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The Winter Meeting of the Nutrition Society, an international conference to mark the 100th anniversary of the birth of Elsie Widdowson, hosted by MRC Human Nutrition Research Cambridge jointly with the Neonatal Society supported by the Royal Society of Medicine was held at Churchill College, Cambridge on 11–13 December 2006

Symposium on 'Nutrition in early life: new horizons in a new century'

Session 6: Infant nutrition: future research developments in Europe EARNEST, the early nutrition programming project: EARly Nutrition programming – long-term Efficacy and Safety Trials and integrated epidemiological, genetic, animal, consumer and economic research

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Increasing evidence from lifetime experimental studies in animals and observational and experimental studies in human subjects suggests that pre- and postnatal nutrition programme long-term health. However, key unanswered questions remain on the extent of early-life programming in contemporary European populations, relevant nutritional exposures, critical time periods, mechanisms and the effectiveness of interventions to prevent or reverse programming effects. The EARly Nutrition programming – long-term Efficacy and Safety Trials and integrated epidemiological, genetic, animal, consumer and economic research (EARNEST) consortium brings together a multi-disciplinary team of scientists from European research institutions in an integrated programme of work that includes experimental studies in human subjects, modern prospective observational studies and mechanistic animal work including physiological studies, cell-culture models and molecular techniques. Theme 1 tests early nutritional programming of disease in human subjects, measuring disease markers in childhood and early adulthood in nineteen randomised controlled trials of nutritional interventions in pregnancy and infancy. Theme 2 examines associations between early nutrition and later outcomes in large modern European population-based prospective studies, with detailed measures of diet in pregnancy and early life. Theme 3 uses animal, cellular and molecular techniques to study lifetime effects of early nutrition. Biomedical studies are complemented by studies of the social and economic importance of programming (themes 4 and 5), and themes encouraging integration, communication, training and wealth creation. The project aims to: help formulate policies on the composition and testing of infant foods; improve the nutritional value of infant formulas; identify interventions to prevent and reverse adverse early nutritional programming. In addition, it has the potential to develop new products through industrial partnerships, generate information on the social and economic cost of programming in Europe and help maintain Europe's lead in this critical area of research.

EARNEST: Early nutrition programming project: European Union

There is increasing evidence that health and development in adult life are influenced or 'programmed' by factors operating during fetal life and infancy. Whilst the general concept of programming has been recognised for several centuries, evidence that nutrition could operate as a programming stimulus or insult is more recent. The pioneering work of McCance and Widdowson in the 1960s demonstrated in animals that nutrition could act during critical windows early in life to affect long-term outcomes. For example, it was found that rats raised in small litters,

Abbreviation: EARNEST, EARly Nutrition programming – long-term Efficacy and Safety Trials and integrated epidemiological, genetic, animal, consumer and economic research.

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and therefore overfed early in postnatal life, develop greater body size as adults (McCance, 1962). Subsequently, Hahn (1984) has shown that rats overfed in the brief suckling period have higher plasma insulin and cholesterol concentrations, while Lewis and Mott (Lewis *et al*. 1986, 1988) have shown that early nutrition in baboons has a major impact on later obesity (Lewis *et al*. 1988) and atherosclerosis (Lewis *et al*. 1986). There is now overwhelming evidence that early nutrition in a variety of animal species, including primates, can influence later CVD, including all components of the metabolic syndrome, learning and behaviour, intermediary metabolism, gut function, bone health, immunity and longevity (Smart, 1986; Dobbing & Sands, 1971; Desai *et al*. 1995; Hales *et al*. 1996; Symonds & Gardner, 2006).

Until recently, evidence of nutritional programming in human subjects has come largely from historical observational studies that have shown associations between small size in early life and adult disease risk (Barker *et al*. 1993; Barker, 1995; Frankel *et al*. 1996; Eriksson *et al*. 1999). These cohorts have been constructed from available maternal or child health records. They have necessarily relied on indirect measures of maternal and infant nutrition (rather than direct measures of maternal or infant diet) and have often lacked detailed data on potential confounding variables. Many of the cohorts were born before the first half of the 20th century, and it is possible that the nature and size of any associations are different in contemporary European populations. While these studies have generated considerable interest, they have been unable to examine direct associations with diet or establish whether associations are causal. Hence, they cannot be used to underpin infant feeding recommendations.

More recently, evidence for nutritional programming has been obtained in experimental studies in human subjects. These studies have demonstrated a causal relationship between nutrition in infancy and later outcomes, including cognitive function (Lucas *et al*. 1989, 1990, 1998) and cardiovascular risk factors (Singhal *et al*. 2001, 2002, 2003). Moreover, the data suggest that the effect size for the influence of early nutrition on later health outcomes is likely to be large. For example, the reported effect of early growth and nutrition on later diastolic blood pressure (effect size approximately 3–4 mmHg) is greater than all other non-pharmacological means of reducing blood pressure such as weight loss, salt restriction or exercise. These data must be considered in the context of the finding that lowering population-wide diastolic blood pressure by only 2 mmHg would be expected to reduce the prevalence of hypertension by 17%, the risk of CHD by 6% and the risk of stroke or transient ischaemic attacks by 15%, and prevent 100 000 vascular events annually among those aged 35–64 years in the USA alone (Cook *et al*. 1995).

Despite rapid progress in the field of programming research over the past decade, 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.

The EARly Nutrition programming – long-term Efficacy and Safety Trials (EARNEST) Project aims to address these issues, using an integrated approach that brings together work from robust experimental studies in human subjects backed by modern prospective observational studies, and mechanistic animal work including physiological studies, cell-culture models and molecular techniques.

The remainder of the present review will cover:

1. the rationale for an integrated approach to nutritional programming research;
2. a description of the component themes of the EARNEST project, with emphasis on those covering research in human subjects. Animal research will be covered in a subsequent paper (Symonds, 2007);
3. integration within the project.

Rationale for an integrated approach to nutritional programming research

As described earlier, evidence for nutritional programming has come from three types of study: animal studies; human epidemiological studies; human experimental studies. Each approach has its advantages and disadvantages, which are summarised in Table 1.

Randomised controlled trials

Randomised controlled trials in human subjects are regarded as methodologically the best approach for underpinning health policy. They equalise unknown as well as known confounders and so can establish causation, they permit quantification of effect size and so can be used to estimate economic benefits and they can detect and quantify adverse effects and thus address safety aspects. Nevertheless, in the context of nutritional programming of disease later in life, they have certain limitations. Many of the disease outcomes will not become apparent for decades, necessitating the use of 'proxies' of later disease risk that can be measured at younger ages. In addition, there is an increasingly-apparent problem of loss to follow-up as the cohort ages, which has implications for study design and initial sample size in such studies.

Historical cohort studies

Historical cohort studies have the advantage of generating rapid results, and often allow investigation of early-life factors and disease end-points (e.g. occurrence of, or death from, IHD). However, the quality of data from pregnancy, infancy and childhood, in particular nutritional exposures, may be suboptimal since the cohorts were not designed for this purpose. Furthermore, since many of these cohorts are from previous generations, the generalisability of the results to modern populations may be questioned.

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Table 1. Advantages and disadvantages of different approaches to investigating nutritional programming

	Advantages	Disadvantages
Animal studies	Short; lifetime studies possible Wide range of interventions possible Availability of tissue, organs and cells Generation of concepts, putative mechanisms and hypotheses to test in human subjects	Questionable extrapolation to human subjects
Human observational studies		
Historical retrospective	Rapid results Disease end-points available Large sample size	Suboptimal data on early nutritional exposures and potential confounders Cannot demonstrate causality of relationships between early exposures and later outcomes Generalisability to modern populations uncertain
Modern prospective	Good records of early nutritional exposures and measurement of confounders Large sample size	Long follow-up required to study disease end-points; need to use proxies for risk of some diseases Cannot demonstrate causality of relationships between early exposures and later outcomes
Human experimental studies	Interventions randomised so can demonstrate causality and effect size, and can be used to underpin policy	Long follow-up required to study disease end-points; need to use proxies for risk of some diseases Potential loss to follow-up with time (implications for initial sample size)

Prospective observational studies

In contrast to historical cohort studies, modern prospective observational studies identify defined (generally large) populations, measure them precisely and follow them up longitudinally. They therefore have better measures of exposure and confounders than historical cohort studies. Of particular relevance to programming research, prospective cohort studies may include very detailed early data, including physiological, biological and social information on the subjects, and are thus better able to control for known confounding factors. Moreover, more-recent prospective studies have contemporary relevance and are not investigating practices and environmental conditions relevant 60–80 years ago. However, these modern cohorts share some of the limitations of experimental intervention trials in terms of the need to use proxies for later disease risk, and cohort attrition with longer-term follow-up.

Animal studies

Animal studies allow rapid exploration of the lifetime consequences of interventions, using a greater variety of nutritional interventions than may be possible in human subjects, with easier collection of tissue and organ samples. They therefore enable more comprehensive investigation of underlying mechanisms of nutritional programming and better identification of the precise developmental windows in which global or specific nutritional interventions contribute to later disease. However, the extrapolation of findings to human subjects may be questioned.

EARly Nutrition programming – long-term Efficacy and Safety Trials

It is clear from these considerations that considerable benefits can be derived from adopting an approach that combines the strengths of these different types of study. Using this approach, the EARNEST project aims to:

1. test early nutritional programming of adult disease risk in human subjects by measuring disease markers in early adulthood in well-conducted randomised controlled trials of specific nutrition interventions in pregnancy and infancy;
2. estimate 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. These observational studies will: allow identification of dietary exposures that can be explored in animal models and eventually tested in future trials in human subjects; study mechanism; confirm whether findings from laboratory studies translate into diet–risk associations in free-living human subjects;
3. use animal, cellular and molecular techniques to study lifetime effects of early nutrition. These studies will seek to refine models of nutritional programming in order to identify mechanisms and critical periods in development when the fetus or infant is most susceptible to nutritional influences. These studies will inform the analyses conducted in the observational studies and help prioritise future trials in human subjects;
4. use developments in functional genomic techniques to further explore the basis of early nutritional programming in clinically-relevant model systems and in the prospective cohort studies (all of which will have collected and stored biological samples).

The EARNEST consortium consists of a multi-disciplinary team of thirty-eight partner institutions from seventeen EU

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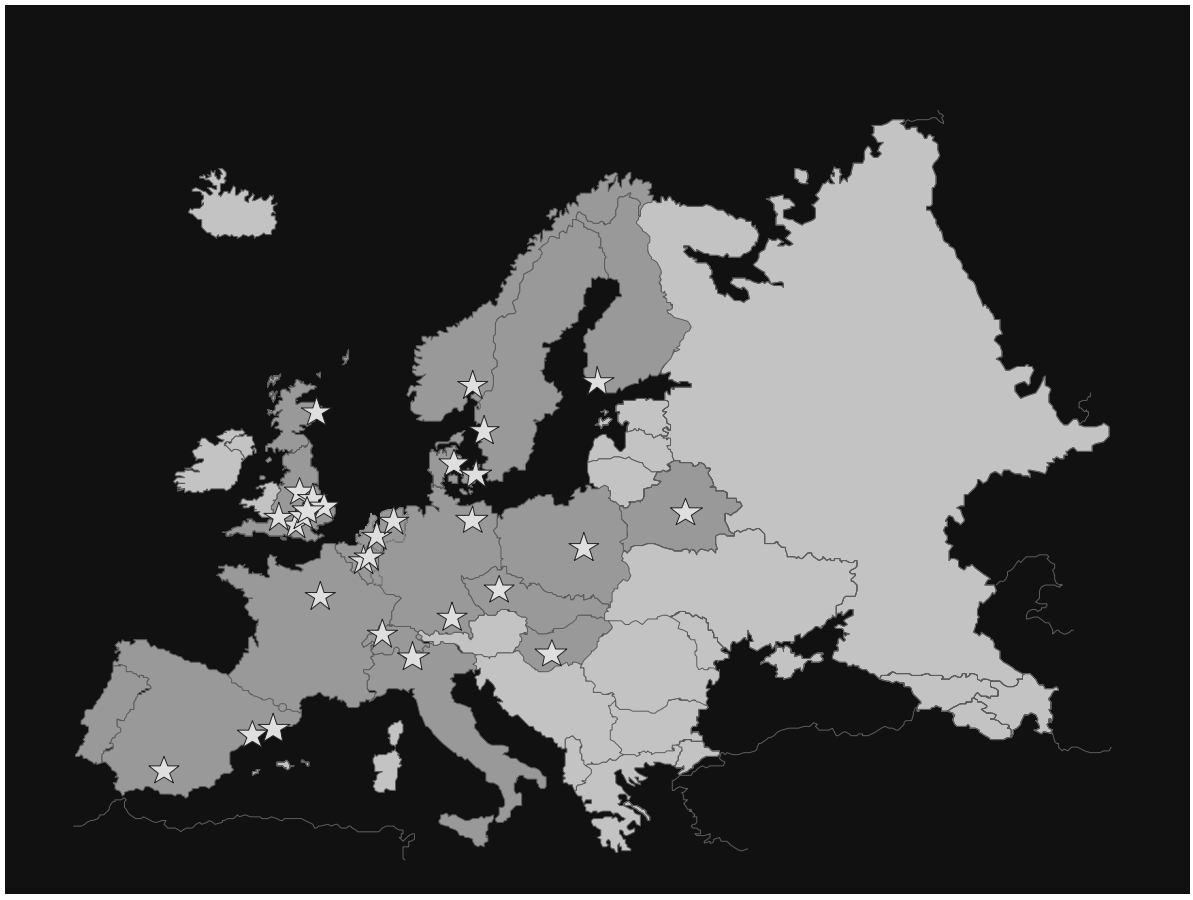

Fig. 1. Location of EARly Nutrition programming – long-term Efficacy and Safety Trials (EARNEST) partners.

Table 2. Component themes in the EARly Nutrition programming – long-term Efficacy and Safety Trials (EARNEST project)

1. Long-term health outcomes of major intervention trials of early nutrition
2. Epidemiological studies to investigate early nutrition programming
3. The mechanism of early nutritional programming
4. Consumer attitudes to issues relating to early nutrition programming
5. Economic- and public health-impact studies
6. Demonstration projects
7. Communication and outreach
8. Training
9. Management and integration

countries (Fig. 1), with a coordinating centre in Munich, Germany. The project commenced in April 2005 and will run for 5 years.

Components of the project

The project consists of nine themes (Table 2). Biomedical studies are complemented by studies of the social and economic importance of programming, and important themes encouraging integration, communication, training and wealth creation. The focus for the remainder of the present review is on those themes involving studies in human subjects.

Theme 1: long-term health outcomes of major intervention trials of early nutrition

The gold standard for underpinning modern practice is the randomised intervention trial. The process of randomisation, given adequate trial size, statistically equalises the distribution of both known and unknown confounders between randomised groups, so that any group differences may reasonably be inferred to be causally related to the intervention. This inference remains valid even for long-term follow up, since with randomisation any post-randomisation influences on the outcome (other than differential effects of the intervention itself) should again be equally distributed between groups. Intervention trials in the field of nutrition are now conducted with the same rigour as pharmaceutical trials. This approach was pioneered for the first experimental studies of nutritional programming in human subjects >20 years ago. Such trials examine both efficacy and safety.

In general, intervention studies are now regarded as methodologically the best approach for establishing health practices. In the case of infant nutrition, a large proportion of European infants are no longer being breast-fed by 3 months and infant formula is recommended for ≤12 months in those infants not breast-fed. Thus, infant formulas are the commonest milk and food used in infancy, and many practical interventions in infancy in non-breast-fed infants inevitably involve manipulation of formula composition.

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Table 3. Summary of studies in theme 1: long-term health outcomes of major intervention trials of early nutrition

Study name or acronym	Intervention	Countries
Interventions in pregnancy		
NUHEAL	DHA with or without folic acid	Hungary, Spain, Germany
Gambian cohorts	Calcium Protein and energy	The Gambia
Arhus study	DHA	Denmark
Breast-feeding and formula interventions		
PROBIT	Breast-feeding promotion	Belarus
EU CHOP	Protein and energy	Germany, Poland, Belgium, Spain, Italy
GINI	Hypoallergenic formula	Germany
UK-CVD	Nutrient-enriched formula	UK
UK-bone	Nutrient-enriched formula Modified-fat blend	UK
LCPUFA supplementation		
Groningen	LCPUFA-supplemented formula	The Netherlands
UK-LCPUFA	LCPUFA-supplemented formula	UK
Prebiotics and probiotics		
NAMI	*Lactobacillus rhamnosus* GG	Finland
Three cohorts	Prebiotics	Italy, Belgium

LCPUFA, long-chain PUFA.

Regulatory authorities now expect formula interventions to be tested using efficacy and safety trials, and would regard observational studies as suboptimal.

The components ('work packages') in theme 1 use nineteen major European pregnancy and infant nutrition intervention trials (see Table 3), some of which now involve follow-up into early adult life, to examine experimentally whether early nutrition influences later obesity risk, CVD risk (blood pressure, insulin resistance, plasma lipid profile), endothelial function (an early stage of the atherosclerotic process), neuro-cognitive outcome, bone health and immune health. Interactions between nutritional interventions and genotype will also be examined. Interventions include the manipulation of macronutrients (e.g. the protein and energy content of maternal diet or infant formula) and specific nutrients such as long-chain PUFA, Ca and probiotics and prebiotics. Integration across the theme will be achieved by the use of many common outcome measures that will be standardised between studies. The aim is that the results of these studies will provide information on optimal maternal and infant nutrition and will allow the formulation of evidence-based nutrition recommendations together with the development or appropriate modification of products for pregnant women and infants.

Theme 2: observational follow-up studies with prospectively-collected information on early diet

Historical observational studies using measures of size at birth and in infancy (as proxy measures of maternal and infant diet) have suggested that nutrition in early life programmes adult disease risk. Some observational and experimental studies (Barker, 1995; Lucas *et al*. 1998; Singhal *et al*. 2001, 2002) have reported associations between direct measures of maternal or infant diet and subsequent disease risk, independent of effects on measures of size. Trials in human subjects are required to establish that observational associations are causal. However, there is a limit to the number of trials that can be conducted, and some experiments in human subjects are not possible because they are not feasible or ethical. There is therefore a need to follow-up observational studies with detailed information on diet in pregnancy and in early childhood to:

1. demonstrate that associations observed in historical cohorts born many years earlier persist in contemporary European populations;
2. describe associations between direct measures of early diet and adult disease risk to guide the design of experiments in animals and human subjects;
3. explore whether the results of animal experiments translate into associations in free-living human subjects;
4. estimate the public health burden of exposures shown to be causal in trials of human subjects;
5. study gene–diet interactions using stored DNA samples.

The work in theme 2 involves follow-up of the largest, most detailed and informative observational studies in contemporary Europeans to test the hypotheses that diet in pregnancy and early childhood predicts later cognitive function, obesity and blood pressure, and that the effect of diet in predicting later adult diseases risk is modified by, and in some cases entirely contingent upon, the underlying genotype. The cohorts participating in this theme are the Avon Longitudinal Study of Parents and Children, the Danish National Birth Cohort and the Norwegian Mother and Child Cohort Study. A further aim is to establish a European network of smaller cohorts that come from populations with different diets or populations with different confounding structures or that have unique dietary or outcome data (approximately 300 000 individuals from multiple European countries (Belgium, Denmark, The Faroe Islands, Finland, France, Germany, Greece, Italy, Norway, Spain, Sweden, The Netherlands, UK).

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Theme 3: animal studies

This theme is covered in a subsequent review (Symonds, 2007).

Theme 4: consumer attitudes to early nutrition programming

Given the importance of early nutrition programming, a major challenge is to promote appropriate infant feeding by parents. However, this objective cannot be accomplished unless both parents and health professionals understand basic infant nutrition and its implications for later health. Parents gain knowledge from a wide range of sources, including health professionals, the media, friends and relatives. The quality of this information may vary and may not necessarily be evidence-based. Little is known about whether the emergence of evidence of the relationship between early diet and later health has influenced the formulation and delivery of current advice on infant feeding practices in Europe.

The aim of the work in this theme is to identify the scope for improving parental knowledge about infant feeding and its long-term health implications, and to suggest means for achieving this objective. The research will collate and evaluate information on current feeding guidelines and advice in different EU countries, focusing particularly on the extent to which they reflect the health consequences of early nutrition, and aims to generate specific information that will allow the development of targeted services and advice to consumers. This work complements that of other studies within the EARNEST consortium, providing information on how research can best be translated into practical and usable guidelines for both health professionals and parents.

Theme 5: economic- and public health-impact studies: a comprehensive health economic analysis of pre- and postnatal nutritional interventions with programming effects on adult health

Health economic evaluation is a prerequisite to convince healthcare providers to implement research findings into national preventive and healthcare systems. These evaluations are innovative, since they will assess the impact of newly-identified preventive measures in a range of European Member States. Given that the interventions potentially provide protection against a variety of adult illnesses and positive long-term health development, healthcare providers, administrators and policymakers may be able to reduce their long-term medical costs by promoting and supporting the right measures.

The work in this theme will consist first of systematic reviews using available data on the validity of the presumed preventive effects from the interventions considered. Modelling of cost–effectiveness analyses, cost–utility analyses and cost–benefit analyses will be performed to decide whether the promotion and supporting of interventions is worthwhile. The costs and benefits will then be adjusted to include new information from the other parts of the project.

Scientific integration

A key aim of the work in this project, and perhaps its most challenging aspect in practice, is to integrate as far as possible the different components. This process is relevant at a number of levels, both between and within themes. For example, the exchange of results between partners involved in animal and human studies is important in order to enable maximum cross-fertilisation of ideas relevant to the design and analysis of ongoing studies. Integration is also important within themes. For example, within theme 1 the protocols and existing follow-up data of the studies are being compared to identify common interventions and outcomes that might usefully be combined for the purpose of hypothesis testing and meta-analysis. Second, many of the proposed follow-up studies to be conducted within themes 1 and 2 use common outcome measures. The identification and standardisation of these outcome measures across different studies at an early stage will facilitate pooling of data and meta-analyses, providing the statistical power necessary to detect meaningful effects of early nutrition on a number of aspects of long-term health.

Summary

The EARNEST project uses an integrated approach to investigate nutritional programming of later health and disease. Whilst each project is individually of high quality, the value of the integrated project is expected to exceed that of the individual parts. The project aims to help formulate policies on the composition and testing of infant foods, improve the nutritional value of infant formulas and identify interventions to prevent and reverse adverse early nutritional programming. Data of this nature are not available elsewhere and may have considerable importance for health policy in Europe, and also worldwide.

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