



STANDARDY MEDYCZNE

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POD PATRONATEM



CENTRUM ZDROWIA DZIECKA

EARNEST - the Early Nutrition Programming Project

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Preface

The EU funded large integrated Project “EAR-NEST” combines follow up data from large randomised, controlled intervention studies in infants, data from observational studies in contemporary cohorts and data from sophisticated animal and in vitro studies. Thus, it provides a unique opportunity for the investigation of the extent to which early nutrition programmes long-term adult health and modulates adult risk for obesity, diabetes, vascular, bone and immune diseases, and cancer, as well as long-term brain development.

Beyond that EARNEST research includes the socioeconomic burden of poor early nutrition and consumer knowledge and attitudes about nutritional programming. This shall enable future public health interventions to prevent and reverse harmful programming. Furthermore, quantitative estimates of potential enhancement of European wealth creation by improved early nutrition are provided.

This article provides an overview about the EAR-NEST objectives, major achievements up to now and the further possibilities and potential impact.

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EARNEST - the Early Nutrition Programming Project (EARNEST)

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Summary

There is now considerable excitement about the possibility that both pre- and post-natal nutrition may affect long-term adult health. This is supported by findings from lifetime experimental studies in animals, historical and prospective observational studies in humans and experimental, hypothesis-testing trials in humans with long-term follow-up.

However, many questions have yet to be resolved:

- What is the extent of early nutrition programming in contemporary European populations with regard to long term health and burden of adult disease?
- Which nutritional exposures lead to programming?
- At what critical time periods (ie where the foetus or infant is most susceptible to nutritional influences) do these exposures act?
- By which mechanisms do these exposures produce programming?
- How effective are interventions to reverse the effects of programming?

The Early Nutrition Programming Project is the short title for “Early nutrition programming – long term follow up of Efficacy and Safety Trials and integrated epidemiological, genetic, animal, consumer and economic research”. The project has the acronym EARNEST and it is a large research pro-

gramme which brings together a multi-disciplinary team of international scientists and leaders in key areas of the early nutrition programming field from major research centres across 16 EU countries. The integrated programme of work includes experimental studies in humans, prospective observational studies and mechanistic animal work including physiological studies, cell culture models and molecular techniques. It is funded by the EC under the Sixth Framework Programme for Research and Technical Development and is co-ordinated by the Ludwig-Maximilians-Universität of Munich. The project started in 2005 and will run to mid-2010. The first results are now available and this article summarises some of them and also gives a taster of those still to come.

KEYWORDS: ■ EARNEST ■ EARLY NUTRITION PROGRAMMING ■ INFANT FEEDING ■ OBESITY ■ ATOPY ■ NEURODEVELOPMENTAL OUTCOME ■ BONE HEALTH ■ PROBIOTICS ■ ANIMAL SCIENCE ■ CONSUMER SCIENCE ■ ECONOMIC MODELLING ■ DISSEMINATION ■ EXPLOITATION.

Background

WHAT IS EARLY NUTRITION PROGRAMMING?

Much attention has been focussed on the influences of nature and nurture on the likelihood of staying healthy or becoming prone to disease; but 'early nutrition programming' is where nature and nurture overlap. Differences in nutritional experience at critical periods in early life, both before and after birth, can programme a person's development, metabolism and health for the future. So Early Nutrition Programming is where an infant's early nutritional environment interacts with the set of genes inherited from their parents to programme their metabolism or their brain development. Actually programming is increasingly being underpinned by the developing science of epigenetics – the science of understanding how genes are turned on or off by 'markings' on the genome which are potentially modifiable by environmental factors.

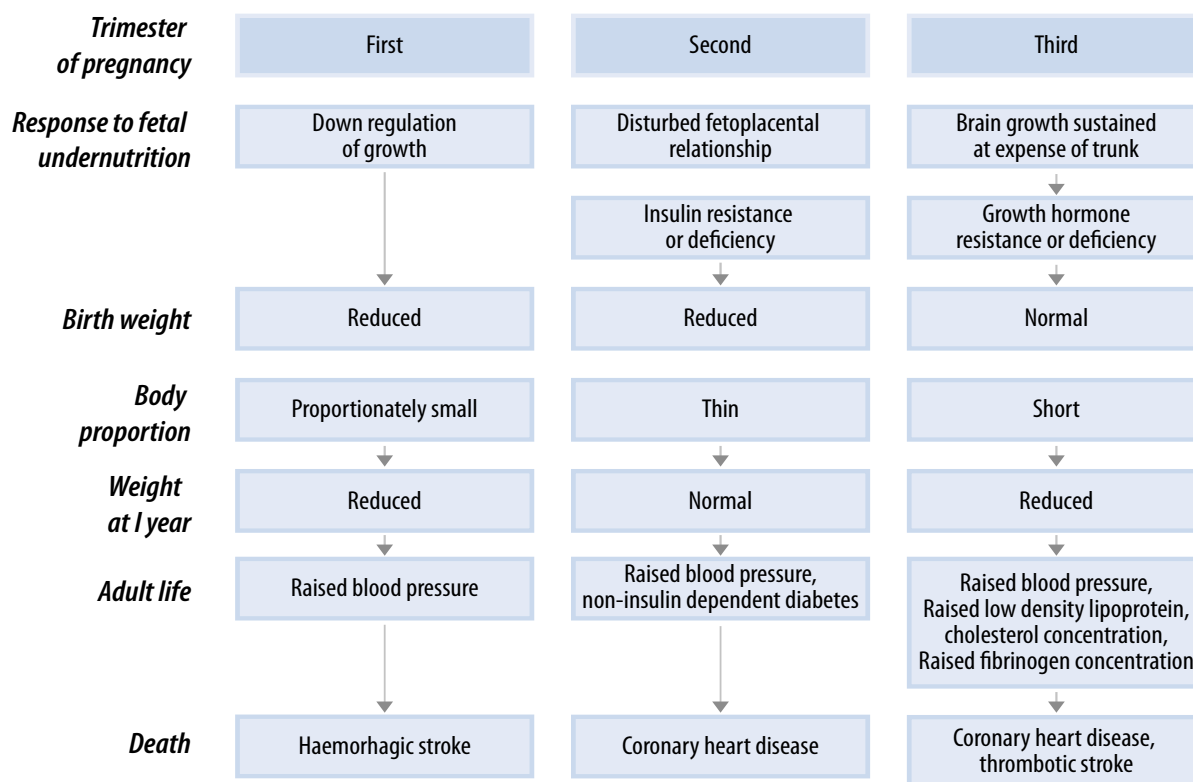
HISTORY OF EARLY NUTRITION PROGRAMMING

The concept that a physiological insult at a critical period during pregnancy can programme lifelong consequences in the development of the

child has been recognised for many years. That variations in the nutritional environment of the developing fetus and infant can also programme development is a more recent understanding. McCance and Widdowson, in Cambridge UK in the 1960s, showed that rats overfed early in postnatal life developed greater body size as adults (Widdowson and McCance 1963). In 1974, Dörner, in East Germany, proposed that the concentrations of hormones, metabolites and neurotransmitters during critical periods of early development were capable of programming brain development and functional and metabolic disturbances in adulthood (Kolelczko et al. 2005). The evidence from animal studies that early nutrition can influence subsequent cardiovascular disease, learning and behaviour, gut function, bone health, immunity and longevity is now overwhelming (Fewtrell and EARNEST 2007).

Until relatively recently, evidence that nutrition during pregnancy and early infancy could programme lifelong outcomes in humans came mostly from historical observational studies, (Barker 1995).

FIGURE 1: FRAMEWORK OF IDEAS IN THE FETAL ORIGINS HYPOTHESIS LINKING FETAL UNDER-NUTRITION WITH LATER ABNORMALITIES (BARKER 1995)



This work was led by David Barker's group and his initial framework of ideas is shown in Figure 1. The variable outcomes of extreme undernutrition during pregnancy, such as that which occurred during famines of the Dutch Hunger Winter and the Leningrad Siege also gave insights into the critical timing of the effects. However these studies suffered from the general problem of observational studies in that they were unable to take into account all the potential confounders. They also did not reflect contemporary lifestyles and dietary patterns. More recently, evidence has been obtained from randomised controlled studies in preterm and 'small for gestational age' babies. These studies have demonstrated a causal relationship between nutrition in infancy and later outcomes, including cognitive function and cardiovascular risk factors. Furthermore, they raised

the possibility that it was the postnatal catch-up growth which led to the adverse outcomes rather than the prenatal growth restriction and that accelerated early growth is the underlying cause of many adverse programming effects (Singhal and Lucas 2004).

Nevertheless several key questions remain:

- What is the extent of early life programming in contemporary populations?
- What are the relevant nutritional exposures?
- What are the critical time periods?
- What are the underlying mechanisms?
- What are effective interventions for preventing or reducing adverse programming effects?

What is EARNEST all about?

PARTNERS AND SCOPE

The Early Nutrition Programming Project, EARNEST, (www.metabolic-programming.org) is an EC funded research programme which aims to address these issues. The EARNEST Research Consortium brings together a multi-disciplinary team of international scientists and leaders in key areas of this field from major research centres across 16 EU countries. The integrated programme of work includes experimental studies in humans, modern prospective observational

studies and mechanistic animal work including physiological studies, cell culture models and molecular techniques (see Figure 2). It is funded by the EC under the Sixth Framework Programme for Research and Technical Development which is contributing 13.4 million euros towards a total cost of 16.5 million euros. The project started in 2005 and will run to 2010 and is being coordinated by Professor Berthold Koletzko of the Children's Hospital, Ludwig-Maximilians-Universität, Munich, Germany.

FIGURE 2: DIAGRAM TO SHOW INTEGRATION WITHIN EARNEST

EARNEST will integrate human, animal, molecular and cell studies

Intervention studies :

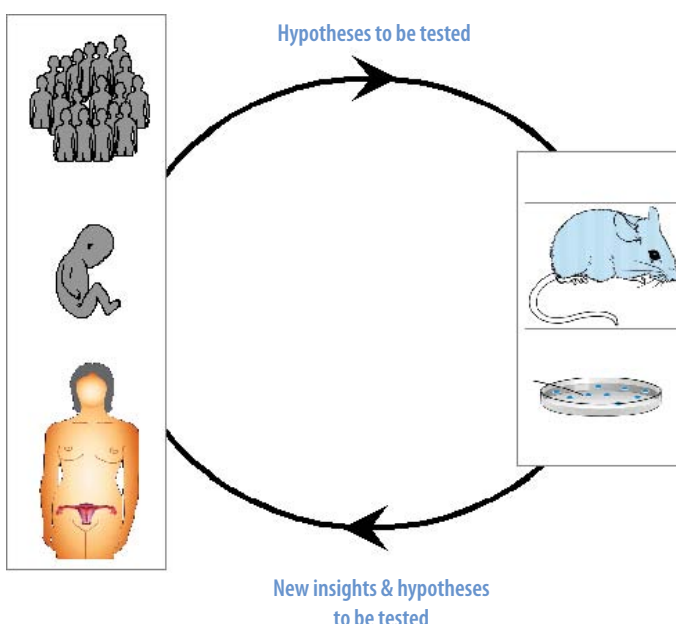
Effect of pre- and post natal interventions on programming mechanisms,

- Birth weight
- Genetic predisposition
- postnatal growth
- Adult disease markers
- Adult disease outcomes

Prospective Observational studies:

Effect of nutrition & genotype on programming mechanisms

- Adult disease markers
- Adult disease outcomes



Animal models and cells:

Effect of pre- and post natal interventions on

- Programming mechanisms,
- birth weight
- Genetic predisposition
- Postnatal growth
- Disease markers
- Disease outcomes

BENEFITS FOR AN INTEGRATED APPROACH TO NUTRITIONAL PROGRAMMING RESEARCH

Evidence for early nutritional programming has come from three types of study - animal studies, human epidemiological studies and randomised controlled trials in mothers and infants. There are considerable benefits from adopting an approach that combines the strengths of these different types of study. EARNEST therefore includes the following approaches which constitute individual Themes within the Early Nutrition Programming Project :

WHAT HAPPENS IN EACH EARNEST THEME?

1) Theme 1 investigates early nutritional programming of adult disease risk in humans by following up previously well-conducted randomised controlled trials of specific nutrition interventions in pregnancy and infancy and measuring disease markers in childhood and early adulthood. Nineteen trials are being followed up as part of EARNEST. Some of these investigated dietary supplementation during pregnancy (e.g. with fish oil) while others compared breast fed infants to infants fed with formulas of different compositions. The age of participants now ranges from five years to adult age. Most of the studied health outcome markers relate to the risk for obesity or cardiovascular disease, but markers for the development of the immune system, bone mass and neurological development are also included.

2) Theme 2 estimates the importance of nutritional programming in contemporary European populations by examining the associations between early nutrition and later outcome in large well-characterised population-based prospective studies with detailed measures of diet in pregnancy and the first years of life.

3) Theme 3 is using animal, molecular and cellular approach to study lifetime effects of early nutrition. The intention is to identify mechanisms and critical periods in development, where the fetus or infant is most susceptible to nutritional influences. The data will be used to develop hypotheses that can be tested in human populations and to provide the underpinning data essential for any future treatment strategies. Theme 3 studies will also inform the analyses conducted in the observational studies and help prioritise future trials in humans. Using developments in functional genomic techniques, they will further explore the basis of early nutrition programming in clinically relevant model systems and in the prospective cohort studies (all of which have collected and stored biological samples).

4) Theme 4 complements these biomedical studies with studies of the social and importance of programming by focussing on consumer science aspects of Early Nutrition Programming.

5) Theme 5 is investigating the economic aspects of Early Nutrition Programming.

6) Theme 6 is devoted to EARNEST's demonstration projects which are looking at the short term and long term health benefits of putting either prebiotics or a biotechnologically produced breast milk lipase into formula for preterm infants.

7) Theme 7 is devoted to dissemination and exploitation of the outputs from the other EARNEST themes.

8) Theme 8 is responsible for the training elements of the Early Nutrition Programming Project.

9) Theme 9 is the management package for EARNEST.

Overviews of EARNEST achievements

WEBSITE

The EARNEST website (www.metabolic-programming.org) (see home page from August 2009 above) contains comprehensive details about the project and its personnel. Brochures and newsletters about the project are available in downloadable pdf format.

CONFERENCES

An international conference “Early nutrition and its later consequences – new opportunities” was organised just before EARNEST started in 2004 and the proceedings were published in 2005 (Koletzko et al.2005). This conference allowed an early announcement of EARNEST’s intentions.

The screenshot shows the homepage of the Early Nutrition Programming Project. The browser window title is "The Early Nutrition Programming Project - Home Page - Windows Internet Explorer". The address bar shows "http://www.metabolic-programming.org/". The page content includes a navigation menu on the left with items like Home page, Research Programme, Consortium, Reports & Newsletters, Members Area, Media Area, Early Nutrition Academy, Publications, News, Conferences, Sign up for regular updates, EU Childhood Obesity Project, Links, and Contact Us. The main content area features a welcome message, project details, and a promotional banner for a 20% discount on a book titled "EARLY NUTRITION PROGRAMMING AND HEALTH OUTCOMES IN LATER LIFE". The book is part of the "ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY" series. The promotional text states: "The Proceedings of the 2007 Budapest Conference have now been published. Click here to get 20% off the list price. Regular Price: € 119.95 Discount Price: € 95.96". On the right side, there is a banner for "THE POWER OF PROGRAMMING International Conference on Developmental Origins of Health and Disease" held from 6th to 8th May, 2010 in Munich, Germany, with a "CLICK HERE FOR FURTHER INFORMATION" link. The background of the website features a scenic view of a city with a large cathedral and mountains in the distance.

The first EARNEST International conference, held in Budapest in April 2007, on “Early nutrition programming and health outcomes in later life: obesity and beyond” attracted over 250 scientists from over 30 countries around the world. The conference was a satellite of the European Congress on Obesity and so focussed on the long term programming effects which might contribute to obesity in later life. The proceedings have recently been published (Koletzko et al.2008).

The final EARNEST conference “The Power Of Programming” will be held in Munich in May 2010. It is planned to publish the proceedings as a

supplement to a major international journal.

THE ‘EARLY NUTRITION (PROGRAMMING) ACADEMY’

The ‘Early Nutrition (Programming) Academy’ (ENA) has been established to extend EARNEST’s activities (primarily training) beyond the EC period of funding. ENA will also be able to raise funds from sponsors to support and enhance the training and research activities beyond that which EARNEST can achieve.

The next sections present some of the research highlights from the individual Themes.

What has EARNEST achieved so far in its clinical studies in Theme 1?

GENERAL

The clinical studies in Theme 1 are based on established cohorts originating from controlled, randomised intervention studies. As many of the proposed follow-ups involve the same outcome measures, the identification and standardisation of these across different studies at an early stage will facilitate pooling of data and meta-analyses later on, providing the statistical power necessary to detect meaningful effects of early nutrition on a number of aspects of long-term health. For example, the two groups involved in the follow-up of LC-PUFA-supplementation trials have identified and agreed on a common battery of detailed cognitive and neurological tests to be used in all subjects at follow-up, which will make future comparisons easier. A similar strategy has been adopted for other outcome measures such as those relating to anthropometry, measurement of body composition and blood pressure. Some of the hypotheses to be tested in Theme 1 are summarised in **Table 1**

Table 1 Hypotheses to be tested in Theme 1

Maternal diet has beneficial long term effects on

- cognitive development
- metabolic risk factors

Prenatal docosahexaenoic acid (DHA) supply is safe regarding

- growth
- infection rates
- adverse effects.

Infant feeding affects long-term adult health outcomes

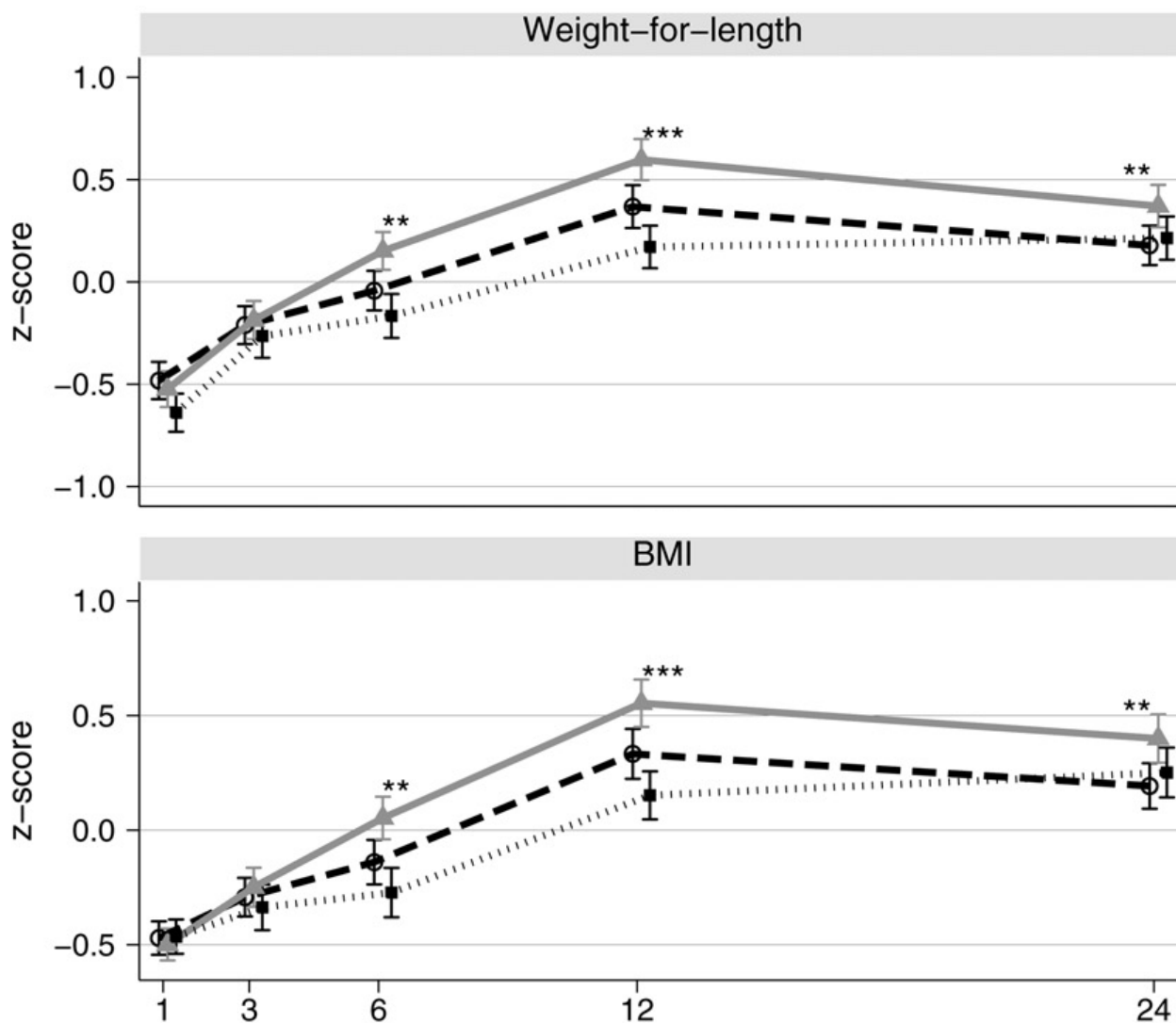
- Postnatal supplementation with infant formula plus long chain polyunsaturated fatty acids (LC-PUFA) affects neurodevelopment at age of 8-12 years.
- Gut microflora are altered in children developing allergic disease.
- Pre- and probiotic interventions during the neonatal period are efficient and safe in the long-term.

CHILDHOOD OBESITY

The first results of the EU Childhood Obesity programme (acronym: CHOP) have now been published. The data from five European countries indicate that infant formulas with lower protein content bring metabolic and endocrine benefits,

as well as body growth rates closer to those of breastfed babies (Koletzko et al.2009). The further follow-up of the children as part of EARNEST will indicate whether these changes are associated with lower risk of childhood obesity at a later age.

FIGURE 3: EU CHILDHOOD OBESITY PROGRAMME SHOWS THAT LOWER PROTEIN INTAKE FAVOURS SLOWER GROWTH (FROM KOLETZKO ET AL.2009, WITH PERMISSION OF AJCN)



LEGEND TO FIGURE 3:

WEIGHT FOR LENGTH AND BMI IN THE LOWER PROTEIN AND HIGHER PROTEIN GROUPS AND IN THE BREAST FED CHILDREN AT BASELINE AND AT 3,6,12 AND 24M OF AGE (KOLETZKO ET AL.2009).

SIGNIFICANTLY DIFFERENT FROM THE LOWER PROTEIN GROUP(ANOVA ADJUSTED FROM BASELINE VALUES) * P<0.05; ** P<0.01; * P<0.001**

The analysis of the dietary records showed that energy intakes were the same in the two groups at 3, 12 and 24 months of age, although they were slightly higher (by around 25kcal/day) at 6 months in the lower protein group. Protein intakes were significantly lower in the lower protein group at 3, 6 and 12 months, but not after that when the intervention ceased. Fat intakes were significantly higher in the lower protein group during the first year.

After 2 years, the infants fed the lower protein formula were the same length but weighed slightly less than the infants fed the higher protein formula and were more similar to the group of breast fed infants. Their weight-for-length and body mass index were significantly less than those fed the higher protein formulas at 6, 12 and 24 months. At 24 months, there was a difference of 0.20 in the adjusted z score between the two intervention groups. The differences in weight, weight for length and BMI emerged by 6 months of age and persisted, even after the intervention ceased and the children went onto similar diets. As there were no significant differences in length between the groups, it is likely that the differences in weight for length and BMI are due to differences in body fat. Whether these small differences in weight and BMI develop into significant differences in the rates of obesity between the groups remains to be seen.

Extrapolating the increase in weight-for-length found in this study using estimates for the increase in obesity associated with higher weight-for-length in infancy seen in previous prospective studies suggests that the children in the higher protein group have a 13% increased risk of being obese in adolescence (Koletzko et al.2009).

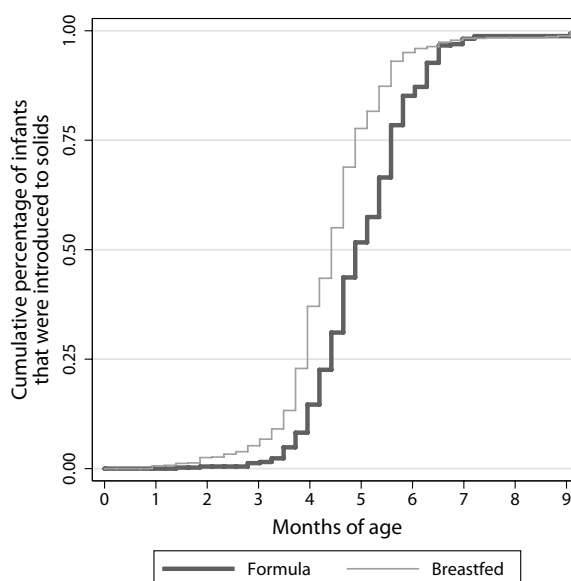
An analysis of the differences in the timing of weaning across the five countries in the study has shown that mothers who breast fed their babies were more likely to follow recommendations to delay the introduction of solid foods than those who gave their babies formula (Schiess et al.2009). Formula fed infants were given solid foods on average 2 weeks earlier than breast fed infants (19 weeks compared to 21 weeks) and were also twice

as likely to be introduced to solid foods before the age of 4 months, the recommended minimum age of solids introduction in Europe. Around 37% of formula fed infants had received solid foods at the age of 4 completed months compared to only 17% of breast fed infants in the study. Only 4% of mothers of formula fed infants delayed the introduction of solid foods to after 6 months, whereas 13% of breast fed babies did not receive solid foods until 6 months. Breast fed babies were also more likely to be given family foods than formula fed infants who were more likely to be given commercial infant foods.

Marked differences in the timing of introducing solids in the five different countries were also seen – mothers in Belgium were more likely to introduce solid foods before 4 months of age in BF infants and before 6 months in FF infants, compared to mothers in Germany. These differences were not due to different recommendations on the introduction of solid foods which are the same in Belgium as in the other countries in the study.

It suggests that there are strong cultural, social and parental influences on the timing of weaning. (see Figure 4)

FIGURE 4: MOTHERS WHO BREAST FED THEIR BABIES WERE MORE LIKELY TO FOLLOW RECOMMENDATIONS TO DELAY THE INTRODUCTION OF SOLID FOODS THAN THOSE WHO GAVE THEIR BABIES FORMULA (FROM SCHIESS ET AL.2009).



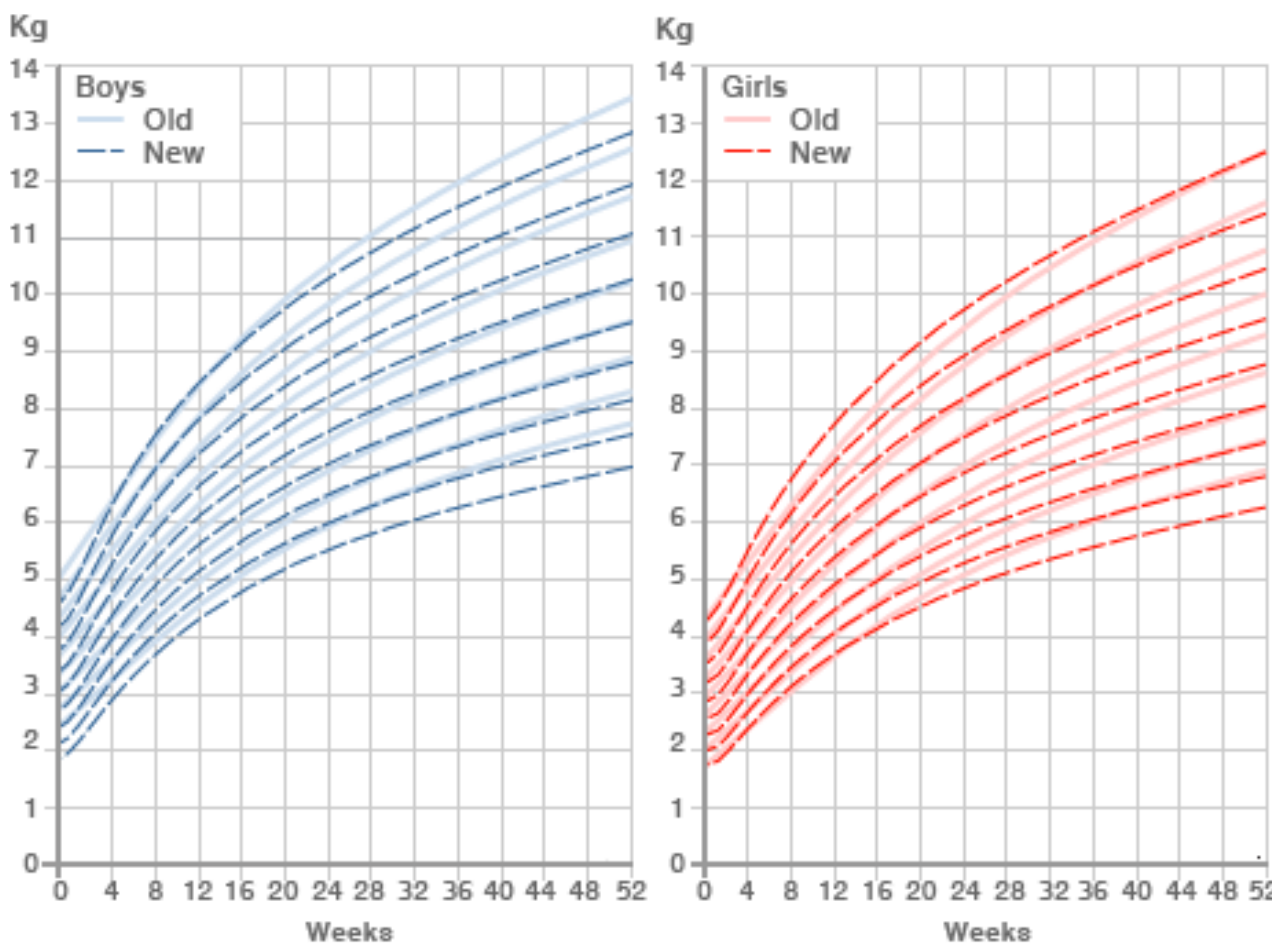
Results from EU Childhood Obesity programme lend support to new WHO Growth Charts

These results showing the different growth trajectories of formula fed and breast-fed infants lends support to the new 2006 WHO growth charts.

These are based on the growth patterns of healthy breast-fed babies (WHO Multicentre Growth Reference Study Group 2009) (see Fig 5). The previous 1990 charts were based on a mixed group of breast and formula fed babies and were actually a description of how infants did grow, rather than how they should grow. Figure 5 compares the two charts as used in UK. The WHO standards are derived from the Multicentre Growth Reference

Study (MGRS) which measured the growth of around 8500 infants from six countries across the globe – Brazil, Ghana, India, Norway, Oman and the USA (WHO Multicentre Growth Reference Study Group 2009). The study included only full-term healthy children who had been exclusively breast fed for at least 4 months and partially breast fed for a year and weaning onto solid foods started by 6 months. The children's growth was followed up for 5 years and the study found that the growth patterns of these children were similar in all countries. The standards produced from this study are therefore applicable to infants from all racial backgrounds.

FIGURE 5: COMPARISON OF OLD UK GROWTH CHARTS AND NEW UK-WHO CHARTS



Results from EU Childhood Obesity programme have implications for the protein content of infant formulas

The introduction of the WHO growth charts is also likely to mean that infant formula companies will alter the composition of their formulas and reduce their protein content to match the growth pattern of breast-fed babies more closely. The results from the CHOP Project might also add as a spur to industry.

The protein content of infant formulas has already been reduced quite substantially over the years but further reductions are likely. The protein content of formulas was as high as 4g/100kcal in the 1970s and came down to around 3g/100kcal in the 1980s as concerns about adequacy of protein intake were lessened. As these concerns have been replaced by concerns about excessive intakes, the protein content of formulas has continued to fall. The minimum protein content stipulated in the 1991 Infant and Follow-on Formula Directive was 2.25g/100kcal and this was reduced to 1.8g/100kcal in the 2006 Directive. The WHO have also reduced their recommendations of the safe level of protein intake for infants less than 12 months of age from their 1985 report (WHO et al. 2007). Those for infants aged up to one month were reduced by about 20%.

Results from EU Childhood Obesity programme indicate mechanisms by which protein exerts its effects.

In the CHOP study additionally to the anthropometric measurements serum and urine parameters were investigated at the age of 6 months. Total and free IGF were significantly increased in infants fed high protein formula when compared to low protein group, IGF-binding protein 2 was decreased in the high protein intake group when compared to low protein intake group. C-peptide/creatinine urine concentration ratio was significantly increased in the high protein group indicating increased insulin release with high protein feeding. The results point consistently to stimulation of IGF axis and insulin release by formula feeding that corresponds to higher BMI observed at the age of 2 years in these children. The data obtained indicate the mechanism of metabolic programming of obesity as well as the time window of programming around 6 months of age.

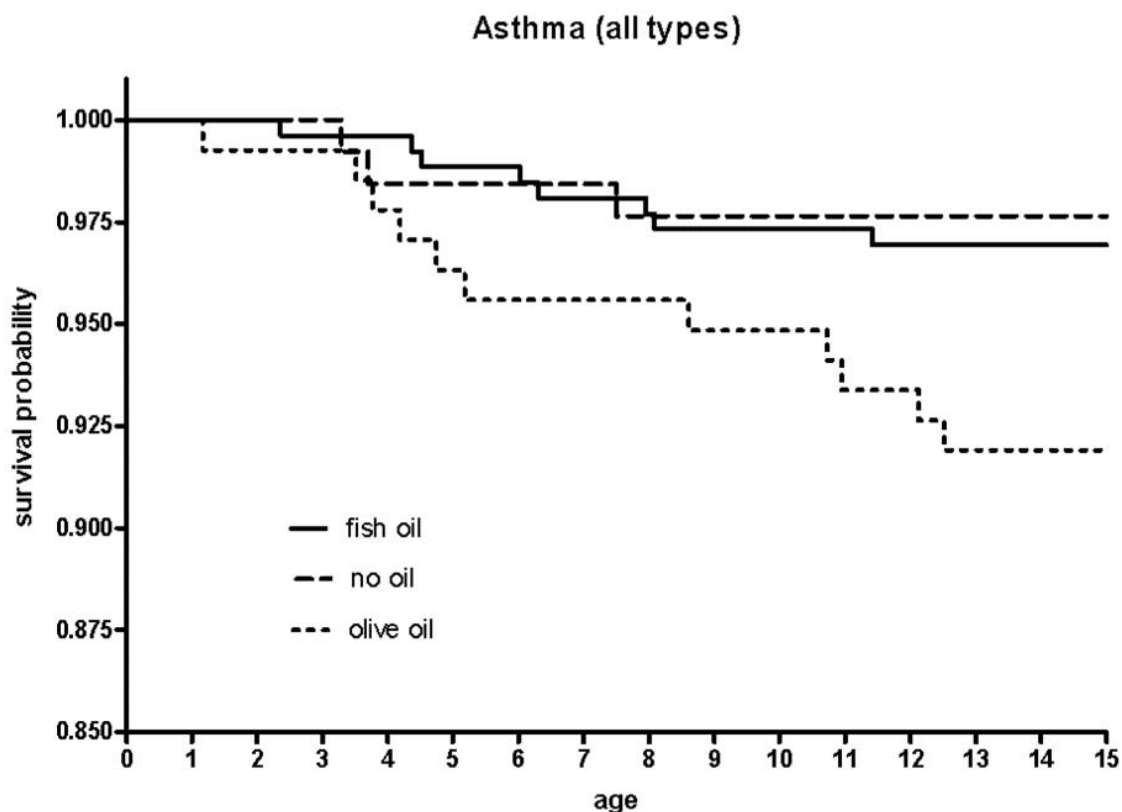
Prebiotics and atopy

One component of EARNEST Theme 1 was a follow up of a study on the effect of supplementing infant formula with a mixture of prebiotics in infants with a high risk of atopy. The researchers found that the group fed the supplemented formula had a lower rate of atopic dermatitis and infections than the unsupplemented group up to the age of 2 years (Moro et al. 2006; Arslanoglu et al. 2008).

FISH OIL AND LATER RISK OF ASTHMA

Another activity was the 16 yr follow-up of children born to mothers in Denmark who had been randomised to a fish oil supplement during the last trimester of their pregnancy. Figure 6 shows a significantly lower risk of asthma in the children compared to those who had been randomised to olive oil supplements. The interpretation of the trial was complicated by the fact that children whose mothers had been given 'no oil' also had a lower risk of developing asthma. Though it is theoretically possible that it was the olive oil supplements that increased the risk of asthma, it is more likely that some of the mothers in the 'no oil' group had voluntarily increased their intake of fatty fish, or even taken fish oil supplements themselves, since all the mothers had been told that the trial was to test the benefits of fish oil on reducing the risk of pre-term delivery and low birth weight.

FIGURE 6: SIXTEEN-YEAR FOLLOW-UP OF OFFSPRING FROM A RANDOMIZED CONTROLLED TRIAL WITH FISH OIL SUPPLEMENTATION IN PREGNANCY. SURVIVAL CURVE SHOWS OCCURRENCES OF ALL TYPES ASTHMA-RELATED DISEASES RECORDED IN THE NATIONAL PATIENT REGISTRY, STRATIFIED BY RANDOMIZATION GROUP (OLSEN ET AL. 2008; WITH PERMISSION OF AJCN).



EARLY DIET AND PEAK BONE MASS

One of the Theme 1 activities was the long term follow up of UK infants born pre term and term and randomised to formulas intended to modify the whole diet or specific nutrients with reference to bone health. After a 20 year follow up the researchers concluded that ‘Infant dietary randomization group did not affect peak bone mass or turnover suggesting the observed reduced final height and LS bone mass, most marked in growth restricted subjects with the lowest birthweight, may not be related to sub-optimal early nutrition. The higher bone mass associated with human milk intake, despite its low nutrient content, may reflect non-nutritive factors in breast milk. These findings may have implications for later osteoporosis risk and require further investigation (Fewtrell et al. 2009).

PROGRAMMING OF ALLERGIES?

Another Theme 1 activity was the long term (10yr) follow up of existing cohorts of infants in several European countries randomised to hypoallergenic formulas with reference to allergies, overweight and food preferences (the GINI Study).

Researchers found that feeding with any kind of hydrolysed formula in infancy was positively associated with a higher acceptance of extensively hydrolysed casein formula after adjusting for sex and study centre (von Berg et al.2008).

What has EARNEST achieved so far in its prospective observational studies in Theme 2?

COLLATION AND COMPARISON OF COHORTS

Observational studies in Theme 2 comprise most of the major longitudinal studies in Europe holding information on dietary exposures in pregnancy and early childhood. A website has been created which gives an overview of all such cohorts in Europe (www.birthcohorts.net), and comparisons of early dietary exposure distributions in these cohorts have been initiated.

A report on the similarities and disparities in intakes of major protein sources during pregnancy has been completed. A draft of a comparative analysis of diet during pregnancy across Europe has also been completed. The assessment of cardiovascular risk parameters of all participants in the Bristol (ALSPAC) cohort at age 15 is completed. The preliminary blood pressure and body composition datasets are now available for exploratory analyses. All the children from the Danish DNBC who were selected on the basis of early diet for cognitive testing at 5 years have been examined.

Detailed comparisons between the Danish (DNBC) and Norwegian (MoBa) cohort databases and calculation of statistical power have shown that for several scientific questions, relating to rare outcomes, it is highly justified to undertake coordinated or pooled analyses. Protocols on coordinated analyses regarding the impact of maternal diet on malformations and childhood cancer have been completed.

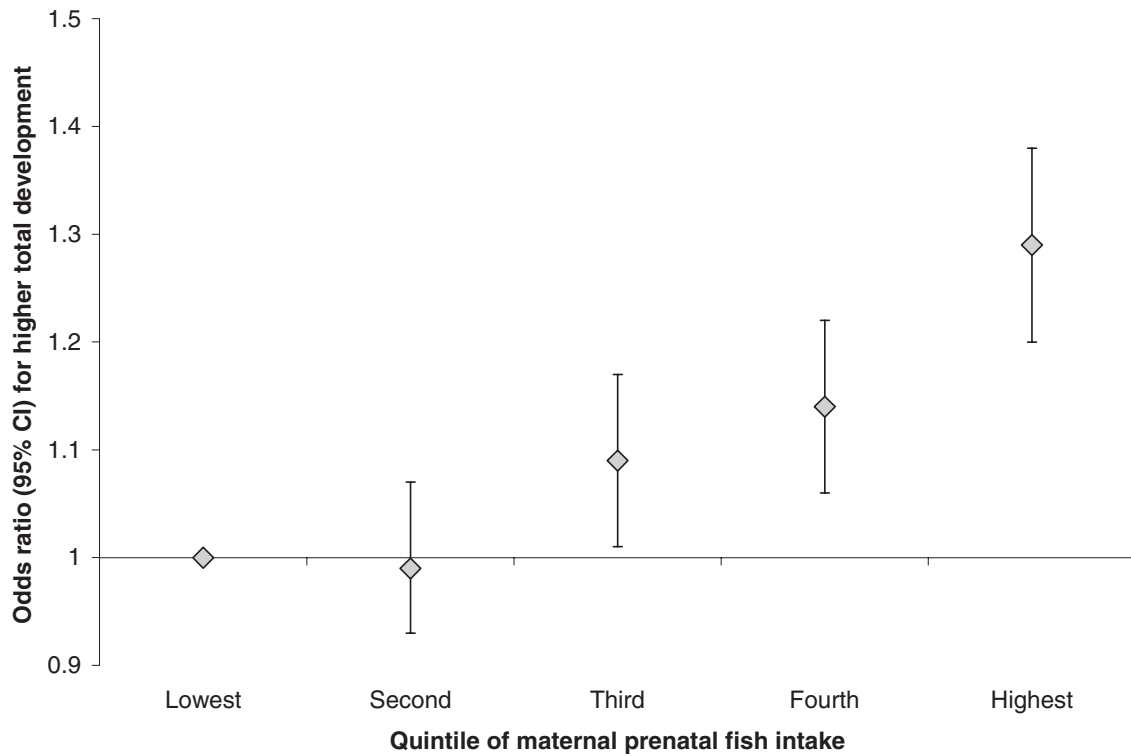
There have been EARNEST publications on parallel studies between Denmark and Norway on 'the possible protective effect of Mediterranean diet on preterm risk' (Haugen et al. 2008) and 'the effect of physical activity in pregnancy and risk of pre-eclampsia' (Mikkelsen et al. 2008). The most important conclusion is that it is possible to undertake complicated, tightly coordinated analyses based on both datasets.

FISH INTAKE AND PSYCHOMOTOR SKILLS

Analysis of data on the maternal diet during pregnancy from the Danish national birth cohort (DNBC) revealed that maternal intake of fish improved psychomotor developmental indices in their offspring, such as being able to climb stairs, remove shoes and socks and orientate a book correctly during the first 18 months of infantile life (Oken et al. 2008). Interestingly this effect was independent of the observed beneficial effect of breast feeding (see Figure 7).



FIGURE 7: ASSOCIATION OF PRENATAL FISH INTAKE WITH TOTAL DEVELOPMENTAL MILESTONES ATTAINED BY CHILDREN AGED 18MO N=25446 CHILDREN IN THE DNBC. ODDS RATIOS ADJUSTED FOR MATERNAL CHILD AND PARENTAL SOCIODEMOGRAPHIC CHARACTERISTICS AS WELL AS FOR BREAST FEEDING DURATION (OKEN ET AL. 2008; WITH PERMISSION OF AJCN).



What has EARNEST achieved so far in its animal, cellular and molecular studies in Theme 3?

In Theme 3, lifetime effects of early nutrition are studied with animal, cellular and molecular techniques. Specific questions in relation to metabolic programming have been addressed using well defined dietary interventions during specified pre or postnatal periods. The majority of the experimental work within Theme 3 has been completed and a substantial number of key papers have been published

POSTNATAL CATCH-UP GROWTH AFTER FETAL PROTEIN RESTRICTION

This research area has recently been reviewed (Ozanne 2009) with focus put on the EARNEST funded results

In a paper entitled “Postnatal catch-up growth after fetal protein restriction programs proliferation of rat preadipocytes”, EARNEST funded authors reported that fetal protein restriction, followed by catch-up growth, induced an increased perigonadal fat mass and programmed a higher capacity for preadipocytes to proliferate (Bol et al. 2008).

A second paper reported that, at 9 months, male offspring who had experienced forced catch-up growth after fetal protein restriction featured an increased relative fat mass, hyperglycaemia, hypercholesterolemia and hyperleptinemia showing, therefore, a higher predisposition to obesity. Significant alterations in the expression of genes playing a role in the differentiation and function of adipose tissue involved in lipid metabolism were also demonstrated (Bol et al. 2009).

LONG-TERM OUTCOMES OF EXPOSURE TO MATERNAL NUTRIENT RESTRICTION AT DIFFERENT STAGES OF GESTATION

Several papers relating to the long-term outcomes of exposure to maternal nutrient restriction at different stages of gestation with regard to the adverse responses following exposure to an obesogenic environment have been published. The major findings have been covered in an invited review which has just submitted to a major journal. This concludes that clinically relevant adaptations, such as primary markers of the metabolic syndrome, are only seen if a nutritional challenge ‘in utero’ is followed by a period of accelerated postnatal growth early in the postnatal period and/or if the offspring become obese.

THE INTERGENERATIONAL CYCLE OF OBESITY

The EARNEST group at King’s College, London investigated whether maternal obesity during pregnancy can programme an increased susceptibility to obesity in their children and so lead to what has been called “an intergenerational cycle of obesity”. They carried out a study on the effects of maternal obesity in mice and found that, at 4 to 6 weeks, the offspring of the obese mothers ate more than the offspring of the non-obese controls despite both being offered the same diet (Samuelsson et al. 2008). By 3 months they had lower levels of physical activity, were fatter, had increased insulin levels and had greater endothelial dysfunction and by 6 months they had increased blood pressure and increased glucose levels. Mater-

nal obesity in the developing mouse, therefore, led to adult offspring adiposity and cardiovascular and metabolic dysfunction.

EFFECTS OF EARLY NUTRITION PROGRAMMING ON BREAST CANCER

Another EARNEST group in Cambridge has looked at the effects of early nutrition programming on breast cancer. Breast cancer risk is increased in women with both low and high birth weight and the suggestion is that poor early growth of the mammary tissue, followed by compensatory growth increases future susceptibility to breast cancer. They carried out a study in which they gave rats an isocaloric low protein diet during pregnancy and lactation (Fernandez-Twinn et al. 2007). The offspring of the low protein dams had poorer initial mammary growth, followed by compensatory growth after 4 weeks and had an increased incidence of early-mammary tumours at 4 months. The period of compensatory growth coincided with increased protein expression of receptors to insulin, IGF-1 and oestrogen.

OTHER INTERESTING NEW FINDINGS

Although final conclusions cannot yet be drawn from many of the studies, several very interesting new findings have emerged:

- administering an antihypertensive drug during the suckling period in rats can alleviate some of the adverse programming effects of maternal undernutrition.
- the importance of adipose tissue in the programming of adult disease eg Fat mice mothers programme fat offspring (Nivoit et al. 2009).
- a high carbohydrate diet given to rats during the weaning period showed programming effects smaller than those seen during the perinatal period
- fetal nutrient restriction in sheep during the period of brain, heart and kidney development has an effect on mitochondrial proteins postnatally (Yakubu et al. 2007).
- folate supplementation may normalise adverse effects on fetal growth in rats against the background of a maternal low protein diet.
- offspring of obese mice show alterations in candidate genes involved in adipogenesis, angiogenesis and inflammation..

The importance of nitric oxide as a signalling me-

chanism for the development of hypertension (Koeners et al. 2007).

- an anti-adipogenic effect of feeding docosahexaenoic acid during gestation in guinea pigs (Aprikian et al. 2007)
- maternal nutrient restriction in pigs causes a long term programming of increased FTO gene expression in the obese offspring

Smaller piglets in a litter have different levels of steroid-metabolising enzymes (McNeil et al. 2007)

Maternal micronutrient restriction during pregnancy increases blood pressure in the offspring (Gambling et al. 2005) and the data suggest that the effect is generated early, rather than late, in pregnancy (Andersen et al. 2006). Interestingly, these data contrast with the low protein data discussed above. This indicates that there are still many things we need to learn about programming and the crucial role of animal experimentation in the programming field.

INTERACTIVE STUDIES BETWEEN THEMES

One of the wider aims of the EARNEST project is to promote interdisciplinary interaction within the consortium. One successful example was between Themes 2 and 3. Data from Theme 3 suggested that maternal iron deficiency resulted in increased blood pressure in the offspring of mice (Gambling et al. 2003). This is particularly important as iron deficiency during pregnancy is common, even in developed societies. Whether the effect was also seen in women was tested using the ALSPAC cohort (Brion et al. 2008). The results did not support the animal studies. At least using the measures available, the data suggested that low iron in the mothers resulted in decreased, not increased, blood pressure in the offspring. There are several possible explanations for the disparity, but the results are particularly important as they point the way to further hypotheses and pathways forward.

EARNEST also pioneered interactions between EC funded projects. Part of the aims of Theme 3 was to utilise the skills available in the Nutrigenomics Network of Excellence. Scientists from EARNEST collaborated with NuGO bioinformaticists, who helped them analyse DNA arrays, interpret the results and then develop further ideas and hypotheses. This was a novel and very effective interaction, resulting in several publications (e.g. (Brion et al. 2008; de Roos and McArdle 2008; Gambling et al. 2008; Fosset et al. 2009; Gambling et al. 2009), with others to follow)

What has EARNEST achieved so far in its consumer science studies in Theme 4?

There has been excellent progress in studying the consumer perceptions and understanding related to the practical implementation of new knowledge about health effects of maternal and infantile nutrition.

Studies in EARNEST Theme 4 have found that the concept of programming by early nutrition is widely acknowledged among scientists and recognized by governmental bodies, but it is not yet reflected in their documents and activities such as in communications aimed directly at parents or in policy documents for professionals (von Rosen-von Hoewel et al. 2007) (Martin-Bautista et al. 2009).

There are also clear inter-country differences in health related behaviours of mothers, infant feeding intentions, and the views of mothers about the relative importance of infant diet on adult health (Schmid et al. 2009). Almost all mothers (95%) agreed that the way they fed their babies was important for their health in their first year of life. However, when mothers were asked about specific long term health conditions such as obesity, high

blood pressure and cancer, they tended to think early diet was less important suggesting that they are not so clear about how early diet might impact later health.

The EARNEST researchers hope to be able to identify gaps in new mothers' understanding of the importance of early diet in different countries and recommend guidance on what sort of advice should be given in each country. The mothers have been followed up when their babies were 8 months old to ask them about their milk feeding and weaning experience; the data is now being analysed.

What has EARNEST achieved so far in its economic studies in Theme 5?

The health economic effects of early nutrition were studied in Theme 5, showing the (monetary) benefit for the society that can be gained by appropriate infant nutrition. The health-economic benefit of the short term lowering of blood pressure after the inclusion of long chain polyunsaturated fatty acids (LCPUFA) into infant formulas and the corresponding lowered risk of hypertension related diseases in later life, were analyzed in a Markov-Model.

This Model (see table 2) shows that the incre-

mental effectiveness of the LCPUFA supplementation is 1.20 quality adjusted life years (Qalys) in comparison with the standard-formula. In terms of cost effectiveness, the LCPUFA 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.

TABLE 2: INCREMENTAL COST EFFECTIVENESS OF LCPUFA IN INFANT FORMULA

	COST (C)(EURO)	INCREASED COST (EURO)		INCREASED EFFECTIVENESS (QALY)	C/E (EURO / QALY)	INCREASED C/E (ICER)
LCPUFA formula	3,250.37		72.303		44.955	
standard formula	3,879.96	629.58	71.099	-1.204	54.571	(Dominated)

The conclusion of this health economic evaluation is that the supplementation of formula with long chain polyunsaturated fatty acids represents an economically worthwhile prevention strategy. So the intervention leads to an economic and medical improvement in comparison to the status quo with the standard formula for infants. So the supplemented formula is the more cost-effective

infant nutrition strategy (Straub et al. 2009).

In the final part of the project, EARNEST researchers will evaluate the economic effects of the lowered obesity risk in later life after improved infant nutrition and compare it with the previous results.

What has EARNEST achieved so far in its demonstration studies in Theme 6?

EARNEST's demonstration projects in Theme 6 are looking at the short term and long term health benefits of adding prebiotics or a biotechnologically produced breast milk lipase into infant formulas and formulas for preterm infants. As these are new clinical trials that have started during Earnest project, results will only be available at the end of the project.

PREBIOTICS

Prebiotics are non-digestible food ingredients that selectively stimulate growth and/or activity of a limited number of beneficial bacteria (bifidobacteria and lactobacilli) residing in the colon, thereby positively affecting host health. Evidence is growing that they have the potential to positively influence the development of the immune system, but there are many details still to be clarified. Within

EARNEST a long-term study is being performed to determine the effect of prebiotics, more specifically of inulin-type fructans, on the incidence of infections and other immune parameters (early development of the immune system) in infants.

BILE SALT STIMULATED LIPASE

The addition of bile salt stimulated lipase (BSSL) to formula for preterm infants also has the potential to benefit the infant in the short and long-term. BSSL is provided by human breast milk and absent from the currently marketed formulas. A clinical trial to evaluate the effects of BSSL on fat absorption in preterm babies has been conducted. Assuming a favourable outcome of this pilot trial within EARNEST, further clinical trials would have to be initiated to fulfil the requirements for a market introduction.

What has EARNEST achieved so far in Dissemination in Theme 7

DISSEMINATION BEYOND SCIENTIFIC JOURNALS

Theme 7 is devoted to dissemination and exploitation so that these findings can be efficiently disseminated beyond scientific publication (Ashwell and Claessens 2005). These include brochures, newsletters, a number of presentations to health professionals, and press releases to the media. These dissemination activities have achieved widespread communication about the results of EARNEST, the personnel involved and the role of the EU in funding the project.

One of the EARNEST website's special features is a searchable publications database which so far contains over 375 recent publications in the field of early nutrition programming, many of which are by members of the EARNEST consortium.

EARNEST scientists have participated in a variety of activities, ranging from "Café Scientifique" sessions to discussion debates and public awareness events. As a grand finale to all the dissemination efforts, EARNEST will take part in the scientific programme of the Eurosciences Open Forum (ESOF) in Turin in July 2010- acknowledged as the most important scientific dissemination event throughout Europe.

MEDIA RELATIONS AND COVERAGE

Each time an EARNEST paper is about to be published, the EARNEST media relations team issue a press release, they target it to media within their extensive database and then capture the media coverage on the paper.

During the last year (2008-9) this strategy has produced 93 press 'cuttings'. These have appeared in 16 different countries including Africa, Brazil, Czech Republic and USA; there have been 14 Cor-dis uploads in 4 languages and 47 online agency uploads (international) including AlphaGalileo, Reuters, Science Daily, EurekAlert. There has been coverage on 19 mainstream media channels including BBC, EUFIC, Le Razon, Telegraph & Daily Mail (UK), and there have been articles in 14 professional/specialist channels including Nursing in Practice, Politiken, MerckSource, Nutrition Bulletin, and Public Service Review.

A NETWORK OF EU DISSEMINATORS STRENGTHENS LINKS BETWEEN EU PROJECTS

The EARNEST dissemination team have taken a major role in an organisation called COMMNET which is a network of scientific communicators from EC funded projects. COMMNET is currently planning the EU Food Science Day to be held in Brussels in November 2009. Experts will discuss topics such as the melamine case, swine influenza, food allergies, nutrition in pregnancy for life-long health, and the impact of infant-feeding on obesity where EARNEST will be represented. This event is therefore a collective effort of ten research networks within CommNet and together they represent more than 2000 European researchers. The goal on this EU Food Science Day is to spread all this important scientific knowledge to a wider audience, including influential stakeholders and policy makers.

What has EARNEST achieved so far with Exploitation in Theme 7

WHO ARE EARNEST'S STAKEHOLDERS?

The Dissemination and Exploitation Consensus Panel (DECP) is a panel of experts drawn from areas with relevant expertise who advise EARNEST on how the results from the project can best be disseminated and exploited and their uses maximised. They have met annually and have discussed how to communicate the results from the project more widely to different groups of stakeholders; they have also considered what drivers and trends will influence the uptake of the EARNEST outcomes by the stakeholder groups (Ashwell and de la Hunty 2009).

The DECP has also discussed health claims extensively since many of the scientific results from EARNEST could be used by the food industry, particularly the infant food industry, on the labels of their products and in their advertising. There is now clear guidance

from EC/EFSA about the scientific evidence required for health claim submission and sufficient EC opinions on submitted claims have now been published to give indications of the type and extent of evidence which can justify approval for the health claim.

HEALTH CLAIMS POSSIBLY ARISING FROM EARNEST RESEARCH

At its 2008 meeting, the DECP discussed 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. They were asked to suggest what alternatives to claims could be used if there were too many negative aspects to making a health claim. The DECP reached a consensus opinion and the results of this discussion have been tabulated in table 3.

TABLE 3: DOES AN EC/EFSA TYPE CLAIM HELP ME TO COMMUNICATE A HEALTH BENEFIT IN A FOOD?– DECP DISCUSSION- VIEWPOINT AS SEEN FROM INDUSTRY PERSPECTIVE AND FROM HEALTH PROFESSIONAL PERSPECTIVE.

INDUSTRY			HEALTH PROFESSIONALS		
YES	NO	ALTERNATIVES	YES	NO	ALTERNATIVES
Will increase market share	Will not differentiate my product if only generic claims can be used	Go for product specific health claims	Will give short cut to get confidence that health claims is backed by good science	Might not realise the credibility of the EFSA process	Need EC/EFSA/national authorities to promote the strengths of the authorisation system.
Opportunities for innovation even with generic	Will not have exclusive rights		Will help to support the concept that nutrition	Might switch the attention away from the balanced	
Should help to persuade consumers to buy	Other companies may make inferior copy product and use same health claims	Lobby for better characterisation of functional ingredients with health claims	Will help to educate consumers on importance of nutrition	Health professionals could be seen to supporting industry if they promote health claims	However good the scheme, might give ammunition to lobby groups to say the EC in pocket of industry.
Should help to influence health professionals (short cut)					
Protection of patented ingredients	Financially expensive to produce evidence, dossiers, alter composition	Self regulation instead. Rely on other companies to report over-claiming	EFSA is a credible source and can be trusted – give greater confidence	They emphasise one diet-health link and do not put it into the context of the whole diet. They are not a substitute for nutrition/dietary education	Clearer, more specific, dietary advice for the weaning period is needed
Competitive advantage greater profit and mark up	Could have counter-productive effect – people see it as a magic bullet and don't need to change diet. People become cynical if there are too many claims	Have more detailed food based dietary guidelines to help put individual claims in context	Saves having to review individual studies themselves	They distract from the breast is best message and might give false reassurance	
Safety for industry – v sensitive area	Will not differentiate my product if only generic claims can be used	Market products on other aspects – cost, taste etc	Can help to summarise evidence for educating parents	People might feel they have to spend more than they need do on buying expensive products	
Distinguishes product			Must be put in perspective though – they are to protect consumers from misleading claims, they are not instructions or recommendations	They have to be interpreted and put into context of whole diet	
Stimulates innovation and research			Makes link between dietary advice to increase or decrease something and food more obvious	Wording, if too specific, might not be understood by parents. Are more general claims more helpful?	
Communicates to gatekeepers – more likely to be trusted by health professionals					
Provides guidance to industry on product composition – spreads improvements					
Can customise claim to appeal to children, parents, health professionals					

What has EARNEST achieved so far with training in Theme 8?

SUMMARY

Training activities cover a wide span. For example, some are devoted to ensuring that EARNEST researchers are using similar techniques for common methodologies; others help them to communicate their findings to a variety of audiences.

HIGHLIGHTS OF TRAINING SESSIONS

The Early Nutrition Academy Symposium, held in Granada in April 2008, on “Demonstrating Early Programming in Human and Animal Models” attracted more than 140 scientists. The symposium was held in between the project meetings of “EARNEST” and the Framework 7 “Nutrimenthe” project and thus provided a great opportunity to exchange and disseminate latest findings (Campoy and Koletzko 2009).

“Pregnancy and Programming” was the theme for the smaller Early Nutrition Academy Winter training School, held in the Sierra Nevada, in February 2009. It attracted 23 delegates and was well received. As well as communicating the science associated with the subject matter, delegates also learned how to communicate to media and trial participants as well as learning how to get a scientific paper published and how to apply for research funds.

CONCLUSIONS

1. In conclusion, the project has made very good progress over the past four years and there are good reasons to be confident that the further

project objectives can be met according to the schedule during the final eighteen months. Furthermore, the “Early Nutrition Academy”, which will pursue the aims of the EARNEST consortium after the duration of the project, continues to demonstrate its inherent potential to support the wider objectives of EARNEST.

Table 4: summarises the major training activities in EARNEST from 2005 until 2009.

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TABLE 4: HIGHLIGHTS OF EARNEST TRAINING

DATE	PLACE	TITLE/TOPIC/EVENT
2005	UK	Workshop in Consumer Science
2005	Spain	Protein and prebiotic Workshop: The role of proteins in infant health and development (ORDESA+AREXIS), Inulin and oligofructose as functional ingredients to improve health
2005	Spain	Gender Workshop
2005	Spain	Internal Website Workshop
2005	Netherlands	Workshop: Neurodevelopmental testing at school age
2005	UK	Short Course: Communicating and Disseminating Research Findings (UNIVBRIS)
2005	Netherlands	Workshop: Neurodevelopmental testing (Hempel) at preschool age (UMCG)
2005	UK	Short Course: Design and Analysis of Randomised Controlled Trials (UNIVBRIS)
2006	Belgium	Anthropometric Workshop
2006	Belgium	Media Training
2006	UK	Consumer Science
2006	Germany	Workshop: Methods of nutrition physiology: Energy and nutrient metabolism using indirect calorimetry and stable isotope techniques in animals
2007	Australia	Earnest Session at DOHaD
2007	Czech	Inter-theme collaboration workshop
2007	Czech	Training courses: Measurement of the Bioelectrical Impedance Vector; Physical Activity and Lifestyle Questionnaire, Culture Fair Intelligence Tests - Scale 1
2007	Germany	Methods for the investigation of energy and substrate metabolism in pigs and mice
2007	Poland	Intima Media Thickness (IMT) training
2007	Belarus	PROBIT III Training Week
2007	Austria	COSPI WORKSHOP Combating Obesity: Strategies for Prevention and Intervention - an Erasmus Intensive Programme)
2008	UK	Training on "Current Techniques in Measuring Body Composition"
2008	Denmark	Basic Principles of Epidemiology
2008	Spain	Unlocking your potential for fame and fortune: press releases, presentations and proposals - everything you need to know about scientific communication
2008	Spain	Physical activity measurement in children in free living conditions. Method: Body monitoring by ArmBrand (Pro2) Sense Wear of Bodymedia®
2009	Poland	ESPGHAN Nutrition Summer School: Paediatric Nutrition: An Evidence-Based Approach
2009	Germany	Cross-Theme Workshop on assessing brain function and behaviour
2009	Spain	ENA Granada Winter School: International Course „Pregnancy and Programming

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