcomes has been achieved, in particular with regards to the impact of the EARNEST supported recommendations on dietary fat intake in pregnant and breastfeeding women which have received global attention and are now being followed by similar advice for example by the European Food Safety Authority and by obstetricians and gynecologists in the US, and with regards to the application of reducing protein content of infant formulas by several infant food manufacturers. These fast translational applications of research findings are expected to have a major benefit for public health and for health economic consequences. With the Early Nutrition Academy, a sustainable platform for further scientific collaboration and training activities has been established that will hold several workshops in 2011 and will continue to support scientific activities in the area of early nutrition in the years to come. The coordinator and the partnership of the EARNEST consortium are very grateful indeed to the European Commission, the member states and the European taxpayers for the opportunity provided and are highly motivated to continue with their best efforts in striving for high quality research work that will benefit the public.